

Identification And Characterization of Regulatory Proteins Involved in Anthocyanin Biosynthesis in *F. vesca* and *R. idaeus*

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INTRODUCTION

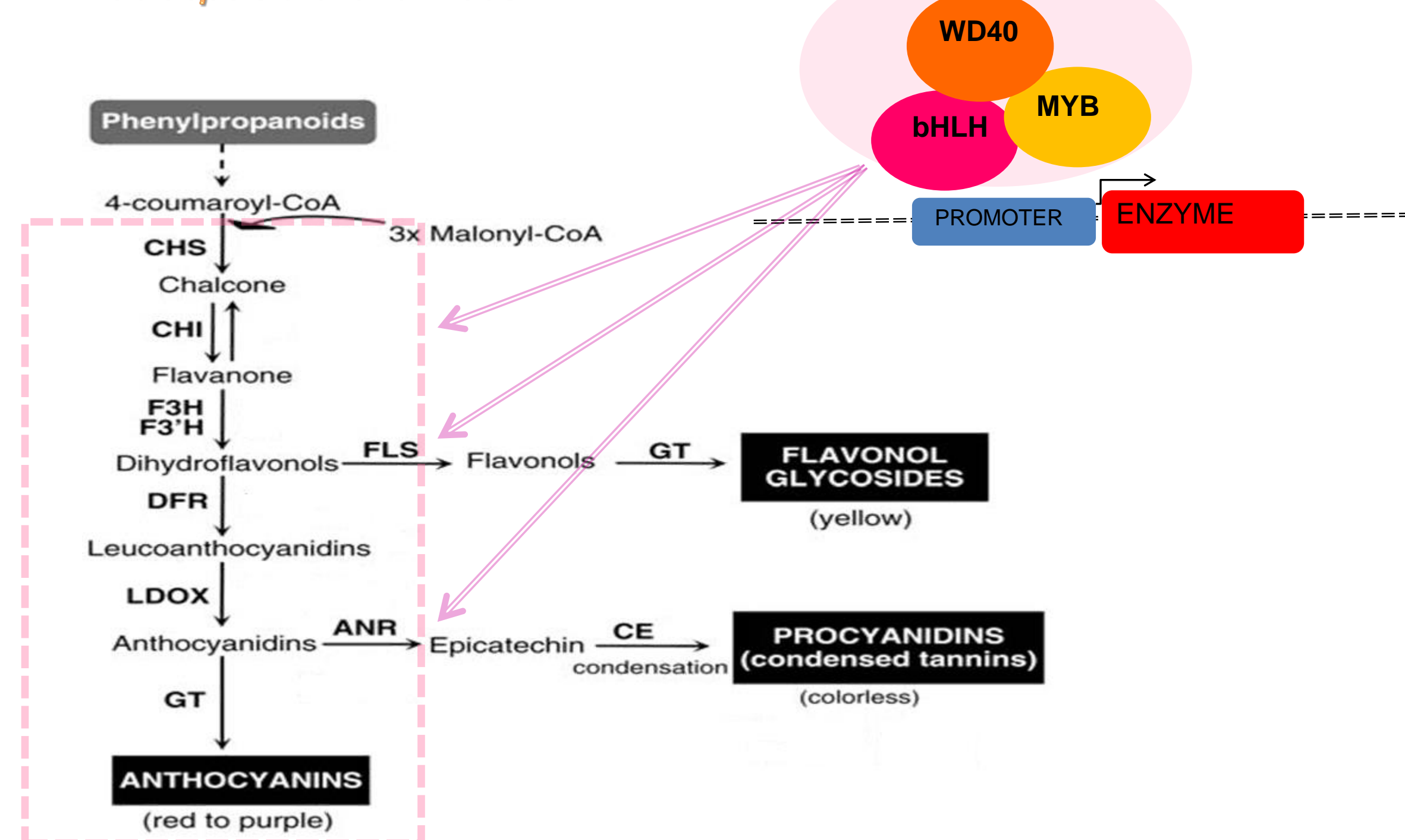
Anthocyanin and proanthocyanin synthesis is regulated through the interaction of the MYB-bHLH-WD40 complex, which has been characterized in several crops from diverse families (1), and some of the regulators have been identified in the Rosaceae species strawberry and apple (2, 3). To date it is not clear which bHLH proteins are involved in this pathway in strawberries and raspberry species, including their correct identification and expression levels during the physiological changes that occur during fruit development and ripening.

