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Characterization of Genes for a Putative Hydroxycinnamoyl-coenzyme A Quinate Transferase and *p*-Coumarate 3′-Hydroxylase and Chlorogenic Acid Accumulation in Tartary Buckwheat

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Supporting Information

ABSTRACT: Tartary buckwheat (*Fagopyrum tataricum* Gaertn.) contains a high level of flavonoid compounds, which have beneficial and pharmacological effects on health. In this study, we isolated full-length cDNAs encoding hydroxycinnamoyl-coenzyme A quinate hydroxycinnamoyltransferase (HQT) and *p*-coumarate 3′-hydroxylase (C3H), which are involved in chlorogenic acid (CGA) biosynthesis. We examined the expression levels of HQT and C3H using real-time RT-PCR in different organs and sprouts of two tartary buckwheat cultivars (Hokkai T8 and T10) and analyzed CGA content using high-performance liquid chromatography. Among the organs, the flowers in both cultivars showed the highest levels of CGA. We concluded that the expression pattern of *FtHQT* and *FtC3H* did not match the accumulation pattern of CGA in different organs of T8 and T10 cultivars. Gene expression and CGA content varied between the cultivars. We presume that *FtHQT* and *FtC3H* levels might be controlled by multiple metabolic pathways in different organs of tartary buckwheat. Probably, *FtC3H* might have a greater effect on CGA biosynthesis than *FtHQT*. Our results will be helpful for a greater understanding of CGA biosynthesis in tartary buckwheat.

KEYWORDS: *chlorogenic acid, gene expression, light, tartary buckwheat*

INTRODUCTION

Buckwheat (Polygonaceae family) includes two species of cultivated buckwheat: common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*Fagopyrum tataricum*). Studies have provided evidence that tartary buckwheat has anticancer, antidiabetic, and antioxidant effects.[1−3] Buckwheat is a rich source of rutin and produces anthocyanins, which are water-soluble pigments, in the leaves, stems, flowers, and roots.[4] Suzuki et al.[4] have obtained new tartary buckwheat cultivars, Hokkai T10 (T10) and Hokkai T8 (T8), which have much higher levels of anthocyanins than common buckwheat. Kim et al.[5] noted that the sprouts of tartary buckwheat, particularly those of T10, are strongly recommended as a new high-rutin food.

Recently, the importance of buckwheat in human health and wellness has increased research interest concerning the synthesis and accumulation of polyphenol compounds such as flavones, flavonols, flavanones, and tannins. Tannins are classified into hydrolyzable, condensed, and complex tannins. Of the hydrolyzable tannins, caffeic acid derivatives contain chlorogenic acid (CGA), caffeetannin, dicaffeoylquinic acid, and rosmarinic acid.[6] Luthar[6] also reported that buckwheat seeds in diploid and tetraploid buckwheat cultivars contained between 0.5 and 4.5% tannin. They consist of a family of esters, including certain hydroxycinnamic acids (such as caffeic acid and ferulic acid), and quinic acid.[7,8] Some researchers have reported that CGA can be relatively well absorbed by people consuming certain plant foods.[9−11]

Owing to these important roles, many studies have focused on the metabolism of flavonoids in recent years. CGA is synthesized by two key enzymes, hydroxycinnamoyl-coenzyme A (CoA) quinate hydroxycinnamoyltransferase (HQT) and *p*-coumarate 3′-hydroxylase (C3H) (Figure 1). These enzymes catalyze the formation and hydrolysis of CGA.[12,13] Lepelley et al.[14] reported that two key enzymes catalyze the formation and hydrolysis of CGA, that is, hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase (HCT) and HQT. HQT enzyme activity has been identified in *Coffea canephora*,[14] *Cynara cardunculus*,[15,16] and *Trifolium pratense*,[17] and HCT has been purified and characterized from tobacco.[13,18] Although the biosynthetic pathways of CGA have not been defined completely, three possible mechanisms have been proposed in plants. In the first, HQT catalyzes the formation of CGA from caffeoyl-CoA and quinic acid.[19] In this pathway, Mahesh et al.[20] pointed out that caffeoyl-CoA is supplied by the

