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INVESTIGATING LEAF-ENVIRONMENT SURFACE MODULATION IN APPLE LEAVES FOR ENHANCING RESILIENCE TO ENVIRONMENTAL CHALLENGES

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agritech National Research Center for Technology in Agriculture

ABSTRACT

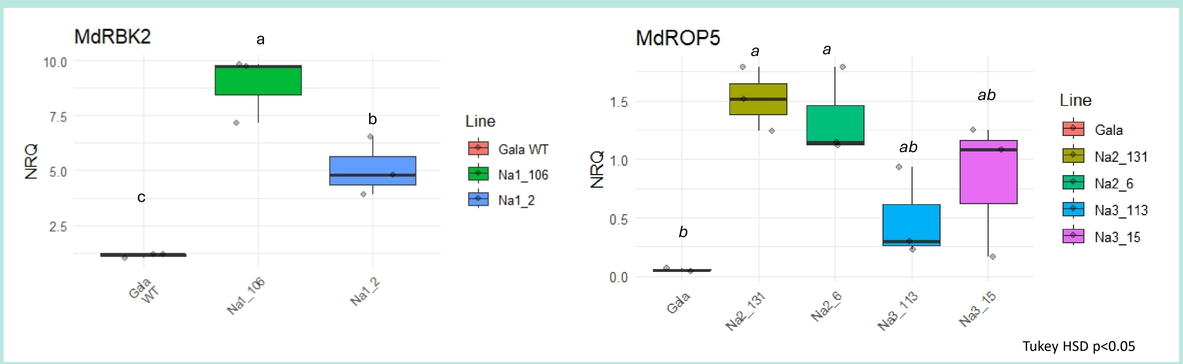
The cuticle, a hydrophobic extracellular layer that covers aerial plant organs, plays a crucial role in plant health and development. It serves as a barrier against pathogens, helps prevent water loss, shields the plant from UV radiation, and supports cellular processes essential for photosynthesis. With the epidermal cells, it forms a specialized tissue that originates from the L1 tissue layer, ensuring effective protection and adaptation to environmental conditions. This research aims to investigate the genetic mechanisms involved in biotic and abiotic stress resistance, by modulating the organization of epidermal cells and influencing the structure and composition of the cuticle. Specific genes, such as ROP and RBK, have been linked to responses to fungal infections in Arabidopsis and bacterial resistance in tomato. In Malus domestica, **MdROP5 is a member of the ROP family, which functions as a molecular regulator of several cellular processes, including stress responses and development**. **ROP proteins** cycle between an **inactive GDP-bound (DN)** and an active **GTP-bound (CA)** state. **MdRBK2, a receptor-like cytosolic kinase, has been identified as an interactor of ROP GTPases in Arabidopsis**. Anatomical evaluations were conducted on **Gala cv. plants overexpressing either the active or inactive forms of MdROP5 and MdRBK2**, revealing their influence on epidermal cell organization. In addition, previous studies have shown **interactions between MdGPAT6 and the *Venturia inaequalis* AvrVf gene in Golden Delicious**, confirmed by BiFC screening. **GPAT6, a membrane-bound enzyme involved in lipid biosynthesis, regulates cutin accumulation and impacts fungal resistance** in *Nicotiana benthamiana* and tomato. A heterologous **subcellular localization study of MdGPAT6** was performed, and **cisgenic Gala overexpressing MdGPAT6** were generated to assess its potential role in enhancing abiotic stress resilience in apples. These findings provide new insights into the molecular pathways regulating epidermal integrity and stress tolerance, with the potential to improve crop protection and stress resilience strategies in apple cultivation.

MdROP5 – MdRBK2

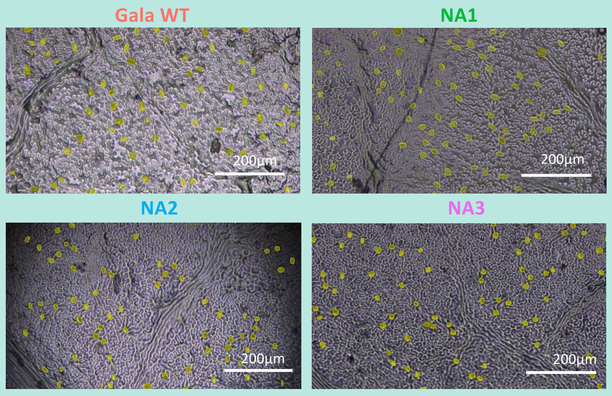
MdROP5 and MdRBK2 overexpressing lines characterization