OBJECTIVES

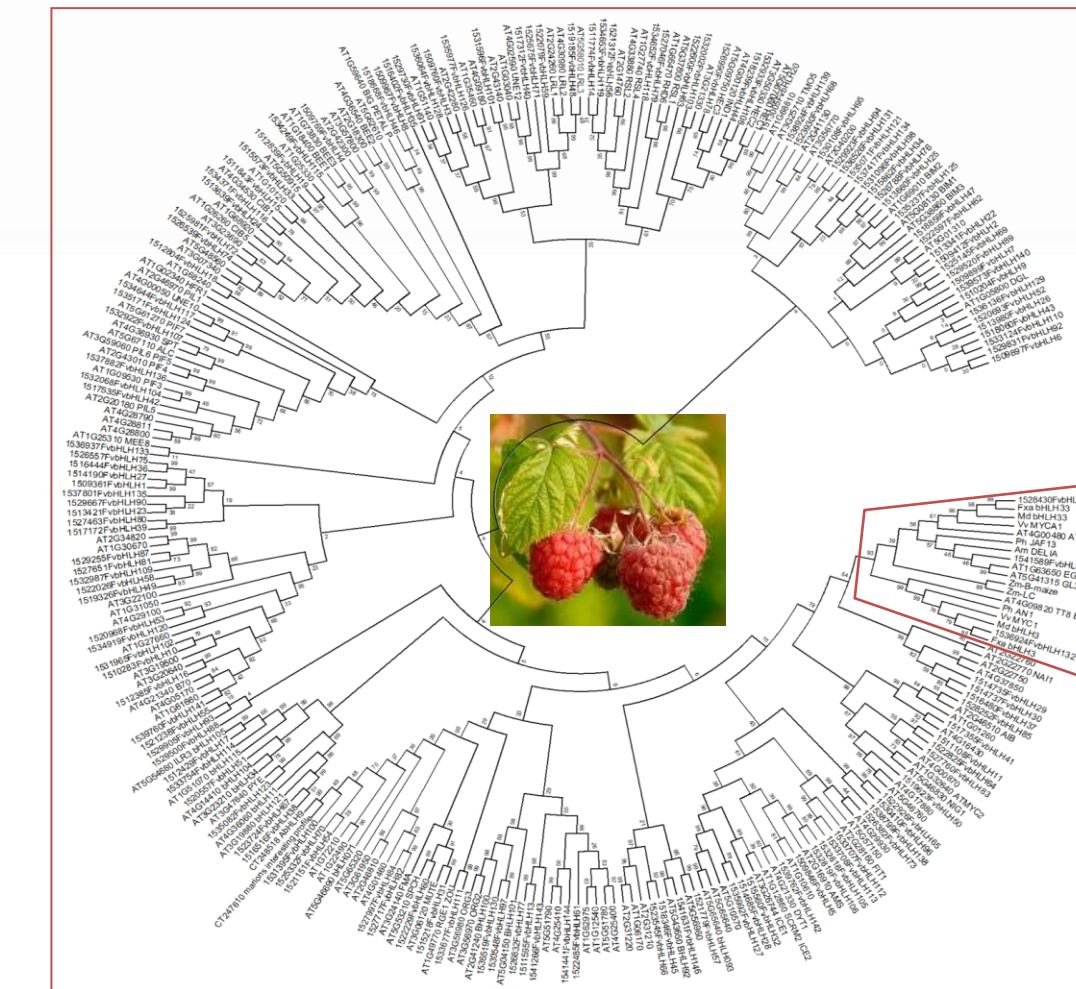
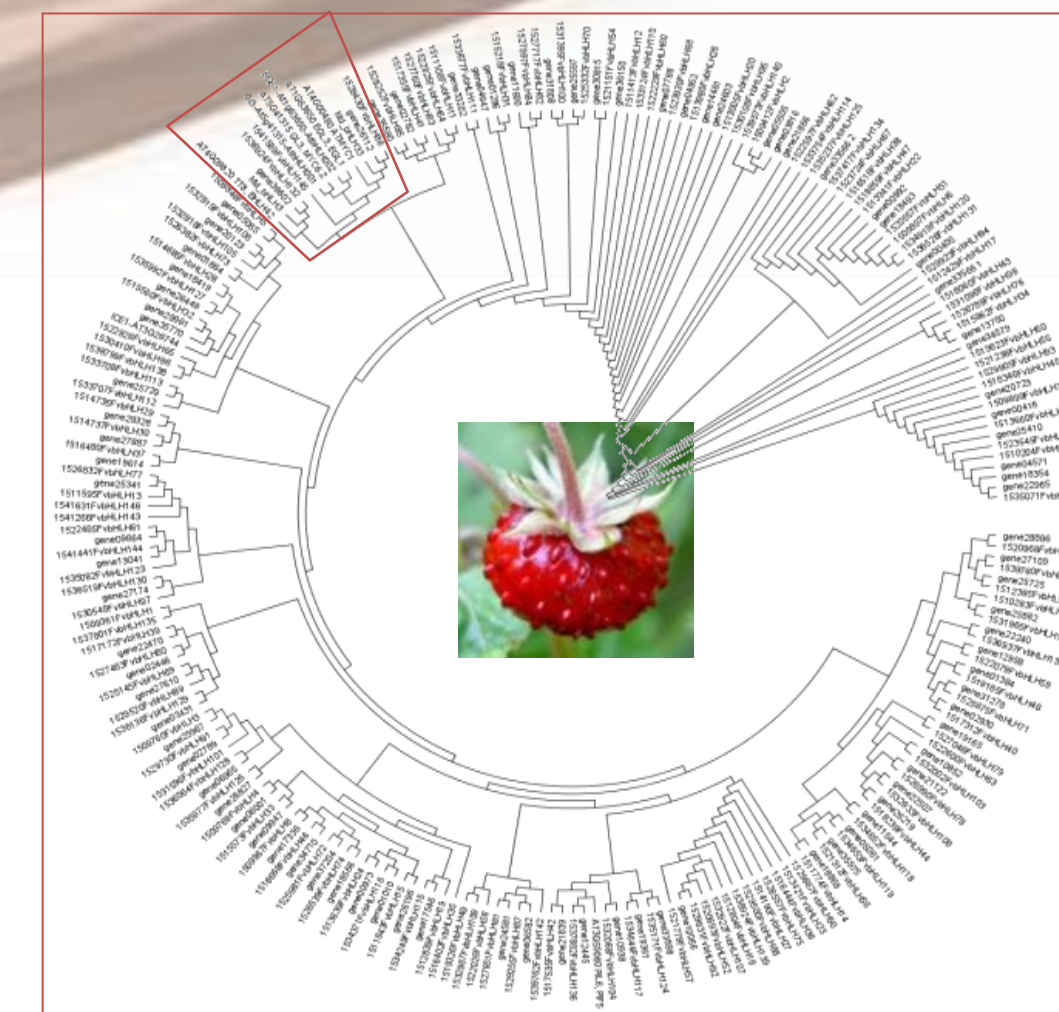
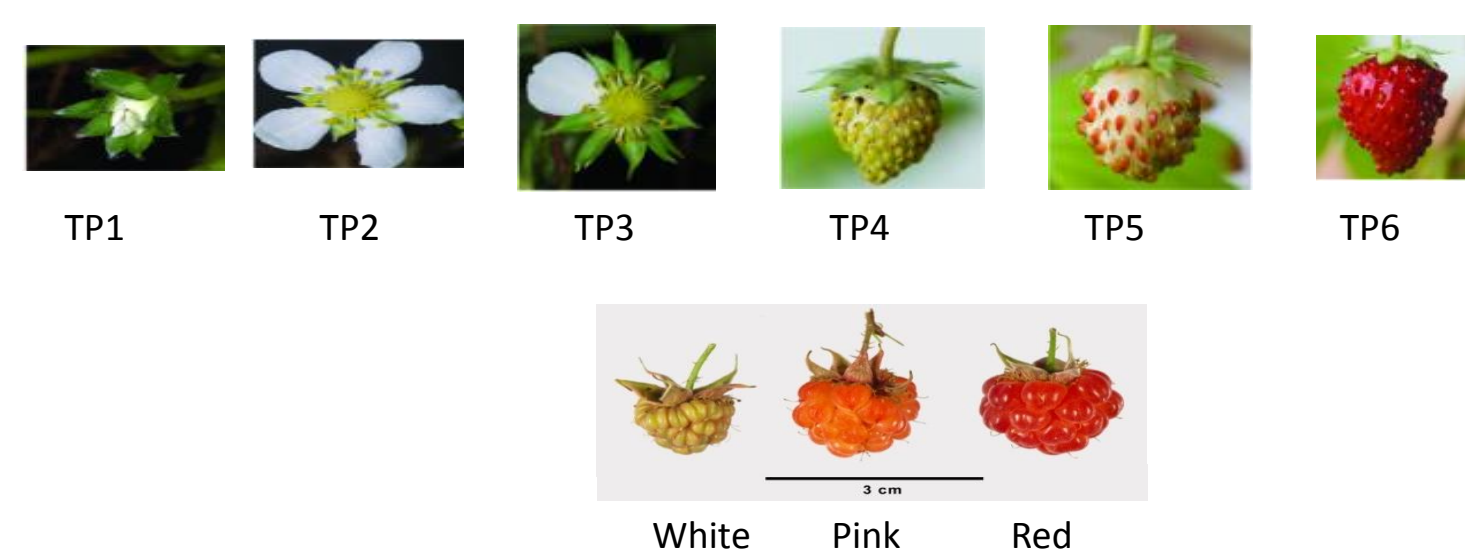
Identification and characterization of regulatory proteins involved in anthocyanin biosynthesis in *F. vesca* and *R. idaeus*

