Two Neighboring CYP450s Orchestrate Quercetin-Mediated Defense and Growth in Tartary Buckwheat

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Introduction

Quercetin, a key flavonoid in Tartary buckwheat (*Fagopyrum tataricum*), plays a dual role in enhancing plant defense and human nutritional health. Through a genome-wide association study (GWAS) of quercetin content, we identified a significant locus containing four tandemly duplicated CYP450 genes. Functional studies revealed that two adjacent homologous genes, '*FtCYP81-a*' and '*FtCYP81-b*', exhibit functional divergence despite their high sequence similarity. '*FtCYP81-a*' is regulated via the jasmonic acid (JA) signaling pathway by the interaction between the *FtMYB2* transcription factor and the virulence protein "RsSpe-Sdh", primarily enhancing quercetin accumulation in roots to improve resistance to *Rhizoctonia solani*. In contrast, '*FtCYP81-b*' is modulated by transcription factor FtNAC2 and specifically influences quercetin levels in seeds, thereby affecting seed size. These findings reveal a novel mechanism by which gene duplication drives functional specialization in secondary metabolism and provide crucial genetic targets for the synergistic improvement of disease resistance and yield in Tartary buckwheat breeding.

Methodology

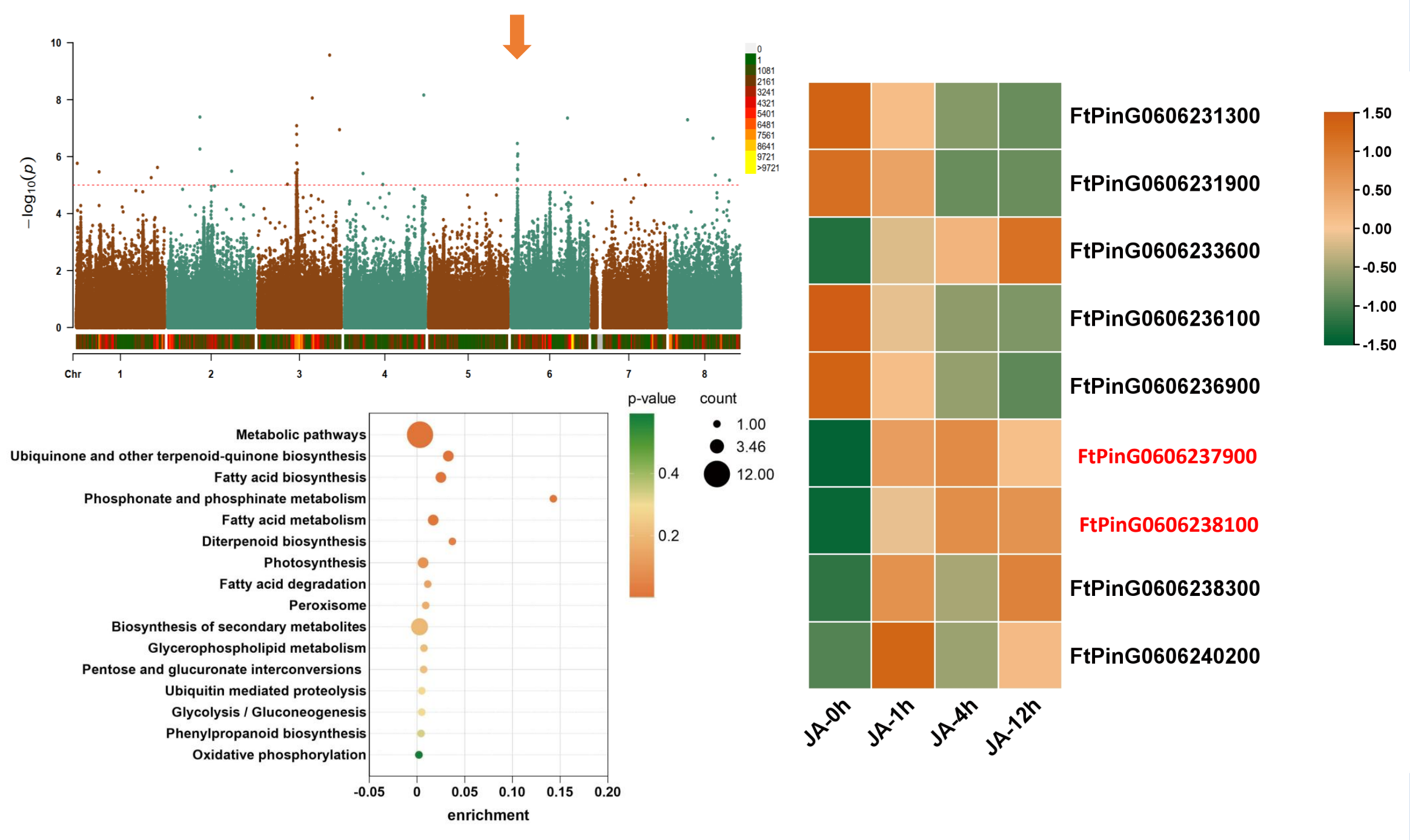
- The identification of candidate genes was achieved by integrating genome-wide association analysis with jasmonic acid transcriptome data.
- Overexpression lines were generated through the use of stable genetic transformation techniques, and their phenotypes were subsequently observed.
- The molecular mechanisms were elucidated through the study of the regulatory pathways.

