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ORIGINAL RESEARCH

Molecular Analysis of Anthocyanin Biosynthetic Genes and Control of Flower Coloration by Flavonoid 3′,5′-Hydroxylase (F3′5′H) in *Dendrobium moniliforme*

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Abstract *Dendrobium moniliforme* is a native species of Korea. The flower of this species is composed of a reproductive column and white perianths including petals, sepals and lip, but the base of the column bears reddish purple pigment spots. Anthocyanins are major pigments that contribute flower color in *Dendrobium*. Three key anthocyanin biosynthetic genes encoding dihydroflavonol 4-reductase (DFR), chalcone synthase (CHS), and flavonoid 3',5'-hydroxylase (F3'5'H) were isolated and analyzed for their expression patterns in floral organs to understand the molecular mechanism underlying flower color development. Quantitative RT-PCR analysis revealed that *F3'5'H* transcripts were highly accumulated in the base of the column compared with those of perianths, but the other two genes showed no significant difference among the floral organs. Microprojectile bombardment using the white perianths revealed that the transient expression of *F3'5'H* gene, but not *DFR* and *CHS* genes, was sufficient to produce reddish purple colored pigmentation. These results suggest that the lack of colors in perianths of *D. moniliforme* is at least due to transcriptional control of *F3'5'H*. The data presented here may improve our understanding of the mechanisms underlying floral color development in *D. moniliforme* and contribute to advances in orchid biotechnology.

Keywords *Dendrobium moniliforme* · Anthocyanin · Flower color · Microprojectile bombardment · Flavonoid 3',5'-hydroxylase (F3'5'H)

Abbreviations

RT-PCR	Reverse transcription-polymerase chain reaction
CHS	Chalcone synthase
DFR	Dihydroflavonol 4-reductases
F3'5'H	Flavonoid 3',5'-hydroxylase
F3'H	Flavonoid 3'-hydroxylase
F3H	Flavanone 3-hydroxylase
ANS	Anthocyanidin synthase
DHK	Dihydrokaempferol
DHQ	Dihydroquercetin
DHM	Dihydromyricetin

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Introduction

The genus *Dendrobium* is the second largest group of the family Orchidaceae, which comprises approximately 1,400 species worldwide (Dressler 1990; Wood 2006). Since species from the genus *Dendrobium* produce valuable floral

traits including colors, morphologies, and scents, they have been regarded as one of the most important commercial orchids that are used for cut flowers and potted plants in the floricultural industry (Yu et al. 2001). In particular, flower color has been regarded as an important characteristic attracting the attention of consumers. To improve flower colors for marketable qualities, a variety of *Dendrobium* hybrids have been created by a traditional breeding strategy through intra- and inter-specific crosses. However, creation or modification of some flower colors is still difficult due to a limitation of gene resources in the species. With recent advances in tissue culture and genetic transformation technology in *Dendrobium*, it has now become feasible to develop specific desired flower colors in relatively short periods at a large scale (Nan and Kuehnle 1995; Chai and Yu 2007).

Many studies have demonstrated that chemical structures of anthocyanins, a class of flavonoids, are primarily responsible for the broad range of flower colors in the *Dendrobium* (Hahlbrock and Griesbach 1975; Holton and Cornish 1995; Winkel-Shirley 2001). Although hundreds of anthocyanins have been reported, they can be classified into three major types: cyaniding (red to magenta), delphinidin (purple to violet), and pelargonidin (brick red to scarlet). Moreover, anthocyanins change their colors depending on vacuolar pH, co-pigmentation, or the formation of a complex with metal ions (Kondo et al. 1992; Yoshida et al. 2003; Shiono et al. 2005; Shoji et al. 2007). The anthocyanin biosynthetic pathway that determines floral pigmentation is generally conserved among plant species, and genes that encode the enzymes regarding this pathway have been mostly isolated and extensively studied (Holton and Cornish 1995; reviewed in Tanaka and Ohmiya 2008).

Flower coloration is also thought to be specified by lack of specific genes that are implicated in anthocyanin biosynthesis (Holton and Cornish 1995; Tanaka and Ohmiya 2008). Thus, plant species usually exhibit a limited number of flower colors, and no species displays all the possible flower colors. For example, roses, carnations and chrysanthemum lack violet/blue varieties due to deficiency of flavonoid 3',5'-hydroxylase (F3'5'H), a key enzyme in the synthesis of delphinidin-based anthocyanins (Holton and Tanaka 1994). *Petunia* and *Cymbidium* lack red/orange varieties due to the absence of dihydroflavonol reductases (DFRs) specificity toward dihydrokaempferol (DHK) substrate, resulting in low accumulation of pelargonidin-based anthocyanins (Forkmann and Ruhnau 1987; Johnson et al. 1999). It was also reported that recessive mutations at the *DFR* gene of maize lead to a colorless aleurone layer (Reddy et al. 1987). CHS represents the first committed step in the anthocyanin pathway that catalyzes the formation of naringenin chalcone from malonyl-CoA and 4-coumaroyl-CoA (Tanaka and Ohmiya 2008). Insertional mutations of *CHS* by transposons resulted in an albino mutant phenotype in the *Ipomoea purpurea* and *I. nil* floral limb (Epperson and Clegg 1987; Habu et al. 1998).

