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► **To cite this version:**

Xuan Xu, Gea Guerriero, Frédéric Domergue, Olga Beine-Golovchuk, Emmanuelle Coco, et al.. Characterization of MdMYB68, a suberin master regulator in russeted apples. Gordon Research Conference / Plant Lipids: Structure, Metabolism and Function, Jan 2023, Galveston, United States. <hal-05265101>

HAL Id: hal-05265101

<https://hal.science/hal-05265101v1>

Submitted on 17 Sep 2025

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Characterization of MdMYB68, a suberin master regulator in russeted apples

LUXEMBOURG
INSTITUTE OF SCIENCE
AND TECHNOLOGY



Laboratoire de
BIOGENÈSE MEMBRANAIRE

Xuan Xu¹, Gea Guerriero¹, Frederic Domergue², Olga Beine-Golovchuk¹, Emmanuelle Cocco¹, Roberto Berni¹, Kjell Sergeant¹, Jean-Francois Hausman¹, Sylvain Legay¹

Apple russeting is mainly due to the accumulation of suberin in the cell wall in response to defects and damages in the cuticle layer. Our previous studies highlighted several MYB transcription factors which might regulate the suberization in russeted or suberized apple fruit skin. Among these, we identified MdMYB68, which was co-expressed with number of well-known key suberin biosynthesis genes (Figure 1). Indeed, its transient expression in *N. benthamiana* triggered the expression of the whole suberin biosynthesis pathway (Figure 2). A lipid content analysis further confirmed that MdMYB68 regulates the deposition of suberin in cell walls (Figure 3 and Figure 4). Furthermore, we also investigated the alteration of the non-lipid cell wall components and showed that MdMYB68 trigger a massive modification of the hemicelluloses and pectins (Figure 5 and Figure 6).

