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	7/8/10

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

Progress is being made on clean-up and transformation of mature citrus tissue using several approaches.

TITLE and CONTACT INFORMATION

Proposal Title Agrobacterium-mediated Genetic Transformation of Mature Citrus Tissue

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Organization University of Florida	*Total Direct Funds (current year)* $224,000

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

Researchers at the USDA Ft. Pierce: Progress this past year in the various components of a mature citrus transformation system is as follows: Source of mature tissue) Four populations of adult phase trees were established in the greenhouse including Valencia sweet orange/Sun Chu Sha (73 trees), Ruby Red grapefruit/US812 (62 trees), US-942 citrange rootstock/Cleo (32 trees), Calamondin (31 trees), and Etrog Arizona 861-S1 citron (67 trees). Decontamination protocol) A decontamination protocol was developed that results in >90% clean explants, sufficient for tissue culture studies and practical applications. In vitro bud emergence and growth) A system was developed for the production of in vitro adult phase shoots from cultured nodes of greenhouse trees. Factors important in bud emergence and growth were identified and a system developed to initiate bud emergence and growth with reduced leaf drop. A manuscript has been prepared that documents this research. Shoot regeneration from mature tissue explants) A system was developed for the production of shoots from cultured internodes from greenhouse trees. Factors (e.g., pre-incubation tissue treatments, plant growth regulators, and incubation conditions) important in bud formation and shoot growth were identified and a system developed that is suitable for sweet orange, grapefruit, calamondin, and US-942 (Note: citron was just recently added so has not yet been tested). The system results in shoot and bud formation in 70-90% of the explants. A manuscript is in preparation that documents this research. Agrobacterium-mediated transformation of mature tissue explants) Transformation of mature internode explants from greenhouse trees has been demonstrated in grapefruit and US-942 using the GUS reporter gene. Though these are the first experiments, the results document that we have a functional mature tissue transformation system. Because explants were stained for GUS activity once shoot buds were observed, we can make no predictions on the efficiency of transformed shoot recovery. Current efforts are now directed toward identifying the factors important for a system of sufficient efficiency for routine transgenic plant production. At the CREC in the Gmitter lab, work is continuing on the use of Thin Cell Layers (TCLs) as explants for mature tissue transformation. Experiments have been done to induce regeneration in the TCLs by manipulating the amount of growth regulators, carbon source and also by pre-treating the TCLs with BA but regeneration is still problematic from these explants. In the Grosser lab, Hamlin mature budwood source trees were grown on a selected complex rootstock that seems to have superior nutrient uptake, and nutrition was provided by a new granular slow release product that has been showing excellent results with nursery and field trees. Six transgenic Hamlin lines stably expressing the GFP gene were regenerated from the 1st experiment using the first flush on the grafted trees as explants. In subsequent experiments, subsequent flushes on the same trees yielded no transgenics, indicating that the regeneration potential diminishes with sequential flushes. In the Machado laboratory in Brazil, regeneration ability of sweet oranges Pera, Valencia, Natal and Hamlin was evaluated by testing: different hormone combinations, the effect of the physiological age of the sprouting, and CTV infected and free tissue. Remarkable differences between genotypes regarding the capacity to transform juvenile tissues with Agrobacterium were observed. Optimum conditions, tissues and genotypes are being used in experiments with mature tissues. In the Moore laboratory in Gainesville, experiments have focused on using small peptides as vehicles to deliver cargos to plant tissues. If these techniques could be worked out they would have a number of applications for citrus transformation, perhaps even eventually allowing the transfer of genes or gene products to existing trees. Experiments this year demonstrated that enzyme (in this case GUS) could be delivered into plant tissues, including whole alfalfa seedlings, mung bean roots and citrus suspension cultures.