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 Genetic Improvement Using Cell-Penetrating Peptides

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
10/1/13	Citrus Research and Development Foundation	Citrus Genomics

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

This quarter work has been divided among between all of the proposal objectives. For the primary objective of transformation via CPPs, a new approach in the transient expression assays has been evaluated in order to enhance the transformation protocol. The new approach has been adapted from Chugh and Eudes, 2008. Instead of 3 days of transfection of citrus seedlings, testing has been done following one hour, two hours, or three hours following incubation of the seedlings with peptide and constructs. Early data indicate that one hour is insufficient for transient expression. As another option to improve upon this method, new and different peptides are being tested, including the classic "Tat" peptide penetratin, and a chimeral peptide "R9-Tat". These experiments did not have as much tissue as normal, since the 'Pineapple' sweet orange (40 of 80, 50%) and the 'Duncan' grapefruit (24 of 240, 10%) both had low germination rates. The next quarter should produce more plant tissue and results from these first experiments. In addition to these new transformation events, a second reporter assay has been run on the 36 'Carrizo' plants. These plants did not report the GUS gene and are presumed negative. DNA from these plants have been purified and a PCR will confirm, but the plants are probably escapes. Finally, work has been done to create plasmid constructs for a silencing experiment. The citrus analog of NPR1 has been sequenced. Using this sequence, we hypothesize that two different constructs can lead to the same desired, silenced effect. The first construct is a full length gene inserted in reverse. The second is a dual vector, where the genes will hairpin due to their complementary nature. Currently, the second vector is half finished, as only one side has been confirmed, and the full-length plasmid needs to be tried again. Finishing these constructs and more transformation on the fresh seedlings will be the focus of next quarter.

PI First Name Gloria

PI Last Name Moore

Email gamoore@ufl.edu

Phone 352-273-4786

Organization University of Florida

Project Number 752rev

Project Duration (years) 3 *Year of Project* 1

Total Funds (current year) $45,417.00

Form PR-12 Quarterly Reports **SUBMIT REPORT**

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.