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)

Third Quarterly Report - Successful development of a specific, inducible, citrus canker-limiting system

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)

In the past quarter, we have succeeded in developing a transgene construct for citrus which is transcriptionally activated by TAL effector proteins delivered by Xanthomonas citri. TAL-induced promoter activation triggers a localized hypersensitive response (HR), a typical plant disease resistance response which generally results in a reduction of bacterial growth.

The transgene was developed based on the two key features of the pepper Bs3 gene; a tightly regulated pathogen-inducible promoter and an encoded protein, Bs3, that triggers an HR and mediates resistance to Xanthomonas. Because it was not know if the activity of Bs3 as an HR-inducing protein would function in citrus, we tested another gene in parallel for the protein known as AvrGf1 from the X. citri Aw strain. Unlike the predominant A strains, Aw strains elicit an HR on all known commercially grown citrus species, specifically due the expression of AvrGf1 (Rybak et al., 2009, Mol Plant Pathol 10:249-262).

Using transient transformation assays, we looked for the production of an HR caused by expression of Bs3 or AvrGf1 in Duncan grapefruit leaves. Indeed, constitutive expression of AvrGf1 produced a robust HR, demonstrating that ectopic expression of AvrGf1 in plants is sufficient to trigger localized cell death. Analysis of constructs in which the AvrGf1 gene is under transcriptional control of the Bs3 promoter demonstrated that in the absence of TAL effectors no reaction was evident on leaves. However upon co-inoculation with X. citri strains containing the TAL effector AvrBs3, the transgene construct produced a robust HR. The tight regulation of the Bs3 promoter and its transcriptional activation by AvrBs3 through its specific recognition sequence is well characterized in pepper and tobacco (Romer et al, 2007, Science 318:645-648), and now confirmed in citrus. We further tested this reaction with X. citri strains that are deficient in their system for delivering effectors into plant cells, and we observed a loss of the HR, confirming that the reaction specifically requires the presence of AvrBs3 in the plant cell. Thus far, we have not observed an HR in response to Bs3 expression in transient assays and continue to test Bs3 in stable transformation assays and particle bombardment experiments. AvrGf1, however, makes an effective alternative to Bs3. Additionally we are in the process of testing a complex Bs3 promoter that has 14 recognition sequences for all currently known X. citri TAL effectors. We will test whether stable citrus transformants containing the complex promoter driving AvrGf1 or Bs3 expression confer an HR in response to a range of X. citri strains.

We have initiated experiments to examine the effect of TAL effector-induced AvrGf1 expression on bacterial growth in grapefruit leaves. In these assays, X. citri is inoculated onto leaves transiently transformed with control or test Bs3 promoter constructs and the growth of bacteria is assessed over time. In our first experiment, we observed that the presence of the Bs3 promoter:AvrGf1construct lowered the amount of an X. citri strain carrying AvrBs3 1000-fold compared to controls lacking the construct. This result is very promising and suggests that the constructs we are developing are capable of conferring disease resistance to citrus canker by restricting X. citri growth in transgenic citrus plants.