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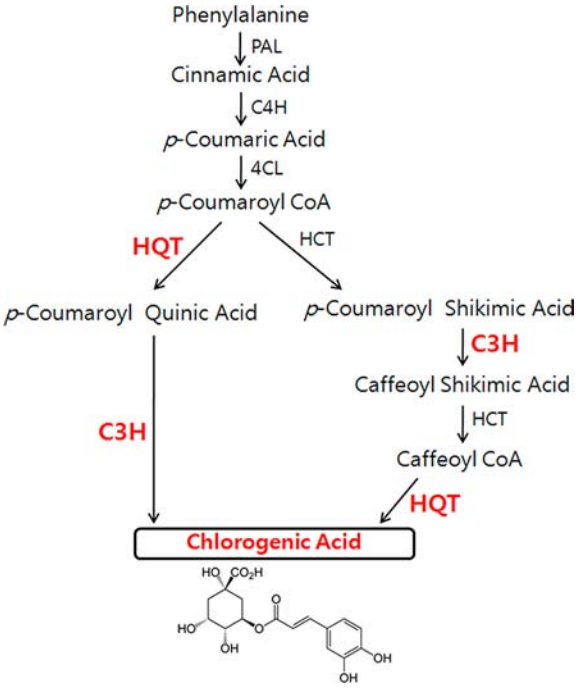

Figure 1. Schematic overview of the CGA pathway in buckwheat (redrawn from André et al.[45]). PAL, phenylalanine ammonia-lyase; C4H, cinnamic acid 4-hydroxylase; 4CL, 4-coumaroyl:CoA-ligase; HQT, hydroxycinnamoyl−coenzyme A quinate hydroxycinnamoyl transferase; HCT, hydroxycinnamoyl-coenzyme A shikimate/quinate hydroxycinnamoyl transferase; C3H, *p*-coumarate 3′-hydroxylase.

combined activity of HCT and C3H via a *p*-coumaroylshikimate intermediate. Moreover, HCT could act either before or after the 3′-hydroxylation step and may contribute to CGA biosynthesis.[16] The second pathway is the hydroxylation of *p*-coumaroylquinate to CGA.[19] In *Arabidopsis thaliana*, HCT and C3H are both active, but CGA does not accumulate,[18] and HCT does not display a close relationship with quinate as an acyl acceptor.[21] Therefore, Sonnante et al.[16] suggested that HCT could be supplemented by HQT in this pathway in some plants. For the third pathway, it was suggested that caffeoyl glucoside serves as an activated intermediate.[22] Niggeweg and colleagues[21] isolated and characterized cDNA encoding HQT from tobacco and tomato; they suggested that HQT is a very important enzyme in the synthesis of CGA in tomato.[21]

When HQT was overexpressed in tomato, the CGA levels increased up to 85% in the leaves, whereas HQT silencing resulted in a 98% reduction of CGA levels in the leaves.[21] Lepelley et al.[14] pointed out that the expression of HQT is more closely correlated with CGA synthesis and accumulation than HCT in coffee. It was found that C3H acts as a cytochrome P450-dependent monooxygenase in various plants such as *Arabidopsis*,[12,23,24] red clover,[17] and globe artichoke.[25] C3H enzyme activity has been characterized in *Ocimum basilicum*,[26] *Ruta graveolens*,[27] and *T. pratense*.[17] In the *Arabidopsis* genome, three C3H genes have been identified, and only one of these genes (C3H1) was expressed in all tissues.[28] Mahesh et al.[20] identified two C3H enzymes, one of which uses both *p*-coumaroylshikimate and *p*-coumaroylquinate with similar efficiency, whereas the other utilizes *p*-coumaroylshikimate only in coffee.

