Breeding citrus rootstocks to mitigate Huanglongbing (HLB, or citrus greening disease)

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Abstract

The often-repeated dogma regarding HLB (Huanglongbing or citrus greening disease) and commercial citrus germplasm is that adequate tolerance or resistance does not exist. However, the UF/CREC citrus improvement broad-based germplasm collection is now undergoing a massive natural HLB screen because of the HLB epidemic currently underway in Florida, and potentially tolerant/resistant scions and rootstocks, some with commercial potential, are being identified. Complex diploid and tetraploid rootstock candidates, not pre-selected for HLB tolerance/resistance, are showing a differential response to HLB in field trials compared with highly susceptible commercial rootstocks, with several showing significantly lower HLB infection rates, and less severe symptoms once infected. Based on this evolving information, we have established a robust rootstock HLB screening program, and we are identifying new rootstock candidates showing potential to protect susceptible scions from this destructive disease. This greenhouse/field screening procedure (referred to as the 'gauntlet') has been underway for the past 3 years, and we will report on promising results obtained so far. Crosses of superior rootstock parents are made at diploid and tetraploid levels. Seed harvested from crosses is planted in bins of calcareous soil (pH 8), inoculated with *Phytophthora nicotianae* and *P. palmivora*. Robust seedlings are selected on the basis of growth rate, health and color, and are then transferred to 4×4 pots in commercial potting soil. The top of each new selected tree goes for seed source tree production, and the remaining liner to the HLB screen. Each hybrid liner is grafted with an HLB-infected budstick of Valencia sweet orange; with the remaining rootstock top removed to force flushing from the HLB-infected sweet orange budstick. Trees are monitored for HLB symptoms, and apparently healthy trees are entered into a 'hot psyllid' house for approximately 8 weeks, followed by field planting at an approved site. The ultimate goal is to develop rootstocks that can be used to establish sustainable, productive groves without the current requirement for efficient psyllid (the HLB vector) control, which is costly and leads to pesticide buildup.

Keywords: disease resistance, fruit quality, somatic hybrid, tetrazyg, tree size control

INTRODUCTION

Huanglongbing (HLB) was first found in Florida in 2005, and since then has become one of the most devastating diseases of citrus trees in the state. More than 90% of the established citrus trees in Florida are thought to be infected. The disease has also been discovered in Texas, and the psyllid vector and a single confirmed positive tree have been found in California. In the last 8 years, citrus acreage and production in Florida have declined significantly, and continued infection will result in severe decimation of the citrus industry. The disease, caused by the phloem-limited bacterium *Candidatus* Liberibacter asiaticus (*C*Las), affects all common commercial cultivars and results in rapid tree decline. Fruit production on infected trees is reduced, with many fruits being small and misshapen, with poor quality. Infected trees exhibit significant pre-harvest fruit drop, up to 50%.

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Significant feeder root loss occurs before major decline symptoms are observed in the foliage of an infected tree. As citrus is Florida's most valuable agricultural commodity, severe reduction in citrus production caused by HLB is already damaging the state's economy, causing the closure of packing houses and juice plants, with a significant loss of jobs, livelihood, and prime agricultural land. In south Texas, where HLB was discovered in 2012, the prognosis is no different from Florida, and so far 20 groves and many residential areas are infected. In one neighborhood, more than 200 infected trees were recently detected. Citrus is also an extremely important and iconic crop in California, and most scientists expect that HLB will eventually begin to spread in California as well. All commercially profitable processing and fresh-fruit cultivars are susceptible.

In apple, rootstock genetics affect scion gene expression related to fire blight resistance, also caused by a gram-negative bacterium (Jensen et al., 2012). Thus, something being produced by the rootstock that affects disease resistance is being translocated to the scion.

This suggests the potential for similar effects in citrus. Rootstocks differentially translocate nutrients, phytohormones (plant growth regulators), micro-RNAs, small proteins (possibly pathogenesis related), and other metabolites to the scion. This could have both direct and indirect, quantitative and quantitative effects on scion gene expression, and possibly *C*Las pathogenesis in citrus, especially with unique complex allotetraploid rootstocks. Thus, the successful development of horticulturally sound rootstocks that can mitigate HLB would provide an ultimate solution, allowing continued production from all current and future commercial scions, regardless of their HLB tolerance. Herein, we report on progress towards achieving this goal.