Genetic studies have identified several transcription factors that affect the expression of multiple anthocyanin structural genes in *Arabidopsis*, maize, snapdragon, *Oncidium* and petunia (Broun 2004; Koes et al. 2005; Quattrocchio et al. 1999; Chiou and Yeh 2008). They are known to belong to three distinct gene families including R2R3-MYB, basic helix-loop-helix, and WD40 repeats. Moreover, other regulatory genes encoding WRKY transcription factor (Johnson et al. 2002), zinc finger protein (Sagasser et al. 2002), MADS domain protein (Nesi et al. 2002), and homeodomain protein (Kubo et al. 1999), have been reported to involve anthocyanin biosynthesis as well as other aspects of plant development. However, their regulatory mechanism is not fully understood since the expression of anthocyanin structural genes is differentially modulated by a regulatory complex composed of different types of transcription factor (Martin and Gerats 1993; Koes et al. 2005).

In this study, we isolated three key anthocyanin biosynthetic genes and analyzed their possible functions with the aims of understanding the molecular mechanism underlying flower pigmentation in *Dendrobium moniliforme*. Our data presented here may be useful for the genetic manipulation of flower pigmentation in the future.

Materials and Methods

Plant Materials and Growth Condition

D. moniliforme was collected from Jindo Island in Korea and cultivated in the greenhouse facility of Chonbuk National University. *D. moniliforme* seeds originated from a single plant were grown on MS agar medium containing 2% peptone for 1 year and then transferred to the greenhouse to produce flowers. Flower buds were harvested just before or after flowering in late May and throughout June. The harvested flower buds were then dissected into petal, sepal, lip and the column including the base, and each organ was combined and kept at −70°C until used. Total RNA was extracted from the combined samples.

Total RNA Isolation and RT-PCR Analysis

Total RNA was isolated using Trizol reagent (Gibco BRL, NY, USA) and then treated with RQ1-DNase I (Promega, Germany) to remove DNA contamination, according to the manufacturer instructions. To examine the expression patterns of anthocyanin biosynthetic genes, total RNA

Table 1 Oligonucleotide primers used in this study for cDNA cloning and RT-PCR analysis

Target gene	Forward primer sequence (5′ to 3′)	Reverse primer sequence (5′ to 3′)
Primers for cDNA cloning		
CHS	AACCATGGCGCCSCCGGCAATGGAAGAG	TCACACCGCACCAGCAATCGGA
DFR	ATGGAGAATGAGAAGAAGGGWCCAGTAGTG	CACTTAACAGCAATCTGYTCTTTAACTTCC
F3′5′H	ATGTCYATCTTCCTCATCRCMWCMCTCYTC	TTAAASAASSCCATAMGCCGCCGSCGSC
Primers for RT-PCR analysis		
CHS	TTCGCCGGCGGCACCGTCCT	AGCCTCCGCCGACCTCCGCC
DFR	GGCCGGCAGCGTTCAGCGAGTG	TGAGTGGGATAAGCTTCTTATCCC
F3′5′H	CGGGGACGGACACCTCCGCCAT	CGGCAACACCAACCCCGGCCCT
Actin	TGCTAGTGGCCGCACGACAGGT	GGGCACCTAAATCTCCCAGCTCC
Primers for quantitative RT-PCR analysis		
CHS	GAAAGACGTCCCAGGCTTGAT1	TGAATACCAAGCGGCTCGAA
DFR	AGGCTGCTTGGGAGTTTGTAAAG	AGGACCCCACCACCAAAGTT
F3′5′H	CATGGTCTTCGCCGATTACG	TTGGAGCCGAGGAGATGGA
Actin	AGCCGAGATCTCACAGACTCCTT	ACGCTCTGCAGTAGTGGTGAAAG

isolated from each floral organ was subjected to semi-quantitative RT-PCR analysis as described previously (Park et al. 2010). PCR conditions were as follows: one cycle of 94°C for 3 min; 20–30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s; and a final extension period of 72°C for 10 min. To use as an internal control, a partial cDNA fragment of the *actin* gene was obtained by RT-PCR using the degenerate primers, 5′-GARAARATGACNCAR ATHATG-3′ and 5′-TCNACRTCRCAYTTCATDAT-3′. After the PCR products were sequenced, primers specific for the *actin* gene were designed (Table 1). Expression levels were precisely quantified by quantitative RT-PCR analysis. One twentieth of the first-strand cDNA was mixed with an equal volume of ×2 SYBR Green Master Mix (Applied Biosystems, USA) and the appropriate primers as listed in Table 1. PCR conditions were as follows: 40 cycles of 95°C for 15 s, 58°C for 30 s, and 72°C for 1 min using the 7500 Real-Time PCR System (Applied Biosystems, USA). Results were analyzed with SDS 1.7 software (Applied Biosystems, USA). Relative expression levels of each gene are normalized to that of actin gene.

