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	6/7/10

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

IVIA has completed the training of the Florida-based manager and developed mature transformation systems for Florida

TITLE and CONTACT INFORMATION

Proposal Title Development of transformation systems for mature tissue of Florida varieties, and strategies to improve tree management

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Phone +34 96 34240070	*Project Duration (years)* 3 *Year of Project* 1
Organization IVIA/CREC	*Total Direct Funds (current year)* $488,000

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

The main objective of our project during this first year was hiring a Florida-based faculty scientist that could be trained under our supervision in Spain, for the purpose of learning the mature tissue transformation technology and transferring it to Florida. Moreover, we had the commitment to establish genetic transformation systems for mature materials from the most important sweet orange varieties grown in Florida and the Carrizo citrange rootstock. The Florida-based faculty scientist was hired (Dr. Cecilia Zapata) on October 2009 and a few weeks later started the training at the Instituto Valenciano de Investigaciones Agrarias (IVIA). She has been trained in all tissue culture techniques associated with mature citrus transformation, starting with preparation of the source of material, and ending with the acclimation of transformants in the greenhouse. She will finalize her second 3-month-stay in our lab next July 2010. In this final stage, we are focusing on improving transformation methods for more recalcitrant types, making molecular analysis of the putative transformants and on starting plant material preparation at the greenhouse.
Transformation experiments were performed with three (3) sweet orange varieties: Hamlin, Valencia and Pineapple (used as readily transformable control). Mature Valencia was very responsive to transformation and organogenic regeneration and transgenic plants have been already acclimated in the greenhouse. Hamlin was more difficult to transform due to quality problems with the starting material and tissue culture media, and procedures specific to this genotype were needed. To date, transformants have been also obtained from this orange type and verified as positive using PCR. The plants are still growing in vitro and will be transferred to the greenhouse within a few weeks. Carrizo citrange transformation experiments were initiated later but putative transformants have been already generated and micrografted in vitro.

The second objective of this project is related with the necessity of implementing new cultural practices to be able to survive with the HLB disease in Florida until a definitive solution is found. We have proposed the use of strategies to control tree size and productivity by genetic modification of either the rootstock or the scion through over-expression of flowering-time or gibberellin biosynthesis genes. This could permit to establish reduced but highly-productive trees at higher planting density which would facilitate flush management and mechanical fruit harvesting. For generating more compact and productive varieties, we are ectopically expressing the flowering time genes FT or AP1 from sweet orange in juvenile sweet orange. Additionally, we are overexpressing these same genes in Carrizo citrange in an attempt to modulate its architecture and reduce its size. More than 10 independent transgenic lines have been generated for each construct and genotype. In both genotypes, overexpression of the FT transgene led to early flowering in vitro and poor regeneration. Once transferred to the greenhouse, transgenic plants continued flowering and consequently their vegetative development was generally very poor, indicating that this transgene could be of interest for other biotechnological application but not to modify the architecture of either a scion or a rootstock. In the case of AP1, some of the transformants showed a compact and branched phenotype. This phenotype remained once plants were established in the greenhouse. We are waiting them to flower and fruit possibly next spring. Moreover, for generating a dwarf-dwarfing rootstock, we are making a construct aimed to induce RNA interference to downregulate the expression of a crucial gene in gibberellin biosynthesis, CcGA20ox1, in Carrizo citrange. We will focus in this sub-objective during the second year of the project.