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An RNA-Seq analysis of the peach transcriptome with a focus on genes associated with skin colour

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Abstract: Red pigmentation of the skin is an important consumer trait in peach (*Prunus persica*). The pigment consists largely of anthocyanin. Here, a transcriptomic contrast, based on RNA-Seq technology, was drawn between a white-skinned (Feitao) and a red-skinned (Qiuxue) peach cultivar. The analysis identified 2407 genes as differentially transcribed in the fruit skin of the two cultivars. Among these were a number of genes known to contribute to anthocyanin synthesis. A quantitative real-time PCR assay was used to validate the RNA-Seq-based estimates of transcript abundance for 14 differentially transcribed genes. Anthocyanin synthesis was observed in the skin of Qiuxue fruit during the late ripening stage, matching the high transcript abundance of the gene encoding UDP glucose: flavonoid 3-O-glucosyltransferase, the final step in the synthesis of anthocyanin.

Keywords: anthocyanin; pericarp colour; *Prunus persica*; sequencing

Peach (*Prunus persica* /L./ Batsch) is an economically valuable fruit and ornamental tree species. The pigmentation of the fruit skin is an important consumer trait; in red-skinned cultivars, the colour develops during maturation and ripening, reflecting both the degradation of chlorophyll and the accumulation of carotenoids and particularly of anthocyanin. The synthesis of anthocyanins has been studied in great detail in a number of plant species, particularly in *Petunia* and *Antirrhinum*. The various genes active in anthocyanin synthesis have been isolated and characterized in a variety of plant species (Rahim *et al*. 2014). As yet, there has been little information regarding the mechanism of anthocyanin synthesis in peach fruit. The recent acquisition of the peach transcriptome provides an opportunity to reveal what genes are involved in anthocyanin synthesis in peach. Here, the genetic basis of skin pigmentation has been addressed.

Fruits were collected from Qiuxue and Feitao trees grown at the Shandong Institute of Pomology, Taian, P.R. China. The skin was separated manually from the flesh of nine fruits sampled at four different stages of fruit development (Figure S1 in electronic supplementary material (ESM)). Anthocyanin, mean fruit mass, firmness and vitamin C (VC) content were detected. Total RNA from skin samples was submitted to Biomarker Technology Co. (Beijing, P.R. China) to sequence. Quantitative real-time PCR (qPCR) analysis was processed.

The skin of Feitao fruit remains green during developmental stages 1 through 3, turning pale yellow during the ripening stage (stage 4). Qiuxue fruit skin is also green during stages 1 and 2, gradually turning red between stages 3 and 4 (Figure 1a). When the skin anthocyanin content was measured, the skin of Feitao contained no detectable amount throughout the period of fruit development. Immature Qiuxue

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fruit skins also contained no detectable anthocyanin, but its content rose gradually through stages 3 and 4 (Figure 1b). At stage 4, there existed differences in fruit quality, including mean fruit mass, soluble solids, titratable acidity, firmness and VC content, but they were not significant at a 5% level (Table S2 in ESM).

Sequencing and subsequent assembly was based on 16.59×10^9 nt of clean sequence, which resolved into 30 211 unigenes. The GC content of the transcriptome was > 45%, and its Q30 was > 91% (Table S3 in ESM). Over 82% of the reads could be mapped onto the current version of the peach genome (Table S3 in ESM). Over 70% of the unigenes were associated with an FPKM > 5, with > 4% registering a value > 100 (Table S4 in ESM). Based on a fold change threshold of 2 and a *P* value of 0.05, 3093 unigenes were designated as differentially transcribed genes (DTGs), including 2005 upregulated and 1088 downregulated Feitao vs Qiuxue. A BLASTx analysis of 3093 DTGs indicated that 3060 DTGs had at least one significant match to an existing gene model (Table S5 in ESM). Based on GO analysis, 2407 DTGs were categorized into 53 functional groups, spread across all three main GO ontologies: cellular component, molecular function and biological process (Figure S2 in ESM). The majority of the DTGs was associated with cell part, cell, binding activity, catalytic activity, cellular process or metabolic process, with only a few associated with extracellular matrix part, nutrient reservoir activity or biological phase. Detailed information was obtained through KEGG analysis, 528 DEGs were categorized into 94 pathways, including six times higher expression patterns pathways ($P < 0.05$). The terms photosynthesis, flavonoid biosynthesis, phenylpropanoid biosynthesis, plant-pathogen interaction, beta-alanine metabolism, and terpenoid backbone biosynthesis were significantly enriched in the upregulated clusters (Table S6 in ESM). Higher expression patterns were exhibited by a number of genes involved in anthocyanin synthesis: notably, these encoded anthocyanidin synthase (ppa008295m), cinnamate-4-hydroxylase (ppa004544m, ppa0018282m), chalcone-flavanone isomerase (ppa011276m), flavanone 3-hydroxylase (ppa007636m), dihydroflavonol 4-reductase (ppa008069m) and leucoanthocyanidin dioxygenase (ppa007738m). An overview of genes involved in anthocyanin synthesis is presented in Figure S3 in ESM. The transcript abundance of genes encoding chalcone synthase (ppa023080m, ppa008402m) and dihydroflavonol 4-reducatase (ppa008069m) was in the amount several times to hundred times higher in the skins of Qiuxue fruit than in those of Feitao (Table S7 in ESM).

