The 8th International *Horticulture Research* Conference
July, 2021

Comparative transcriptomic analysis provides new insights into graft compatibility/incompatibility in *Citrus*

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ABSTRACT

Grafting is a useful cultivation technology to resist abiotic and biotic stresses and is an integral part of citrus production. However, some widespread utilized rootstocks may still cause graft incompatibility in the orchard. *Citrus grandis* (L.) Osbeck cv. Hongmian miyou is mutated from 'Guanxi miyou', but these two scions showed different compatible with available *Poncirus trifoliata* rootstock. In the present study, the morphological and cytological development before and during foliage etiolation was first investigated, and the crucial developmental stages were defined. Afterward, with combined physiological and biochemical data and transcriptome profiling, two rate-limiting genes, *NCED3* (9-cis-epoxycarotenoid dioxygenases 3) and *NCED5*, responsible for abscisic acid accumulation were highlighted. Later, correlation analysis between co-expression modules and traits indicated that abscisic acid is the most likely inducer of the expression of stresses-related genes. In addition, the excessive starch accumulation was observed in leaf lamina and midribs of graft incompatible plant leaves. Taken together, our work provides a new insight into the role of the carotenoid and abscisic acid biosynthesis genes in regulation and contributing to the graft incompatibility, and will further efforts to define and deploy useful genes to study the mechanisms underlying citrus rootstock- scion interactions.

RESULTS

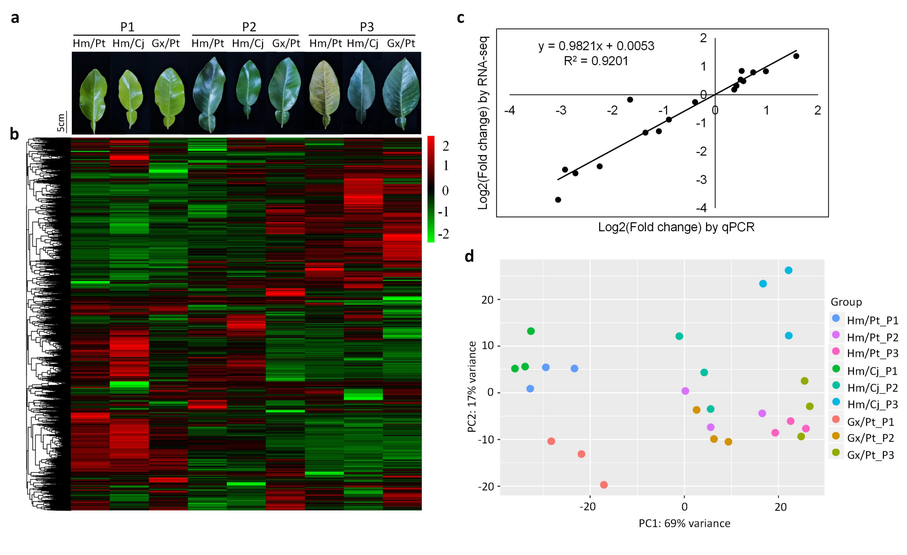

Fig. 1 Transcriptome analysis of leaves. a Leaf samples at three phases (P1, P2 and P3). b Hierarchical clustering of unigene expression. c Correlation of expression changes observed by RNA-seq (Y-axis) and qPCR (X-axis). d Principal component analysis (PCA) of the samples sequenced by RNA-seq. X-axis and Y-axis represent the first and second component. Dots with the same color indicate same sample, different replicates. Red ovals enclose same timepoint.

