CITRUS ADVANCED TECHNOLOGY PROGRAM

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

▶ INSTRUCTIONS

(●) *Quarterly Report* ( ) *Annual Report* ( ) *Final*

Required: What is the "headline" for this report (e.g. a one-sentence "newspaper headline" describing what you accomplished)

Pholem-specific promoter used to express resistance gene

Proposal Title

Engineering citrus for resistance to Liberibacter and other phloem pathogens

Today's Date	Sponsoring Organization (drop-down)	Category (drop down)
8/22/11	Citrus Research and Development Foundation	Transgenic/Metagenomic Mediation of Resistance

REPORT UPDATE (500 words; summarize your accomplishments)

Objective 1: Transform citrus with constitutively active resistant proteins (R proteins) that will only be expressed in phloem cells. The rationale is that by constitutive expression of an R protein, the plant innate immunity response will be at a high state of alert and will be able to mount a robust defense against infection by phloem pathogens. Overexpression of R proteins often results in lethality or in severe stunting of growth. By restricting expression to phloem cells we hope to limit the negative impact on growth and development.

Results: The transgenic plants containing AtSUC2/snc1 and AtSUC2/ssi4 mutants, as well transgenic control plants are growing in the laboratory of Dr. Orbovic at the UF Citrus Research Facility (Lake Alfred) until they are ready for the next level experiments.

Objective 2: Develop a method to elicit a robust plant defense response triggered by psyllid feeding. By further restricting expression of the R protein to a single cell that is pierced by the insect stylet, we anticipate that a defense can be mounted without a manifestation of a dwarf phenotype.

Results: The vast majority of T1 and T2 transgenic Arabidopsis plants expressing snc1 and ssi4 mutant coding sequences under the control of the AtSUC2-940 promoter have wild type phenotypes. Although the AtSUC2 promoter has been reported to be phloem-specific, we have found that it often does not maintain this tissue-specific pattern of expression in transformed Arabidopsis. However, despite the likelihood of expression in tissues other than phloem, only a few transformants showed any negative developmental or growth abnormalities. This lack of a negative phenotype in Arabidopsis provides a basis for optimism for similar results in transformed citrus.

Our working hypothesis is that expression of the constitutive R proteins (mutants) in the phloem will active components of the innate immunity response to provide enhanced protection from Liberibacter infection in phloem cells. In order to monitor the activation state on the innate immunity system, we will cross the R protein transformants with transformed Arabidopsis lines containing pathogen-inducible promoters driving GUS reporter genes. We cloned the PR2 (also known as BGL2), and PR5 pathogen-inducible promoters in front of the GUSplus gene in pCAMBIA 2301. They were sequenced, transformed via electroporation into Agrobacterium tumefaciens strain GV3101 and introduced into Arabidopsis (strain GV3101) through the floral dip protocol in order to generate stable transgenic lines. We currently await the T-1 seeds from these transformations. In parallel, we acquired BGL2-GUS (in pBI101 vector; from Dr. Xinnian Dong from the Duke University) stable transgenic line to use as an alternative donor. The introduction of our R protein constructs into reporter lines by crosspollination will be faster and more efficient than transformation by agrobacterium. Being able to monitor constitutive activation of the innate immunity system by GUS will provide a test of the hypothesis that our constructs will activate pathogen-inducible promoters and will allow us to select lines that have strict phloem-specific expression for further study.

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SUBMIT REPORT

CITRUS RESEARCH AND DEVELOPMENT FOUNDATION, INC.