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Differential Expression of Anthocyanin Biosynthetic Genes and Anthocyanin Accumulation in Tartary Buckwheat Cultivars 'Hokkai T8' and 'Hokkai T10'

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Supporting Information

ABSTRACT: Six genes involved in anthocyanin biosynthesis in tartary buckwheat have been cloned, namely, *FtC4H*, *Ft4CL*, *FtCHI*, *FtF3H*, *FtF3′H*, and *FtANS*, which encode cinnamate 4-hydroxylase (C4H), 4-coumarate:CoA ligase (4CL), chalcone isomerase (CHI), flavones 3-hydroxylase (F3H), flavonoid 3′-hydroxylase (F3′H), and anthocyanidin synthase (ANS), respectively. Then, these cDNAs were used, along with previously isolated clones for phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS), to compare gene expression in different organs, flowering stages, and maturing seeds of tartary buckwheat cultivars 'Hokkai T8' and 'Hokkai T10'. Quantitative real-time polymerase chain reaction analysis showed that these anthocyanin biosynthetic genes were most highly expressed in the stems and roots of Hokkai T10. The *FtANS* gene was more highly expressed than other genes during flowering and maturing seeds. In addition, the anthocyanin concentration was higher in 'Hokkai T10' than in 'Hokkai T8'; however, naringenin chalcone, a flavonoid, was absent from 'Hokkai T10' seedlings based on fluorescence microscopy.

KEYWORDS: Anthocyanin, biosynthetic pathways, *Fagopyrum tataricum*, rutin, tartary buckwheat

INTRODUCTION

Buckwheat belongs to the family Polygonaceae and includes two species of cultivated buckwheat: common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*Fagopyrum tataricum*). Common buckwheat is cultivated around the world, whereas tartary buckwheat is cultivated mainly in southern China, northern India, Bhutan, and Nepal.[1,2] Tartary buckwheat has several beneficial pharmacological and biological effects, such as anticancer, antidiabetic, and antioxidant activities.[3−5] In addition, tartary buckwheat is also a richer source of rutin, a flavonol glycoside that prevents ultraviolet-induced DNA damage and disease, than common buckwheat.[6,7] For example, tartary buckwheat seeds contain more rutin [0.8−1.7% dry weight (DW)] than common buckwheat seeds (0.01% DW).[8] In fact, the amount of rutin in tartary buckwheat can be as high as 3% of the DW of the vegetative parts of the plant. In comparison, the amount of another flavonoid, quercetin, in tartary buckwheat is only 0.01−0.05% DW.[8]

Buckwheat also produces other flavonoids, such as anthocyanins, which are water-soluble pigments in the leaves, stems, flowers, and roots. The petals of a common buckwheat cultivar (*F. esculentum* 'Moench') contain four anthocyanins: cyanidin 3-*O*-glucoside, cyanidin 3-*O*-rutinoside, cyanidin 3-*O*-galactoside, and cyanidin 3-*O*-galactopyranosyl-rhamnoside. The concentration of anthocyanins in the petals of buckwheat cultivars determines the color of the flowers. For example, the 'Gan-Chao' cultivar, which has red flowers, contains 4.69 μg of anthocyanins (mainly cyanidin 3-*O*-rutinoside) per petal, whereas the 'Kitawasesoba' cultivar, which has white flowers, only has 0.06 μg of anthocyanins per petal. Furthermore, the total amount of anthocyanins increases with flower development.[9] Recently, a tartary buckwheat cultivar 'Hokkai T10', which was obtained through ethyl methane sulfonate (EMS) mutagenesis of 'Hokkai T8', was developed that contains much higher levels of cyanidin 3-*O*-glucoside and cyanidin 3-*O*-rutinoside in its sprouts than its parent varieties.[9−11] In 'Hokkai T10', the amount of cyanidin 3-*O*-rutinoside was as high as 6.3 mg g^{-1} DW 10 days after sowing (DAS) but only 0.17 mg g^{-1} in 'Hokkai T8'.[10] In comparison, the amount of rutin, the major flavonoid in tartary buckwheat sprouts, was 42 mg g^{-1} at 10 DAS.[12]

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There have only been a few molecular studies of the flavonoid biosynthetic pathway in tartary buckwheat. For example, it is known that rutin and anthocyanins share the same initial biosynthetic steps (see Figure S1 in the Supporting Information).[13] In this study, the full-length cDNA of flavone 3-hydroxylase (*FtF3H*) and partial cDNAs of cinnamate 4-hydroxylase (*FtC4H*), 4-coumarate:CoA ligase (*Ft4CL*), chalcone isomerase (*FtCHI*), flavonoid 3′-hydroxylase (*FtF3′H*), and anthocyanidin synthase (*FtANS*) from tartary buckwheat have been cloned. These clones, along with previously isolated clones for phenylalanine ammonia lyase (PAL) and chalcone synthase (CHS), have been used to compare the expression of anthocyanin biosynthetic genes in different organs of tartary buckwheat cultivars 'Hokkai T10' and its parent cultivar 'Hokkai T8'. In addition, the anthocyanin and flavonol contents in seedlings of these two cultivars have been analyzed.