Isolation of Full-Length cDNA Clones

Three micrograms of total RNA isolated from the column of base was reverse transcribed using the Impron II Reverse

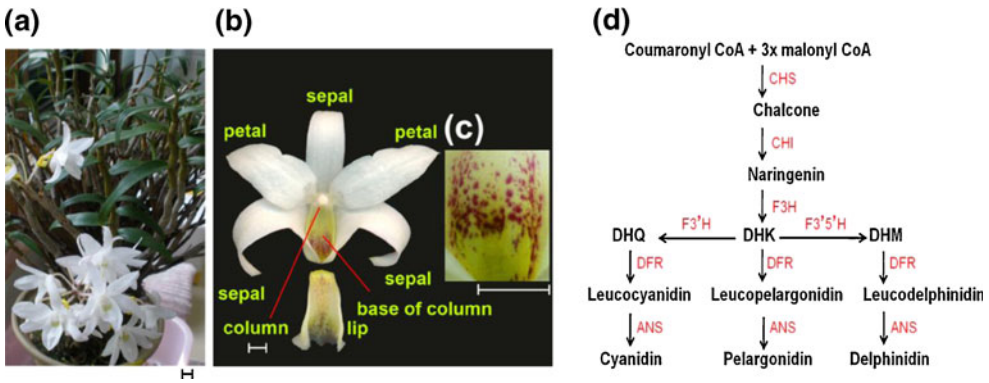

Fig. 1 Structure of *Dendrobium moniliforme* flower and anthocyanin biosynthetic pathway. **a** Whole plant phenotype of *D. moniliforme*. **b** Representation of different floral organs including three sepals, two petals, lip, column, and the base of the column. Lip was detached from the flower to show the base of the column. **c** Magnification image of the base of the column. *Scale bars* indicate 5 mm. **d** Proposed model for anthocyanin biosynthetic pathway in orchid (Tanaka and Ohmiya 2008). Names of enzymes are abbreviated as follows: *CHS* chalcone synthase, *CHI* chalcone flavanone isomerase, *F3H* flavanone 3-hydroxylase, *F3′H* flavonoid 3′-hydroxylase, *F3′5′H* flavonoid 3′,5′-hydroxylase, *DFR* dihydroflavonol 4-redutase, *ANS* anthocyanidin synthase

Transcription System (Promega) as described previously (Park et al. 2010). Then, one tenth of the first-strand cDNA samples were used as a template for RT-PCR using the degenerated primers as listed in Table 1. Degenerated primers were designed to amplify the full-length anthocyanin biosynthetic genes based on an alignment of 5' and 3'-end sequence of orchid genes that are previously deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank): *chalcone synthase* (*CHS*; AM286424, AM490639, AY741319, DQ462460, FM209429, and FM209430), *dihydroflavonol 4-reductase (DFR;* AY741318, FJ426271, FM209431, and FM209432), and *flavonoid 3',5'-hydroxylase (F3'5'H;* DQ923127 and DQ148458). PCR conditions were as follows: one cycle of 94°C for 3 min, 40 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 2 min and a final extension period of 72°C for 10 min. The resulting PCR products were recovered from agarose gel electrophoresis and cloned into a pBluescript-T vector as described previously (Park et al. 2010). All full-length clones were confirmed of their nucleotide sequence by DNA sequencing. GenBank accession numbers are HQ412558 (CHS), HQ412559 (DFR), and HQ412560 (F3′5′H). The BLASTn and BLASTp algorithm in the NCBI database (http://www.ncbi.nlm.nih.gov/database, accessed on Jan 13, 2009) was

