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	1/18/10

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

Citrus genome sequencing has begun, to provide tools in the fight against HLB

TITLE and CONTACT INFORMATION

Proposal Title International citrus genome consortium (ICGC): Providing tools to address HLB and other challenges

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Phone 8639561151	*Project Duration (years)* 3 *Year of Project* 1
Organization UF-CREC	*Total Direct Funds (current year)* $1,300,000

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

Funding is now in place among all the partners of the International Citrus Genome Consortium (US, Brazil, Spain, France, and Italy) to move forward with the project to sequence a haploid citrus genome. This genome sequence, when completed, will be THE reference genome for citrus, as it will be of the highest quality technically possible. DNA samples for sequencing have been prepared, and the strict quality control standards required by the sequencing centers (JGI in the US, Genoscope in France, and IGA in Italy) have been met. DNA samples have been shipped to the three centers, and sequencing has begun at Genoscope. Unfortunately, library preparations at JGI failed to achieve the needed insert size, so new DNA preps will be sent from Spain to JGI in January 2010.The Brazilian group remains in negotiations with JGI over contract language, but resolution is anticipated no later than February 2010. A meeting was held at Genoscope (Paris) with representatives from the sequencing partner nations, to revisit plans and progress. Progress was reported on the genetic linkage map, on haploid transcriptome sequencing, and genome sequencing (GS has completed 2.1x already); most importantly, a new time-line was produced that could lead to availability of gene sets by October 2010.

Two sets of plants of sweet orange, rough lemon, and Volkamer lemon, representing the more susceptible and more tolerant types respectively, were inoculated with HLB in an environmentally controlled greenhouse. Plants were monitored for symptom development and for Las by qPCR. RNA samples were prepared from all plants at regular intervals, from inoculation through symptom development to be used in microarray experiments. Hybridization of RNA with Affymetrix and Agilent citrus chips (the latter developed by us at UF) has been completed and data sets have been generated; these are currently being analyzed to compare gene expression profiles over the time course.Though funding to the collaborators at UCR was delayed, they proceeded with their objective to update the HarvEST-Citrus EST database, including EST sequences from colleagues in Brazil and Japan, to provide an improved database for gene expression studies; currently there are more than 465,000 publicly available ESTs. Likewise, the collaborator in Spain was delayed in receipt of funds, but he has worked with us in preliminary experiments to test the feasibility of tissue-specific gene expression analysis that may be conducted.

The sweet orange genome sequencing project is a collaboration between UF, JGI, and Roche/454 using next-gen sequencing platforms. Sequencing runs have been completed, and we now have >30x sweet orange genome coverage. In addition, 1.2 million ESTs were produced by one Titanium run of an RNA library from leaf tissue, to aid in subsequent assembly, gene prediction, and annotation. Six preliminary assemblies of the genome sequence have been attempted using various versions of 454's assembly program, Newbler. To this point, the work has yielded fragmented assemblies upon which gene prediction models had a difficult time to work. Roche/454 will continue their efforts at assembly. Both genome sequences, when assembled and annotated, will be housed in a new database, Tree Fruit GDR, which was funded by an SCRI grant to include citrus. Further, the sequences will be available also through the JGI plant genome portal, and will be deposited with NCBI, as well. The plans to exploit genome sequence information for a better understanding of the interactions of citrus plants with the pathogen causing HLB are ultimately most dependent on having the genome assembled and annotated; for this reason, our main focus will be on accomplishing that goal, while continuing to conduct experiments using microarray analyses and deep transcriptome sequencing.