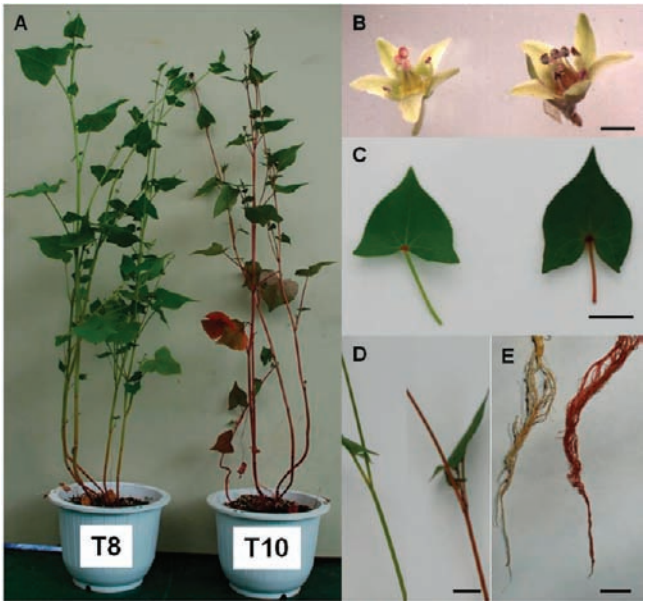

Figure 1. Tartary buckwheat cultivars 'Hokkai T8' and 'Hokkai T10': (A) whole plants, (B) flowers, (C) leaves, (D) stems, and (E) roots. The scale bar represents 1 mm in panel B and 1 cm in panels C, D, and E.

MATERIALS AND METHODS

Plant Materials. Two cultivars of tartary buckwheat, 'Hokkai T8' and 'Hokkai T10', were bred by the National Agricultural Research Center (Hokkaido, Japan). 'Hokkai T8' was derived from the Russian cultivar 'Rotindatum', and 'Hokkai T10' was produced by ethyl methanesulfonate mutagenesis of 'Hokkai T8'.[10] The 'Hokkai' cultivars were germinated in a growth chamber for 10 days at 25 °C and approximately 60% humidity. Then, the seedlings were transferred to a greenhouse (25 °C and 50% humidity) and grown for 6 weeks until they flowered. Plant samples were collected until the completion of flowering and separated into roots, stems, leaves, and inflorescences for RNA extraction. To investigate the expression level of anthocyanin biosynthetic genes, three stages during flowering and maturing seeds, petals, and unmatured seeds were harvested for each growth stage and classified according to fresh weights and sizes for petals and seeds, respectively.[9]

RNA Extraction and Polymerase Chain Reaction (PCR) Analyses. Total RNA was isolated from these organs from three different plants of each cultivar using the RNeasy Plant Mini Kit (Qiagen, Valencia, CA). Then, 1 μg of total RNA was reverse-transcribed using the Superscript II First Strand Synthesis Kit (Invitrogen, Carlsbad, CA) and an oligo$(dT)_{20}$ primer. The resulting cDNA was used as a template for rapid analysis of cDNA ends (RACE) PCR and quantitative real-time reverse transcription PCR (qRT-PCR).

The primers for *FtF3H*, *FtC4H*, *Ft4CL*, *FtCHI*, *FtF3′H*, and *FtANS* (GenBank accession numbers HQ003252, HQ003249, HQ003250, HQ003251, HQ003253, and HQ003254, respectively) were designed from the conserved sequences of known orthologous sequences (see Table S1 in the Supporting Information). In addition, the primers for *FtPAL* and *FtCHS* were obtained from previously isolated clones (GenBank accession numbers GQ285125 and GU172165, respectively). Gene expression was normalized to that of the histone H3 gene.[14] Real-time PCR reactions were carried out in triplicate on a MiniOpticon system (Bio-Rad Laboratories, Hercules, CA) with the QuantiTect SYBR Green PCR Kit (Qiagen). The PCR protocol was as follows: denaturation for 5 min at 95 °C, 40 cycles of denaturation for 15 s at 95 °C, annealing for 15 s at 56 °C, and elongation for 20 s at 72 °C.

