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	3/11/10

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

Citrus Transformation Facility is in the "Full speed ahead" mode

TITLE and CONTACT INFORMATION

Proposal Title Increasing the Capacity of the University of Florida's CREC Core Citrus Transformation Facility (CCTF)

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Phone 863-956-1151	*Project Duration (years)* 3 *Year of Project* 1
Organization CREC-University of Florida/IFAS	*Total Direct Funds (current year)* $85,000

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

The funding that was received allowed the Core Citrus Transformation Facility (CCTF) to have best year since it opened. As such, CCTF has continued to serve research community by providing reliable service through the production of transgenic citrus plants, primarily for NAS/FCPRAC funded projects of other researchers that do not have citrus transformation capacity.

The initial objective of this project has been met in a very short period of time. The employment of the additional OPS technician allowed for the increase in the amount of work that is being performed. In the induction phases of transformation process, increment was adjusted to the maximum available capacity of the laboratory. The number of explants that is being processed has increased from 2000 per week to about 2800. However, major improvement achieved due to addition of new employee is that there are no back-logs in the selection phase of the experiments. Number of shoots or soil-adapted seedlings that can be inspected/assayed for the presence of reporter gene or used in the PCR reaction is about 200 per week. Increased capacity to inspect shoots and soil-adapted plants resulted in higher numbers of detected plants. Most recently, new method for the detection of foreign DNA in plants that requires minute amounts of tissue as a source of template was adopted by the facility. Application of this method has further facilitated our rate of detection of transgenic plants because screening can be done on a small shoots before they get micro-grafted on the rootstock plants. This is very important for those orders where binary vectors have no reporter gene. Also, labor that was earlier invested into "shotgun" grafting of many shoots where at least some would be positive is now diverted to care of confirmed transgenic plants. As a consequence, the delivery time for production of PCR-confirmed transgenic plants is made shorter than in the past.

The work performed within last 12 months included production of plants transformed with 21 binary vectors. They are: pCL2, pAF1, p6Cass, p pSUC-LIMA1, pLIMA, pLIMA-Sn, pNPR1, pPiTA, pCIT1070, pCIT108, pCIT108p, pCIT108p3, pCIT108p17, pN1*, pC5*, pF3*, pCN1, pCAMBIA2301, pTLAB21, pTLAB32, and pSuperNPR1. Out of these 21 vectors, 17 carry genes associated with improvement of citrus resistance against pathogens. Three vectors (so-called "empty") were used to produce transgenic plants without the gene of interest. These plants represent obligatory control plants for comparisons between transgenic material and wild-type plants. Only one binary vector included gene that can affect citrus development. Transgenic plants that were produced belong to seven cultivars: sweet oranges-Hamlin and Valencia, grapefruits-Duncan and Flame, rootstocks-sour orange and Carrizo, and Mexican lime. All together, CCTF produced more than 450 transgenic plants during the first year funding cycle.

Relevance of this project to the overall effort to break the HLB cycle and fight against canker remains high. Transgenic plants produced within last 12 months are from orders placed by seven different clients. Six out of seven clients are faculty presently involved in research projects associated with NAS/FCPRAC funded efforts to produce Citrus plants resistant/tolerant to huanglongbing (HLB) or canker. Some of transgenic Duncan grapefruit plants that CCTF produced for one of the recent orders may represent a breakthrough in the fight against Citrus canker. In a challenge experiments with canker-inducing bacteria, these plants exhibited significant increase in resistance to this disease.

Within the last three and-a-half months, the CCTF facility received a large number of new orders. Therefore, CCTF continues to be an irreplaceable element in the fight against Citrus diseases and especially HLB. Activities of the CCTF on a few orders that have to do with improvement of Citrus not associated with disease resistance are continuing as well.