

GENETIC TRANSFORMATION TO IMPROVE THE PIERCE'S DISEASE RESISTANCE OF EXISTING GRAPE VARIETIES

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INTRODUCTION

Genetic engineering offers the possibility of introducing a gene that will confer increased Pierce's disease (PD) tolerance to existing grape varieties. If other research strategies being considered for PD management are not sufficiently successful, genetic engineering may be the only viable alternative.

In many crops, disease resistance is achieved by identifying sources of resistance within the crop species or its wild relatives and then moving the resistance to the crop by several cycles of conventional breeding. This approach produces entirely new varieties. In California, new varieties of table grapes and raisins are readily accepted but new winegrape varieties are not. As in Europe, the California wine industry is based on the classic wine cultivars and wines are identified by the grape cultivar name. Thus new wine cultivars are incompatible with the way wine is marketed and there is little interest in them in the California wine industry.

We are investigating the use of genetic engineering to alter the disease response of existing grape varieties without otherwise changing their viticultural or enological characteristics. Legal authorities in Europe and the U.S. have expressed the opinion that a genetically engineered winegrape produced by the addition of a gene to a traditional wine variety may be permitted to retain the original variety name if it is indistinguishable from the original version in appearance and flavor.

Genetic engineering methods are now well established for many major crops-thousands of field trials of transgenic crops have already been conducted and many such plants have now been commercialized. Many of these transgenic crop plants carry introduced genes for disease resistance. Perhaps the most dramatic example is that of the papaya industry in Hawaii, the state's second largest fruit crop. Papaya producers were faced with the complete devastation of their industry by papaya ringspot virus disease. A genetically engineered resistant papaya was developed (Luis et al., 1997) and is now the predominant papaya variety in Hawaii, well accepted by both growers and consumers alike. The Hawaiian papaya industry has been rescued by genetic engineering.

The xylem vessels of PD-infected grapevines are blocked by a polysaccharide substance that may be a product of cell wall breakdown. The origin and composition of this substance are the subjects of other research projects. Plant cell wall degradation is an early step in the development of many plant diseases and, of several cell-wall-degrading enzymes produced by pathogens, the best known are the polygalacturonases (PGs). Many plants have polygalacturonase inhibiting proteins (PGIPs) that inhibit pathogen PG enzymes and enhance plant defense response. The expression of a pear PGIP gene in tomato has been shown to reduce the development of fungal disease (Powell et al., 2000). The discovery that the *Xylella fastidiosa* genome appears to encode a polygalacturonase and several other cell wall degrading enzymes (Simpson et al., 2000), suggests that PGIP might reduce vascular plugging in PD-affected grapevines or otherwise restrict the development of the disease. We are studying the Pierce's disease response of transgenic grapevines expressing pear PGIP.

OBJECTIVES

1. Evaluate the effect of an introduced polygalacturonase inhibitory protein (PGIP) gene on the development of Pierce's disease in transgenic grapevines.
2. Evaluate the effect of promoters and signal sequences on the targeting of transgene products to xylem tissue.

RESULTS AND CONCLUSIONS

Proembryogenic cultures of *Vitis vinifera* cvs. Chardonnay and Thompson Seedless were transformed with *Agrobacterium tumefaciens* containing a pear PGIP gene construct under the control of the CaMV 35S promoter. Fifty-nine independent putatively transformed lines were obtained and plants were regenerated. Plants from 37 of these lines have been transferred to the greenhouse. Of 25 lines that have been tested to date, 18 are positive for PGIP activity. PGIP activity was not detected in untransformed controls. Western blot analysis demonstrated the presence of the protein in roots, leaves and young stems of the transgenic plants but not in untransformed controls. The post-translational glycosylation of the enzyme is apparently similar to that of the endogenous PGIP in pear fruit. Plants from some lines have been inoculated with *Xylella fastidiosa* and will be evaluated for Pierce's disease symptoms.

New gene constructs are being made that combine the pear PGIP gene with regulatory sequences from a cucumber gene that encodes a xylem-specific protein. These will be used in a new round of transformation experiments to determine whether the cucumber sequences enhance the presence of PGIP in the xylem of transgenic grapevines.

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