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	10/5/09

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

Second Quarterly Report - focus on citrus transformation

TITLE and CONTACT INFORMATION

Proposal Title Genetic Resistance to Citrus Canker Conferred by the Pepper Bs3 Gene

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Organization Two Blades Foundation	*Total Direct Funds (current year)* 208,880

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

Assessment of Bs3 activity in citrus remains our primary initial objective. We have three approaches under development:
1) Transient transformation by Agrobacterium infiltration. We have been working to establish a reproducible Agrobacterium-based citrus transformation system. Our first efforts have focused on leaf infiltrations with fluorescent (GFP) reporter gene constructs and examination by confocal microscopy. The method has not performed adequately thus far, with the appearance of fluorescent materials in the GFP channel which may be phenolic compounds. Although we can subtract out autofluorescence, these compounds are still visible and complicate the interpretation of the experiments. Consequently we resynthesized gene constructs with the GUS reporter gene. We continue to test the GFP constructs with appropriate controls, but we have now also begun the use of the GUS constructs. We have also tested transient transformation by dip inoculation of various citrus plant parts, as well as infiltration of intact citrus fruits. Most of the testing has been with 35S: GUS marker gene constructs, and we have observed GUS staining of albedo in sweet orange and other citrus fruits. We will continue to examine the Bs3 GFP and GUS constructs in the fruit assay to assess the function of both marker genes and Bs3 by these approaches.
2) Transient transformation using particle bombardment. We have initiated this additional transient approach to test a standard assay for resistance gene function - co-bombardment of reporter genes with or without a resistance gene that cause cell death. In these assays, a GUS reporter gene produces individual transformed cells that turn blue, whereas in the presence of the R gene, the blue signal is lost due to a highly localized hypersensitive response. These experiments require a different set of gene constructs, which have now been made and will be testing with a particle gun at UF.
3) Stable transformation using Agrobacterium. Our stable transformation protocol requires citrus seedlings, which must be germinated from seasonally-available seeds. Seeds are now available, and the first experiments have been set up. These experiments included the following constructs: (i) 35S:Bs3-GFP (constitutively expressed Bs3 gene) (ii) 35S:Bs3m-GFP (constitutively expressed inactive Bs3 gene) and (iii) Bs3:Bs3-GFP (the Bs3 gene under control of its own promoter). We will assess GFP expression, cell death, and growth of X. citri. First results will be available later in October.

In addition to the transformation work, we have completed assembly of a multi X. citri strain-inducible Bs3 gene construct. The original Bs3 promoter, which has an activation site for AvrBs3, was modified to contain fourteen additional activation sites for TAL effectors from all four X. citri A strains, as well as A*, Aw, C and B strains (Table 2 of our Project Narrative). This promoter is currently used to drive the GUS reporter gene, will be used in planta to test which X. citri strains activate the complex promoter. The design is facilitated by the recent discovery of a simple code between amino acid residues in TAL effectors and specific DNA bases defining protein-DNA interaction, in the manner of zinc finger proteins (in press, Science). The results will inform commercial constructs with Bs3 conferring disease resistance.

2Blades has gained the capability to do whole genome sequencing in our new laboratory, and we will begin sequencing of several X. citri strains to examine TAL effector sequence diversity.

Coordination of the project is facilitated by participation of all the parties in regular conference calls to discuss results and next steps.