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)

Diverse transgenic strategies mobilized for HLB resistance

Proposal Title

Production of Transgenic Commercial Cultivars Resistant to HLB and Canker

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
6/7/11	Citrus Research and Development Foundation	Transgenic/Metagenomic Mediation of Resistance

REPORT UPDATE (500 words; summarize your accomplishments)

Huanglongbing (HLB) and Citrus Bacterial Canker present serious threats to citrus production in the US. Insertion of transgenes conferring resistance to these diseases or the HLB insect vector is a promising solution. Genes for antimicrobial peptides (AMPs) with diverse promoters are used to generate numerous transformants of rootstock and scion genotypes. New promoters and/or transgenes are being regularly introduced with more than a thousand new transformation attempts on citrus epicotyl sections each week. Plants from the initial round of scion transformations are now replicated and are being exposed to HLB, using graft inoculations and CLas infected psyllids in greenhouse and field environments. Challenge with HLB through exposure to infected ACP (D. Hall collaboration) is being conducted on a replicated set of 33 independent Hamlin transformants, 5 Valencia transformants, 4 midseason transformants, and 3 non-transformed controls.

A series of promoters were tested with the GUS gene. The three vascular-specific promoters show expression only in phloem and xylem, while other promoters show broad expression in tested tissues. Sucrose synthase promoter from Arabidopsis drives high GUS expression more consistently than citrus SS promoter or a phloem promoter from wheat dwarf virus. A ubiquitin promoter from potato drives unusually consistent and high GUS activity. Use of this promoter may reduce the number of independent transformants needed. A new ubiquitin promoter from citrus (Belknap) is being tested.

CLas sequence data target a transmembrane transporter (Duan collaboration),as a possible transgenic solution for HLB-resistance. In E. coli expressing the CLas translocase, two exterior epitope-specific peptides suppressed ATP uptake by 60+% and are being tested further for suppression of CLas in culture, before creating transgenes. ARS-Albany (Belknap) collaboration is providing genes from Carrizo citrange sequence generated using USDA and now CA-CRB funds, and other citrus genomic data, to permit transformation and resistance using citrus-only sequences; citrus-derived T-DNA border analogues have been shown to be effective in producing transgenic Carrizo and tobacco and are being tested in citrus scions. Sequence data are being mined for citrus AMPs and defensins to test in-vitro and ultimately in-planta. Anthocyanin production genes,give bright red shoots (UGray collaboration) and are being tested as a visual marker for transformation, as a component of a citrus-only transgenic system.

Transgenes have been developed to suppress (using an RNAi strategy) a lectin-like protein produced in the phloem of HLB-infected citrus. It is possible that suppression of this protein may significantly reduce disease symptoms.

High throughput evaluation of HLB resistance will require the ability to efficiently assess resistance in numerous plants. Graft-inoculation, controlled psyllid-inoculation, and "natural" psyllid inoculation in the field are being compared. The first trial has been in the field for 30 months and a repeated trial has been in the field for 18 months. Leaf samples have been collected monthly and PCR analysis of CLas conducted.

Several new collaborations are being explored to feed new HLB-suppressing transgenes and novel strategies into the citrus transformation pipeline.

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Organization USDA/ARS/USHRL

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Total Funds (current year) $104,325.00

SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.