Gene Editing Promises New Disease Resistant Citrus Trees

In the past few decades, two bacterial diseases — Huanglongbing (HLB) and citrus canker— have decimated the Florida citrus industry by killing millions of trees, costing the state billions in lost revenue, and reducing production by 80%. HLB disease has spread to Alabama, California, Georgia, Louisiana, Mississippi, South Carolina, and Texas. There are currently no economical solutions for these industry-threatening diseases. Growers are applying large amounts of pesticide to combat the disease; this is unsustainable and has little to no effect.

"It is of the utmost urgency that a solution to HLB disease be identified. Not only are the livelihoods of all Florida growers at stake, but the disease threatens 50,000 jobs statewide, as well as the continuance of Florida's signature crop," says Bob Behr, CEO of Florida's Natural.

Biotechnology start-up Soil Culture Solutions, LLC (d/b/a Soilcea), in conjunction with the Univ. of Florida (UF), is attempting to solve this problem by developing disease-resistant citrus trees using CRISPR precision breeding. CRISPR precision breeding is a powerful tool for breeding new resistant varieties that the U.S. Dept. of Agriculture (USDA) can classify as non-GMO. This process mimics natural evolution, where edits occur that delete the DNA that causes susceptibility to diseases, allowing the plant to adopt the beneficial change slowly through natural selection.

By targeting susceptibility genes discovered in the UF lab of Nian Wang, Soilcea has developed new citrus varieties that have proven resistance to canker and have shown early resistance to HLB. With funding from the National Science Foundation (NSF), Soilcea developed innovative, proprietary protocols and regeneration methods to create the new citrus varieties. The company tested for HLB resistance by grafting their CRISPR-edited trees onto HLB-diseased trees and found that the disease did not spread to the edited trees.

"In order to deploy our new citrus varieties, we are using protoplast transfection and regeneration to produce non-GMO orange trees with improved resistance genes for commercialization," explains Dusica Coltrane, Lead Protoplast Scientist at Soilcea.

Soilcea has a successful working protocol for protoplast transfection and regeneration. The approach begins with editing the protoplasts (plant cells without cell walls) through polyethylene-glycol-mediated transfection. After receiving plasmids for the desired DNA mutation, the protoplasts undergo a series of media and temperature rotations until microcallus (cell clusters of 0.5–1.0 mm) formation occurs. This is followed by shoot and root regeneration, testing to confirm edits, and finally obtaining the edited citrus plant. Since there is no foreign DNA added into the protoplast, this process is classified as non-GMO by the USDA. This protocol has been repeated and has the capacity for expansion to large-scale production.

Soilcea is in the process of partnering with growers and nurseries to rapidly test and distribute the edited trees. Clay Pederson from Agromillora Florida, part of a world-leading tree nursery, says, "The research Soilcea is doing to create disease-resistant citrus trees is crucial to the survival of the Florida citrus industry, and we are excited to partner with Soilcea to propagate and deliver disease-resistant citrus trees to the growers." Bob Behr from Florida's Natural wrote in a letter of support, "We believe the CRISPR-edited citrus trees that Soilcea is creating with technologies licensed from Dr. Nian Wang's lab at UF could finally be that long-needed viable solution that stems the continual decimation of citrus worldwide."

With their CRISPR precision breeding technology, Soilcea anticipates creating new citrus varieties that offer greater disease-resistance and require fewer pesticides. Yianni Lagos, CEO of Soilcea, says, "We are excited to partner with growers and nurseries to get new canker- and HLB-resistant trees in the field, and to he [illegible] the Florida citrus industry." CEP

▲ (a) Florida citrus production has experienced a $2 billion production decline due to HLB and citrus canker diseases. Source: USDA National Agricultural Statistics Service. (b) Soilcea used CRISPR precision breeding to delete DNA that makes trees susceptible to HLB. To test for HLB-resistance, the edited plants, as well as several control plants, were grafted onto a citrus [illegible]

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