Genetic Breakthroughs

By Rick Dantzler, CRDF chief operating officer

The Citrus Research and Development Foundation (CRDF) was founded in 2009 by Florida citrus growers to find a solution to HLB. Working alongside primarily University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) and U.S. Department of Agriculture-Agricultural Research Service scientists and administrators, nearly every avenue in search of a solution was pursued, but answers proved elusive. As frustration grew, tempers flared as growers saw generational citrus families make the difficult decision to walk away from their groves, and industry infrastructure crumbled.

Then, in 2021, a CRDF board member in some of his last remarks as he was leaving the board because of term limits, said that the only thing he saw that could save the industry was injecting oxytetracycline (OTC) in trees. History will mark that day as historic because it provided — at least for CRDF — the green light to begin working on the process of getting OTC approved by regulators. In record time, approval and commercial acceptance were received. New therapies involving plant growth regulators soon followed, and the beneficial results of individual protective covers were confirmed. The bridge to short and midterm survival was now in place.

However, long-term survival has always meant developing sufficiently tolerant or, better yet, resistant citrus germplasm. Now, after nearly 20 years, non-genetically modified, HLB resistant/tolerant citrus trees may be with us.

Nian Wang and his team at UF/IFAS have successfully knocked out two genes (not in the same plant) — EDS1 and DMR6 — from the genome of Hamlin and Valencia. This is believed to bestow resistance or a high level of tolerance.

EDS1 directly controls 1) callose deposition and sieve element plugging in the phloem and 2) reactive oxygen species (ROS) accumulation caused by the HLB pathogen. These factors compromise phloem functions and lead to HLB disease damage. Knocking out EDS1 reduces callose deposition, sieve element plugging and ROS, thus preventing HLB disease symptoms and keeping trees productive. An added benefit of editing EDS1 is its relationship with the PUB21 gene, a gene believed responsible for susceptibility to HLB infection. EDS1 positively regulates the PUB21 gene, so editing EDS1 negatively affects PUB21.

The DMR6 gene attacks HLB from a different approach. Editing DMR6 reduces or eliminates SA-5 hydroxylase, an enzyme which degrades salicylic acid. By reducing or eliminating SA-5 hydrolase, the level of salicylic acid is increased so much, especially during pathogen infection, that, unlike knocking out EDS1 which reduces salicylic acid, it confers resistance to several pathogens by enhancing the killing effect of the plant's natural defenses, including *C*Las and canker. Knockout of DMR6 has increased disease resistance against bacterial, fungal and oomycete pathogens in many crops already.

To be clear, there are only a few of these plants in existence, and they are still in the greenhouse. Pulling the trees out of juvenility quickly remains an issue, but if they work in the field as expected, we may have found what we've been looking for.