

High throughput expression profiling of xylem sap proteome of susceptible and resistant tomato genotypes revealed networks of metabolic, defense as well as cell wall metabolic and signalling proteins

Diwakar Dahal*, Andreas Pich and Kerstin Wydra

* Email: dahal@ipp.uni-hannover.de

Background

Xylem sap in vascular plants plays a pivotal role of water and minerals transport from roots to above ground plant parts. The composition and conduction of sap is, therefore, considered as the key determinant of physiological states and activities of the aerial organs. Xylem conducting elements lose both nuclei and its cytoplasmic contents during their functional maturation however, the presence of proteins was revealed in the sap.

Aim

The current study aimed to provide broad spectrum characterization of tomato xylem sap proteome from healthy susceptible and resistant plants differing in resistance to bacterial wilt.

Materials and Methods

Tomato genotypes

- Hawaii7996 (resistant) and WVa700 (susceptible)

Xylem sap

- Collected under Root-Pressure-Exudate system
- Sap pooled from several plants of each genotype

Protein sample preparation

- Centrifugal concentration of xylem sap
- Sap protein separation with 1-D SDS gradient PAGE
- In-gel protein digestion with trypsin
- Peptides separation with nano-HPLC

Protein identification

- MALDI TOF/TOF MS analysis
- MASCOT search using MSDB database

Results and Discussion

Several physiologically important groups of protein were identified (Figure 1).

Peroxidase, cell wall associated proteins, proteases, and defense related proteins were conserved in sap of many plants and indicates their role in the growth, development, and differentiation of xylem elements during the functional maturity acquisition.

The occurrence of many signalling and transport proteins is expected for root to shoot communication that are vital for the growth and development of whole plant.

Large number of hypothetical proteins could provide novel functions after performing additional experiments.

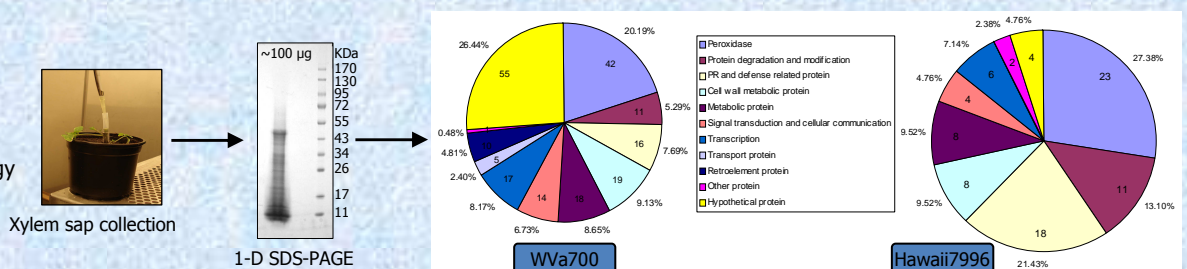
The sap showed majority of proteins with secretion signals that guides them to the extracellular compartments.

Interestingly, sap also contained large number of protein without secretion signals indicating the existing the moonlighting proteins.

Xylem sap proteome comparison (Hawaii7996 Vs WVa700)

- High number of proteins in susceptible genotype including signalling and transcription related proteins
- Higher percentage of defense related proteins, peroxidase, proteins degradation/modification enzymes and metabolic proteins in resistant genotype

Figure 1. Overview of the xylem sap analysis methodology and the results



Putative functional groups of proteins identified in the sap of two genotypes

- Number of identified proteins of each group (Inside Pie)
- Percentage of each group of protein (Outside Pie)

Conclusion

Xylem sap proteome from tomato plant provided the overview of various functions of the xylem sap with respect to whole plant physiology.

The protein profiles information has also created a platform which can further be used for the comparative and quantitative proteomics investigating the plant pathogen interactions at the molecular level.