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CHARACTERIZATION OF RIPE FRUIT EPIDERMIS-SPECIFIC TRANSCRIPTION FACTORS IN STRAWBERRY

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Abstract: The epidermis is the external cell layer in direct contact with the environment, and it plays essential biological roles. Transcriptome analysis (RNA-seq) of *Fragaria vesca* fruit receptacles at four ripening stages (green, white, turning and red) and of different tissue types of receptacles (pith, vascular bundles, cortex and epidermis) at two ripening stages (green and red) allowed us to infer tissue- and stage-specific Gene Regulatory Networks (GRN). Due to the potential role of the epidermis in defense and in the differential anthocyanin accumulation pattern that shows *F. vesca* fruits at the ripe stage (the skin is red, while the inner part is white), we have focused on the GRN of the epidermis of red receptacles. In this study, we aim at the functional characterization of two transcription factors (TFs) that constituted the main hubs of this GRN: a MYB-like gene, and a member of the NAC family of TFs. MapMan analysis of the genes constituting the GRN in ripe epidermis showed that wax and flavonoid biosynthesis were significantly overrepresented functions in the epidermis of red receptacles. Using the Luciferase/Renilla (Luc/Ren) system, the interaction of the MYB and NAC TFs with their wax-related putative targets was validated. To experimentally validate the target genes of these two TFs, their binding sites were mapped genome-wide using DAP-seq analyses. Consistently, MYB bound to a set of genes involved in cuticle formation and flavonoid biosynthesis, while a number of genes involved in solute transport were enriched among the NAC direct targets. Finally, protein interaction assays showed that MYB physically interacts with two members of the bHLH TF family specifically expressed in the red epidermis of receptacles, leading to the subcellular relocalization of one of them from the cytoplasm to the nucleus. Currently, we are phenotyping transgenic overexpressing lines of MYB and generating CRISPR/Cas9

mutant lines to functionally characterize these two TFs.

Key words: Strawberry, Epidermis, Transcription Factor, Gene Regulatory Network

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