DEVELOPING TRANSCRIPTIONAL PROFILES AND GENE 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 used highly resistant and susceptible genotypes from a *Vitis rupestris* x *V. arizonica* population segregating for Pierce's disease (PD) resistance. We developed a functional genomic approach to specifically identify PD-related transcriptional profiles from susceptible and resistant responses. About 5,000 expressed clones have been sequenced and annotated from forward and reverse subtractions of cDNA libraries. These expression profiles derived from the stem, leaf and shoot tissues of resistant and susceptible genotypes throughout the course of disease development provide informative details of molecular events associated with PD. Currently we have identified 63 up/down regulated genes in response to *Xf* infection in both genotypes. To further characterize genes involved in the host-pathogen interaction at different tissues and stages of disease development, we are constructing a set of genes for microarray-based global gene expression analysis. Currently, we are analyzing the first 20 candidate genes using the Taq-Man gene expression assay method. These research efforts will help identify spatial and temporal gene expression involved in the defense response and signaling recognition in PD susceptible and resistant grapes.

INTRODUCTION
The impact of Pierce's disease (PD) on the California grape industry has been exacerbated by the recent introduction and establishment of a more effective vector, *Homalodisca coagulata*, the glassy-winged sharpshooter. Host plant resistance is a critical component of integrated crop management. Traditional breeding has been the main strategy in developing disease/pest resistant plants and is underway in the Walker laboratory. The goal of this breeding program is to develop resistant cultivars, map and develop DNA-based markers for resistance screening, and finally identify resistance genes. Breeding efforts confirm that resistance is inheritable, and molecular mapping has linked DNA markers to *Xylella fastidiosa (Xf)* resistance (see Reports from Walker's grape breeding projects). Once the resistance genes are confirmed, it will be possible to incorporate PD resistance genes from grape species into traditional grape cultivars. However under conventional breeding procedures, several generations will be required to exclude undesirable characteristics from wild species and non-*vinifera* cultivars. In order to speed up resistance gene identification and elucidate the molecular basis of resistance and pathogenicity to *Xf*, we propose here to develop a functional genomic approach for PD research.

Suppression Subtractive Hybridization (SSH) is a powerful tool for comparing two populations of mRNA and elucidates clones of genes that are expressed in one population, but not in the other (e.g. infected vs. control). By using this molecular technique, we are able to selectively enrich these differentially expressed genes, clone and sequence them. This technique has a number of powerful aspects. It is a high efficiency for cloning pathogen-induced genes while removing or reducing constitutively expressed housekeeping genes. 2) The system works particularly well with paired comparisons within a population of segregating siblings. 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 likely reflect the molecular basis of the resistance and susceptibility responses. 3) The SSH cDNA technique normalizes expressed cDNAs during library construction and therefore significantly increases the chance of cloning genes that are expressed but at very low abundance. This is particularly important because many pathogen-related genes might be expressed at low abundance, and limited to particular tissues or cell types at certain times (Caturla et al., 2002). Some of these genes are less likely to be cloned if a standard EST cloning method is used.

OBJECTIVES
1. Construct twelve tissue-specific reciprocal SSH cDNA libraries from highly resistant and highly susceptible genotypes.
2. Sequence and annotate expressed genes. Identify differentially expressed genes associated with disease development and resistance. Make annotated sequenced genes available to public.
3. Conduct expression gene profile analysis using Microarrary and Taq-Man gene expression technology. Identify genes associated with pathogenicity and genes linked to *Xf* resistance. Elucidate metabolic pathways involved in the pathogenicity and resistance mechanism(s).

RESULTS

Objective 1

RNA Sample Preparation

A pair of highly resistant (#9621-67) and highly susceptible (#9621-94) sibling genotypes selected from segregated population of *Vitis rupestris* x *V. arizonica* were used for this study. Samples were collected from leaf, stem and shoot of infected and non-infected, resistant and susceptible plants at 1, 3, and 5 days after inoculation, followed by 4 collections at 7-day intervals, and then by 4 additional collections at 14-day intervals. The total time from the first inoculation to last sampling was more than 90 days. We used our recently developed a grape RNA extraction protocol for grape stem, leaf and shoot RNA isolation. The average yields of total RNA are 15, 40 and 70 µg/g tissue respectively. mRNAs were further purified from total RNA using the Dynabeads Oligo$(dT)_{25}$ method. About 2-3 µg mRNA was obtained from each sample for constructing cDNA libraries.

cDNA Library Construction

We used our modified version of the CloneTech SSH library construction kit (CLONTECH-Laboratories, 1999) to construct twelve reciprocal SSH cDNA libraries (Table 1). Cloned cDNAs were transformed and quality of each library was evaluated before preparing plasmid DNAs for sequencing work.

