



## VARIATION IN METABOLOME AND GENE EXPRESSION OF *Citrus sinensis* x *Citrus limonia* IN RESPONSE TO HUANGLONGBING

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Huanglongbing (HLB), also known as citrus greening disease, is the most destructive disease affecting citrus crops. It is caused by the phloem-restricted bacteria *Candidatus Liberibacter asiaticus* (CLas), *C. Liberibacter africanus*, and *C. Liberibacter americanus*, and is transmitted by the insect vector *Diaphorina citri*. Currently, there are no commercially available rootstocks or scion varieties resistant to the disease, and infected plants cannot be cured. Despite extensive research efforts, the molecular mechanisms underlying CLas infection and colonization in citrus remain poorly understood. In this study, the interaction between citrus and CLas was investigated using LC-HRMS/qTOF in both positive and negative ionization modes. Samples of central and lateral roots from healthy and CLas-infected *C. sinensis* x *C. limonia* plants grown in a greenhouse were subjected to metabolomic analysis. Feature-Based Molecular Networking (FBMN) on the GNPS platform enabled the annotation of metabolites that were significantly altered in response to CLas infection. Significant differences, determined using the paired t-test, were observed in the concentrations of demethylsuberosin, ostenol, 5-methoxyselesin, xanthyletin, selesin, and limonin. These compounds contributed to the classification of samples into four distinct groups in the PCA: central roots, lateral roots, lateral roots of healthy plants and lateral roots of infected plants. In addition, gene expression analysis of candidate key enzymes in the prenylated coumarin biosynthetic pathway DXR, DXS2, PT1, and PT2, was performed by qPCR in *C. sinensis* x *C. limonia* lateral roots. CLas infected plants exhibited downregulation of DXR (2-fold), DXS2 (5.7-fold), and PT1 (3.6-fold), whereas PT2 expression was upregulated 2.6-fold compared to healthy controls. Overall, our findings demonstrate that CLas infection induces metabolic changes and modulates the expression of genes involved in prenylated coumarins biosynthesis. The results indicate that the plant mounts a biochemical response to bacterial infection by suppressing the biosynthesis of linear pyranocoumarins and favoring the production of angular pyranocoumarins, which are prenylated at C-8.

**Keywords:** metabolomics, gene expression, Citrus, Huanglongbing

