CITRUS ADVANCED TECHNOLOGY PROGRAM

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

▶ SELECT PERIOD September 2021

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Delivery of Verified HLB-Resistant Transgenic Citrus Cultivars

Today's Date: 09/29/2021

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

Category (drop down): Plant Improvement

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

Objective 1, Mthionin Constructs: Assessment of the Mthionin transgenic lines is ongoing. As the most proven of our transgenics, we continue to use them as a reference in detached leaf assays, as well as studying them in established greenhouse and field trials. The first MThionin field trial (45 plants, WT or transgenic Carrizo on rough lemon scions) has shown transgenics maintaining higher average CLas CT values (2.5 CT higher @ 18 months), but with a high degree of variability. The larger second MThionin planting (205 total grafted plants of transgenic Hamlin scions, transgenic Carrizo rootstock, or WT/WT controls) is producing encouraging results; with the transgenic Hamlin on WT Carrizo having statistically better trunk diameter, tree height and canopy volume compared to controls. Leaf samples from the second planting are collected and undergoing CLas quantification.

The Mthionin construct has also been extensively transformed into additional varieties; with 10 confirmed transgenic lines of US-942 and 44 putative lines of Valencia and Ray Ruby undergoing expression analysis.

Objective 2, Citrus Chimera Constructs: Detached leaf assays, with CLas+ ACP feeding, have been conducted to screen citrus lines expressing chimera constructs TPK, PKT, CT-CII, TBL, BLT, LBP/'74', '73', and '188' (as well as scFv-InvA, scFv-TolC, Topaz and Onyx). Testing of all 35s driven Carrizo lines is complete and the analysis of phloem specific and scion-types is well underway. This work has already identified numerous lines with significant effects on CLas transmission and increased ACP mortality (up to 95% from TBL, 96% from PKT, 60% from BLT and 70% from TPK) and decreased transmission of CLas into the leaf. The ACP endosymbionts show a reduction in titer that may indicate a mode of action for ACP mortality when feeding on CLas-killing transgenics (outlined in Objective 4) as these are also gram-negative bacteria and ACP are dependent on their nutritional contributions. The best performing of these lines have been moved forward into greenhouse trials as described below.

Initial ACP-inoculated greenhouse trials on 8 lines of citrus Thionin-LBP chimeras ('73', and '74') showed a statistically significant reduction (13x) in CLas titer for '74' transgenics vs WT. However, many plants of both treatments remained CLas negative due to low inoculation efficiency. In June, 150 plants representing the best performing 6 lines of `74' and 7 lines of `188' were no-choice caged ACP inoculated using a new protocol to improve transmission rates. At 3 months, control plants tested positive at twice the rate of the earlier inoculation; 6-12 month tissue samples have been collected and are undergoing qPCR analysis.

PI First Name Ed

PI Last Name Stover

Email ed.stover@usda.gov

Phone 772-462-5951

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% Completion of Objectives 85.00%

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.