CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD December 2016

○ *Quarterly Report* ● *Final*

Proposal Title

Diaprepes control using a plant based insecticidal transgene approach

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
04-03-17	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.)

Our project aims to provide durable long term resistance to Diaprepes using a plant based insecticidal transgene approach. A number of plant derived insecticidal transgenes, each driven by a root specific promoter were incorporated into Carrizo citrange. As part of this project we cloned four insecticidal genes and two plant derived promoters. Constructs were initially tested on N. benthamiana for confirmation of transgene activity. Carrizo citrange was subsequently transformed utilizing the conventional Agrobacterium mediated transformation process. A total of 123 putative transgenic lines were generated. PCR screening identified 74 of these lines to be transgenic. 54 of these transgenic lines had a high level of transgene expression as determined by qPCR.

We have also identified a number of putative root specific genes from Citrus clementina by data mining the phytozome database. Of them several putative sequences were selected for characterization using qPCR. RNA was extracted from mature and juvenile roots from non-transgenic Swingle citrange. Each of the identified sequences were characterized in these different tissues. In addition, transcript levels in the leaves were also measured. The Cic1867m gene was determined to be very root specific and a 1.2 kb fragment of its promoter was cloned from Clementine genomic DNA using PCR. Deletion analyses identified a 0.8 kb fragment from that promoter fragment to be sufficient for root specific activity and transgenic plants were produced using this promoter.

Cuttings from all the better performing lines have been made and are being rooted in the mist bed. These clones will be sized up for Diaprepes feeding experiments. Clonally propagated plants will be force fed with Diaprepes neonates - when available and root damage / insect mortality evaluated.

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Organization University of Florida/CREC

Project Number 925

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

Total Funds (current year) $41797

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

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.