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Characterization of the ripe fruit epidermis-specific FvMYB29- FvbHLH transcription factors complexes in woodland strawberry

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Transcriptome changes during strawberry fruit ripening have been previously described using either complete fruits or achenes (real fruits) and receptacles (fleshy part) separately. Our group aimed to identify tissue- and stage-specific patterns in *Fragaria vesca* coupling Laser Capture Microdissection (LCM) receptacles with RNA-seq analysis. In particular, we have focused on the Gene Regulatory Network (GRN) at the epidermis in ripe fruits. This constitutes the external cell layer of the strawberry fruit, which is in direct contact with the environment. Furthermore, and in contrast to receptacles of the octoploid species, it is the only part of the *F. vesca* red fruits that accumulate anthocyanins. Consistently, a MapMan functional analysis of this GRN showed enrichment in flavonoid and wax biosynthesis genes.

Three out of the several ripe epidermis-specific TFs were selected to study their biological role, one of them belonging to the MYB family (FvMYB29), and two bHLH-like proteins (FvbHLH22 and FvbHLH67). Protein interaction assays revealed that the FvMYB29 protein physically interacts independently with the two FvbHLHs forming a heterocomplex. Furthermore, these two bHLHs might participate together in this FvMYB29-FvbHLHs heterocomplex, as evidenced by an *in vivo* assay. Genome-wide binding sites of these TFs were identified by DAP-seq, revealing that genes involved in flavonoid biosynthesis and cuticle composition are among the FvMYB29 targets, which were validated by transactivation assays (Luciferase/Renilla system), while the bHLH TFs did not bind to

DNA by themselves. Interestingly, transactivation assays combining FvMYB29 and the two FvbHLHs separately or together showed that the latter modulates the transcription of these targets by FvMYB29.

Stable *FvMYB29* overexpression led to pleiotropic phenotypes evident in both vegetative and reproductive organs compared to the wild type. These plants exhibited significantly smaller leaves, crown elongation, taller inflorescences with more branching, earlier flowering, and a higher rate of fruit abortion due to a reduction in pollen content and viability. As expected from the enrichment in cuticle formation-related genes among the FvMYB29 targets, the overexpression lines showed a misregulation of genes related to cutin and wax biosynthesis in ripe fruits and leaves, which is consistent with a higher content in epicuticular waxes, in particular alkanes, aldehydes, and esters, on the abaxial surface of their leaves compared to those of the control. All these results support the role of the FvMYB29-FvbHLH TF complex as an important regulator of cuticle structure in *F. vesca*. We are currently analyzing RNAi and CRISPR lines for these three TFs to further investigate their biological role and the consequences on gene expression of their interactions.