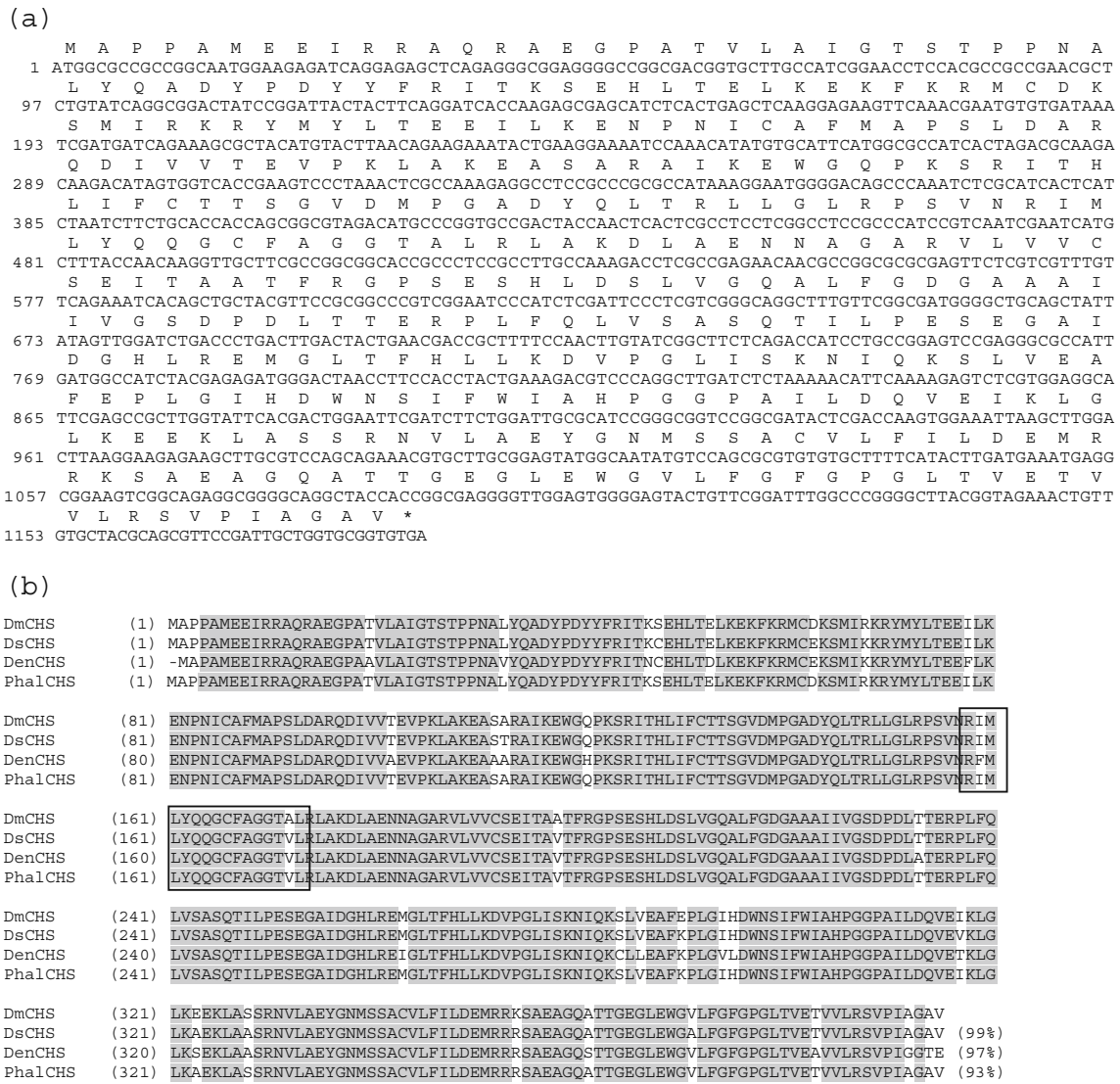

Fig. 2 Nucleotide and deduced amino acid sequence of *DmCHS* and alignment of the deduced amino acid sequences with other homologues. **a** Nucleotide and deduced amino acid sequence of *DmCHS*. The positions of nucleotides are given on the left. GenBank accession number for *DmCHS* is HQ412558. **b** The alignment of deduced amino acid sequences of *DmCHS* with homologues from *Dendrobium* hybrid cultivar Sonia (*DsCHS*; CAM32716), *Dendrobium* hybrid (DenCHS; AAU93767), and *Phalaenopsis* hybrid (*PhalCHS*; AAY83389). *Shaded regions* show identical amino acids. *Box* indicates active site of chalcone synthases. The positions of amino acids are given on the *left*

