CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD September 2014

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Screening and Cloning of Resistance Related Genes by RNA-Seq in Huanglongbing (HLB) Resistant and Susceptible Citrus Breeding Lines

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
10/16/14	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.)

The objectives of this project are: 1) to generate transcriptome profiles of both susceptible and resistant citrus responding to HLB infection using RNA-Seq technology; 2) to identify key resistant genes from differentially expressed genes and gene clusters between the HLB-susceptible and HLB-resistant plants via intensive bioinformatics and other experimental verifications; and 3) to create transgenic citrus cultivars with new constructs containing the resistant genes.
A total of 25 samples for RNA-Seq, including resistant/tolerant vs. susceptible plants were sequenced and analyzed.
We mapped the RNA-Seq data to a reference genome, C. clementina using the bioinformatics program STAR. About 85% of the raw reads could be uniquely mapped. The transfrags of each library were assembled with cufflinks and merged with cuffmerg. 24,275 genes of the originally predicted genes had been found to be expressed and a total of 10,539 novel transfrags were identified with cufflinks, which were missing from the original reference genome annotation.
Some of the NBS genes were found to be expressed. For C. clementine and C. sinensis, there were 118,381 and 214,858 mRNAs or ESTs deposited in GenBank and 93 out of 607 and 221 out of 484 NBS related genes match one or more ESTs respectively. The number of ESTs varied from 1 to 25. The expression abundance of each gene was measured by FPKM. The distribution curves of density of FPKM of 6 samples are very similar, indicating that the gene expression is similar and the quality of sequencing is high.
We also performed the principal component (PC) analysis study on the expressions of six samples. The results showed that the gene expressions were significantly different in resistant vs. susceptible citrus. A total of 686 differentially expressed (DE) genes between two groups using FDR threshold of 0.1 were identified. Among them, 247 genes were up-regulated and 439 were down-regulated in tolerant citrus trees. We performed Gene Ontology (GO) enrichment analysis of DE genes. Genes associated with beta-amyrin synthase, cycloartenol synthase and Camelliol C synthase were significantly up-regulated in the HLB tolerant citrus trees while terpene synthase genes (CiClev10014707, Ciclev10017785) were down-regulated in the tolerant citrus trees. Some PR-protein genes were significantly up-regulated in the resistant citrus trees, including several TIR-NBS-LRR genes. Many cell wall degradation-related genes, such as cellulose synthase/transferase, cellulase and expansins were up-regulated in the susceptible citrus trees. Some glucan hydrolase genes were also up-regulated in the resistant citrus trees. These genes may play important roles in symptom development. The DE genes were also enriched in two classes of RLKs, LRR-RLKs and DUF26-RLKs. We have experimentally verified the expressions of 14 up-regulated genes and 20 down-regulated genes on three HLB-tolerant 'Jackson' and three HLB-susceptible 'Marsh' trees using real time PCR. 11 of 14 up-regulated genes and 18 of 20 down-regulated genes were validated. Further characterization is underway for these differentially expressed genes and their potential roles in HLB progression. Meanwhile we are making constructs of a few selected genes for citrus transformation.

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Form PR-12 Quarterly Reports **SUBMIT REPORT**

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