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	7/16/09

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

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 Moreira2	*Total Direct Funds (current year)*

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

Main goal
- Analysis of transcriptome of sweet orange Hamlin during the infection of Candidatus Liberibacter spp.

Main activities
Experiment set up – From February to June 60 plants of sweet orange Hamlin were grafted into Rangpur lime and established at screen house. They will be grafted with infected budwoods in vegetative development of the plants and grafting of the budwoods. I have a pure source of Candidatus Liberibacter asiaticus (CLas) kept under screen house but source of Ca. Liberibacter americanus (CLam) has been difficult to keep, because the bacteria have low transmission efficiency with infected budwoods. Infected plants in the field have decreased in the last years, and CLas seems to be more pathogenic than CLam. The experiments will be changed from the original version in order to improve the quality of results. Besides the time course for sampling of infected and health leaves, tissue of bark (enriched with phloem tissue) in the infected branches will also be collected and compared with leaves. A concern in the experiments is regard with time of infection that can really be associated to the interaction plant with the bacteria. Preliminary we consider 0, 2, 6, 12, 18,24, 30, 45, 60, and 90 days after grafting. I am evaluating to consider three main pools. One before the bacteria can be detected by RT-qPCR (0, 2, 6, 12, 18 days); other pool of samples collected at 24, 30, 45 and 60 days. Sample after 90 days will be considered alone, since at that time severe damages caused by the disease could disturb the results. Thus the experiments could be conduced with three pools of leaves + control, three of bark + control, with tissue infected with both bacteria individually.
Array design - The arrays and the hybridization will be conduced as service by Nimblegen / Roche. A set of arrays was already tested in other experiment with suitable quality. The database was 32,000 unigenes of sweet orange (CitEST). Each unigene was represented 6 times with oligo probes of 50 to 60 bases. Therefore, an array has a density of 180,000 spots. Procedure for RNA isolation with high quality is established.
RT-qPCR – Although the experiments with arrays were not finished, we are looking for good candidates as house keeping genes in citrus. Among them β-tubulina, expansin-like B1, and ankirin.
Some troubles – Although FCPRAC sent a check with part of financial support in Abril 2009, until now the check could not be paid. The bank cannot explain what is going on, but they promised in August the funds would be available. Actually all activities were until now conduced with funds of other projects.