JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

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Accumulation of Phenylpropanoids and Correlated Gene Expression during the Development of Tartary Buckwheat Sprouts

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Supporting Information

ABSTRACT: Buckwheat sprouts are considered an excellent dietary source of phenolic compounds. The time duration and amount of light for sprouting strongly affect the nutritional quality of sprouts. In this study, these two factors were investigated in two cultivars of tartary buckwheat sprouts: Hokkai T8 and T10. The transcriptional levels of flavonoid biosynthetic genes were investigated in light/dark- and dark-treated sprouts. Among the main flavonoid biosynthesis structural genes, *FtPAL*, *Ft4CL*, *FtF3H*, *FtDFR*, and *FtANS* exhibited higher transcriptional levels than others as compared to that of a housekeeping gene (histone H3) during sprouting; *FtF3′H1*, *FtF3′H2*, *FtFLS2*, and *FtANS* were substantially upregulated at 2, 4, and 6 days in light/dark-treated T10 sprouts than in dark-treated ones. However, *FtDFR* was downregulated in 8 and 10 day old light/dark-treated sprouts of both cultivars. High-performance liquid chromatography (HPLC) analysis revealed that increasing the culture time did not affect the accumulation of flavonoids or anthocyanins. However, light contributed the production of anthocyanins in Hokkai T10 sprouts. The anthocyanins included cyanidin 3-*O*-glucoside, cyanidin 3-*O*-rutinoside, and delphinidin-3-*O*-coumarylglucoside, which were identified by HPLC and electrospray ionization−tandem mass spectrometry. Instead of anthocyanins, Hokkai T8 sprouts produced large amounts of 4 flavonoid *C*-glycosylflavone compounds in both light/dark and dark conditions: orientin, isoorientin, vitexin, and isovitexin. These results indicate that these two types of tartary buckwheat sprouts have different mechanisms for flavonoid and anthocyanin biosynthesis that also vary in light/dark and dark conditions.

KEYWORDS: *tartary buckwheat sprouts, flavonoid biosynthesis pathway, gene expression, anthocyanins, LC-MS/MS*

INTRODUCTION

As a dicotyledonous crop of the Polygonaceae family, buckwheat is well-known as a healthy food because it contains large amounts of protein, amino acids, fatty acids, vitamin B, trace elements, and dietary fiber.[1−3] In particular, buckwheat has a high content of flavonoid compounds, which have beneficial and pharmacological effects on health. Rutin and quercitrin, both alone and in combination with other flavonoids, are known to have antioxidant activity, antiedema effects, and antibacterial effects; they also antagonize capillary fragility, decrease the permeability of the vessels, and reduce the risk of arteriosclerosis.[4−7]

Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) is widely grown and used in the mountainous regions of Southwest China (Sichuan) and the Himalayan region (i.e., Bhutan, Northern India, and Nepal) primarily because it is well adapted to cold conditions. Although tartary buckwheat is not consumed widely because of its bitter taste, tartary buckwheat seeds and sprouts contain more rutin than common buckwheat.[4,8,9] Furthermore, tartary buckwheat also possesses a substantially higher anthocyanin content than common buckwheat.[10,11]

Sprouts have received attention as functional vegetables because of their beneficial nutritional components, including amino acids, fiber, minerals, carbohydrates, and protein.[12] Many reports state that the cereal or vegetable type sprouts, such as radish and broccoli, wheat, soybean, lentil, barley, brown rice, and quinoa, contain more beneficial components and exhibit higher beneficial activity than do nongerminated seeds.[13−18] Buckwheat sprouts have also received great interest mostly because of their antioxidant components (polyphenols), although phototoxic substance (fagopyrin) contained in green parts of buckwheat plants.[19] Seed sprouts of both common and tartary buckwheat are also excellent sources of nutrients and phenolic compounds in addition to having a good balance of amino acids, minerals, and potential antioxidative activity.[11,20,21] It is reported that the main flavonoid compound, rutin, doubles after germination.[22] Also, tartary buckwheat sprouts are an excellent dietary source of phenolic compounds.[9] The sprouts of tartary buckwheat, especially the Hokkai T10 variety, which was developed from Hokkai T8 by chemical treatment, have a higher anthocyanin content than common buckwheat and are

Received: April 4, 2012
Revised: May 15, 2012
Accepted: May 15, 2012
Published: May 15, 2012

recommended as a new functional food for a rich dietary anthocyanin source.[11]

The nutritional quality of sprouts is influenced by many factors such as salt stress, germination time and techniques, the type of illumination, exogenous elicitors, and sugars.[20,23−26] Previously, we reported that different sucrose concentrations influence the synthesis of rutin in buckwheat sprout cultures.[27] In this study, we investigated flavonoid and anthocyanin production between tartary buckwheat T8 and T10 light- and dark-treated sprouts. In addition, we studied the dynamic transcriptional levels of flavonoid biosynthetic genes (Figure 1).

