APPLICATION OF *AGROBACTERIUM RHIZOGENES*-MEDIATED TRANSFORMATION STRATEGIES FOR (A) RAPID HIGH THROUGH PUT SCREEN FOR GENETIC RESISTANCE TO PIERCE'S DISEASE IN GRAPE THAT MAINTAINS THE CLONAL INTEGRITY OF THE RECIPIENT HOST, AND (B) RAPID SCREENING FOR VIRULENCE DETERMINANTS IN *XYLELLA FASTIDIOSA*

***Principal Leaders*:**

David Gilchrist
CEPRAP & Dept. of Plant Pathology
University of California
Davis, CA 95616

George Bruening
CEPRAP and Dept. of Plant Pathology
University of California
Davis CA

James E. Lincoln
CEPRAP
University of California
Davis, CA

Andrew Walker
Dept. of Viticulture and Enology
University of California
Davis CA

Cooperators:

Bruce Kirkpatrick
Dept. of Plant Pathology
University of California
Davis CA

Edwin Civerolo
Dept. of Plant Pathology
University of California
Davis CA

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This brief report will summarize the results that have been accomplished for the two-month period August 1 to October 1, 2001 dating from the receipt of funds.

INTRODUCTION

The goal of this project is to identify novel genes from either grape or heterologous plants that, when expressed in grape, will lead to disruption of infection or spread of the xylem-limited bacteria, *Xylella fastidiosa.* There is no useful genetic resistance in commercially used grape clones, and introgression of resistance from grape relatives by sexual crossing introduces substantial genetic variation. Introgression of resistance would be most useful if it were introduced directly into vegetative tissue without requiring recurrent selection to attempt to return to the original host genotype. We have developed a functional screen for cDNAs that block either bacterial multiplication, movement or symptom expression using an *A. rhizogenes* mediated transformation strategy that also enables the direct introgression of cloned resistance genes into a susceptible host plant while maintaining the clonal integrity of the recipient plant following transformation.

OBJECTIVES

The research plan includes (a) a rapid functional screen for genes that confer resistance to Pierce's disease (PD) in transformed grape tissue and (b) an analysis of potential pathogenicity factors of the PD causative agent, the bacterium *X. fastidiosa*. Approximately 85% of the research effort is devoted to sub-objective (a) and 15% to sub-objective (b).

Sub-objective (a): Screening for resistance genes in *Agrobacterium rhizogenes*-induced *X. fastidiosa*-infected hairy root cultures. (85% effort)

The goal is to rapidly identify resistance genes in grape genotypes that block any one of several required steps in the infection and spread of *X. fastidiosa* in the xylem.

RESULTS AND CONCLUSIONS

Transformation with *A. rhizogenes*

The method of delivery of the cDNA libraries into grape is now established in our laboratory. In the pasts two months we

have confirmed that grape is readily transformed by *A. rhizogenes* and that foreign genes (green fluorescent protein, GFP), including our new cDNA libraries, can be expressed readily in grape by this method. In the case of the roots expressing GFP driven by the 35S promoter, all roots were highly fluorescent when viewed under a fluorescence microscope.

Transformation of infected grape with *A. rhizogenes*

We have established *Xylella* infections in the xylem of *V. vinifera* (Chardonnay) and transformed test genes into roots derived from infected stem sections by *A. rhizogenes*. Transformed root induction occurred equally well on both infected and healthy stem sections. Interestingly, and perhaps fortuitously, the roots from the healthy stem sections remain alive and growing after 2 months, however the roots that emerged from the infected stem sections appeared normal for about two weeks but then they stopped growing and eventually died. We have now repeated this result and believe it constitutes a direct assay for genes from the resistant background that block movement into or accumulation of bacteria in the very young roots that leads to root stress and eventually death. If this works, as now visualized, we will be able to screen the cDNA libraries without the GFP-transformed *X. fastidiosa*.

