CITRUS ADVANCED TECHNOLOGY PROGRAM

QUARTERLY & FINAL REPORTS: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

SELECT PERIOD December 2015

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Accelerating Citrus Gene Discovery for HLB Tolerance/Resistance

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
2/15/16	Citrus Research and Development Foundation	Citrus Genomics

REPORT UPDATE (500 words-It is not necessary in this public report to disclose your institution's proprietary information or intellectual property.)

Citrus Huanglongbing (HLB) poses the greatest threat to the survival of the Florida and US citrus industry. Research to incorporate HLB resistance/tolerance into citrus has been recommended by the National Research Council as one of the top priority topics for addressing the HLB threat. A number of Poncirus and Citrus cultivars have been recently found to be tolerant to HLB. Identification, characterization and validation of candidate genes responsible for HLB-resistance are of the remarkable value to develop new HLR-resistant/tolerant citrus varieties. More than 70 different accessions of Citrus species and relatives have been observed in a field planting in Florida (with cooperation of Dr. E. Stover, USDA). Leaf samples were collected from about 450 trees. The titer of CLas in the HLB-infected plants was determined by TaqMan probe-based qPCR, and the HLB symptom severity on the plants was evaluated by experienced researchers. These accessions showed diverse responses (susceptible, tolerant and resistant) against HLB. Many HLB-tolerant citrus relatives were revealed, including Citrus latipes, Poncirus trifoliata, Severinia buxifolia and Microcitrus australis.

Interestingly, S. buxifolia plants did not show severe HLB symptoms yet had relatively low Ct values when assayed for CLas titer. Two general trends observed so far were increased Ct values on the same trees in the summer than in the spring or fall and the inconsistent relationships between stem diameter and shoot growth and HLB severity across a diverse group of Citrus, Poncirus, and distant relatives.

To identify sequence polymorphisms in candidate genes and assess their association with HLB tolerance/resistance, the genomes of 15 citrus accessions have been sequenced to about 25x coverage (9 to 11 Gb/genome) and the genome of one Poncirus trifoliata accession has been sequenced to about 50x coverage (~21 Gb).

We have detected 614 nucleotide-binding site - leucine-rich repeat (NB-LRR) genes with more than 50% coverage in the re-sequenced genomes, and 149 NB-LRR genes with more than 95% coverage in re-sequenced genomes. No NB-LRR genes were lost in the re-sequenced genomes. This superfamiliy of genes represents the largest group of disease resistance genes in plants, conferring resistance to bacterial, fungal, viral and nematode diseases. Most of the NB-LRR genes confer disease resistance in other plants in a race-specific fashion, but some render broad-spectrum and durable quantitative disease resistance. Single nucleotide polymorphisms (SNPs, one of the most common sequence polymorphisms) were found in 330 NB-LRR genes of Poncirus trifoliata DPI-50-7, 98 NB-LRR genes of Poncirus trifoliata Flying Dragon, 156 NB-LRR genes of Poncirus hybrid US-897 and 107 NB-LRR genes of Poncirus US-812. We also detected 72 genes from another two gene families that have more than 90% coverage in the DPI-50-7, Flying Dragon, US-812, and US-897. Real-time PCR was performed with HLB-resistant and HLB-susceptible samples to detect the relative change in the expression of these candidate genes. Results so far showed that several candidate genes were up-regulated with higher expression values in HLB-tolerant Poncirus genotypes when compared with HLB-susceptible citrus genotypes.

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