Small deletion in a photosynthesis-related gene is involved in anthocyanin accumulation in the mesocarp of bf blood-flesh peaches

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Small deletion in a photosynthesis-related gene is involved in anthocyanin accumulation in the mesocarp of bf blood-flesh peaches

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Little is known about mechanisms by which light controls anthocyanin biosynthesis in fruit-flesh. Here, we studied the molecular bases of a photosynthesis related gene segregating with the recessive blood-flesh trait (bf) in peach (Prunus persica). This gene is furthermore linked with red midrib of leaves and a reduced height of tree. For this study, a fine mapping (positional cloning) approach was coupled with a candidate gene strategy to gather a bundle of converging evidences [A]. The sequence polymorphism analysis of the genes present in this interval allowed the identification of only one candidate gene (CG) in the LG4 with a deletion of 21 bp in each. A deletion KASPP marker was designed in the CG and its link to BF phenotype validated in different genetic backgrounds [B]. A comparative RNAseq study [C] of fruit flesh from bf and non-bf cultivar was revealed co-expressed genes and the results was validated by qPCR [D]. The cg was overexpressed in flesh of the bf cultivar compared to the non-bf cultivar and this overexpression was especially correlated with expression level of HYS (photosynthesis-related gene), PpWDA40 (regulatory gene) and PpUPGT (structural gene). Yeast two-hybrid analysis was performed to confirm physical interactions between proteins detected by RNAseq study [E].

A. Genetic and physical mapping of the bf locus and candidate gene approach by NGS-Illumina sequencing

1600 trees comprising a F2 segregating population dubbed 173° (cross O’Hearn (non-blood-flesh) x Super Tardive (blood-flesh) 1/2), at INRAE GAFL, were used to fine map the bf locus - the bf region < 8 kb included 8 predicted genes - the predicted genes were checked in v 2.0 with Augustus (http://augustus.ur-square.org) - according to the annotation file, all were potentially good candidate genes for bf

B. A 21 pb mutation is present in the candidate gene (cg) for all bf cultivars tested

A bioinformatic analysis of the genomic sequence of the blood-flesh nectarine Montarsa (NECTAVIGNE Montarsa clone S7634) was performed to study the nucleotidic and proteic sequences of the 8 genes. - among the 8 genes, 3 had a sequence polymorphism - only 1 CG of the 3 genes had a variation (INDEL) in the coding sequence - a deletion of 21 bp in exon3 was observed in CG by IGV software

C. RNA-Seq data generation and expression analysis

RNAseq study was carried out on flesh from 2 blood-flesh (bf) and non blood-flesh (non-bf) cultivars at 4 fruit development stages, from 60 days after blooming up to fruit maturity - 2 cultivars x 4 times (T1) x 5 biological replicates (fruit lots) = 40 samples
We employed a RNAseq approach to collect genome wide expression data and identify differentially expressed genes. 40 libraries including biological and technical replicates were sequenced by Illumina platform (GetPlaGe, Toulouse) which generated 145 Gbp
Data has been implemented using the Snakemake workflow and post analyses were performed by DiCoExpress pipeline

D. Validation by qRT-PCR

We observed a similar variation between anthocyanin variation and expression level of CG - accumulation of two anthocyanins into fruit flesh during fruit growth from 60 DAB to fruit maturity (extraction and identification by HPLC) - expression level of CG in Montarsa (red) and non blood-flesh (green) cultivar according a kinetic of development of fruit from 60 DAB to fruit maturity

E. Protein-protein interaction study

Yeast two-hybrid analysis was performed to confirm physical interactions between proteins synthesized by the genes detected by the RNAseq study. This analysis showed the CG interacted with HYS (photosynthesis-related gene), PpWDA40 (regulatory gene) and Pp MYB10.2 (factors transcription) with a strong interaction

We also observed HYS interacted with MYB10.1 and MYB10.2 and with PpWDA40 with a less strong interaction than CG
PpWDA40 interacted with MYB10.2.

Hypothesis of regulation genes

Conclusion

We observed an accumulation of anthocyanins in mesocarp and midvene of blood-flesh genotypes. Fine mapping and rescreening showed that a small deletion in a unique candidate gene was linked to anthocyanin accumulation in the mesocarp of bf blood-flesh peaches. We validated the deletion KASPP marker (diagnostic marker) in various backgrounds. In the RNAseq study, we observed an over-expression of CG in the mesocarp and midvein of blood-flesh cultivars compared to non blood-flesh cultivars (Lovell for example). In addition, we found a cluster of genes linked to the Photosystem I and II overexpressed in times 2 and 3 in blood-flesh, which corresponds to the peak of synthesis of anthocyanins in the mesocarp of bf cultivars. The protein-protein interaction study confirmed the links between the product of the CG and the product of the photosynthesis-related genes and of transcription factors.