Reference

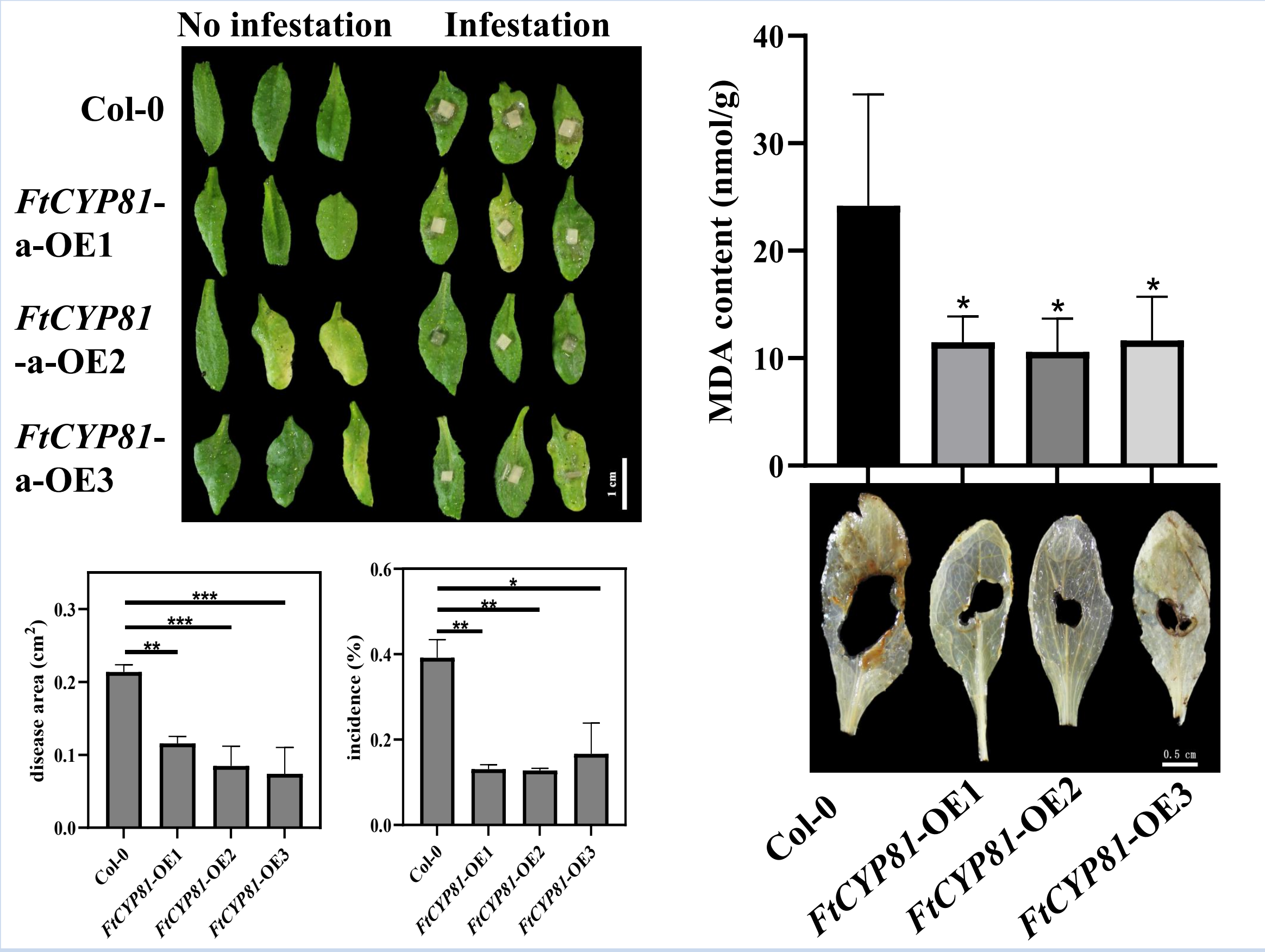
[1] Zhang K, He M, Fan Y, Zhao H, Gao B, Yang K, Li F, Tang Y, Gao Q, Lin T, Quinet M, Janovská D, Meglič V, Kwiatkowski J, Romanova O, Chrungoo N, Suzuki T, Luthar Z, Germ M, Woo SH, Georgiev MI, Zhou M*. Resequencing of global Tartary buckwheat accessions reveals multiple domestication events and key loci associated with agronomic traits. Genome Biology. 2021, 22(1):23