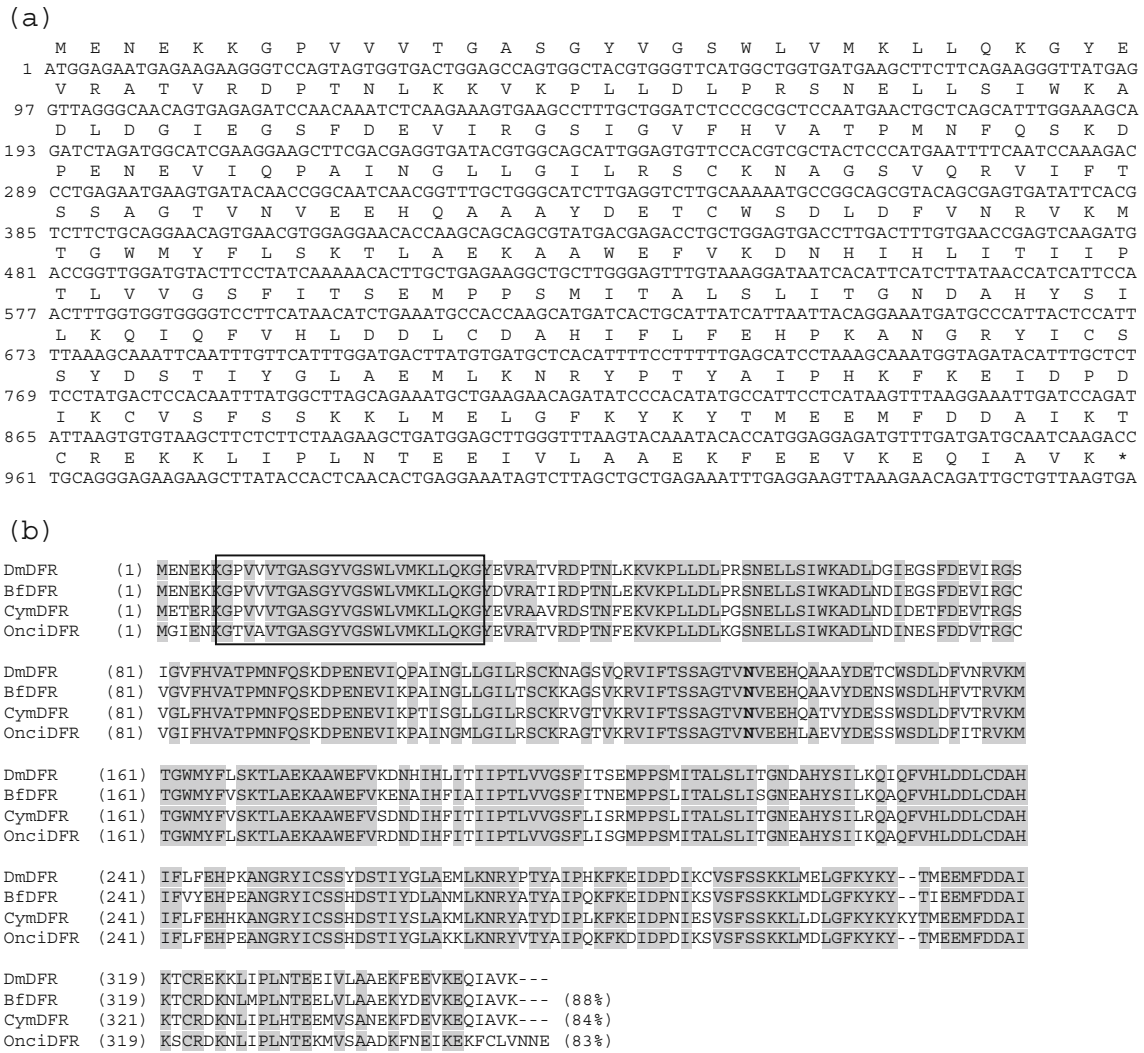

Fig. 3 Nucleotide and deduced amino acid sequence of *DmDFR* and alignment of the deduced amino acid sequences with other homologues. **a** Nucleotide and deduced amino acid sequence of *DmDFR*. The positions of nucleotides are given on the left. GenBank accession number for *DmDFR* is HQ412559. **b** The alignment of deduced amino acid sequences of *DmDFR* with homologues from *Bromheadia finlaysoniana* (*BfDFR*; AAB62873), *Cymbidium* hybrid (*CymDFR*; AAC17843), and *Oncidium* Gower Ramsey (*OnciDFR*; AAY32602). *Shaded regions* show identical amino acids. *Box* indicates the NADP binding site. *Bold character* indicates the Asn of active site in the 135 residue. The positions of amino acids are shown on the *left*

used to detect similarities between cloned gene sequences and previously deposited sequences.

Gene Constructs

To construct the transient expression vector, the full-length *CHS*, *DFR*, and *F3′5′H* were placed between the *Cassava Vein Mosaic Virus* (CsVMV) promoter and the *Nos* terminator in the CsV vector (Verdaguer et al. 1996). Briefly, the full-length genes in pBluescript-T vector were digested with *XbaI* I and *Kpn* I, and then ligated into the same sites of the CsV vector to yield *pCsVMV::CHS*, *pCsVMV::DFR*, and *pCsVMV::F3′5′H*. To test promoter activity and bombardment efficiency, the *pCsVMV::GUS* construct was included. All constructs were confirmed by DNA sequencing.

Bombardment with Particle Inflow Gun

Near mature flowers were carefully excised from the plant using a razor and placed on the center of MS agar plate. DNA constructs were coated onto gold particles (1.5–3 μm diameter; Sigma-Aldrich, USA) as described previously (Akashi et al. 2002). A biolistic helium device was used to microproject the DNA-coated gold particles into the perianths of *D. moniliforme* flower. Conditions for bombardment were as follows: reduced air pressure of −0.1 MPa, target distance of 12 cm, and helium pressure of 3.5 bar