Cloning of cDNAs Encoding Anthocyanin Biosynthetic Enzymes. The GeneRacer Kit (Invitrogen) was used to synthesize single-stranded cDNA. Then, the PCR products were subcloned into a TOPO TA vector (Invitrogen) and sequenced at the National Instrumentation Center for Environmental Management (NICEM, Seoul

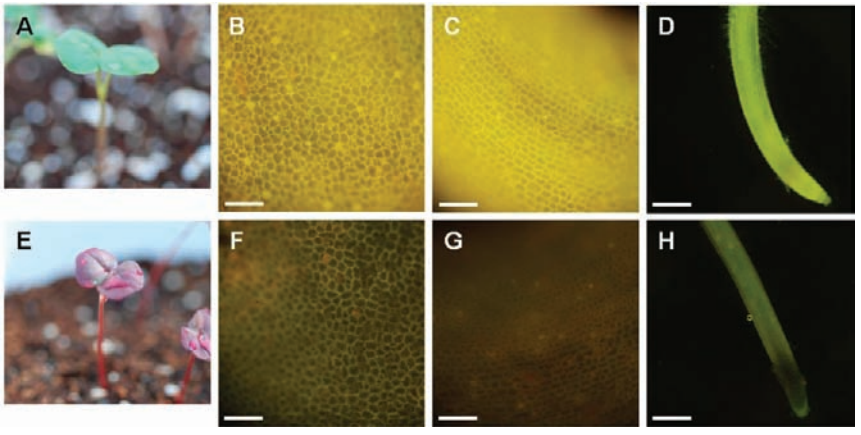

Figure 2. Anthocyanin and flavonoid contents in tartary buckwheat cultivars (A−D) 'Hokkai T8' and (E−H) 'Hokkai T10' seedlings. Anthocyanin accumulation in (A) 'Hokkai T8' and (E) 'Hokkai T10' seedlings. Flavonoid staining in (B and F) cotyledons, (C and G) cotyledonary nodes, and (D and H) roots of 'Hokkai T8' and 'Hokkai T10'. The characteristic bright yellow fluorescence of chalcone-naringenin is strongest in the cotyledons and cotyledonary nodes of (B and C) 'Hokkai T8' but is weaker in (F and G) 'Hokkai T10'. The characteristic bright green fluorescence of kaempferol is strongest in the roots of (D) 'Hokkai T8' but is weaker in (H) 'Hokkai T10'. The scale bars in panels B, C, F, and G represent 100 μm, and those in panels D and H represent 500 μm.

