DIRECTING POTENTIAL ANTI-*XYLELLA* GENE PRODUCTS TO THE XYLEM OF TRANSGENIC GRAPEVINES

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ABSTRACT
The purpose of this research was to transform *Vitis vinifera* cultivars with the pear polygalacturonase inhibiting protein (PGIP) gene in order to analyze its effect in developing resistance to PD in transgenic plants. A second goal was the transformation of grapevine with several green fluorescence protein (GFP) constructs carrying sequences expected to enhance secretion from the cell to evaluate the effect of signal sequences on the targeting of transgene products to xylem tissue. Some of the transgenic lines expressing *pgip* exhibited reduced PD symptoms, which suggests that *Xyllela* polygalaturonase might be inhibited in transgenic plants. Tests will be conducted in the future to evaluate the development of PD in the field. We also found that the pear PGIP was secreted into the xylem. This is relevant to PD because *X. fastidiosa* is a xylem-limited bacteria. It is also very important that the transgene product was observed to move through the graft union and thus is transmitted to the scion, implying that a few transgenic rootstocks could be used with any scion variety. Fluorescence in plants transformed with GFP fused to the signal peptide sequences of tricosanthin and XSP30 was only detected inside the cells. The absence of fluorescence in the apoplast could be related to GFP expression itself instead of failure of TCS and XSP30 signal peptides.

INTRODUCTION
Genetic engineering offers the possibility of introducing genes that will improve tolerance to Pierce's disease in existing grape varieties without otherwise changing their viticultural or enological characteristics.

One of our targets is a gene coding for a pear PGIP cloned in the Labavitch lab (Stotz et al. 1993). PGIP's are plant cell wall proteins that specifically inhibit fungal polygalacturonases (PG). By inhibiting PGs, PGIP's interfere directly with host cell wall degradation and may prevent degradation of pectic oligomeric elicitors that are inducers of the plant defense response. Their role in plant defense suggests that they may be useful for engineering transgenic plants resistant to pathogen infection. Powell et al. (2000) showed that transgenic tomato plants transformed with the pear PGIP gene exhibited reduced susceptibility to infection with *Botrytis cinerea*. The fact that *Xylella fastidiosa*, the causal agent of PD in grapevines, has genes putatively encoding PG and other cell wall-degrading enzymes (Simpson et al., 2000) led us to hypothesize that PGIP could confer tolerance against *Xylella* in grapes. In order to test this hypothesis, pre-embryogenic calluses originating from anthers of *Vitis vinifera* cvs. 'Thompson Seedless' and 'Chardonnay' were cultivated with *Agrobacterium tumefaciens* harboring binary plasmid pDU94.0928, that contains the pear PGIP gene under the control of the CaMV 35S promoter.

We are also investigating the targeting of transgene products to xylem tissue. Because *X. fastidiosa* is xylem limited, it will be essential that any anti-*Xylella* gene product be present in the xylem in an effective concentration. In order to study protein secretion in grape, pre-embryogenic calli originating from anthers of *Vitis vinifera* cvs. 'Thompson Seedless' and 'Chardonnay' were cultivated with *Agrobacterium tumefaciens* carrying three gene constructs that included the coding sequence for a synthetic GFP (Maximova et al 1998) and GFP fused with amino-terminal of the secreted protein tricosanthin (TCS) (Krishnan 2000) or the xylem specific protein XSP30 (Masuda et al, 1999), all under the control of the Ca MV 35S promoter.

OBJECTIVES
1. Characterize the role of the transgene in the delayed development of PD in transgenic grapevines that express the pear PGIP.
2. Measure the abundance of marker gene product in the xylem sap of transgenic plants and non-transgenic scions grafted into transgenic rootstocks.
3. Evaluate the effect of signal sequences on the targeting of transgene products to xylem tissue.

RESULTS AND CONCLUSIONS

Effect of PGIPs on the development of PD in transgenic grapevines

Active PGIP was found in leaves, roots and stems of transgenic plants obtained from independent transformation events but not in untransformed controls. Secretion of the protein was confirmed with the presence of active PGIP in the xylem sap of transgenic plants and untransformed scions of 'Chardonnay' and 'Thompson Seedless' grafted on transgenic rootstocks. PG inhibition remained high in 10x diluted xylem sap (Figure 1). Five to seven plants of each line were mechanically inoculated with the Temecula strain of *X. fastidiosa.* The development of PD was delayed in some transgenic lines with high PGIP activity, which exhibited reduced leaf scorching, lower *Xylella* titers and better re-growth after pruning than the untransformed controls.

