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Gene Note

Self-interaction of DcMYB1 proteins involved in stress-inducible *DcPAL1* expression

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Abstract DcMYB1 is a member of the R2R3-type MYB protein family in carrot and can activate expression of the *DcPAL1* gene induced by stresses such as the dilution effect, UV-B irradiation and elicitor treatment. To understand the regulatory mechanism of DcMYB1 we screened cDNA for proteins interacting with DcMYB1 as bait in a yeast two-hybrid system. Several positive cDNA, which were isolated as prey, encoded DcMYB1, indicating that DcMYB1 could interact with itself. The interaction of full-length DcMYB1 protein with partial DcMYB1 amino acid sequences derived from DcMYB1 cDNA deletion mutants was observed in yeast and carrot protoplasts, suggesting the self-interaction of R2R3-type MYB protein of DcMYB1 *in vivo*.

Key words: MYB, phenylalanine ammonia-lyase, protein–protein interaction, transcription factor.

Many MYB genes exist in plant genomes, e.g., 190 MYB-related genes have been found in the whole genome of *Arabidopsis* (Riechmann et al. 2000). The MYB gene family is classified into three types according to the number of imperfect repeats in the DNA binding domain; these include one-repeat (1R), two-repeat (R2 and R3) and three-repeat (R1, R2 and R3) types. The R2R3-type MYB subgroup forms the largest family among the three subgroups in plant genomes (Romero et al. 1998; Stracke et al. 2001; Dias et al. 2003). Some R2R3-type MYB proteins play important roles in controlling the expression of genes involved in secondary metabolism in tissues and organs during developmental processes, such as development of the seed epidermis and flower petals. Other R2R3-type MYB proteins are involved in gene expression, induced by abiotic and biotic stresses, that is mediated through signal transduction pathways from stress cues (Bodeau and Walbot 1992; Grotewold et al. 1994; Sugimoto et al. 2000; Vailleau et al. 2002; Abe et al. 2003). R2R3-type MYB proteins have been well studied in relation to the regulation of the expression of genes for enzymes involved in phenylpropanoid and flavonoid biosynthetic pathways regulating pigmentation in differentiated plant tissues and organs in accordance with developmental stages (Bodeau and Walbot 1992; Grotewold et al. 1994; Quattrocchio et al. 1999; Borevitz et al. 2000; Grotewold et al. 2000; Nesi et al. 2001). The C1 protein in maize, which was the first MYB protein identified in plants (Paz-Ares et al. 1987), regulates the expression of genes coding for the enzymes involved in the anthocyanin (flavonoid) biosynthetic pathway. C1 requires the unique partner, R, which is a basic helix-loop-helix (bHLH) protein, to up-regulate genes in the anthocyanin biosynthetic pathway (Roth et al. 1991; Grotewold et al. 2000). The close partnership between C1 and R indicates that the ectopic expression of C1 and R *in planta* induces the biosynthesis and accumulation of anthocyanins from the coordinated expression of most of the genes encoding enzymes involved in anthocyanin biosynthesis (Grotewold et al. 1998). Furthermore, in yeast, C1 could have transactivating activity when the R protein is coexpressed and interacts physically with C1 (Grotewold et al. 2000; Hernandez et al. 2004). A similar close partnership has been demonstrated between the MYB protein encoded by *TT2* and the bHLH protein encoded by *TT8* in *Arabidopsis thaliana* (Roth et al. 1991; Nesi et al. 2000; Nesi et al. 2001; Baudry et al. 2004). In contrast, the ectopic expression of the *PAP1* gene, which encodes the other MYB protein in *Arabidopsis*, without the expression of any partners, can promote the synthesis and accumulation of anthocyanin in whole plants by coordinated expression of most of the genes for the enzymes involved in the phenylpropanoid and

