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	1/13/10

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 generated

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 (AtMKK7) gene in citrus to increase disease resistance (Transgenic approach).
(2) Select for citrus mutants with increased disease resistance (Non-transgenic approach).

For objective 1, the AtMKK7 gene has been subcloned into the CTV-based expression vector and transition expression of MKK7 in citrus leaves is ongoing. The AtMKK7 gene has also been subcloned into the plant binary vector pBI1.4T (a pBI121 derivative) and transformed into citrus using the Agrobacterium-mediated approach. The AtMKK7 transgenic plants are growing. Conformation of the presence of the AtMKK7 gene in the transgenic plants by PCR and analysis of the expression levels of AtMKK7 in each transgenic line are underway. Resistance of the transgenic lines to citrus canker and greening (HLB) will be characterized when the transgenic plants are ready.

For objective 2, Hamlin suspension cells have been used 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 have tested the resistance of hypocotyls of citrus seedlings to the selective compound sodium iodoacetate and found that citrus hypocotyls are very sensitive to this inhibitor. A concentration of 0.2 mM could completely inhibit the growth of the calli generated from hypocotyls. We will use 0.2 mM of sodium iodoacetate in selection of the hypocotyl-derived calli. We have done irradiation for the first batch of Duncan grapefruit cuttings on 11/2/09. The irradiation dosage was 40G. We found that the irradiated cuttings generated significantly fewer shoots than the control and calli were formed on both irradiated cuttings and the control. The shoots and calli generated on both the irradiated cuttings and the control will be transferred onto selective medium containing 0.2 mM of sodium iodoacetate. We are preparing another batch of explants for irradiation.