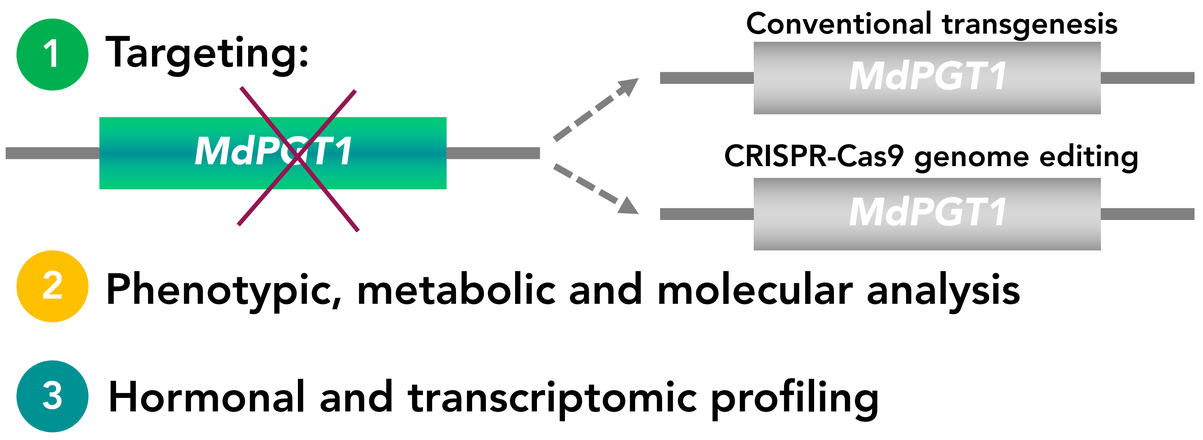
Transcriptome and metabolic profiling provide insights into the role of *MdPGT1* in the phloridzin-mediated effect on apple development

Simón MIRANDA[1,2], Stefano PIAZZA[1], Axel MITHÖFER[3], Floriana NUZZO[1], Luisa PALMIERI[1], Alessandro CESTARO[1], Mickael MALNOY[1]*, Stefan MARTENS[1]*

[1]Research and Innovation Centre, Edmund Mach Foundation, Trento, Italy.
[2]C3A Center Agriculture, Food and Environment - PhD programme in AgriFood and Environmental Sciences, University of Trento, Italy.
[3]Max Planck Institute for Chemical Ecology, Jena, Germany.

Introduction and Methodology

Dihydrochalcones (DHCs) constitute a class of metabolites derived from the phenylpropanoid pathway, which have been shown to exert beneficial properties both in human health and plants. Among DHCs, phloridzin is the most abundant compound accumulated in apple (*Malus domestica*), and the biosynthetic steps towards its production have been recently elucidated. In order to get a better understanding of DHCs functions in apple, this work aimed to study the effect of silencing *MdPGT1*, encoding a phloretin-specific glycosyltransferase that catalyses the final step to produce phloridzin. Towards this aim, *MdPGT1* was targeted by traditional transgenesis and precise CRISPR-Cas9 genome editing. Then, metabolic, transcriptomic and hormonal analyses were performed in silenced apple lines.