Abbreviations: AD, activation domain; BD, binding domain; bHLH, basic helix-loop-helix; *Dc*, *Daucus carota*; *luc*, firefly luciferase gene; PAL, phenylalanine ammonia-lyase; SD, synthetic dropout.
This article can be found at http://www.jspcmb.jp/

anthocyanin biosynthetic pathways (Borevitz et al. 2000). However, a recent report indicates that the PAP1 protein causes up-regulation of *TT8* gene expression (Tohge et al. 2005), and the interaction of PAP1 proteins with bHLH TT8 proteins has been reported in a yeast two-hybrid system (Zimmermann et al. 2004). These results imply that the transactivation of the genes for enzymes involved in anthocyanin biosynthesis by *PAP1* might require the TT8 protein as a partner. Furthermore, the duet of TT2 MYB and TT8 bHLH proteins requires another partner protein, the WD40 repeat protein of TTG1, to act as the most powerful trio in the chorus of transcription machinery that regulates the gene expression of enzymes involved in the phenylpropanoid and anthocyanin biosynthetic pathways (Baudry et al. 2004; Grotewold et al. 2000; Zimmermann et al. 2004). All these results suggest that the regulatory mechanisms of MYB proteins, for the transactivation of genes encoding enzymes involved in the phenylpropanoid and anthocyanin (flavonoid) biosynthetic pathways in plants, require other factor(s) to show full promoting activity.

DcMYB1 is an R2R3-type MYB protein of carrot. *DcMYB1* gene expression is rapidly induced at the transcriptional level in response to the dilution effect, UV-B irradiation and elicitor treatment in suspension-cultured carrot cells. DcMYB1 regulates *DcPAL1* gene expression induced by these stresses. The *DcPAL1* promoter region has four box-L-like sequences (boxes L0, L1, L3/4, L5), which are AC-rich elements that play an important role in the regulation of *DcPAL1* gene expression (Takeda et al. 1997; Takeda et al. 2002; Maeda et al. 2005). For the activation of transcription from the *DcPAL1* promoter in the transient expression system of carrot protoplasts, DcMYB1 does not require the coexistence of other partner factor(s), such as a bHLH protein (Maeda et al. 2005). However, by analogy with other MYB proteins that require partner(s) to show full transactivation activity, it is possible that other partner factor(s) exist that associate with DcMYB1 to induce full transactivation activity. Therefore, we have undertaken to isolate and identify proteins that interact with the DcMYB1 protein in carrot using a yeast two-hybrid system.

A carrot cDNA library in a pAD-GAL4.2-1 plasmid for prey was prepared with mRNA from suspension-cultured carrot cells treated with the dilution effect using the HybriZAP Two-Hybrid cDNA Gigapack Cloning Kit (Stratagene, CA, USA), as previously reported (Maeda et al. 2005). The Matchmaker Two-Hybrid System (Clontech, CA, USA) was used to screen cDNA encoding for proteins that interacted with DcMYB1. First, the entire coding sequence of DcMYB1 cDNA was amplified by polymerase chain reaction (PCR) and cloned into a bait construct plasmid pGBKT7 (Clontech). The pGBKT7 plasmid was then introduced into the yeast cell line AH109 and plated on synthetic dropout (SD) selection medium that lacked tryptophan, adenine and histidine (SD (-Trp/-Ade/-His) medium). The yeast cells grew on the SD (-Trp/-Ade/-His) medium without any prey, indicating that DcMYB1, which was fused with the GAL4 binding domain (GAL4BD) via the GAL4 binding site in front of the *HIS3* and *ADE2* yeast reporter genes, could activate the *HIS3* and *ADE2* reporter genes by its activation domain (data not shown). Deleted coding sequences of *DcMYB1* cDNA were generated by PCR and introduced into the pGBKT7 plasmid. The pGBKT7 plasmid was then transformed into yeast AH109 cells and plated on SD (-Trp/-Ade/-His) medium. The DcMYB1 protein, with a C-terminal 21 amino acid deletion consisting of an acidic amino acid cluster estimated to be the activation domain, could not activate the *HIS3* and *ADE2* reporter genes in yeast AH109 cells without prey (data not shown). This result indicated that the C-terminal 21 amino acid sequence acted as a transcriptional activation domain in yeast. This result was consistent with the general structure of the activation domain of R2R3 MYB proteins, which comprises an acidic amino acid cluster located at the C-terminus of the protein (Stracke et al. 2001). Therefore, the construct with the deleted C-terminal 21 amino acids (designated DcMYB1-1/212) was used as a bait for yeast two-hybrid screening. The prey plasmids prepared from the cDNA library and the bait plasmid were cotransformed into yeast AH109 cells by a lithium acetate method and approximately 2×10^6 transformants were plated on SD (-Trp/-Leu/-Ade/-His) medium. Yeast transformants of colonies that grew on the SD (-Trp/-Leu/-Ade/-His) medium were picked up and streaked on selection medium containing 5-bromo-4-chloro-3-indolyl-α-D-galactopyranoside (X-α-Gal) to test α-galactosidase activity of another reporter gene, *MEL1*, which catalyzes X-α-Gal to produce a blue dye. Clones that produced blue colonies were selected as positive. As a result, 14 positive clones highlighted by both selection markers were isolated. Nucleotide sequence analysis revealed that five of 10 clones, from which plasmids were isolated and nucleotide sequences analyzed, encoded DcMYB1 (Table 1). This result indicated that DcMYB1 could interact with DcMYB1-1/212 in yeast.

