Phosphorus starvation linked to symptoms of citrus disease Huanglongbing in new study

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The citrus disease Huanglongbing (HLB), meaning "yellow shoot disease" in Chinese and also called citrus greening in English-speaking countries, is the most destructive disease threatening the citrus industry worldwide. Powerful diagnostic tools and management strategies are desired to control it.

A new study, 'Small RNA profiling reveals phosphorus deficiency as a contributing factor in symptom expression for citrus Huanglongbing disease', published online today (Friday) in the journal *Molecular Plant* profiled small Ribonucleic Acids (sRNAs) from both diseased and healthy plants and found that some of these tiny molecules could potentially be developed into early diagnosis markers for HLB. More importantly, the study demonstrates that the diseased trees suffered from severe phosphorus (P) deficiency and that application of phosphorus solutions to the diseased trees significantly alleviated HLB symptoms and thus improved fruit yield in a three-year field trial in southwest Florida.

HLB is caused by the bacteria of the genus "*Candidatus Liberibacter*". Symptoms of the disease include blotchy mottled leaves, sections of yellow and underdeveloped vegetative growth, premature fruit drop, and in some cases, off-flavoured fruit with aborted seeds. In areas affected by HLB, citrus management costs have increased dramatically in the last few years: in the US alone, annual HLB management-related costs have totalled around $1.2 billion and it has been estimated that HLB management has increased citrus production costs by 40-50% in affected areas. These high costs are largely due to the need for more additional treatments to mitigate the disease's effects, and therefore early, rapid, and robust detective methods are required, especially presymptomatic diagnosis.

The study's author, Dr Hailing Jin of The University of California, Riverside, explains, "sRNAs are important gene expression modulators, some of which regulate plant responses to microbial infections. To study the expression of citrus sRNAs in response to HLB, we grafted 19 greenhouse-grown healthy sweet orange plants with HLB-positive bark or leaf pieces. Both donor and receptor trees tested negative for other graft-transmissible pathogens of citrus. As controls, five plants were mock-inoculated with pathogen-free healthy tissue. Samples were collected at 10- and 14-week post inoculation/grafting for small RNA profiling. Leaves were also collected continuously at later points to ensure that the tissue used for sRNA libraries was from the diseased trees. sRNAs ranging from 18 to 28 nucleotides were isolated, cloned, and sequenced."

This resulted in the discovery of ten new microRNAs (miRNAs), along with 76 conserved miRNAs, and many small interfering miRNAs. Several of these sRNAs were found to have been induced specifically by HLB, which means that they can potentially be developed into early diagnosis markers for the disease. This is important because if trees can be diagnosed with HLB and treated before symptoms start to become apparent, then money spent by the global citrus industry on additional treatments might be saved.

In particular, miR399, which is induced by P starvation in other plant species, was discovered to be induced by HLB infection in the diseased citrus trees. It is known that miR399 is important for P translocation in plants by suppressing a P signalling pathway gene. The study found that the phosphorus level in leave of HLB-positive plants was on about 65% of that in healthy plants, suggesting that HLB is associated with P starvation in the citrus plants, which may in turn contribute to

the expression of HLB symptoms.

Dr Jin writes, "we hypothesized that applying P solutions to the Las-positive trees would reduce HLB symptoms and improve tree performance." Phosphorus solutions were applied to HLB-positive sweet orange trees in a field trial three times per year for more than three years. After two years of treatment, the diseased trees displayed the significantly reduced HLB symptoms.

"Compared with the mock-treated plants, the P-treated trees had a greener appearance and more vigorous growth. Fruit yield increased approximately two-fold compared with the mock-treated plants."

It should be noted that the application of phosphorus solutions did not cure the trees, but this study suggests that additional phosphorus application may help the diseased trees to look healthier and improve fruit yield. This, along with the potential use of miRNAs to diagnose infected trees earlier, could have real, practical significance given the global economic importance of citrus plants and the rising costs of HLB management.

More information: *Molecular Plant*, DOI: 10.1093/mp/sst002

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