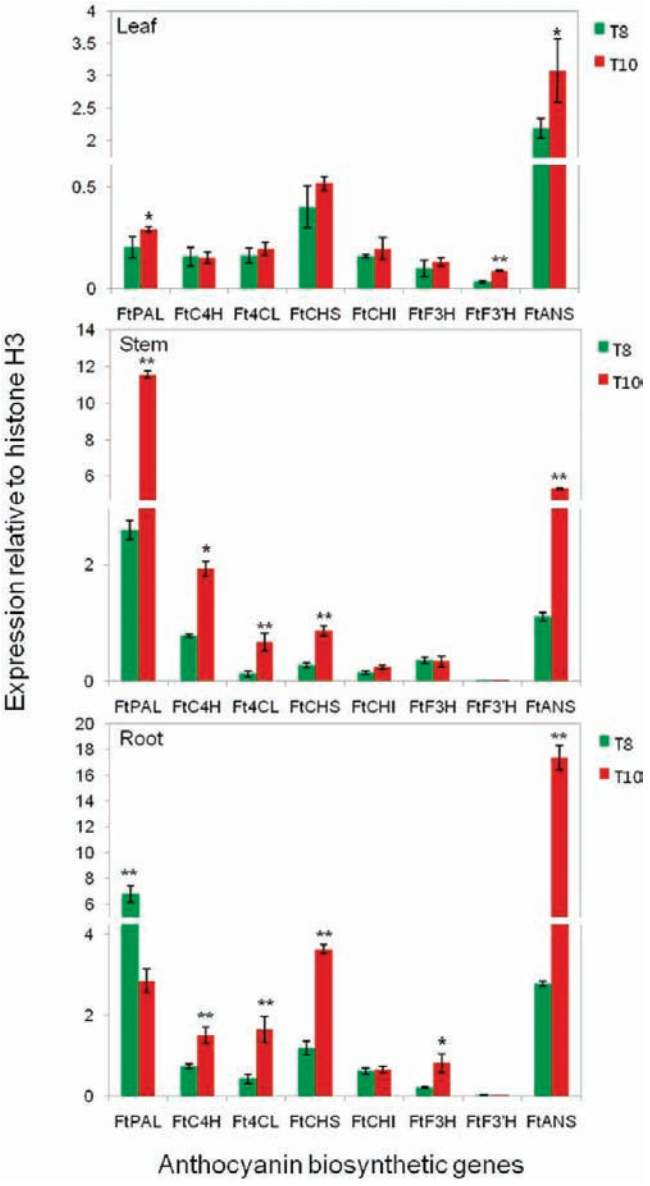

Figure 3. Differential expression of anthocyanin biosynthetic genes in different organs of tartary buckwheat cultivars 'Hokkai T8' and 'Hokkai T10'. The height of each bar represents the mean of three measurements from three different plants of each cultivar, and error bars indicate the standard deviation. Asterisks indicate significant differences between 'Hokkai T8' and 'Hokkai T10' cultivars by Student's *t* test (∗, $p < 0.05$; ∗∗, $p < 0.01$).

National University, Korea). The sequence data were used to design new primer pairs for RACE PCR (Table S1 in the Supporting Information). All PCRs were initiated with the hot start method using the RACE cDNA template, and the products were subcloned into TOPO TA vectors and sequenced as described above.

Sequence Analyses. Sequence similarities were calculated with the Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/BLAST). Multiple sequence alignments were constructed using the MultAlin program (http://bioinfo.genotoul.fr/multalin/multalin.html).

Flavonoid Staining. Flavonoid staining was performed as described by Peer et al.[15] Briefly, seedlings were stained for 20 min with 0.25% (w/v) diphenylboric acid-2-aminoethyl ester (DPBA) and 0.005% Triton X-100. Then, they were visualized with a fluorescence microscope (BX51, Olympus, Japan) equipped with a fluoroscein isothiocyanate (FITC) filter. Images were captured with a DP71 digital microscope camera (Olympus, Japan).

Quantification of the Anthocyanin Content. The anthocyanin content of samples was quantified as described by Ronchi et al.[16] Briefly, anthocyanins were extracted from individual organs with a fixed volume of 1% HCl in methanol overnight at 4 °C. Subsequently, the extracts were mixed with two-thirds of distilled water and centrifuged for 3 min at 13 000 rpm. The anthocyanins in the aqueous phase were isolated by adding 1 volume of chloroform, and then the absorbances at 530 and 657 nm were measured spectrophotometrically. Finally, the total anthocyanin concentration was calculated according to the formula: concentration = $[A_{530} - 0.33 \times (A_{657})]\ g^{-1}$ fresh weight (FW). Results were expressed as the mean of three independent measurements.

Statistical Analysis. Each result shown was the mean of three replicated treatments from three different plants of each cultivar. Significance between two cultivars for each gene was evaluated by Student's *t* test. $p < 0.05$ was considered to indicate statistical significance.

RESULTS

Isolation and Sequence Analysis of Anthocyanin Biosynthetic Genes from *F. tataricum*. The 1340 base pair (bp) *FtF3H* sequence contained a 1104 bp open-reading frame that encoded 367 amino acids. A BLAST analysis showed that the deduced amino acid sequence of this gene shared 79−84% identity and 86−92% similarity to the F3H sequence from *Gossypium hirsutum* (GenBank accession number ABM64799), *Dimocarpus longan* (ABO48521), *Pyrus communis* (AAM18084), *Rubus coreanus* (ABW74548), and *Camellia sinensis* (AAT68774). The sequence similarities between the deduced amino acid sequences of the other isolated partial genes and their plant orthologues are shown in Figure S2 in the Supporting Information.

Anthocyanin Content in 'Hokkai T8' and 'Hokkai T10'. Both visual inspection and chemical analyses of the 'Hokkai' cultivars showed that 'Hokkai T10' had more red pigments than 'Hokkai T8', most likely because of anthocyanins (Figure 1 and see Figure S3 in the Supporting Information). All of the organs of 'Hokkai T10' contained 2.6−6 times more anthocyanins than those of 'Hokkai T8' (see Figure S3 in the Supporting Information). For example, the total anthocyanin content of the flowers of 'Hokkai T10' was 4.5 times that of 'Hokkai T8'. These results showed that anthocyanin accumulation was cultivar-specific.

