Figure 1A (left): Rootstock candidate challenged by stick-grafting with HLB-positive Valencia scion. **Figure 1B** (right): 1-year-old gauntlet trees. The middle tree, showing good HLB tolerance, is Valencia/Sugar Belle x S10-15-9, infected with *Candidatus* Liberibacter asiaticus before planting.

Progress in developing improved rootstocks to mitigate HLB

By Jude Grosser, Manjul Dutt and Fred Gmitter

Exploiting citrus genetic diversity is the key to defeating HLB. Plant species have survived for millennia with evolving, hostile pathogens. This is possible through natural selection within genetically diverse populations. Tolerant or resistant individuals survive and intermate, get through the bottleneck, and the species evolves.

Current citriculture is based on extremely limited genetic diversity, approaching monoculture, and this has contributed to the current HLB crisis. Facilitated by biotechnology, citrus breeders are exploiting the broad and unique genetic diversity from elite parents, followed by robust screening. As citriculture utilizes rootstocks, it is possible that a resistant or highly tolerant rootstock could mitigate the disease in any commercial scion. This would be the ultimate solution to HLB!

THE GAUNTLET

University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) citrus breeders developed a high-throughput "gauntlet" approach to screen hundreds of new diverse rootstock candidates annually from carefully designed crosses.

First, seedlings are challenged by high pH, calcareous soil and phytophthora to effectively remove weak individuals. Then, each candidate rootstock is stick-grafted with Valencia cut from HLB-positive field trees (Figure 1A). Those rootstock candidates showing robust growth and good health are planted into a challenging field site at the U.S. Department of Agriculture (USDA) Picos Farm (Fort Pierce, in cooperation with Ed Stover) and grown with minimal psyllid control.

From more than 16,000 hybrids, over 800 have made it to the field. These are evaluated annually, and several promising candidates have been identified (Figure 1B). Superior gauntlet selections are being propagated by rooted cuttings or tissue culture micropropagation (in collaboration with Agromillora Florida Inc.) for advanced trials.

One such trial now underway is the 10-acre DeLuca Preserve trial funded by a UF/IFAS Jumpstart grant. This replicated trial will feature nine new rootstock candidates and UFR-4 as a control. The trial includes five of the best new scions from

the UF/IFAS breeding program, including OLL-20 sweet orange, UF-1859 sweet orange-like hybrid, N11-7 dark red grapefruit, UF-914 red grapefruit hybrid and 18A-9-39 Gator Bites tangerine.

Selection will focus on rootstock candidates with the best ability to confer HLB tolerance to infected scions, and precocious bearing of high-quality fruit, to address the young tree production and fruit-quality problems in the industry. Several candidates have shown no root *Candidatus* Liberibacter asiaticus (*C*Las) for two consecutive years, while suppressing *C*Las in the Valencia scion. Patterns are emerging that can help decipher the underlying genetic mechanisms of tolerance. Continued efforts will generate horticulturally sound rootstocks with HLB tolerance exceeding what is available in the current commercial portfolio.

XXX-639 MUTANT

Following a rootstock/controlled-release fertilizer nutrition experiment in the greenhouse, funded by the Citrus Research and Development Foundation (CRDF), UF/IFAS researchers had HLB-positive Valencia on multiple rootstocks. Many of the trees looked reasonably healthy. They were planted in fall 2019 in a commercial grove, under a Division of Plant Industry permit. All trees had high *C*Las titers when planted (Ct values ~24).

One set of trees was on X-639 rootstock, a hybrid of Cleopatra and trifoliate orange. Two years later, most trees were still alive but growing slowly, and a few had died. However, one X-639 tree was growing vigorously, approximately twice as big as any others, bearing nearly 50 fruit (Figure 2).

Researchers wanted to determine if the rootstock was a zygotic hybrid, possibly explaining the different performance. Simple sequence repeat (SSR) marker analysis showed this rootstock matched the standard X-639. However, flow cytometry analysis showed that there was less measurable DNA than in standard X-639, suggesting possible deletion mutations.