MdMYB68 is co-expressed with suberin biosynthesis genes in russeted apples

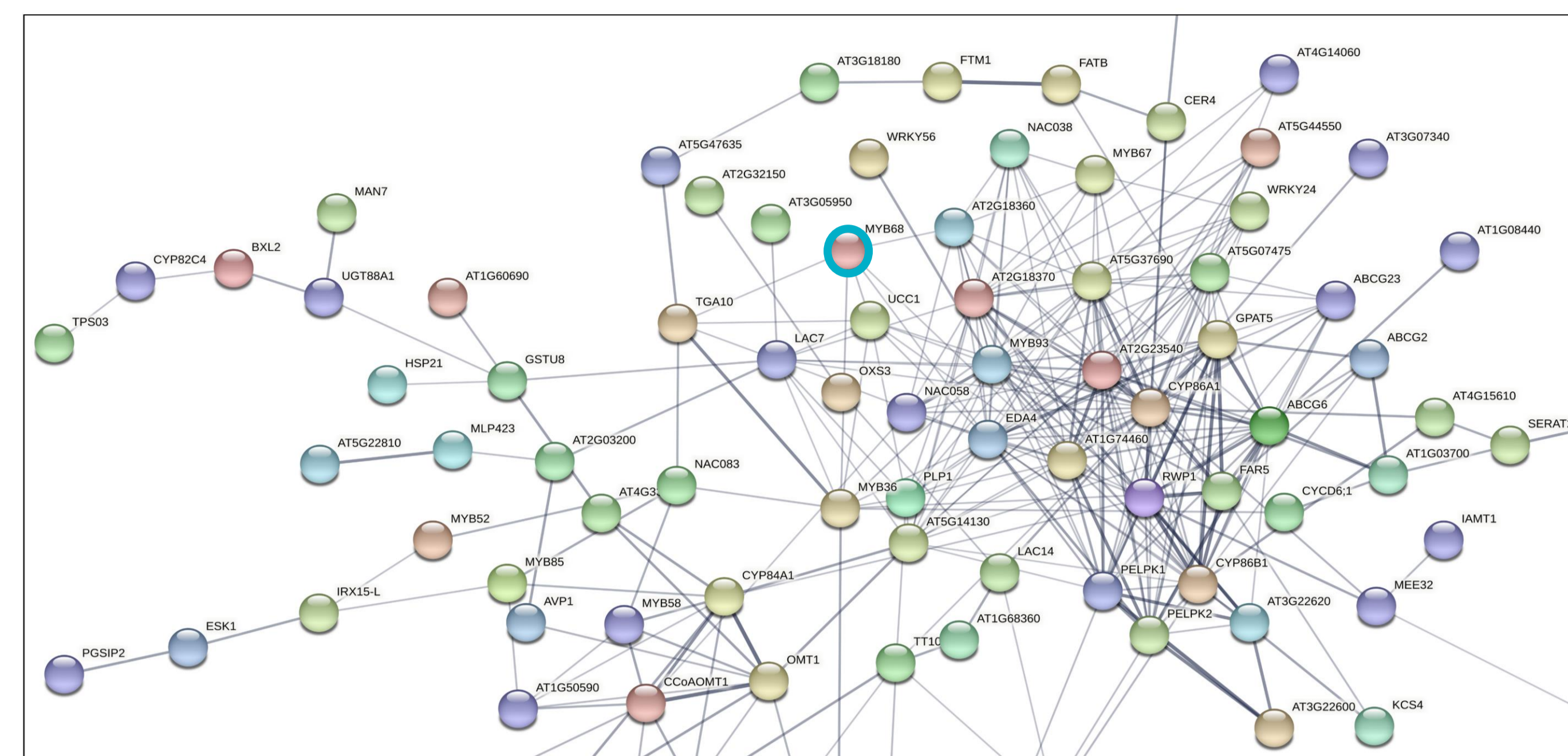


Figure 1: Subset of the gene interaction matrix obtained from genes significantly upregulated in russeted apple fruit skin compared to waxy apples ($-3 < \log_2(\text{ratio}(\text{russeted/waxy})) < 3$) (Legay et al., 2015; André et al., 2022). Grey lines represent the gene interaction confidence (ranged from 0 to 1): thickest lines display confidence scores higher than 0.9, thinnest lines display confidence scores between 0.4 and 0.7.

MdMYB68 triggered the expression of phenylpropanoid, lipid biosynthesis, and cell wall modification

