

# State of the CRISPR Tree

By Rick Dantzler, CRDF chief operating officer



**T**he question growers most often ask me is: What is the state of the CRISPR tree? Since a breeding solution is the most likely way to put HLB behind us, it is a good question.

Clustered regularly interspaced short palindromic repeats (CRISPR) is based on a process that bacteria use to destroy other bacteria in which an enzyme — guided by RNA — can cut DNA in a particular area. For plants, this process can knock out a gene by removing one or a few nucleotides, making it nonfunctional. CRISPR mutations insert no foreign sequences into citrus and thus should be considered non-GMO.

The Citrus Research and Development Foundation (CRDF) funded initial work developing the CRISPR technology for citrus, and there are several labs now working on different approaches to creating trees resistant or tolerant to HLB. One lab has already created canker-resistant trees and is in the process of moving these trees into commercial production.

CRDF also funded the work that led to identification of potential “susceptibility” genes that allow *Candidatus Liberibacter asiaticus* to infect citrus that are the CRISPR targets of Soilcea, a biotech company commercially developing HLB-resistant citrus. This is a good example of how public sector research works. CRDF provided funding to the University of Florida Institute of Food and Agricultural Sciences to develop the technology and product, and the private sector acquired what had been developed and commercialized it. CRDF is discussing with Soilcea ways that we can assist in getting its trees into the field.

A problem with CRISPR and traditional citrus breeding is that plants initially have a juvenile phase that can last for years, which is why another CRDF project is the use of the citrus tristeza virus vector to quickly induce maturity. Regardless of the issues remaining with CRISPR, we can't let up. The next step is to get new creations into the field to determine if they will work in a commercial growing environment. There is a chance that a tree possessing enough tolerance or perhaps even resistance has already been developed. However, trees have the genes they do because they need them. So, until we get such trees in the field, we can't tell what will happen when certain genes are silenced.

CRDF is supporting several parallel approaches, too. One is a modification of production of transgenic citrus called “cisgenics.” This is a process where all the sequences inserted into the tree are from citrus or citrus relatives that can be crossed into commercial citrus. The result is a tree that could have been produced by traditional crosses but produced in a shorter time, which may allow it to be considered non-GMO.

CRDF is also funding the production of transgenics that could provide resistance to HLB. Although these trees would be GMOs and require normal U.S. Department of Agriculture and Environmental Protection Agency regulations, until CRISPR or cisgenic trees are proven to be effective against HLB, we should pursue this strategy as a backup. Again, of paramount importance is to get these trees into the field for testing in commercial growing environments.

