COMPARATIVE PROTEOMIC ANALYSIS OF STEM TISSUE AND XYLEM SAP FROM PIERCE'S DISEASE RESISTANT AND SUSCEPTIBLE GRAPEVINES

Project Leaders:
Hong Lin
Crop Diseases, Pests, and Genetics Research Unit
USDA, ARS
Parlier, CA 93648

Felix Fritschi
Crop Diseases, Pests, and Genetics Research Unit
USDA, ARS
Parlier, CA 93648

Cooperators:
Litao Yang
Crop Diseases, Pests and Genetics Research Unit
USDA, ARS
Parlier, CA 93648

Andrew Walker
Department of Viticulture and Enology
University of California
Davis, CA, 95616

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ABSTRACT
Both xylem sap and stem tissue are key components in the *Xylella fastidiosa (Xf)*-grapevine interaction. In this research we investigate protein expression in xylem sap and stem tissue of highly tolerant and susceptible grape genotypes. Ten sequential samplings of stem tissues have been conducted from infected and non-infected grapevines ranging from 1 day to 12 weeks post inoculation. Protein extraction and analyses on these tissues has recently been initiated. Plants for xylem sap extraction are currently being grown in the greenhouse. Xylem sap will be extracted multiple times post inoculation from *Xf* and water-inoculated plants. Differentially expressed proteins in both stem tissue and xylem sap will be identified and investigated in more detail in the coming months. Results obtained will deepen our understanding of host-pathogen interactions, a key component in fighting Pierce's disease (PD).

INTRODUCTION
Xylem sap is very important for *Xf* growth *in planta*. Be it as individual cells at the beginning of an infection or later in biofilms, *Xf* not only obtain their nutrients from xylem sap but also are in constant contact with it. Andersen et al. (2004a) and Toscano et al. (2004) reported that the source of xylem sap affects *Xf* growth rates and growth characteristics. Results from bioassays conducted in our lab also indicate that xylem sap collected from various PD resistant *Vitis* genotypes has dramatically different effects on *Xf* growth in comparison with controls consisting of artificial media (PW) and xylem sap from 'Chardonnay' (*V. vinifera*).

To date, numerous studies have investigated inorganic and organic solutes in grape xylem sap showing that xylem sap chemistry is a function of temperature and fertilization, and changes over time (Andersen and Brodbeck, 1989a, 1989b, 1991; Andersen et al., 1995, 2004b). Although some xylem sap compounds have been suggested to be related to the susceptibility, i.e. [P]*[citrate] to [Ca]*[Mg] ratio (Andersen et al. 2004a), a complete understanding of the influence of xylem sap chemistry on the host pathogen interaction is missing. Specifically, as of now, the protein composition of the xylem sap has not been investigated in detail.

The direct contact between sap and *Xf* makes xylem sap a promising venue to interfere with a successful pathogen colonization of the host. In other host plant - pathogen systems, extracellular/apoplastic proteins were found to be responsive to disease pressure and in some instances important in suppressing disease development (Ceccardi et al., 1998; Guo et al., 1993; Nemec, 1995; Reimers and Leach, 1991; Reimers et al., 1992; Rep et al., 2002; Young et al., 1995). Combined with the evidence for xylem sap effects on *Xf* growth, these examples suggest that the analysis of the xylem sap proteome is likely to result in the identification of protein(s) influencing the interaction of grapevines and *Xf*. Identified proteins may provide information to develop approaches and/or be part of strategies to improve grapevine tolerance or resistance to *Xf*. In addition, the identification of PD-specific proteins would allow the production of specific antibodies which may potentially be used for serological diagnostic tests for PD.

Recent findings of genotypic differences in symptomology and *Xf* populations in stems (canes) of resistant and susceptible grapevine genotypes highlight the importance of this tissue in the host-pathogen interaction. Krivanek et al. (2005) developed a cane maturation index (CMI) to quantify the uneven cane maturation manifested in the green-island symptoms that arise in PD infected plants. They found "green-island" formation as measured by the CMI to correlate better with PD resistance then other phenotypic symptoms. The irregular nature of the symptoms suggests that localized, rather than systemic signals are responsible for the spatial patterns observed. Thus, signal and signal recognition as well as signal transduction events appear to occur localized in stem tissue since a systemic signal transaction and recognition is unlikely to result in the characteristic green-island symptomology. Furthermore, in a companion study Krivanek and Walker (2005) found that, in resistant genotypes, *Xf* populations in stem internodes were significantly smaller than in leaves. In contrast, corresponding samples from susceptible genotypes were not significantly different. The two studies highlight the importance

of plant-pathogen interactions occurring in the stem for PD susceptibility characteristics of the different genotypes. Therefore, detailed examination of stem proteins extracted from susceptible and resistant genotypes of *Xf* infected and healthy plants is a very promising approach to identify important components involved in host-pathogen interactions as well as the plant response.