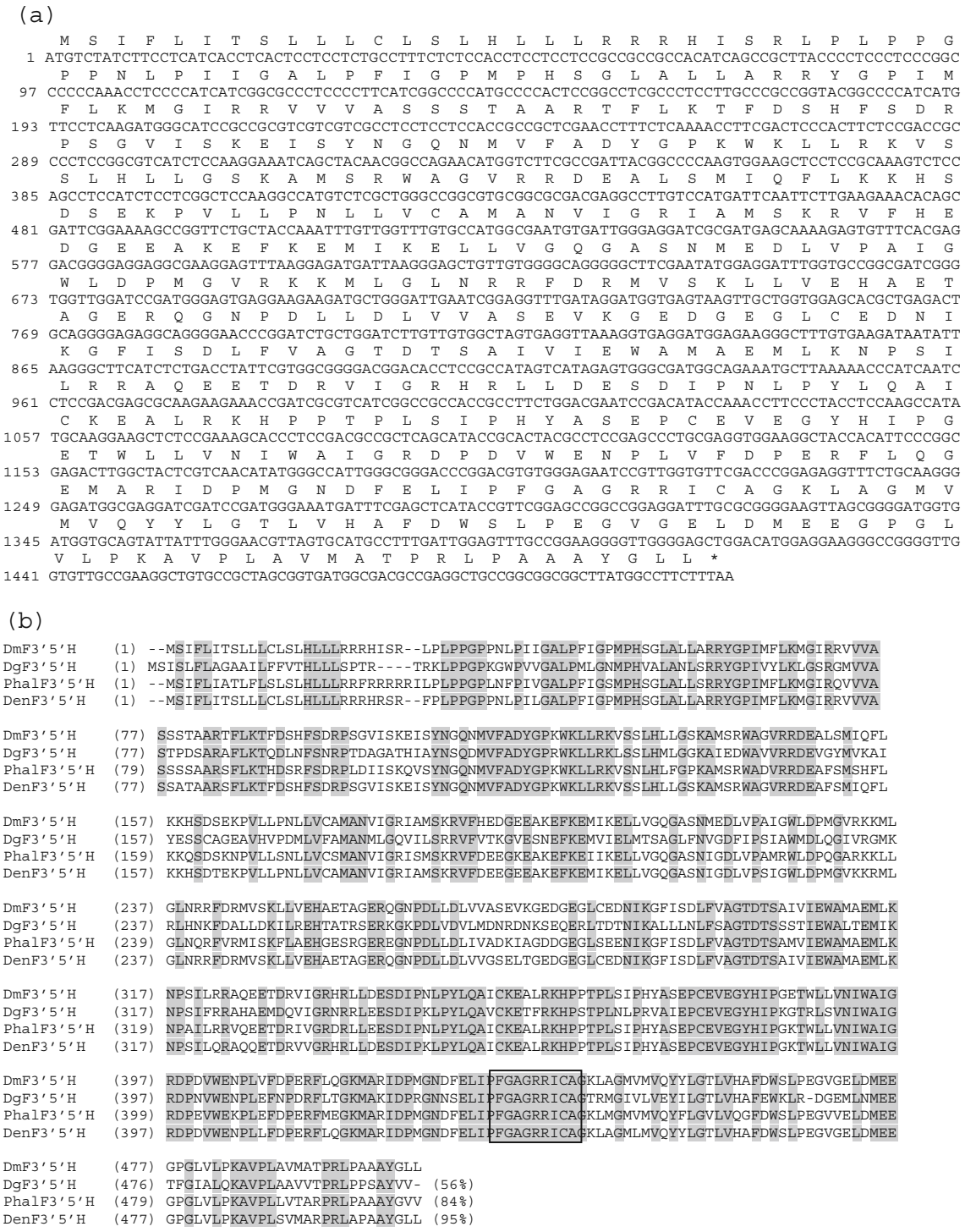

Fig. 4 Nucleotide and deduced amino acid sequence of *DmF3'5'H* and alignment of the deduced amino acid sequences with other homologues. **a** Nucleotide and deduced amino acid sequence of *DmF3'5'H*. The positions of nucleotides are given on the *left*. GenBank accession number for *DmF3'5'H* is HQ412560. **b** The alignment of deduced amino acid sequences of *DmF3'5'H* with homologues from *Delphinium grandiflorum* (*DgF3'5'H*; AAX51796), *Phalaenopsis* hybrid (*PhalF3'5' H*; AAZ79451), and *Dendrobium* hybrid (*DenF3'5'H*; ABI95365). Shaded regions show identical amino acids. *Box* indicates the Cytochrome P450 cysteine heme-iron ligand signature. The positions of amino acids are shown on the *left*

(50 psi). The bombarded floral organs were then incubated on MS agar medium in 12-h light/12-h dark cycle at 25°C. Three to five days after bombardment, the development of pigmentation was analyzed in the perianth organ. GUS activity was measured by histochemical staining as described (Koo et al. 2007). Pigmentation color was analyzed and

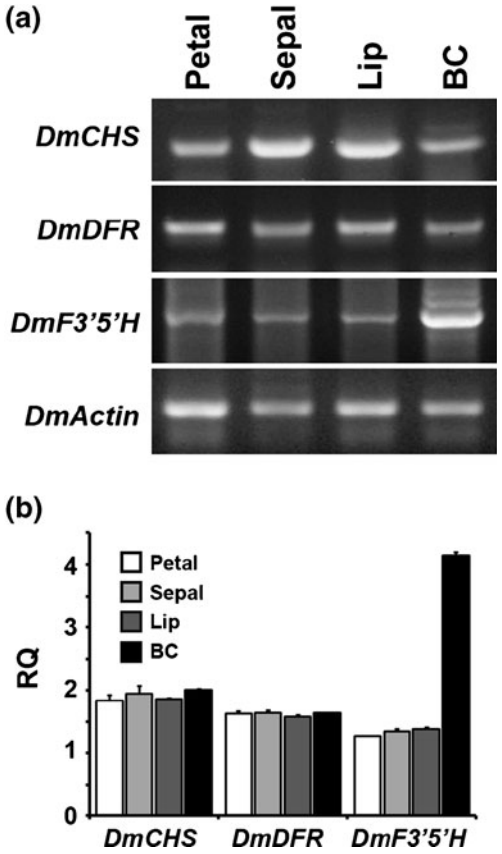

Fig. 5 Expression analysis of three anthocyanin biosynthetic genes in *Delphinium moniliforme* floral organs. **a** Semi-quantitative RT-PCR analysis. First-stranded cDNA was synthesized using total RNA extracted from petals (*Petal*), sepals (*Sepal*), lip (*Lip*), and column including the base (*BC*). *Actin* (*DmActin*) gene was included in the experiment as an internal control. The PCR amplified products were analyzed by 1.5% agarose gel electrophoresis. **b** Quantitative RT-PCR analysis. One twentieth of first-strand cDNA mixture was used as template. Relative quantity of each gene expression is represented by to one fourth level of *actin* mRNA. *Error bars* indicate SD (n=3)

presented as value according to PCCS 199a Harmonic Color Charts (COJI, Japan).

