CITRUS ADVANCED TECHNOLOGY PROGRAM

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

SELECT PERIOD March 2012

(●) *Quarterly Report* ( ) *Final*

Proposal Title

Anti-psyllid artifical miRNAs are being evaluated in plants

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
10/14/13	Citrus Research and Development Foundation	Vector Management

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

We are developing and evaluating optimal RNA interference constructs in attempts to target Diaphorina citri, the psyllid vector of Ca. Liberibacter asiaticus, the causal agent of citrus greening. We are using two psyllid species for our work, D. citri and Bactericera cockerelli, the latter being the potato psyllid. Using B. cockerelli offers the opportunity to use herbaceous plants and make more rapid progress that can be applied to citrus and D. citri. So far, 7 pAMIRNAs targeting the BC-ATPase gene (pAMIRA1, pAMIRA1c, pAMIRA2, pAMIRA2c, pAMIRA3, pAMIRA3c, and pAMIRA2PE) and 4 pAMIRNAs targeting the GFP gene (pAMIRG1, pAMIRG1c, pAMIRG2, and pAMIRG2c, as controls) were constructed into the viral vector of geminivirus, and cloned into the binary vector pCB301. Our miRNA Northern blot analysis showed that in Nicotiana benthamiana, plants, the Tomato bushy stunt virus (TBSV) p19 silencing suppressor was needed in order to have high amountsof artificial miRNA (amiRNA) accumulation, while in Nicotiana tabacum, p19 triggered cell death and caused necrosis at the infiltrated area, which resulted in low amiRNA accumulation. The clones that are producing amiRNAs in N. tabacum, tested by Northern blots, are being used for psyllid feeding experiments. The target mRNA expression levels in the tested psyllids will be examined by RT-real time PCR. The amiR-TAV-pCB301 constructs will also be infiltrated into different citrus plants, and then tested by Northern blot analysis to assess for amiRNA accumulation. To mimic endogenous primary miRNAs, all the artificial primary miRNA constructs were in vitro transcribed by the mMESSAGE mMACHINE High yield capped RNA transcription kit (Ambion). The in vitro transcripts were added into a 15% sucrose artificial diet, and used for in vitro psyllid feeding tests. 11 pAMIRNAs were also cloned into the agroinfection-compatible Tobacco mosaic virus expression vector. To test if the amiRNAs can be delivered by TMV viral vector, we will do miRNA Northern blot analysis to confirm the amiRNA accumulation in plants. Using different agroinfiltration and plant viral systems to deliver amiRNAs will help us to identify the optimal versions to apply to citrus in order to target Diaphorina citri.

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

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.