Accumulation of Flavonoids and Anthocyanins Are Inversely Related in 'Hokkai T8' and 'Hokkai T10'. DPBA fluoresces in the presence of flavonoids, such as naringenin-chalcone (bright yellow) and kaempferol (bright green).[17] DPBA staining of 'Hokkai T8' seedlings showed bright yellow fluorescence in the cotyledons and hypocotyls and bright green fluorescence in the roots (panels B−D of Figure 2). In contrast, 'Hokkai T10' seedlings exhibited less fluorescence in these parts (panels F−H of Figure 2), which coincided with the locations of greater anthocyanin accumulation (see Figure S3 in the Supporting Information).

Expression of Anthocyanin Biosynthetic Genes in Different Organs of *F. tataricum*. The expression levels of *FtPAL*, *FtC4H*, *Ft4CL*, *FtCHS*, *FtCHI*, *FtF3H*, *FtF3′H*, and *FtANS* in different organs of 'Hokkai T8' and 'Hokkai T10' were investigated. In leaves, all of these genes were upregulated in 'Hokkai T10'. In the stems, the expression of all of the genes, except *FtF3H*, was higher in 'Hokkai T10' than in 'Hokkai T8'

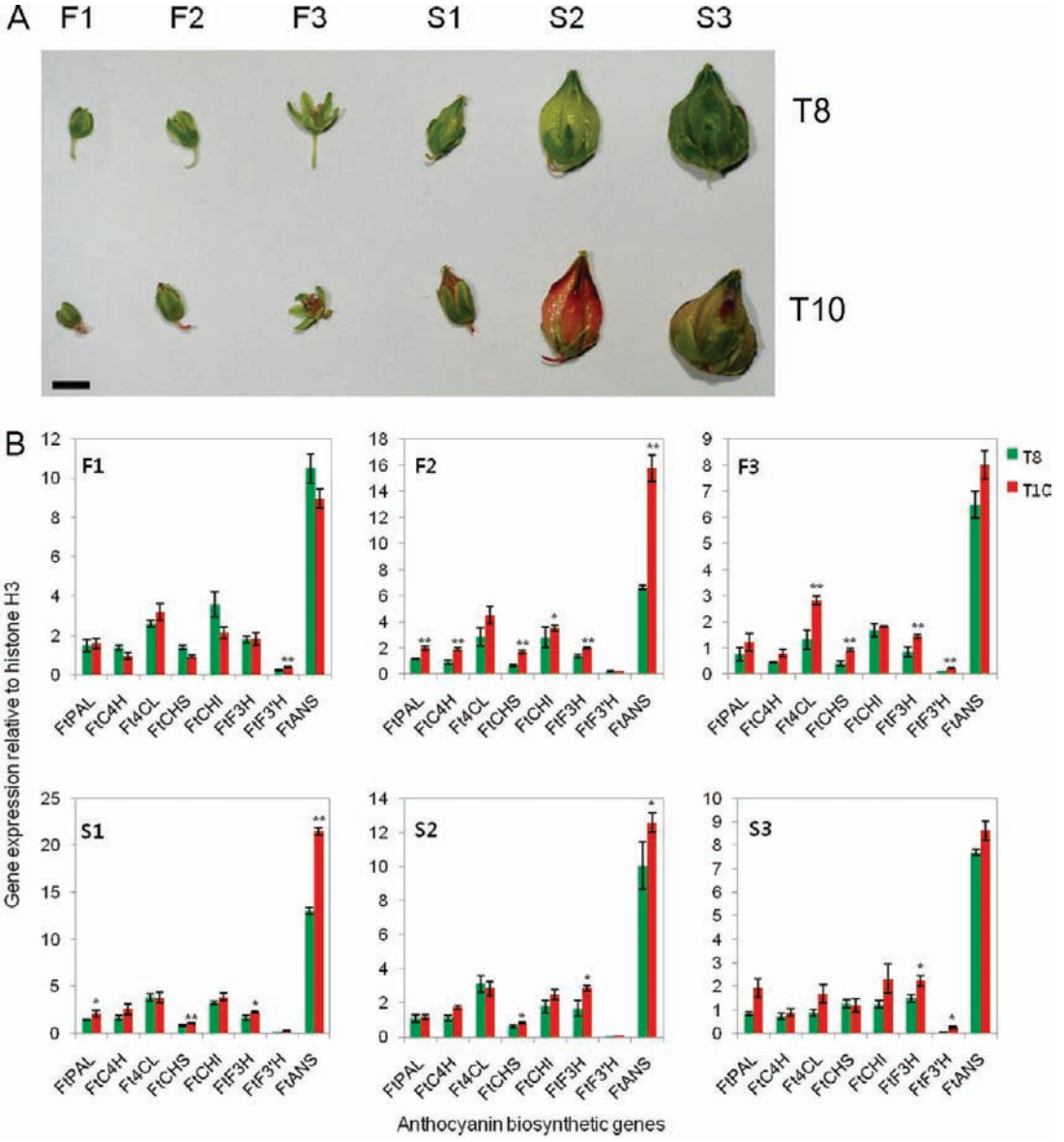

Figure 4. Anthocyanin biosynthetic genes in flowering stages and maturing seeds of tartary buckwheat cultivars 'Hokkai T8' and 'Hokkai T10'. (A) Picture of flowering stages (F1−F3) and maturing seeds (S1−S3). (B) Differential expression of anthocyanin biosynthetic genes in different flowering stages and maturing seeds of tartary buckwheat cultivars 'Hokkai T8' and 'Hokkai T10'. The height of each bar represents the mean of three measurements from three different plants of each cultivar, and error bars indicate the standard deviation. Asterisks indicate significant differences between 'Hokkai T8' and 'Hokkai T10' cultivars by Student's *t* test (∗, $p < 0.05$; ∗∗, $p < 0.01$).

(Figure 3). In particular, in stems, the expression of *FtPAL* and *FtANS* was about 5-fold higher in 'Hokkai T10' than in 'Hokkai T8'. In roots, the expression of *FtANS* was significantly higher in 'Hokkai T10' than in 'Hokkai T8', whereas the expression of *FtPAL* was higher in 'Hokkai T8' than in 'Hokkai T10' (Figure 3).

Expression of Anthocyanin Biosynthetic Genes in Different Flowering Stages and Maturing Seeds of 'Hokkai T8' and 'Hokkai T10'. To investigate the control of anthocyanin biosynthesis in *F. tataricum*, we examined the expression levels of anthocyanin biosynthesis genes in different flowering stages of *F. tataricum*. During flowering (F1−F3) and maturing seeds (S1−S3) (Figure 4A), most of the genes were expressed more in 'Hokkai T10' than in 'Hokkai T8', except stage F1 (Figure 4B). The *FtANS* gene was more highly expressed than other genes during flowering and maturing seeds. When we studied gene expression in different organs, our result showed that the expression of *FtCHI*, *FtF3′H*, and *FtANS* was higher in 'Hokkai T10' than in 'Hokkai T8' and the expression of the other genes was higher in 'Hokkai T8' than in 'Hokkai T10' (Figure 3).