Objective 2

Sequencing cDNA Library

Unlike a standard cDNA library, an SSH library selectively clones differentially expressed genes. Depending on the complexity of expression in each expression source, each library usually does not require very deep sequencing. To minimize sequence diminishing return while covering as many genes as possible, 480 (96 x 5) clones were first sequenced from each library. Based on the results of the numbers of contigs and sequence redundancy from each library, more sequences were adjusted to ensure good coverage for all libraries.

Sequence Data Processing

Sequence trace files were scored with cutoff scores of PHRED 20. The FASTA files were trimmed of vector sequences and filtered of non-target sequences such as rRNA and *E. coli*. After contig assembly, BLASTX and BLASTN analyses were performed against the NCBI protein and EST databases, *Arabidopsis* and grape genomic databases. As preliminary annotation, orthologous analysis of *Vitis* expressed genes to *Arabidopsis* is based on the expected values. We grouped the results into three classes as high similarity with E value of $<e^{-30}$ or less, no significant match with E value between $<e^{-6}$ and $<e^{-4}$d and no hit. The "no hit" class is likely to contain *Vitis* specific expressed genes. According to the BLAST reports, we are dividing these contigs into categories according to biological functions such as pathogenesis, disease defense, heat shock, signaling, oxidative metabolism and so on. A possible metabolic role will be assigned to each sequence file.

Objective 3

While we are processing our PD specific transcriptional profile database and designing a set of candidate genes for global gene expression analysis, we identified 63 up/down-regulated transcripts in response to *Xf* infection in both resistant and susceptible genotypes (Table 2). Some of these are putatively involved in pathogenesis, defense response and signal transduction (Figure 1). We used Taq-Man expression analysis method to analyze the first 20 genes. An example of gene expression analysis is presented in Figure 2.

CONCLUSIONS

Characterizing the molecular basis of the grape response to *Xf* is important toward understanding mechanisms of PD resistance and pathogenesis. Expression profiles provide a useful framework for the next step of expression analysis that will help to further dissect genes underlying metabolic pathways involved PD responses.

Table 1. Forward and reverse SSH cDNA library construction for both resistant and susceptible genotypes.

Genotypes	Resistant or susceptible genotype		
Tissues	Leaf	Stem	shoot
Forward subtraction	Infected ← health	Infected ← health	Infected ← health
Reverser subtraction	Infected → health	Infected → health	Infected → health

Table 2. Summary of up-regulated and down-regulated transcripts between resistant and susceptible genotypes among three tissues following of *Xf* infection

Genotypes	Tissue	Up Regulated	Down regulated
Resistant (9621-67)	Stem	8	6
	Leaf	1	2
	Shoot	16	3
Susceptible (9621-94)	Stem	8	5
	Leaf	3	2
	Shoot	7	2

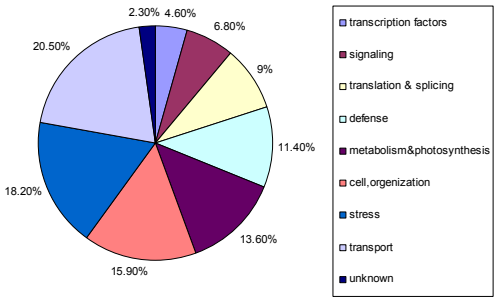

Figure 1. Functional category of putative genes of among 63 differentially expressed transcripts.

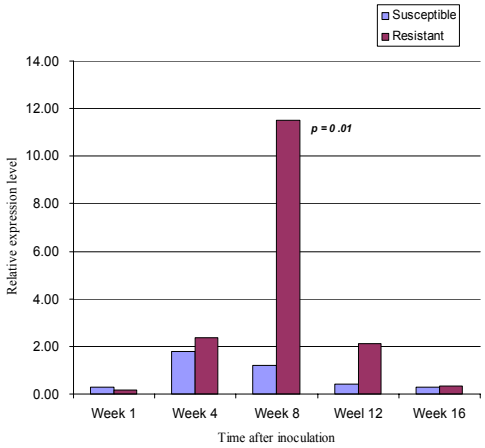

Figure 2. Taq-Man gene expression analysis was used to analyze expression during PD development. Here is an example of the putative pathogenesis-related gene, which increased more than 10 times the transcriptional levels in the 8th week after inoculation in the susceptible genotype (9621-94) as compared to the resistant genotype (9621-67).

REFERENCES

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