Figure 1. HLB-tolerant sour orange + Rangpur somatic hybrid seed tree used as a rootstock breeding parent.

New rootstocks in the citrus breeding pipeline

By Jude Grosser, Fred Gmitter and Kim Bowman

Two citrus breeding programs have identified several rootstocks that can currently be planted with confidence when combined with appropriate scions, including mid- and late-season oranges, and grown with emerging enhanced nutrition programs. These citrus breeding programs are at the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) Citrus Research and Education Center (CREC) and the U.S. Department of Agriculture (USDA) Agricultural Research Service (ARS). New websites with valuable information and data from historic and ongoing field trials are now available at https://citrusresearch.ifas.ufl.edu/rootstock-field-data/ (UF/IFAS) and https://citrusrootstocks.org (USDA/ARS).

Researchers are working hard to develop the next generation of rootstocks that will allow for sustainable and profitable citriculture with any commercial scions, including more HLB-susceptible sweet oranges, grapefruits and Honey Murcotts. The key to success is screening a tremendous amount of genetic diversity and identifying patterns in rootstock hybrids that can transmit their tolerance/resistance through the graft union to mitigate or prevent the disease in any grafted scion. This would be the ultimate solution to the HLB problem worldwide.

Plant species in nature have thrived for millennia in the presence of evolving, hostile pathogens. They have exploited their own inherent genetic diversity, and through the process of natural selection, there are usually at least a few tolerant or resistant individuals that overcome the threat. They intermate and drive the species through the bottleneck, allowing recovery of the

species and continued evolution.

In citrus, this process has been largely interrupted by man, with citriculture now approaching monoculture — leading to the problem that has brought us all together. Facilitated by biotechnology, citrus breeders can artificially reinstate this process by using the broad and unique genetic diversity from elite parents to create new genetic combinations, followed by robust screening for desired characteristics.

RUNNING THE ROOTSTOCK GAUNTLET

UF/IFAS researchers have developed the Gauntlet High-Throughput Screening Program. Since HLB has become endemic in Florida, the UF/IFAS program has screened more than 13,000 rootstock hybrids (diploid and tetraploid) in search of rootstocks that can mitigate or eliminate HLB in grafted commercial scions. Such rootstocks would be the ultimate solution, as they minimize the need for psyllid control and could avoid the use of antibiotics, genetically modified organisms, etc.

Seed from diploid and tetraploid rootstock crosses of superior parents are planted in flats of high pH, calcareous soil (brought to the CREC from the Indian River) and inoculated with both *Phytophthora nicotianae* and *P. palmivora* (with assistance from Diane Bright in the Jim Graham/Evan Johnson lab). Vigorous dark-green rootstock hybrids that can handle the calcareous soil and phytophthora are grafted with *C*Las-positive Valencia budsticks (which generally results in a 99 percent infection rate). *C*Las-positive trees are grown out directly from the infected budwood to produce trees for field evaluation at the USDA Picos Farm in Fort Pierce. This is a quick gauntlet screening method to determine if the hybrid rootstock can mitigate the disease in the Valencia scion. Several promising rootstock hybrids have already been identified, and genetic patterns are emerging.

PATTERNS OBSERVED

The first pattern observed was the good performance of a large number of hybrids that combined the feeder root preservation characteristic of UFR-4 (formerly Orange 19) and its sibling Orange 14 with parents containing a

Figure 2. 1.5-year-old 'gauntlet' tree of Valencia/Sugar Belle x S13-15-16 infected with *CLas* before planting.

high-phloem-regeneration capacity from the lemon group (e.g., Volkameriana, Milam and rough lemon). One of the best hybrids in this group, tetraploid hybrid A+Volk x Orange 19-11-8, produces abundant nucellar seed. It has already been planted in rootstock trials and shows good early performance.

A second pattern observed has been the overall excellent performance of rootstocks produced from parents previously selected for abiotic stress tolerance, especially salinity tolerance. Pummelo x Cleopatra mandarin and Pummelo x Shekwasha mandarin hybrid parents (half pummelo and half mandarin, so they can be considered sour orange-types) have been used in crosses with each other and with other HLB-tolerant rootstocks including US-812 (Sunki mandarin x trifoliate orange) and salt-tolerant X639 (Cleopatra x trifoliate orange).

The good performance of many of these hybrids in the gauntlet screening suggests that there is overlap for the pathways controlling abiotic and biotic stress tolerance. The top-performing rootstock in the gauntlet so far is a hybrid of (pummelo x Shekwasha) x (pummelo x Cleopatra). The asymptomatic original gauntlet tree grew vigorously and cropped early and heavily two consecutive seasons (third and fourth years after planting). Since no seed tree is available, this selection has been entered into tissue-culture micropropagation for further evaluation.

