By Michael Rogers

Trekking toward HLB-resistant trees

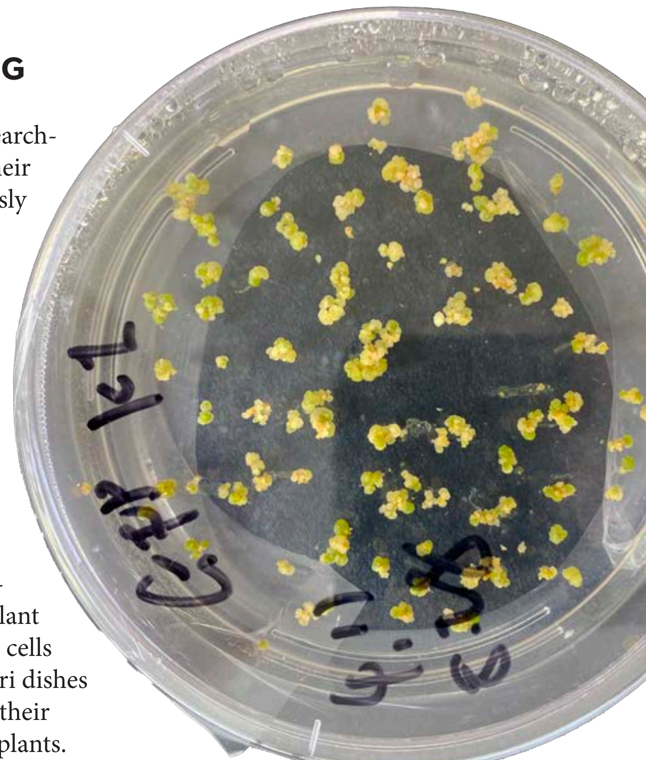
Nian Wang examines gene-edited non-transgenic Hamlin orange plants in the greenhouse.

Much has been discussed recently about the promise and potential of utilizing CRISPR technology to create HLB-resistant trees for Florida's citrus growers. The primary objective in citrus gene editing is to develop new varieties that are resistant to HLB disease while remaining non-transgenic.

To achieve this goal, researchers make alterations to or eliminate specific genes within the plant responsible for disease symptoms. This results in a distinction from transgenic plants, where genes are added to confer disease resistance. Developing new citrus varieties through non-transgenic gene-editing methods eliminates the additional layer of regulatory and public scrutiny associated with transgenics (GMOs). This is why the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) is placing increased emphasis on gene-editing research programs.

TIME-CONSUMING PROCESS

While UF/IFAS researchers continue to refine their methods for expeditiously and efficiently developing gene-edited plants, the current techniques employed are meticulous and time-consuming, involving multiple stages of work. The gene-editing process begins at the microscopic level, where specific genes are modified within individual plant cells. These edited plant cells are then cultured in petri dishes with media to facilitate their growth into new citrus plants.



Citrus embryos grow in a petri dish six to seven months after gene editing.

The transition from a plant cell, invisible to the naked eye, to a young seedling takes approximately 12 months. Once the plants reach this developmental stage, they undergo PCR analysis to confirm the accuracy of the gene edits. Less than 1% of the developed plants will exhibit the desired edits, while the rest are discarded. The "potential winners" are mass-produced in greenhouses for subsequent field trials. Several years of field evaluations are necessary to determine definitively if researchers have successfully developed a new variety resistant to HLB.

Nian Wang of UF/IFAS has refined the CRISPR editing process to create non-transgenic gene-edited citrus plants. He has successfully generated four lines of non-transgenic trees that have exhibited resistance to canker in greenhouse studies. These plants are currently being moved to replicated field trials to evaluate their resistance against both canker *and* HLB. It will take several years of field testing before their performance against both diseases can be ascertained.

In an effort to expedite the process, budwood has been provided to the Florida Department of Agriculture and Consumer Services Division of Plant Industry (DPI) to "clean up" these lines, which typically takes a couple of years. This will trim the time needed to produce clean lines for growers. If these lines are successful in the field, then DPI will already have them "cleaned up" and ready to go to growers.

ADDITIONAL EFFORTS

Other UF/IFAS faculty members are also employing biotechnology to develop transgenic disease-resistant citrus varieties. One such faculty member, Zhonglin Mou in Gainesville, has multiple lines of transgenic Hamlin orange and Duncan grapefruit that reportedly exhibit strong tolerance to HLB. While this year marks the commencement of fruit data collection from these lines, the process of deregulation for release of these transgenic varieties to growers has already begun. This has not been done before, so the amount of time it will take is unknown.

While UF/IFAS continues its extensive research efforts, it is important to note that these projects will take time to reach completion. In the interim, there are various therapies available to help sustain commercial citrus operations. In addition to new UF/IFAS sweet orange varieties with better HLB tolerance, practices such as employing individual protective covers, trunk injections, application of PGRs like gibberellic acid, and, of course, maintaining vigilance with nutrition and irrigation can support groves.

For more information, please visit citrusresearch.ifas.ufl.edu and consider tuning in to the monthly All In For Citrus podcast.

Michael Rogers is a professor and director of the UF/IFAS Citrus Research and Education Center in Lake Alfred.

More Molecules Under Study

By Rick Dantzler, CRDF chief operating officer

At its September meeting, the Citrus Research and Development Foundation (CRDF) board took numerous actions which were driven by the thought that it is possible that the bacterium (*C*Las) that causes HLB could develop resistance to oxytetracycline (OTC) before the holy grail — a sufficiently resistant or tolerant tree — is available to growers. We believe that will be longer than many believe because the bacterium is isolated in the phloem of the tree. But it makes sense to have alternatives ready because we don't know when or if that will occur.

First, the board agreed to fund the testing of five molecules identified by Kranthi Mandadi of Texas A&M University as having high efficacy in killing *C*Las. Mandadi developed the hairy root assay in which citrus roots are infected with HLB, and potential killing agents are taken up by these roots. This allows the effectiveness of the killing agents to be measured. He has screened thousands of candidates and has identified five which have demonstrated high levels of effectiveness. We believe these molecules can survive the federal regulatory process. They will be tested in the field by Ute Albrecht of the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS).

Second, Ozgur Batuman, UF/IFAS, was funded to inject five antimicrobials — including streptomycin — in rotation with OTC. Why streptomycin? It was discovered in earlier studies that there may be a synergistic effect between OTC and streptomycin, meaning that the reduction in titer was nearly 100%. High kill rates of bacteria are a good way to prevent resistance from developing.

Third, the board put out to bid two scopes of work, each involving novel molecules.

The first is to test an antibacterial and a pesticide brought to us by Henry Yonce and Tom Minter. The antimicrobial is a derivative of rifampicin but is *not* used in human health. The pesticide is thought to work on a wide variety of pathogens. They will be tested as standalone products and in combination with each other. Using an antimicrobial in combination with a pesticide could be the next wave of efficiency in treating numerous pathogens at once.

The second scope of work tests the injection of OTC in a pH neutral solution. If this works, it could be a game-changer because a pH neutral solution may better allow pesticides and other products to be added to it. Residue testing will be key since a lower pH helps keep residue levels below regulatory thresholds, and the last thing we need to do is pursue products that don't work as well in this regard.

The board also invited a full proposal from Yu Wang, UF/IFAS, to test a long list of natural molecules that she has discovered have antibacterial effects.

The last funding decision made at this meeting was to fund the latest iteration of the Citrus Research and Field Trial program so that we can test therapies on a large scale and assist growers at the same time.

CRDF Grower Dollars at work for you

Column sponsored by the Citrus Research and Development Foundation