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	6/4/10

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* 1
Organization Centro de Citricultura Sylvio Moreira	*Total Direct Funds (current year)* 47,400,00

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

Narrative

Experiments – From February to June 60 plants of sweet orange Hamlin were grafted into Rangpur lime and established at screen house. After reached 30 cm tall, they were grafted with budwoods infected with Ca. Liberibacter americanus (CLam) and budwoods infected with Ca. Liberibacter asiaticus (CLas). All budwoods were checked by conventional PCR and RT-qPCR by the presence of the bacteria. Healthy plants are the negative control. After the plants were confirmed bacteria positive (RT-qPCR) they were pruned and transferred to a growth chamber at 22 to 24 oC and photoperiod of 16/8 hours. Approximately 40 days after pruning 15 cm branches were were collected. Leaves and barks were separately grinding in liquid nitrogen. Concentration and integrity of total RNA were evaluated before hybridization experiments in microarray plataform (Roche Nimblegen).

Hybridization experiments - The RNA samples were sent to Roche NimbleGen Systems, where cDNA synthesis and Cy3 labeling was performed. Hybridization, scanning, and image analysis of the arrays were performed according to the manufacturer's recommendations. The oligo-array includes 32,000 genes of sweet orange with six replicas of each one with density of 340,000 spots. Preliminary results were presented in the previous report.

Next steps – Array hybridizations with plants infected with Ca. L. asiaticus, but symptomless. Array experiments with symptomatic plants infected with Ca. L. asiaticus and plants infected with Ca. L. americanus. Statistical analysis of the global gene expression experiments. Primers design for specific gene expression during the infection with both bacteria. First draft of the manuscript for publication.

Expected results and practical application

The main expected result is the knowledge on the gene expression pattern in sweet orange during the process of infection by Candidatus Liberibacter spp. Such knowledge will enlarge our understanding on the basic aspects of the disease and may be help to approach new technologies for control, especially technology based on gene silencing or gene over expression.

Revised Budget for the Second Year

The last part (15 %) of the approved budget will be used for the payment of the temporary Post doctor working in the project. 10 % will to be deducted for the Department, according with the contract (Considerations 6.2).