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*	Management	5/17/10

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

Gene expression analysis of host response in the fruit peel and completion of deep transcriptome profiling in young leaves

TITLE and CONTACT INFORMATION

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

Principal Investigator Abhaya M. Dandekar	*PI Last Name* Dandekar
Email amdandekar@ucdavis.edu	*FDACS Contract Number*
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 progress 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 have performed the bioinformatic analysis of the deep sequencing runs (Illumina Genome Analyzer II, read lengths up to 85 bp pair-ended) for the four types of fruit samples of the experimental design: symptomatic, asymptomatic and apparently healthy from an infected orchard and healthy plants from a disease-free location. We have also completed the deep sequencing runs for the same samples of mature and young leaves: 32-36 million reads were obtained for the immature leaves (95-97% high quality reads after quality trimming) and 32-34 million reads (96-97% high quality reads) obtained for the mature leaves. The bioinformatic analysis of these data is ongoing. In regards with the immature fruit set, the RNA have been extracted and cDNA libraries have been constructed. The runs for these samples are scheduled to take place in the next weeks. For the validation of the mature fruit transcriptome analysis, Taqman Real Time assays have been performed for genes involved in important differentially regulated pathways such as hormone signaling and biosynthesis (gibberellin, ethylene, cytokinin), photosynthesis, transcription factors, terpenoid metabolism. Other assays have been designed to validate the transcriptome results of the mature fruits and related to energy metabolism, hormone, sugar and starch metabolism. In anticipation of the leaf analysis we have initiated the designed of 13 Taqman Real Time assays based on previously published leaf responses to HLB obtained with microarrays. Other Taqman assays will be designed once the leaf samples will be analyzed to validate the transcriptome profile obtained by the deep sequencing.

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 validated more than 10 Taqman Real Time assays for early HLB-regulated genes developed as biomarkers to distinguish infections at asymptomatic stage in the fruit. These genes belong to sucrose metabolism, photosynthesis, cell wall, lipid, volatile pathways, gibberellin signaling, ethylene response, transcription factors. These biomarkers provided a valuable insight into fruit peel responses to HLB. A significant number of regulated genes in the symptomatic fruits were involved in transport functions. An upregulation of light reactions was observed with the corresponding increased regulation of sucrose and starch metabolism. Genes encoding heat shock proteins appear to be major hubs in a protein-protein network analysis we conducted. The downregulation of these genes would stimulate protein misfolding processes and disrupt source-sink communications. Pathways encoding plant growth regulators like gibberellin and cytokinin were downregulated, whereas ethylene, jasmonate and salicylic acid metabolism and signaling were induced and these could affect fruit metabolism. WRKY, AP2-EREBP and bZIP transcription factors were highly regulated at different stages of disease. Several receptor kinase genes (LRR protein) seemed to be involved in reception of the signal triggering the host response. While cell cycle and organization were downregulated, protein modifications and RNA processing were induced in symptomatic fruits. Interestingly, genes involved in ATP synthesis were upregulated in mature fruits and agreed with previous findings of the presence of an ATP translocase gene in the pathogen genome (Duan et al., 2009). Taqman assays have been designed and analysis are on going to confirm these preliminary results.