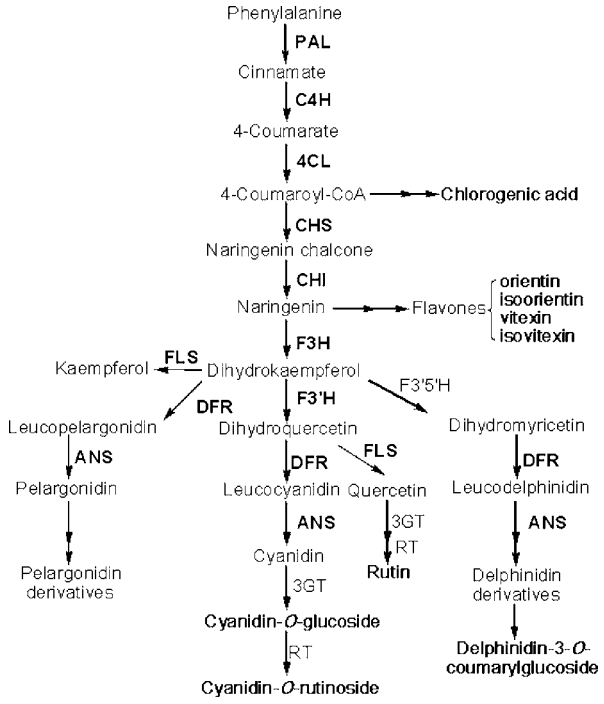

Figure 1. Schematic representation of flavonoid biosynthesis in *F. tataricum*. PAL, phenylalanine ammonium lyase; C4H, cinnamic acid 4-hydroxylase; 4CL, 4-coumarate-CoA ligase; CHS, chalcone synthase; CHI, chalcone isomerase; F3H, flavanone-3-hydroxylase; F3′H, flavonoid-3′-hydroxylase; F3′5′H, flavonoid-3′5′-hydroxylase; FLS, flavonol synthase; DFR, dihydroflavonol reductase; ANS, anthocyanin synthase; 3GT, flavonoid 3-*O*-glucosyltransferase; and RT, 3-*O*-rhamnosyltransferase. Boldface words indicate that the genes or compounds were detected in this study.

By analyzing both the chemical compounds and the biosynthetic gene expression of buckwheat sprouts, we aim to elucidate the mechanism of flavonoid and anthocyanin accumulation. Our study may enhance the theoretical basis for further study of buckwheat.

MATERIALS AND METHODS

Chemicals and Solvents. The external standards chlorogenic acid, orientin, isoorientin, vitexin, isovitexin, rutin, and quercetin were purchased from Extrasynthese (Genay, France); cyanidin 3-*O*-glucoside was purchased from Fujicco Co., Ltd. (Kobe, Japan); and high-performance liquid chromatography (HPLC)-grade acetonitrile (CH_3CN) and methanol (MeOH) were purchased from J. T. Baker Chemical Co. (New Jersey). Formic and acetic acid were provided by Kanto Chemical Co., Inc. (Tokyo, Japan) and Junsei Chemical Co., Ltd. (Kyoto, Japan), respectively.

Plant Materials and Culture Conditions. Two tartary buckwheat (*F. tataricum* Gaertn.) cultivars, "Hokkai T8" and "Hokkai T10", were bred by the Hokkaido Agricultural Research Center (Hokkaido, Japan). Hokkai T10 was produced by ethyl methanesulfonate mutagenesis of Hokkai T8, which was derived from the Russian cultivar "Rotundatum".[11] Tartary buckwheat seeds were dehulled, surface-sterilized with 70% ethanol for 30 s and 4% (v/v) bleach solution for 15 min, and then rinsed several times in sterile water. These seeds were placed on sucrose-free quarter-strength sterilized Murashige−Skoog medium (1/4 MS) solidified with 0.8% agar. The two cultivars were germinated in a growth chamber under light/dark (16/8 h) or dark conditions at 25 °C and approximately 60% humidity. Samples were harvested after 2, 4, 6, 8, and 10 days, measured for their length and fresh weight, frozen in liquid nitrogen, and stored at −80 °C until chemical analysis.

