AMERICAN VINEYARD FOUNDATION

CALIFORNIA COMPETITIVE GRANT PROGRAM IN VITICULTURE AND ENOLOGY

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PROJECT TITLE: Genetic transformation to improve the Pierce's disease resistance of existing grape varieties

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SUMMARY

We now have an efficient transformation system and are able to routinely produce transgenic plants of several grape cultivars. We have performed numerous transformation experiments with marker genes carry regulatory sequences that might be expected to cause the gene products to be secreted from the cell. We have obtained a xylem-specific protein gene from a research group in Japan in order to use its xylem-specific regulatory sequences. We have introduced a PGIP gene that has previously been shown to have disease reducing effects in other plants.

OBJECTIVES OF PROPOSED RESEARCH

1. Further improve a genetic transformation protocol in our laboratory to routinely produce transgenic vines of important grape varieties, particularly Chardonnay.

2. Introduce into an existing variety a gene encoding an antimicrobial compound shown to be effective against the causal agent of Pierce's disease.

3. Regenerate transgenic vines and determine whether they exhibit improved tolerance to Pierce's disease.

EXPERIMENTS CONDUCTED AND RESEARCH ACCOMPLISHMENTS

We have been able to establish embryogenic lines suitable for *Agrobacterium tumefaciens* genetic transformation and to develop an efficient transformation and regeneration system. Basically, pro-embryogenic calli are obtained from immature anthers cultured in PIV medium (Franks et al. 1999) and maintained by transferring to fresh PT medium (Hanson et al. 1999). For inoculation, a dilute Agrobacterium culture is dropped onto individual clumps of calli and incubated for 48 h. Transformed cells are then selected in PT medium supplemented with 100 $mg.l^{-1}$ kanamycin and 300 $mg.l^{-1}$ cefotaxime. After 8-12 weeks putative transformed calli are transferred to WP medium (Lloyd and McCown 1980) supplemented with 100 $mg.l^{-1}$ glutamine, 100 $mg.l^{-1}$ asparagine, 100 $mg.l^{-1}$ arginine and 0.5 $mg.l^{-1}$ (2.22 uM) BA to induce embryo germination.

After having successfully expressed GUS in plants of Thompson Seedless, we have focused on the transformation of cultivars Chardonnay and Thompson Seedless and the rootstock Saint George using constructs that may be relevant for PD resistance. They include a gene that codes for a pear polygalacturonase inhibitor protein (PGIP) and two types of fusions of the green fluorescence protein (GFP) with the amino and carboxy-terminal of a ribosome-inactivating protein (RIP) from *Trichosanthes kirilowii*. PGIPs are localized in plant cell walls and play an important role in prevention of the penetration of microorganisms in several species (Glinka and Protsenko 1998, Lang and Dornenburg 2000). Although the chemical composition of the substance that occludes xylem vessels in PD affected plants is not yet known, PGIP might inhibit the breakdown of plant or bacterial cells walls that contribute to the occlusion. The fusion of GFP to the RIP secretory sequences will permit determination of the level of accumulation of the protein in the xylem sap, providing useful information for future studies related to the delivery of anti-Xylella gene products into the xylem. The GUS gene is also present in all the constructs and all the genes are under the control of CaMV35S promoter.

Inoculations with these constructs started on May 2000. At present, we have obtained 52 plants from independent transformation events (Table 1). A larger number is expected, taking into account the large number of embryos that are still germinating. All the plantlets tested for GUS-activity exhibited positive blue coloration. Fluorescence was also detected in embryos as expected (Figure 1).

Table 1. Number of transformed plants obtained with each construct

Thompson Seedless				**Chardonnay**			
PGIP	GFP	N-GFP	N-GFP-C	PGIP	GFP	N-GFP	N-GFP-C
13	13	2	17	1	3	-	3

In the near future we plan to test in vitro transformed plants for PGIP activity and to transfer the plants to the greenhouse for further analysis, specially for the presence of GFP in the xylem sap. In relation to this, we were encouraged to find GUS activity in xylem sap of transgenic apples and almonds transformed with a standard CaMV35S-GUS construct, although sample contamination with disrupted cells should not be discarded. In addition new transformation experiments will be

performed using a fusion of GFP with the secretion sequence of a xylem sap protein cloned from *Cucumis sativus* (Masuda et al. 1999).