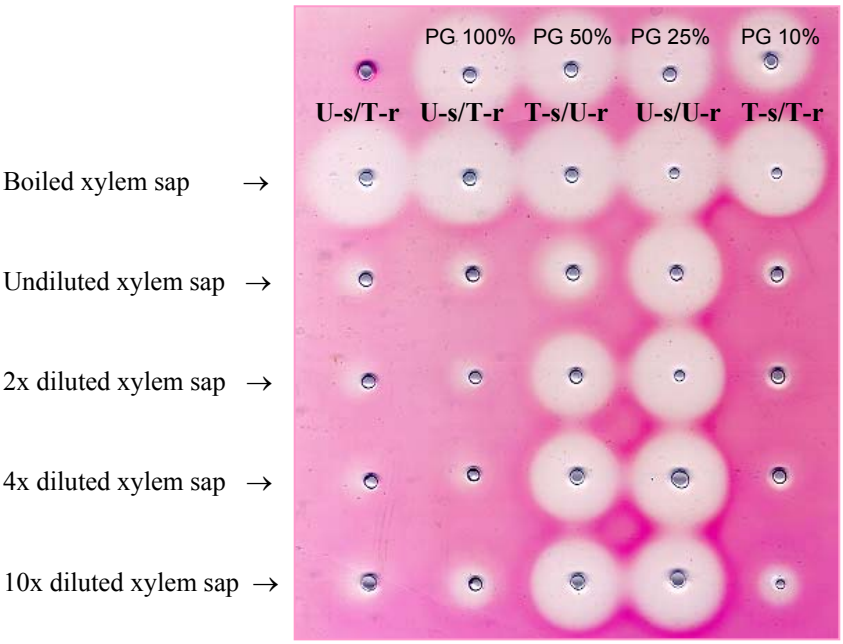

Figure 1. PGIP activity in xylem sap obtained from different graft combinations. A clear zone is indicative of PGs degrading the glycosydic bonds present in the polygalacturonic substrate. First row corresponds to 100, 50, 25 and 10% PG dilutions. Second row are 100% PG with boiled xylem sap samples, rest of the rows correspond to 100% PG with different dilutions of xylem sap. First and second column of treatments are replicate plants. U is untransformed, T is Thompson Seedless transgenic line 77, s is scion and r is rootstock.

Effect of signal sequences on the targeting of transgene products to xylem tissue

Strong fluorescence was found in embryos, roots, stems and leaves of plants transformed with *gfp* and *xsp30-gfp* but the levels of fluorescence observed in *tcs-gfp* transformants were very low. In all cases fluorescence was detected only inside the cells.

CONCLUSIONS

Although all lines tested were susceptible to *X. fastidiosa*, the development of PD was delayed in some transgenic lines with high PGIP activity. Whether these results can be attributed to the inhibition of *Xylella* PG is unclear. The improved growth of PD transgenic grapevines might account for a delay of PD movement along the stem but more information is needed to explain these results. Also it will be important to determine whether the transgenic plants grown in the field exhibit the same characteristics. We are currently growing at least 20 plants per line in the greenhouse to be transplanted to the field this coming spring. Nevertheless, the expression of PGIP has provided useful data regarding the control of the disease. First, our results showed that the pear PGIP is secreted and reaches the xylem. This is relevant to PD because *X. fastidiosa* is xylem-limited and any anti-*Xylella* gene product must be present in the xylem. Second, the signal peptide of the pear PGIP could be used to direct other anti-*Xylella* products to the xylem. And third, the fact that the transgene product moves through the graft union and is transmitted to the scion implies that a few transgenic rootstocks could be used with any scion variety provided that the anti-*Xylella* compound is synthesized in effective concentration in the roots.

Fluorescence in plants with GFP fused to the leader sequences of TCS and XSP30 was detected only inside the cells. The simplest explanation is that these signal peptides were not recognized by the grape secretory machinery but other interpretations are also likely. Alternative possibilities are that the protein was secreted but was not folded properly, or that it is less stable in the apoplast or that it was retained in the ER/Golgi compartments. Consequently more analysis is needed,

e.g., Western blotting of apoplastic fluid, before ruling out the use of the TCS and XSP30 signal peptides for the delivery of anti-*Xyllela* compounds to the xylem.

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