HOW ANTHOCYANINS ARE PRODUCED

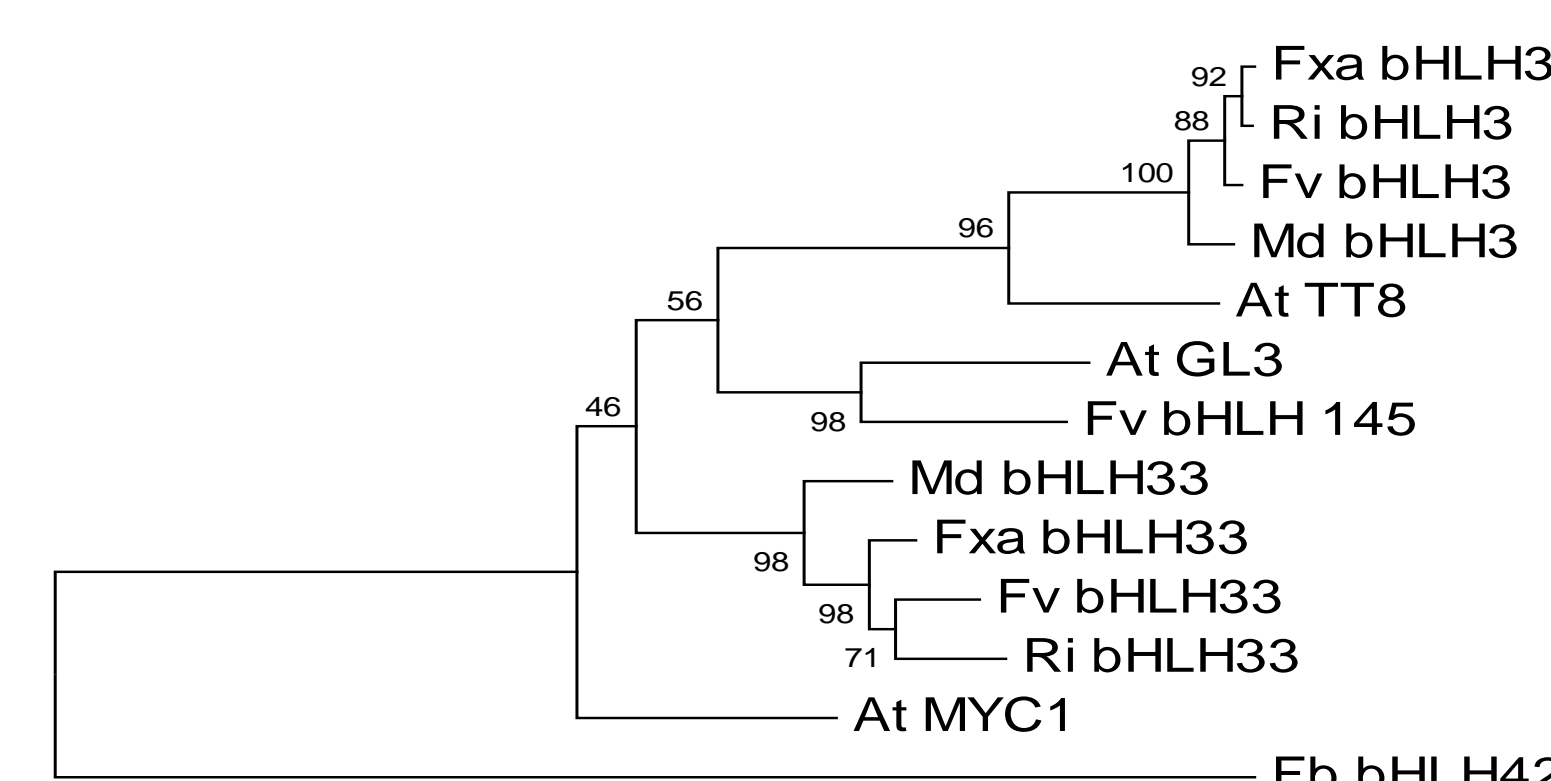
Arabidopsis thaliana model



Fruit development



152 *A. thaliana* previously identified bHLH proteins (4), 14 bHLH proteins involved in anthocyanin regulation from closely related species such as *M. domestica* and *F. ananassa* (2,3,5), 142 *F. vesca* gene models and 97 *R. idaeus* gene models, were compared to establish the phylogenetic association between these candidate genes.