Total RNA Extraction and Quantification of Gene Expression. Total RNA was isolated from different *F. tataricum* sprouts using the RNeasy Plant Mini Kit (Qiagen, Valencia, CA). For quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), 1 μg of total RNA was reverse-transcribed using the Superscript II First Strand SynthesisKit (Invitrogen, Carlsbad, CA) and an oligo $(dT)_{20}$ primer. Transcription levels were analyzed by real-time PCR. The gene-specific primer sets were designed for real-time PCR as described previously.[28] Gene expression was normalized to that of the histone H3 gene as a housekeeping gene.[29] Real-time PCR reactions were performed 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: initial denaturation for 5 min at 95 °C and 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. The PCR results were calculated as the mean of three replicates. Statistical differences between treatments were evaluated according to standard deviations (SDs).

Flavonoid and Anthocyanin Extraction. The materials were freeze-dried and ground into a fine powder. Flavonoid and anthocyanin extraction was performed as described previously.[9] Briefly, for the flavonoid analysis, 10 mg samples were extracted with 1 mL of MeOH containing 10% phosphoric acid [0.1% (v/v)], vortexed at room temperature for 5 min, and stored at 37 °C for 3 h in an incubator with 5 min of vortexing after each hour. After centrifugation at 1000*g* for 5 min, the supernatant was filtered through a 0.45 μm PTFE syringe filter (Advantec DISMIC-13HP, Toyo Roshi Kaisha, Ltd., Tokyo, Japan) for HPLC analysis. The anthocyanins of buckwheat sprouts (100 mg of powder) were obtained by sonication extraction with MeOH containing 5% formic acid (v/v) for 20 min followed by centrifugation at 12000 rpm at 4 °C for 15 min. The supernatant was filtered through a 0.45 μm PTFE syringe filter for HPLC analysis.

Quantitative HPLC Analysis for Flavonoids. The HPLC analysis of flavonoids was performed on a Shimadzu class-VP HPLC system (Kyoto, Japan) equipped with a Capcell PAK ODS column (250 mm × 4.6 mm, 5 μm; Shiseido, Tokyo, Japan). The mobile phase consisted of a mixture of (A) MeOH:water:acetic acid (5:92.5:2.5, v/v/v) and (B) MeOH:water:acetic acid (95:2.5:2.5, v/v/v). The initial mobile phase composition was 0% solvent B, followed by a linear gradient from 0 to 80% of solvent B over 48 min, and then holding at 0% solvent B for an additional 10 min. The column was maintained at 40 °C, the flow rate was 1.0 mL/min, the injection volume was 10 μL, and the eluted components were monitored at 350 nm. Different compounds were quantified on the basis of peak areas and calculated as equivalents of representative standard compounds. All quantities are expressed as milligrams per gram dry weight.

Quantitative HPLC Analysis for Anthocyanins. The anthocyanin filtrate was analyzed using an Agilent Technologies 1200 Series HPLC (Agilent Technologies, Palo Alto, CA) equipped with an a Synergi 4 μ POLAR-RP 80A column (250 mm × 4.6 mm, i.d., particle size 4 μm; Phenomenex, Torrance, CA) equipped with a Security Guard Cartridges Kit AQ C18 column (4 mm × 3 mm, i.d.). The mobile phase consisted of a mixture of (A) water/formic acid (95:5, v/v) and (B) acetonitrile/formic acid (95:5, v/v). The gradient program, which was largely modified from our previous work,[30] was as follows: 0−8 min, 5−10% solvent B; 8−13 min, 10−13% solvent B; 13−15

Figure 2. Development of *F. tataricum* sprouts from 2 to 4 days. Two and 4 day old Hokkai T8 and T10 sprouts under light/dark and dark culture. L, light/dark; D, dark.

