# 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 | 1/15/10 |

**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

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| *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)**

Each group of collaborators on this project are exploring a different approach to achieve the goal of the project: genetic transformation of mature citrus tissue obtained from trees grown under the challenging conditions present in climates such as that in Florida, thus shortening the time from transformation to evaluation in the field of citrus trees containing promising genes. USDA Ft. Pierce: Development of an in vitro shoot induction and multiplication process to serve as a source of in vitro adult phase tissue explants for transformation. An in vitro tissue culture system was developed using adult phase citrus nodes from calamondin, grapefruit, and sweet orange. Various types and amounts of plant growth regulators, salts and agar concentrations were evaluated for shoot growth. However, grapefruit in particular showed a large amount of leaf abscission in two weeks. Therefore ethylene action was inhibited using silver nitrate and vented lids to prevent abscission. Using a combination of these techniques, we have established a medium and procedure for the in vitro system. The appropriate preincubation tissue treatments, plant growth regulator types and concentrations, and incubation conditions for shoot organogenesis from intermodal explants from greenhouse grown citrus plants were evaluated. As a result, we have identified a protocol that provides shoot regeneration from mature tissue for a majority of explants. Shoots are being micrografted to month old rootstock seedlings. Following this success, we have initiated transformation with Agrobacterium carrying the GUS reporter gene. CREC Grosser: Transgenic lines of 'Hamlin' sweet orange stably expressing the GFP gene have been produced. PCR analysis of 4 of these lines has validated the presence of the C. sinensis codon optimized CEMA antibacterial gene. In all cases, explants for transformation were grown on the vigorous experimental rootstock tetrazyg 'Orange 19'. Three of these lines are large enough and have been propagated by the ex vitro micrografting technique onto vigorous rootstocks. Optimization of protocol for Florida specific conditions is currently underway. Seed from several experimental tetraploid rootstocks selected for vigor and enhanced nutrient uptake were planted to provide liners for a 2010 study to investigate rootstock effects on mature tissue transformation efficiency. CREC Gmitter: Thin cell layers (TCLs) are being investigated as explants. In order to overcome the inhibitive effect of the contamination reduction medium additive PPM on the TCLs, axillary buds from the first flush were cultured onto medium with the addition of PPM and the shoots obtained from these axillary buds were used as the explant source for TCLs cultured onto medium without the addition of PPM. However, these explants have not yet shown any regeneration even though no contamination or browning has been observed. Experiments are being carried out to induce regeneration in the TCLs by manipulating the amount of growth regulators and carbon source. Brazil Machado: The Brazilian collaborator has been delayed in starting the project due to difficulties in getting funding from UF to his institution in Brazil. This has been rectified and he has now initiated experiments. Gainesville Moore: Since all of the other collaborators are making progress on developing in vitro systems, we have focused our efforts on the evaluation of cell penetrating peptides (CPPs) and the possibility of using them as vehicles to transport compounds into mature citrus tissue without the necessity of a tissue culture step. CPPs have been best characterized in mammalian and bacterial systems but there are new and surprising reports that the compounds can also work in plants. CPPs are short peptides that, when present either attached or not attached to another compound, allow the compound to be taken up by cells. Compounds that have been taken up with certain CPPs and under certain circumstances include DNA molecules, RNA molecules, proteins, and antibodies. We are presently evaluating various CPPs in model plant systems to determine how to visualize uptake and identify the most promising CPPs to evaluate in citrus.