Previously, we reported that different sucrose concentrations influence the synthesis of rutin in buckwheat sprout cultures.[29] We also described the production of flavonoids and anthocyanins in tartary buckwheat T8 and T10 light- and dark-treated sprouts.[30] In addition, the carotenoid content of Chinese cabbage sprout was reportedly affected by light.[31] Therefore, in the present study, to gain information on the impact of mRNA levels of genes involved in CGA biosynthesis and also to determine CGA content during the development of tartary buckwheat sprouts under light and dark conditions, we isolated and characterized two full-length cDNA sequences encoding HQT and C3H from two tartary buckwheat cultivars. The expression patterns of HQT and C3H in sprouts and different organs were examined using real-time reverse-transcription polymerase chain reaction (RT-PCR), and CGA levels were ascertained using high-performance liquid chromatography (HPLC).

MATERIALS AND METHODS

Preparation of Plant Materials. T8 and T10 tartary buckwheat samples were provided by the Hokkaido Agricultural Research Center (Hokkaido, Japan). Their seeds were surface sterilized with 70% ethanol for 1 min and with a 4% (v/v) bleach solution for 20 min and then rinsed several times in sterile water. For biological repeats, we used three plastic magenta boxes per sample and harvested seedlings

Table 1. Primers Used in This Study

primer name	sequence (5′ → 3′)	length (bp)
FtHQT-ORF(F)	ATGGAGAAGAAGATGATAATCAACGTGAG	1308
FtHQT-ORF(R)	TCATATTTCATAGAGGTACTTTGAGAATAGCTTC	
FtC3H-ORF(F)	ATGCTCCTCCTCGTTTCCATTG	1524
FtC3H-ORF(R)	TTACATATCCACAGGTACCCTTTTGTAC	
FtHQT-RT(F)	ATGTTTGGCGTTGTGTTTGT	138
FtHQT-RT(R)	TGGTGGTGAAGATCACGTTT	
FtC3H-RT(F)	GGCTATCATTGAGGAGCACA	137
FtC3H-RT(R)	TCATGTCCCAAAGAAGTCCA	
FtH3 (F)	GAAATTCGCAAGTACCAGAAGAG	158
FtH3 (R)	CCAACAAGGTATGCCTCAGC	
FtC3H-5′RACE(R)	TGATTTTCAGGAGAGATGCAATCTTTGTGAATGG	

including roots in each plastic box at 0, 3, 6, 9, and 12 days after sowing (DAS). Seedlings were grown under light condition (16 h light/8 h darkness) and dark condition (24 h darkness) at 25 °C. Seeds were used as 0 DAS. In addition, to study the different organs, the seeds were sown on May 10, 2012, and T8 and T10 plants (with 12 in each pot filled with the perlite-mixed soil to reduce error variation) were cultured in the Chungnam National University greenhouse (25 °C and 50% humidity). Each organ was collected after 6 weeks. Sprouts and different organs (seed stages 1, 2, and 3; flowers, stems, leaves, and roots) were ground with a mortar and pestle in liquid nitrogen as preparation for gene expression studies and CGA analysis.

Isolation of HQT and C3H cDNA from *F. tartary*. Two different methods were used to extract the total RNA. Total RNA of sprouts was isolated using an easy BLUE total RNA Kit (iNtRON, Korea). For complex polysaccharides, each different organ was extracted according to a minor modified cetyltrimethylammonium bromide (CTAB) method.[32] RNA quantity and quality were measured by a NanoDrop ND-1000 spectrophotometer and assessed by running 1 μg of RNA on 1.2% formaldehyde RNA agarose gel, respectively. First-strand cDNA was synthesized from 1 μg of total RNA in a total volume of 20 μL using a ReverTra Ace-α-(Toyobo, Osaka, Japan) Kit and oligo $(dT)_{20}$ primer according to the manufacturer's instructions. The synthesized cDNA was used as the template for real-time RT-PCR and rapid amplification of cDNA ends (RACE) PCR. A full-length cDNA of putative HQT was obtained from next-generation sequencing platforms (NGS) (Roche/454 GS_FLX+ and Illumina/Solexa HiSeq2000) (unpublished data) of *F. tataricum* of SolGent Co. (Daejeon, Korea). C3H partial sequences were obtained from NGS data, and a full-length cDNA was obtained using 5′ RACE PCR. 5′ RACE was carried out following the methods described in the 5′ RACE system kit (Life Technologies, USA). The primer pairs used for 5′ RACE PCR, real-time RT-PCR, and ORF PCR are given in Table 1. cDNA of T10 flower was used as the template for RACE or ORF PCR for C3H and HQT. The PCR conditions were as follows: 94 °C for 3 min, 94 °C for 30 s, 68 °C for 30 s, and 72 °C for 1 min for 30 cycles, followed by one step at 72 °C for 7 min. The PCR products were then analyzed by agarose gel electrophoresis. The fragment of the expected size was then cloned into pCR4-TOPO using TOPO TA Cloning Kit (Life Technologies, USA) according to the instructions given by the manufacturer. After sequencing confirmation, full-length HQT and C3H sequences were aligned using the MultAlin online program (http://bioinfo.genotoul.fr/multalin/multalin.html) and separately subjected to NCBI's Basic Local Alignment Search Tool (BLAST) search.