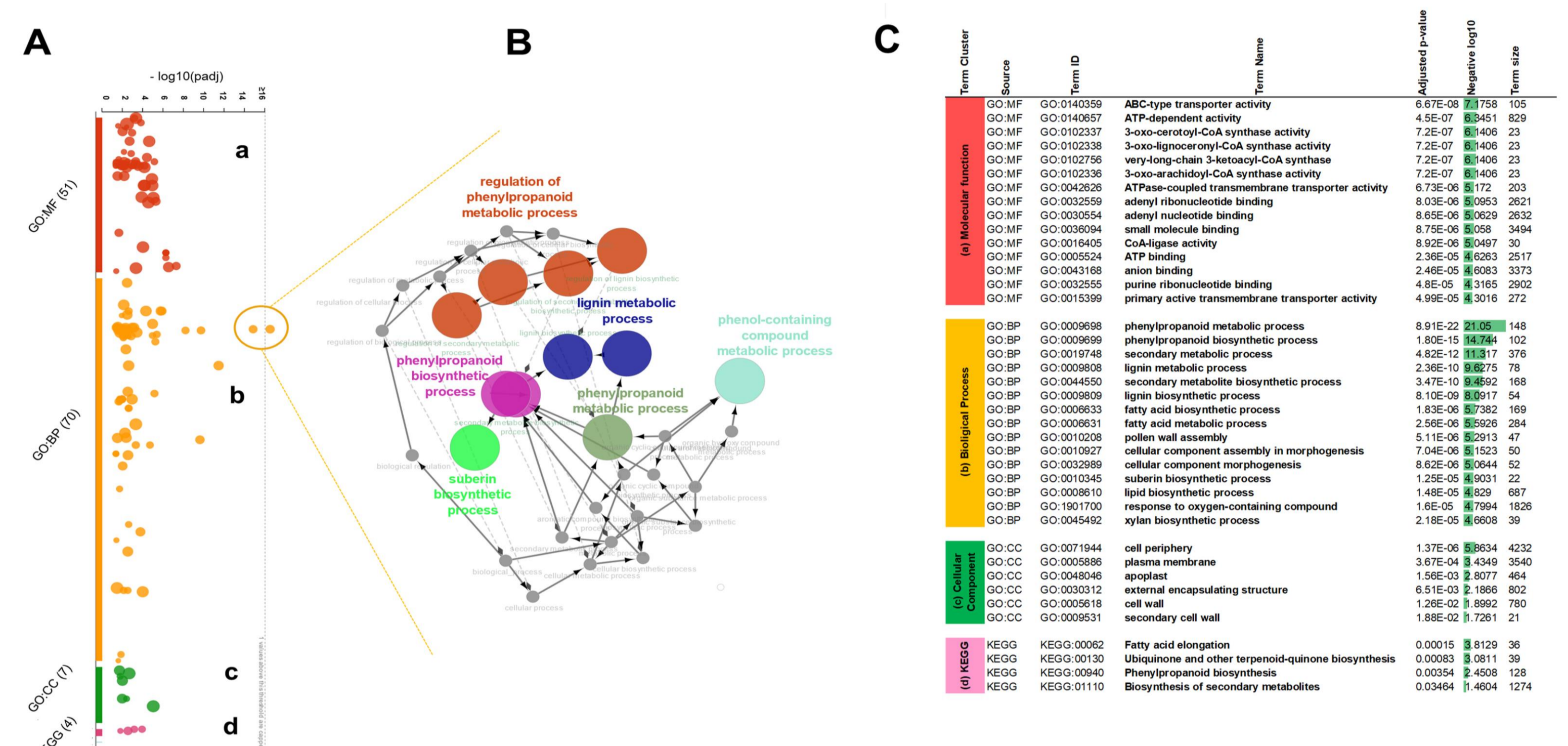


Figure 2: Gene ontology enrichment analysis of upregulated genes obtained 4 days after MdMYB68 transient expression in *Nicotiana benthamiana* leaves. A: An interactive Manhattan plot visualizes enriched GO term cluster within plant response to MYB68 overexpression in *N. benthamiana*. The x-axis shows the functional terms from 2840 upregulated genes. B: Expanded view and network mapping of the genes corresponding to GO:0009698 and GO:0009699 terms, indicating the highest p-value. The gene network highlights interaction of most regulated biological processes revealed significant stimulating in the phenylpropanoid fatty acid pathway. C: Table of most significant gene ontology terms enriched after MYB68 overexpression.

MdMYB68 triggered the ectopic deposition of aliphatic suberin in *N. benthamiana* leaves

