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	5/11/10

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

Annual Report - Successful transient resistance to citrus canker

TITLE and CONTACT INFORMATION

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

Principal Investigator Diana Horvath	*PI Last Name* Horvath
Email dmh@2blades.org	*FDACS Contract Number* 102
Phone 847 425 1277	*Project Duration (years)* 3 *Year of Project* 1
Organization Two Blades Foundation	*Total Direct Funds (current year)* 208,880

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

Our group made outstanding progress in the first year of FCATP funding, accomplishing all of our original first year goals and making significant progress on our second year goals already. In brief, we determined the binding sites for all fourteen known Xanthomonas citri TAL effectors and combined these into new Bs3 promoter constructs. Thirty-two constructs and controls were prepared in the Lahaye lab with different Bs3 promoter and Bs3, AvrGf1, and reporter gene combinations and sent to Gainesville.

Constructs were introduced into grapefruit in both transient and stable transformation experiments:
- A transient Agrobacterium transformation and assay system was successfully developed for grapefruit leaves. In this system, promoter constructs were introduced into Agrobacterium strains and infiltrated into leaves. Infiltrated areas were assessed over several days for the appearance of a hypersensitive response (HR) produced by the Agrobacterium strain or following co-inoculation with strains of X. citri carrying various citrus TAL effector/Pth A genes. We demonstrated that only the combination of specific X. citri strains and Bs3 constructs containing binding sites recognized by TAL effectors in those strains produced a strong HR.
- Stable transformations were carried out with nine different promoter constructs, producing more than 300 plantlets on tissue culture, with more than 50 rooted and transferred to soil. There are many more transformed epicotyls in progress. Positive transformants will be identified and tested by X. citri inoculation.

In addition, we tested our constructs in growth assays to assess their effect on X. citri growth in grapefruit leaves. Whereas the original Agrobacterium strains used in our study had little to no effect on X. citri growth and Agrobacterium strains carrying the Bs3 promoter constructs had a small effect on X. citri growth, the population of X. citri on leaves co-inoculated both with Agrobacterium containing Bs3 constructs and X. citri strains with corresponding TAL effectors was dramatically reduced (see below). Population studies will be carried out on stable transformants to identify lines for product development.

Growth of X. citri at 6 days post inoculation on grapefruit leaves, following transient transformation treatments (average from two experiments):

Pre-inoculation treatment:			X. citri growth (Log 10 CFU/ml):
Agro strain	BS3 promoter construct	TAL effector	
-	-	-	8.50
+	-	-	8.41
+	+	-	7.43
+	+	+	4.80

Lastly, we have begun testing the responses of the diverse accessions of X. citri available to the Jones lab.