

DEVELOPING TRANSCRIPTIONAL PROFILES AND MICROARRAY EXPRESSION ANALYSIS OF GRAPE PLANT RESPONSE TO *XYLELLA FASTIDIOSA*

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ABSTRACT

The goal of the project is to characterize the molecular events in the grape / *Xylella fastidiosa* (*Xf*) interaction. We are developing a genomic approach to identify transcriptional pathways correlated with susceptibility and resistance. Highly resistant and susceptible genotypes were selected from a *Vitis rupestris* x *V. arizonica* population. Reciprocal suppression subtractive hybridization (SSH) cDNA libraries for both resistant and susceptible genotypes that represent the complete spectrum of gene expression profiles in response to the *Xf* infection are being constructed. This strategy is efficient for cloning and identifying differentially expressed genes associated with signal recognition/transduction and potentially regulated interactions between pathogens and host plants. Based on annotation results, a subset of candidate genes will be selected for cDNA microarray expression analysis. The information derived from this study will help reveal the details of metabolic pathways of host responses and molecular mechanism(s) of grape resistance and susceptibility to *Xf*.

INTRODUCTION

The Pierce's disease (PD) threat to the California viticulture industry has been exacerbated by the recent introduction and establishment of the glassy-winged sharpshooter. Host plant resistance is critical component of integrated crop management and molecular genetic techniques are now available to optimize the development of *Xf* resistant plants. The sequences of four *Xf* strains from citrus, grape, almond and oleander are now available on Genebank (Simpson et al., 2000; Van Sluys et al., 2003) and provide new insights into all aspects of PD research. Information derived from the sequences allows us to understand the molecular basis of the bacterial pathogenicity. Characterization of host responses to *Xf* infection at the molecular level is an important step toward understanding mechanisms of host pathogenicity and resistance. It is clear that PD resistance exists in grape plants (Mortensen; 1967, 1977). Genetic breeding strongly supports inheritable PD resistance. Molecular mapping has linked DNA markers to *Xf* resistance (see Reports from Walker's grape breeding projects). However, details regarding how the genetic information transfers from DNA to RNA and eventually to functional gene products, and details on the molecular basis of pathogen recognition and subsequent activation of defense response in grape plants are very limited.

SSH (suppression subtractive hybridization) is a powerful tool that enables researchers to compare two populations of mRNA and obtain clones of genes that are expressed in one population, but not in the other (e.g. resistant genotypes vs. susceptible genotypes, infected vs. control). By using this molecular technique, we are able to selectively enrich these differentially expressed genes and clone them. There are several advantages of using this technique:

1. This approach is capable of removing most housekeeping genes during library construction and therefore increases the efficiency of cloning pathogen-induced genes.
2. The system works well with paired comparisons between segregated genotypes. In the case of PD, we used highly resistant and susceptible sibling progenies from a *V. rupestris* x *V. arizonica* cross. Thus the differences in gene expression patterns between genotypes and between experimental treatments likely reflect the molecular basis of resistance and susceptibility.
3. The SSH cDNA libraries approach normalizes expressed cDNAs during library construction and therefore significantly increases the chance of cloning expressed genes that are in low abundance. This is particularly important because many pathogen-related genes are expressed at low abundance, and limited to particular tissues or cell types (Caturla et al., 2002). Some of these genes are less likely to be cloned if a standard EST cloning method is used.

We will perform BLAST (Basic Local Alignment Search Tool) search from publicly available grape ESTs resources, and orthologous analysis against *Arabidopsis* and other plant databases, to annotate sequence information derived from SSH cDNA libraries. Differentially expressed genes associated with *Xf* resistance will be selected and analyzed using cDNA microarray technology. This high throughput process allows the parallel assessment of gene expression for thousands of genes. Combining SSH, cDNA microarray, and bioinformatic tools is an innovative way to effectively dissect gene expression profiles of grape plants in response to *Xf*. These gene expression patterns underlying metabolic pathways can help

to elucidate possible mechanisms involved in resistance and pathogenicity (Gu and Martin, 1998). This project is in conjunction with the PD resistance breeding/mapping program. We will also develop DNA molecular markers from resistance genes, which will help accelerate the PD resistance breeding program.

OBJECTIVES

1. Construct tissue-specific reciprocal Suppression Subtractive Hybridization (SSH) cDNA libraries from two sets of plants (resistant genotype vs. susceptible genotype; infected tissue vs. non-infected tissue).
2. Sequence and annotate expressed genes. Identify differentially expressed genes associated with disease development and resistance. Submit annotated sequenced genes to public domain.
3. Expression gene profile analysis using cDNA Microarray technology. Identify genes associated with pathogenicity and genes linked to *Xf* resistance. Elucidate metabolic pathways involved in the pathogenicity and resistance mechanism(s).

RESULTS AND CONCLUSIONS

The project was funded in May 2003. Research is under way. Currently, we have completed green house experiment and working toward cDNA library construction.

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