Results and Discussion

Isolation of Three Anthocyanin Biosynthetic Genes from *D. moniliforme*

D. moniliforme is a species of genus *Dendrobium* found in the southern areas of Korea. Like other orchids, the *D. moniliforme* flower consists of a reproductive column and three types of perianth organs including three outer tepals (called sepals), two inner tepals (petal) and a labellum (lip) (Fig. 1a–c). Interestingly, the base of the column, which is fused with the column, bears vivid reddish purple spots compared with white perianths. Color value is v23 according to the PCCS Harmonic Color Charts. This may suggest that white perianths of *D. moniliforme* are due to transcriptional control of spatially regulated pigment biosynthetic genes and/or their regulatory genes.

To test this hypothesis, we initially attempted to isolate the genes that are involved in the anthocyanin biosynthetic pathway since anthocyanins have been reported to mainly contribute diverse flower colors in *Dendrobium* (Arditti and Fisch 1977). In particular, sense and antisense expression of *CHS*, *DFR*, and *F3'5'H* (Fig. 1d) have been the most exploited to modulate flower color and/or intensity (van der Krol et al. 1990; Courtney-Gutterson et al. 1994; Jorgensen et al. 1996; Tanaka et al. 1998). To isolate these genes in *D. moniliforme*, degenerate primers were designed based on conserved regions of the previously reported sequences in GenBank (see "Materials and Methods").

Using RT-PCR with degenerate primers, a full-length cDNA encoding CHS was cloned by amplifying a 1,188 bp sequence from the base of the column and designated *DmCHS* (Fig. 2a). BLASTP searches with the deduced amino acid sequences of *DmCHS* showed 99%, 97% and 93% identity with those of *Dendrobium* hybrid cultivar Sonia (accession no. CAM32716; Pitakdantham et al. 2010), *Dendrobium* hybrid (accession no. AAU93767; Mudalige-Jayawickrama et al. 2005), and *Phalaenopsis* hybrid (accession no. AAY83389; Han et al. 2006), respectively (Fig. 2b). An essential catalytic site for CHS and possible binding site for the 4-coumaryl-CoA group was found in the sequence as a consensus pattern of "R-[LIVMFYS]-x-[LIVM]-x-[QHG]-x-G-C-[FYNA]-[GAPV]-G-[GAC]-[STAVK]-x-[LIVMF]-[RAL]" (Lanz et al. 1991).

In the same manner, a full-length cDNA encoding DFR was isolated from the base of the column and designated as *DmDFR* (Fig. 3a). The length of *DmDFR* was 1,056 bp encoding for a protein of 147 amino acids. The protein sequence of DmDFR showed 88%, 84%, and 83% identity to those from *Bromheadia finlaysoniana* (AAB62873), *Cymbidium* hybrid (AAC17843), and *Oncidium* Gower Ramsey (AAY32602; Mudalige-Jayawickrama and Kuehnle 2006) (Fig. 3b). In addition, the consensus sequence of the NADPH-binding domain, found in the DFR superfamily (Baker and Blasco 1992), was conserved in the N-terminal region of DmDFR. Although DFR catalyzes the conversion of DHK, DHQ, and DHM to leucoanthocyanidins (Fig. 1a), certain types of DFR in some plants lack the ability to reduce DHK. For example, *Petunia* DFR was reported to lack the ability to convert DHK to leucopelargonidin (Forkmann and Ruhnau 1987) due to a substitution of Asn to Asp at the 134 residue of the active site (Beld et al. 1989; Johnson et al. 2001). In DmDFR, amino acid residue 135 (corresponding 134 residue of petunia or Gerbera) is an Asn as observed in other *Dendrobium* species (Mudalige-Jayawickrama et al. 2005), implying that DmDFR contains functionally active Asn residue in 135 position.

The *F3′5′H* clone, designated as *DmF3′5′H*, is 1,512 bp in length encoded a protein of 504 amino acid residues (Fig. 4a). F3′5′H catalyzes the hydroxylation of DHK at both 3′ and 5′ positions of the B-ring that leads to the formation of DHM and subsequently to the production of delphinidin-based pigments (purple to violet) as shown Fig. 1a. Sequence comparison revealed that deduced amino acid sequence of DmF3′5′H shared 56%, 84%, and 95% identity compared with those from *Delphinium grandiflorum* (AAX51796), *Phalaenopsis hybrid* (AAZ79451), and *Dendrobium* hybrid (ABI95365) (Fig. 4b). F3′5′H belongs to the cytochrome P450 superfamily (Holton et al. 1993). The consensus sequence of cytochrome P450 signature controlling flower color was well conserved in DmF3′5′H. Taken together, these data strongly suggest that the three isolated cDNA clones may be typical types of anthocyanin biosynthetic genes.

