Variation in promoter of a CYP450 gene contributes to flavonoids and pathogen resistence in Tartary buckwheat

Wang J, Zhang K, He Y, ZHAO H, SHI Y, MURIEL Q, ZHOU M, PURCARO G, FAUCONNIER M.

LIÈGE université Gembloux Agro-Bio Tech

中国农业科学院 CHINESE ACADEMY OF AGRICULTURAL SCIENCES

Introduction

Buckwheat is rich in flavonoid compounds, which not only confer pharmacological value to humans but also modulate the plant ability to adapt to environmental stresses. This study identified a cytochrome P450 gene (*FtCYP81*) associated with quercetin through a genome-wide association analysis[1], which exhibited higher levels of quercetin in hairy root overexpression lines. The gene was significantly induced in the jasmonic acid transcriptome[2], and the results revealed that the OE-*FtCYP81* line in Arabidopsis exhibited enhanced resistance to *Rhizoctonia. solani*. Further analysis of the promoter sequence showed that a 277-bp deletion in the Hap.2 affected its expression levels, leading to variations in flavonoid content and pathogen resistance among different buckwheat cultivars. Indeed, the upstream region contains a MYB binding element that can directly interact with *FtMYB2*, thereby activating the expression of *FtCYP81* and increasing flavonoid content while enhancing disease resistance. In summary, our study provides insights into the potential relationship between flavonoids and disease resistance, offering promising candidates for the development of new buckwheat varieties with superior agronomic traits.

Methodology

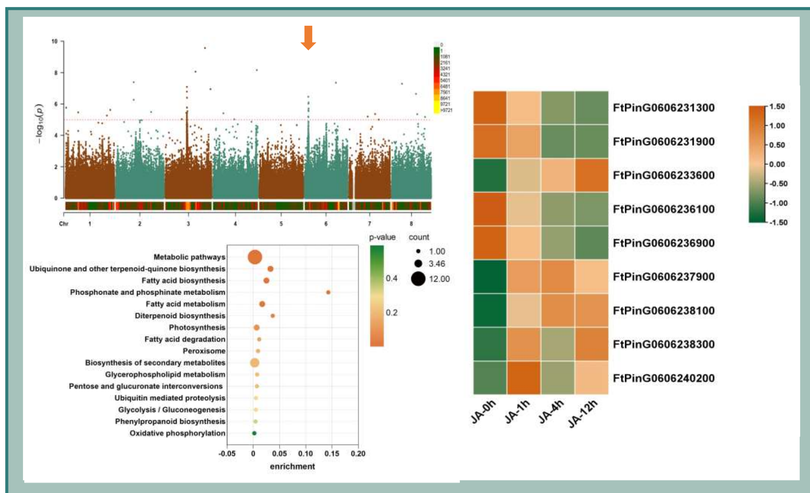
- The identification of candidate genes was achieved by integrating genome-wide association analysis with jasmonic acid transcriptome data.
- Overexpression lines were generated through the use of stable genetic transformation techniques, and their phenotypes were subsequently observed.
- The molecular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

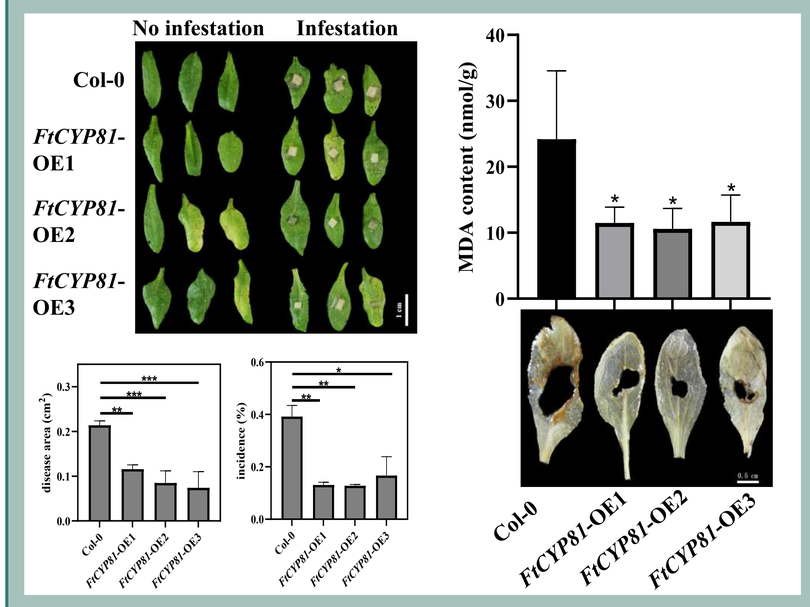
[2] He Y, Zhang K, Li S, Lu X, Zhao H, Guan C, Huang X, Shi Y, Kang Z, Fan Y, Li W, Chen C, Li G, Long O, Chen Y, Hu M, Cheng J, Xu B, Chapman M, Georgiev M, Fernie A, Zhou M*. Multi-omics analysis reveals the molecular mechanisms underlying virulence in Rhizoctonia and Jasmonic acids-mediated resistance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

