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

Engineering Citrus for Canker Resistance

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
1/11/2016	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.)

Objective 1: Assess canker resistance conferred by the PAMP receptors EFR and XA21
Three constructs were used for genetic transformation of Duncan grapefruit and sweet orange as part of a previous grant: EFR, EFR coexpressed with XA21, and EFR coexpressed with an XA21:EFR chimera. Seven transgenics have survived and passed a PCR screen, and these have been grafted onto rootstocks. Grafted plants are currently growing, and will be tested for responsiveness to the elf18 ligand for EFR and for canker resistance. To ensure that there will be sufficient events to analyze to come to a conclusion about the effectiveness of these genes, we have initiated more transformations in Duncan grapefruit at the Core Citrus Transformation Facility at UF Lake Alfred. In addition, we have added the recently-identified Cold Shock Protein Receptor (CSPR) to the transformation queue. Selection is underway, but the GFP marker is not expressed in citrus, and the protocol is being optimized.

Objective 2: Introduction of the pepper Bs2 disease resistance gene into citrus
Two constructs were created to co-express Bs2 with other R genes that may serve as accessory factors for Bs2. These constructs were provided to the Lake Alfred transformation facility, but the transformation attempts have so far been unsuccessful, possibly due to negative effects of the constructs in Agrobacterium or in citrus. Troubleshooting of these transformations with a negative control construct is underway.

Objective 3: Development of genome editing technologies (Cas9/CRISPR) for citrus improvement
The initial target for gene editing is the citrus homolog of Bs5 of pepper. The recessive bs5 resistance allele contains a deletion of two conserved leucines. The citrus Bs5 homologs were sequenced from both Carrizo citrange and Duncan grapefruit, and conserved CRISPR targets were identified. For proof of concept, we are targeting mutating the native citrus Bs5 alleles while simultaneously replacing the gene with the effective resistance allele. Two editing constructs have been created, one targeting a site overlapping the two conserved leucines, and one targeting two flanking sites to create a deletion in Bs5. Both constructs have been verified to function by co-delivery into Nicotiana benthamiana leaves with another construct carrying the targeted DNA from Carrizo or Duncan varieties. These constructs have been prioritized for transformation into Carrizo citrange, and transformations are underway at UC Davis. Transformants with mutations in Bs5 that contain the replacement bs5 allele will be selected and tested for canker resistance.

PI First Name Lynne

PI Last Name Reuber

Email tlr@2blades.org

Phone 470-222-5230

Organization Two Blades Foundation

Project Number 15-022

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

Total Funds (current year) $147,538

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

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.