CITRUS ADVANCED TECHNOLOGY PROGRAM

QUARTERLY & FINAL REPORTS: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

SELECT PERIOD September 2013

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Citrus Core Transformation Facility as a platform for testing of different genes and/or sequences that have potential to render Citrus plants tolerant or resistant to diseases

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
10/8/13	Citrus Research and Development Foundation	Transgenic/Metagenomic Mediation of Resistance

REPORT UPDATE (500 words-It is not necessary in this public report to disclose your institution's proprietary information or intellectual property.)

Within the last year and-a-half, researchers trying to find solution to HLB through production of transgenic plants have used every possible approach that offered some prospect for production of tolerant/resistant citrus plant. As a result, the Core Citrus Transformation Facility (CCTF) has indeed become the platform for testing the effect of different DNA sequences ("genes") on transgenic plants that could result in possible alteration of their ability to sustain pathogen attack. Since most of this work in its nature is theoretical, it is not well known how the introduction of certain gene into Citrus will affect production of transgenic plants. Some genes may be easily introduced into model organisms or may cause weak effect on their phenotype. However, very often situation is rather different in Citrus. From one client, CCTF has received a group of orders that all have common DNA sequence combined with other, different DNA sequences. Those orders consisted of six vectors that were supposed to be introduced into three different citrus cultivars in certain combinations. Since the time when these orders were placed and 9/20/2013, altogether 70 co-incubation experiments were performed with more than 38000 explants. Despite all this work, no transgenic plants were produced. From another research group came an order to produce plants with the gene that severely affected the phenotype of transgenic plants. Transgenic shoots were stunted and extremely bushy, making our efforts to graft them impractical. Work on this order stopped after about 30 PCR-positive shoots were produced at which time the agreement was reached with the client who will try to place new order where this gene will be controlled by an inducible promoter.

The unintended consequence of the efforts of researchers to find the "gene" that may render Citrus plants tolerant/resistant to HLB by using CCTF as a testing platform is low number of transgenic plants produced in the CCTF. In the last quarter, only 20 plants were produced. They belong to the following orders: pHGJ2 vector-one plant, pHGJ3 vector-seven plants, pHGJ4-three plants, pN5-five plants, pBI121-one plant, pN7-one plant, pW14 one plant, and pMED14 one plant.

The second (out of ordinary) reason that contributed to low productivity within this quarter was the massive contamination in one of the common growth rooms where CCTF also keeps germinated plants. This contamination took place in June and July and wiped out 18% of our cultures from that period.

Out of three orders for transgenic plants placed in the previous quarter, two were withdrawn. Within this quarter, six orders were placed. Work with two vectors is to proceed only to the early step in shoots production. For additional three vectors, the goal is to establish possible effect of introduced genes on genome activation/transformation success rate. Because of the presence of GFP as a reporter gene in these vectors, the work on these orders may also be completed at the phase of transformation when fully developed shoots are harvested from explants.

PI First Name Vladimir

PI Last Name Orbovic

Email orbovic@ufl.edu

Phone 863-956-8729

Organization CREC-U. of Florida/IFAS

Project Number 579

Project Duration (years) 3 *Year of Project* 2

Total Funds (current year) $85,000.00