Construction of cDNA libraries

Isolation of grape mRNA proved to be a considerable challenge, not only for us but also for Dr. Cook's research group who have similar needs for clean RNA preparations from grape. In the past two months we have developed a procedure that now enables high yields of high quality RNA from grape. Dr. Cook's group now uses the procedure successfully; details of the procedure can be obtained by email. We have now begun the library construction from both healthy and infected grape tissue. The mRNA is being converted into cDNA and cloned into a binary plant transformation vector, a derivative of pBIN19, with the CaMV 35S promoter for high level, constitutive expression. Libraries are being made from *Vitis vinifera* (Chardonnay), *Muscadinia rotundifolia* (Cowart), and *Vitis shuttleworthii* (Hanes City) as indicated in the original proposal. These materials are being used in Dr. Walker's research and the libraries will be available to his group. The first screens will be done with the Cowart cDNA library with susceptible Chardonnay as a recipient host.

Transformation of *X. fastidiosa* with the green fluorescent protein (GFP)

Using the technique developed by Zhang et al. (2000), Guilhabert and Kirkpatrick introduced a kanamycin resistance gene into *X. fastidiosa*; by electroporating a Kan cartridge with inverted repeats and a bound transposase that catalyzes the insertion of the Kan resistance gene. However, our current results indicate that this method, although effective at generating very low level Kan resistance, is not effective in introducing GFP with expression at a level necessary to visualize the bacteria in the xylem of grape. We have now constructed a modified transposon that expresses Kan resistance and GFP from T7 RNA polymerase promoters. Also present at the very end of the transposon is a promoter-less T7 RNA polymerase gene. Following electroporation, we expect random insertions into the genome some of which will have integrated in a manner whereby a low level of T7 RNA polymerase (higher levels are toxic) is expressed, resulting in a large amount of Kan resistance and GFP expression. This method is currently being tested in *E. coli*.

Sub-objective (b) Identification of virulence genes in *X. fastidiosa* (15% effort)

Objective (b) is designed to identify those genes of the *Xylella fastidiosa* bacterium whose products are essential for virulence. The research described under objective b is directed by Dr. Bruening.

RESULTS AND CONCLUSIONS

A program aimed at recovering *X. fastidiosa* mutants requires a rapid simple assay for such mutants. Beginning in July of 2000, we developed an assay for biological activity of *X. fastidiosa* that requires only two days. Cell suspensions (0.1 to 0.4 A600 turbidity) are pressure infiltrated into inter-vein panels of 3-5 cm long leaves of *Chenopodium quinoa*. A chlorosis with small yellow to white spots, limited to the infiltrated area, develops in about 40 hr. Some tested *Xanthomas* species, which are closely related to *Xylella*, evoked no reaction on *C. quinoa*, whereas a few caused chlorosis or late necrosis (observations of Dr. Edwin Civerolo). Pseudomonads evoked necrosis. All six of the virulent *X. fastidiosa* strains tested to date induce chlorosis with spots on *C. quinoa*. More than 500 *X. fastidiosa* transposon mutants from the laboratory of Dr. Bruce Kirkpatrick were tested. None of these mutants failed to give chlorosis, suggesting that no transposon mutant tested has an insert in any region of the bacterial genome needed for chlorosis induction or that the factor(s) needed for chlorosis induction are essential to *X. fastidiosa*.

We observed that the chlorosis-inducing activity survived being heated to 100°C for 6 min or being incubated in detergent solution at 65°C for 30 min. These results suggested that the activity does not require a protein but might, for example, be due to a polysaccharide. However, incubation with any of three proteases eliminated or nearly eliminated the activity, suggesting that a protein component of unusual stability to heating and detergent contributes to the chlorosis-inducing activity. Experiments designed to identify the putative protein component of the *X. fastidiosa*-derived activity are in progress and will take advantage of genomic information available. Other research in progress explores the possible biological relationships between chlorosis on *C. quinoa* and the leaf inter-veinal chlorosis that is one of the symptoms of Pierce's disease in grape.