Florida Citrus Advanced Technology Program

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

Instructions Complete the fields based on your project specs. When finished, save the form to your local disk using a unique name. Then, go to **http://research.fcprac.com**, and log in with your user name and password using Researcher Login in the lower left. Find this project title and click on **Submit a Report**. Update your profile information if needed, then upload this report as directed.

2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
(●) *Quarterly Report* ( ) *Annual Report* ( ) *Final*	HLB Pathology	11/13/09

WHAT IS THE "HEADLINE" FOR THIS REPORT (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Transcriptome profiling of citrus response to HLB disease in mature fruits

TITLE and CONTACT INFORMATION

Proposal Title Identification and modeling of early responses to HLB infection to improve disease management

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Phone (530) 752-7784	*Project Duration (years)* 1 *Year of Project* 1
Organization University of California, Davis	*Total Direct Funds (current year)* $298,538.00

REPORT UPDATE (650 words; provide details about your headline)

The project has two specific aims. We outlined below the progresses made for each of them.
Specific Aim 1: Identify sweet orange responses to Huanglongbing disease (HLB) through deep transcriptome profiling using new DNA sequencing technologies.
We are analyzing both leaves and fruits at two different developmental stage (immature and mature) comparing control plants (healthy plants in healthy location) with other three types of plants (apparently healthy, asymptomatic, symptomatic) in an infected location.
We just completed the sampling of the immature fruits sampling a disease-free orchard in Lake Alfred (Polk County, FL) and an infected orchard close to South Apopka (Orange County, FL). The RNA isolations from the entire peel tissue (albedo and flavedo) are currently under way using the method developed by Albrecht and Bowman (Plant Sciences, 2008, 175:191-206) pooling four different fruits of the five biological replicates (different trees) for each treatment.
We have started the transcriptomic analysis from the entire set of the four types of mature fruits combining same amounts of of RNA from the five biological replicates. The cDNA libraries were constructed following the mRNA-sequencing sample preparation protocol (Illumina Inc.). The complexity and quality of each library was determined using a high sensitivity chip from Agilent Bioanalyzer. We have run the 4 mature fruit cDNA libraries on the Solexa Genome Analyzer II to obtain read lengths up to 125 bp pair-ended (one sample for each lane). The raw Illumina (Solexa) reads/sample ranged between 25 and 31 million. Trimming was carried out using Velvet assembling into contigs (Zerbino and Birney, Genome Research, 2008, 18:821-829). Alignment of the individual reads and contigs to the Citrus sinensis unigene set (15,808 sequences; NCBI Unigene Build #11, 4/20/09) was performed using BWA (Li and Durbin, Bioinformatics, 2009, 25:1754-1760). We further processed the obtained datasets with the SAM Tools utility to efficiently align short sequencing reads against a large reference sequence (Li et al., Bioinformatics, 2009, 25:2078-9). An optimization of the read length/run is underway to define the optimal conditions for the next runs. We are currently in the process of defining the number of reads mapping to each unigene. We have developed a Taqman Real Time PCR gene expression assay to validate these results and to confirm specific biomarkers that strongly correlate with early disease detection.
Specific Aim 2: Define and validate gene networks and identify host (sweet orange) response biomarkers regulated by HLB at different stages of infection.
We have re-analyzed published data on gene expression in HLB infected leaves (Albrecht and Bowman, Plant Sciences, 2008, 175:191-206; Kim et al., Phytopathology, 2009, 99(1):50-57) using functional enrichment and gene set analysis employing several bioinformatic tools (blast2go, pathexpress, mapman and cytoscape). These analyses have identified HLB-regulated pathways such as sucrose and starch metabolism, phenylpropanoids, hormone-related pathways (jasmonate, ethylene and gibberellins). We compared these results with other stress-induced responses such as citrus bacterial canker disease (Cernadas et al., 2008, 9(5):609-631) and 'puff' physiological disorder (unpublished data). We have a predicted protein-protein interaction network inferred from an Arabidopsis knowledgebase that identified the most interactive proteins encoded by HLB-responsive genes such as Heat Shock Protein 81-1 and fatty acid hydrolase 1. HLB regulation of other "hub" proteins such as plant-glycogenin like starch initiation protein and carbohydrate transmembrane transporter reflect the transcriptional regulation observed in sugar metabolism that in turn cause the unbalance in source-sink communications.