# RESISTANCE TO PIERCE'S DISEASE BY TRANSGENIC EXPRESSION OF PLANT-DERIVED ANTI-APOPTOTIC GENES

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## ABSTRACT
The goal of this research is to identify, clone, and express plant genes that can functionally suppress the symptoms of Pierce's disease (PD) in grape. It is well established that several relatives of grape, including *Vitis californica*, and other host plants can harbor otherwise lethal titers of *Xylella fastidiosa (Xf)* without exhibiting PD symptoms. Hence, *Xf* does not have to kill in order to colonize the vascular system and, death-based symptoms are not necessary for *Xf* to exist as benign endophyte. We now have identified several grape genes from a programmed cell death (PCD) suppressive functional cDNA library screen that block PCD when over-expressed in the presence of known pathogen-derived chemical inducers of PCD. These genes were then put into grapes transgenically being driven by the constitutive 35S promoter. First cycle experiments indicate that three of these genes have homology to genes from other sources whereby their presence is associated with situations in which PCD is suppressed. For example, VVPR1A, is expressed in situations in which PCD is blocked in humans, nematodes, hookworms and several plant species. The images presented here indicate that the first three of these putative anti-apoptotic genes tested suppress PCD induced by *Xf* and the commensurate PD disease symptoms. In addition, the data confirm that the bacteria remains present in the asymptomatic transgenic plants at a titre level equivalent to that supported by *V. californica* and at levels that were lethal to untransformed or GFP-transformed grape plants used as controls.

## INTRODUCTION
We confirmed before beginning this project that the symptoms of PD are due to the induction of apoptosis or PCD. In animals PCD is regulated by many genes but the functional regulators in plants are unknown. We then designed a conditional life-death screen to attempt to identify plant genes that could modulate the activity of the signaling interactions between the bacteria and the grape plant that lead to PCD. The questions posed were: a) are there genes in the plant that respond to *Xf* signals by triggering PCD in certain grape genotypes, b) can this response be blocked genetically, and 3) if so, does this then allow the bacteria to return to the endophytic state, leaving the plant otherwise unaltered but free of disease symptoms or 4) does suppressing PD symptoms negatively affect the ability of the bacteria to colonize the vascular system?

The basic premise is that strategies for disease suppression and characterizing the bacterial-plant interaction were high priority areas in the Pierce's Disease/GWSS Research Program and as noted in the NAS report. Pierce's Disease is defined as plants expressing several symptoms resulting from cell death (leaf scorch) or changes in tissue differentiation (green islands) with the potential to kill plants over time once the infection is established. However, it also is established that several relatives of grape, including *Vitis californica*, and other host plants can harbor otherwise lethal titers of *Xf* without exhibiting PD symptoms.

The goal of this project is to identify novel genes from cDNA libraries of either grape or heterologous plants that, when over expressed in grape, will prevent infection, spread or symptom development due to the presence of *Xf* in the xylem.

## OBJECTIVES
1. Produce grape transgenic plants over-expressing candidate anti-apoptotic plant genes obtained through conditional life-death cDNA library screens.
2. Measure the effect of blocking PD symptoms with anti-apoptotic transgenes on bacterial population and movement *in planta*.
3. Determine grape gene expression changes in transgenic compared with non-transgenic plants infected with *Xf*.

## RESULTS
### Genes identified as potential anti-PCD genes from the conditional life-death screen.
Previous funding on this project lead to the development of a functional cDNA screen to identify plant genes, which when over-expressed as transgenes, suppress cell death triggered by chemical inducers of PCD. The genes in Table 1 have been described in earlier reports to this symposium. These genes were then transferred to the UC Davis plant transformation facility to insert these genes in cv Freedom and Thompson Seedless, chosen for their high relative susceptibility and ease of transformation. Within the past year we have obtained 42 Freedom and 195 Thompson seedless independent transgenics with a variety of these anti-PCD genes being expressed from the 35S, a strong constitutive promoter.

**Grape transgenic plants over-expressing candidate anti-apoptotic plant genes** (Tables 2 and 3). All plants were tested in the laboratory for the presence of the test gene and expression of the gene by Northern analysis before moving to the greenhouse. Individual plants were then cloned into ramets of each line and tested again for expression of the transgene before being inoculated with *Xf*.

**Cv.Freedom grape plants expressing anti-apoptotic genes were inoculated** after creating ramets of these transgenic lines. The transformed plants were individually inoculated March thru June of 2007. The inoculation method was by needle puncture of the stem to allow uptake of 10-20 ul of *Xf* at $2x10^8$ cfu/ml of the Temecula strain. The plants were monitored for symptoms and bacterial movement by PCR. They were scored for disease severity in September 2007 using a 5 point scale (1=dead and 5= asymptomatic) (Figure1 and Table 4) and photographed.