DISCUSSION

Our results that the total anthocyanin content was higher in 'Hokkai T10' than in 'Hokkai T8' are consistent with previous studies.[11] For example, Suzuki et al.[9] showed that the total anthocyanin concentrations at 14, 28, and 45 DAS in the leaves of 'Hokkai T10' were 5-fold higher than those of 'Hokkai T8'. This cultivar-specific difference may be due to differential gene expression in different plant organs (Figures 3 and 4B). Specifically, the most highly expressed genes were *FtPAL*, *FtC4H*,

Ft4CL, *FtCHS*, *FtCHI*, *FtF3H*, and *FtANS* in the flowers, *FtPAL*, *FtCHS*, and *FtANS* in the leaves, *FtPAL*, *FtC4H*, *Ft4CL*, *FtCHS*, and *FtANS* in the stems, and *FtC4H*, *Ft4CL*, *FtCHS*, *FtF3H*, and *FtANS* in the roots. The expression of all of the genes examined in this study was higher in the lower parts of the plant (i.e., stems and roots) than the higher parts of the plant (i.e., flowers and leaves). Although we did not measure the flavonoid compounds in different organs of 'Hokkai T8' and 'Hokkai T10', the inverse relationship between the expression of flavonoid biosynthesis genes and the accumulation of their products may be accomplished in *F. tataricum* similar to *F. esculentum*.[18] Because 'Hokkai T10' is an EMS mutant of the 'Hokkai T8' cultivar, the mutagen drastically altered the levels of anthocyanin biosynthesis[11] and expression level of genes (our results). Although our results did not explain directly, EMS mutagenesis changed the nucleotide sequences of transcription factors (e.g., MYBs), which activate the anthocyanin biosynthetic genes.[19]

According to the proposed flavonoid biosynthesis pathway in tartary buckwheat (see Figure S1 in the Supporting Information), *FtF3H* and *FtANS* are critical enzymes for producing rutin and cyanidin 3-*O*-rutinoside. Because rutinose, the sugar moiety of cyanidin 3-*O*-rutinoside, is also part of the structure of rutin, cyanidin glycosyltransferases and glycosidases in the cotyledons of tartary buckwheat that recognize this moiety probably have similar substrate specificities as those reported for flavonoid 3-*O*-glycosyltransferase and flavonol 3-*O*-glycosidase, respectively.[20−22]

