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Expression Analysis of Anthocyanin Biosynthetic Genes in Different Colored Sweet Cherries (*Prunus avium* L.) During Fruit Development

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Abstract To identify the key enzymes involved in anthocyanin synthesis in sweet cherry (*Prunus avium* L.), the differences in anthocyanin biosynthetic gene expressions were investigated in the samples (mix of peel and flesh) of a red-colored cultivar ('Hongdeng') and a bicolored cultivar ('Caihong') during fruit development. The expression of six anthocyanin synthetic genes in cherry (*PacCHS*, *PacCHI*, *PacF3H*, *PacDFR*, *PacANS*, and *PacUFGT*) was analyzed by quantitative real-time PCR and Western blot analysis. Meanwhile, the changes in anthocyanin contents were measured by ultra-performance liquid chromatography. The expression of anthocyanin synthetic genes and the anthocyanin contents were much higher in 'Hongdeng' than in 'Caihong' fruits. Gene transcription and translation and anthocyanin accumulation all started approximately at the end of the pit-hardening period and reached a maximum at maturation. All six genes were significantly correlated with anthocyanin accumulation in 'Hongdeng' and *PacCHS* had the highest direct effect. However, only *PacUFGT* was significantly correlated with anthocyanin accumulation in 'Caihong'. Anthocyanin biosynthesis in sweet cherry seems to be regulated mostly at the transcript levels. CHS appears to be the key enzyme involved in anthocyanin synthesis in 'Hongdeng', while UFGT is involved in anthocyanin synthesis in 'Caihong' fruits.

Keywords Sweet cherries · Anthocyanin synthetic genes · qRT-PCR · Western blotting · Ultra-performance liquid chromatography

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Introduction

Sweet cherry (*Prunus avium* L.) is a very popular temperate fruit. It contains high levels of anthocyanins (Kim and others 2005; Usenik and others 2008), which are beneficial to humans because they have antioxidant functions, are able to protect against coronary heart disease, and are also involved in the body's defense against pathogens and ultraviolet radiation (Gronbaek and others 1995; Knekt and others 1997; Guendez and others 2005; Nhukarume and others 2010). Knowledge of the molecular mechanism of anthocyanin biosynthesis is of great importance in order to improve anthocyanins in food crops.

Chalcone synthase (CHS) is the first committed enzyme in the anthocyanin pathway. It catalyzes the synthesis of tetrahydrocannabinol (THC) from one molecule of 4-coumaroyl CoA and three molecules of malonyl CoA (Tanaka and others 2008). Fukusaki and others (2004) reported that flower color in the garden plant *Torenia hybrid* was

successfully modulated by RNA interference (RNAi) of the *CHS* gene, with which the original blue flower color changed to white and pale colors. THC is isomerized to the colorless (2S)-naringenin by chalcone isomerase (CHI). Li and others (2006) found that transgenic tobacco plants overexpressing sense *SmCHI* produced up to five times more total flavonoids compared to wild-type tobacco plants. (2S)-Naringenin is hydroxylated at the 3-position by flavanone 3-hydroxylase (F3H) to form dihydroflavonol [(2R, 3R)-dihydrokaempferol, and so on]. A study showed that the *F3H* gene was expressed in any grape plant tissues that accumulate flavonoids, in particular, the skin of ripening red berries, which synthesizes most of the anthocyanins (Castellarin and others 2006). Dihydroflavonols are reduced to corresponding 3,4-cisleucoanthocyanidins by the action of dihydroflavonol 4-reductase (DFR). Northern blot analysis showed that the expressions of *F3H* and *DFR* were higher in flowers of alfalfa (*Medicago sativa* L.) than in roots and nodules; the purple flowers contained more anthocyanins (Charrier and others 1995). Anthocyanidin synthase (ANS, also called leucoanthocyanidin dioxygenase) catalyzes the synthesis of colored anthocyanidins. The study conducted by Jaakola and others (2002) demonstrated the coordinated expression of flavonoid biosynthetic genes (including *ANS*) in relation to the accumulation of anthocyanins, proanthocyanidins, and flavonols in the developing fruits of bilberry. Anthocyanidins are initially 3-glucosylated by the action of UDP-glucose:flavonoid (or anthocyanidin) 3GT and then turned into anthocyanins. The synthesis of anthocyanin in grape involves the expression of *CHS*, *F3H*, *DFR*, *ANS*, and *UFGT* genes. During grape ripening (after veraison), UFGT activity was concomitant with the increase in anthocyanin content (Mori and others 2005). However, *UFGT* expression was not detected in white grapes (Boss and others 1996).