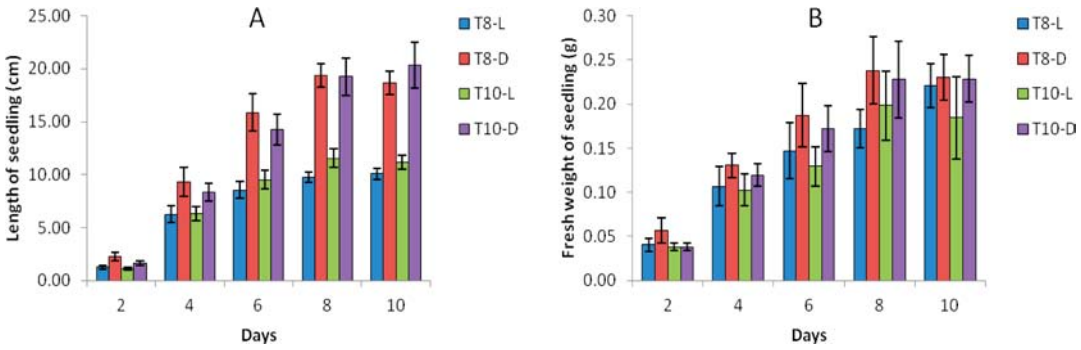

Figure 3. Growth of *F. tataricum* sprouts from 2 to 10 days. (A) The length of sprouts. (B) The fresh weight of Hokkai T8 and T10 sprouts from 2 to 10 days. Blue, red, green, and purple indicate T8-light, T8-dark, T10-light, and T10-dark sprouts, respectively.

min, 13% solvent B; 15−18 min, 13−15% solvent B; 18−25 min, 15% solvent B; 5% solvent B at 25.1 min; and finally 5% solvent B constant for 10 min (total 35 min). Detection was performed at 520 nm, and the column oven temperature was 40 °C. The flow rate was set at 1.0 mL/min, and the injection volume was 10 μL. The anthocyanin content was calculated by comparing the HPLC peak area with that of an authentic standard (cyanidin-3-*O*-glucoside).

HPLC-MS/MS Analysis for Anthocyanins. Anthocyanins were identified using an API 4000 Q TRAP tandem mass spectrometer system (Applied Biosystems, Foster City, CA) in the positive ion mode ($[M + H]^+$) equipped with an Agilent 1200 series HPLC system. Solvent A was water/formic acid (99:1, v/v), and solvent B was acetonitrile/formic acid (99:1, v/v). All HPLC conditions were the same as the HPLC quantitative analysis for anthocyanins described above. However, the LC eluate was directly introduced into the ESI interface with splitting at a flow rate of 200 μL/min. The MS/MS parameters were as follows: nebulizer and auxiliary gas, nitrogen; source temperature (TEM), 550 °C; scan range, 100−1300 amu (start−stop); scan time, 4.8 s; curtain gas, 20 psi (N_2); nebulizing gas, 50 psi; heating gas, 50 psi; ion spray voltage, 5500 V; declustering potential, 100 V; and entrance potential, 10 V.

Statistical Analysis. All results in the figures and tables are expressed as the mean of three replicates. Differences between treatments were analyzed according to SDs. Data are expressed as the mean ± SD of three independent replicates.

RESULTS AND DISCUSSION

Buckwheat Sprout Growth under Light/Dark Conditions. The fresh weight and length of buckwheat sprouts were measured from 2 to 10 days after sowing (DAS). The phenotypes of Hokkai T8 and T10 were completely different: Hokkai T10 had a red color from the cotyledon to roots (Figure 2). However, the trends of growth were similar between these two buckwheat cultivars (Figure 3). The length and weight increased with time. After 8 days of culture, the sprouts under the dark condition were almost twice as long as those under the light culture condition (Figure 3A); however, fresh weight did not increase significantly as compared to 10 day old sprouts (Figure 3B). These results indicate that maximum biomass for commercial purposes can be achieved for buckwheat sprouts cultured under darkness for around 8 days, which is concordant with the findings of a previous report.[9]

