

Understanding the NPR1 Gene

By Rick Dantzler, CRDF chief operating officer



Last month, I highlighted the work of Professor Nian Wang's University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) lab in silencing two genes — EDS1 and DMR6 — from Hamlin and Valencia as possible solutions to the citrus disease huanglongbing (HLB). This month, the focus is on the gene NPR1 and the work of Zhonglin Mou, UF/IFAS professor, to overexpress it in Hamlin. Unlike highly experimental trees in which EDS1 and DMR6 are silenced, Hamlin trees overexpressing NPR1 have been in the ground in Florida since 2019. Another 50 trees exposed to hot psyllids in the greenhouse are soon to be planted in the field, and all appear to be extremely tolerant of HLB.

NPR1 is a key protein that helps plants activate their immune system. Scientists first discovered NPR1 in *Arabidopsis* mutant plants that failed to respond to salicylic acid (SA), a natural chemical that causes plants to trigger their defenses against disease.

When NPR1 does not function properly, the plant cannot respond to SA, cannot switch on important defense genes and becomes more vulnerable to infections. NPR1's main job is to act like a "master switch" that turns on many immune-related genes, helping the plant fight off harmful microbes.

HLB is caused by the bacterium *Candidatus Liberibacter asiaticus* (CLAs). HLB attacks the plant's phloem, the tissue that transports sugars and nutrients from the leaves to other parts of the plant, like the roots and fruit.

When citrus trees are infected with CLAs, they mount a very strong immune response. But this response can be too aggressive. The infected phloem accumulates high levels of reactive oxygen species (ROS), which are toxic molecules, and produces too much callose, a sticky, sugar-like substance that clogs the phloem. This overreaction leads to cell damage, phloem collapse and blocked nutrient flow. As a result, the tree shows symptoms such as yellowing leaves, stunted growth, deformed fruit and early fruit drop.

NPR1 helps regulate this immune response. When more NPR1 is present in citrus trees, it prevents the immune system from overreacting by reducing excessive ROS production and limiting callose buildup. This keeps the phloem healthy and functioning, allowing the plant to grow and produce fruit, even when infected with CLAs.

Importantly, NPR1 does not weaken the plant's natural immunity. In fact, overexpressing NPR1 improves tolerance to HLB and strengthens the plant's resistance to other diseases like citrus canker. By fine-tuning the immune system, NPR1 helps citrus trees stay healthy and resilient under disease pressure, according to Mou.

Citrus has its own NPR1 gene, but to overexpress it, the NPR1 gene from *Arabidopsis* is added, making the tree particularly potent at fighting off CLAs. The results are impressive, but it makes the resulting tree genetically modified. Nevertheless, there appear to be no detrimental effects to the tree, and all tests to date support its safety profile.



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