Although many studies have focused on the anthocyanin synthetic genes such as *CHS*, *CHI*, *F3H*, *DFR*, *ANS*, and *UFGT* in apples, pears, and so on (Espley and others 2007; Mano and others 2007; Feng and others 2010), there are few reports about these genes in cherries. Wang and others (2010a) compared *ANS* and *CHS* expression levels in the cherry fruit of 'Stella' (a red fruit cultivar) with those in 'Rainier' (a bicolored cultivar) during fruit development.

Our previous research indicated differences in constituents and contents of phenolic components between red-colored cherries and bicolored cherries (Liu and others 2011). In this study, to identify the key enzymes involved in anthocyanin synthesis in sweet cherry, the anthocyanin content and the expression of anthocyanin synthetic genes at the mRNA and protein levels were investigated in the red-colored cultivar 'Hongdeng' and the bicolored cultivar 'Caihong' during the fruit development period.

Materials and Methods

Plant Materials

The sweet cherry (*Prunus avium* L.) cultivars 'Hongdeng' and 'Caihong' were cultivated at the Beijing Institute of Forestry and Pomology (Beijing, China) under field conditions. The fruits of the red-colored cultivar 'Hongdeng' had red-colored flesh and peel at maturation, whereas the bicolored 'Caihong' had only a small amount of red color in the peel. In 2010 and 2011, cherry fruits at different developmental stages were collected. The collecting dates were 5, 14, 19, 22, 25, 28, 31, 34, 37, 40, and 43 day after full bloom (DAFB). The seeds were immediately separated from peel and flesh. The peel and flesh of the cherry are very difficult to separate from each other, especially in young fruit, so a mixture of peel and flesh was used as samples for all the experiments. All the samples were frozen in liquid nitrogen and stored at −80 °C for subsequent analysis.

Extraction of Anthocyanins

Anthocyanins were extracted according to the method of Liu and others (2011). The cherry sample (0.5 g) was ground into a homogenate and extracted using 7.5 ml of methanol solution (containing 0.5 % hydrochloric acid) at −20 °C for 24 h. After centrifugation (10,000×*g*, 4 °C) for 15 min, the supernatant was collected for analysis. The anthocyanin contents were measured immediately after extraction to avoid degradation. The extracted supernatant was filtered through a 0.22-μm filter and then 10 μl was injected into the ultra-performance liquid chromatography (UPLC) analyzer.

Quantification of the Anthocyanins

The UPLC system used was an ACQUITY UPLC series (Waters, Milford, MA, USA) with a diode array detector (DAD). The anthocyanins were analyzed at 530 nm. Chromatographic separations were performed with an ACQUITY UPLC BEH C18 (Waters) column (2.1 × 100 mm, i.d. = 1.7 μm) placed in a column oven at 35 °C. The method used was that of Liu and others (2011) with some modifications. Two solvents [acetonitrile (solvent A) and 0.1 % trifluoroacetic acid in water (solvent B)] were used with a constant flow rate of 0.2 ml/min. For the elution program, the following proportion of solvent B was used: 0–8 min, 95–75 % B; 8–12 min, 75–60 % B; 12–15 min, 60–10 % B; and 15–17 min, 10–95 % B. The anthocyanin contents were assessed from peak areas and calculated as equivalents of standards. Cyanidin-3*O*-rutinoside

(Sigma-Aldrich Chemical Co., St. Louis, MO, USA) was used as the equivalents of standards.

RNA Extraction and Quantitative Real-time PCR (qRT-PCR) Analysis

Total RNA was extracted from 1.0 g of peel and flesh using the hot borate method (Wan and Wilkins 1994). Synthesis of first-strand cDNA from 1 μg of total RNA isolated from each sample was conducted using M-MLV reverse transcriptase (Promega, Madison, WI, USA) with an oligo-dT primer and a random primer.