The transcript abundance of 14 flavonoid synthesis-related DTGs was assayed by qPCR to validate the RNA-Seq-based assessment. When the two independent estimates of fold change were subjected to a linear regression, the overall correlation coefficient was 0.898 in the R^2 statistic, which confirmed the reliability of the RNA-Seq data (Figure S4 in ESM). The temporal variation in the transcript abundance of 14 genes was also monitored by analysing fruit harvested at stages 1 through 4 over two consecutive fruiting seasons. The genes behaved similarly in the two years. Genes encoding enzymes active in the upstream pathway (Figure 2), for example *CHS*, *CHI*, *F3H* and *DFR*, were transcribed similarly in the skin of Feitao and Qiuxue: their transcripts increased

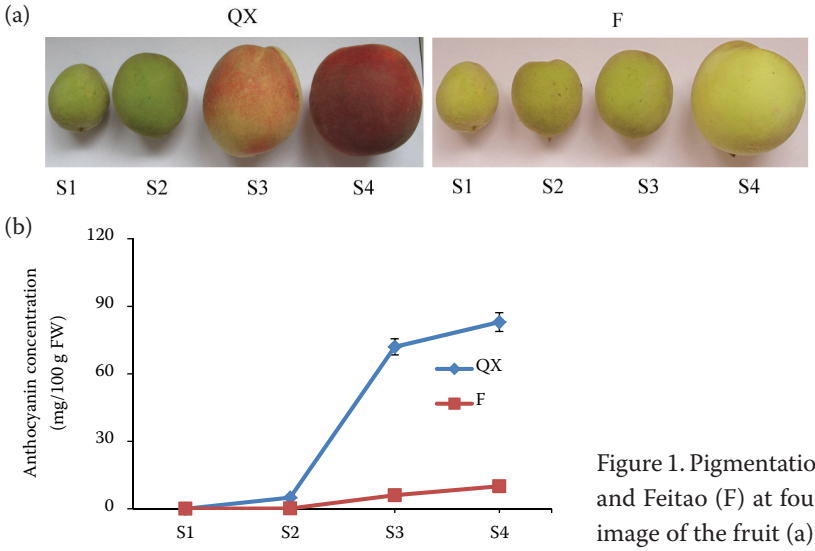

Figure 1. Pigmentation of the peach fruit skin of cv. Qiuxue (QX) and Feitao (F) at four stages of fruit development (S1–S4): an image of the fruit (a), anthocyanin content (b)