The third pattern observed is the good performance of hybrids made with the somatic hybrid of sour orange + Rangpur lime as a parent. This parent has shown exceptional Sugar Belle-type HLB tolerance (Figure 1, page 8) but is not adequate by itself. This is due to lack of vigor as a rootstock, and it cannot be propagated by seed.

Hybrids made with more vigorous parents such as the somatic hybrid Amblycarpa + Hirado Buntan pummelo are performing very well in the gauntlet. Since sour orange and Rangpur lime have historically been important rootstocks worldwide, such hybrids are expected to inherit good rootstock characteristics regarding soil adaptation and fruit quality.

SUGAR BELLE'S REMARKABLE ROLE

The CREC team is exploring the potential of HLB-tolerant Sugar Belle in rootstock breeding. Sugar Belle cannot be used directly as a rootstock because it is susceptible to phytophthora diseases and does not come true-to-type from seed. Since Sugar Belle expresses its HLB tolerance on any rootstock, why can't this equation be flipped, creating a rootstock that can support any commercial scion in the HLB-endemic world?

Thus, numerous rootstock hybrids have been made crossing Sugar Belle with complementary rootstock parents. These hybrids are being screened for their ability to transmit HLB tolerance to Valencia sweet orange. The early results are remarkable, as several of the new rootstock hybrids grafted with *C*Las-positive Valencia are growing in the field like normal non-infected trees, especially a hybrid of Sugar Belle with a salt-tolerant Hirado Buntan pummelo x Shekwasha mandarin hybrid (Figure 2). One or more of these Sugar Belle rootstock hybrids could play a significant role in the future success of the Florida citrus Industry.

SUPERSOUR HYBRIDS

The USDA citrus rootstock breeding program has implemented many similar strategies to develop and evaluate new hybrid rootstocks. These rootstocks have potential to create healthier and more productive trees, which are better able to overcome HLB disease. Aiming to improve on the outstanding rootstock traits of sour orange, thousands of hybrids have been created that recombine the ancestral species of sour orange, mandarin and pummelo with new germplasm from trifoliate orange and other species that can transmit greater tolerance to citrus tristeza virus (CTV) and other valuable traits. Hundreds of these SuperSour hybrids have

appeared outstanding in initial testing. They have been entered into Phase 1 replicated trials in several locations to evaluate field performance over multiple years under HLB-endemic conditions and with normal field production care.

In a strategy to incorporate the best of existing rootstock germplasm into new material, a series of new SuperSour rootstock hybrids has also been created with US-942, US-812, US-802 and US-897 in their parentage. These hybrids integrate the CTV-resistance and HLB-tolerance from the US rootstocks into SuperSour-type material. Some of these hybrids are looking promising in early field trials.

For the best of those new USDA rootstocks being prepared for release to industry, work is underway to establish seed sources or alternative propagation methods so that expanding commercial use can move swiftly following release. The core of the USDA rootstock development program relies upon replicated field trials over multiple years that compare new rootstocks to commercial standard rootstocks. Progress and results from these trials are posted on the previously mentioned USDA/ARS website, providing an easy way to see how different commercial rootstocks compare in real-world field performance. Also included are updates on the many exciting new rootstocks being evaluated.

COLLABORATION TO ACHIEVE GOALS

Information already available from trials shows that the choice among currently available rootstocks for a planting can result in large differences in tree health and survival, fruit yield and fruit quality. This has a corresponding large effect on profitability from the grove. Results from ongoing trials with newer rootstocks demonstrate that the next crop of rootstocks to be released in two to three years is likely to produce an even better combination of tree health, yield and fruit quality.

Although the programs are housed in different institutions, there is abundant crosstalk and substantial collaboration by the breeders, in service to the industry. This is exemplified by shared HLB Multi-Agency Coordination Group grant projects for numerous advanced field trials already in the ground. In addition, a collaborative National Institute of Food and Agriculture-supported project is aimed at generating new genetic information and tools to make the rootstock breeding process more streamlined and precise.

Researchers have not forgotten about the primary rootstock breeding objectives before HLB became the dominant problem. Much of the germplasm being utilized in breeding efforts also has the genetics needed to produce rootstock hybrids that can handle blight, the diaprepes/phytophthora complex and other stresses and diseases. The goals are aligned with the industry to make Florida citrus a viable and sustainable industry going forward!

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