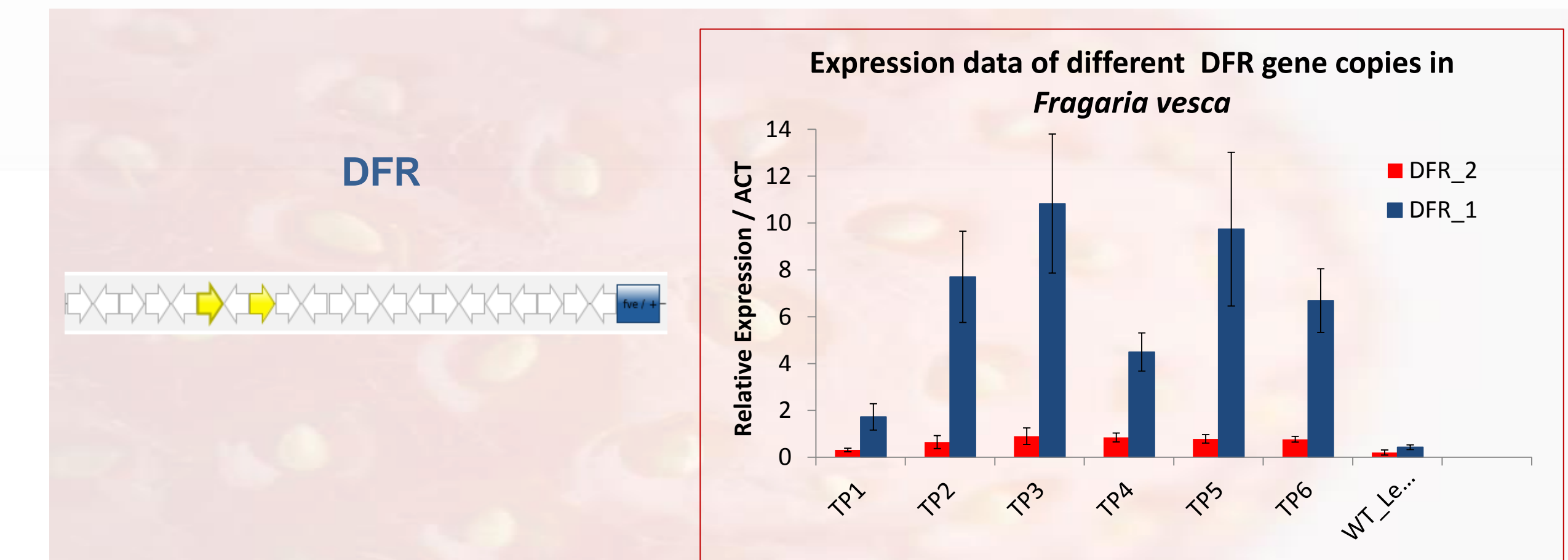
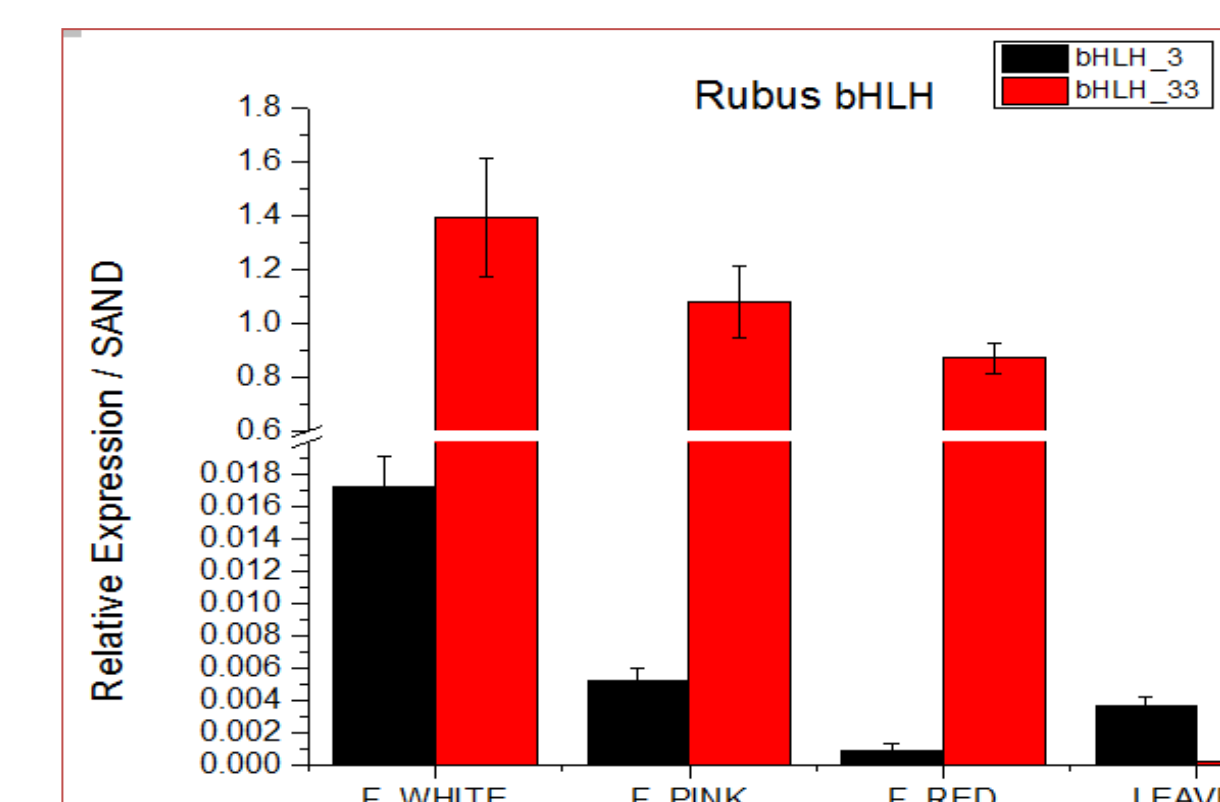
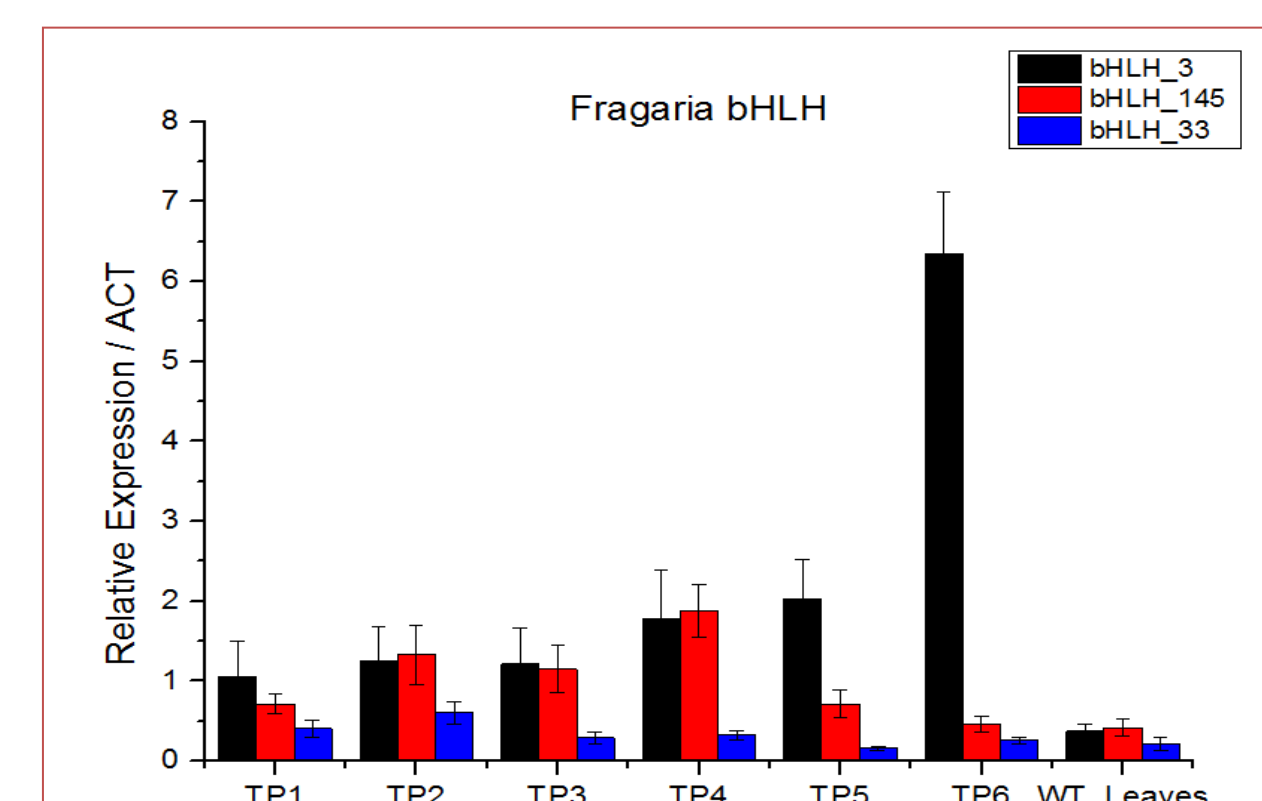