[2] He Y, Zhang K, Li S, Lu X, Zhao H, Guan C, Huang X, Shi Y, Kang Z, Fan Y, Li W, Chen C, Li G, Long O, Chen Y, Hu M, Cheng J, Xu B, Chapman M, Georgiev M, Fernie A, Zhou M*. Multi-omics analysis reveals the molecular mechanisms underlying virulence in Rhizoctonia and Jasmonic acids-mediated resistance in Tartary buckwheat. Plant Cell. 2023, https://doi.org/10.1093/plcell/koad118.

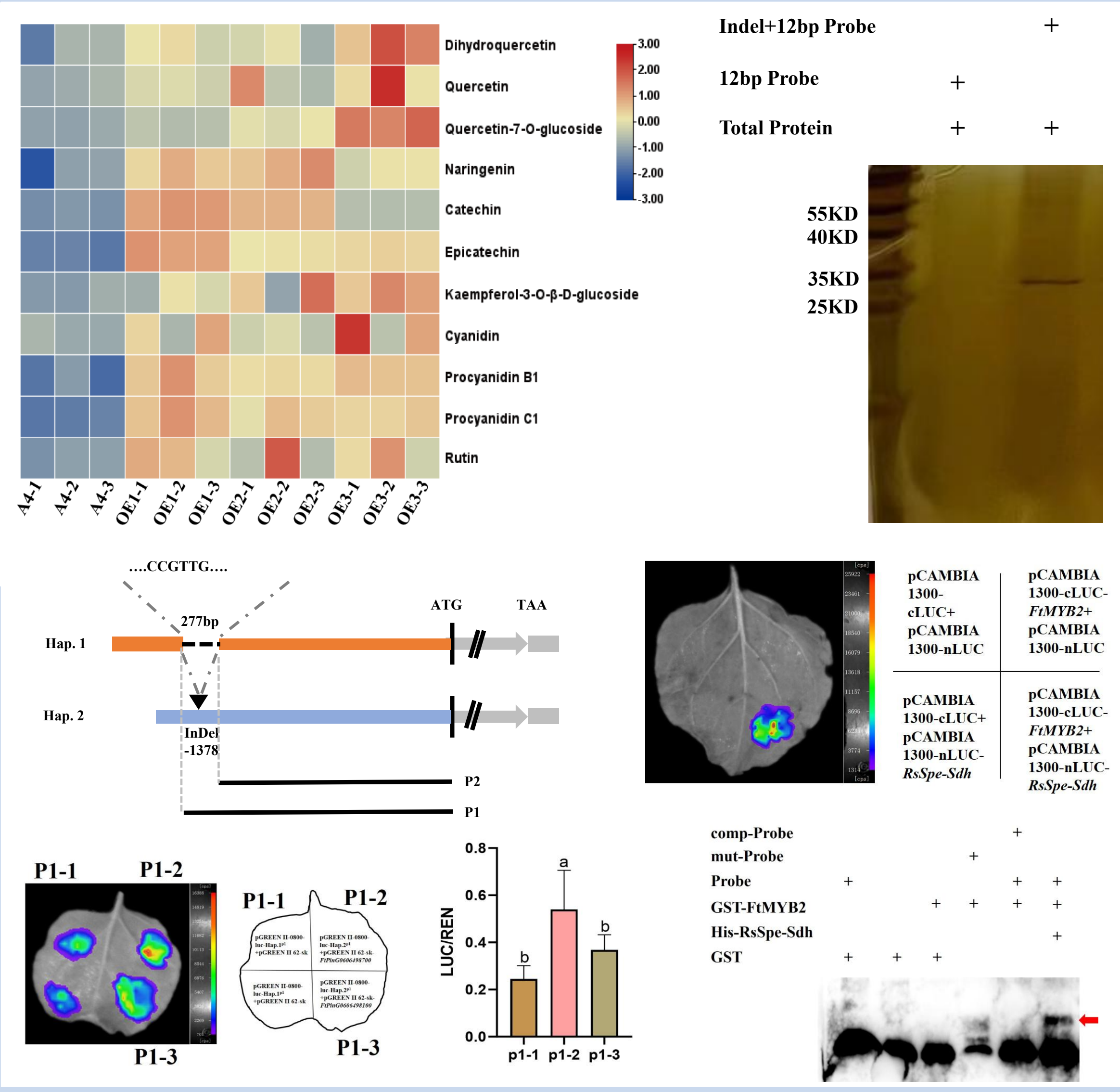
Results



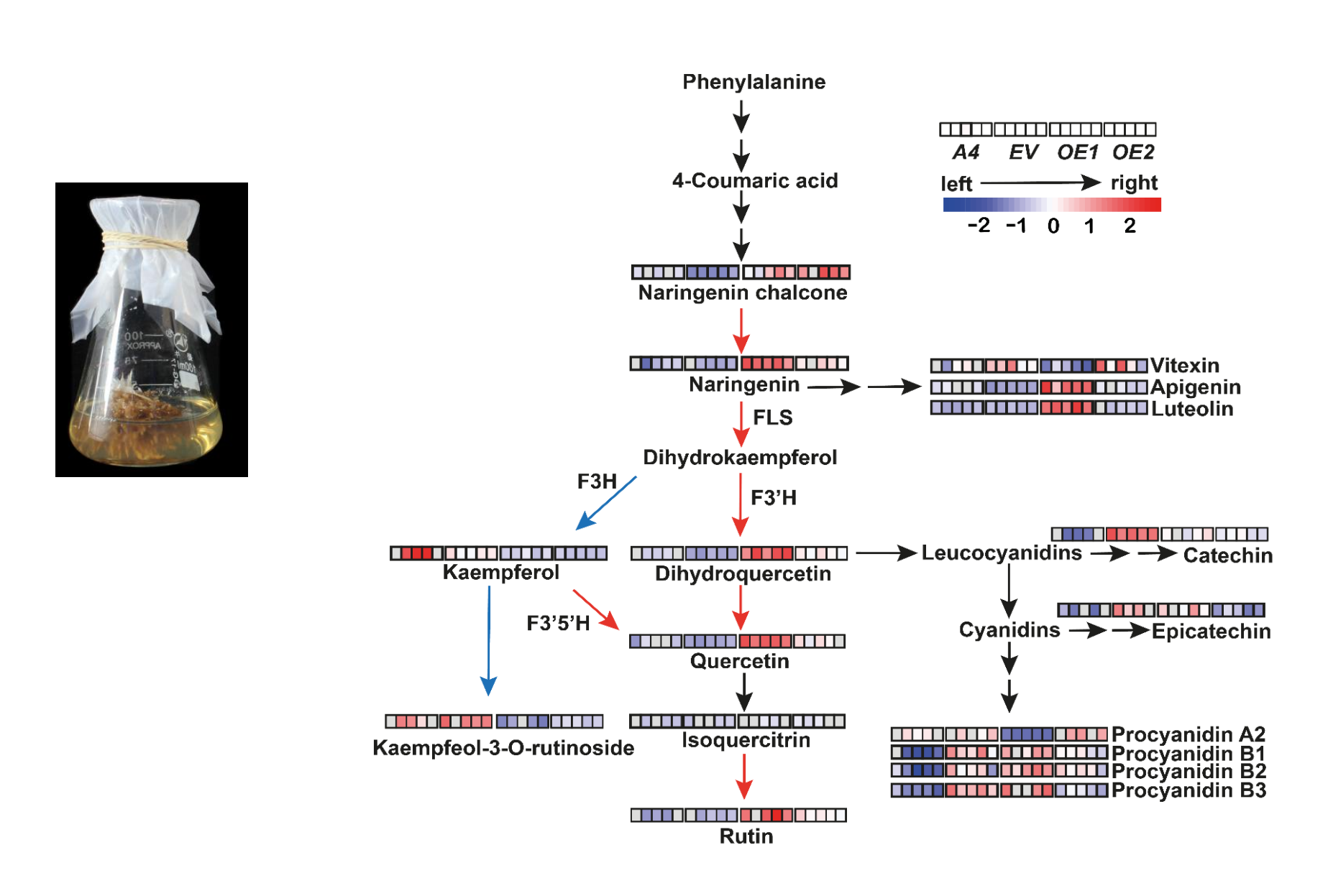
1. Two P450 gene potentially associated with quercetin and significantly induced by jasmonic acid was identified based on GWAS and JA transcriptome.