Quantitative Real-Time RT-PCR Analysis. For real-time RT-PCR, the TM Calculator program (http://frodo.wi.mit.edu/primers3) was used to compute the PCR annealing temperatures. Real-time RT-PCR assay was carried out in a total volume of 20 μL, containing 10 μL of 2× SYBR Green real time PCR master mix (Toyobo), 0.5 μM (each) of specific primers, and 5 μL of cDNA diluted 20-fold. Thermal cycling conditions were as follows: 95 °C for 3 min, followed by 40 cycles of 95 °C for 15 s, 72 °C for 20 s, and annealing temperature of 55 °C for 30 s. PCR products were analyzed by Bio-Rad CFX Manager 2.0 software. The reaction was performed in triplicate. Expression of these genes was calculated according to the method of relative quantification using the histone H3 gene (GenBank No. HM628903) as the reference.[30,33,34]

Bioinformatic Analysis. Sequence similarities were calculated with the BLAST (http://blast.ncbi.nlm.nih.gov/). The deduced amino acid sequences of *FtHQT* and *FtC3H* were aligned using BioEdit (biological sequence alignment editor). Theoretical molecular weights (MW) and p*I* values were calculated with the Compute pI/M_w tool (http://ca.expasy.org/tools/pi_tool.html). Gene-specific primers were designed using an online program (https://www.genscript.com/ssl-bin/app/primer) (Table 1). The putative target location of the plant was predicted online through PSORT (http://wolfpsort.org/).

Estimation of CGA. 3-(3,4-Dihydroxycinnamoyl)quinic acid (CGA) analysis was carried out by HPLC. Sprouts and different organs were ground into a fine powder using a mortar and pestle. Approximately 100 mg of powdered samples was extracted in 80% (v/v) methanol at room temperature for 60 min. Thereafter, the extracts were centrifuged, and the supernatant was filtered with a 0.45 μm Acrodisc syringe filter (Pall Corp., Port Washington, NY, USA) for HPLC analysis. CGA analysis was performed with a C18 column (μBondapak C18 10 μm, 125 Å, 3.9 × 300 mm). The mobile phase was a gradient prepared from mixtures of methanol and 0.5% acetic acid. The flow rate was maintained at 0.8 mL/min. An injection volume of 20 μL was used for detection at 280 nm wavelengths. CGA was quantified on the basis of peak areas and calculated as equivalents of representative standard compounds. All quantities are expressed as milligrams of dry weight, and all samples were analyzed in triplicate.

Statistical Analysis. The data were analyzed by using the computer software Statistical Analysis System (SAS version 9.2). All data are given as the mean and standard deviation of triplicate experiments. Treatment mean comparisons were performed with the least significant difference (LSD) test.

