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*	Canker Pathology	2/26/10

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

Cybrids of Red grapefruit inherit resistance from kumquat via transfer of mitochondria and chloroplast genes

TITLE and CONTACT INFORMATION

Proposal Title Characterization of canker resistance in citrus plants created by 'Somatic Cybridization' without citrus transformation

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Organization UF, Citrus Research and Education Center	*Total Direct Funds (current year)* $122,717

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

Objective 1: A comparative study of grapefruit (C. paradisi) cv. Duncan, a very susceptible host, and two resistant cultivars of kumquat (Fortunella spp.), 'Meiwa' and 'Nagami', evaluated the mechanisms involved in the resistance of kumquat to the citrus canker. Xcc inoculum densities of 104 to 108 cfu/ml were infiltrated into immature leaves in the greenhouse (in planta) and into detached leaves incubated on water agar plates (in vitro). At higher bacterial inoculum density, kumquat cultivars developed a hypersensitive (HR)-like reaction in the infiltrated area, within a period of 72 h in vitro, and 96-168 h in planta. No symptoms or a few small necrotic spots developed in kumquats at the lower inoculum density. Susceptible grapefruit infiltrated with the same inoculum densities produced no visible tissue alterations at 72 h after inoculation and required 120 h or longer to develop water-soaking, hypertrophy and hyperplasia typical of canker lesions in compatible hosts. Phenotype of the lesions, bacterial population growth, anatomical changes in the infiltrated tissue and early expression of genes related to programmed cell death are indicative of HR that reduces growth of Xcc in the inoculation site and the further development of disease. Microarray produced differential expression of genes in grapefruit and Nagami kumquat inoculated with Xcc. In comparison kumquat expression changes were more marked than those of grapefruit. 172 kumquat and 140 grapefruit ESTs were sequenced and 52.5% of the sequences in the EST library were up regulated at 4 hrs in KN that shared significant sequence similarity with putative senescent related protein, DNA damage related kinases (CHKI), chloroplast binding proteins, chlorophyll carrying proteins, lipoxygenases, multiple complex forming proteins (SKP1), membrane associated peptidases, detoxification related enzymes (such as glutathione S-transferase, ribonucleases, and metallothionein-like protein), transposase, ethylene synthesis and chlorophyll related genes. In DG, sequence identities included major facilitator superfamily (MFS-1) phosphorylases and transferase, lectin related protein precursors, aspartyl tRNA synthethase, phosphatase 2A inhibitor, NADH dehydrogenase, catalase, chloroplast heat shock proteins. Genes associated with hypersensitive responses found in the citrus EST database, like lipoxygenase, superoxide dismutase (SPD), and glutatione peroxidase (GLP), were expressed early in kumquats, but not in grapefruit. A study is underway to confirm whether similar responses occur in the somatic cybrid of rough lemon with maternal 'Meiwa' kumquat.

Objective 2: Validate the inheritance or resistance for cybrids of susceptible Red grapefruit. The in vitro assay was used to evaluate the leaf resistance of 22 cybrids of Ruby red grapefruit with 'Meiwa' kumquat compared to grapefruit and kumquat parents. 'Meiwa' reduced lesions per leaf 88% and populations of Xcc were 7 log cfu per inoculation site for grapefruit compared to 2.5 log units for kumquat. All cybrids significantly reduced lesion number compared to grapefruit. The range of reduction varied widely from 20% to 88%. The most resistant cybrid produced an equivalent reduction in lesions produced by HR-resistant 'Meiwa'. Populations of Xcc in cybrids also varied widely from 7 to 2.2 log units. Thus, resistance in cybrids may be inherited at different levels depending which sets of genes for resistance/HR contained in the mitochondrion or chloroplatst genomes are transferred to grapefruit in the cybridization process. In year 2 of the project, Cybrids of early oranges and more Cybrid lines of red grapefruit will be constructed and analyzed for phenotypic and genotypic inheritance of resistance using in vitro assay and HR and mitochondrion gene markers. The current set of Ruby red grapefruit cybrids will be grown out for observation of true-type-morphology and planted in canker-affected locations for observation of vigor and fruiting characteristics, as well as resistance to canker.