To analyze which region of the DcMYB1 protein played an important role in interactions with other DcMYB1 proteins using the yeast two-hybrid assay, DcMYB1 was fused with the GAL4 activation domain (AD-DcMYB1) to form a prey construct, as reported previously (Maeda et al. 2005). Several deleted DcMYB1 proteins fused with GAL4BD were used as bait constructs (Figure 1, left side). Using DcMYB1 cDNA as a template, and primers with appropriate nucleotide sequences, partial cDNA fragments of DcMYB1 cDNA were generated by PCR and then

Table 1. cDNA clones isolated from a carrot cDNA library using a yeast two-hybrid screening method on the selection medium containing X-α-Gal.

Clone No.	Growth on SD (-Ade/-His/-Trp/-Leu)	α-galactosidase assay test	Annotation
1	+	+	40S ribosomal protein
2	+	+	(sequence could not be determined)
3	+	+	Lycopersicon esculentum clp-like energy-dependent protease
4	+	+	homology not found
5	+	−	
6	+	+	DcMYB1
7	+	+	–
8	+	+	DcMYB1
9	+	+	DcMYB1
10	+	+	DcMYB1
11	+	+	–
12	+	−	–
13	+	+	Nicotiana tabacum phosphoribosylaminoimidazole carboxylase protein
14	+	+	–
15	+	+	60S ribosomal protein
16	+	+	DcMYB1

Sixteen colonies were first identified on the selection medium from the screening. These 16 colonies were then replated on SD (-Trp/-Leu/-Ade/-His) medium containing X-α-Gal. Among the positive clones of 14 colonies, plasmid DNA was obtained from 10 clones and their nucleotide sequences and homology to sequences reported in databases were determined, shown as annotations in the table.