Results

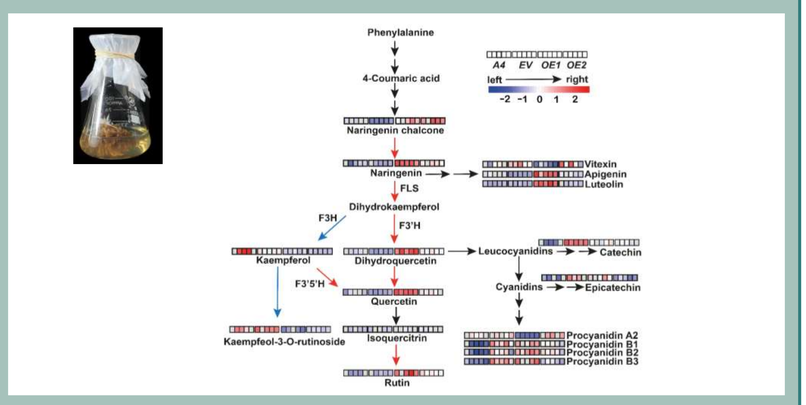


1. A P450 gene potentially associated with quercetin and significantly induced by jasmonic acid was identified based on GWAS and JA transcriptome.

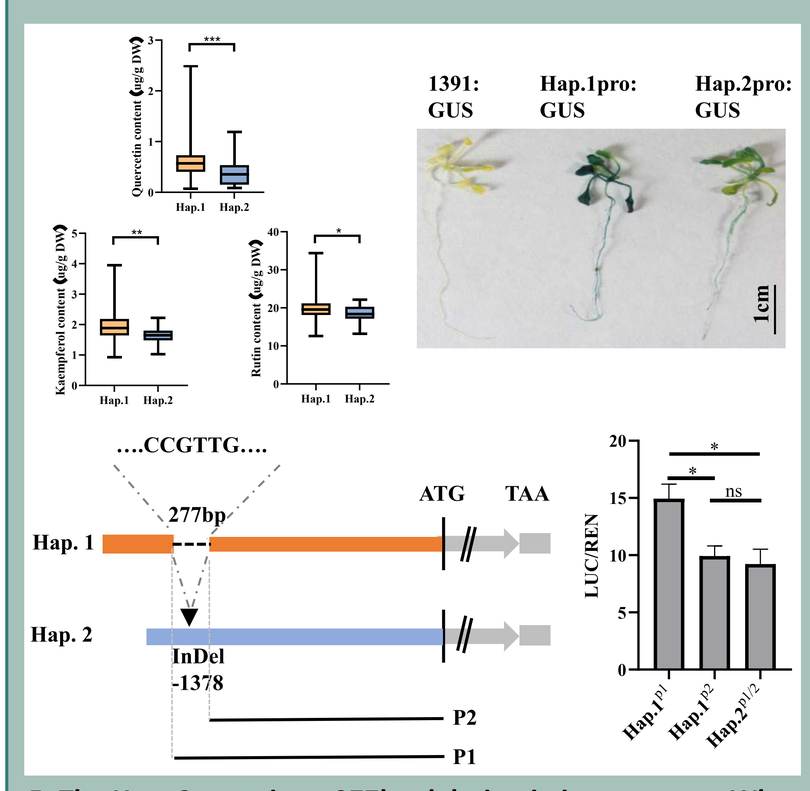
2. This gene belongs to the CYP81 family and is capable of rapidly responding to *R.solani* infection. The content of quercetin and gene relative expression in Hap. 1 are both higher than those in Hap. 2.