In conclusion, we cloned and characterized six anthocyanin biosynthetic genes, namely, *FtC4H*, *Ft4CL*, *FtCHI*, *FtF3H*, *FtF3′H*, and *FtANS*, from tartary buckwheat. We also compared the anthocyanin content in different organs of two cultivars, 'Hokkai T8' and 'Hokkai T10'. The results of this study have applications for further research about anthocyanin biosynthesis in tartary buckwheat, as well as the metabolic engineering of transgenic buckwheat plants for the production of medicinally useful compounds.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Primers used for RACE PCR and real-time PCR (Table S1), flavonoid biosynthetic pathway (Figure S1), protein sequence alignments (Figure S2), and accumulation of anthocyanin in different organs from two varieties 'Hokkai T8' and 'Hokkai T10' (Figure S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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■ ABBREVIATIONS USED

4CL, 4-coumarate:CoA ligase; ANS, anthocyanidin synthase; C4H, cinnamate 4-hydroxylase; CHI, chalcone isomerase; CHS, chalcone synthase; DAS, days after sowing; DPBA, diphenylboric acid-2-aminoethyl ester; F3H, flavone 3-hydroxylase; F3′H, flavonoid 3′-hydroxylase; FITC, fluoroscein isothiocyanate; PAL, phenylalanine ammonia lyase; RACE, rapid analysis of cDNA ends; qRT-PCR, quantitative real-time reverse transcription polymerase chain reaction

■ REFERENCES

(1) Bonafaccia, G.; Gambelli, L.; Fabjan, N.; Kreft, I. Trace elements in flour and bran from common and tartary buckwheat. *Food Chem.* **2003**, *83* (1), 1–5.

(2) Xuan, T. D.; Tsuzuki, E. Allelopathic plants: Buckwheat (*Fagopyrum* spp.). *Allelopathy* **2004**, *13*, 137–148.

(3) Guo, X.; Zhu, K.; Zhang, H.; Yao, H. Purification and characterization of the antitumor protein from Chinese tartary buckwheat (*Fagopyrum tataricum* Gaertn.) water-soluble extracts. *J. Agric. Food Chem.* **2007**, *55* (17), 6958–6961.

(4) Yao, Y.; Shan, F.; Bian, J.; Chen, F.; Wang, M.; Ren, G. D-*chiro*-Inositol-enriched tartary buckwheat bran extract lowers the blood glucose level in KK-A^{y} mice. *J. Agric. Food Chem.* **2008**, *56* (21), 10027–10031.

(5) Liu, C.-L.; Chen, Y.-S.; Yang, J.-H.; Chiang, B.-H. Antioxidant activity of tartary (*Fagopyrum tataricum* (L.) Gaertn.) and common (*Fagopyrum esculentum* Moench) buckwheat sprouts. *J. Agric. Food Chem.* **2008**, *56* (1), 173–178.

(6) Gaberscik, A.; Voncina, M.; Trost, T.; Germ, M.; Björn, L. O. Growth and production of buckwheat (*Fagopyrum esculentum*) treated with reduced, ambient, and enhanced UV-B radiation. *J. Photochem. Photobiol., B* **2002**, *66* (1), 30–36.

(7) Rozema, J.; Björn, L. O.; Bornman, J. F.; Gaberscik, A.; Häder, D. P.; Trost, T.; Germ, M.; Klisch, M.; Gröniger, A.; Sinha, R. P.; Lebert, M.; He, Y. Y.; Buffoni-Hall, R.; de Bakker, N. V. J.; van de Staaij, J.; Meijkamp, B. B. The role of UV-B radiation in aquatic and terrestrial ecosystems—An experimental and functional analysis of the evolution of UV-absorbing compounds. *J. Photochem. Photobiol., B* **2002**, *66* (1), 2–12.

(8) Fabjan, N.; Rode, J.; Kosir, I. J.; Wang, Z.; Zhang, Z.; Kreft, I. Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) as a source of dietary rutin and quercitrin. *J. Agric. Food Chem.* **2003**, *51* (22), 6452–6455.

(9) Suzuki, T.; Kim, S.-J.; Mohamed, Z. I. S.; Mukasa, Y.; Takigawa, S.; Matsuura-Endo, C.; Yamauchi, H.; Hashimoto, N.; Noda, T.; Saito, T. Structural identification of anthocyanins and analysis of concentrations during growth and flowering in buckwheat (*Fagopyrum esculentum* Moench) petals. *J. Agric. Food Chem.* **2007**, *55* (23), 9571–9575.

