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	10/14/09

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

The Arabidopsis MKK7 has been transformed into citrus and the citrus materials for screen has been established.

TITLE and CONTACT INFORMATION

Proposal Title Transferring disease resistance technology from a model system to citrus

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Phone 352-392-0285, 863-956-1151 ext. 1361	*Project Duration (years)* 3 *Year of Project* 2nd
Organization University of Florida	*Total Direct Funds (current year)* $100,000

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

This is a 3-year project with 2 main objectives:

(1) Over-express the Arabidopsis MAP kinase kinase 7 (MKK7) gene in citrus to increase disease resistance (Transgenic approach).
(2) Select for citrus mutants with increased disease resistance (Non-transgenic approach).

For objective 1, we have subcloned the Arabidopsis MKK7 gene into the CTV-based expression vector and transition expression of MKK7 in citrus leaves is underway. We have also subcloned the Arabidopsis MKK7 gene into the plant binary vector pBI1.4T (a pBI121 derivative) and transformed the MKK7 gene into citrus using the Agrobacterium-mediated approach. The MKK7 transgenic plants are growing and characterization of the MKK7 transgenic citrus plants is underway.

For objective 2, we have decided to use the Hamlin suspension cells as starting materials for the selection. The Hamlin cell suspension culture has been scaled up in Murashige and Tucker (MT) liquid medium. Several flasks of the culture are maintained for subculture. To determine the concentrations that will be used in the selection, the Hamlin cells from the suspension culture were grown on MT medium plates supplemented with different concentrations of sodium iodoacetate ranged from 0 to 0.2 mM. Hamlin suspension cells were found to be highly sensitive to the inhibitor. A concentration of 0.1 mM of sodium iodoacetate could completely arrest their growth. Therefore, 0.1 mM of sodium iodoacetate has been used in the selection.

We are also testing hypocotyls of citrus seedlings because plants can be easily regenerated from hypocotyl-derived callus cells. Hypocotyls were grown on MT medium plates supplemented with different concentrations of sodium iodoacetate ranged from 0 to 0.4 mM. Hypocotyls were also very sensitive to the inhibitor. A concentration of 0.2 mM could completely inhibit the growth of calli generated from hypocotyls. We will use 0.2 mM of sodium iodoacetate in selection of hypocotyl-derived calli. We are currently generate mutations in the hypocotyls using fast neutron-mediated mutagenesis. Calli will be generated from fast neutron-treated hypocotyls. The hypocotyl-derived callus cells will be selected on MT medium plates supplemented with 0.2 mM sodium iodoacetate.