Suppression of Pierce's Disease symptoms by transgenic expression of plant-derived anti-apoptotic genes

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Pierce's Disease (PD) is recognized in grape (*Vitis vinifera*) when plants infected with *Xylella fastidiosa* express several symptoms resulting from cell death (leaf scorch, matchsticks etc.) or changes in tissue differentiation (green islands). It also is established that several relatives of cultivated grape and numerous other host plants can harbor high titers of *Xylella fastidiosa* in the xylem without exhibiting symptoms of Pierce's Disease. These observations point to two interesting aspects of this plant-bacterial interaction, namely that *X. fastidiosa* is capable of living within the xylem of some plants as a benign endophyte and secondly, in certain situations, their presence can elicit death in cells contiguous to the xylem but not in all cases. We subsequently determined that the leaf scorch symptoms in grape exhibited morphological hallmarks of apoptosis or programmed cell death (PCD), which is a genetically conserved molecular mechanism leading to cell death in animals and plants. In this context, recent published information from our laboratory established 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 apoptosis or PCD (1). Furthermore, we determined that transgenic expression of genes, known to block apoptosis in both animals and plants, can be used to block plant disease symptoms, essentially protecting the plant against the pathogen signals that result in disease (2,3). If such transgene-directed blocking of the death of symptoms of PD can be achieved, this would be the functional equivalent of maintaining the bacteria in an endophytic relationship without triggering the symptoms of PD.

Currently, several laboratories including our own are conducting systematic studies of the molecular basis of susceptibility of plants to a range of pathogens including bacteria and fungi. The objective of our studies with grape is to identify genes that have the potential to block the expression of disease symptoms in grape to Pierce's Disease (PD). Such genes could effectively create cells that are refractory or insensitive to the signals expressed by pathogens that lead to susceptibility. This approach parallels investigations now widely reported and accepted in human medicine whereby genes, signaling pathways and chemical signals expressed by animal pathogens initiate or block infection by activating or blocking apoptosis through constitutive genes or signaling pathways present in all cells. This has lead broadly to searches for chemical and genetic approaches to block apoptosis as a means to suppress several degenerative human diseases.

In our approach to studying PD, the goal was to attempt to isolate potential anti-PCD genes from grape or heterologous plants. We first developed cDNA libraries from susceptible and resistant grape cultivars. The construction of a grape cDNA library initially proved much more difficult than we had experienced in making libraries from 4 other plant species. Eventually, we developed an efficient protocol for generating full-length cDNA libraries from grape using an antioxidant cocktail during homogenization and CsCl gradient purification of RNA. The inserts for the libraries were cloned into the binary vector B5 for transformation into the Agrobacterium rhizogenes, with the A. rhizogenes then used to transfer the cDNA library inserts into grape. The A. rhizogenes-based transformation procedure results in the induction of transformed roots from infected (or healthy) vegetative tissue sections following co-cultivation with the transforming bacteria. Each emerging root is an independent transformation event, contains a single new DNA insert, and from which the transgene can be re-isolated by PCR for characterization. This technique is a functional cDNA library screen (each root contains a different cDNA library member) for genes from grape libraries that block bacterial multiplication, movement, or symptom expression. The library was screened in sets of 50,000 cDNAs. As potential cloned resistance genes became available they also will be used to identify homologues from the Chardonnay cDNA library that may provide resistance by simple alteration in expression level within the homologous host in a time and tissue specific manner (4).

Summary:
Several relatives of grape and other asymptomatic plants can harbor high titers of X. fastidiosa without exhibiting PD symptoms. We have established that leaf scorch PD symptoms in grape result from apoptosis or programmed cell death (PCD). Clearly, X. fastidiosa does not have to kill in order colonize the vascular

system. So, a key question addressed by this research is; are there genes in the plant that respond by triggering programmed cell death in certain grape genotypes, can this response be blocked genetically, and, if so, does this then allow the bacteria to return to the endophytic state, leaving the plant otherwise unaltered and disease symptom free? We have identified from a functional cDNA library screen several grape genes that block PCD when over-expressed (Table 1). Preliminary experiments indicate that one of these genes, VVPR1A, is expressed in situations in which PCD is blocked in humans, nematodes, hookworms and several plant species. We are testing the hypothesis that over expression of genes like VVPR1A can block both PCD induced by X. fastidiosa and disease symptoms associated with PD in both detached branch or leaf uptake assays and in inoculated whole plants.

References:

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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

* Northern positive transgenic plants available at this time in cvs. Freedom and Chardonnay.