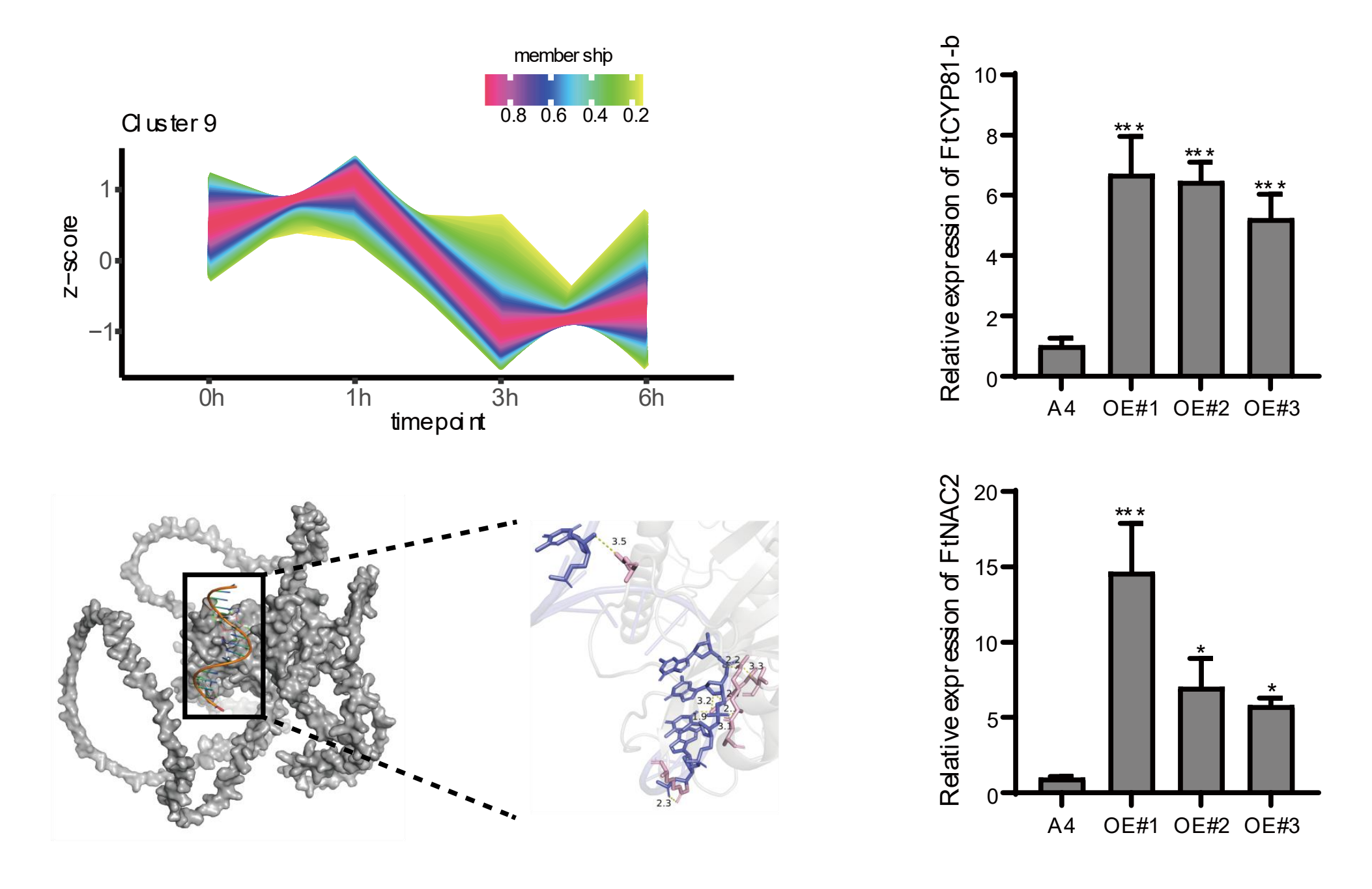
2. Overexpression of *FtCYP81-a* in *Arabidopsis* results in increased resistance against *R. solani* in comparison to the wild type.



