CITRUS ADVANCED TECHNOLOGY PROGRAM

QUARTERLY & FINAL REPORTS: Control of Citrus Greening, Canker & Emerging Diseases of Citrus

SELECT PERIOD September 2013

(●) *Quarterly Report* ( ) *Final*

Proposal Title

TAL Effector induced resistance to Xanthomonas

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
10/15/13	Citrus Research and Development Foundation	Transgenic/Metagenomic Mediation of Resistance

REPORT UPDATE (500 words-It is not necessary in this public report to disclose your institution's proprietary information or intellectual property.)

Function of individual X. citri transcription activator like effectors (TALEs):
- Xanthomonas citri strain 2090 from Florida does not contain a typical 17.5 RVD TALE essential for typical pustule formation, but does contain pthA3 15.5 RVD. We cloned this gene and expressed in Xanthomonas citri subsp. citri 306 Δ pthA1-4 and tested transiently with the 14 EBE promoter:GUS construct in grapefruit leaves. Activity was very low for this TALE, suggesting it may play a marginal role.
- Xanthomonas citri strain #93 carries two TALEs - pthC 14.5 RVD and pthC2 17.5 RVD. These were tested in the transient assay and found to weakly activate the 14 EBE promoter:GUS construct in grapefruit leaves. We used an in silico tool - Talvez (http://bioinfo.mpl.ird.fr/cgi-bin/talvez/talvez.cgi, Pérez-Quintero et al., 2013) to scan citrus genomes for TAL effector binding sites, which suggests that PthC2 has differential ability to activate host genes compared to PthC.

Transformation:
Whereas we observed the expected behavior of gene constructs in transient assays, we have been unable to isolate stable transgenic citrus lines with functional gene constructs. We continue to explore multiple approaches to overcome this technical issue:
1. We tested Carrizo for transformation with the 4 EBE promoter:avrGf1 and challenged with Xanthomonas citri subsp. citri strain 306 and 306 transconjugant carrying avrGf1 into young leaves. The carrizo genotype gave significant resistance for transconjugant Xcc 306: avrGf1 with pathogenicity test infiltrated at 10-3 cfu/ml bacterial suspension.
2. The 14EBE promoter construct efficiency is being tested in tomato; binary vector was engineered with NosT: ProBs314EBE :avrBs4: NosT in T-DNA. AvrBs4 when expressed in tomato results in hypersensitive reaction. The Binary construct in agrobacterium strain Agl-1 was used for transforming tomato Bonny Best and large Red Cherry varieties. Transformant screening is in progress for assessing resistance to bacterial spot disease.
3. We sent two of our constructs for testing in parallel at a contract transformation lab The facility at UC Davis compared the 14 EBE:GUS and 14 EBE:AvrG1 constructs with their standard control plasmid in both Carrizo and tobacco. They found that both of our constructs gave a far lower transformation efficiency than their standard in both tobacco and citrus, and transformants with GUS recovered so far have not showed GUS activity. These results indicate that a likely source of the difficulty we have encountered is the vector.
4. To date we have prepared four promoter constructs in another vector that we use commonly and have good success with. The first set of these transformants are in soil, and we will be able to test the integrity of the inserts by PCR in a few weeks. Preliminary histochemical screening for successful transformation in putatively transgenic shoots of grapefruit, sweet orange and Carrizo showed that transformation has been successful and that transformation was significantly higher in Carrizo than in grapefruit or sweet orange.

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Form PR-12 Quarterly Reports **SUBMIT REPORT**

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.