Na1: OverExpressing MdRBK2

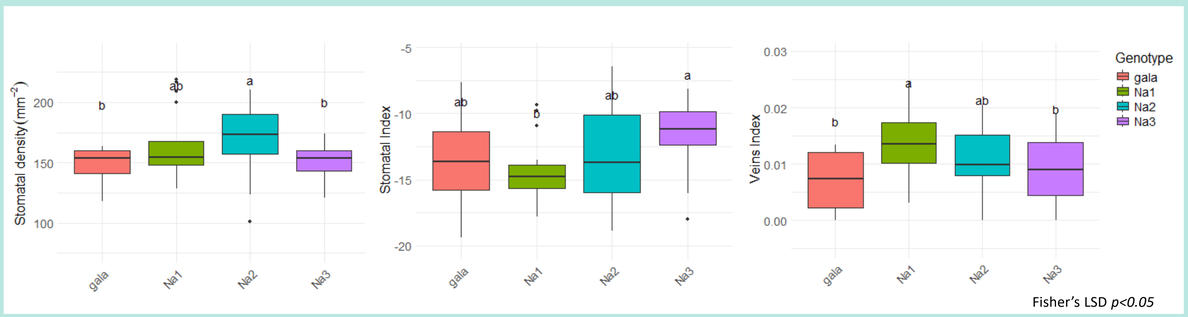
Na2: OverExpressing MdROP5_Active
Na3: OverExpressing MdROP5_Inactive



Stomatal count and epidermal cells phenotyping of the mutant lines

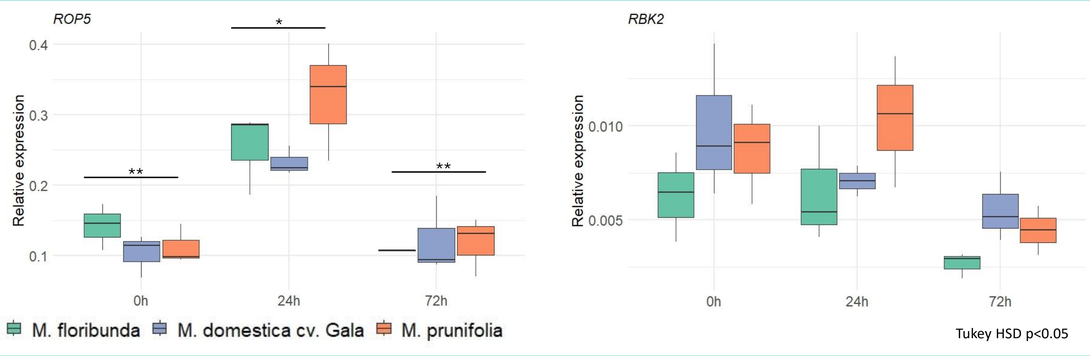


Biodock
End-to-end deep AI for biological images



MdROP5_Inactive lines exhibit a behavior that closely resembles the WT and show significant differences compared to MdRBK2 lines. Despite having a significantly different stomatal density from WT, MdROP5_Active lines display a comparable stomatal index, suggesting a higher total cell number per mm² in MdROP5_Active relative to WT.

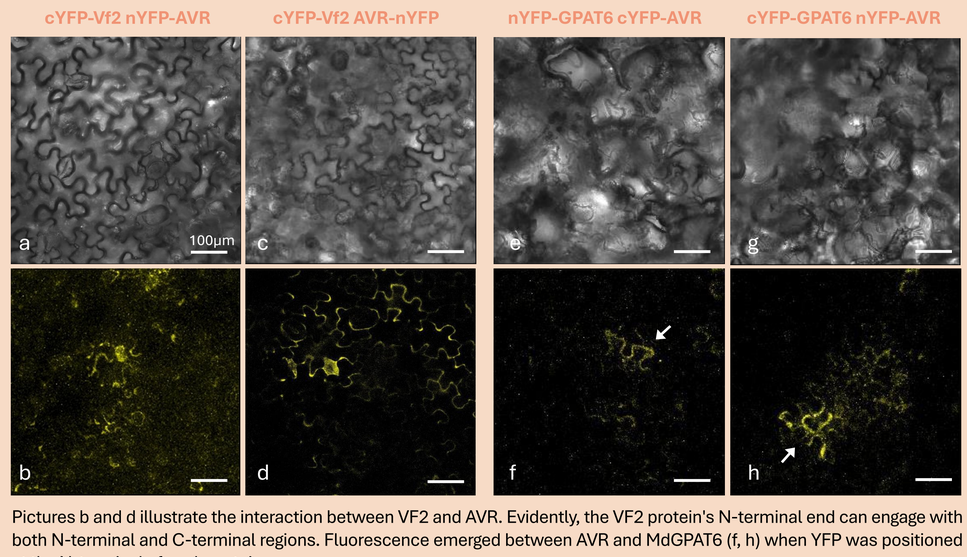
MdROP5 and MdRBK2 modulation under *V. inequalis* infection



MdROP5 shows significant modulation in both Gala cv. and wild Malus species at 24 hours after inoculation with *V. inaequalis*. No significant differences are observed for MdRBK2 expression.

