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	8/4/09

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

Genetic transformation of Murraya paniculata is an ongoing challenge

TITLE and CONTACT INFORMATION

Proposal Title Development of transformation techniques for Murraya, to engineer a deadly trap plant

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Phone 1-863-956-1151	*Project Duration (years)* 2 *Year of Project* 1
Organization Citrus Research & Education Center	*Total Direct Funds (current year)* 35,000

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

The seeds of Murraya paniculata were procured from the USDA-ARS National Clonal Germplasm Repository for Citrus and Dates, as well as from local sources in Florida. Seeds have been available only periodically for our experiments. These seeds have been germinated in vitro and used to establish cultures for experiments designed first to improve the efficiency of plantlet regeneration from epicotyl explants, which is a prerequisite for experiments to develop successful genetic transformation protocols for Murraya. Following this step, we conducted a first round of transformation experiments using the plant regeneration protocol that was developed. A tissue culture medium designated as M10 (Murashige and Skoog's (MS) standard medium supplemented with predetermined levels of BA and NAA) was used as the regeneration medium for all the transformation experiments. In efforts to standardize the genetic transformation protocol, experiments were carried out with plasmids previously used successfully for citrus genetic transformation in our lab; specifically we used pCAMBIA2301 and pGreen0029 harbored in Agrobacterium tumefaciens strain AGL-1. Various factors were tested in efforts to develop a standard protocol for transformation, such as varying OD values of the Agrobacterium cultures, the duration of explant incubation time, duration of co-cultivation, and the amount of antibiotic used for selection of transgenic shoots and for Agrobacterium removal. Unfortunately, we were not successful in recovering transgenic plantlets from any of these experimental treatments. Currently, we have designed and implemented new experiments using a number of different Agrobacterium strains to which Murraya may be more susceptible, as well as other plasmid vectors that may be more compatible with this system. Our original intention was to test methods and materials that are routinely used successfully for citrus transformation, to avoid additional and possibly lengthy experiments to assess various other Agrobacterium strains and plasmid constructs. We did so, according to plans, but found that the assessment of other strains and plasmids is unavoidable.