CITRUS ADVANCED TECHNOLOGY PROGRAM

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

▶PROJECT QUARTER END October 2023 ● *Quarterly Report* ○ *Final*

Proposal Title

Evaluating Novel Gene-edited Duncan Grapefruit Mutants for Resistance to Huanglongbing (HLB)

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
11/14/2023	Citrus Research and Development Foundation	Plant Improvement

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

1. Please state project objectives and what work was done this quarter to address them:

Knocking out disease susceptibility genes (*S* genes) has resulted in broad disease resistance in multiple crops. The bottleneck to using CRISPR for engineering HLB resistance in citrus has been the lack of suitable and validated gene targets. Identifying suitable gene targets has been the most urgent task for achieving HLB resistance through gene editing. We are the first group in the world that have knocked out two S genes, DMR6 and SWEET1, in HLB-susceptible `Duncan' grapefruit and produced dmr6 and *sweet1* mutants. The objectives of this project are to evaluate the resistance of four `Duncan' mutants' after graft inoculation (Objective 1a) and exposure to infected Asian citrus psyllids (ACP) (Objective 1b,) and to assess potential side effects of these mutations on citrus plant growth and morphology (Objective 2). The overall goal of this project is to determine the effectiveness of editing DMR6 and SWEET1 for engineering HLB resistance in citrus.

Experiment 1 is being conducted to achieve Objective 1a. Four `Duncan' mutants and one control wildtype line were grafted onto HLB-free sour orange rootstock in two batches. The first batch of clonal plants were graft-inoculated with CLas-positive buds or CLas-free buds (mock inoculation) on June 17th, 2023, resulting in 29 graft-inoculated plants and 10 mock-inoculated plants. These plants have been grown in a temperature-controlled greenhouse. Data on plant height, trunk diameter below and above graft union, HLB symptoms, and CLas titer were collected on June 6th (before inoculation) and September 21st, 2023 (3 months post inoculation). The Ct value of these mutants and wildtype was approximately 40, indicating undetectable CLas in these inoculated plants. The second batch of clonal plants (total 34, with 4 to 8 per mutant) were propagated onto sour orange on July 24th, 2023. These plants have grown to a height of 5 to 12 inches.

Experiment 2 and Experiment 3 are being conducted for Objective 1b and 2, respectively. These experiments require approximately 20 clonal plants of similar stem diameters for each mutant or wildtype line. To produce the required clonal plants for these objectives, 50 to 75 cuttings were taken from each mutant stock plant and stuck into potting mix in early July 2023. The cuttings were rooted under an intermittent misting system in a secure greenhouse for 3 months. For each mutant, six to 37 cuttings have rooted and produced new shoots. To produce additional clonal plants for Objective 1b and 2, another batch of cuttings were taken on November 3, 2023. These cuttings are being rooted in Oasis Rootcubes.

2. Please state what work is anticipated for next quarter:

Objective 1a (Experiment 1) -1st batch of inoculated plants: Since this batch of inoculated mutant and wildtype plants have not shown any HLB symptoms or CLas titer 5 months after inoculation, we suspect that the graft inoculation with hot citrus buds might not have provided sufficient CLas inoculum. We plan to re-inoculate these plants using a different method, i.e., by exposing the plants to infected ACP. These plants will be trimmed to induce a new flush of tender shoots for infected ACP feeding. Subsequently, leaf samples will be collected, DNA will be extracted, and qRT-PCR

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

% Completion of Objectives (FDACS requirement) 20.00%

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