The cDNA was used as a template for qRT-PCR. Amplification of specific regions of targeted genes and real-time detection of amplicon production were conducted using an ABI 7500 qRT-PCR system (Applied Biosystems, Carlsbad, CA, USA) with the primers (Table 1) designed according to the sequences of the anthocyanin biosynthetic genes in sweet cherries (*PacCHS*, *PacCHI*, *PacF3H*, *PacDFR*, *PacANS*, *PacUFGT*). The following genes were cloned by our laboratory and their GenBank accession numbers are *PacCHS*, JF748833; *PacCHI*, F740091; *PacF3H*, JF740092; *PacDFR*, JF740093; *PacANS*, JF740094 and *PacUFGT*, JF740090.

Ultra SYBR® Mixture (with ROX) (CWBIO, Beijing, China) was used to perform the qRT-PCR. The reaction solutions contained 20 μM of forward primer, 20 μM of reverse primer, 2 ng of cDNA template, 10 μl of Ultra SYBR Mixture and 6 μl of water for a total volume of 20 μl. The reactions were carried out under the following conditions: 95 °C for 5 min (1 cycle) and 95 °C for 10 s and 60 °C for 30 s (40 cycles). A melting curve analysis was also undertaken.

PCR amplification of a single product of the correct size for each gene was confirmed by agarose gel electrophoresis and double-strand sequencing.

Protein Extraction and Western Blotting

Total proteins were extracted according to the method of Zheng and others (2009) with modifications. The extracted proteins were separated by SDS-PAGE in a 10 % polyacrylamide gel as described by Laemmli (1970). The same amount of total protein (8 μg) was loaded per lane. After electrophoresis, the proteins were electrotransferred to nitrocellulose (NC) (0.45 mm, PALL) using a transfer apparatus (Bio-Rad, Hercules, CA, USA) according to Isla and others (1998). For Western blot analysis, immunological detection of proteins on the NC membrane was carried out using primary polyclonal CHS, CHI, F3H, DFR, ANS, and UFGT antibodies in a 1/200 dilution with alkaline phosphatase conjugated anti-rat IgG antibody from goat (CWBIO, Beijing, China; 1/1,000 dilution) as a secondary antibody at 25 °C. Then the membrane was stained with 5 ml of 3,3,5,5-tetramethylbenzidine for 5–30 min. The color change was monitored during the staining period.

Statistical Analysis

Results presented in the tables and figures are shown with standard deviations. Correlation analysis and path coefficient analysis were statistically assessed using SPSS ver. 13.0 and SigmaPlot 8.0. The test of statistical significance was based on the total error criteria with a confidence level of 95.0 and 99.0 %.

Results

Changes in Anthocyanin Contents during Sweet Cherry Fruit Development

Anthocyanin levels were measured in each sweet cherry fruit sample. No anthocyanins were detected in 'Hongdeng' or 'Caihong' sweet cherry fruits before 31 DAFB. Soon after 31 DAFB, the anthocyanin contents increased rapidly, especially in the 'Hongdeng' cherry fruits (Fig. 1). The highest anthocyanin content was reached about 40 DAFB for 'Hongdeng' and 43 DAFB for 'Caihong'. The anthocyanin content in 'Hongdeng' [maximum 659.0 mg/100 g FW (fresh weight)] was significantly higher than in 'Caihong' (maximum 51.2 mg/100 g FW), which was not surprising as 'Hongdeng' is a red-colored cultivar and 'Caihong' is a bicolored cultivar.

Table 1 Primers used in qRT-PCR experiments

Gene	Forward primer	Reverse primer
PacCHS	5′-GGTGCTCGTGTTCTTGTTGTG-3′	5′-ACTGTCGGGAAGGATGGTTTG-3′
PacCHI	5′-TCCACCGTCAGTCAAACCACC-3′	5′-TCCAGCCCCCTCACCCCT-3′
PacF3H	5′-GATTGTGGAGGCTTGTGAGGAT-3′	5′-GTAAATGGCTGGAGACGATGAA-3′
PacDFR	5′-CGAAGTGACCAAGCCAACAATAA-3′	5′-GCAGAGGATGTAAACACCAGC-3′
PacANS	5′-GGGATTGGAAGAAGGGAGGC-3′	5′-ATTTGGAACACACTTGGCAGAG-3′
PacUFGT	5′-GGTGTTTGATGTGGCTGATGG-3′	5′-GCTGGTTGTAAAGTTGTGGGG-3′
18s rRNA	5′-AGTCGGGGGCATTCGTATTT-3′	5′-CCCTGGTCGGCATCGTTTAT-3′