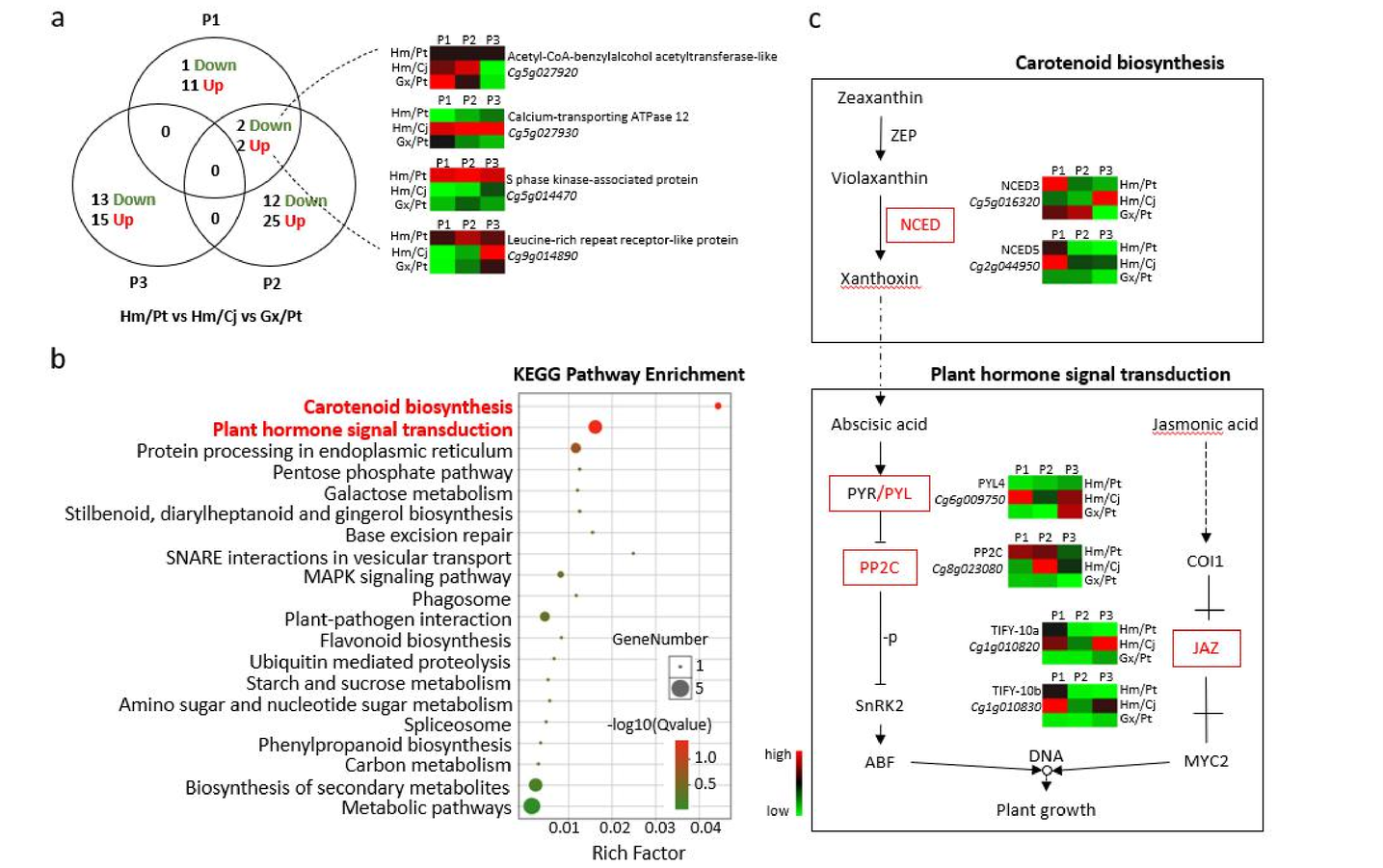

Fig. 2 Genes differentially expressed analyses. a Venn diagrams of genes differentially expressed between the Hm/Pt and controls at the same developmental stage. b KEGG enrichment analysis of the DEGs. c expression of DEGs in carotenoid biosynthesis and plant hormone signal transduction pathways. Heatmap color indicates FPKM value.

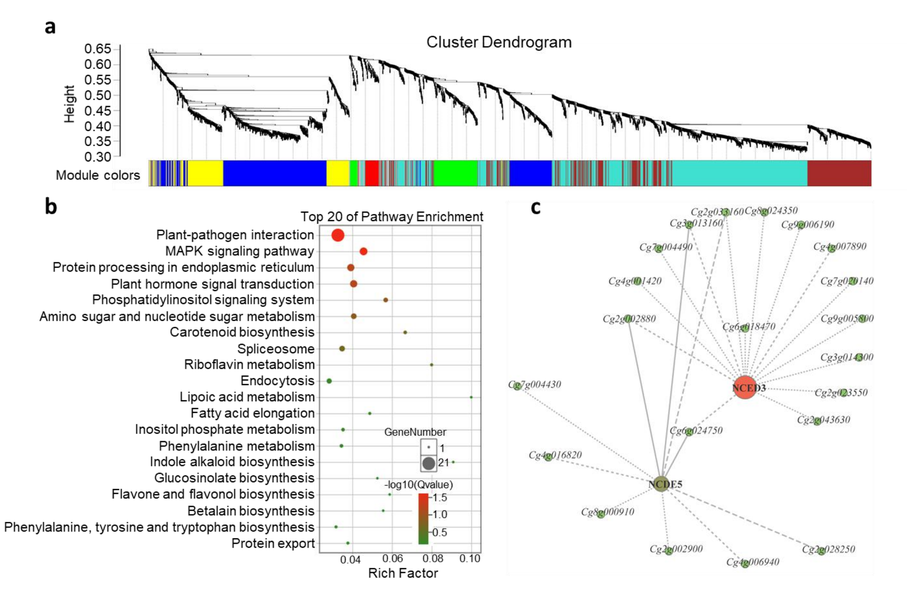

Fig. 3 WGCNA of differentially expressed genes. a Hierarchical cluster tree showing co-expression modules identified by WGCNA. Each leaf in the tree is one gene. The major tree branches constitute 7 modules labelled by different colors. b KEGG enrichment analysis of the genes in the module blue. c genes whose expression is highly correlated in the module blue.