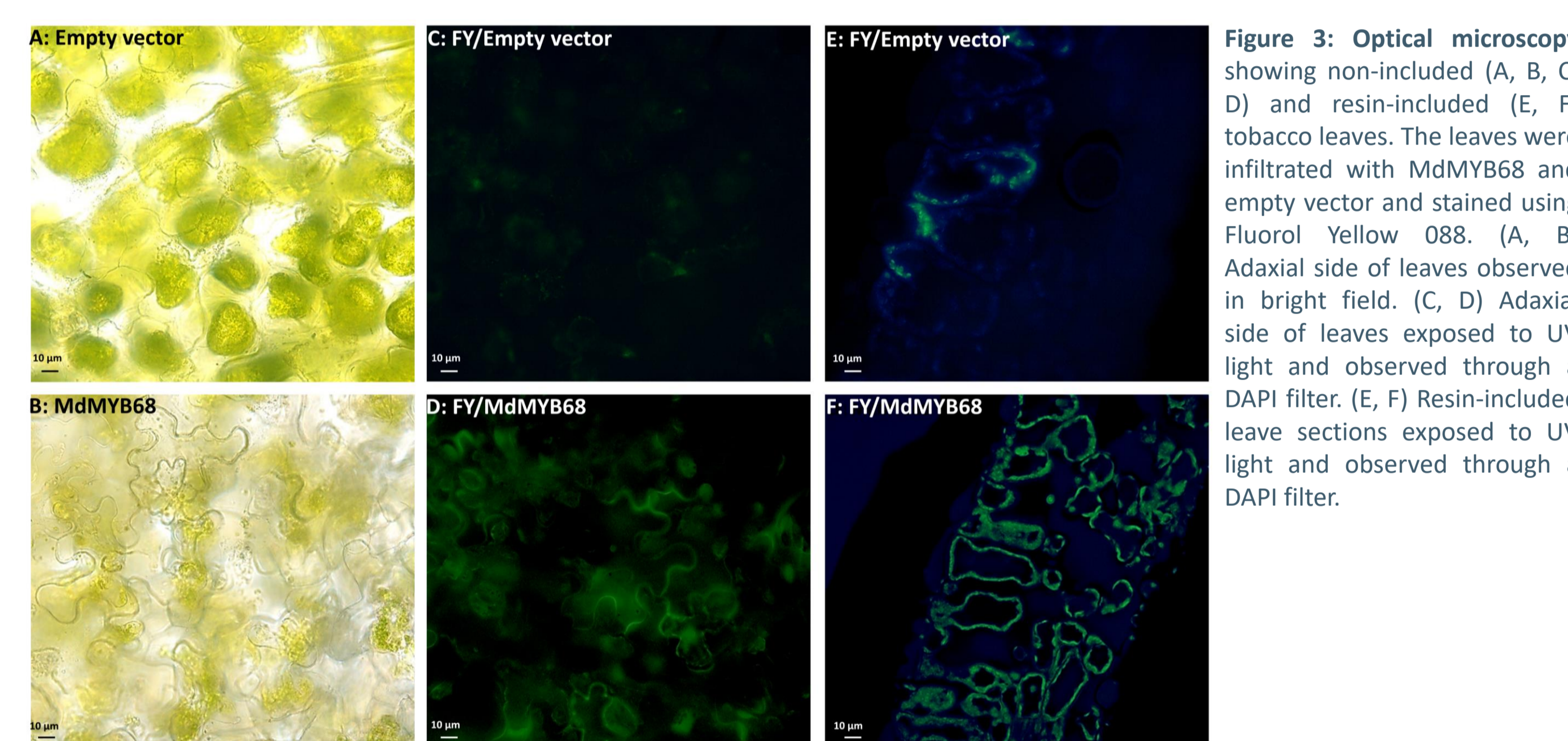


Figure 3: Optical microscopy showing non-included (A, B, C, D) and resin-included (E, F) tobacco leaves. The leaves were infiltrated with MdMYB68 and empty vector and stained with Fluorol Yellow O88. (A, B) Adaxial side of leaves observed in bright field. (C, D) Adaxial side of leaves exposed to UV light and observed through a DAPI filter. (E, F) Resin-included leaf sections exposed to UV light and observed through a DAPI filter.