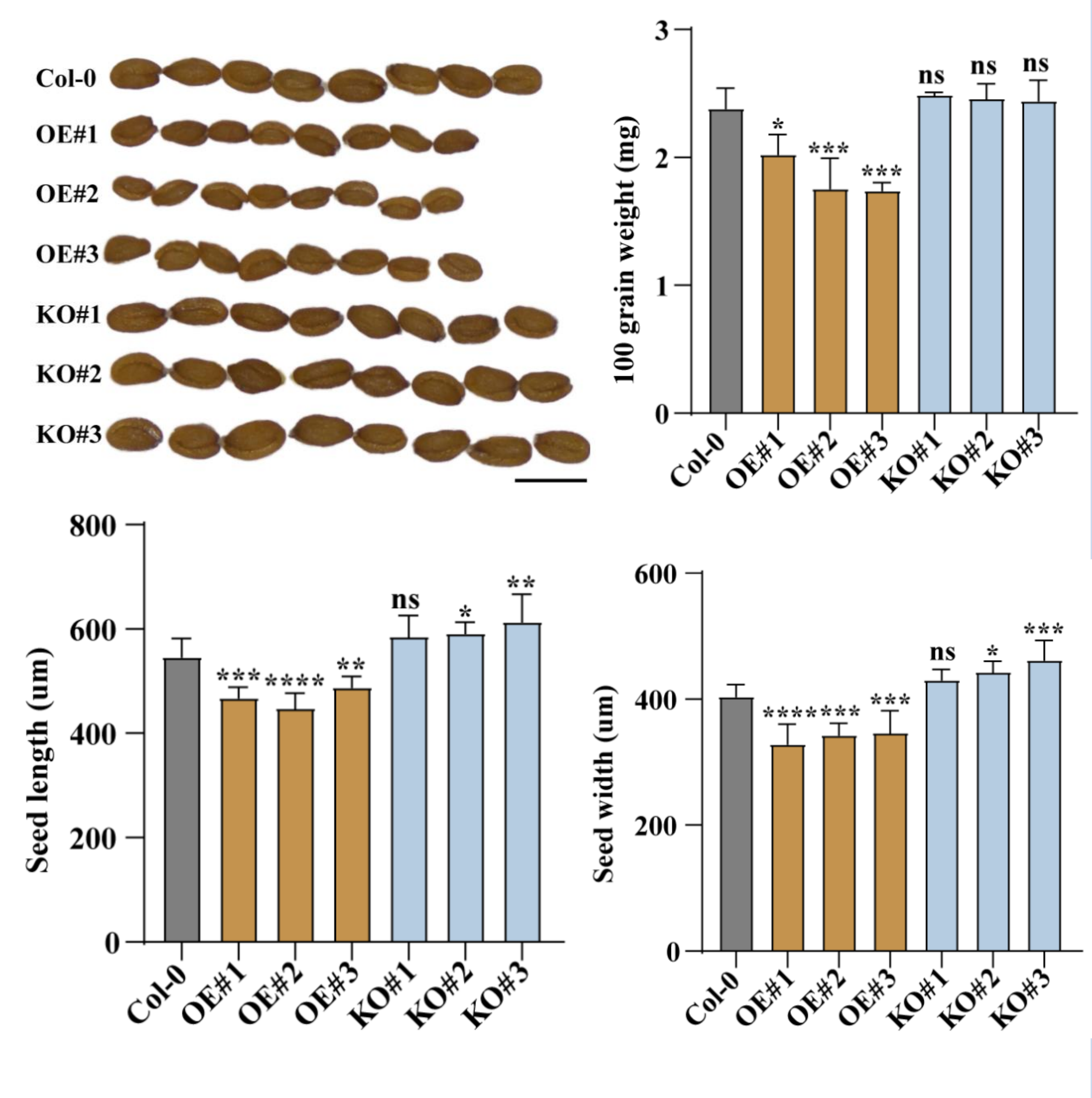
3. *FtCYP81-a* is regulated by the interaction between the FtMYB2 transcription factor and the virulence protein "RsSpe-Sdh", enhancing quercetin accumulation.



4. The overexpression of *FtCYP81-b* in Tartary buckwheat hairy root, the contents of quercetin, rutin, naringenin increase.