Expressions of Flavonoid Biosynthetic Genes in Hokkai T8 and T10 Sprouts. After germination, the transcriptional levels of a series of flavonoid and anthocyanin biosynthetic genes were investigated in Hokkai T8 and T10 by quantitative RT-PCR analysis. The expression patterns of *FtPAL*, *FtC4H*, *Ft4CL*, *FtCHS*, *FtCHI*, *FtF3H*, *FtF3′H*, *FtFLS*, *FtDFR*, and *FtANS*, which encode phenylalanine ammonium lyase (PAL), cinnamic acid 4-hydroxylase (C4H), 4-coumarate:CoA ligase (4CL), chalcone synthase (CHS), chalcone isomerase (CHI), flavanone-3-hydroxylase (F3H), flavonoid-3′-hydroxylase (F3′H), flavonol synthase (FLS), dihydroflavonol reductase (DFR), and anthocyanin synthase (ANS), respectively, are shown in Figure 4. As compared to other genes, *FtPAL*, *Ft4CL*, *FtF3H*, *FtDFR*, and *FtANS* exhibited higher transcriptional levels in both seeds and sprouts from 2 to 10 DAS relative to that of the histone H3 gene. In addition, the transcription of *FtANS* was 30 and 22 times greater than that of the histone H3 gene in light/dark-treated Hokkai T8 sprouts at 2 DAS. In particular, most of the genes in the flavonoid biosynthetic pathway were upregulated at 2 DAS except *FtPAL* and *FtF3′H2*. In general, flavonoids and anthocyanins shared a similar pathway from phenylalanine to dihydrokaempferol (Figure 1), while the genes from *PAL* to *F3H* were not significantly different between Hokkai T8 and T10 sprouts under light/dark or dark culture.

