CHANGES IN THE FLAVONOID PATHWAY DURING THE LAST FOUR WEEKS OF FRUIT RIPENING IN AN APPLE CULTIVAR SHOWING BICOLORED FRUIT FLESH

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Introduction: Red-fleshed apples are increasingly important for consumer preference and marketability, not only due to aesthetic reasons, but also to the health benefits associated particularly with red pigments. 'Baya® Franconia' is a modern and aromatic red-fleshed apple cultivar with an attractive bicolored fruit flesh. It was bred at the Bavarian Centre of Pomology and Fruit Breeding. The fruit of the apple develops a broad red colored stripe below the skin that turns into a marbled pattern towards the core of the fruit. Red fruit flesh was shown to be controlled by the transcription factor *MdMYB10* [1], in concert with bHLH transcription factors. For a better understanding of the coloration of the fruit flesh in this variety we analyzed RNASeq data of white and red parts of the fruit flesh during fruit ripening. Selected genes were also analyzed by reverse transcription quantitative PCR (RT-qPCR).

Materials and methods: Three apples were harvested four weeks prior to maturity, and a further three at maturity. The flesh was divided into red and white tissue from which RNA was isolated. RNA was subjected to RNASeq. Reads obtained were mapped against the apple genome [2]. Differentially expressed reads were extracted, functionally annotated, and analyzed for differences in genes involved in the flavonoid pathway, thereby including structural and regulatory genes, as well as genes involved in transport processes. These genes include three major structural genes (flavanone 3β-hydroxylase (FHT), dihydroflavonol 4-reductase (DFR) and anthocyanidin synthase (ANS)), as well as the *MdMYB10* gene which influences the onset of pigmentation in apple fruits. Primers for hydroxycinnamoyltransferase (HCT) and caffeic acid O-methyltransferase (OMT) were designed from the transcripts derived from RNA-seq and annotated to the respective genes. The transcription of all genes was measured by RT-qPCR using *MdActin* as a reference gene. In addition to the RT-qPCR, high performance liquid chromatography was utilized to observe differences between the fruit tissues regarding the phenolic profile.

Results and discussion: Red and white colored flesh from the ripe apple fruits did not show many differences in the expression of flavonoid pathway genes. Most notably, white flesh showed higher flavonoid methyltransferase expression, OMT in particular, than the red parts.

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Red parts showed a somewhat higher expression of the early anthocyanin pathway genes (up to FHT), but no differential expression of downstream genes or genes opening side branches, such as flavonol synthase, anthocyanidin reductase or regulatory genes.
Differentially colored flesh from apples harvested four weeks before maturity, in contrast, showed clear differences in the expression of flavonoid pathway genes. In comparison to the white parts, the whole pathway in the red tissues, particularly the late pathway was induced. On the other hand, white parts showed increased expression of a lignin biosynthesis gene, HCT in particular, that was shown to be an interconnection between the two pathways [3]. Expression analysis of the genes involved in the flavonoid anthocyanin pathway revealed differences not only between red and white tissue fruits but also between unripe and ripe fruits. This result points at a later upregulation of the anthocyanin pathway in the white parts of apple flesh. The next steps will include the determination of the commencement of the anthocyanin accumulation.

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