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
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-reductase (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...
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 $16 \times 10^9$ 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-
reflects 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.

References


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