Phylogenetic analysis of the bHLH subgroup III (4) involved in anthocyanin biosynthesis.

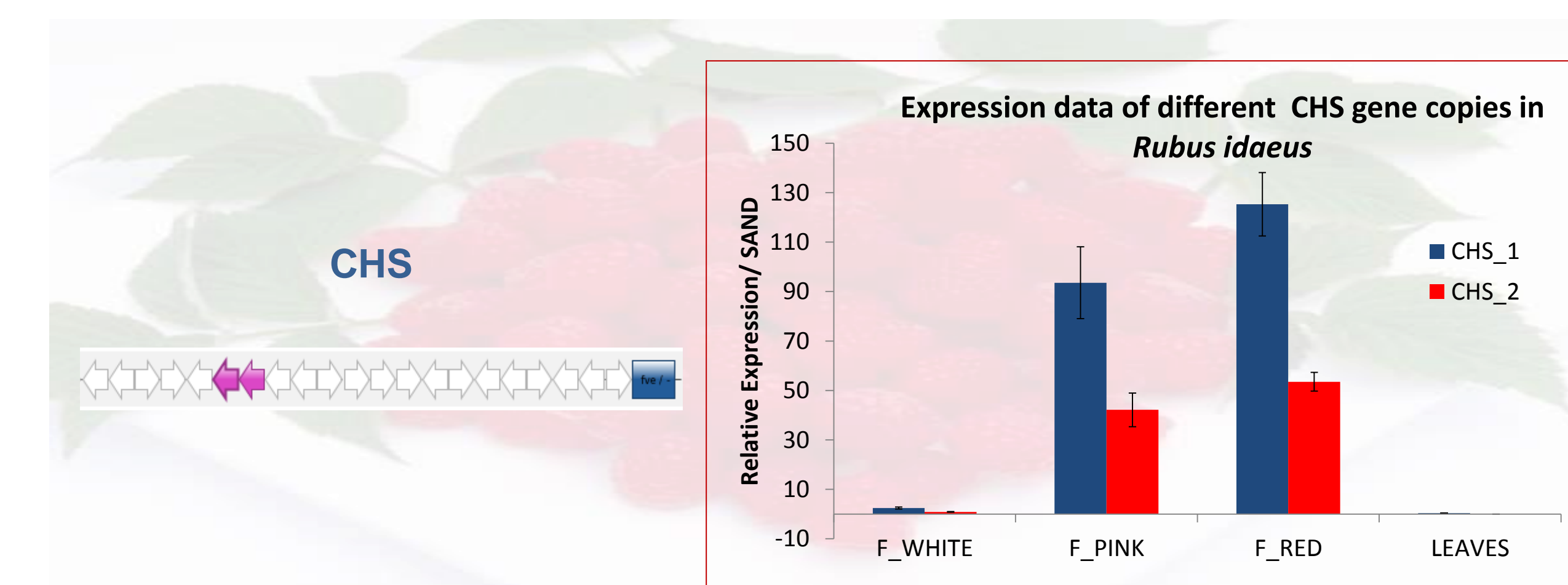
Candidate bHLHs involved in anthocyanin regulation

After the protein sequence/structure (domain) and phylogenetic tree analysis, a total of 5 gene models were found (3 for *F. vesca* and 2 for *R. idaeus*).