RESULTS AND DISCUSSION

Isolation and Sequence Analysis of HQT and C3H from Tartary Buckwheat. The full-length cDNA sequences of *FtHQT* and *FtC3H* have been deposited in GenBank (KC404851 and KC404850, respectively). The HQT obtained from the NGS platforms (Roche/454 GS_FLX+ and Illumina/Solexa HiSeq2000) (unpublished data) of *F. tataricum* was composed of a 1308 bp open reading frame (ORF) encoding a 435 residue protein from a full-length sequence of 1555 bp. The theoretical molecular weight (MW) and isoelectric point values were 48.65 kDa and 6.28, respectively. From BLAST analysis of the deduced amino acid sequence of HQT, *FtHQT* shared homology with *Hibiscus cannabinus* HCT (82% identity, 91% similarity), *Coffea arabica* HCT (81% identity, 90% similarity), *C. arabica* HQT (81% identity, 90 similarity), and *Populus trichocarpa* HQT (80% identity, 90% similarity). The *FtHQT* protein sequence possessed two conserved BAHD acyltransferase domains (HXXXDG and DFGWG) that were previously associated with plant acyltransferases (Supporting Information, Figure S1).[35,36] Sonnante et al.[16] found *cis*-regulatory elements present in other genes of the phenylpropanoid pathway in the upstream region of *HQT1* and *HQT2* from artichoke using the PLACE database. *FtC3H* cDNA was isolated from the flowers of *F. tataricum* T10 by using RACE with partial sequence obtained from NGS data. Its gene contained a 1524 bp ORF of 1544 bp and encoded a polypeptide of 507 amino acids with a MW of 57.95 kDa. *FtC3H* showed the greatest homology with *Eucalyptus globules* C3H (84% identity, 93% similarity), *P. trichocarpa* C3H (83% identity, 91% similarity), *Populus alba* × *Populus grandidentata* C3H (83% identity, 91% similarity), *Platycodon grandiflorus* C3H (81% identity, 92% similarity), and *Cynara cardunculus* C3H (82% identity, 92% similarity) (Supporting Information, Figure S2). The encoded FtC3H had a valine at position 75, as does CYP98a44v2 of red clover,[17] and the heme-binding sequence PFGXGRRXCX, which is highly conserved among the cytochrome P450 enzymes of higher plants, was also found between amino acid positions 432 and 441 (Supporting Information, Figure S2). The putative target location of *FtHQT* was predicted as the cytosol and chloroplasts, whereas *FtC3H* was located in the vacuoles and chloroplasts.

***FtHQT* and *FtC3H* Gene Expression during Sprout Development under Dark and Light Conditions in T8 and T10 Cultivars.** The transcript levels of *FtHQT* and *FtC3H* during the development of tartary buckwheat sprouts under light and dark conditions were investigated. Real-time RT-PCR

