CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD March 2014

(●) *Quarterly Report* ( ) *Final*

Proposal Title

TAL Effector induced resistance to Xanthomonas

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
4/15/14	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.)

This quarter we have made good progress on our transformation approaches:

1. Stable transformations in citrus using new vectors.
Transformation experiments were carried out in Duncan grapefruit and carrizo with 5 constructs in a preferred vector backbone, including an empty vector control . 52 carrizo transformants were selected and analyzed by PCR. The genes of interest that were screened for included avrGf1, avrGf2, and NPTII. PCR results showed that 5 of 6 transformed with the avrGf1 gene were positive and 9 of 30 plants were positive for the avrGf2 gene. NPTII was detected in 22 of the 52 carrizo plants. 6 out of 7 Duncan plants were positive for NPTII. These results demonstrate improved transformation efficiency using this vector backbone.

Further epicotyl transformation experiments were carried out with both 'Duncan' grapefruit (624 segments) and 'Pineapple sweet orange (136 segments) with 6 constructs. These segments were placed on media without selection (kanamycin). This modification was done to see if transformation efficiency would improve. Rooting experiments and transplanting segments are ongoing. Of 1,507 transformants selected so far, 168 putative transgenic shoots of grapefruit, sweet orange and Carrizo were confirmed as transgenic by GUS assay (35S:GUS construct in vector) this period. To date, although no GUS positive or chimeric shoots have been observed for the sweet orange cultivar, Duncan grapefruit and carrizo citrange had totals of 4 and 134 GUS positive shoots, respectively. We will continue to screen and progress positively transformed plants for further analysis. Pathogenicity tests will be carried out as soon as plants reach adequate size.

2. Stable transformation in test systems
Tobacco:
N. tabacum lines transformed with the 14 EBE promoter fused to GUS, generated at the UC Davis transformation facility, were tested by GUS assay in the presence and absence of the X. citri effector PthA4, delivered as a 35S expression construct via Agrobacterium. Nine of 10 of the lines showed GUS expression only in the presence of PthA4. This system is now established as functioning and will provide a rapid means of testing the ability for individual and combinations of X. citri effectors to induce the promoter construct. We are in the process of harvesting seed for full scale screening.

Tomato:
A second test system was designed and tested in which the 14 EBE promoter was fused to the avrBs4 gene capable of inducing a hypersensitive reaction in tomato. Positive transformants of Bonny Best and large Red Cherry tomato cultivars were confirmed using DNA dot blot. T0 transgenic tomato were screened for pathogenicity reaction with X. euvesicatoria strains (8510 avrBs3 transconjugant & Race 9) and X. gardneri, and several Bonny Best and large Red Cherry were found to be resistant. This demonstrates that this test system, in which resistance is induced by the effectors AvrBs3 and AvrHah1, is functional for conferring stably-transformed transgenic disease resistance. We are harvesting seed from transgenic plants and are in the process of testing seedlings for resistance.

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

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.