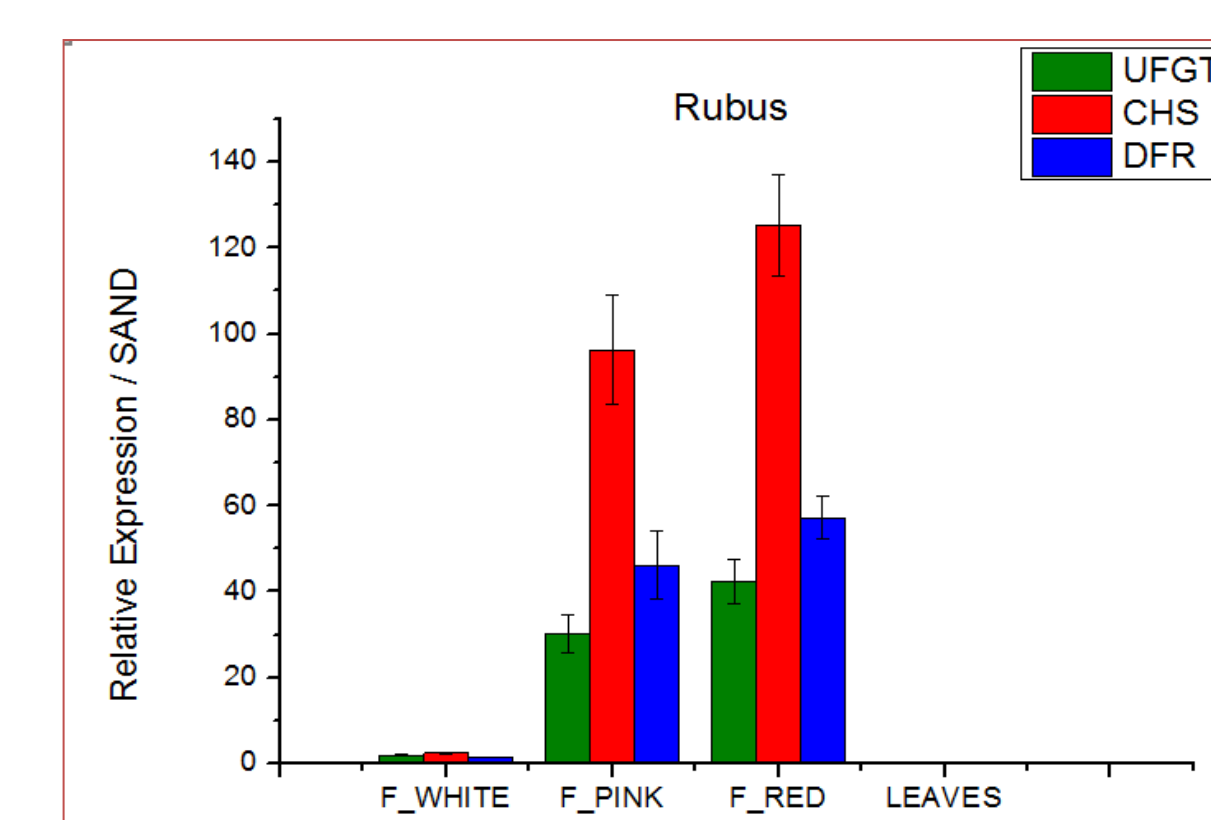
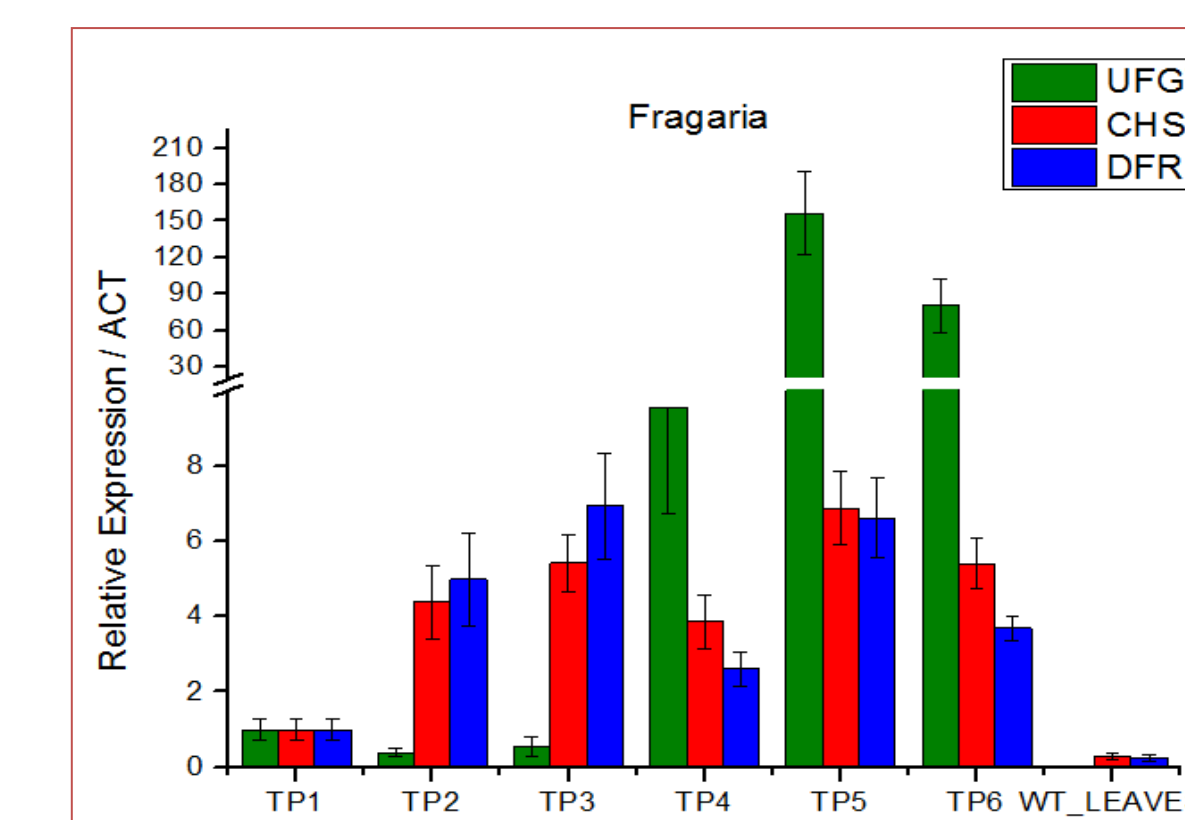
The tree shows the homology of the candidates with previously identified bHLH proteins from *M. domestica* (3). A new bHLH candidate was identified which has no known homolog in apple but which maps close to GL3 in the phylogenetic tree (FvbHLH145).



