CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD September 2014

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Engineering PAMP-receptor mediated broad spectrum resistance to HLB and canker

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
10/15/14	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.)

Objective 1: Generate functional EFR variants (EFR+) recognizing both elf18-Xac and elf18-CLas.
In order to perform screening on complex EFR mutant libraries required to discover mutants which respond to elf18-CLas ,we have been developing a FACS-based screen. To this end we have generated a number of reporter lines (using GFP) in both suspension cultures and transgenic Arabidopsis plants. The reporter lines are driven by the FRK1, WRKY30 and PER4 promoters. We have tested two PER4p:GFP cell suspension lines for responsiveness to elf18, and both of these give clear induction of the reporter gene following treatment. However, when protoplasts were produced of these lines, elf18 responsiveness was no longer observed. We are currently retesting these lines to ensure the buffer conditions and EFR expression is correct. In addition, plant and cell suspension lines transformed with FRK1p:GFP and WRKY30p:GFP are also in the process of being tested.

In addition to the mutagenesis approach, we screened the Nordborg collection of Arabidopsis ecotypes for sensitivity to elf18-CLas or reciprocal chimeric peptides of elf18-Ecoli and elf18-CLas. Of this collection, none show ROS in response to either elf18-CLas or the chimeric peptides. We did observed one line (Se-0) which had enhanced response to the CLas-Ecoli-elf18 chimera. This chimera has some activity in Col-0, but only at high concentrations. Further testing of this ecotype revealed that it also enhanced ROS to elf18-Ecoli and to flg22, indicating that it was not a variant of EFR which was causing the enhanced ROS. Indeed the sequence of EFR from Se-0 contains no non-synonymous SNPs.

We have been also investigating the possibility of targeting other PAMPs. To this end we conducted a bioinformatic comparison of known PAMPs with those in C. Liberibacter asiaticus. From these search we identified CSP22 (Felix & Boller, JBC 2003, 278:6201) as a potential candidate, since it is conserved in the sequence required for recognition. We are currently waiting for delivery of the CLas-CSP22 peptide to test.

Objective 2. Generate functional XA21-EFR chimera (XA21-EFRchim) recognizing axYS22-Xac.
These constructs have been constructed and tested and a manuscript is under revision.

Objective 3: Generate transgenic citrus plants expressing both EFR+ and XA21-EFRchim.
Transformation experiments are ongoing; to date, a total of 5,781 'Duncan' grapefruit and 956 sweet orange segments have been collectively transformed with the constructs EFR, EFR-XA21, EFR-XA21-EFRchim and pCAMBIA2201 (empty vector control). A total of 580 and 219 grapefruit and sweet orange shoots, respectively, were transferred to rooting media. These shoots were first analyzed histochemically for GUS expression. The results show that collectively 6 grapefruit shoots were GUS positive with the constructs EFR-XA21, EFR-XA21-EFRchim and pCAMBIA2201 and 1 sweet orange shoot GUS positive with the construct EFR-XA21-EFRchim. Other grapefruit shoots (47) collectively stained partially (chimeric) for GUS with the constructs EFR, EFR-XA21, EFR-XA21-EFRchim and pCAMBIA2201, while 5 sweet orange shoots stained chimeric for GUS with the constructs EFR, EFR-XA21 and EFR-XA21-EFRchim. Chimeric shoots were those segments with less than 85% of blue staining.

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

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.