Florida Citrus Advanced Technology Program

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

Instructions Complete the fields based on your project specs. When finished, save the form to your local disk using a unique name. Then, go to **http://research.fcprac.com**, and log in with your user name and password using Researcher Login in the lower left. Find this project title and click on **Submit a Report**. Update your profile information if needed, then upload this report as directed.

2009-2010 REPORT	CATEGORY (drop-down)	TODAY'S DATE (m/d/yr)
○ *Quarterly Report* ◉ *Annual Report* ○ *Final*	Genetics	3/11/10

WHAT IS THE "HEADLINE" FOR THIS REPORT (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Significant progress towards faster flowering and fruiting of genetically engineered processing oranges and specialty fruits

TITLE and CONTACT INFORMATION

Proposal Title Accelerating the Commercialization of Transformed Juvenile Citrus

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Organization University of Florida	*Total Direct Funds (current year)* $178,000

REPORT UPDATE (650 words; provide details about your headline)

Juvenile citrus transformation is at least an order of magnitude more efficient and less cultivar specific than mature tissue transformation. If the juvenile period in subsequent trasngenic plants can be overcome quickly, commercialization could be on a time-frame similar to transgenics from mature tissue transformation. Significant progress was made on all primary objectives, and a new research result from a parallel project was obtained that will impact his project significantly - early flowering (11 months after planting) on several completely juvenile scion hybrids in our breeding program was achieved in the RES (Rapid Evaluation System, funded by NVDMC). Work is in progress (in the RES) to test the horticultural manipulations utilized to achieve this result on commercial sweet orange, grapefruit and mandarin scions most important for our industry, for subsequent use with HLB-resistant transgenic lines.

The Agrobacterium-mediated juvenile citrus transformation protocol was improved to increase speed and efficiency: utilizing a combination of improved media, a new anti-oxidant treatment, and a modified micro-grafting technique, we are able to consistently recover and replicate more transgenic citrus plants in half the time.

Alternative transformation methodology: A protocol was developed for the direct transformation of embryogenic callus, and numerous transgenic plants were recovered from OLL-8 sweet orange, W. Murcott tangor,(Afourer/Nadercott), and Ponkan tangerine. This technique clearly extends transformation methodology to other important polyembryonic commercial citrus cultivars, particularly those that are recalcitrant to Agro-bacterium mediated transformation (ie. fresh market mandarin types that are important in Calfornia).

Transformation of Selected Precocious or Potentially HLB-Avoiding Sweet Oranges: Using the improved protocol, transgenic plants of high quality precocious Vernia sweet orange somaclones C2-1-1, C2-1-2 and C2-2-1, Rhode Red Valencia clones avoiding HLB infection (B4-79 and B10-68) in a heavily HLB-infected Martin County grove, and a high quality processing somaclone OLL#8, containing the LIMA anti-bacterial gene were produced. The OLL somaclones are showing mature tree juice quality in juvenile trees. Non-transgenic seedlings of these clones are being grown for evaluation in the RES to determine the effect of scion genetics on length of juvenility.

Rootstock Effect on Length of Juvenility: Juvenile sweet orange scion (OLL-8, a high quality Valencia-type with high solids and enhanced juice color) grafted to 6 selected precocious rootstocks from our breeding program and Carrizo as a control were single-stemmed and planted in the RES. Rootstock effect on the speed of juvenile citrus flowering will be determined.

Transfer of genes to induce precocious flowering: We have cloned each of the genomic sequences of ciFT1, ciFT2, and ciFT3 (Arabidopsis Flowering Locus T genes) into a plasmid vector in which their expression is constitutively driven by the 34FMV promoter. Transgenic Carrizo plants have been produced with all of the ciFT constructs, and are being prepared for evaluation. The occurrence of in vitro flowering also suggested that replacement of the constitutive 34FMV promoter with an inducible promoter may provide a better system for controlling precocious flowering, particularly when ciFT3 serves as the transgene. We have obtained vectors for an estradiol inducible system and the experiments to test inducibility in citrus have begun.