Changes in Gene Expression during Sweet Cherry Fruit Development

To study the molecular mechanism behind anthocyanin accumulation during fruit development, *PacCHS*, *PacCHI*, *PacF3H*, *PacDFR*, *PacANS*, and *PacUFGT*, which are related to sweet cherry anthocyanin synthesis, were cloned from sweet cherry fruit and antibodies to each gene were obtained. Western blot analysis and qRT-PCR were performed to analyze the relative-fold expression of these genes during fruit development. The results showed that the transcripts of all six genes were detected throughout the entire fruit development period, but the expression patterns were different among the genes (Fig. 2). The expression of *PacCHS* in 'Hongdeng' increased at 31 DAFB and reached a maximum at 40 DAFB, indicating a close association with the accumulation of anthocyanin. The increase in the expression of *PacCHI* and *PacF3H* started about 3–4 days later than that of *PacCHS* in 'Hongdeng' and the increase in the expression of *PacDFR* and *PacANS* was even later at about 37 DAFB. All the expression peaks occurred at 40 DAFB. The increased expression time points for the first five genes (*PacCHS*, *PacCHI*, *PacF3H*, *PacDFR*, and *PacANS*) were in the same order as the anthocyanin synthesis pathway. However, the sixth gene, *PacUFGT*, was an exception. Its expression increased at 22 DAFB, which was earlier than the start of anthocyanin accumulation.

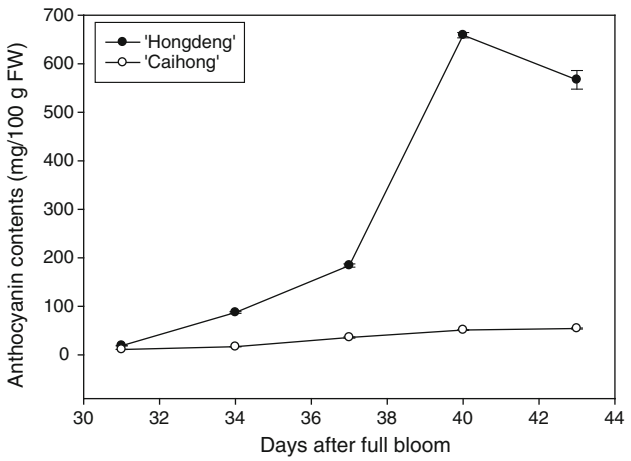

Fig. 1 Changes in anthocyanin contents during fruit development of sweet cherry 'Hongdeng' and 'Caihong'. *FW* fresh weight. Standard errors are indicated ($n = 3$)

The expression patterns of these genes in 'Caihong' were different from the patterns in 'Hongdeng' (Fig. 2). The expressions of the first five genes (*PacCHS*, *PacCHI*, *PacF3H*, *PacDFR*, and *PacANS*) were almost unchanged during the entire fruit development period. Expression levels were also low compared to those in 'Hongdeng'. Only *PacUFGT* had an expression maximum at the same time as the anthocyanin accumulation peak. Its expression increased at 28 DAFB, which was earlier than the start of anthocyanin accumulation.

The Western blot results were consistent with qRT-PCR (Fig. 3). In 'Hongdeng' cherry fruits, the CHS protein was detected at 34 DAFB and CHI and F3H proteins were detected at 40 DAFB. These were later than the transcript increase times. The DFR protein was detected at 37 DAFB, when transcript levels had started to increase. The proteins associated with the last two genes (ANS and UFGT) were detected prior to the transcript level increases, particularly the UFGT. All six genes caused a maximum protein production peak at 40 DAFB. No visible signals were obtained from 'Caihong' except for the UFGT, which confirmed the results of the qRT-PCR analysis.

