

TESTING TRANSGENIC GRAPEVINES FOR RESISTANCE TO PIERCE'S DISEASE

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ABSTRACT

Numerous genes involved in plant disease defense have been isolated and when introduced and expressed in transgenic plants, fungal and bacterial diseases have been greatly reduced. This strategy is especially appropriate for grapevines, where the industry is rooted in traditional European grapes with strong name recognition and very high disease susceptibility. Our laboratory has developed a set of transformed grapevines harboring genes that produce anti-microbial peptides (AMPs). Seventy-six 'Chardonnay' lines transformed with the magainin-type genes, mag-2 and MSI99, and with a PGL class gene, were produced. Some are now growing in tissue culture, in the greenhouse, and in the field (Vidal et al. 2003). AMPs are particularly effective against bacteria, and act by disrupting the cell membranes. The primary goal of this project is to study the potential resistance to Pierce's disease (PD) of magainin and PGL-producing vines. In doing so, it becomes important to characterize the expression of these genes in each line. We are also studying whether AMPs can move from a transgenic rootstock to a non-transgenic scion. If so, it might be possible for engineered rootstocks to be used as a means to affect disease development in a range of scion varieties. Our data to date show that between 1 and 4 copies of the foreign gene can be found in each line studied, and that the inserted genes were correctly transcribed in 46 of 76 lines tested (via RT-PCR). An antibody was produced that can detect the presence of both mag-2 and MSI99 peptides. Using this antibody, AMP production was verified in leaf extracts of in vitro vines harboring mag-2 and MSI99 genes. Studies of PD resistance and movement of transgenic proteins from rootstocks to scions are at a very early stage in this newly funded project.

INTRODUCTION

Numerous genes involved in plant disease defense have been isolated (Punja 2001; Mourges et al. 1998). When disease resistance genes are introduced and expressed in transgenic plants, fungal and bacterial diseases have been greatly reduced (Mourges et al. 1998; Punja 2001; Van der Biezen 2001). This disease control strategy is especially appropriate for clonally-propagated crops, such as grapevines, where the industry is rooted in traditional European grapes with strong name recognition and very high disease susceptibility. Moreover, cross-breeding cannot produce disease resistant forms of elite varieties because other characteristics would be altered and varietal identity would be lost.

Our laboratory has developed a set of transformed grapevines in which we have determined that anti-microbial peptides (AMPs) are transcribed. Seventy-six 'Chardonnay' lines transformed with the magainin-type genes, mag-2 and MSI99, and with a PGL class gene, were produced (Vidal et al. 2003). These are now growing in tissue culture in the greenhouse, and in the field. Magainins are small peptides with strong inhibitory activity against numerous bacteria and fungi (DeGray et al. 2001; Zasloff et al. 1988; Smith et al. 1998; Smith et al. 2001). The MSI99 peptide expressed in tobacco and banana was recently shown to be highly effective against several pathogens (Chakrabarti et al. 2003). In studies conducted in 2002 and 2003, we determined that some AMP-transgenic lines of 'Chardonnay' are significantly more resistant to tumorigenic strains of crown gall (*Agrobacterium vitis*). It is logical to think that these plants might have improved resistance to other bacterial diseases as well.

Some AMP producing genes such as *Shiva-1* are effective against Pierce's disease (PD), according to a recently issued patent (Scorza and Gray 2001) but the subject warrants further study. Scorza and Gray described a trial of two lines of 'Thompson Seedless' expressing the Shiva-1 peptide; both eventually succumbed to PD, but one showed milder symptoms, which did not include the typical signs of marginal leaf burn when compared to the non-transformed control plant. Based on this data, the

above-mentioned patent was issued covering the usage of all AMP genes in the development of *Xylella fastidiosa* (*Xf*) resistant transgenic vines. However, data are not available in the literature to determine if mag-2, PGL, and MSI99 peptides are effective *in planta* against *Xf*. It is the purpose of this proposal to study the potential resistance of our magainin and PGL-producing 'Chardonnay' vines to PD.