Examination of the protein content of stem tissue and xylem sap is a new approach with distinct advantages complementing other strategies currently pursued in the fight against PD. Using this approach, we focus on key components: stem and xylem sap protein content rather than the entire grapevine proteome. In addition, regulatory mechanisms including transcriptional, post-transcriptional, and translational events which can constitute significant confounding effects in functional genomics approaches, are already integrated in the proteomic approach. Furthermore, it is possible to identify post-translational protein modifications (e.g. phosphorylation, acetylation, methylation, glycosylation, cleavage, etc.) which play key roles in protein function.

OBJECTIVES

1. Identify xylem sap and stem proteins differentially expressed in healthy grapevines from resistant and susceptible genotypes.
2. Identify xylem sap and stem proteins induced by *Xf* in resistant and susceptible grapevines.
3. Determine the relationship of identified proteins to PD.

RESULTS

Highly susceptible (9621-94) and resistant genotypes (9621-67) selected from a segregating population of *V. rupestris x V. arizonica* as well as *vinifera* grape, Chardonnay are being used in this study. An expression experiment was conducted in the greenhouse where treatment and control grapevines were mechanically inoculated with *Xf* suspension and culture medium respectively. Leaf and stem tissues were then collected at day one, two, and five post inoculation, and subsequently at three one-week and four two-week intervals for a total of ten collections with three biological replicates in both treatment groups. Leaf and stem tissues collected at each time point were immediately stored at –80°C for later protein extraction.

Stem and leaf protein extraction and 2-DE (Figure 1) have recently been initiated and will be conducted over the coming few months. A new set of plants (same genotypes and treatments as above) was recently established in the greenhouse and will be used to extract xylem sap and investigate protein expression pattern in the xylem sap.

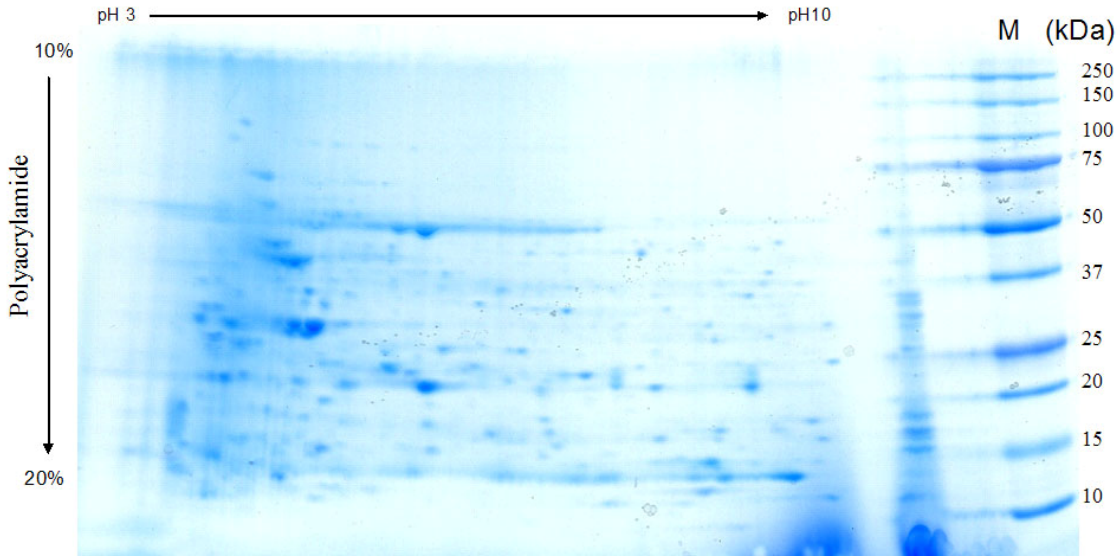

Figure 1. Grape leaf protein profiles from two dimensional SDS-PAGE gel. Electrophoresis was carried out using a Bio-Rad Criterion™ Cell and gel was stained by BioSafe Coomassie blue.

CONCLUSION

We have initiated a study to investigate stem and xylem sap protein expression in one highly resistant and two susceptible grape genotypes. Investigation of the xylem sap and stem proteome are of particular interest because of the importance of both xylem sap and grape stem in the host-pathogen interaction. Examination of the interaction between *Xf* and grapevine hosts at the protein level is of particular importance since there often is a lack of correlation between gene and protein expression. This study will complement ongoing efforts in transcriptional level of gene expression analyses and provide a more integrative picture of the nature of PD resistance.

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