Fruit development



Fruit development



Expression data of anthocyanin pathway genes

Three main genes of the anthocyanin pathway - chalcone synthase (CHS), dihydroflavonol reductase (DFR) and flavonoid-3-O-glucosyltransferase (UFGT) were analysed in *Fragaria* and *Rubus* fruit developmental stages using qPCR. The expression pattern shows an increase of the activity of these genes during fruit ripening and color production, confirming the activity.

PRELIMINARY CONCLUSIONS AND FUTURE WORK

According to the phylogenetic analysis and protein sequences alignments, 3 bHLH candidates were identified for *F. vesca* and 2 for *R. idaeus*.

Further analysis on promoter activation for these candidates will be done to confirm their participation in anthocyanin regulation.

A new anthocyanin related bHLH candidate was found for *Fragaria vesca*.

Acknowledgment

GPMF and Fondazione Edmund Mach for PhD scholarship funding as part of the project "Identification and characterization of regulatory proteins involved in anthocyanin biosynthesis in *F. vesca* and *R. idaeus*". Plant And Food Research, for all the training opportunities, and academic and technical support.

References:

- Feller, A., Macherey, K., Braun, E.L. and Grotewold, E. (2011) Evolutionary and comparative analysis of MYB and bHLH plant transcription factors. *Plant J.* 66, 94–116.
- Schaart, J. G., Dubos, C., Romero De La Fuente, I., van Houwelingen, A. M. M. L., de Vos, R. C. H., Jonker, H. H., Xu, W., Routaboul, J.-M., Lepiniec, L., Bovy, A. G. (2013) Identification and characterization of MYB-bHLH-WD40 regulatory complexes controlling proanthocyanidin biosynthesis in strawberry (*Fragaria × ananassa*) fruits. *New Phytol.* 197, 454–467.
- Espley, R.V., Hellens, R.P., Putterill, J., Stevenson, D.E., Kutty-Amm, S. and Allan, A.C. (2007) Red colouration in apple fruit is due to the activity of the MYB transcription factor, MdMYB10. *Plant J.*, 49, 414–427.
- Heim, M.A., Jakoby, M., Werber, M., Martin, C., Weisshaar, B. and Bailey, P.C. (2003) The basic helix-loop-helix transcription factor family in plants: a genome-wide study of protein structure and functional diversity. *Mol. Biol. Evol.* 20, 735–747.
- Lin-Wang, K., Bolitho, K., Grafton K., Kortstee, A., Karunairatnam, S., McGhie, J., Espley R.V., Hellens R.P., Allan A. (2010). An R2R3 MYB transcription factor associated with regulation of the anthocyanin biosynthetic pathway in Rosaceae. *BMC Plant Biology* 2010, 10:50

Using the available *F. vesca* published genome data and *R. idaeus* genomes under construction, we identified possible bHLH regulatory proteins involved in anthocyanin biosynthesis from *F. vesca* and two from *R. idaeus*.

These candidate genes were evaluated for sequence/structure homology with previously characterized bHLH proteins to determine the phylogenetic relationship amongst them and to identify subgroups of interest. In addition, we analyzed gene expression of these bHLH proteins as well as of putative biosynthetic key gene candidates during fruit development. Preliminary results shown here suggest which bHLH proteins are involved in anthocyanin biosynthesis in these fruits.

FRUIT RIPENING AND bHLH TRANSCRIPTION FACTORS

Expression data for bHLH candidate genes

F. vesca bHLH expression pattern shows an increase of the activity during fruit development for two candidates, especially bHLH 3, results that are consistent with former studies (2, 3, 5). As expected for anthocyanin related genes, the leaf expression was very low compared with flower and fruit tissues. Interestingly, *Rubus* bHLH candidates show opposite expression patterns, with high expression in the earliest developmental stage (white fruit), which decreases in pink and red fruits. Further gene expression studies as well as functional analysis will shed light onto the contradictory data obtained to date.