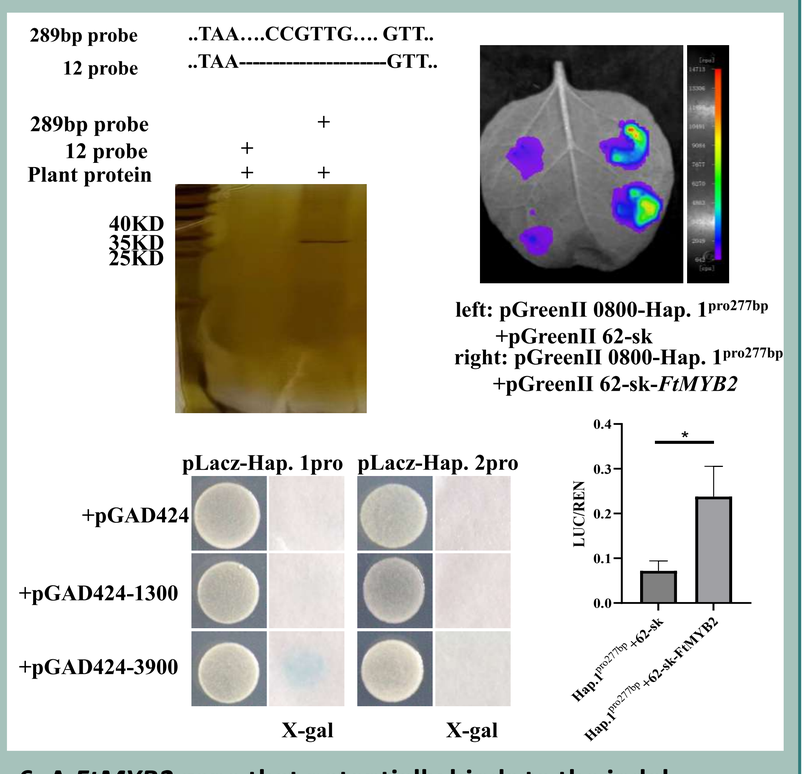
3. Overexpression of *FtCYP81* in Arabidopsis results in increased resistance against *R. solani* in comparison to the wild type.



4. In overexpressed hairy root of Tartary buckwheat, the contents of quercetin, rutin, naringenin increase.

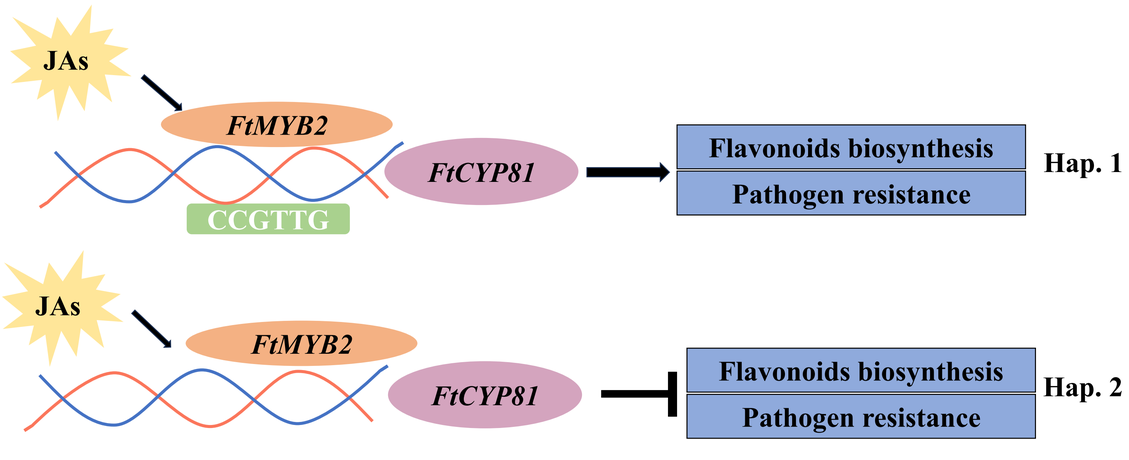


5. The Hap. 2 contains a 277bp deletion in its promoter. When this indel segment is deleted from the Hap. 1 promoter, the promoter activity no longer differs between Hap. 1 and Hap. 2.



6. A *FtMYB2* gene that potentially binds to the indel fragment was identified using DNA pull-down combined with yeast one-hybrid. Transactivation assays indicate that this gene can activate the expression of the *FtCYP81* promoter.

Conclusion



- In the Hap. 1 haplotype, the *FtMYB2* gene responds to JA signaling, binds to the *FtCYP81* promoter, and activates gene expression. The expression of *FtCYP81* promotes the synthesis of flavonoids such as quercetin and enhances resistance against pathogens.
- however, in the Hap. 2 haplotype, the deletion of a 277bp indel within the promoter prevents the positive activation of the *FtCYP81* promoter.

国家留学基金管理委员会 CHINA SCHOLARSHIP COUNCIL