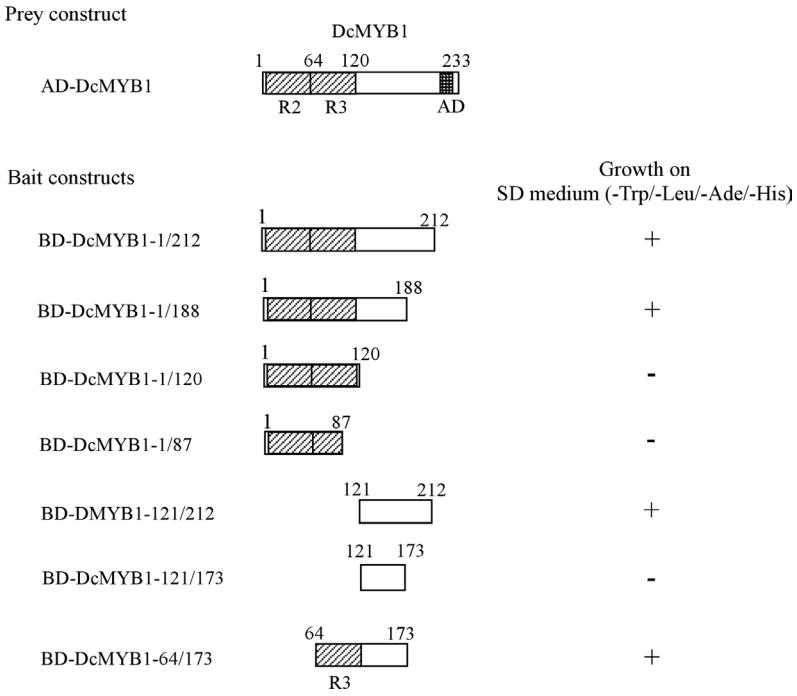

Figure 1. Interaction between full-length DcMYB1 protein and truncated DcMYB1 protein in yeast. The full-length DcMYB1 cDNA and partial cDNA fragments of DcMYB1 with different lengths generated by PCR, shown as boxes, were introduced into pAD-GAL4.2-1 as a prey plasmid and into pGBKT7 as a bait plasmid, respectively. The prey and bait plasmids were cotransformed into yeast strain AH109 and plated onto synthetic dropout selection medium lacking tryptophan, leucine, adenine and histidine (SD (-Trp/-Leu/-Ade/-His) medium) and containing X-α-Gal, to test nutritional phenotype and α-galactosidase activity. +, growth on SD medium forming blue colonies; −, no growth on SD medium; R2 and R3 indicate R2 and R3 imperfect repeat sequences of the MYB domain (DNA binding domain).

introduced into the pBGKT7 plasmid with in-frame translational fusion to the GAL4BD sequence. Both prey and bait constructs were introduced into yeast AH109 cells and the transformed yeast cells were plated on SD (-Trp/-Leu/-Ade/-His) medium containing X-α-Gal to identify the interaction between DcMYB1 and truncated DcMYB1 by judging colony formation and blue coloring of colonies (Figure 1). The C-terminus truncated constructs, BD-DcMYB1-1/188 and BD-DcMYB1-1/212, could not activate the reporter genes without the GAL4AD fusion protein (data not shown) because they lacked the C-terminal 21 amino acid sequence. When

