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

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ABSTRACT

In silico mining of EST data, Real Time PCR, and Affymetrix GeneChip technology was used to characterize the transcriptional response of *Vitis vinifera* to the Pierce's disease (PD) pathogen *Xylella fastidiosa (Xf)*. We have determined that susceptible *V. vinifera* responds to *Xylella* infection with a massive re-direction of gene transcription. This transcriptional response includes the up regulation of transcripts for phenlypropanoid and flavonoid biosynthesis, ethylene production, adaptation to oxidative stress, and homologs of pathogenesis related (PR) proteins. In addition to highlighting potential metabolic and biochemical changes that are correlated with disease, the results suggest that susceptible genotypes respond to *Xylella* infection by induction of limited defense response.

A long-standing hypothesis states that PD results from pathogen-induced drought stress, with the consequent development of disease symptoms. To test this hypothesis, we compared the transcriptional and physiological response of plants treated by pathogen infection, low or moderate water deficit, or a combination of pathogen infection and water deficit. We determined that the transcriptional response of plants to *Xylella* infection is not the same as the response of healthy plants to moderate water stress. However, there is an apparent synergistic interaction between water stress and disease, such that water stressed plants exhibit a stronger physiological and transcriptional response to the pathogen. Qualitative and quantitative estimates of gene expression derived from the Affymetrix gene chip were confirmed by a combination of Real Time PCR and *in situ* hybridization analysis with ~20 candidate marker genes.

Real Time PCR analysis involving six marker genes was used to survey the specificity of *Xylella*-induced gene expression under field conditions. The results demonstrate that the marker genes are up-regulated in response to *Xylella* infection but not in response to the other pathogens assayed, including common viral, nematode and fungal pathogens, or by *Phylloxera* infestation or herbicide damage. Similarly, moderate drought stress did not result in increased transcript levels for these marker genes. By contrast, each of the marker genes was strongly induced in non-infected leaves where the vascular system was compromised by biotic or abiotic factors, including girdling by insect damage and severe drought stress leading to death. We hypothesize that an aspect of xylem dysfunction, but not drought stress per se, is one trigger for *Xylella*-induced gene expression.

INTRODUCTION

All organisms adapt to external stressors by activating the expression of genes that confer adaptation to the particular stress. In the case of Pierce's disease (PD), such genes are likely to include those coding for resistance or susceptibility to *Xylella fastidiosa (Xf)*.

Genomics technology offers an opportunity to monitor gene expression changes on a massive scale (so-called "transcriptional profiling"), with the parallel analysis of thousands of host genes conducted in a single experiment. In the case of PD of grapes, the resulting data can reveal aspects of the host response that are inaccessible by other experimental strategies. In May of 2004, the first Affymetrix gene chip was made available for public use, with ~15,700 *Vitis* genes represented. This gene chip has been developed based primarily on a collaboration between the Cook laboratory and researchers at the University of Nevada-Reno (Goes da Silva et al., 2005). With the arrival of the Affymetrix gene chip, we are poised to make a quantum leap in the identification of host gene expression in response to *Xf*.

In addition to enumerating differences between susceptible and resistant genotypes of *Vitis*, this research is testing a long-standing but largely untested hypothesis that pathogen-induced drought stress is one of the fundamental triggers of PD symptom development. The utility of this type of data will be to inform the PD research community about the genes and corresponding protein products that are produced in susceptible, tolerant and resistant interactions. Differences in the transcriptional profiles between these situations are expected to include host resistance and susceptibility genes, and thus