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 endoplasmatic recticulum (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.

OBJECTIVES

1. Obtain partial sequences of proteins found in grape xylem exudates and search cDNA databases for signal sequence identification and selection.
2. Design and construct chimeric genes by fusing the selected signal sequences to a sequence coding for a mature secreted protein (pPGIP).
3. Transform grapevines with the chimeric genes via *Agrobacterium tumefaciens* and *A. rhizogenes*.
4. Evaluate the efficiency of the different signal sequences in targeting protein products to the xylem tissue of grapevine through the:
   4.a. analysis of the expression and secretion of pPGIP in transiently transformed grapevines.
   4.b. analysis of the expression and secretion of pPGIP in grapevines bearing roots transformed via *A. rhizogenes*.

RESULTS

Peptide spectrum and Blast analysis showed that the proteins found in grape xylem exudates are secreted and share function similarities with proteins found in xylem exudates of other species (5). cDNA sequences of 5 of them were found in the TIGR *v. vinifera* gene index. However, it was possible to predict the signal peptide in 2 contigs only (TC 39929 and TC 45857, annotated as Chi1b and similar to NtPRp27 respectively). Based on their sequences, we designed primers that were used to amplify the predicted fragments from genomic DNA of 'Chardonnay' and 'Cabernet Savignon'. Those fragments were fused to the mature pPGIP gene through the gene splicing by overlap extension method (SOE) (6) and cloned into the pCR2.1-TOPO vector. These two chimeric genes will be ligated into a plant expression vector containing the 35S cauliflower mosaic virus promoter and the octopine synthase terminator and the resultant expression cassettes will be then ligated into the binary vector pDU99.2215, which contains an *nptII*-selectable marker gene and a *uidA* (β-glucuronidase, GUS) scorable marker gene. The resultant binary vectors will be transformed into the disarmed *A. tumefaciens* strain EHA105 by electroporation.

SOE was also used to produce the following chimeric genes:

1) pPGIPsignal peptide(sp)-GFP
2) XSP30sp-mpPGIP
3) RAmysp-mpPGIP
4) pPGIPsp-mpPGIP
5) mpPGIP

Construct 1 will help to elucidate if GFP is secretion competent in grape. In construct 2, mpPGIP has been fused to the signal sequence of cucumber XSP30, which is a xylem-specific protein. In construct 3 mpPGIP has been fused to the signal sequence of rice amylase 3 (Ramy), which has been very effective in secretion of human α1-antitrypsin in rice cell cultures (7). Constructs 4 and 5 will be controls. All five genes have been ligated into the plant expression vector described above and then ligated into binary vector pDU99.2215 and the resulting plasmids have been transformed into the disarmed *A. tumefaciens* strain EHA 105.

The next step will be the permanent and transient transformation of *V. vinifera* 'Thompson Seedless' followed by the analysis of the expression and secretion of pPGIP.

CONCLUSIONS

Through the study of the proteins present in xylem exudates of 'Chardonnay', we have found 2 good candidates to investigate the effect of using grape signal sequences on xylem targeting. In addition we have produced 2 other chimeric genes containing the signal peptide of a xylem-specific protein in cucumber and the signal sequence of rice amylase. The results obtained with transient and permanent transformations with these genes will provide, in the short term, valuable information for the identification of signal peptides that will deliver proteins to grapevine xylem with high efficiency. In the long term, the development of an efficient secretory system will be essential to target therapeutic proteins to the xylem of grapevine. In addition, the results of this research will also be applied in functional studies that are intended to target the products of *Xylella fastidiosa* and glassy-winged sharpshooter genes to the xylem.

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FUNDING AGENCIES
Funding for this project was provided by the University of California Pierce's Disease Grant Program.