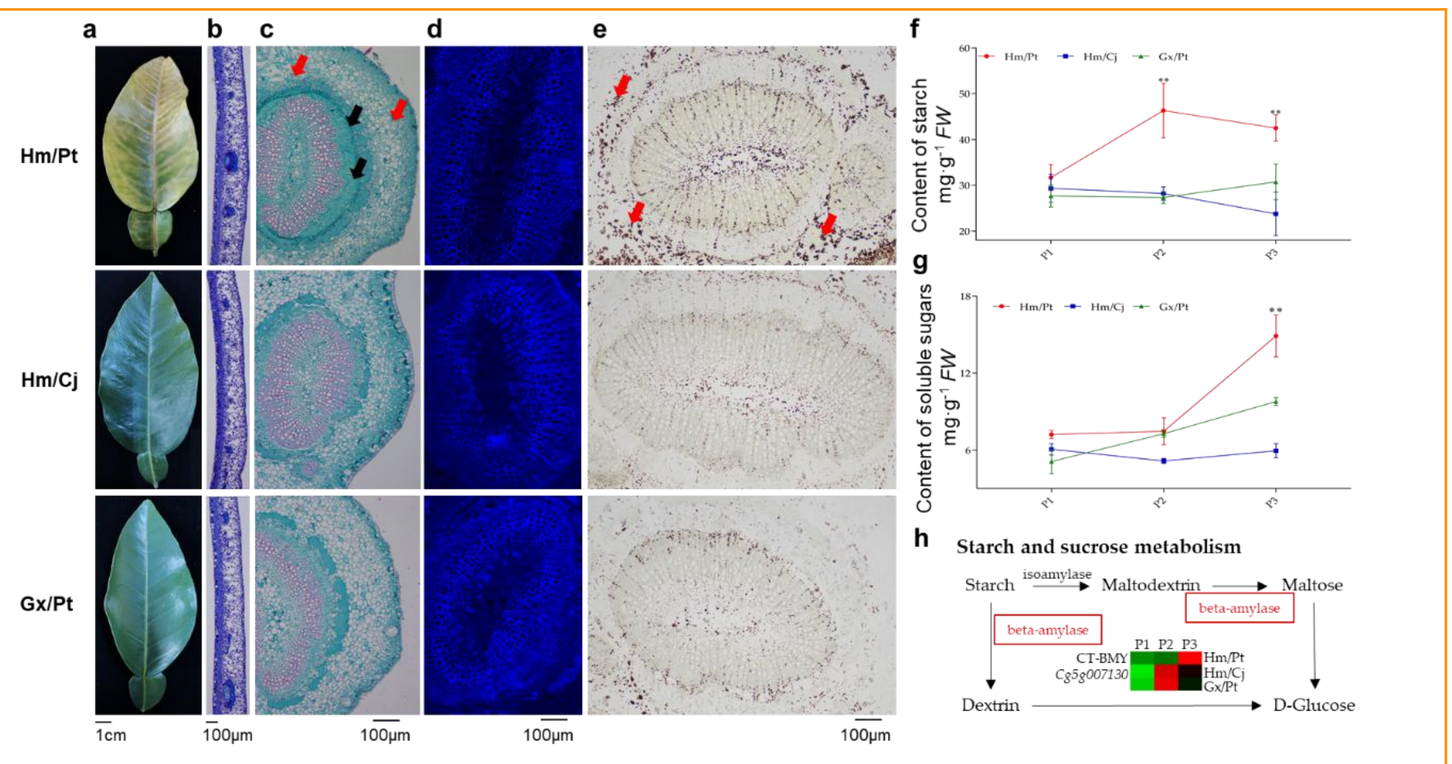

Fig. 4 Transverse section showing changes of leaf of different graft combinations. a Leaf samples at P3. b Cross section of leaf. c Midrib section was observed and photographs taken under light microscope. d Epifluorescence photomicrographs of phloem. e Starch grains were dyed blue. f-g The content of starch and soluble sugars. h Transcript abundance changes of starch and sucrose metabolism pathways. Asterisks represent remarkable differences compared to the control (*$p < 0.05$, **$p < 0.01$), analyzed using Student's t-test. Heatmap shows the log10 (FPKM+0.01) of selected differentially expressed transcripts. The black arrows indicate the parenchyma cells and red arrows indicate starch accumulation.

CONCLUSION

In this study, we compared the transcriptomes of the leaves of graft incompatible/ compatible combinations. The results indicated that a few DEGs may cause graft incompatible happen. The current study revealed the physiological and molecular mechanisms underlying the etiolation process in citrus, and will help elucidate the mechanisms of graft incompatibility in citrus.

ACKNOWLEDGEMENTS

This work was financially supported by Sichuan Science and Technology Program (2019YFH0061, 2020ZHCG0027, 2019NZZJ0015).