MdMYB68 expression altered the cell wall monosaccharide composition

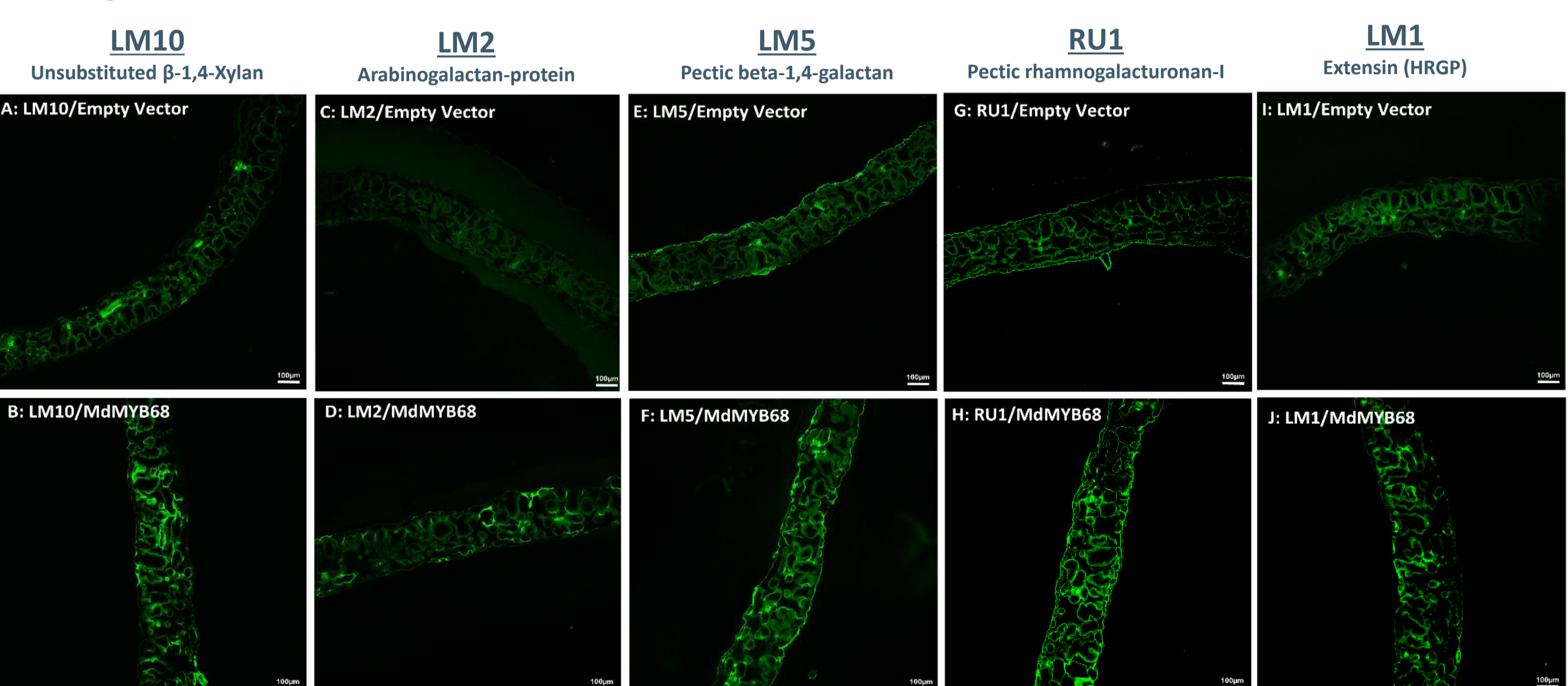


Figure 5: Immunodetection performed in *N. benthamiana* leaves infiltrated with the Empty vector (top) and MdMYB68 (bottom) constructs using the following antibodies: LM10 specific for unsubstituted β -1,4-Xylan (A and B), LM2 specific for carbohydrates containing β -linked glucuronic (C and D), LM5 specific for β -1,4-D-galactan (E and F), RU1 specific for unbranched regions of rhamnogalacturonan-I (G and H), LM1 specific for extensins/HRGP (I and J).

