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	4/15/10

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

Principal Investigator Gloria A Moore	*PI Last Name* Moore
Email gamoore@ufl.edu	*FDACS Contract Number* NAS144
Phone 352.392.4711x221	*Project Duration (years)* 3 *Year of Project* 1
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 have established an in vitro system for growing mature tissue buds in vitro as a tissue source for transformation. One severe problem that they ran into was leaf drop. They have been able to reduce leaf drop substantially (though not eliminate it entirely) and are preparing a manuscript to document this work. The other area where they have made progress is shoot regeneration from mature tissue derived from greenhouse trees. We are preparing a manuscript on this as well. We have started transformation experiments but have run into some Agrobacterium overgrowth issues and are looking into that now. However, given the relatively high levels of shoot regeneration (Valencia, Ruby Red grapefruit, Calamondin, and US-942) that they are seeing they are anticipating that transformation should be pretty straightforward. Their best shoot regeneration is from greenhouse trees. Though they get shoot regeneration of in vitro derived shoots they do not believe that it is quite sufficient for routine transformation (Note: They are still working this part out, so it is not yet clear if in vitro tissue is a viable source of tissue for transformation). At the CREC in the Gmitter lab, work in continuing on the use of Thin Cell Layers (TCLs) as explants for mature tissue transformation. Experiments were done this quarter 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. Therefore they have recently additionally initiated experiments with internodal segments from greenhouse grown plants and are also planning to do preliminary experiments to establish axillary bud transformation. In the Grosser lab at the CREC, transformation experiments were tried this quarter with secondary citrus flushes. These were unsuccessful. Since they obtained positive results with first primary flush tissues, the results suggest that primary flush works better (which is in agreement with what the Spanish group has reported). In the Moore laboratory in Gainesville, experiments continued 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 quarter demonstrated that enzyme (in this case GUS) could be delivered into plant tissues, including whole alfalfa seedlings, mung bean roots and citrus suspension cultures. Work is now underway to determine whether DNA uptake can be achieved.