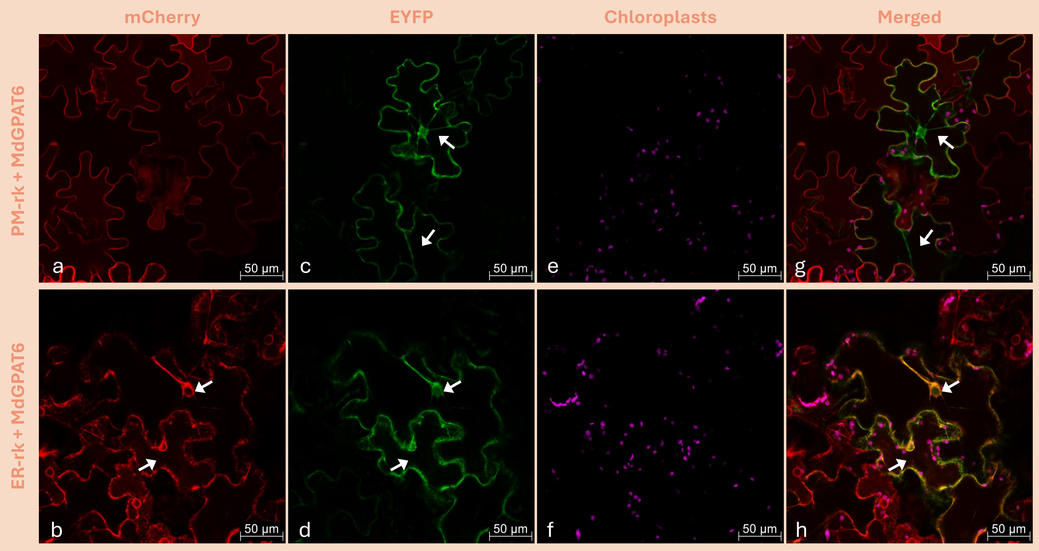
MdGPAT6

AVR and MdGPAT6 show an interaction pattern



Pictures b and d illustrate the interaction between VF2 and AVR. Evidently, the VF2 protein's N-terminal end can engage with both N-terminal and C-terminal regions. Fluorescence emerged between AVR and MdGPAT6 (f, h) when YFP was positioned at the N-terminal of each protein.

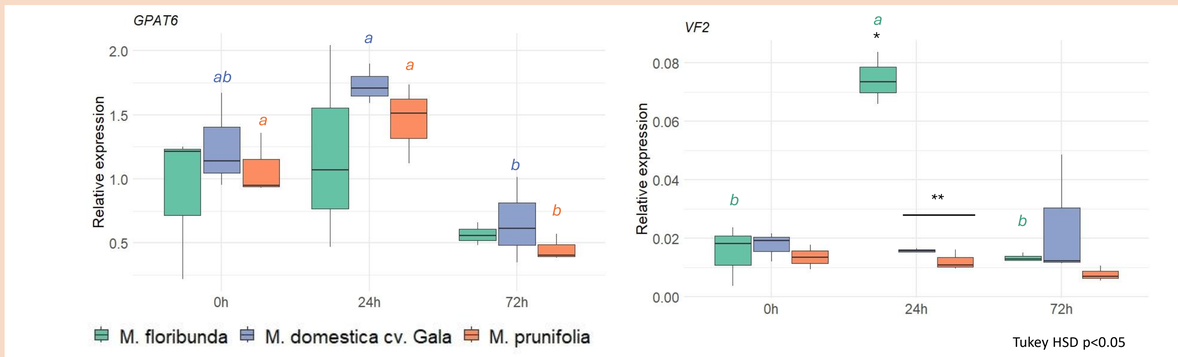
MdGPAT6 localized in the ER



MdGPAT6 subcellular localization was observed by introducing *MdGPAT6*-EYFP into *N. tabacum* epidermal cells with ER and PM markers fused to mCherry. mCherry (a, b), EYFP (c, d), chloroplast visualization (e,f) and merged images (g, h) were used to demonstrate localization patterns. Overlapping fluorescence signals were visible in the ER-rk + MdGPAT6 sample (h).

MdGPAT6 cisgenic lines production

MdGPAT6 and Vf2 modulation under *V. inequalis* infection



MdGPAT6 is significantly modulated in non-resistant genotypes Gala cv. and *M. prunifolia* after inoculation with *V. inaequalis*. VF2 expression peak is reached at 24 hours after inoculation in resistant *Malus floribunda* as expected.

FUTURE PERSPECTIVES

Water stress experiment on GPAT6 cisgenic lines in a phenotyping platform under controlled conditions and RNAseq analysis of sampled plants.
Chemical analysis of the cuticle components through GC-MS.