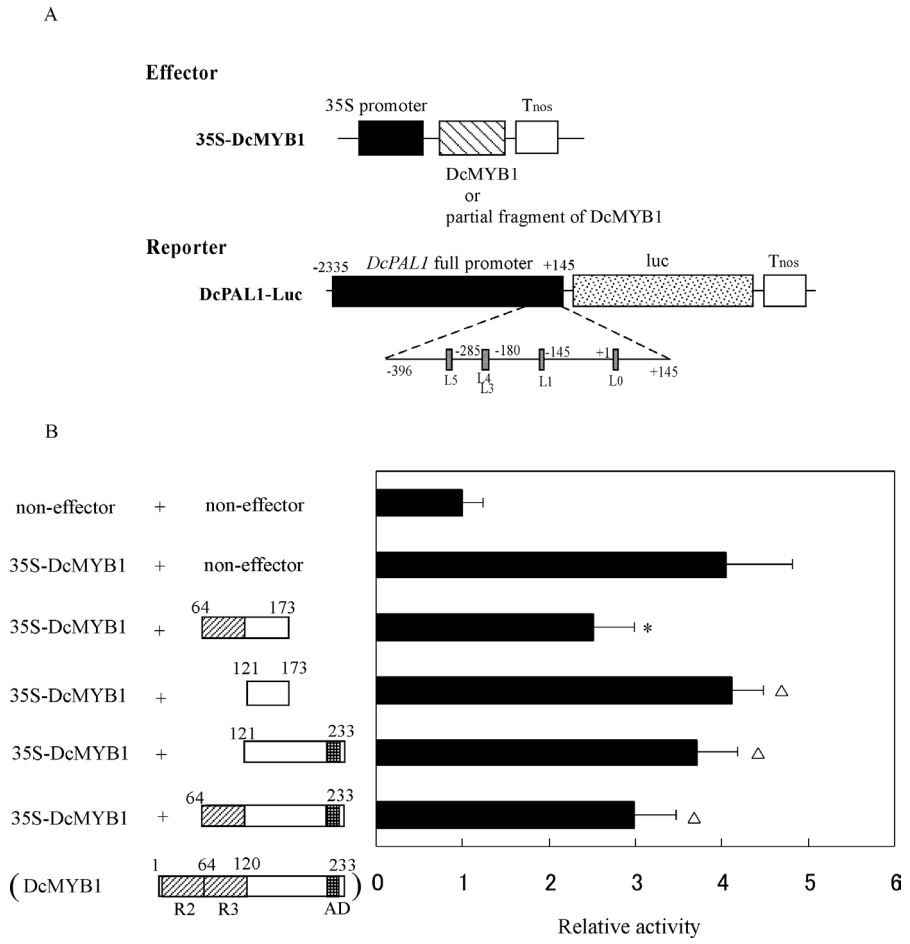

Figure 2. Dominant-negative effect of partial DcMYB1 protein amino acid sequence on full-length DcMYB1 protein activation of *DcPAL1* promoter activity in carrot protoplasts. A, effector and reporter plasmid constructs. B, relative LUC activity, driven by the *DcPAL1* promoter, as the reporter in carrot protoplasts. LUC activity was activated by the DcMYB1 protein, derived from 35S-DcMYB1 that was affected by coexpression of partial amino acid fragments of DcMYB1 derived from cDNA fragments (shown as boxes) driven by the 35S promoter. Average and standard errors of LUC activity were obtained from three independent transfection assays. Statistical comparison using Student's *t* test indicated a significant difference between the control (second row, 35S-DcMYB1 + noneffector) and DcMYB1-64/173 (asterisk; $p<0.05$); however, there was no difference between the control and other truncated *DcMYB1* proteins (triangles; $p>0.05$).

BD-DcMYB1-1/188 and BD-DcMYB1-1/212 were coexpressed in yeast with the full-length DcMYB1 fused with GAL4AD (AD-DcMYB1), activation of the reporter genes occurred, and the yeast cells grew on SD (-Trp/-Leu/-Ade/-His) medium containing X-α-Gal and formed blue colonies (Figure 1). This indicated that the amino acid region from 188 to 233 might not contribute to interactions with DcMYB1. When BD-DcMYB1-1/120 and BD-DcMYB1-1/87 were coexpressed in yeast, the yeast cells did not grow (Figure 1), indicating that the N-terminal amino acids region from 1 to 120 plays a less important role in the interaction of a partner DcMYB1 protein with GAL4AD. The coexpression of BD-DcMYB1-121/212 and BD-DcMYB1-64/173 resulted in the formation of blue yeast colonies on SD (-Trp/-Leu/-Ade/-His) medium containing X-α-Gal, indicating that the region of most importance in the interaction of DcMYB1 protein with a partner DcMYB1 protein is located from amino acids 64 to 212. However, the BD-DcMYB1-121/173 construct, which contained an overlapping region between amino acids 64 to 212, did not interact with DcMYB1 in yeast and resulted in no yeast cell growth on the SD (-Trp/-Leu/-Ade/-His) medium. These results indicate that the amino acid sequences of the proximal region, from amino acids 121 to 173 as a core sequence, play an important role in the interaction of DcMYB1 with other DcMYB1. More detailed analysis using fine and critical mutants, including serial and internal deletions or amino acid substitutions, would help determine the region(s) for self-interaction of DcMYB1 in yeast.