Results

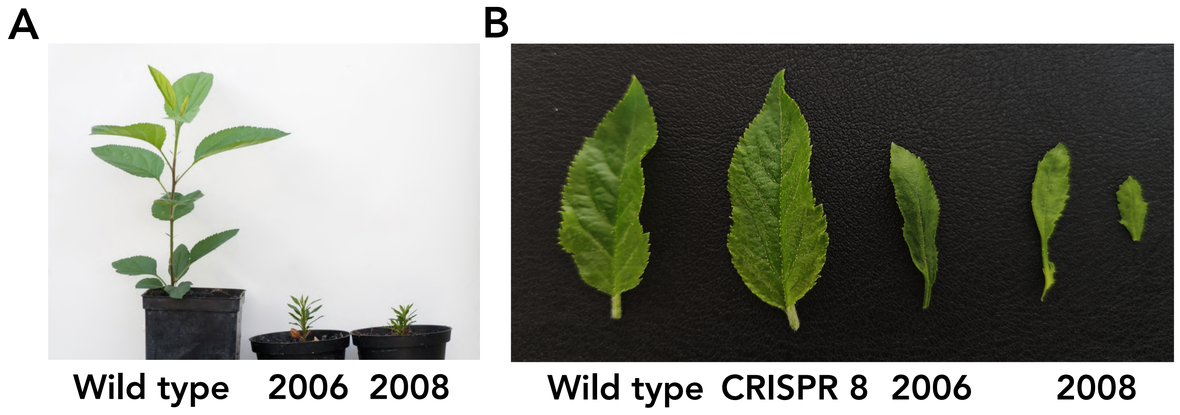


Figure 1. Stunted phenotype and leaves morphology is altered in *MdPGT1* silenced lines. *A*, Growth habit of Golden Delicious (*wild type*) and transgenic lines (*2006*, *2008*). *B*, Fully expanded leaves of representative phenotypes, including a genome-edited line (CRISPR 8), which exhibits leaves similar to wild type phenotype.

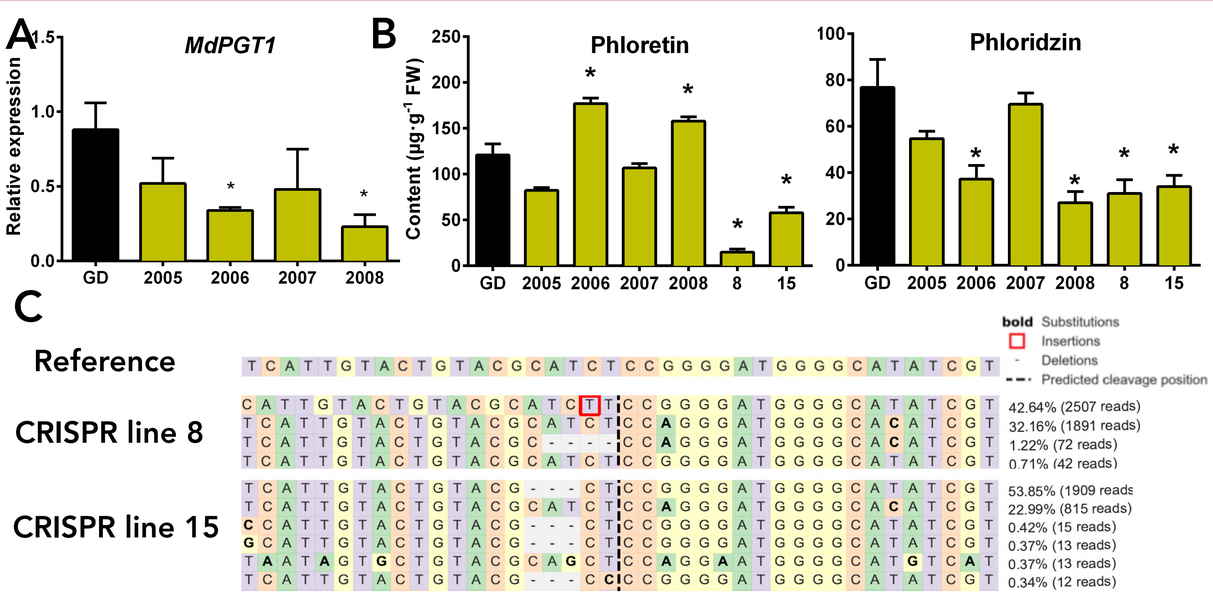


Figure 2. Molecular analysis of apple lines indicate reduced *MdPGT1* expression along with differential accumulation of phloretin and phloridzin. *A*, RT-qPCR of *MdPGT1* expression in Golden Delicious (*GD*), transgenic lines (*2005 - 2008*). ***B***, Levels of phloretin (precursor) and phloridzin (product) in transgenic and CRISPR-Cas9 edited lines (*8* and *15*). ***C***, Illumina sequencing result in part of *MdPGT1* coding sequence, for lines 8 and 15. A second allele in *MdPGT1* CDS was not edited. Dashed line indicates the site of cleavage of Cas9; red boxes and – represent insertions and deletions.