The mutant rootstock (referred to as XXX-639) has been recovered, and propagations are underway for advanced trials. The recovered rootstock has also tested completely negative for *C*Las, suggesting an ability to suppress *C*Las replication. Comparing genome sequences of the mutant with the original shows how they differ and can possibly determine the mechanisms of enhanced tolerance.

SUPER-ROOT MUTANTS

Beth Lamb, the tissue culture wizard who manages the citrus micropropagation lab at Phillip Rucks Nursery, has been propagating UFR rootstocks in tissue culture. She has discovered mutant lines with more prolific root systems and slightly different morphology. Mutants identified from four UFR cultivars are moving now into field trials.

Among these, the most interesting is Clone #28

Figure 2A (top): Three 18-month-old Valencia trees, planted with HLB. The middle tree was selected for unusual vigor and productivity. **Figure 2B** (middle): Mutant X-639 tree cut to recover the rootstock. **Figure 2C** (bottom): Mutant X-639 rootstock sprouts recovered

Figure 3A (left): 2.5-year-old tree of new OLL clone on Fast Eddy super-root mutant rootstock candidate. **Figure 3B** (right): 9-year-old tree of Vernia on MG-11 rootstock with no symptomatic fruit and high soluble solids. The rootstock has been recovered for genetic testing and propagation.

from UFR-1, named Fast Eddy (previously known as Fast 28), due to its root vigor and fast growth. UFR-1 has reasonably good HLB tolerance, but early trial performance of Fast Eddy suggests that it may be more tolerant.

Multiple resets with new OLL sweet orange scions are all doing well (Figure 3A), and 2.5-year-old trees made 5.1 pounds solid per box this season. Two-year-old trees with red grapefruit and Murcott scions also are performing well at the Eagle Lake Tri-State trial.

Super-root mutants may be another good source of genetic variation for improved HLB rootstock tolerance.

Fast Eddy also may be a deletion mutant; genome sequence analyses will take place, as with XXX-639. These super-root mutants may be another good source of genetic variation for improved HLB rootstock tolerance, and propagations are underway for advanced replicated trials.

SUPERIOR ZYGOTIC ROOTSTOCK RECOVERY

Commercial rootstocks are generally propagated by nucellar seed, to provide uniform trees in the grove. However, some rootstocks also produce zygotic seedlings that are not true-to-type. Nurseries try to rogue these out before grafting, as most produce inferior trees. However, it is possible that a zygotic seedling could be superior to the mother clone and end up being a better rootstock. Some zygotic liners look like the typical rootstock, escape rogueing and get planted in the field. Thus, these rare escape trees are screened naturally in groves.

When superior trees showing potentially greater HLB tolerance from rootstocks prone to produce zygotic progeny are identified, DNA from roots are isolated and genetic markers are used to determine if the rootstock is true-to-type or zygotic. If zygotic, a rootstock's superior performance could be due to better genetics. The MG-11 (diploid pummelo x mandarin hybrid) rootstock selection shown in Figure 3B has been recovered; genetic testing is underway to determine if it is zygotic. If so, it will be propagated for advanced trials.

This is being done with older commercial trees still showing exceptional HLB tolerance, and some zygotics derived from Swingle and other commercial rootstocks have been identified for further study. This approach is more promising now that more time has passed, as there are fewer alternative reasons why an older tree might still be doing so well.

CONCLUDING REMARKS

Researchers know they are looking for the needle in the haystack, but accumulating evidence indicates that exploiting genetic diversity in rootstock germplasm can lead to a truly viable answer. Continued selection, propagation and movement of new rootstock hybrids from the approaches outlined above into advanced trials should lead to subsequent identification and commercialization of improved and truly HLB tolerant (or even possibly resistant) rootstocks. This will contribute to the revival and long-term sustainable profitability of the citrus industry.🍊

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