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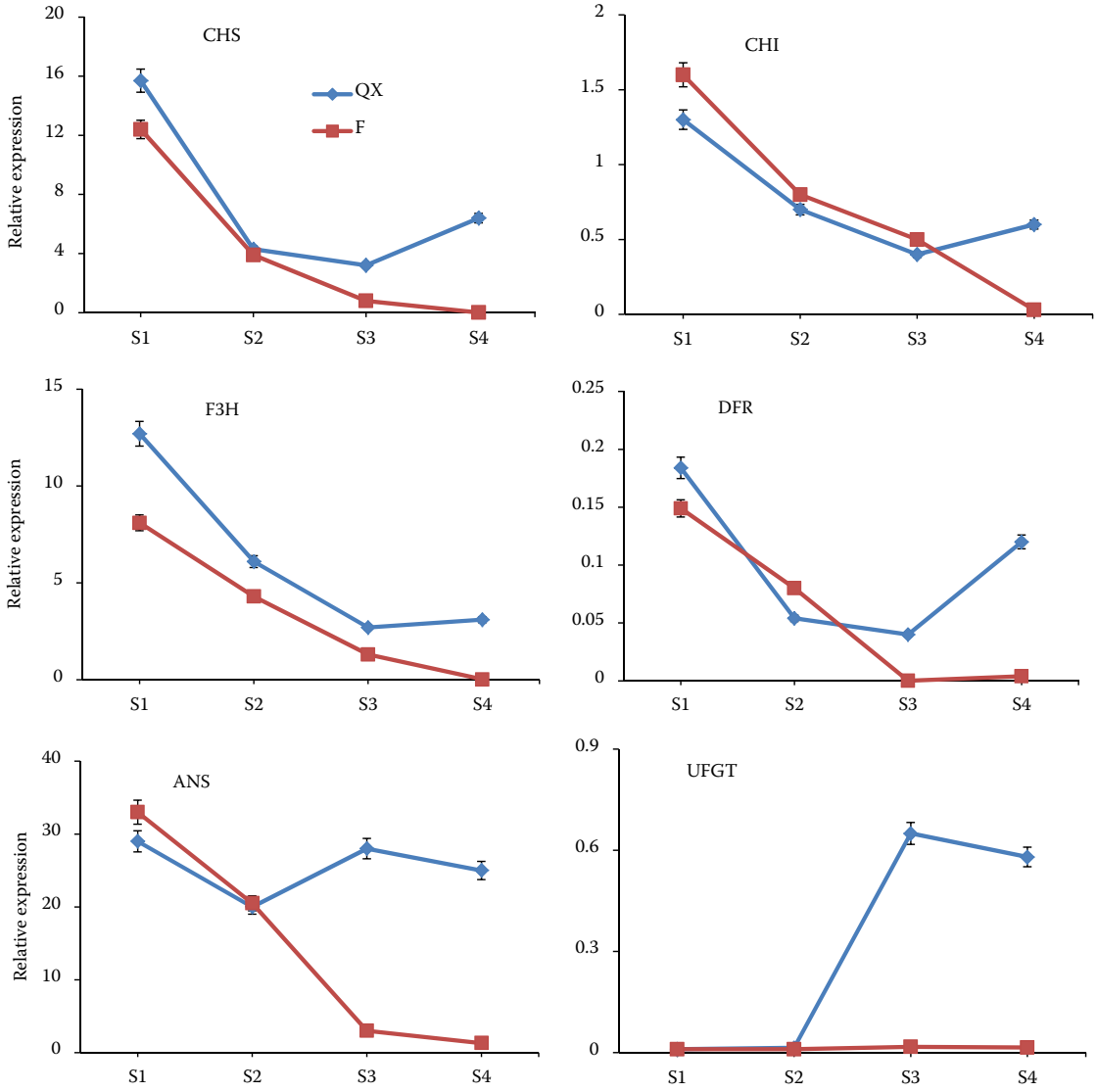

Figure 2. Transcription profiling based on qPCR of genes involved in anthocyanin synthesis in the peach fruit skin of cv. Qiuxue (QX) and Feitao (F) at four stages of fruit development (S1–S4)
CHS – chalcone synthase; CHI – chalcone isomerase; F3H – flavanone 3-hydroxylase; DFR – dihydroflavonol 4-reducatase; ANS – anthocyanidin synthase, UFGT – UDP-flavonoid glucosyltransferase

in abundance during stage 1, peaked at stage 2, and then fell away between stages 3 and 4. The gene *ANS* behaved in the same manner in the skin of Feitao, but in Qiuxue, a peak in abundance was reached during stage 3. The abundance of *UFGT* transcript was low in Feitao skin throughout fruit development, and was also low in Qiuxue skin during stages 1 and 2, but it later increased. Although the transcription of *CHS*, *DFR* and *ANS* was high in Qiuxue skin during stages 3 and 4, only *UFGT*, the product of which catalyzes the final step of anthocyanin synthesis, showed a match between the transcription level and skin anthocyanin content. The implication is that *UFGT* is the key enzyme limiting anthocyanin synthesis in the peach fruit skin.

High throughput sequencing technologies have revolutionized the characterization of transcriptomes, including those of the leading fruit species (Liu *et al.* 2012; Zhang *et al.* 2012; Dai *et al.* 2013). Here, the RNA-Seq platform was able to deliver more than 16 × 10^9 nt of *bona fide* peach transcript sequence, of which > 82% could be mapped onto the current version of the genome sequence (Verde *et al.* 2013). The red colour characteristic of the skin of some peach cultivars re-

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flects the accumulation of anthocyanin, the flavonoid responsible for pigmentation in most plant organs (Welch *et al*. 2008). Consistent with the anthocyanin accumulation in the skin of Qiuxue fruit, the level of *CHS* transcription was demonstrably higher than in the fruit of Feitao, which mirrors the situation obtained in red fruits of a number of Rosaceae species (Tsuda *et al.* 2004). We found that the abundance of *UFGT* transcript is much lower in Feitao than in Qiuxue. The implication is that in peach at least, *UFGT* is regulated differently from other flavonoid pathway genes, and that anthocyanin synthesis is controlled at a later stage than it was suggested previously (Kobayashi *et al.* 2002). Anthocyanin concentrations were previously measured by ultraviolet spectrometry in the peach (Zhou *et al*. 2013). In the current study, anthocyanin was not found at the beginning of Qiuxue fruit development, and it only appeared at stage 3 and increased to a great extent at stage 4. And white-skinned Feitao did not show any anthocyanin in the skin throughout fruit development. The results were consistent with RNA-Seq and qPCR studies.

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