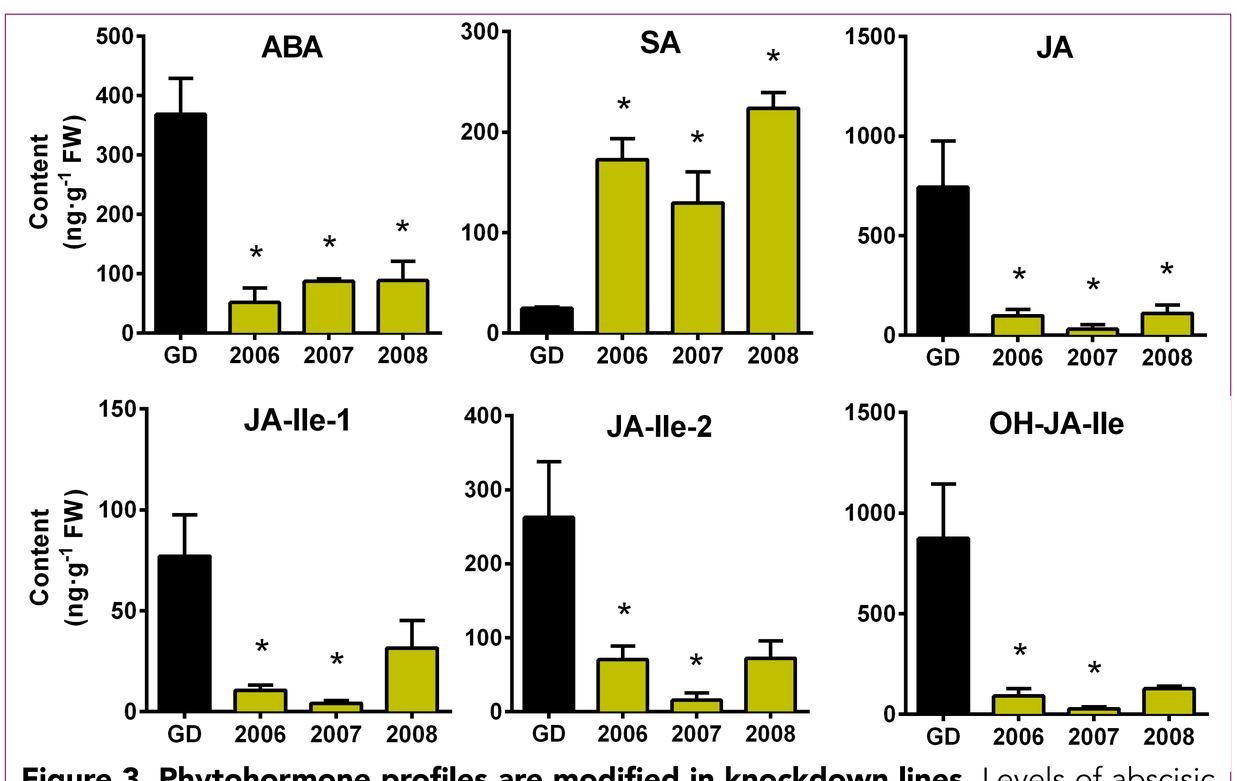


Figure 3. Phytohormone profiles are modified in knockdown lines. Levels of abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA), JA-Isoleucine 1, 2 (JA-Ile-1, JA-Ile-2) and hydroxy-JA-Ile (OH-JA-Ile) were analysed in Golden Delicious (*GD*), transgenic lines (*2005 – 2008*). *, $p < 0.05$, Mann-Whitney U-Test (n = 4)