(10) Kim, S.-J.; Maeda, T.; Sarker, M. Z. I.; Takigawa, S.; Matsuura-Endo, C.; Yamauchi, H.; Mukasa, Y.; Saito, K.; Hashimoto, N.; Noda, T.; Saito, T.; Suzuki, T. Identification of anthocyanins in the sprouts of buckwheat. *J. Agric. Food Chem.* **2007**, *55* (15), 6314–6318.

(11) Suzuki, T.; Watanabe, M.; Iki, M.; Aoyagi, Y.; Kim, S.-J.; Mukasa, Y.; Yokota, S.; Takigawa, S.; Hashimoto, N.; Noda, T.; Yamauchi, H.; Matsuura-Endo, C. Time-course study and effects of drying method on concentrations of γ-aminobutyric acid, flavonoids, anthocyanin, and 2″-hydroxynicotianamine in leaves of buckwheats. *J. Agric. Food Chem.* **2009**, *57* (1), 259–264.

(12) Kim, S.-J.; Zaidul, I. S. M.; Maeda, T.; Suzuki, T.; Hashimoto, N.; Takigawa, S.; Noda, T.; Matsuura-Endo, C.; Yamauchi, H. A time-course study of flavonoids in the sprouts of tartary (*Fagopyrum tataricum* Gaertn.) buckwheats. *Sci. Hortic.* **2007**, *115* (1), 13–18.

(13) Winkel-Shirley, B. Flavonoid biosynthesis. A colorful model for genetics, biochemistry, cell biology, and biotechnology. *Plant Physiol.* **2001**, *126* (2), 485–493.

(14) Timotijevic, G. S.; Milisavljevic, M. D.; Radovic, S. R.; Konstantinovic, M. M.; Maksimovic, V. R. Ubiquitous aspartic proteinase as an actor in the stress response in buckwheat. *J. Plant Physiol.* **2010**, *167* (1), 61–68.

(15) Peer, W. A.; Brown, D. E.; Tague, B. W.; Muday, G. K.; Taiz, L.; Murphy, A. S. Flavonoid accumulation patterns of transparent testa mutants of *Arabidopsis*. *Plant Physiol.* **2001**, *126* (2), 536–548.

(16) Ronchi, A.; Farina, G.; Gozzo, F.; Tonelli, C. Effects of a triazolic fungicide on maize plant metabolism: Modifications of transcript abundance in resistance-related pathways. *Plant Sci.* **1997**, *130* (1), 51–62.

(17) Solfanelli, C.; Poggi, A.; Loreti, E.; Alpi, A.; Perata, P. Sucrose-specific induction of the anthocyanin biosynthetic pathway in *Arabidopsis*. *Plant Physiol.* **2006**, *140* (2), 637–646.

(18) Li, X.; Park, N. I.; Xu, H.; Woo, S.-H.; Park, C. H.; Park, S. U. Differential expression of flavonoid biosynthesis genes and accumulation of phenolic compounds in common buckwheat (*Fagopyrum esculentum*). *J. Agric. Food Chem.* **2010**, *58* (23), 12176–12181.

(19) Du, H.; Zhang, L.; Liu, L.; Tang, X.-F.; Yang, W.-J.; Wu, Y.-M.; Huang, Y.-B.; Tang, Y.-X. Biochemical and molecular characterization of plant MYB transcription factor family. *Biochemistry (Moscow)* **2009**, *74* (1), 1–11.

(20) Suzuki, T.; Honda, Y.; Funatsuki, W.; Nakatsuka, K. Purification and characterization of flavonol 3-glucosidase, and its activity during ripening in tartary buckwheat seeds. *Plant Sci.* **2002**, *163* (3), 417–423.

(21) Suzuki, T.; Honda, Y.; Mukasa, Y. Effects of UV-B radiation, cold and desiccation stress on rutin concentration and rutin glucosidase activity in tartary buckwheat (*Fagopyrum tataricum*) leaves. *Plant Sci.* **2005**, *168* (5), 1303–1307.

(22) Suzuki, T.; Kim, S.-J.; Yamauchi, H.; Takigawa, S.; Honda, Y.; Mukasa, Y. Characterization of a flavonoid 3-*O*-glucosyltransferase and its activity during cotyledon growth in buckwheat (*Fagopyrum esculentum*). *Plant Sci.* **2005**, *169* (5), 943–948.