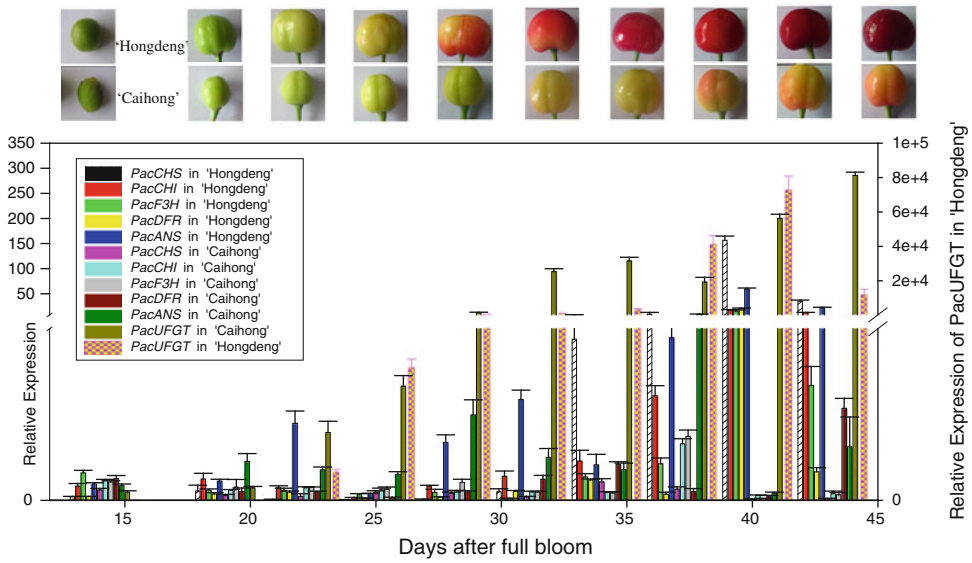

Fig. 2 qRT-PCR analysis of *PacCHS*, *PacCHI*, *PacF3H*, *PacDFR*, *PacANS*, and *PacUFGT* in 'Hongdeng' and 'Caihong' during fruit development. Expression of each gene is presented as a relative fold change. The transcript values at 5 DAFB were set at 1

Correlation Analysis and Path Coefficient Analysis

Correlation analysis and path coefficient analysis were conducted between gene expression levels and anthocyanin content using SPSS ver. 13.0. The results indicated that the expressions of all six genes were significantly correlated with anthocyanin accumulation in 'Hongdeng' fruits and four of them were at the 0.01 level of significance (Table 1). The results of the path coefficient analysis showed that *PacCHS* had the highest direct effect ($P_1 = 2.269$). *PacCHI* showed significantly higher positive indirect effects via *PacCHS* ($P_{21} = 0.9161$) on anthocyanin content in 'Hongdeng', whereas *PacF3H* ($P_{31} = -0.5563$), *PacDFR* ($P_{41} = -1.2884$), and *PacANS* ($P_{51} = -0.4844$) had negative indirect effects. Only the expression level of *PacUFGT* ($r^2 = 0.9371$) was significantly correlated with anthocyanin content in 'Caihong' (Table 2). The path coefficient calculation for 'Caihong' showed that *PacUFGT* ($P_6 = 1.577$) had significantly higher direct effects on anthocyanin content than the other genes.

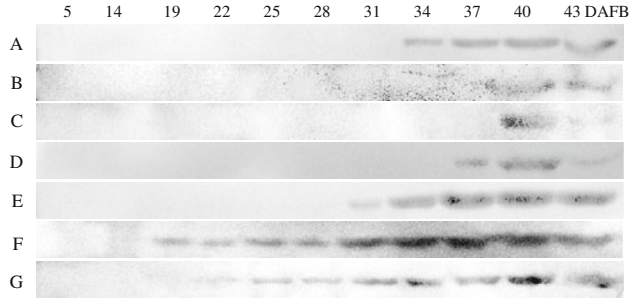

Fig. 3 Western blot of the six genes in cherries. **a–f** The protein accumulation of CHS, CHI, F3H, DFR, ANS, and UFGT in 'Hongdeng' cherry fruits during fruit development. **g** The protein accumulation of UFGT in 'Caihong' cherry fruits during fruit development. *DAFB* days after full bloom