dx.doi.org/10.1021/jf301449a | J. Agric. Food Chem. 2012, 60, 5629−5635

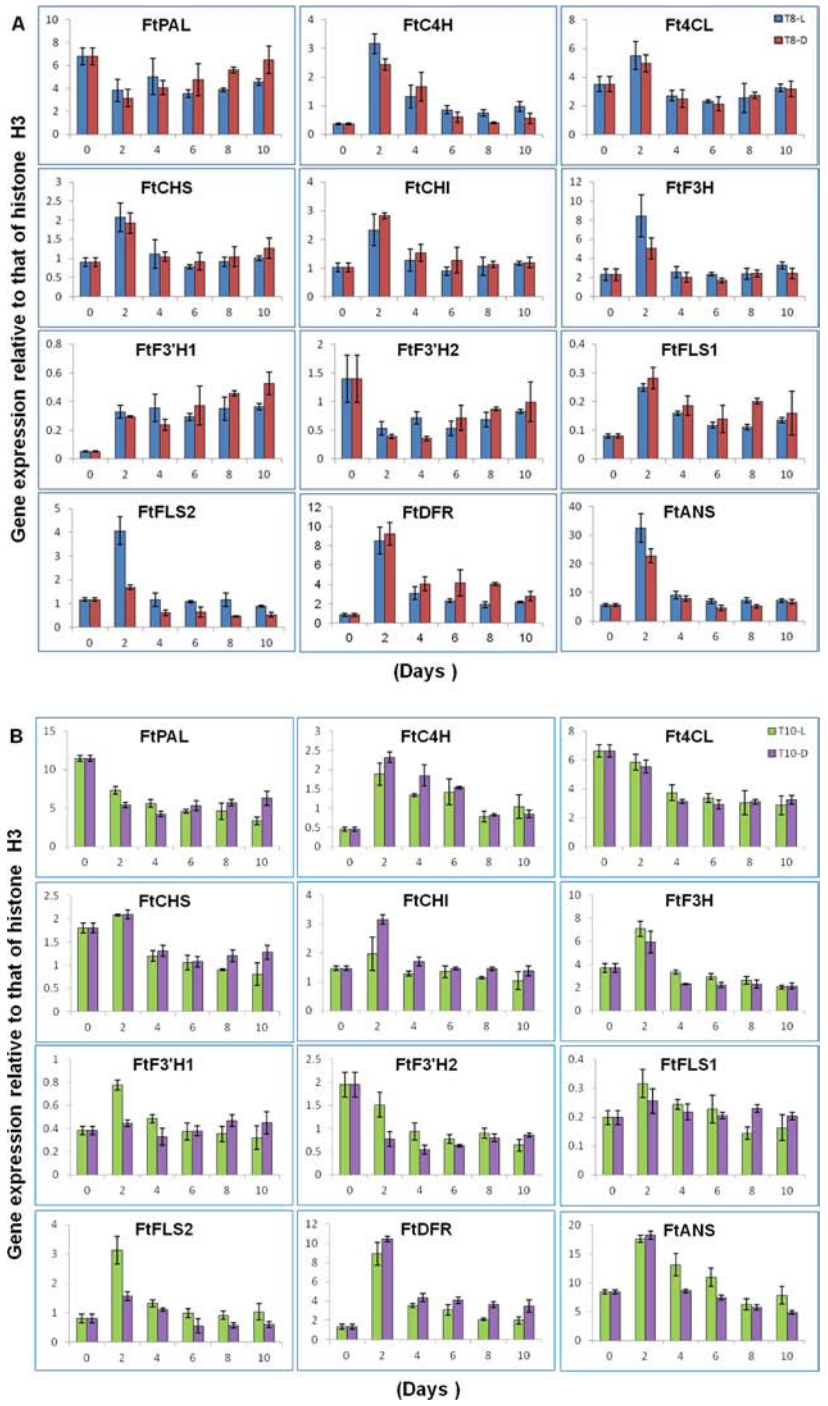

Figure 4. Expression of flavonoid and anthocyanin biosynthetic genes during the development of *F. tataricum* sprouts under light or dark culture. (A) T8 sprouts. (B) T10 sprouts. Blue and red indicate light/dark and dark conditions, respectively. The abscissa axis indicates DAS; the longitudinal axis indicates the expression levels of genes relative to that of histone H3. Each value is the mean of three replicates, and error bars indicate SDs.

Dihydrokaempferol plays an important role in synthesizing the derivatives of quercetin, kaempferol, and anthocyanins. Meanwhile, *FLS* and *F3′H* can invert dihydrokaempferol, forming quercetin or kaempferol branches. Interestingly, although two isomer genes of *FLS* and *F3′H* were investigated, only *FtFLS2* exhibited higher expression levels in both cultivars under the light/dark condition. The transcriptional levels of both *FtF3′H1* and *FtF3′H2* were obviously induced by light/dark treatment by 2 and 4 days in T10 sprouts (Figure 4); this indicates that *FtFLS2*, *FtF3′H1*, and *FtF3′H2* are upregulated by light, possibly contributing to the biosynthesis pathway fluxing toward quercetin or kaempferol branches. In this pathway, *DFR* and *ANS* play important roles in the biosynthesis of anthocyanins; this contrasts with *FtDFR*, which was upregulated under the dark condition after 4 days of culture as well as *FtANS*, which was upregulated by the light/dark condition and exhibited significantly higher transcript levels in all seedling cultures. Considering the position of *ANS* in the anthocyanin pathway, some anthocyanins may accumulate in high levels in tartary buckwheat.

The transcription factor MYB plays an important role in the metabolic pathways of plants. In this study, one transcription