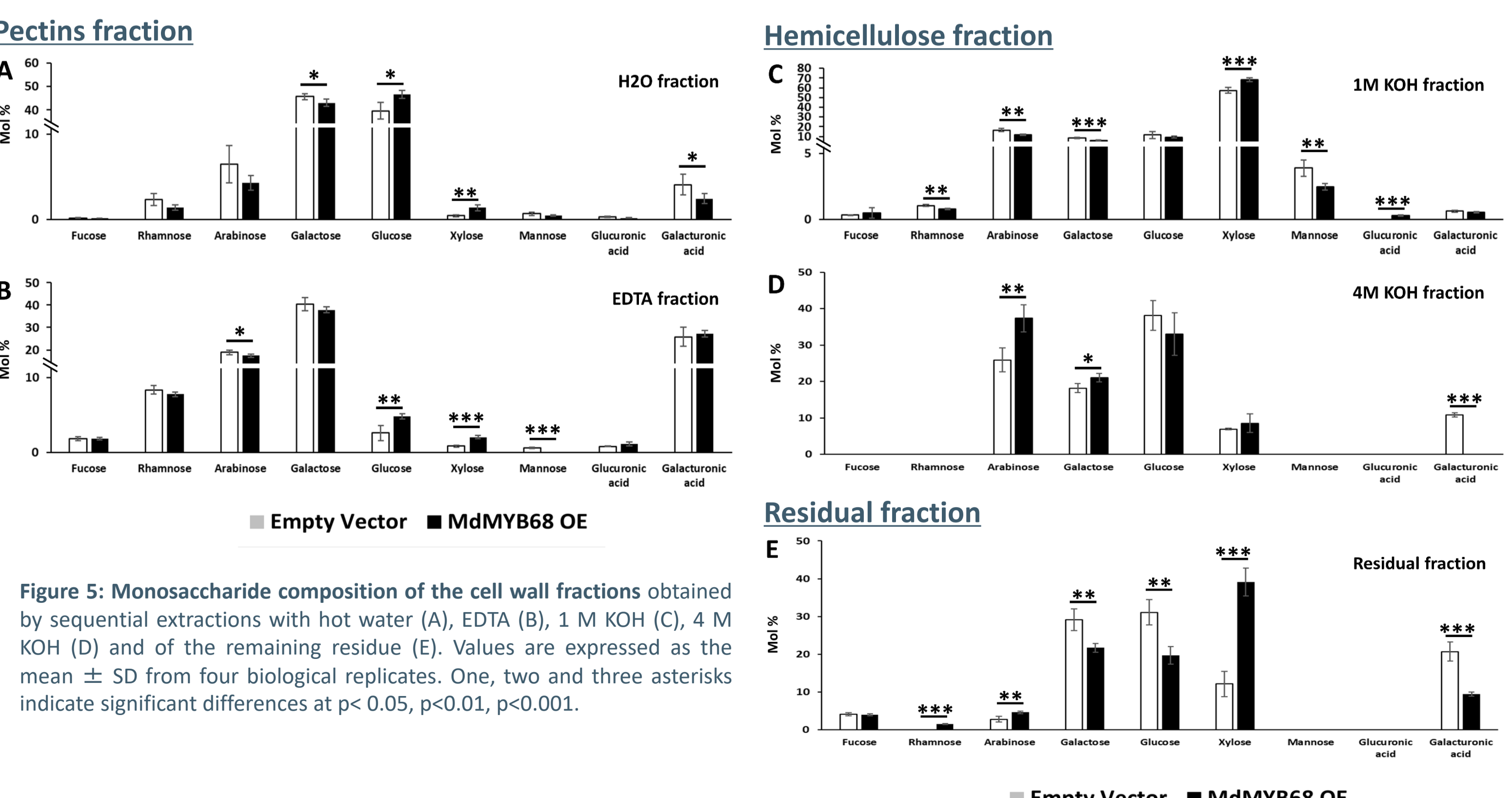


Figure 6: Monosaccharide composition of the cell wall fractions obtained by sequential extractions with hot water (A), EDTA (B), 1 M KOH (C), 4 M KOH (D) and of the remaining residue (E). Values are expressed as the mean \pm SD from four biological replicates. One, two and three asterisks indicate significant differences at $p < 0.05$, $p < 0.01$, $p < 0.001$.