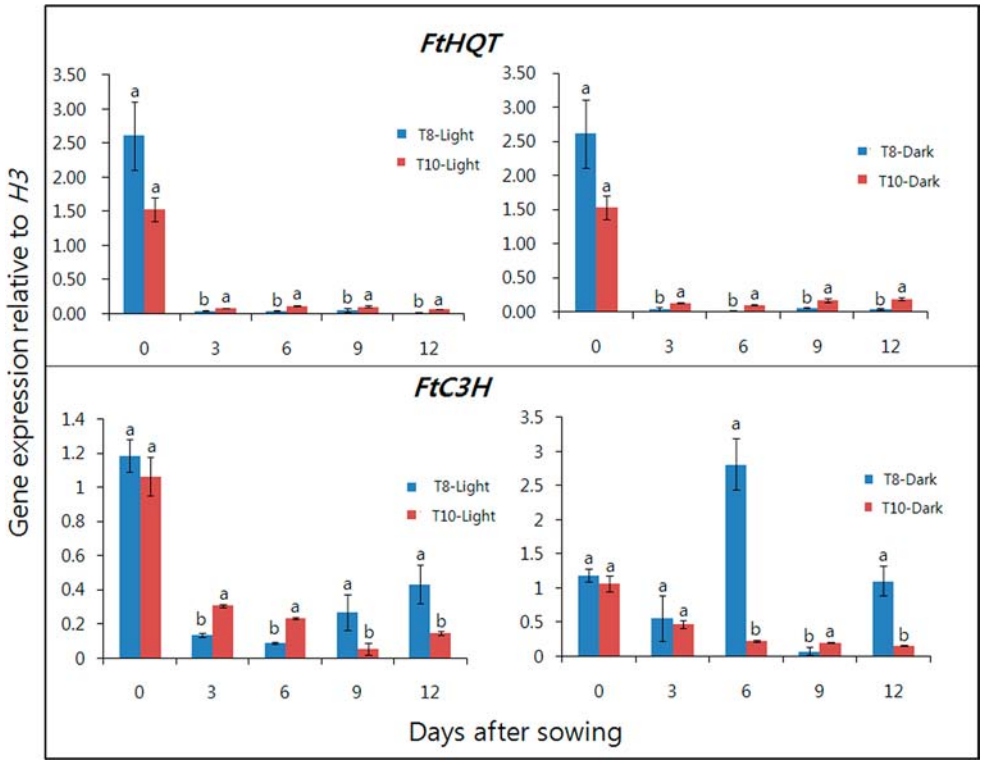

Figure 2. Relative expression levels of *FtHQT* and *FtC3H* in *F. tataricum* T8 and T10 sprouts. The height of each bar and the error bars show the mean and standard error, respectively, from three independent measurements. Mean values indicated by the same letter are not significantly different according to the least significant difference test (p = 0.05).

analysis with samples taken at 3 day intervals indicated that *FtHQT* and *FtC3H* were expressed at different DAS starting from 0 DAS to 12 DAS under dark and light conditions (Figure 2). The expression levels of *FtHQT* did not differ significantly from 3 to 12 DAS for both cultivars in dark and light conditions. Under light conditions at 6 DAS, the expression of *FtHQT* in T10 was 3-fold higher than in T8, and *FtHQT* expression in T10 was 6.3-fold higher than in T8 under dark conditions at 12 DAS. *FtC3H* was expressed at higher levels under dark conditions than under light conditions in both cultivars. Furthermore, in both cultivars, *C3H* was highly expressed at 0 DAS in light conditions. The expression levels of *C3H* in T8 under light conditions gradually increased from 6 to 12 DAS, whereas its expression levels decreased gradually from 3 to 9 DAS and then increased at 12 DAS in T10. Under light conditions, the expression levels of *C3H* in T8 at 9 and 12 DAS were 5.4- and 2.9-fold higher, respectively, than in T10. Conversely, the expression levels of *C3H* in T10 at 3 and 6 DAS were 2.2- and 2.5-fold higher, respectively, than in T8. The mean values of *FtHQT* and *FtC3H* between T8 and T10 were significantly different according to the LSD test (Figure 2). In dark conditions, the expression levels of *C3H* at 6 DAS were the highest in T8, displaying 13-fold higher levels than in T10. Under dark conditions, the transcript levels of *C3H* gradually decreased from 0 to 12 DAS in T10.

The transcript levels of *FtHQT* were the highest at 0 DAS in both cultivars. In a previous study of phenylpropanoid genes, only *F. tataricum* phenylalanine ammonia-lyase and flavonoid 3′-hydroxylase 2 displayed high levels of transcription at 0 DAS in T8 and T10 tartary buckwheat sprouts under light and dark conditions.[30] Apart from at 0 DAS, the transcript levels of T10 *FtHQT* were higher than T8 *FtHQT* levels in light and dark conditions, but they were not significantly different according to the LSD test. The expression levels of *CYP98a22*[27] were strongly increased (3-fold) in ultraviolet (UV) light-treated leaves, and it was suggested that CYP98a22 is involved in the response against several stresses in *R. graveolens*.[27] In addition, the expression of *C3H* (*CYP98a49*)[25] in *C. cardunculus* was induced using UV-C light. As mentioned in the Introduction, C3H is a cytochrome P450 enzyme; therefore, *FtC3H* might be affected more than *FtHQT* in light conditions.

