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

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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 target genes is a pear *pgip* cloned in the Labavitch lab (Stotz et al. 1993). PGIPs are proteins containing a leucine-rich repeat domain that are targeted to the plant cell wall and that specifically inhibit fungal polygalacturonases (PGs). By inhibiting PGs, PGIPs directly interfere with host cell wall degradation and may thus prevent degradation of pectic oligomeric elicitors that are inducers of the plant defense response. Their role in plant defense response 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 Pierce's disease (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, proembryogenic calluses originating from anthers of *Vitis vinifera* cvs. Thompson Seedless and Chardonnay were co-cultivated with *Agrobacterium tumefaciens* strain EHA 101 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. We have obtained a xylem-specific gene from cucumber, XSP30, from colleagues in Japan (Masuda et al. 1999). We have fused its leader sequence to a GFP marker gene, the expression of which is readily detectable in the laboratory (Maximova et al. 1998), in order to study its ability to target the expression of marker gene products to the xylem stream of grapevines.

OBJECTIVES

1. Evaluate the effect of PGIPs on the development of Pierce's disease in transgenic grapevines.
2. Evaluate the effect of several signal sequences on the targeting of transgene products to the xylem.

RESULTS AND CONCLUSIONS

Effect of PGIPs on the development of Pierce's disease in transgenic grapevines

We have produced 50 transgenic lines that have been transferred successfully to the greenhouse, all from independent transformation events. The presence of the gene and the protein has been confirmed by PCR and Western blotts respectively, and high levels of enzyme activity have been found in crude extracts from leaves.

A group of lines has been tested against *X. fastidiosa*. Five to seven plants of each line were mechanically inoculated with the Temecula strain of this pathogen. Additional plants were inoculated with *Xylella*-free buffer or left untreated. Untransformed plants were subjected to the same treatments. The development of Pierce's disease symptoms was delayed in some lines by several weeks (Figure 1). These lines are currently being evaluated for bacterial growth by ELISA and hydraulic conductance. Infection experiments on the rest of the lines are underway.

In addition we have found PGIP activity in the xylem sap of the transgenic plants but not in untransformed controls. These results suggest that the presence of PGIP in the xylem might interfere with cell wall degradation, preventing vascular occlusion and bacteria movement and/or favoring the accumulation of elicitor-active molecules. We will carry out grafting experiments to determine the transmissibility of the gene product in scion xylem sap.

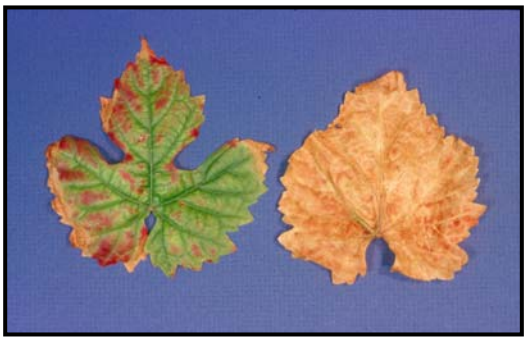

Figure 1a (left). Thompson Seedless vines 3 months after inoculation with *Xylella fastidiosa*. Vine on right is untransformed and has lost all of its leaves. Center vine is a transgenic vine expressing pear PGIP. Vine on left is untransformed and was inoculated with buffer only.

Figure 1b (top). Thompson Seedless leaves 3 months after inoculation with *Xylella fastidiosa*. Leaf on right is from an untransformed control vine. Leaf on left is from a transgenic vine expressing pear PGIP.

Effect of several signal sequences on the targeting of transgene products to the xylem:

A fusion between the leader sequence of a *Cucumis sativus* xylem sap protein (XSP30) and GFP was done in the Dandekar lab. Proembryogenic calluses of cvs. Chardonnay and Thompson Seedless were transformed in May 2002 and are being cultured in germination medium. The strong fluorescence detected in germinating embryos and their periphery (Figure 2) indicates high levels of enzyme synthesis and suggests that GFP is being secreted but additional analysis is needed to determine its subcellular localization.

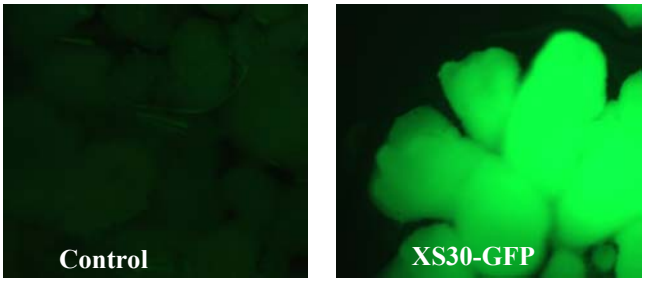

Figure 2. GFP fluorescence of embryos of 'Thompson Seedless' transformed with *35S-XS30-gfp.*

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