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	5/4/10

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

Principal Investigator Fred G. Gmitter Jr.	*PI Last Name* Gmitter
Email fgg@crec.ifas.ufl.edu	*FDACS Contract Number* 66
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 the local sources in Florida. Seeds have been available only periodically for our experiments. These seeds were germinated in vitro and used to establish cultures for experiments designed first to improve the efficiency of plantlet regeneration from epicotyl explants through adventitious organogenesis. 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 pCAMBIA2301 and pGreen0029 harbored in Agrobacterium tumefaciens strain AGL-1 and pTLAB21 harbored in Agrobacterium tumefaciens strain EHA101. 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 and despite substantial efforts, we were not successful in recovering transgenic plantlets from any of these experimental treatments.

To overcome this regeneration barrier, consequently, we have pursued in parallel the regeneration of Murraya directly from axillary buds obtained from in vitro grown seedlings. A variety of conditions were screened including those relating to the amount of growth regulators, basal medium and carbon source; as a consequence, conditions suitable for micropropagation of axillary buds, as well as induction of multiple shoot regeneration from axillary buds, have been standardized. Prior to genetic transformation experiments, the amount of antibiotics required to inhibit micropropagation culture growth has been determined using non-transformed axillary bud cultures. To study the response of the explants to wounding, wounded (nicked and/or pricked) non-transformed axillary buds have also been cultured. The intact, nicked and/or pricked axillary buds have been co-cultivated with plasmids pCAMBIA2301 and pGreen0029 harbored in Agrobacterium tumefaciens strain AGL-1 and pTLAB21 harbored in Agrobacterium tumefaciens strain EHA101. An experiment was performed using two different sizes of explants while the OD600 of Agrobacterium cultures, duration of co-cultivation and amount of antibiotic were kept constant. The size of the intact as well as the wounded explants influenced their survival. Putative regenerants will be tested for the presence of marker genes. Depending on the response of these axillary buds, various treatments such as OD600 of Agrobacterium cultures, duration of incubation time, duration of co-cultivation and amount of antibiotic used will be carried out to define the requirements for successful genetic transformation of Murraya.