Discussion

Anthocyanin contents have been shown to differ between the red cherry cultivar and the bicolored cultivar (Usenik and others 2008; Liu and others 2011). However, the molecular basis of this variation is not clear. This study was carried out using the red-colored sweet cherry cultivar 'Hongdeng' and the bicolored cultivar 'Caihong'. UPLC analysis revealed that the anthocyanin content in 'Hongdeng' was significantly higher than in 'Caihong' (Fig. 1). This was because the flesh (mesocarp) of 'Hongdeng' was as red as the peel (exocarp), whereas there was very little red in the peel of the 'Caihong' fruit and none in the flesh. Consistent with anthocyanin content, 'Hongdeng' was associated with higher transcriptions of the genes studied than 'Caihong' at each fruit development stage. Takos and others (2006) reported that the transcript levels of the *MdCHS*, *MdF3H*, *MdDFR*, *MdLDOX*, and *MdUFGT* genes, which are required for apple anthocyanin synthesis, were lower in the peels of nonred than in red-peeled cultivars. A similar study in cherries was also carried out by Wang and others (2010a). The results showed that *ANS* and *CHS* expression levels were much higher in the cherry fruits of 'Stella' (a red fruit cultivar) compared to 'Rainier' (a bicolored cultivar) during the last two stages of fruit development. Their results were confirmed by this study. The expression levels of all six genes studied were much higher in 'Hongdeng' than in 'Caihong' (Fig. 2). Our results also showed that the first five genes (*PacCHS*, *PacCHI*, *PacF3H*, *PacDFR*, and *PacANS*) were barely expressed during the entire fruit development period in 'Caihong' (Fig. 2). A similar finding by Kim and others (2003) showed that the transcription of genes during the later stages of anthocyanin biosynthesis in green apples was barely detectable by RNA gel blot analysis.

The two types of cherry fruits used in this study both started accumulating anthocyanin at approximately the end of the pit-hardening period, when the fruit barely enlarges

Table 2 Direct and indirect effects of the six major genes on anthocyanin accumulation in 'Hongdeng' fruits

Genes	Correlation coefficients with anthocyanin content	Direct effect trait on anthocyanin content	Indirect effect traits by the genes					
			PacCHS (1)	*PacCHI* (2)	*PacF3H* (3)	*PacDFR* (4)	*PacANS* (5)	*PacUFGT* (6)
PacCHS (1)	0.8820**	2.2690		0.9161	−0.5563	−1.2884	−0.4844	0.0260
PacCHI (2)	0.9976**	0.9618	2.0712		−0.5047	−1.0949	−0.4590	0.0232
PacF3H (3)	0.9840**	−0.5234	2.4003	0.9099		−1.3301	−0.4989	0.0262
PacDFR (4)	0.6918*	−1.3929	2.2355	0.8317	−0.5245		−0.4837	0.0257
PacANS (5)	0.9207**	−0.4892	2.2469	0.9164	−0.5683	−1.2099		0.0248
PacUFGT (6)	0.7453*	0.0301	2.0538	0.7391	−0.4857	−1.1751	−0.4169	

* indicates significant differences at the 0.05 level of significance ($R^2 = 0.6319$); ** indicates significant differences at the 0.01 level of significance ($R^2 = 0.7646$)

Table 3 Direct and indirect effects of the six major genes on anthocyanin accumulation in 'Caihong' fruits

Genes	Correlation coefficients with anthocyanin content	Direct effect trait on anthocyanin content	Indirect effect traits by the genes					
			PacCHS (1)	*PacCHI* (2)	*PacF3H* (3)	*PacDFR* (4)	*PacANS* (5)	*PacUFGT* (6)
PacCHS (1)	−0.2543	−0.0595		−0.5955	1.2362	0.0237	−0.3658	−0.4934
PacCHI (2)	0.1917	−1.4748	−0.0228		3.2828	0.0482	−1.3144	−0.3273
PacF3H (3)	−0.0586	3.2665	−0.0215	−1.5184		0.0589	−1.4211	−0.4230
PacDFR (4)	0.5088	−0.2068	0.0065	0.3251	−0.8592		0.1359	1.1073
PacANS (5)	0.2523	−1.4297	−0.0151	−1.3176	3.0769	0.0196		−0.0818
PacUFGT (6)	0.9371**	1.5771	0.0195	0.3128	−0.8987	−0.1495	0.0759	

