APPLICATION OF *AGROBACTERIUM RHIZOGENES*-MEDIATED TRANSFORMATION STRATEGIES FOR A RAPID HIGH THROUGHPUT SCREEN FOR GENETIC RESISTANCE TO PIERCE'S DISEASE IN GRAPE THAT MAINTAINS THE CLONAL INTEGRITY OF THE RECIPIENT HOST

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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, spread or symptom development of the xylem-limited bacteria, *Xylella fastidiosa.* There is no useful genetic resistance in commercially preferred 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 *Agrobacterium rhizogenes* mediated transformation strategy. This system enables the direct introgression of cloned resistance genes into a susceptible host plant while maintaining the clonal integrity of the recipient plant following transformation. In working with symptomatic grape leaf tissue for isolation of RNA for development of cDNA libraries, we examined the pattern and form of symptom development cytologically. The cytological pattern of symptom development suggested a similarity to tissue death in other plant systems that we have been studying for several years. As a consequence of our preliminary cytological studies we concluded that the death that was occurring in the pre-symptomatic and symptomatic areas of leaves on infected plants borne changes that we associate with the activation of a programmed cell death process that exhibits the morphological hallmarks of apoptosis, a widely studied gene mediated fundamental process of development and disease in animals and in plants. We have therefore included as a second objective an examination of the molecular basis of cell death in pre-symptomatic and symptomatic tissues along with the immediate assessment of the effect of expressing anti-apoptotic transgenes in Pierce's disease (PD) infected tissues on the development of death related symptoms in grape. The research plan includes a rapid functional screen for genes that confer resistance to PD in transformed grape tissue. 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.

OBJECTIVES

1. Transformation of grape with *Agrobacterium rhizogenes* for cDNA library screening.
2. Construction of a series of cDNA libraries from healthy and infected grape tissues exhibiting foliar symptoms.
3. Examine the morphological and cytological features of cell death in symptomatic leaves.
4. Investigate the potential of blocking PD symptom expression with anti-apoptotic transgenes.

RESULTS AND CONCLUSIONS

Transformation of healthy grape with A. rhizogenes:

The method of delivery of the cDNA libraries into grape is now established in our laboratory. We have confirmed that grape is readily transformed by *A. rhizogenes* and that foreign genes (e.g. GFP) and our new cDNA libraries, can be expressed readily in grape by this method. The proof of concept 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 the GFP gene 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 10 days but then they stopped growing and eventually died with the death beginning at the root tip. We have now repeated this result numerous times and conclude that 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 death, due either to signals from the bacteria or plant-expressed signals triggered by the presence of the bacteria in the vascular system of the root.

The pattern of death in the root tips is identical to the pattern we have observed and have published on in several host-pathogen systems that is characteristic of pathogen and toxin-induced death. This observation, which we believe is highly significant, suggests that the mechanism of cell death in PD is a form of programmed cell death with morphological features of apoptosis. We also have found in several other systems that we can simultaneously block this programmed cell death and disease using both anti-apoptotic transgenes and cell permeable chemicals (Richael et al. 2001). These results are described in two recent reports. The first report deals with anti-apoptotic chemicals (Richael et al. 2001), and the second report of this approach using an anti-apoptotic transgene to engineered broad spectrum disease resistance was published recently in the Proceeding of the National Academy of Sciences (Lincoln et al. 2002).

Construction of cDNA libraries:
The construction of a grape cDNA library proved much more difficult than originally assumed. We have to date constructed approximately 150,000 independent grape cDNAs cloned into a plant transformation binary vector, CB404, which is a derivative of pBIN19 and uses the CaMV 35S promoter for high level, constitutive expression. We will proceed to generate the 500,000 independent grape cDNAs needed for our complete screen. We have begun to move the cDNA library into *Agrobacterium rhizogenes* to transform infected grape explants for the purpose of finding cDNAs that will block the death of infected tissues. We intend to screen small batches of the library at first in order to ensure that the entire procedure is as efficient as possible. The first library constructed is from both healthy and infected *Vitis vinifera* (Chardonnay) grape leaves. Libraries are being made from *Muscadinia rotundifolia* (Cowart) and *V. shuttleworthii* (Hanes City) as indicated in the original proposal. These resistant source plant materials are being used in Dr. Walker's research and the libraries will be available to his group.

Transformation of the baculovirus anti-apoptotic gene p35 gene into infected grape:
In order to test the hypothesis that programmed cell death (PCD) mechanisms are responsible for the death that occurs in roots from *Xylella* infected grape stems, we directly transformed the baculovirus p35 gene into infected grape tissue explants in a manner similar to that reported by Lincoln et al (2002). Expression of p35 transgene in PD infected tissue explants blocked the *Xylella* induced root death, which indicates that signals directly from the bacterium or from the plant but induced by the presence of the bacterium trigged the root death which can be blocked by anti-apoptotic transgenes. Based on previous screens of cDNA libraries of tomato for endogenous anti-apoptotic genes we have 15 potential genes from tomato to test immediately in the *A. rhizogenes* transformed grape systems. Homologues of the tomato genes are currently being cloned from grape so that we will have the authentic grape genes to use also in the very near future.

REFERENCES
Lincoln J.E., C. Richael, B. Overduin, K. Smith, B.D. Hall, R.M. Bostock, and D.G. Gilchrist. 2002. Expression of the anti-apoptotic baculovirus p35 gene in tomato results in inhibition of cell death and a decreased susceptibility to a variety of pathogens. Proc. Natl. Acad. Sci. (Online Early Edition, 25 October, 2002).

Richael, C., J.E. Lincoln, R. Bostock and D.G. Gilchrist. 2001. Caspase inhibitors reduce symptom development in compatible plant-pathogen interactions and limit pathogen multiplication *in planta*. Physiol. Mol. Plant Pathol. 59: 213-221.

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Biological Control of Pierce's Disease