FUNCTIONAL GENOMICS OF THE GRAPE-*XYLELLA* INTERACTION: TOWARDS THE IDENTIFICATION OF HOST RESISTANCE DETERMINANTS

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INTRODUCTION

Pierce's disease (PD), caused by the bacterial pathogen *Xylella fastidiosa* (Xf), is one of the most destructive diseases of grapevines (Purcell and Hopkins, 1996). All genotypes of *Vitis vinifera* are susceptible to the PD pathogen and only certain non-vinifera species (e.g., *V. shuttleworthii* and *Muscadinia rotundifolia*), typically not suitable for wine production, are able to resist or tolerate this pathogen. Development of resistant varieties through classical breeding is complicated by the desire to retain varietal phenotypes in cultivated species, and by the generally poor agronomic properties (e.g., fruit quality) of these non-vinifera species. An alternative approach for developing disease resistant germplasm is to characterize the molecular basis of resistance and susceptibility in *Vitis* species, and to use this information to design rational strategies for crop protection. In this project we are pursuing a genomics approach to identify transcriptional pathways that are correlated with susceptible or resistant interactions in *Vitis* and *Muscadinia* species. The comparison of these two distinct interactions should reveal functional elements of the host resistance response, or conversely host functions that confer susceptibility.

The experimental strategies outlined below use genomics technology (e.g., cDNA sequencing to create Expressed Sequence Tags [ESTs] and transcriptional profiling using micro arrays) to identify genes in *Vitis* species that may be causal to host susceptibility (in the case of *V. vinifera*) or resistance/tolerance (in the case of *M. rotundifolia*). Such information will considerably increase our knowledge of the grape-*Xylella* interaction and potentially provide the basis for developing resistance to the PD pathogen in *V. vinifera*. A side benefit of these activities will be derivative information, such as a public database of grape ESTs, information for molecular marker development (e.g., SSR and SNP information), and anticipated public access to a grape oligonucleotide microarray.

OBJECTIVES

1. cDNA libraries will be produced from infected and non-infected grape genotypes. Library construction will focus on susceptible *V. vinifera* and related species (e.g., a *Vitis rupestris* x *Muscadinia rotundifolia* cross) that are tolerant/resistant to *Xylella* infection.
2. Sequencing reactions will be completed for a total of 60,000 cDNA clones obtained from the above libraries (30,000 from *V. vinifera* and 30,000 from the *V. rupestris* x *M. rotundifolia* cross). The resulting sequence information (i.e., Expressed Sequence Tags (ESTs)) will be submitted to the National Center for Biotechnology Information (NCBI).
3. An analysis pipeline and web-accessible database will be developed for the grape transcriptome. The initial focus of the database will be on the minimum gene set expressed during the grape-*Xylella* interactions.
4. Transcriptional profiling will be conducted to characterize host gene expression in susceptible and resistant/tolerant grape-*Xylella* interactions.

RESULTS AND CONCLUSIONS

We are taking an EST sequencing and transcriptional profiling approach to develop a detailed picture of the molecular events that underlie host susceptibility and host resistance to the pathogen *Xylella fastidiosa* in *Vitis* species. Currently we have constructed eight cDNA leaf libraries from infected and non-infected plants of *Vitis vinifera* at host developmental stages corresponding to key steps in disease development and in excess of 100,000 clones have been picked and archived for further analysis. DNA sequencing reactions are being completed and analyzed for a total of 30,000 cDNA products from these pathogen-related libraries. A similar strategy is being implemented to sequence and characterize an additional 30,000 cDNA sequences from related species of grapes that are resistant to *Xylella* infection. In collaboration with Dr. Andrew Walker, we will characterize PD-resistant progeny from a cross between *V. rupestris* and *M. rotundifolia*. In association with the National Center for Genome Resources (NCGR) we are implementing an online relational database (the X-Genome Initiative, XGI) as a means to organize and annotate the EST information resulting from the projects. As a temporary measure, we have established an in-house data analysis pipeline, consisting of EST contig assembly by means of the

PHRED/PHRAP algorithm, and BLASTN analysis against the entire *Arabidopsis* coding sequence and all publicly available sequences from *V. vinifera*. BLAST reports are stored on line and provide a simple homology-based analysis of the grape EST dataset. Subsequent to cDNA sequencing and electronic data mining we will employ a functional genomics strategy to monitor host gene expression during grape development using oligonucleotide microarrays. This work will be done in collaboration with the Department of Biochemistry, University of Nevada, Reno (as part of an NSF Plant Genome grant). The two projects are coordinating an international effort to develop a 70-mer oligonucleotide microarray. For purposes of this project, the array will provide a means to analyze the expression of thousands of grape genes during the grape-*Xylella* interaction. We anticipate that the strategies outlined above will define the transcriptional response of susceptible and resistant *Vitis* and *Muscadinia* species to infection by *Xylella fastidiosa*. This information will significantly advance our knowledge of grape-*Xylella* interactions, and it may reveal transcriptional pathways that are causal to host susceptibility or resistance/tolerance.

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