** indicates significant differences at the 0.01 level of significance ($R^2 = 0.7646$)

and the stony endocarp (pit) undergoes hardening (DeJong and Grossman 1995). Anthocyanin accumulation in both cultivars reached a peak at maturation. The anthocyanin peak for 'Caihong' was a little later than that of 'Hongdeng' because 'Caihong' sweet cherries are harvested later than 'Hongdeng'. The increase in the transcripts of the first five genes in 'Hondeng' was closely associated with the increase in accumulation of anthocyanin, which occurred right after the pit-hardening period ended and reached a peak at fruit maturation. The protein accumulation started to increase later than the transcript accumulation. Although these results showed that the dates when proteins of the last two genes (ANS and UFGT) could be detected were about 3–7 days earlier than their transcript times, the sharply increasing time points for protein production were still consistent with the increase in transcript levels. When the transcripts of the last two genes were not increasing sharply, they were able to produce enough proteins to be detected. Therefore, the protein amounts were determined by the transcript levels.

There have been few reports about the protein accumulation of anthocyanin biosynthetic genes. Wang and others (2010b) detected changes in ANS protein levels during grape berry development. Although the ANS protein accumulation pattern of grape was different from that of cherry in this study, both studies indicated that protein accumulation is in accord with their transcript accumulation. These results suggest that anthocyanin biosynthesis in sweet cherry seems to be mostly regulated at the transcript level.

Although *PacUFGT* had a much higher expression level than the other five genes (Fig. 2) and had a significant correlation with anthocyanin contents in 'Hongdeng' (Tables 2, 3), transcript and protein accumulation of this gene started much earlier than the anthocyanin accumulation. This suggested that UFGT may promote other substrates that accumulated earlier than did the anthocyanins. For example, the flavonols were synthesized before the anthocyanins (Winkel-Shirley 2001), and they also combined sugars, a process that is catalyzed by the UFGTs.

Correlation analysis indicated that the expression of all six genes had a significant correlation with anthocyanin accumulation in sweet cherry 'Hongdeng'. A previous report confirmed that the expression of cherry *CHS* (an early step in the anthocyanin biosynthesis pathway) and cherry *ANS* (a later step) was upregulated and correlated with cherry color (Wang and others 2010a). However, sometimes correlation coefficients give misleading results because the correlation between two variables may be due to a third factor. Therefore, it is necessary to analyze the cause-and-effect relationship between dependent and independent variables (Sidramappa and others 2008). In this study, path coefficient analysis (Dewey and Lu 1959) was used to investigate the nature of the relationship between dependent and associated independent variables. The variables with high positive correlations and high direct effects could be used as selection criteria in selection programs (Sadeghi and others 2011). In this study, *PacCHS* had the highest direct effect on anthocyanin accumulation in 'Hongdeng', indicating that to obtain an increase of anthocyanin content, *PacCHS* should be considered first. Our results also showed that *PacCHI* had positive indirect effects via *PacCHS* on anthocyanin content in 'Hongdeng' cherry, whereas *PacF3H*, *PacDFR*, and *PacANS* might inhibit these effects. For 'Caihong' cherry, *PacUFGT* was the only gene that had a significant correlation with anthocyanin accumulation. Others have reported that the MYBs, which is a large family of transcription factors associated with anthocyanin pathway, play a critical regulatory role in anthocyanin biosynthesis (Espley and others 2007; Feng and others 2010; Wang and others 2010a), and our laboratory is conducting a study to determine the role of MYBs in cherries.

Conclusion

The transcription and translation levels of anthocyanin synthetic genes and anthocyanin accumulation were all much higher in the red fruit sweet cherry 'Hongdeng' than

in the bicolored cultivar 'Caihong'. Expression mainly started approximately at the end of the pit-hardening period. Anthocyanin biosynthesis in sweet cherry seems to be mostly regulated at the transcript levels. The starting time points of gene transcription and gene expression levels were different among the six anthocyanin synthetic genes. CHS could be the key enzyme for anthocyanin contents in 'Hongdeng', whereas UFGT plays a major role in 'Caihong'.

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