METHODS, RESULTS AND DISCUSSION

Rootstock improvement is slow and difficult, because an improved rootstock requires a multitude of traits, including: wide adaptability; tolerance of high-pH, calcareous and heavy soils; resistance to *Citrus tristeza virus*- (CTV-) induced quick decline; resistance to citrus blight; high yields of good fruit quality; optimum tree size for new production systems; ability to tolerate mechanical damage inflicted by *Diaprepes* larvae and to resist secondary infections of *Phytophthora nicotianae*, *P. palmivora* and other invading microorganisms, and must be capable of vigorous root growth following *Diaprepes* damage; tolerance of salinity in some areas; nematode resistance; cold-tolerance; and now tolerance/resistance to HLB. The UF/CREC Citrus Improvement Program has had a major effort in rootstock breeding and evaluation for the past 30 years. We have numerous trials (both replicated and observational) planted across the state with more than 30 industry collaborators. The majority of these trials have now been under heavy HLB pressure for the past 7-10 years, and rootstock differences are beginning to emerge. We are observing rootstock differences in rates of infection and disease severity once infected (Table 1) from rootstock hybrids that were not pre-selected for this trait. This suggests that there is indeed genetic diversity among our rootstock germplasm for the ability to mitigate HLB in trees grafted with susceptible commercial scions. This also suggests that, with focused selection for HLB tolerance/resistance, progress should be much faster regarding the degree of tolerance/resistance that can be achieved. Our rootstock breeding efforts can be broken down into the following three categories, and HLB-tolerant/-resistant selections are emerging in all three categories.

Table 1. Rootstock data from 5-year-old trees in the St. Helena trial, Dundee, FL.

Scion	Rootstock	Solids box^{-1} (lb)		Yield (boxes tree^{-1})			Cumulative yield (boxes)	Trees with symptoms as of March 2013	Number of trees in trial	Percentage with HLB as of March 2013 (5 years)
		2012	2013	2011 (35 mo.)	2012 (47 mo.)	2013 (59 mo.)				
Somatic hybrids										
Valquarius	UFR-6 CH+50-7	5.64	5.43	0.50	0.78	1.94	3.22	25	60	42%
Vernia	UFR-6 CH+50-7	5.67	6.01	0.40	0.63	1.41	2.44			
Tetrazyg										
Valquarius	UFR-1 Orange 3	5.50	4.87	NS	0.72	2.23	2.95	15	60	25%
Vernia	UFR-1 Orange 3	5.61	6.28	0.31	0.67	1.33	2.31			
Vernia	UFR-2 Orange 4	5.47	5.93	0.35	0.25	1.38	1.98	22	73	30%
Valquarius	UFR-2 Orange 4	4.57	5.37	NS	0.75	1.73	2.48			
Valquarius	UFR-3 Orange 15	4.84	5.05	NS	0.81	1.97	2.78	6	43	14%
Vernia	UFR-3 Orange 15	5.46	5.82	0.37	0.38	1.82	2.57			
Vernia	UFR-4 Orange 19	5.79	6.07	0.54	0.71	1.73	2.98	30	129	23%
Valquarius	UFR-4 Orange 19	4.65	5.07	NS	0.65	1.59	2.64			
Valquarius	UFR-5 White 4	5.76	5.72	0.33	0.56	1.80	2.69	20	72	28%
Vernia	UFR-5 White 4	5.89	5.34	0.42	0.25	1.93	2.60			
Diploid										
Valquarius	UFR-13 FG 1731	5.83	6.81	NS	0.68	2.20	2.88	1	5	20%
Valquarius	UFR-14 FG 1733	5.12	5.63	NS	0.67	2.77	3.44			
Vernia	Swingle*	5.11	5.79	0.33	0.85	1.08	2.26	14	20	70%
Valquarius	Swingle*	NS	5.61	NS	NS	1.50	1.50			
Vernia	Cleo*	4.79	5.51	NS	0.50	0.83	1.33	6	16	38%
Valquarius	Cleo*	NS	5.21	NS	NS	1.70	1.70			
Vernia	R. Lemon*	3.67	na	NS	0.78	na	0.78	12	18	67%
Valquarius	Volk*	NS	4.12	NS	NS	2.58	2.58	18	20	90%
Vernia	Volk*	3.60	4.73	0.40	1.13	0.83	2.36			
Valquarius	Kuharske*	NS	5.75	NS	NS	2.20	2.20	56	65	86%
Vernia	Kuharske*	4.34	5.83	0.15	0.75	1.08	1.98			

NS, No significant fruit; na, data not available; *, control commercial rootstock.