Tissue-Specific Expression of *FtHQT* and *FtC3H* in Different Organs of T8 and T10. The expression levels of *FtHQT* and *FtC3H* were analyzed using quantitative real-time RT-PCR in the flowers, stems, leaves, seed stages 1, 2, and 3, and roots of T8 and T10 cultivars (Figure 3). *FtHQT* transcriptions were found at relatively low levels in all tissues in both cultivars, and their levels did not differ significantly between the cultivars. However, there was significant variation in the expression levels of *FtC3H* between the cultivars. The expression levels of *FtC3H* were the highest in the stems of cultivar T8, with 4.6-fold higher expression levels than in T10. The second and third highest expression levels of *FtC3H* were found in the flowers and leaves, respectively, with cultivar T8 displaying 3.8- and 2.2-fold higher expression levels, respectively, than in the same organs of T10. The transcription patterns of *HQT* and *C3H* during grain development in *C. canephora* were not significantly different from each other.[14] Peng et al.[37] described that the highest expression levels of *HQT* mRNA were found in the flower buds of *Lonicera japonica*. The mRNA levels of *Arabidopsis REF8*, which encodes C3H, was the highest in the stem.[23] In addition, the highest levels of red clover *C3H* (CYP98A44) transcripts were observed in the flowers and stem.[17] *HQT* and *C3H1* transcripts

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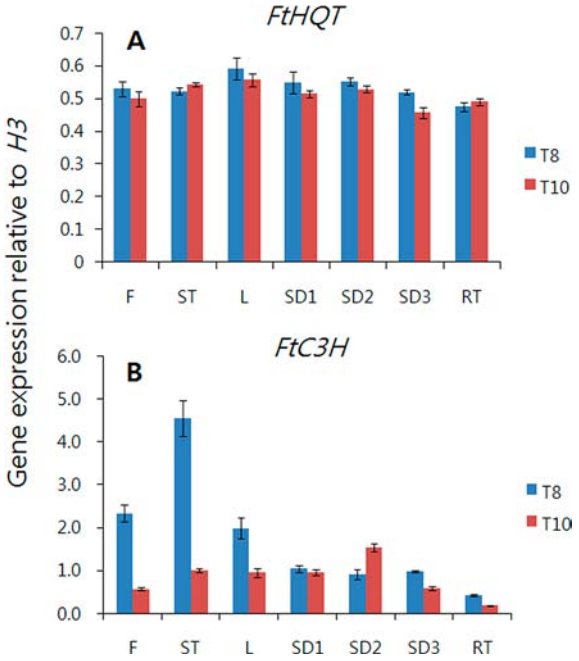

Figure 3. Relative expression levels of *FtHQT* and *FtC3H* in different organs of *F. tataricum* T8 and T10. F, flowers; ST, stems; L, leaves; SD1, SD2, and SD3, seed stages 1, 2, and 3, respectively; RT, roots. The height of each bar and the error bars show the mean and standard error, respectively, from three independent measurements.

were detected in all tissues examined, but there were significant variations in their levels during grain development in *C. canephora*.[7]

These high expression levels in the flowers and stem of T8 were consistent with the findings of previous studies.[17,23,37] Very recently, HQT expression was reportedly not regulated at the transcriptional level, but HQT enzyme activity was a key determinant of developmental changes in potato phenylpropanoid metabolism during tuber development.[38] Mahesh et al.[20] and Sonnante et al.[16] isolated and characterized C3H and HQT isoforms from coffee tree and artichoke, respectively. In *Nicotiana* species, the transient and stable expression of *HQT1* resulted in the increased production of CGA and cynarin (1,3-dicaffeoylquinic acid).[20]

We found that the transcript levels of *FtHQT* were similar among different organs, whereas *FtC3H* showed different levels. According to the transcript levels observed in several plants, we suggest that *HQT* and *C3H* are differentially regulated in different tissues of different plant species. Additionally, we presume that *FtHQT* isoforms exist and these isoforms contribute to CGA biosynthesis and transcription in T8 and T10 tartary buckwheat through different metabolic pathways.

