

MICROARRAY ANALYSIS OF GLOBAL GENE EXPRESSION OF *VITIS VINIFERA* IN RESPONSE TO *XYLELLA FASTIDIOSA* INFECTION

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ABSTRACT

In previous years we have analyzed gene expression profiles of Pierce's Disease (PD) resistant and susceptible genotypes of *Vitis arizonica* hybrids in response to infection by *Xylella. fastidiosa* (*Xf*), the bacterium causing PD. Here we report gene expression of the PD susceptible European grapevine, *V. vinifera* in response to *Xf* infection. RNA was extracted from healthy and infected leaf tissues at 4, 8 and 10 weeks post inoculation. Isolated RNA was converted into cDNA and used for microarray-based global gene expression analysis. Data analysis results indicated that there were a total of 2,385 differentially expressed transcripts from early (4 week), middle (8 week) and late (10 week) stages of disease development. Of these 2,385 transcripts, 1,050 transcripts were up-regulated (2 to 100 fold) and 1,335 transcripts were down-regulated (0.5 to 0.02 fold) across the three stages. Comparative analysis of the differentially regulated transcripts has identified common and distinctive features of the host response from *V. arizonica* hybrids and *V. vinifera* to *Xf* infection that are important for understanding of PD resistant and susceptible mechanisms. An online relational database is now publicly available that has *Vitis* transcriptome data along with other relevant information and bioinformatics tools.

INTRODUCTION

The impact of PD on the California grape industry has been significant since the introduction and establishment of a more effective vector, *Homalodisca coagulata*, the glassy-winged sharpshooter (Almeida and Purcell 2003). Development of resistance in grape is stymied by the relatively limited amount of genetic and molecular information regarding genotype specific resistance to PD infection (Davis et al. 1978). From genotypic screening and genetic mapping studies, it was concluded that a dominant allele controls PD resistance and recently, Krivanek et al. (2006) identified a major quantitative trait locus that controls PD resistance and denoted it as 'Pierce's disease resistance 1' (*PdR1*). The above studies confirm that the genetic basis of PD resistance in grapes varies from tolerance to resistance and suggest that host responses to the pathogen are genotype dependent. Our recent studies further confirmed that PD response differs between resistance and susceptible genotypes at molecular and physiological levels (Lin et al., 2007; Fritschi et al, 2007). Further, in the PD resistant genotypes, differential responses between stem and leaf tissues were also noted (Krivanek and Walker, 2005). The results from these studies prompted study of genome-wide molecular basis of this host / pathogen interaction.

Plants respond to pathogen attack through a variety of signaling pathways consisting of a large number of regulatory as well as effector genes. Microarrays facilitate automated analysis of transcriptional profiling data to enable an understanding of such gene function and interactions. The goal of this study was to identify and characterize the molecular events in the grape/*Xf* interaction using genome wide transcriptome profiling between resistant and susceptible genotypes and among the different tissue types.

OBJECTIVES

1. Perform a microarray gene expression analysis.
2. Develop a grape transcriptional relational database.

RESULTS AND DISCUSSIONS

Objective 1 - Microarray gene expression analysis.

Experimental set-up: Total RNA from leaf tissues of *V. vinifera* from 4, 8 and 12 weeks post-infection with *Xf* was hybridized to nine slides in a two-color experiment using the monochromatic dyes Cy5 and Cy3. For each time point, there were three slides (biological replicates) including a dye flip.

Data analysis: For each gene there were 54 data points per each stage (18 per slide x 3 biological replications) of disease development. Data representing raw spot intensities generated by the GenePix software were first normalized with RMA algorithm (Robust multichip average) and data was further subjected to quantile normalization. SAM software was used to identify statistically significant genes expression changes using a cut-off value of two-fold differential expression and a q-value of 0.5. Clustering of the significantly differentially expressed genes was carried out using TMEV software.

Overview of transcriptional responses: A total of 2,385 transcripts and 1,955 individual genes from early (4 week), middle (8 week) and late (10 week) stage of infection were differentially regulated. Of these 2,385 transcripts, 1,050 transcripts are up-regulated (2 to 100 fold) and 1,335 transcripts are down-regulated (0.5 to 0.02 fold) across the three stages (Table 1). Below we briefly describe the expression pattern of the differentially expressed genes.

Table 1. Summary of the gene expression profile of *V. vinifera* infected with *Xf*. Expression profile data for the spotted genes was generated using the RMA algorithm (Robust multichip average) and was further subjected to quantile normalization. SAM software was used to identify statistically significant genes expression changes using a cut-off value of 2-fold differential expression and a q-value of 0.5.