dx.doi.org/10.1021/jf301449a | J. Agric. Food Chem. 2012, 60, 5629−5635

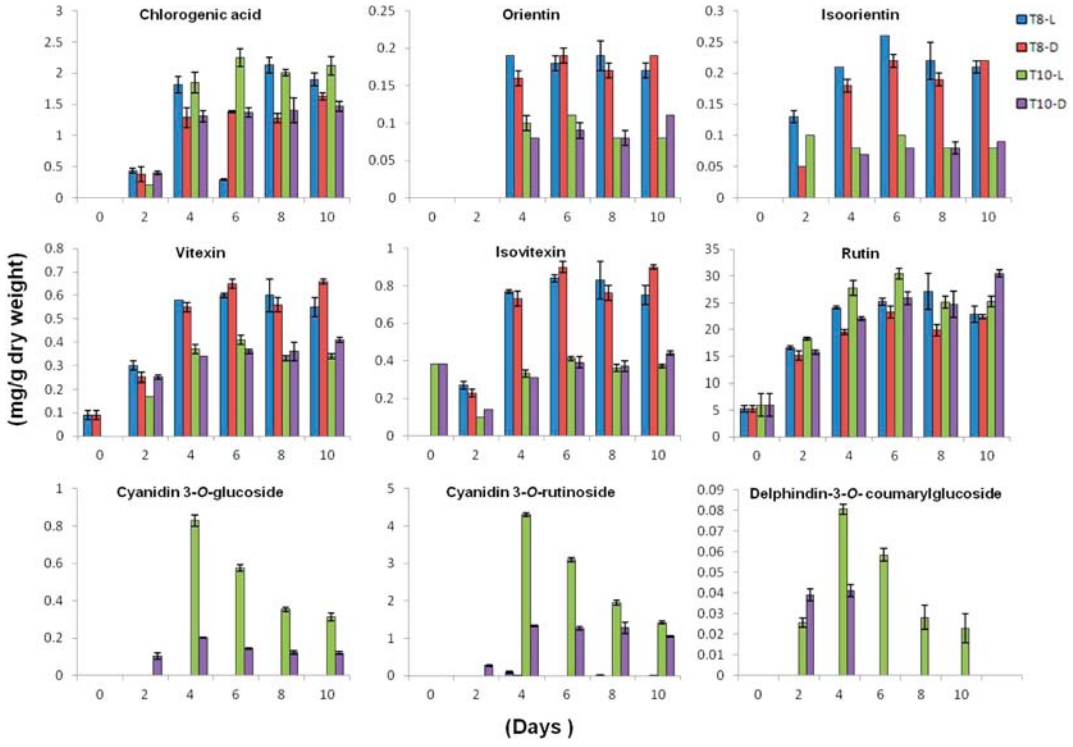

Figure 5. Accumulation of flavonoids and anthocyanins in *F. tataricum* sprouts. Blue, red, green, and purple indicate T8-light, T8-dark, T10-light, and T10-dark sprouts, respectively. Each value is the mean of three replicates, and error bars indicate SDs.

factor, *FtMYB*-like gene (GenBank accession number: HQ 214132) was also detected by qRT-PCR (Supporting Information, Figure 1). The expression of *FtMYB*-like gene was markedly induced in light/dark-treated sprouts; in particular, the expression of this *MYB*-like gene was substantially more upregulated in T10 in light/dark-treated sprouts from 2 to 8 days than in dark-treated ones. In general, regulatory genes regulate a series of structural genes to influence secondary metabolites in plants; for example, *AtMYB12* can affect the main genes of the flavonol biosynthetic pathway or caffeoylquinic acid biosynthetic pathway, leading high accumulation of these two polyphenols in *Arabidopsis thaliana* or tomato.[31,32] Furthermore, the heterologous coexpression the MYC-type leaf color (LC) gene and MYB type C1 (colorless) gene from maize can induce more kaempferol type flavonols and pelargonidin type anthocyanins in ripening tomato fruit since LC/C1 regulates most of the structural genes in the metabolic pathway of tomato.[33] Combining the results of the expression analysis of structural genes and transcriptional pattern of *FtMYB*-like gene in light/dark-treated buckwheat sprouts suggests that the transcription factor *FtMYB*-like gene regulates some structural genes such as *FtF3′H*, *FtFLS2*, *FtDFR*, and *FtANS* (Figure 4) in light/dark-treated buckwheat sprouts. Furthermore, *FtMYB*-like gene is potentially involved in the regulation of flavonoids and/or anthocyanin biosynthesis in light/dark-treated buckwheat sprouts.

Flavonoids in Buckwheat Sprouts. The accumulation of flavonoids and anthocyanins including chlorogenic acid, four *C*-glycosylflavones (i.e., orientin, isoorientin, vitexin, and isovitexin), and rutin was investigated in Hokkai T8 and T10 sprouts (Figure 5). Chlorogenic acid and these four *C*-glycosylflavones were rare in Hokkai T8 and T10 seeds but appeared after 2 days of culture. Chlorogenic acid tended to be induced by light in Hokkai T8 and T10 from 4 DAS. Meanwhile, the four *C*-glycosylflavones increased substantially in these two kinds of buckwheat sprouts; Hokkai T8 had twice as much as Hokkai T10. In addition, the rutin content of sprouts was significantly greater than that in ungerminated seeds. Furthermore, light/dark-cultured sprouts contained slightly higher rutin levels from 4 to 8 days among both Hokkai T8 and T10.

Identification and Accumulation of Anthocyanins in Buckwheat Sprouts. Cyanidin is the only reported aglycone present in tartary buckwheat sprouts, while the major anthocyanin is cyanidin 3-*O*-rutinoside,[10,34] which may mainly contribute to the red color of tartary buckwheat sprouts. Anthocyanins exhibit maximum absorption between 500−535 nm in the UV−visible spectrum with different aglycone-based derivatives including pelargonidin (502−506 nm), cyanidin (512−520 nm), peonidin (517−520 nm), delphinidin (525 nm), petunidin (526−529 nm), and malvidin (530 nm).[35] According to the HPLC graphs, there were three prominent anthocyanin peaks in 4 day old Hokkai T10 sprouts at 520 nm—an anthocyanin-specific wavelength (Supporting Information, Figure 2). Two anthocyanins, cyanidin 3-*O*-glucoside (*m*/*z* 449.1, $[M + H]^+$) (Supporting Information, Figure 4A) and cyanidin 3-*O*-rutinoside (*m*/*z* 595.1, $[M + H]^+$) (Supporting Information, Figure 4B), were identified by comparing the MS spectral data (Supporting Information, Figure 3) and HPLC profiles.[11] Moreover, the third peak, which was much smaller, was putatively identified as delphinidin-3-*O*-coumarylglucoside (Supporting Information, Figure 4C)[36−38] having molecular ions *m*/*z* 611.1 ($[M + H]^+$) and product ions *m*/*z* 465.1 ($[M + H]^+$) and 302.9 ($[M + H]^+$), suggesting the loss of w fragmented ions, *m*/*z* 146 and *m*/*z* 162, and the delphinidin aglycone (*m*/*z* 302.9, $[M + H]^+$). The structures and molecular weights of these three anthocyanin compounds are shown in

