

## EVALUATION OF SIGNAL SEQUENCES FOR THE DELIVERY OF TRANSGENE PRODUCTS INTO THE XYLEM

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**Reporting Period:** The results reported here are from work conducted July 2005 to September 2005.

### ABSTRACT

*Xylella fastidiosa* (*Xf*), a gram-negative bacterium, is the causative agent of Pierce's disease in grapevines. Because *Xf* is xylem-limited, it will be essential that any anti-*Xylella* gene product be present in the xylem in an effective concentration. Work on understanding the mechanism of how proteins are targeted to this plant compartment will be relevant for the delivery of therapeutic proteins into the xylem. In addition, it will be a useful tool for *Xylella* and glassy-wing sharpshooter (GWSS) gene function studies.

We collected xylem exudate from grapevines and analyzed its protein composition by two-dimensional gel electrophoresis. Peptide spectrum and Blast analysis showed that the proteins found in the exudates are secreted proteins that share function similarities with proteins found in xylem exudates of other species. The corresponding cDNA sequences of 5 of them were found in the TIGR *Vitis vinifera* gene index. The signal sequences of xylem proteins Chi1b and similar to NtPRp27 were fused to the mature pear polygalacturonase inhibiting protein (pPGIP)-encoding gene. The expression of these chimeric genes will be evaluated in transient and permanent transformations in order to evaluate their ability to target pPGIP to the xylem. The results of this research will not only be applied in projects that test anti-*Xylella* gene products that should be delivered into the xylem but also in functional studies that are intended to target the products of *Xf* and GWSS genes to the xylem.

### INTRODUCTION

Signal peptides control the entry of virtually all proteins to the secretory pathway, both in eukaryotes and prokaryotes. They comprise the N-terminal part of the amino acid chain and are cleaved off while the protein is translocated through the membrane of the endoplasmic reticulum (1). Generally, signal peptides are interchangeable and secretion of non-secreted proteins becomes possible by the fusion of a signal peptide at the N-terminus of the mature protein; however, changing the signal sequence of recombinant proteins can affect the degree of protein production (2).

In previous research, we fused the sequence coding for the signal peptide of XSP30, a xylem-specific protein from cucumber (3), to the green fluorescent protein (GFP) reporter gene. Contrary to what we expected, fluorescence was only detected inside the cells. Our results suggested that either the XSP30 signal peptide is not recognized by the grape secretory machinery or GFP is not secretion competent. If the first hypothesis is correct, signal sequences obtained from proteins present in grape xylem sap would constitute better candidates for delivery of transgene products to the xylem.

Interestingly, we have also found that the product of the pPGIP encoding gene from pear fruit, heterologously expressed in transgenic grapevines, is present in xylem exudates and moves through the graft union (4). These results show that pPGIP is secretion competent in grapes and constitutes a good alternative to GFP. We intend to use the sequence encoding the mature pPGIP fused to the signal peptides to be analyzed.

We have collected xylem exudate from plants of *Vitis vinifera* 'Chardonnay' and analyzed its protein composition by two-dimensional gel electrophoresis. The purpose of this project is to fuse the signal sequences of these grape xylem sap proteins to the mature pPGIP-encoding gene in order to evaluate their ability to target pPGIP to the xylem.