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	1/17/10

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

Over a thousand host responses are specifically regulated in peel tissues of mature citrus fruit in response to HLB

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 outline below the progress made for each of them.
Specific Aim 1: Identify sweet orange responses to Huanglongbing disease (HLB) through deep transcriptome profiling using next generation DNA sequencing technologies.
Transcriptome analysis was conducted using Illumina Genome Analyzer II. We obtained read lengths up to 125 bp pair-ended for the entire set of mature fruits analyzing the four types of samples: symptomatic, asymptomatic and apparently healthy from infected locations and healthy plants from disease-free location. 25 and 31 million reads/sample were obtained and 12,8-18.99 million reads were mapped to the NCBI citrus Unigene set (15808). Using velvet assembly we obtained 158,656 contigs that were BLASTed to the following databases : JGI 1.2x citrus genomic sequences (78.6% of contigs had hits), NCBI C. sinensis Unigene set (45.7% of contigs had hits), TAIR9 protein sequences (53.6% of contigs had hits) poplar protein sequences (58.7% of contigs had hits). About 1156-1734 of the citrus unigenes were differentially regulated in pair wise comparisons of symptomatic, asymptomatic, and apparently healthy fruits with control fruits. We have also completed construction of three of the four cDNA libraries from immature leaves. We are planning to obtain the last sample (control) to soon run this set of samples before the analysis of the mature leaves and immature fruits. Additional RNA was isolated from both mature fruits and leaves for the validation of the results using qRT-PCR.
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 analyzed transcriptome data using BLAST2go, mapman, pathexpress software. A gene set enrichment analysis has revealed the following HLB-regulated pathways: starch metabolism, lignin, stilbene biosynthesis, phenylpropanoid, sucrose metabolism, coumarin, salicylic acid and hormone metabolism. A pair wise comparison between the four types of samples has identified several genes that can be used to distinguish infections before the symptoms appear. After qRT-PCR validation these genes can be confirmed as biomarkers for asymptomatic diagnosis. A list has been made including genes belonging to sucrose metabolism, photosynthesis, cell wall metabolism, lipid metabolism and certain volatile metabolite pathways. Terpene metabolism was highly regulated in apparently healthy compared to HLB-infected fruits. Jasmonate, salicylic acid, ethylene and auxin were the key hormones involved in host response to HLB-infection in the fruit peel tissues. Among transcription factors, WRKY, NAC, MYB, EREBP, bZIP were mostly highly regulated after the infection proceeding from the asymptomatic stage. With respect to cell biology processes the most significant up regulation was observed in pathways related to, protein modification, protein degradation, transport, metal handling pathways, while cell division and cell cycle and organization were down regulated. Up regulation was observed in HLB symptomatic fruits for genes involved in Photosystem II and redox chain reactions as well as ubiquitin dependent protein degradation. The following receptor like proteins were found to be involved in the host responses in the fruit: leucine-rich-repeat protein receptors, DUF26, WAK, thaumatin. We are conducting a network analysis of citrus responses based on a Arabidopsis knowledge base of protein-protein interactions to determine which genes play key roles in the cascade of HLB-regulated pathways (hubs or connecting hubs). Such genes will be excellent candidates as biomarkers for diagnosis and as targets for therapeutics. Currently, qRT-PCR has been performed to validate 6 genes belonging to starch, sucrose, hormone metabolism and additional analysis will include up to 20-30 genes to identify biomarkers for asymptomatic host responses in fruit to HLB.