To confirm that DcMYB1 proteins interact with each other in carrot, the effect of the expression of partial fragments of DcMYB1 protein on the up-regulation of *DcPAL1* promoter activity by full-length DcMYB1 protein binding to box-L-like sequences was analyzed using transient assays in protoplasts prepared from suspension-cultured carrot cells. The effector and

reporter constructs, 35S-DcMYB1 and the *DcPAL1* promoter-*luc*, respectively, were used (Figure 2A); these were constructed as previously reported (Maeda et al. 2005). Partial cDNA fragments of *DcMYB1* cDNA were generated by PCR using *DcMYB1* cDNA as a template and primers, and adding appropriate nucleotide sequences for initiation and termination of translation, including the first methionine for N-terminal deletion and a stop codon for C-terminal deletion. The truncated constructs were prepared by replacing full-length *DcMYB1* cDNA with the PCR-generated cDNA fragments in a 35S-DcMYB1 plasmid. The experimental procedures for preparation of the protoplasts from suspension-cultured carrot cells, transfection of the constructs into the protoplasts using a polyethylene glycol-mediated transfection method, and culturing of transfected protoplasts followed by measurement of LUC activity in the extract prepared from the protoplasts, were as previously reported (Maeda et al. 2005).

As previously reported (Maeda et al. 2005), DcMYB1 protein in carrot protoplasts binds to four box-L-like sequences in the *DcPAL1* promoter region and activates *DcPAL1* promoter activity reported by LUC activity (Figure 2B, second row). A reduction in *DcPAL1* promoter activity was observed from coexpression with *DcMYB1*-64/173 (Figure 2B, third row). It is unlikely that this reduction was caused by interception of binding of the full-length DcMYB1 to box-L-like sequences with the R3 domain of DcMYB1-64/173 because expression of the R2-deleted mutant protein of DcMYB1, DcMYB1-64/233, in the protoplasts without any other effectors could not activate *DcPAL1* promoter activity. This indicates that the lack of the R2 domain and the solo R3 domain in DcMYB1 may result in a loss of binding activity to box-L-like sequences (data not shown). Therefore, the reduction of DcMYB1 activation by DcMYB1-64/173 might be caused by a dominant-negative interaction of DcMYB1-64/173 with full-length DcMYB1. That is, because DcMYB1-64/173 lacks the activation domain and could interact with full-length DcMYB1 proteins, thus lowering the number of DcMYB1 activation domains able to access the transcriptional machinery on the *DcPAL1* promoter. The interaction of DcMYB1-64/173 with DcMYB1 in carrot protoplasts was consistent with that of the interaction of BD-DcMYB1-64/173 with AD-DcMYB1 in yeast. A clear interaction between BD-DcMYB1-1/212 and BD-DcMYB1-121/212 with AD-DcMYB1 was observed in yeast (Figure 1), but coexpression of full-length DcMYB1 in carrot protoplasts with DcMYB1-64/233 or DcMYB1-121/233, including the region from amino acids 121 to 212, showed a slight reduction in *DcPAL1* promoter activity in comparison with coexpression with DcMYB1-64/173 (Figure 2B, fifth and sixth rows, respectively). Although statistical analysis did not show a significant difference between the activities of *DcMYB1*-64/233 or DcMYB1-121/233 and the control, a reduction in activity of DcMYB1-64/233 and DcMYB1-121/233 was observed in independent reproducible experiments. However, we could not confirm that the reduction was statistically significant because the LUC activity for DcMYB1-64/233 and DcMYB1-121/233 varied in each experiment with large standard errors, despite the clear and remarkable reduction by DcMYB1-64/173 that was observed with small standard errors in each independent experiment (data not shown). To obtain clearer results we prepared a reporter construct that contained five GAL4 binding sites in front of the 35S-minimal promoter conjugated with the *luc* gene, an effector construct as a bait that consisted of GAL4BD translationally fused to a truncated DcMYB1 sequence driven by the 35S promoter, and an effector construct of 35S-DcMYB1 as a prey that contained an interaction domain with the bait of the truncated DcMYB1 and an activation domain of DcMYB1. A two-hybrid assay using these constructs in carrot protoplasts was performed; however, we did not succeed in getting clear results (data not shown). However, our results presented here support the belief that DcMYB1–DcMYB1 interaction does physically occur in carrot protoplasts.