Expression Analysis of Anthocyanin Biosynthetic Genes in Floral Organs

Colorless perianths of *D. moniliforme* flower could be explained by several reasons. These may include the spatio-temporal regulation of anthocyanin biosynthetic genes and/or their regulatory genes, or mutational effects on the structural genes encoding enzymes for anthocyanin biosynthesis. However, we focused on the first possibility since reddish purple pigmentation spots were locally accumulated in the base of the column of the flower (Fig. 1c, d).

To determine the expression patterns of three anthocyanin biosynthetic genes in floral organs in detail, semi-quantitative RT-PCR was performed using total RNA isolated from sepals, petals, lip and column including the base. CHS controls first step in anthocyanin biosynthesis, thus we assume that its expression is lowered or absent in the perianths of the *D. moniliforme* flower. In contrast to our expectation, the *CHS* transcript level was slightly higher or not significantly different in sepals, lip, and petal compared with the base of the column (Fig. 5a, b), respectively. In addition, the expression of *DFR* exhibited no significant differences among floral organs. However, the *F3′5′H* transcripts level in the base of the column was significantly higher than that of other floral organs. Quantitative RT-PCR analysis also confirmed that *F3′5′H* expression is approximately 3-fold higher in the base of the column compared with other organs (Fig. 5b). Therefore, the result suggested that colorless of the perianth organs was attributed to a lowered expression of *F3′5′H* expression. This, in turn, may suggest that the *F3′5′H* expression is controlled by spatially regulated transcriptional factor(s) in *D. moniliforme* flower.

Development of Pigmentation by Transient Expression of DmF3′5′H

As shown in RT-PCR analysis (Fig. 5), pigment accumulation in the base of column may be due to the preferential expression of *DmF3′5′H*. Thus, we expected that expression of this gene would complete the anthocyanin pathway and produce anthocyanin compounds to display the similar pigment in perianth. To test this, the full-length *DmF3′5′H* as well as *DmDFR* and *DmCHS* genes were individually cloned into CsV999 vector (Verdaguer et al. 1996) that contained the constitutive *CsVMV* promoter and *NOS* terminator. Since the activity of CsVMV promoter was

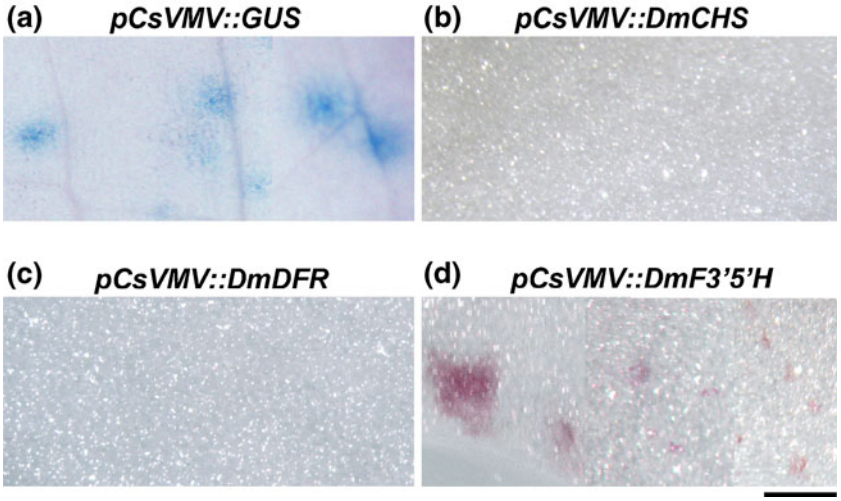

Fig. 6 Development of reddish purple spots in white perianth of *D. moniliforme* flower by transient expression of *DmF3′5′H* gene. The constructs including *DmCHS* (**b**), *DmDFR* (**c**), and *DmF3′5′H* (**d**) were bombarded into perianth organs. Bacterial *GUS* gene was included as a control (**a**). Transient expression of bombarded genes were driven using the constitutive CsVMV promoter. After 3 to 5 days of incubation, GUS activity or pigmentation was examined under the stereomicroscope. Representative images from two independent experiments were shown. *Scale bar* indicate 1 mm

not tested in *Dendrobium* flower before, *pCsVMV::GUS* construct was also included to validate promoter activity and bombardment efficiency. These constructs were then bombarded into white perianths (Fig. 6). After incubation on MS agar for three to five days, distinct GUS staining spots were observed in the perianths bombarded with *pCsVMV::GUS* construct, suggesting that *CsVMV* promoter was active in perianths of *D. moniliforme* flower. Under the same condition, bombardment with *pCsVMV::DmF3′5′H* resulted in development of reddish purple pigment spots in perianth organs, corresponding to v23 or v24 value of PCCS 199a color charts. However, perianth organs bombarded with *pCsVMV::DmCHS* or *pCsVMV::DmDFR* did not show noticeable signs of pigmentation during the assay period. These results indicated that expression of *DmF3′5′H* could completely rescued the absence of anthocyanin biosynthesis in perianth organs.

In this study, we demonstrated that transcriptional activation of *DmF3′5′H* gene is critical for coloration in the perianths of *D. moniliforme* flower. Therefore, it is interesting to determine which regulatory component(s) is responsible for spatial expression of *DmF3′5′H* gene in *D. moniliforme* flower.

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