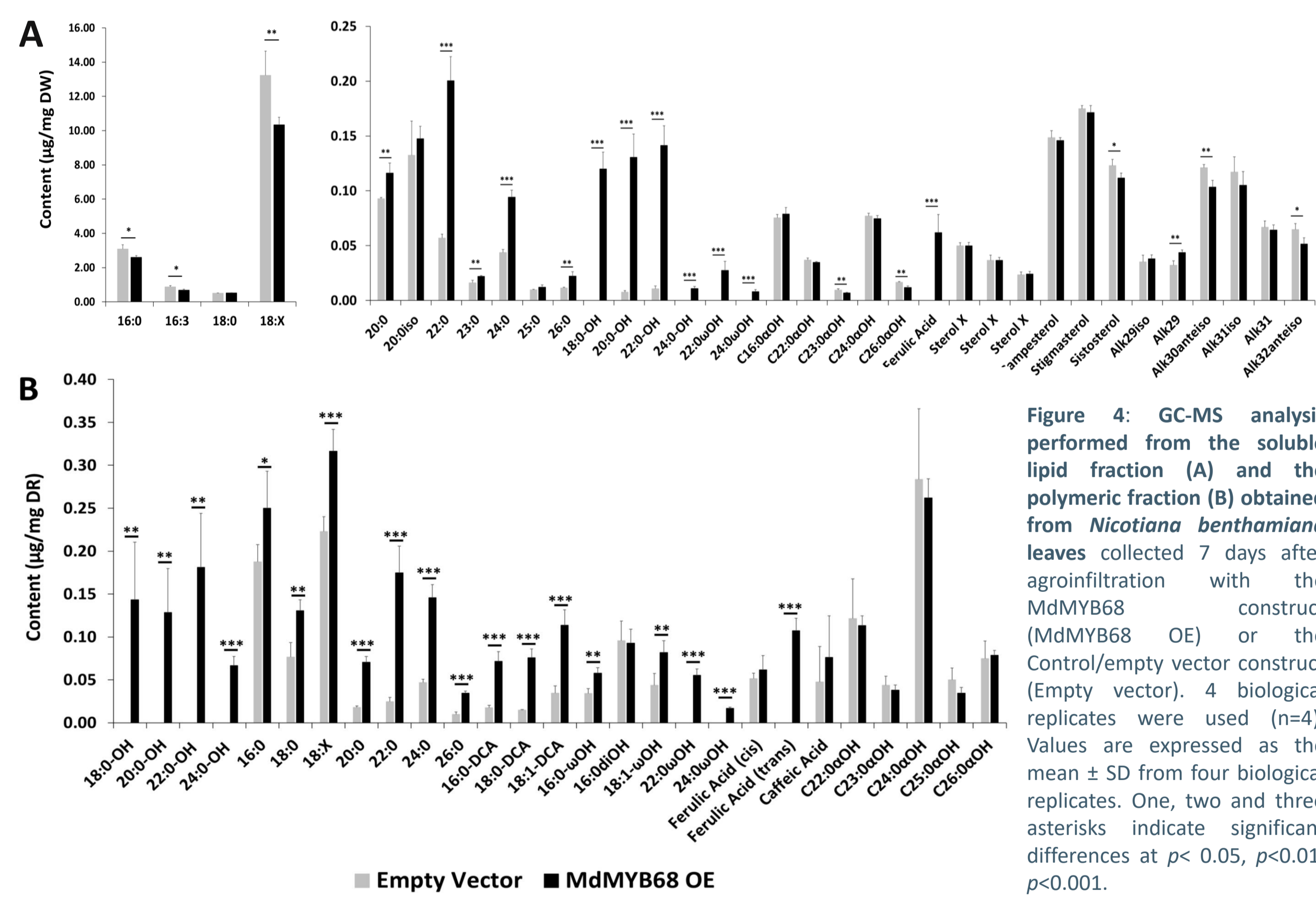


Figure 4: GC-MS analysis performed on the soluble lipid fraction (A) and the polymeric fraction (B) obtained from *Nicotiana benthamiana* leaves collected 7 days after agroinfiltration with the MdMYB68 OE construct (MdMYB68 OE) or the Control/empty vector construct (Empty vector). 4 biological replicates were used (n=4). Values are expressed as the mean \pm SD from four biological replicates. One, two and three asterisks indicate significant differences at $p < 0.05$, $p < 0.01$, $p < 0.001$.

Take home message:

- MdMYB68 is major regulator of the suberin deposition in apple fruit skin
- MdMYB68 triggers the expression of pathways associated with the biosynthesis of the suberin aromatic and aliphatic components, namely phenylpropanoids and lipids; and genes associated with the cell wall modification.
- We here described the fate of the different cell wall component during the suberization process: MdMYB68 is able to alter the pectin and hemicellulose, particularly xylans composition