**The effect of anti-apoptotic transgenes on *Xf* bacterial populations and movement *in planta* was measured** by RealTime quantitative PCR of the stem of primary branches (Table 4). It is essential to determine the effect of blocking PCD-based symptoms in the transgenic plants on the bacterial multiplication and spread in terms of the overall impact of the transgenes. Based on initial experiments to ascertain which tissue to sample for *Xf* quantitation, we have sampled the stem of primary branches of individual plants. This also allows repeated sampling of an individual plant over the course of the experiment. We find that *Xf* bacterial concentrations are similar for all of the asymptomatic plants including *V. californica*

**Table 1.** Plant anti-apoptotic genes, derived from functional screen of cDNA libraries, for transformation into grape plants

| Construct | Gene | Source |
|---|---|---|
| CBWG8 | glutathione-S-transferase | Chardonnay |
| CB390 | metallothionein | Chardonnay |
| CB456 | Nematode induced gene | Chardonnay |
| CBWG23 | unknown function | Chardonnay |
| CBWG29 | unknown function | Chardonnay |
| CBWG33 | unknown function | Chardonnay |
| CBWG71 | cytokine-like gene | Chardonnay |
| CBWG75 | germin-like gene | Chardonnay |
| CBPR1A | VVPR1A | Chardonnay |
| CBI35 | Intron p35 (anti-PCD control gene) | baculovirus |
| CBP14LD*# | P14 (homolog of PR1A) | tomato |
| CB376# | Mycorrhizal induced gene | tomato |

**Table 2.** VARIETY: Freedom

| Genotype | # of Independent Transformants | # of Plants |
|---|---|---|
| FR - CBP14 | 16 | 293 |
| FR - CB456 | 7 | 112 |
| FR – CBGFP control | 10 | 140 |
| FR - CB390 | 9 | 126 |
| FR-Walker control | 1 | 122 |
| FR-FPS control | 1 | 39 |
| total | 44 | 832 |

**Table 3.** VARIETY: Thompson Seedless now available with putative anti-PCD genes under control of the 35S promoter.

| Genotype | # Lines or Independent Transformants | # of Plants |
|---|---|---|
| TS – CBPR1A | 24 | 30 |
| TS CBP14LD | 27 | 27 |
| TS - CB376 | 28 | 29 |
| TS - CB456 | 27 | 31 |
| TS - I35 | 14 | 15 |
| TS - SGFP-RIN | 10 | 12 |
| TS – CB390 | 22 | 24 |
| TS - CBWG23 | 23 | 25 |
| TS - CBWG71 | 20 | 23 |
| TS – Control | 7 | 72 |
| TS - GFP | 1 | 1 |
| total | 203 | 289 |

**Table 4**

| Genotype | # of Inoculated independent transformants | # of Plants evaluated to date | Category 3-5 plants similar to images in Figure 1 | Mean bacterial load per gm of stem in asymptomatic category 5 branch |
|---|---|---|---|---|
| FR - CBP14 | 5 | 32 | 58% | $10^6$ |
| FR - CB456 | 2 | 15 | 71% | $10^6$ |
| FR - CB390 | 3 | 24 | 76% | $10^6$ |
| FR – CBGFP (control) | 4 | 29 | 0% | not applicable |
| *V. californica* | asymptomatic host | 3 | 100% | $10^4$ |

**CONCLUSIONS**

Genetic strategies for disease suppression and information characterizing the bacterial-plant interaction are high priority areas in the Pierce's Disease/GWSS Research Program and the NAS report. The 2007 RFP for research on Pierce's Disease lists as one focused area of research the effect of blocking disease symptoms, defined primary as cell death, initially of foliar tissue with leaf scorch and matchsticks, culminating under heavy disease pressure in death of the entire plant. The overall goal of our research is to determine the molecular basis for the symptomatic cell death and attempt to genetically block the Xylella- triggered cell death as a means of blocking the deleterious effects of the disease. We have identified novel genes from cDNA libraries of either grape or heterologous plants that, when over expressed in grape, prevented symptom development due to the presence of *Xf* in the xylem. We have previously reported that susceptibility of several plants to a range of pathogens depends on the ability of the pathogen to directly or indirectly trigger the activation of genetically determined pathways leading to apoptosis or PCD. The anti-PCD genes isolated through earlier research in our laboratory are the focus of the current studies that now demonstrate that blocking PCD initiated by *Xf* can block disease rending the grape plant an asymptomatic endophytic host. The current experiments provide initial information that the effect of the genes appears to be on symptom expression and not a direct effect on the bacteria. Therefore, the symptom suppressive genes do not act as antibiotics and do not affect the natural endophytic ecology of the bacteria in the xylem. In essence, an endophyte gone bad has been returned to the state of a benign endophyte.

**REFERENCES**

Gilchrist, D.G., and J.E. Lincoln. 2006. Resistance to Pierce's Disease by transgenic expression of plant-derived anti-apoptotic genes. San Diego, CA November 27-29

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**Figure 1.** Anti-PCD genes expressed in transgenic grape (cv. Freedom) plants suppress symptom appearance in PD susceptible plants without affecting the presence of *Xf* in the asymptomatic branches. The transgenic control plants and non-transgenic plants were uniformly killed under the same conditions with the same level of initial inoculum.