dx.doi.org/10.1021/jf301449a | J. Agric. Food Chem. 2012, 60, 5629−5635

Supporting Information, Figure 4. This is the first time a delphinidin derivative, delphinidin-3-*O*-coumarylglucoside, has been reported in tartary buckwheat sprouts.

In contrast to the accumulation of flavonoids, the HPLC results show that these three anthocyanin compounds accumulated in greater amounts in Hokkai T10 light/dark-cultured sprouts; 4 day old seedlings contained nearly 4-fold more individual anthocyanin compounds than those grown under the dark condition (Figure 5). The greatest amounts of cyanidin 3-*O*-glucoside, cyanidin 3-*O*-rutinoside, and delphinidin-3-*O*-coumarylglucoside were 0.83, 4.30, and 0.08 mg/g (cyanidin 3-*O*-glucoside equivalent) in 4 day old Hokkai T10 sprouts under light/dark culture, respectively; these decreased noticeably with culture time to 0.31, 1.43, and 0.02 mg/g at 10 days, respectively, although they did not decrease drastically in dark-cultured sprouts. Meanwhile, only cyanidin 3-*O*-rutinoside was detected in trace amounts in Hokkai T8 sprouts.

Combining the HPLC results and main transcriptional patterns of flavonoids biosynthetic genes indicates that the flavonoid biosynthesis differs between Hokkai T8 and T10. In Hokkai T8 sprouts, the flavonoid biosynthesis pathway mainly focuses on flavone biosynthesis. In contrast, the flavonoid biosynthesis pathway strongly favors anthocyanin biosynthesis in Hokkai T10 mutants. Therefore, chemical treatments to make new lines, such as methanesulfonate mutagenesis, suppress the side branches of flavone biosynthesis; this will result in naringenin production, which provides more substrates to the dihydrokaempferol pool (Figure 1). Therefore, such treatments may act on the flavonoid pathway in a similar manner to that of some genetic engineering technologies.[39,40] Moreover, the pathway efficiently flows toward the biosynthesis of anthocyanins, especially cyanidin derivatives, in Hokkai T10 sprouts. Further study is required to adequately explain the differential flavonoid biosynthesis mechanisms in tartary buckwheat Hokkai T8 and T10.

■ ASSOCIATED CONTENT

Supporting Information

Figure depicting expression of *FtMYB*-like gene in tartary buckwheat sprouts, figure depicting a typical chromatograph of tartary buckwheat at 520 nm, figure depicting LC-MS/MS ion graphs of 4 day old tartary buckwheat Hokkai T10 sprouts under light/dark culture, and figure depicting structures and major cleavage of anthocyanins identified in *F. tataricum* sprouts. This material is available free of charge via the Internet at http://pubs.acs.org.

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Funding

This study was supported by Technology Development Program for Agriculture and Forestry, Ministry for Food, Agriculture, Forestry, and Fisheries, Republic of Korea

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

HPLC, high-performance liquid chromatography; PAL, phenylalanine ammonium lyase; C4H, cinnamic acid 4-hydroxylase; CHI, chalcone isomerase; CHS, chalcone synthase; 4CL, 4-coumarate-CoA ligase; DFR, dihydroflavonol reductase; F3H, flavanone-3-hydroxylase; F3′H, flavonoid-3′-hydroxylase; F3′5′H, flavonoid-3′5′-hydroxylase; FLS, flavonol synthase; ANS, anthocyanin synthase; 3GT, flavonoid 3-*O*-glucosyltransferase; RT, 3-*O*-rhamnosyltransferase

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