Expression Pattern	Microarray Results		
	4 weeks	8 weeks	12 weeks
Upregulated	86 (2 to 6 fold)	100 (2 to 100 fold)	864 (2 to 60 fold)
Downregulated	300 (0.12 to 0.49-fold)	162 (0.05 to 0.49-fold)	873 (0.02 to 0.49-fold)

1. Early disease development response

Out of the 1,955 transcripts that were differentially regulated, 158 were exclusively up or down-regulated four weeks after infection. Of these, 33 genes were up-regulated (2 to 5.4-fold) and 125 genes were down-regulated (-2 to -5.9-fold). Some of the up-regulated genes included ABC transporter, adenosine 5' phosphosulfate reductase, polygalacturonase and the down-regulated ones included flavonol synthase, calcium binding protein and heat shock protein 18.

2. Mid-disease development response

There were 57 transcripts that were exclusively up or down-regulated eight weeks after infection. Of these, 17 genes were up-regulated (2 to 2.8-fold) and 40 genes were down-regulated (-2 to -9-fold). Some of the up-regulated genes included chlorophyll A-B binding protein, Glycoside hydrolase family 1 genes. The down-regulated ones included pectinesterase and Serine carboxypeptidase family protein coding genes.

3. Late disease development response

Similarly, after 12 weeks of infection, 1,345 transcripts were exclusively up or down-regulated 12 weeks after infection. Of these, 713 genes were up-regulated (2 to 47-fold) and 632 genes were down-regulated (-2 to -37.5-fold). Some of the up-regulated genes included CBF-like transcription factor, pathogenesis-related protein 5-1, Tyrosine protein kinase, chitinase genes. The down-regulated ones included subtilase, heat shock protein 22, sulphate transporter and phosphate transporter, desiccation-related protein and salicylic acid-binding protein 2.

4. Over lapping transcriptional response

In addition, there were three genes that were differentially regulated in early and mid stages of disease development, 167 genes that were differentially regulated in mid and late stages, and 190 genes that were differentially regulated in early and late stages of disease development. 35 genes were found to be differentially regulated at all the three stages.

5. Comparison of *V. vinifera* vs. *V. arizonica* transcriptional responses

Comparison of the PD susceptible *V. vinifera* transcriptional responses to *Xf* infection with the responses observed from *V. arizonica* hybrids that are resistant (9621-67) and susceptible (9621-94) genotypes suggests common as well as distinct responses. Transcripts such as WRKY transcription factor 30, CBF like transcription factor, NDR-1 like protein, phi-1 (an *AvrPto-Pto*, or AP responsive gene) are commonly upregulated in *V. vinifera* and the resistant (9621-67) genotypes (Figure 1A). Similarly, genes such as polygalacturonase inhibitor like protein, amino acid carrier, sulfate transporter-2, integral membrane protein Nrampl, cholone phosphate cytidyltransferase and CXE carboxylesterase are commonly downregulated between the *V. vinifera* and the susceptible (9621-94) genotypes. On the other hand, there are genes that are differentially regulated in *V. vinifera* species alone. This includes homologues of metallothionein like proteins, aspartic proteinase 2, starch phosphorylase, putative purine permease, and few hypothetical proteins that are down-regulated only in the *V. vinifera* species (Figure 1B). Similarly, transcripts of genes such as Glycoside hydrolase family-1, dirigent protein oxidase, nucleotide sugar epimerase, ubiquitin protein ligase, NAF protein kinase are only up-regulated in the *V. vinifera* species.

Objective 2 - Develop of a grape transcriptional relational database

VitisExpDB is an online MySQL-PHP driven relational database that houses annotated expressed sequence tags (ESTs) and gene expression data for *V. vinifera* and non-*vinifera* grape varieties. Currently, the database has over 300,000 EST sequences derived from 8 species/hybrids, their annotation details and gene ontology based structured vocabulary. The database has information on probe sequence and annotation features of the 60-mer gene expression chip consisting of ~20,000 non-redundant set of ESTs. There is data on 14 processed global microarray expression profile sets. Data from 12 of these Expression profile sets have been mapped onto metabolic pathways. A web interface with multiple search indices and hyperlinked result features has been developed. Several online bioinformatics tools have been added. In addition, users

can submit their ESTs to the database. VitisExpDB database is available at http://cropdisease.ars.usda.gov/vitis_at/main-page.htm