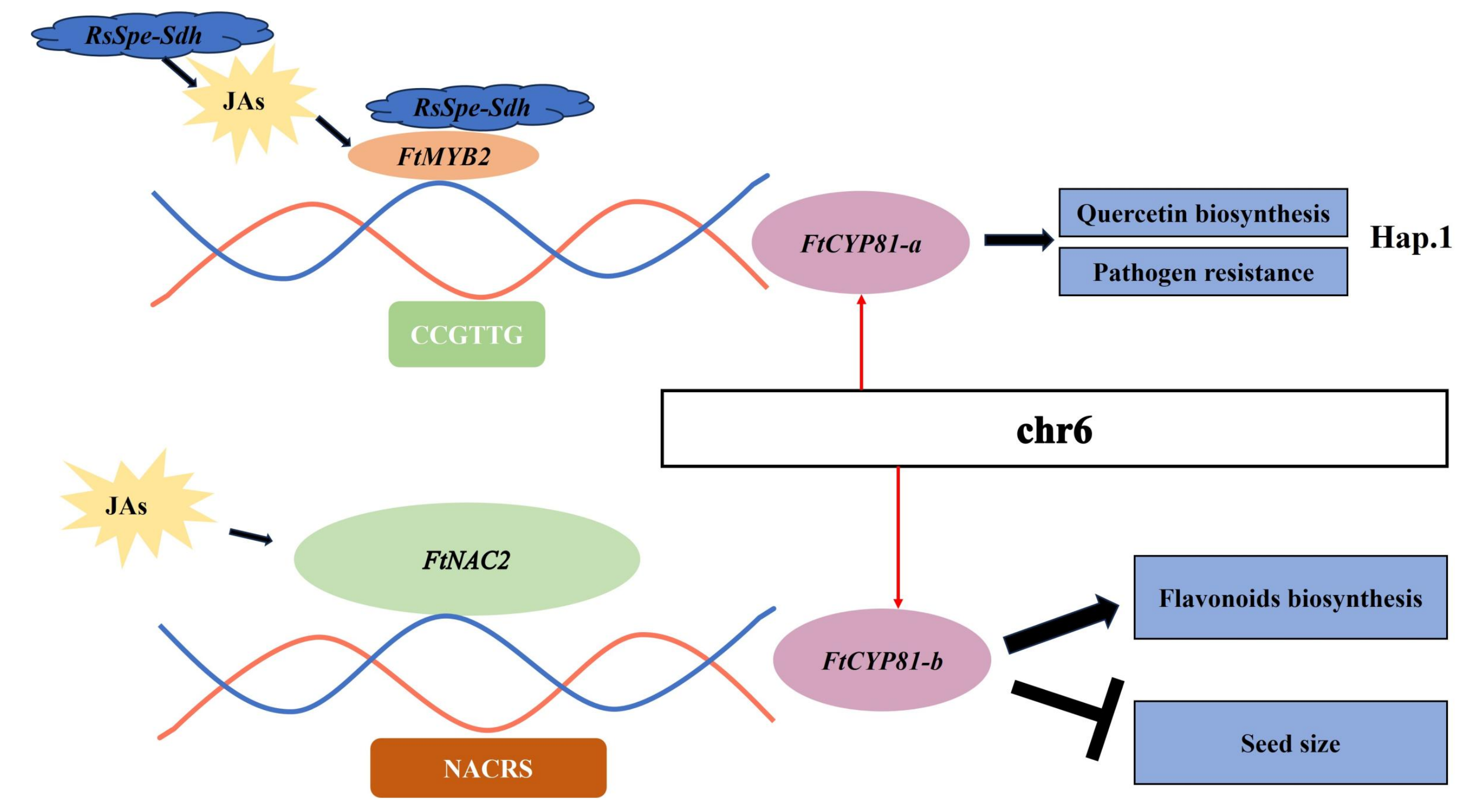


5. *FtCYP81-b* is regulated by *FtNAC2* but not by *FtMYB2*. *FtNAC2* upregulates gene expression by binding to and activating its promoter.



6. *FtCYP81-b* overexpression resulted in shorter, narrower, and smaller seeds in *Arabidopsis thaliana*, while knockout lines showed the opposite effect.

Conclusion



- Upon *Rhizoctonia solani* infection, jasmonic acid signaling is rapidly activated. The fungal effector protein RsSpe-Sdh interacts with the host transcription factor FtMYB2, directing it to the MRS cis-element in the *FtCYP81-a* promoter to activate gene expression. This leads to quercetin accumulation that enhances ROS scavenging capacity and pathogen resistance..
- Simultaneously, FtCYP81-b is independently regulated by *FtNAC2* under JA signaling. While its overexpression promotes quercetin biosynthesis, it reduces seed size and grain weight, revealing a trade-off between defense and growth.

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