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CHARACTERIZATION OF RIPE FRUIT EPIDERMIS-SPECIFIC TRANSCRIPTION FACTORS 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. In order to perform a more detailed study, we have performed a tissue- and stage-specific transcriptome analysis in receptacles of *Fragaria vesca* fruits, allowing us to infer Gene Regulatory Networks (GRN) in each tissue and stage. In the study, we have focused on the epidermis at the ripe stage, since it plays an important role in defense, as it is the external cell layer in direct contact with the environment, and, in contrast to receptacles of the commercial species, it is the only part of the fruit that accumulates anthocyanins. MapMan analysis of the GRN in ripe epidermis showed that wax and flavonoid biosynthesis were significantly overrepresented functions. Three out of the several TFs found among the main hubs in this GRN were selected to study their biological role, one of them belonging to the MYB family, and two bHLH genes. Protein interaction assays revealed that the MYB protein physically interacts with the two bHLHs, leading to the subcellular relocalization from the cytoplasm to the nucleus in one of them. DAP-seq analyses showed that the bHLH TFs do not bind DNA by themselves, but that genes involved in cuticle formation and flavonoid biosynthesis are among the MYB targets, which were validated by a transactivation assay using the Luciferase/Renilla system. Consistently, MYB-overexpressing stable lines exhibited an upregulation of genes related to cuticle and wax biosynthesis in ripe fruits, and an accumulation of higher amounts of epicuticular waxes in young leaves compared to the WT. We are currently establishing RNAi and CRISPR lines for these three ripe-epidermis specific TFs to further investigate their biological role and performing analyses to understand the effect on gene expression of the interaction between them.

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