Analysis of CGA Content in Different Organs of T8 and T10. Previously, we examined the levels of CGA in T8 and T10 sprouts;[30] CGA and four *C*-glycosylflavones (i.e., orientin, isoorientin, vitexin, and isovitexin) were rare in T8 and T10 seeds, but were detected after 2 days of culture. In addition, CGA content tended to be induced by light in T8 and T10 cultivars from 4 DAS.[30] In this study, we also analyzed the CGA content of T8 and T10 sprouts, and our findings (data not shown) were in agreement with previous results.[30] Therefore, we describe CGA content only in the different organs of T8 and T10 cultivars (Figure 4). Cultivar T8 CGA contents were higher than in T10 in all tissues, except the stem. Among the

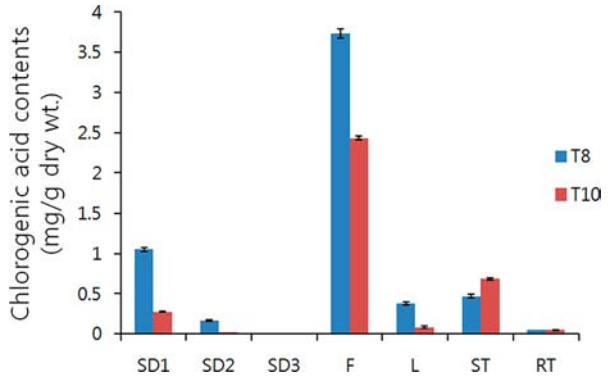

Figure 4. Chlorogenic acid content in different organs of T8 and T10. F, flowers; ST, stems; L, leaves; SD1, SD2, and SD3, seed stages 1, 2, and 3, respectively; RT, roots. The height of each bar and the error bars show the mean and standard error, respectively, from three independent measurements.

organs, the flowers of both cultivars showed the highest levels of CGA, and T8 CGA content [3.73 mg/g dry weight (DW)] was somewhat higher than in T10 (2.43 mg/g DW). T8 CGA content (0.37 mg/g DW) in the leaves was 4.6-fold higher than in T10 (0.08 mg/g DW). The stem of T10 (0.68 mg/g DW) contained slightly higher CGA levels than T8 (0.47 mg/g DW). Common and tartary buckwheat flowers reportedly have the highest content of phenolic compounds, including CGA, gallic acid, catechin hydrate, caffeic acid, epicatechin, and rutin, in different organs.[33,39] Shinozaki et al.[40,41] and Hirai et al.[42,43] reported that CGA and some other phenylpropanoids accumulated in the cotyledons under conditions of poor nutrition, low temperature, or high-intensity light. In addition, Ishimaru and collegues[44] pointed out the close correlation between CGA content and flowering response, in which the number of flower buds increased in parallel with CGA content.

Overall, the expression pattern of *FtHQT* and *FtC3H* did not match the accumulation pattern of CGA in different organs of T8 and T10. Gene expression and CGA content varied between the cultivars. We suggest that *FtHQT* and *FtC3H* might be controlled by multiple metabolic pathways in different organs of tartary buckwheat. Probably, *FtC3H* might be more affected than *FtHQT* in CGA biosynthesis. CGA, which acts as an antioxidant in plants and protects against degenerative, age-related diseases in animals when given as a dietary supplement, was abundant in the flowers of T8 and T10 cultivars. This indicates that the flowers of T8 and T10 could be considered a valuable source of health information for CGA.

The characterization of *FtHQT* and *FtC3H* will be helpful for a greater understanding of CGA biosynthesis in tartary buckwheat. To explain adequately the CGA biosynthesis mechanisms in tartary buckwheat Hokkai T8 and T10, the enzyme activities of *FtHQT*, *FtHCT*, and *FtC3H* should be examined in the near future.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional figures. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

HPCL, high-performance liquid chromatography; CGA, chlorogenic acid; PAL, phenylalanine ammonia-lyase; C4H, cinnamic acid 4-hydroxylase; 4CL, 4-coumaroyl:CoA-ligase; HQT, hydroxycinnamoyl-coenzyme A quinate hydroxycinnamoyl transferase; HCT, hydroxycinnamoyl-coenzyme A shikimate/quinate hydroxycinnamoyl transferase; C3H, *p*-coumarate 3′-hydroxylase

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