CITRUS ADVANCED TECHNOLOGY PROGRAM

QUARTERLY & FINAL PROGRESS REPORT FORM: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

SELECT PERIOD September 2022

Quarterly Report ● Final

Proposal Title

Using a Multipronged Approach to Engineer Citrus for Canker Resistance

Today's Date 09/08/2022

Sponsoring Organization (drop-down) Citrus Research and Development Foundation

Category (drop down) Horticultural & Management

ABSTRACT (750-word Executive Summary-public report-do not disclose proprietary information or intellectual property)

This project is a continuation of previously funded CRDF grants to TWO BLADES focused on utilizing multiple strategies to produce canker-resistant citrus plants plus the addition of a new strategy using gene editing. The project has focused on transforming Duncan grapefruit with genes that express EFR or a gene construct designated ProBs314EBE:avrGf2 that is activated by citrus canker bacteria virulence factors. We also are in the process of testing citrus that has been transformed to modify the bs5 gene to enhance resistance to the citrus canker bacterium.

Objective 1. To determine if Bs3-generated transgenic grapefruit plants are resistant to diverse strains of the citrus canker bacterium in greenhouse experiments and to the citrus canker bacterium in field experiments in Fort Pierce. In late March, 2019, in the field at Fort Pierce in collaboration Dr. Ed Stover, the transgenic material was planted. Citrus canker has developed on plants in the field and the trees were rated for disease in November 20, 2021and there was moderate disease on Duncan grapefruit trees but none on JJ5. We rated the plots again on July 28 (not June as mentioned in May report) 2021 and there were similar trends as in November 2020 although disease was lower given significant defoliation in the plots. We have also analyzed JJ5 for response to strains from Dr. Nian Wang to determine if those strains with unusual characteristics in terms of targeting the susceptibility gene could overcome the JJ5 resistance. Interestingly we noted unique phenotypes in plants inoculated with these strains although they were not typical disease reactions but more of a watersoaked appearance. We noted that theygrew to higher populations in inoculated tissue, although the disease phenotype was very weak and not typical of canker. We also used a 5' Race kit to determine the transcription start in our JJ5 construct and observed that all strains tested activated trasncription of our As for developing a different transgenic with ProBs314EBE:avrGf2, we have placed our constuct in a different vector that is acceptable for future transgenic purposes. The previous constructs contain an additional selectable marker that allowed for identifying putative transgenics with a higher success rate. Given that there was concern about the additional marker, the new construct contains only NPT as a selectable marker. We have created a second construct for Vladimir Orbovic given the first attempt was not successful.The construct was transferred to Vladimir Orbovic, who was tasked with creating additional transformants in sweet orange. He identified several putative transgenic trees that weregrown and tested for disease reaction. Three of the putative transgenics were shown to elicit a hypersensitive reaction when infiltrated with a bacterial suspension of a *Xanthomonas citri* strain. Currently, we are maintaining the transgenic plants in our greenhouse. We are hopeful that these transgenic trees will be of use in the near future given that these particular ones do not express GFP. We are in the process of publishing this work.

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Organization University of Florida

Sponsor Project Number 18-013

Project Duration (years 3 *Year of Project* 3

% Completion of Objectives 90.00%

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.