CITRUS ADVANCED TECHNOLOGY PROGRAM

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

▶ INSTRUCTIONS

(●) *Quarterly Report* ( ) *Annual Report* ( ) *Final*

Required: What is the "headline" for this report (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Callose phloem blockage factor, citrus transformed to prevent

Proposal Title

Characterizing the Roles of Callose and Phloem Proteins in HLB Symptom Development

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
9/20/10	Citrus Research and Development Foundation	Infection Responses

REPORT UPDATE (500 words; summarize your accomplishments)

Samples for phloem plugging determination were collected from HLB infected mature field grown trees and from young greenhouse grown trees. A total of 29 trees and 4 cultivars were sampled; 22 trees were field grown. Callose plugs were 2.7 and 2.4 times more prevalent than phloem protein 2 (PP2) plugs in the field and greenhouse samples, respectively. Three Murcott field trees has a ratio of 13 callose plugs per each PP2 plug. For grapefruit, Valencia and Hamlin orange samples, the ratio range was 1.8 to 4.2 callose/PP2 plug. This data suggests that blocking callose production may be more productive than blocking PP2 production.

Transformation experiments were initiated in efforts to produce plants that over-express the citrus ß-1,3-glucanase gene, both with the constitutive 35S promoter (p35S – BG-35T) and the Suc 2 phloem specific promoter (pSuc2-BG-35T). Using a modified Agrobacterium-mediated transformation protocol, 26 transgenic sweet orange shoots were regenerated containing p35S – BG-35T (Valencia and Vernia sweet oranges) and 24 containing pSuc2-BG-35T (Valencia and Vernia). All have been micro-grafted to Carrizo citrange to expedite their greenhouse evaluation. Twenty transgenic Carrizo shoots were also recovered containing p35S – BG-35T.

Construction of plasmids for protoplast transformation has also been completed for the ß-1,3-glucanase gene, and protoplast transformation experiments using new robust sweet orange suspension lines are being initiated. Protoplast transformation generally results in higher transgene copy numbers than Agrobacterium-mediated transformation.

Twenty-eight genes containing a signal peptide (identified by SignalP) were cloned in TMV30b GFP viral vector. Thirteen constructs were successfully assayed in tobacco plants for symptom expression. Two (AS7 and AS13) out of the 13 showed interesting symptoms, and hence were selected for further characterization. AS 7 produced wilting and death of the whole tobacco plant within 2-3 weeks. AS13 transformed plants developed phyllody, stunting and very clear growth defects. The symptoms were significantly different from the infection using the empty vector (TMV 30BGFP). The expression of these genes in planta was checked using RT-PCR. The leaf, petiole and roots of tobacco plants infected with AS7 were collected after ~3weeks for observation under the light microscope. AS7 caused very clear phloem collapse in the petiole, after 3 weeks of inoculation. These symptoms were not observed earlier, 10 days after infection.

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Contract Number 2

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

Total Funds (current year) $125,000.00

SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.