Results

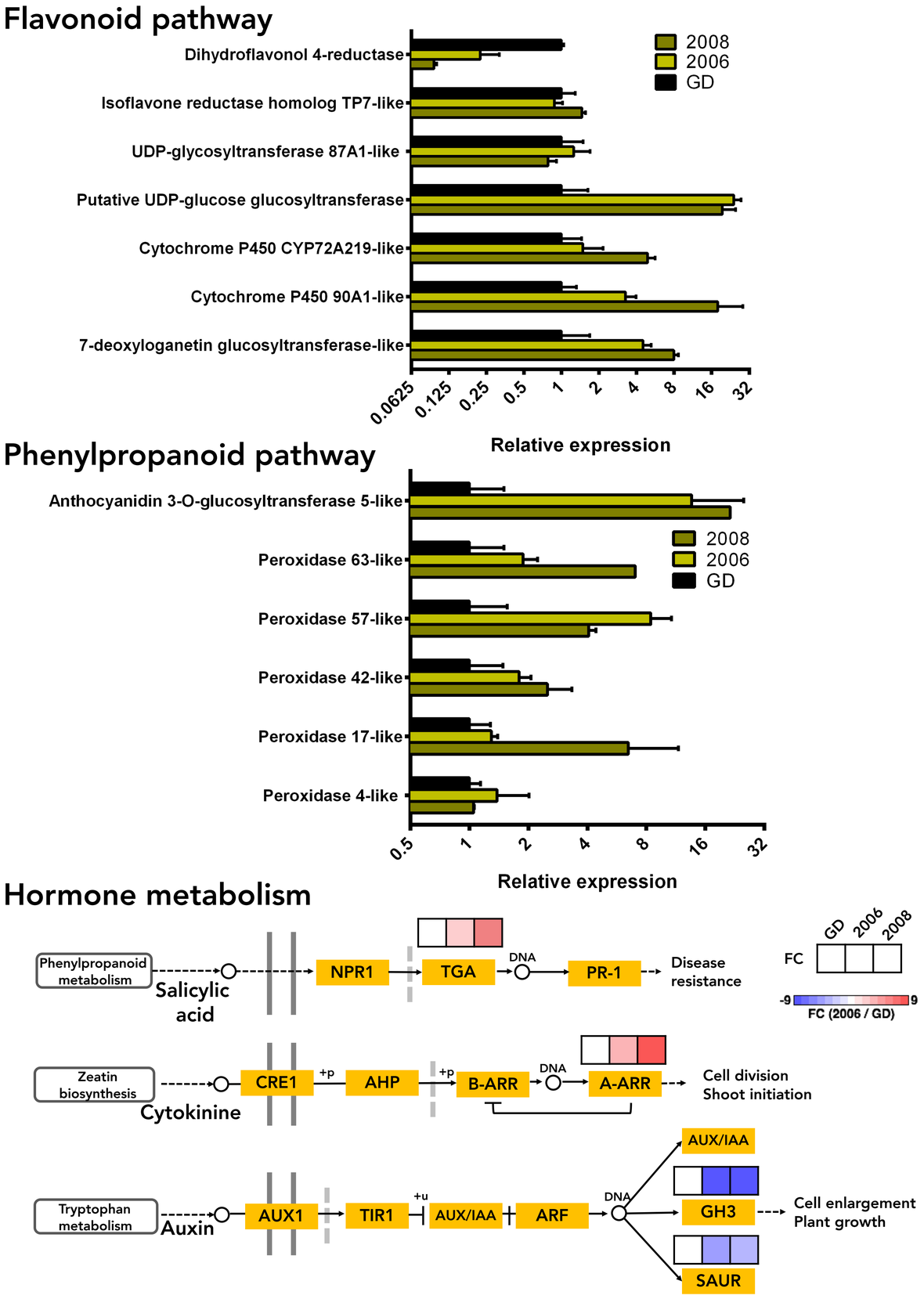


Figure 4. Transcriptomic modulation of flavonoid and phenylpropanoid pathways and hormone metabolism in targeted lines. A RNA-seq profiling was performed in a silenced line and Golden Delicious. Then, candidate differentially expressed genes involved in flavonoid, phenylpropanoid and hormone metabolism were validated by RT-qPCR. Expression levels of genes involved in were normalized to *EF1 alpha* expression. In hormone pathways, boxes represent the fold change (FC) in a color scale, from downregulation (blue) to upregulation (red) in line 2006 compared to GD.

Conclusions

- We obtained edited apple plants and transgenic lines showing a reduction in *MdPGT1* expression.
- These lines exhibited reduced levels of phloridzin and increased levels of phloretin at different extent.
- ABA, JA, JA-Ile and JA-derivatives decrease in knockdown plants, whereas SA is significantly higher.
- RNA-seq analysis and validation by RT-qPCR contributed to elucidate transcriptional modifications in silenced lines.
- Some key regulators in SA, cytokinine and auxin metabolism were transcriptionally modulated, suggesting a correlation between hormonal levels and physiological effects observed in silenced lines.

* Correspondence should be addressed to: Stefan Martens (stefan.martens@fmach.it) and Mickael Malnoy (mickael.malnoy@fmach.it).