The interaction of MYB proteins with other transcription regulatory factors has been reported in several plant species, such as *Arabidopsis* where the MYB protein TT2 interacts with the bHLH protein TT8 and the WD40 protein TTG1 to form TT2-TT8-TTG1 that regulates gene expression related to proanthocyanidin biosynthesis (Baudry et al. 2004). In contrast to these reports, the present study is the first report to show the self-interaction of R2R3-type MYB proteins in yeast and carrot protoplasts. In animals, the self-interaction of MYB proteins of B-myb and c-myb has been reported, with the leucine zipper motif C-terminal region of c-myb playing an important role in c-myb self-interaction (Nomura et al., 1993; Kim et al., 1999). However, the amino acid sequence of the C-terminal region of DcMYB1 did not have any similarity to that of animal myb proteins, such as the leucine zipper motif, indicating that DcMYB1 proteins in plants interact with each other in a different manner to that of animal myb proteins.

In our previous report on the regulatory mechanism of *DcPAL1* gene expression, we showed that binding of DcMYB1 proteins to all four box-L-like sequences located on the proximal promoter region of the *DcPAL1* gene might play an important role in the activation of full promoter activity because DcMYB1 activation of the mutated *DcPAL1* promoter, which lacks one of the four box-L-like sequences, was reduced in carrot protoplasts (Maeda et al. 2005). Here, expression of the truncated DcMYB1-64/173 proteins, which lack the DNA binding

domain and the activation domain, had a dominant-negative effect on *DcPAL1* promoter activity by reducing the activation of the promoter by full-length DcMYB1 protein in carrot protoplasts. The amino acid sequence of the proximal region from amino acids 121 to 173 of DcMYB1 should play an important role in the interaction of DcMYB1 with other DcMYB1 proteins in yeast. As shown in Figure 2, the reduction of the activation of the *DcPAL1* promoter by coexpression of DcMYB1-64/233 and DcMYB1-121/233, which include amino acids 121 to 173 and lack the DNA binding domain, might be caused by dominant-negative interaction in carrot protoplasts. Obstruction of the self-interaction of DcMYB1 proteins might occur to prevent the activation domain of DcMYB1 from effectively approaching or acting the transcriptional machinery on the *DcPAL1* promoter. These results suggest that DcMYB1 self-interaction might play an important role in the regulatory mechanism of *DcPAL1* gene expression to bring out its full activity, together with DcMYB1 binding to all four box-L-like sequences located on the proximal promoter region of the *DcPAL1* gene.

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References

Abe H, Urao T, Ito T, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003) *Arabidopsis* AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. *Plant Cell* 15: 63–78

Baudry A, Heim MA, Dubreucq B, Caboche M, Weisshaar B, Lepiniec L (2004) TT2, TT8, and TTG1 synergistically specify the expression of BANYULS and proanthocyanidin biosynthesis in *Arabidopsis thaliana*. *Plant J* 39: 366–380

Bodeau JP, Walbot V (1992) Regulated transcription of the maize Bronze-2 promoter in electroporated protoplasts requires the C1 and R gene products. *Mol Gen Genet* 233: 379–387

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

Dias AP, Braun EL, McMullen MD, Grotewold E (2003) Recently duplicated maize R2R3 Myb genes provide evidence for distinct mechanisms of evolutionary divergence after duplication. *Plant Physiol* 131: 610–620

Grotewold E, Drummond BJ, Bowen B, Peterson T (1994) The myb-homologous *P* gene controls phlobaphene pigmentation in maize floral organs by directly activating a flavonoid biosynthetic gene subset. *Cell* 76: 543–553

Grotewold E, Chamberlin M, Snook M, Siame B, Butler L, Swenson J, Maddock S, Clair GS, Bowen B (1998) Engineering secondary metabolism in maize cells by ectopic expression of transcription factors. *Plant Cell* 10: 721–740

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

Hernandez JM, Heine GF, Irani NG, Feller A, Kim MG, Matulnik T, Chandler VL, Grotewold E (2004) Different mechanisms participate in the R-dependent activity of the R2R3 MYB transcription factor C1. *J Biol Chem* 279: 48205–48213

Kim T, Jung H, Min S, Kim KT, Ha H (1999) B-myb proto-oncogene products interact *in vivo* with each other via the carboxy-terminal conserved region. *FEBS Lett* 460: 363–368

