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*	Genetics	3/3/10

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

Global gene expression of sweet orange infected with Ca. Liberibacter spp.

TITLE and CONTACT INFORMATION

Proposal Title Analysis of transcriptome of citrus infected with Ca. Liberibacter asiaticus and Ca. L. americanus.

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Phone 55 19 35461399	*Project Duration (years)* 2 *Year of Project* 2
Organization Centro de Citricultura Sylvio Moreira	*Total Direct Funds (current year)*

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

Plant material -To get healthy and infected tissue around 60 virus-free plants of sweet were grafted into Rangpur lime, and kept in screen-house until use.
Challenge with Candidatus Liberibacter spp -After reached 30 cm tall, a batch of 25 plants were grafted with budwoods infected with Ca. Liberibacter americanus (CLam) and another 25 were grafted with budwoods infected with Ca. Liberibacter asiaticus (CLas). All budwoods were checked by conventional PCR and RT-qPCR by the presence of the bacteria. Ten healthy plants were kept in the same conditions as control of no infected tissue. Every 30 days after grafting samples were collected and evaluated for bacteria. Since the plants were confirmed to be positive for the bacteria they were drastically pruned and transferred to a growth chamber at 22 to 24 oC and photoperiod of 16/8 hours.
Total RNA-When the branches were around 15 cm (approximately 40 days after pruning) they were collected, and leaves and barks were separately grinding in liquid nitrogen. RNA was isolated using RNeasy Plant Mini Kit (Qiagen) according to manufacturer's instructions. Genomic DNA contamination was removed from the total RNA using the RNase-free DNase (Qiagen) following the manufacturer's instructions. The concentration of total RNA was determined using a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies). RNA integrity was verified using a Bioanalyzer 1000 (Agilent, Palo Alto, CA). Synthesis of cDNA and array hybridization: The RNA samples were sent to Roche NimbleGen Systems, where cDNA synthesis and Cy3 labeling was performed. Equal amounts of total RNA for each sample were converted to double stranded cDNA using the SuperScript II cDNA Conversion Kit (Invitrogen, Carlsbad, CA). Because this method uses an oligo dT primer, RNAs lacking polyA tails are likely to be under-represented. Hybridization, scanning, and image analysis of the arrays were performed by Roche NimbleGen Systems according to the manufacturer's recommendations. The oligo-array includes 32,000 unigenes of sweet orange with six replicas of each unigenes with density of 340,000 spots. The preliminary results presented above were from the hybridization of RNA of plants infected with Ca. L. americanus. Other results with Ca. L. asiaticus were not evaluated yet. A set of primers for validation by RT-qPCR were designed.
Ca. L. asiaticus and Ca. L. americanus were first detected 90 and 150 days, and the symptoms were observed 120 to 150 and 220 days after grafting, respectively. The rate of infection of Ca. L. asiaticus reached 95 % of the grafted plants whereas Ca. L. americanus was just detected in 30 % of the plants. Comparing two biological replicas of infected and healthy plants about 600 genes were up regulated in plants infected with Ca. L. americanus. Those genes were clustered in unclassified proteins (45%), metabolism (11%), protein fate (6%), classification not yet clear-cut (6%), cellular communication/signal transduction mechanisms (5%), transport facilitation (5%), cell rescue, defense and virulence (5%), transcription (4%), protein with bindind function or cofator requirement – structural or catalytic (3%), subcellular localization (3%), control of cellular organization (2%), cellular transport and transport mechanisms (1%), energy (1%), systemic regulation of/interaction with environment (1%), storage protein (1%), and others categories presented inferior percentages to 1 % (cell cycle and DNA processing, development (systemic) and protein activity regulation). 200 genes were down regulated and they grouped into unclassified proteins (43%), metabolism (14%), subcellular localization (8%), transcription (6%), protein with binding function or cofator requirement [structural or catalytic] (7%), transport facilitation (5%), energy (5%), protein fate (4%), cellular communication/signal transduction mechanism (3%), %), control of cellular organization (1%), development (1%) and other categories.