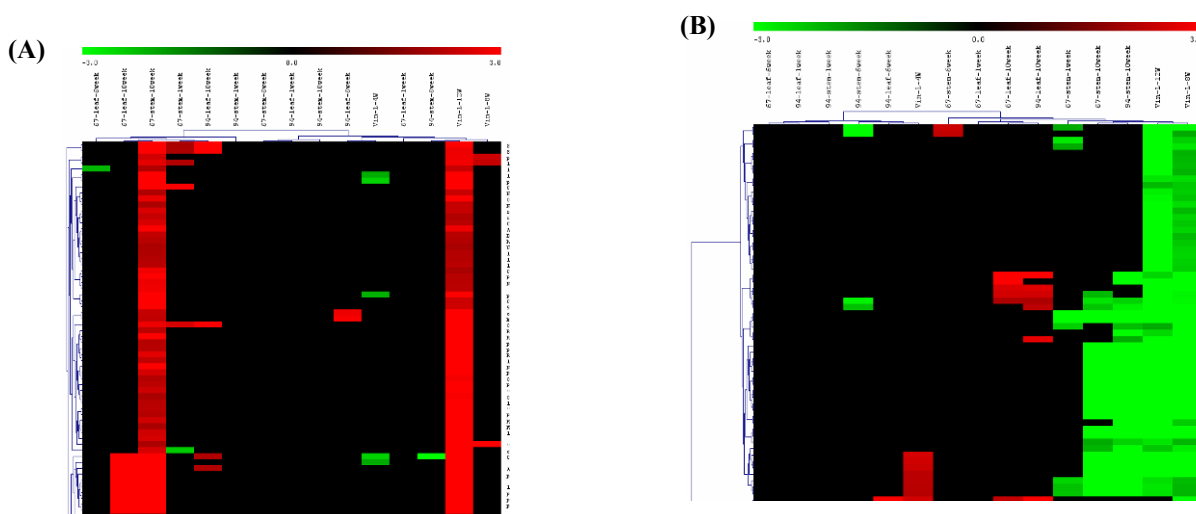


Figure 1. Expression profiling of the differentially regulated transcripts. Red indicates transcriptional activation and green represents repression. Transcripts that are not significantly regulated are shown in black. (A) Transcripts that are up-regulated in *V. vinifera* and the resistant genotype (9621-67) of the *V. arizonica* hybrids, (B). Transcripts that are downregulated only in the *V. vinifera* species

Database architecture and Web interface

The server uses Red Hat Enterprise Linux 4 RPM (x86). The relational database was developed using MySQL 4.0 as the back end. The website is powered by an Apache server. A number of useful query interfaces for data mining, analysis and visualization have been developed. This includes simple and advance search forms that facilitate either single query or multiple query search options for both EST and microarrays components. The EST component of the database can be searched using GB number, GI number, Gene Ontology ID, enzyme number or putative function as a key word. Other additional parameters can be included to build a stringent query. A separate web page is provided for listing the homologous gene sets in the major *Vitis* varieties using nWayComp. To query the microarray data, a simple form can be used where the user can enter a GB number, an array ID(s) or a putative function. Alternately, an advance search form is designed where the user can build stringent Boolean searches such as a cut-off expression value or select a particular stage of the experiment, tissue or genotype, or based on Arabidopsis gene ID, for data retrieval. Under the microarray warehouse, a separate HTML page has been designed that has hyperlinked icons to various metabolic pathways. There are 25 different pathways for each of the studied 12 microarray experiments.

Online data analysis tools

Several online tools have been developed that will either interact with the database such as BLAST, CLUSTALW, Tandem Repeats Finder (TRF), and Cluster, or work independently such as CAP3, and other BioPHP modules. Annotated *Vitis* databases such as EST and microarray probe sequence sets have been added to the BLAST database that will help the grape scientific community for quick and efficient identification and annotation of their ESTs. The developed VitisExpDB also includes other online software tools such as bioPHP modules and CAP3 software that will help the user in carrying out DNA, protein and microarray data manipulation. An EST sequence submission form has been developed where users can submit their sequence directly to the database.

CONCLUSIONS

Characterizing the molecular basis of the grape response to *Xf* is critical to understanding the mechanisms of PD resistance and pathogenesis. Based on our transcript profiling, it is clear that grape plant response to *Xf* infection is different among species, tissues and between resistant and susceptible genotypes, and early and late stages. While a broad spectrum and presumably non specific plant response was observed in *V. vinifera* species as well as in the susceptible *V. arizonica* genotype, a majority of this did not overlap with the resistance genotype response.

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