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	5/3/10

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

Citrus Genome Sequencing: Full Speed Ahead!

TITLE and CONTACT INFORMATION

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

Principal Investigator Fred G. Gmitter Jr.	*PI Last Name* Gmitter
Email fgg@crec.ifas.ufl.edu	*FDACS Contract Number* 71
Phone 8639561151	*Project Duration (years)* 3 *Year of Project* 1
Organization UF-CREC	*Total Direct Funds (current year)* 13000000

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

Funding is now in place from the International Citrus Genome Consortium partners (US, Brazil, Spain, France, and Italy), and the project to sequence a citrus genome is well underway. This will be THE reference genome sequence for citrus; it will be of the highest quality technically possible, by virtue of its haploid condition and the use of Sanger sequencing technology. DNA samples 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, and sequencing is ongoing at Genoscope and IGA. Unfortunately, library preparations at JGI failed, so new DNA preps have been sent to JGI recently; a backup plan has been established for timely delivery of new samples, should current samples prove inadequate. The HudsonAlpha Institute in Alabama, a JGI affiliate, will sequence the Brazilian part of the collaboration; this brings Drs. J Schmutz and J. Grimwood, two world authorities in sequencing, to the project. A meeting was held last December at Genoscope (Paris) with representatives from the partner nations, to revisit plans and progress on the genetic linkage map, and haploid transcriptome 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. The sweet orange genome sequencing project is collaboration between UF, JGI, and Roche/454 using their next-gen sequencing platform. Sequencing runs have been completed, yielding 30x sweet orange coverage. In addition, 1.2 million ESTs were produced from an RNA library from leaf tissue, and several additional libraries for sequencing are being constructed with RNAs from 16 different biotic and abiotic conditions, to broaden gene coverage to aid subsequent assembly, gene prediction, and annotation. Six preliminary genome sequence assemblies were attempted using various versions of 454's assembly program, Newbler, yielding fragmented assemblies upon which gene prediction models could not work. Roche/454 continued their efforts, and a 7th and useful assembly has been produced; currently gene prediction and annotation have begun. 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. 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. Two sets of plants of sweet orange and rough lemon, representing 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 RT-PCR. RNA samples were prepared from all plants at regular intervals, from inoculation through symptom development, to be used in microarray experiments. Hybridization with Affymetrix and Agilent citrus chips (the latter developed by us at UF) has been completed and data sets have been generated; these are under analysis to compare expression profiles over time; some genes clearly up-or-down regulated differentially have already been confirmed by RT-PCR. Our collaborators at UCR have updated the HarvEST-Citrus database, including sequences from colleagues in Brazil and Japan, to provide an improved database for gene expression studies containing more than 465,000 publicly available ESTs. We are working with our collaborator in Spain on experiments to examine tissue-specific gene expression. New experiments have been designed and are being implemented.