Sour orange types

A major objective of our rootstock breeding has been to develop a replacement rootstock for sour orange, which is a hybrid of pummelo and mandarin (Grosser et al., 2004). Pummelo × mandarin hybrids are resistant to citrus blight, still a major breeding objective in Florida. Sour orange is widely adapted and produces fruit of excellent quality; but it is susceptible to CTV-induced quick decline disease, causing it to go from being the world's most popular rootstock to now just minimal use. We have been trying to exploit what we know about sour orange genetics in breeding efforts at both the diploid and tetraploid levels. Sour orange can be used directly as a breeding parent in diploid crosses, as a fusion parent in somatic hybridization (Grosser and Gmitter, 2011), and as a partial parent in tetraploid crosses. In such crosses, genetic resources beyond pummelo and mandarin can be included in selected parents. We have also been focusing on resynthesizing pummelo × mandarin hybrids similar to sour orange. This is also being done at both the diploid and tetraploid levels, using pummelo and mandarin parents selected for superior genetics. For example, we have produced hundreds of hybrids combining pummelo with 'Shekwasha', 'Cleopatra' and 'Amblycarpa' mandarins. Several hybrid rootstocks in this category are showing good tolerance/resistance to HLB, including diploids 46x20-04-37 and 46x20-04-48 ('Hirado Buntan' pink pummelo × 'Cleopatra'), and tetraploid Amblycarpa+HBJL-2 pummelo (Figure 1, left). Further evaluation of these and other selections is underway.

Figure 1. Left: grapefruit tree on tetraploid HLB-tolerant rootstock Amblycarpa+HBJL-2B. Right: 'Gauntlet' tree grown from HLB-infected 'Valencia' on tetrazyg rootstock A+VolkxOrange 19-11-31. Both trees adjacent to trees heavily impacted by HLB.

Citranges

Citrange rootstocks are hybrids of sweet orange and trifoliate orange. They represent a widely utilized group of rootstocks worldwide due to their ability to produce high yields of high-quality fruit; these include Carrizo, Kuharske, C-35 and Benton. We have produced many new citranges that are currently under evaluation, and several are showing enhanced tolerance to HLB compared with the commercial rootstocks. Six of these new citranges were selected for tissue culture propagation (underway), as the seed source trees no longer exist.

Tetraploids for tree size control (use in ACPS, advanced citrus production systems)

Tetraploid rootstocks produced from either somatic hybridization or by conventional breeding of tetraploid parents (somatic hybrids or zygotic hybrids), called 'tetrazygs', almost always provide some level of tree size control (Grosser and Gmitter, 2011; Grosser et al., 2011). Thus, they are an excellent source of new rootstocks to facilitate ACPS featuring high-density plantings. The 'UFR' hybrids described in Table 1 are all complex allotetraploids that show improved tolerance to HLB and good promise for ACPS. These hybrids have been released under a 'fast track release' program for large-scale industry evaluation.

The 'Gauntlet': screening rootstock hybrids for HLB

As mentioned above, we have been conducting rootstock breeding at both the diploid and tetraploid levels by standard crossing methods. Approximately 3 years ago, we established the 'Gauntlet' screening method to rapidly identify any new hybrids with the potential to mitigate HLB in grafted commercial scions. Seed from rootstock crosses is planted in calcareous soil inoculated with *P. nicotianae* and *P. palmivora*. Robust seedlings are transferred to citripots. Tops of selected hybrids are used to propagate seed trees via rooted cuttings; thus subsequent seed trees can be planted in the field on their own roots, avoiding grafting to other rootstocks that might compromise their health and survivability. Remaining liners are grafted with 25 cm budsticks of HLB-infected sweet orange, which are forced to flush to produce a new tree top. Use of large budsticks rather than smaller traditional buds minimizes the possibility of escapes. Trees showing few or no symptoms in growth from the infected sweet orange are selected for further challenge in a 'hot' psyllid house until visible psyllid damage is observed. Promising trees are then transferred to a challenging field test site (the current site is the USDA Picos Farm in Fort Pierce, FL; DPI permit issued to move pathogen-infected material). Rootstocks showing the ability to mitigate HLB after 2 years in the field (HLB symptomless trees with good growth; PCR status monitored yearly) are propagated by having clean seed trees entered into tissue-culture-based propagation schemes for large-scale testing (one pathogen-free seed tree of each field-planted gauntlet rootstock selection will be maintained in a certified greenhouse until this determination, to avoid the additional 2-year meristem-tip culture process used by the state of Florida's Parent Tree Program). Significant progress has already been made, as 20 of 150 hybrid rootstocks that have been in the field for more than 1 year look quite promising (Figure 1, right). Hybrids successfully making it through the hot psyllid house and planted in spring of 2014 include 45 complex tetraploids, 15 robust Flying Dragon hybrids (all with potential for use in ACPS), and 10 diploid sour orange-like hybrids.

CONCLUSIONS

Rootstock improvements regarding HLB are likely to come in stages. The first stage consists of rootstocks that reduce the frequency of HLB infection, and reduce the severity of the disease once infected. These will still require efficient psyllid control and optimized production systems. The second stage will hopefully consist of rootstocks that can completely mitigate the disease, regardless of scion. Psyllid control may not be necessary. No horticultural performance data would be available on such selections initially, but such hybrids should have good rootstock pedigree. We have initiated an extensive rootstock HLB screening program in efforts to exploit this possibility. The goal is eventually to develop horticulturally superior rootstocks that will not require psyllid control to overcome HLB, that can also facilitate sustainable ACPS.

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