CITRUS ADVANCED TECHNOLOGY PROGRAM

QUARTERLY & FINAL REPORTS: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

▶ INSTRUCTIONS

(●) *Quarterly Report* ( ) *Annual Report* ( ) *Final*

Required: What is the "headline" for this report (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Genetic transformation of Murraya: an elusive target.

Proposal Title

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

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
1/26/11	Citrus Research and Development Foundation	Transgenic/Metagenomic Mediation of Resistance

REPORT UPDATE (500 words; summarize your accomplishments)

Quarterly report April 2011: Reserach to explore optimal various parameters for genetic transformation of Murraya was carried out, including assessments of shoot sensitivity to the selection agent kanamycin using untransformed shoots, determinations of bacterial growth curves, and appropriate and effective antibiotic concentrations for bacterial selection. Using the recently optimized protocol for organogenic shoot regeneration from appropriate seedling tissues, transformation experiments have been initiated using the plasmid pTLAB 21 harbored in Agrobacterium tumefaciens strain EHA 101. This vector contains the genes for kanamycin resistance as a selection agent and GFP (green fluorescent protein) as an easily observed marker. Various factors, including a range of OD values (cell density or concentration in liquid culture) of Agrobacterium cultures, the duration of explant incubation in bacterial cultures, duration of co-cultivation period, and the composition of co-cultivation and regeneration media were tested, and we established an optimal, standardized transformation protocol. Optimal conditions for transformation using shoot tips and lateral buds, to develop an alternative method using a different tissue source should the organogenic approach prove too difficult or inefficient for transformation, were also explored. Regeneration of buds and some shoots occurred from organogenic cultures of longitudinally cut seedling epicotyl segments, following these transformation experiments. Observations of the regenerating cultures revealed several buds and shoots displaying green fluorescence, indicating successful genetic transformation. Their growth was monitored, as well as the stability and uniformity of GFP expression over time. Nearly all of these transformation events proved to be either chimeric or transient, so further production of new transgenic events is being pursued.

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Organization UF-CREC

Contract Number 66

Project Duration (years) 2 *Year of Project* 2

Total Funds (current year) $35,000.00

SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.