CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD December 2015

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Engineering Citrus for Canker Resistance

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
1/15/16	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. Putative transgenics are currently being verified by PCR in the Jones lab, and five PCR positive plants have been identified so far. To ensure that there will be sufficient events to analyze to come to a conclusion about the effectiveness of these genes, we will initiate more transformations in Duncan grapefruit at the Core Citrus Transformation Facility at UF Lake Alfred. EFR, XA21, and XA21 + EFR constructs have been re-created with the inclusion of a GFP marker for identification of transformants.

Objective 2: Introduction of the pepper Bs2 disease resistance gene into citrus
Constructs are being created in the Staskawicz lab to express Bs2 under the 35S promoter and under a resistance gene promoter from tomato. Constructs are also being created in which Bs2 is co-expressed with other R genes that may serve as accessory factors for Bs2. Constructs with tagged Bs2 have been confirmed to function in transient assays, and have been transformed into Arabidopsis. Protein expression will be confirmed by immunoblot. GFP is currently being added to the constructs to facilitate selection of transformants in citrus.

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 homolog was sequenced from both Carrizo citrange and Duncan grapefruit, and conserved CRISPR targets were identified. Four CRISPR constructs are being created in the Staskawicz lab:
C1) A construct targeting two sites that will produce a 100 bp deletion in Bs5 in both Carrizo and Duncan (the bs5 transgene will be added);
C2) A construct targeting a site overlapping the two conserved leucines;
C3) C2 with the addition of a bs5 repair template for Carrizo that will not be cut; and
C4) C2 with a similar repair template for Duncan grapefruit.

C1 and C2 have been tested by co-delivery into Nicotiana benthamiana leaves with another construct carrying the targeted DNA from Carrizo or Duncan varieties. "C1" clearly cut the target sites of both varieties, causing 100-bp deletions. Sequence analysis confirms that "C2" cuts the target site in Carrizo. Considering this site is identical in both Duncan alleles, we expect it to cut Duncan as well. And, considering "C3" and "C4" are built from "C2," we expect them to target the cut site as well. Sequence analysis is underway to confirm these expectations. In addition, to aid in the selection of positive transgenics, we are currently adding a GFP reporter into each CRISPR construct.

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Project Number 15-022

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

Total Funds (current year) $161,930.00

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

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.