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ROLE OF RIPE FRUIT EPIDERMIS-SPECIFIC FvMYB29-FvbHLH TRANSCRIPTION FACTOR COMPLEXES IN STRAWBERRY

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Transcriptome changes during strawberry fruit ripening have been previously reported using either complete fruits or achenes (actual fruits) and receptacles (fleshy part) separately. However, we have identified genes with tissue- and stage-specific patterns in the receptacles of *Fragaria* vesca coupling Laser Capture Microdissection with RNA-seq analysis. In the study, we have focused on the Gene Regulatory Network (GRN) at the epidermis in ripe fruits, since it is the external cell layer in direct contact with the environment and it plays an important role in defense, and, in contrast to receptacles of the commercial species, it is the only part of the fruit that accumulates anthocyanins. Consistently, a Mapman/GO functional analysis of this GRN showed enrichment in genes involved in flavonoid and wax biosynthesis.

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 with the two FvbHLHs. 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 was validated by transactivation assays (Luciferase/Renilla system), while the bHLH TFs did not bind to DNA by themselves. However, transactivation assays with different combinations of FvMYB29 and the two FvbHLH showed that the latter modulates the activation of transcription of the targets. Consistently with the role of FvMYB29 in the cuticle formation, stable *FvMYB29*-

overexpressing lines showed a misregulation of genes related to cutin and wax biosynthesis in ripe fruits and leaves. Furthermore, *FvMYB29*-overexpressing fruits presented cuticular nanoridges, that could be explained by an alteration in the cell wall or cuticle structures. On the other hand, young leaves of *FvMYB29*-overexpression lines showed denser epicuticular waxes in the abaxial surface and an alteration in wax composition but compared to 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.