LITERATURE CITED

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Lang C. and Dornenburg H. 2000 Appl. Microbiol. Biotechnol 53:366-375
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OUTSIDE PRESENTATIONS OF RESEARCH

None

RESEARCH SUCCESS STATEMENTS

Grape transformation is now routine in the Meredith lab. We can produce transgenic plants of both scion and rootstock cultivars. We have detected xylem expression of transgene products. We have obtained regulatory sequences that will further direct gene products to the xylem, where Xylella resides. We are working cooperatively with other laboratories that are studying PD disease mechanisms and Xylella biology. As more knowledge is gained in these groups, we will be in a good position to select genes that have potential to improve tolerance to Pierce's Disease.

FUNDS STATUS

All 2000-01 funds have been expended.

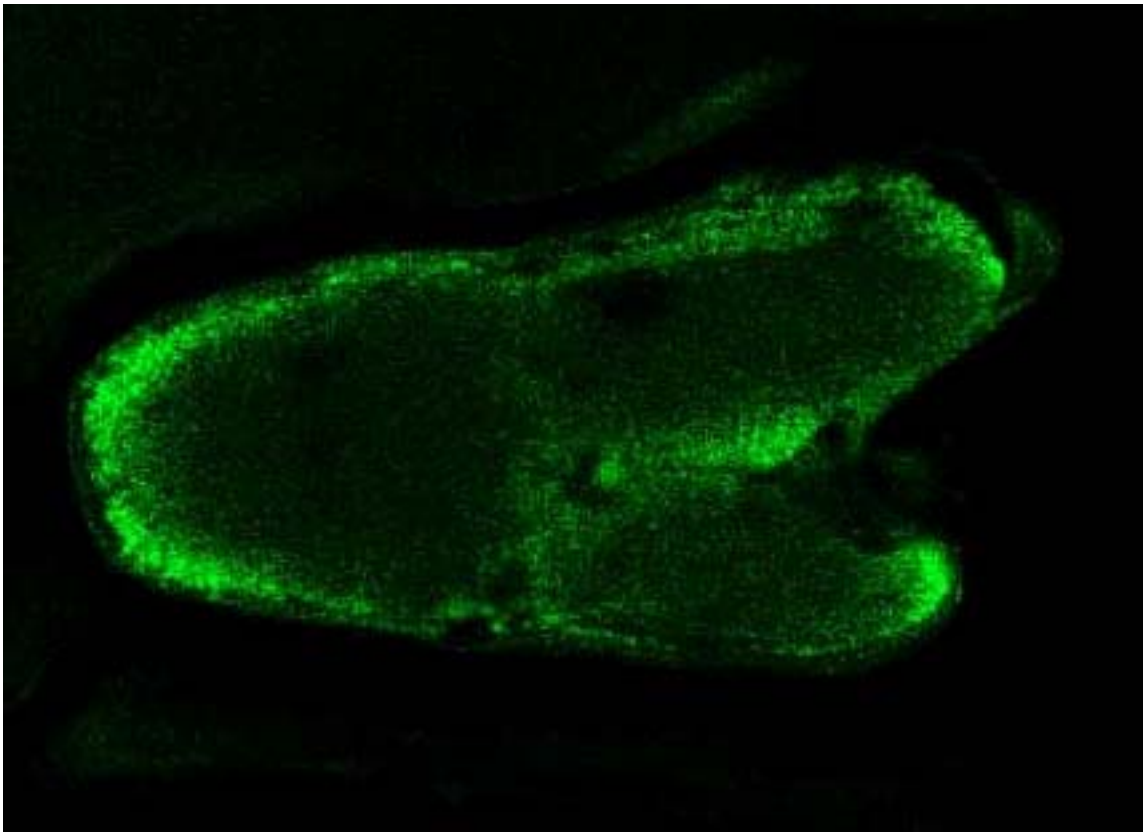

Figure 1. Transgenic grape somatic embryo expressing the introduced GFP (green fluorescent protein) gene.