Maeda K, Kimura S, Demura T, Takeda J, Ozeki Y (2005) DcMYB1 acts as a transcriptional activator of the carrot phenylalanine ammonia-lyase gene (*DcPAL1*) in response to elicitor treatment, UV-B irradiation and the dilution effect. *Plant Mol Biol* 59: 739–752

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

Nesi N, Jond C, Debeaujon I, Caboche M, Lepiniec L (2001) The *Arabidopsis TT2* gene encodes an R2R3 MYB domain protein that acts as a key determinant for proanthocyanidin accumulation in developing seed. *Plant Cell* 13: 2099–2114

Nomura T, Sakai N, Sarai A, Sudo T, Kanei-Ishii C, Ramsay RG, Favier D, Gonda TJ, Ishii S (1993) Negative autoregulation of c-Myb activity by homodimer formation through the leucine zipper. *J Biol Chem* 268: 21914–21923

Paz-Ares J, Ghosal D, Wienand U, Peterson PA, Saedler H (1987) The regulatory c1 locus of *Zea mays* encodes a protein with homology to myb proto-oncogene products and with structural similarities to transcriptional activators. *EMBO J* 6: 3553–3558

Quattrocchio F, Wing J, van der Woude K, Souer E, de Vetten N, Mol J, Koes R (1999) Molecular analysis of the *anthocyanin2* gene of petunia and its role in the evolution of flower color. *Plant Cell* 11: 1433–1444

Riechmann JL, Heard J, Martin G, Reuber L, Jiang C, Keddie J, Adam L, Pineda O, Ratcliffe OJ, Samaha RR, Creelman R, Pilgrim M, Broun P, Zhang JZ, Ghandehari D, Sherman BK, Yu G (2000) *Arabidopsis* transcription factors: genome-wide comparative analysis among eukaryotes. *Science* 290: 2105–2110

Romero I, Fuertes A, Benito MJ, Malpica JM, Leyva A, Paz-Ares J (1998) More than 80 R2R3-MYB regulatory genes in the genome of *Arabidopsis thaliana.* *Plant J* 14: 273–284

Roth BA, Goff SA, Klein TM, Fromm ME (1991) C1- and R-dependent expression of the maize *Bz1* gene requires sequences with homology to mammalian myb and myc binding sites. *Plant Cell* 3: 317–325

Stracke R, Werber M, Weisshaar B (2001) The R2R3-MYB gene family in *Arabidopsis thaliana*. *Curr Opin Plant Biol* 4: 447–456

Sugimoto K, Takeda S, Hirochika H (2000) MYB-related transcription factor NtMYB2 induced by wounding and elicitors is a regulator of the tobacco retrotransposon Tto1 and defense-related genes. *Plant Cell* 12: 2511–2528

Takeda J, Ozeki Y, Yoshida K (1997) Action spectrum for induction of promoter activity of phenylalanine ammonia-lyase gene by UV in carrot suspension cells. *Photochem Photobiol* 66: 464–470

Takeda J, Ito Y, Maeda K, Ozeki Y (2002) Assignment of UVB-

responsive cis-element and protoplastization-(dilution-) and elicitor-responsive ones in the promoter region of a carrot phenylalanine ammonia-lyase gene (*gDcPAL1*). *Photochem Photobiol* 76: 232–238

Tohge T, Nishiyama Y, Hirai MY, Yano M, Nakajima J, Awazuhara M, Inoue E, Takahashi H, Goodenowe DB, Kitayama M, Noji M, Yamazaki M, Saito K (2005) Functional genomics by integrated analysis of metabolome and transcriptome of *Arabidopsis* plants over-expressing an MYB transcription factor. *Plant J* 42: 218–235

Vailleau F, Daniel X, Tronchet M, Montillet JL, Triantaphylides C, Roby D (2002) A R2R3-MYB gene, *AtMYB30*, acts as a positive regulator of the hypersensitive cell death program in plants in response to pathogen attack. *Proc Natl Acad Sci USA* 99: 10179–10184

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