OBJECTIVES

1. Quantify the expression of AMPs (anti-microbial peptides) in transgenic 'Chardonnay' vines.
2. Evaluate resistance to Pierce's disease among these transgenic vines.
3. Determine the extent to which an AMP transgenic rootstock can confer PD resistance to the scion.

RESULTS

Objective 1: Because AMPs are very small (~2.7 kD) and easily degraded by host proteases, expression was studied by RT-PCR (for detection of mRNA products from the inserted gene sequences) and ELISA (for direct detection of the presence of peptides). Out of 76 transgenic lines tested by RT-PCR, 46 lines (11 mag-2, 11 MSI99, 8 PGL and 16 mag-2 + PGL) were positive for transcription of the expected mRNA. For mag-2 (23 amino acids) and MSI99 (22 amino acids) peptides, an antibody was developed (by Sigma-Genosys) that recognizes an antigenic sequence common to both peptides. In a series of preliminary ELISA tests using leaf extract from *in vitro* plants, the peptide could be detected in a number of lines, in agreement with the RT-PCR results. However we were unable to detect the peptide consistently, suggesting the methodology requires some improvement. To improve the ELISA procedure, young leaves from actively growing greenhouse plants will be used immediately after collection.

Objective 2: In a preliminary study, 31 transgenic lines (4 plants/line) were inoculated with two different *Agrobacterium vitis* strains to test for resistance to crown gall disease. Among these lines, 6 harboring mag-2, 5 with MSI99, 5 with PGL and 5 with the combination of mag-2 + PGL showed statistically significant gall size reductions ($P < 0.05$) compared to non-transformed controls. From among these crown gall resistant lines, the most promising 16 (4 lines per gene construct) were selected and sent in early October, 2003, to Dr. Walker for PD resistance testing. These are currently being grown in an isolated greenhouse at U.C. Davis awaiting sufficient growth to begin inoculations with *Xf*. PD symptoms are expected to appear 8 weeks after inoculation, and stem and leaf symptoms will be recorded 12 and 16 weeks following inoculation.

Objective 3: Plans are to test the hypothesis that rootstocks can transmit peptides to the scion in late winter or early spring, 2004. In the case of the mag-2 and MSI99 transformants, the AMP gene was fused to a signal peptide to allow product secretion into intercellular spaces. These products are likely to be xylem mobile and may have the ability to move upwards in the plant. Their small molecular size may further facilitate movement within the plant. These transgenic 'Chardonnay' are not being considered for use as commercial rootstocks. However, if they are capable of suppressing PD, efforts to engineer appropriate grape rootstocks would then be justified.

To test this hypothesis, transgenic 'Chardonnay' vines will be used as rootstocks with non-transgenic 'Chardonnay' as scions. Since we will be using young potted plants, approach grafting will be used to connect the plants. Transgenic and normal 'Chardonnay' plants will be planted in the same 15 cm pot, and, after about 4 weeks, there will be enough shoot growth to produce a tongued approach graft and connect the two plants. The unions will heal in about 4 weeks and the non-transgenic 'Chardonnay' stems will be cut so that they become scions on the transgenic 'Chardonnay' rootstock. Shoot growth on the scion 'Chardonnay' should be ready for *Xf* inoculation in about 4 weeks and the scions will be needle inoculated with the Stag's Leap *Xf* strain. Symptom expression will be evaluated as above. We will also culture *Xf* from stem pieces to further quantify the levels of *Xf* in the 'Chardonnay' scions. ELISA techniques will be used to detect the presence of the rootstock-produced AMP in the leaves and stems of the scions. If an effect is detected, it will be very important to carry out rootstock/scion tests using hardwood cuttings and under field conditions.

CONCLUSIONS

This project is at a very early stage, yet it is progressing very well. We are developing an ELISA technique to detect magainin-like peptides in young tissues, with promising results to date. PD resistance studies are about to get underway, and studies regarding the concept that a rootstock can be used to transmit disease-fighting substances to the scion are planned to take place in the coming months. If successful, this project could result in the development of transgenic versions of important cultivars that resist Pierce's disease while maintaining all their important varietal characteristics. In addition, we hope to learn whether rootstocks could be used to improve the resistance of the scion.

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