Final Report for CDFA Agreement Number 09-0780

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Project Title: Field evaluation of grape plants expressing PR1 and UT456 transgenic DNA sequences for protection against Pierce's Disease.

Principal Investigator	David Gilchrist	Department of Plant Pathology, UC Davis	dggilchrist@ucdavis.edu
Co-Principal Investigator	James Lincoln	Department of Plant Pathology, UC Davis	jelincoln@ucdavis.edu

Reporting Period: The results are inclusive for the entire grant period March 1, 2010 to February 28,2013

ABSTRACT

The objective is to evaluate transgenic grape plants and grape rootstocks expressing two DNA constructs designated PR1 and UT456 genes in field sites in Solano and Riverside Counties for resistance to *Xylella fastidiosa* (Pierce's Disease strain) (Xf). Infection at the Solano site will use mechanical inoculation and will depend on natural inoculation at the Riverside site where endemic sharpshooters carry Xf. The basis for this experiment derives from four previous inoculation experiments in a controlled greenhouse over a two year period, involving more than 300 transgenic plants of PR1 and UT456 indicated that suppression of PD symptoms and reduction in bacterial titer was consistent in the transgenic compared with untransformed control plants. The Solano field experiment is conducted in two phases. The first phase started in 2010 to evaluate clonal copies of the fully transformed ungrafted plants that exhibited suppressed PD symptoms and low bacterial titers. The second phase began in 2011 with planting the untransformed Thompson Seedless scions grafted onto the most resistant of the PR1 and UT456 plants as rootstocks. Over the course of the 3 year field evaluation at both sites, test plants in the first planting will include ungrafted conventional Thompson Seedless and Freedom plants as controls to be compared with the transformed plants. Controls in the second phase will include, untransformed rootstocks grafted to the untransformed scions, which will be compared to equivalent combinations expressing the test genes grafted to untransformed PD susceptible scions. Data collected in 2012 from both sites indicate that the bacteria are present in all plants at the Riverside site and in the mechanically inoculated plants at the Solano site. Some plants in Riverside are showing symptoms of leaf death while the plants in Solano remain healthy in appearance. Clearly, at least one more year of evaluation is needed to begin to develop an assessment of the possible field efficacy of the transgenes. Quantitative data collection is in progress at both sites.

INTRODUCTION

Susceptibility in most plant-microbe interactions depends on the ability of the pathogen to directly or indirectly regulate genetically determined pathways leading to apoptosis or programmed cell death (PCD). The role of altered cell stability in disease through an evolutionarily conserved program involving programmed cell death occurs in both animals and plants. Functionally, the induction of PCD results in an orderly dismantling of cells while maintaining integrity of the plasma membrane until internal organelles and potentially harmful contents including phenolics, reactive oxygen and hydrolytic enzymes have been rendered harmless to contiguous cells. Processed in this manner, the cell contents can serve as nutrients for microbial cells when they are present in the immediate environment of the pathogen (2). In the case of *Xf* and many other plant pathogenic bacteria, the bacteria live predominantly as endophytes or epiphytes but occasionally as pathogens. The relative susceptibility of the individual plant species is determined by unknown genetic factors. Presumably, sensitivity to the presence of the bacteria, expressed as cell death-dependent symptoms, is the result of signals expressed

by the bacteria that lead to activation of PCD, as appears to be the case with PD. Our research has focused on the effect of altering the expression of two different plant DNA sequences (PR1 and UT456). Both of these putative anti-PCD sequences protected both against PD symptoms and limited bacterial titer four to six orders of magnitude below that reached in untransformed control vines of the susceptible cultivar Thompson Seedless and the commercial rootstock Freedom, In the past year we constructed transformed rootstocks (Freedom and Thompson Seedless) expressing PR1 or UT456 grafted to untransformed Thompson Seedless and winegrape scions to be tested for efficacy of protection across a graft union. Initial greenhouse inoculation experiments indicated that the protection by PR1 and UT456 does move across the graft union. In summary, experimental results to date confirm progress in identifying DNA transcripts of grape which, if regulation of the natural transcripts is altered in transgenic plants, result in the suppression of symptoms of PD with an associated limitation in bacterial titer to levels generally associated with a benign endophytic association. Initial data on potential for transmission of protection by these anti-PCD sequences across a graft union to protect an untransformed wild type scion is positive.

OBJECTIVES 2011-2012

A. The overall objective is to evaluate transgenic grape plants and grape rootstocks expressing two DNA constructs designated PR1 and UT456 genes in a field site in Solano County for resistance to the Pierce's Disease strain of Xylella *fastidiosa* (*Xf*) following mechanical inoculation.

B. The field experiments in Solano County will be conducted in two phases. The first phase of the field experiment started in 2010 will evaluate clonal copies of the fully transformed ungrafted PR1 and UT 456 plants that exhibited suppressed PD symptoms and low bacterial titers. These experiments will consist of sets of inoculated and uninoculated control plants. All plants to be inoculated will be infected by stem puncture with ~20,000 *Xf* bacterial cells per inoculation site. Inoculations were done July 2011 and repeated in June of 2012.

C. The second phase of the Solano County field planting began in 2011 with planting the untransformed commercial scions grafted onto the most resistant of the PR1 and UT456 plants as rootstocks.

D. The field experiment in Riverside County was planted in the Spring of 2011. The planting consisted of clonal copies of the fully transformed ungrafted plants expressing PR1 or UT 456 that were planted in 2010 in Solano County. These Riverside plants will not be inoculated with *X. fastidiosa* but will be exposed to infection via natural populations of the Glassywinged sharpshooter Xf vector.

RESULTS AND DISCUSSION

A. *The first phase of the field experiment started in 2010 to evaluate clonal copies of the fully transformed ungrafted PR1 and UT 456 plants that exhibited suppressed PD symptoms and low bacterial titers (2010-2013).*

This phase took place as planned with the planting occurring on July 12, 2010. Plants were placed in plastic sleeves to protect against sunburn and wind damage. The young plants had all emerged from the sleeves within two months and appeared to be growing normally. Selections of canes to form cordons were made in spring 2011. Test plants were planted in a complete randomized block design. Field maps were prepared prior to planting and each plant is labeled with a permanent metal tag. Evaluation of the experimental plants for plant morphology, symptoms of Pierce's Disease infection, and the presence of the bacteria will be a time course evaluation by visual monitoring of symptom development and sampling inoculated tissue (mainly leaves and stems) for *Xf* by quantitative PCR (qPCR) assays. A comparative quantitative determination by qPCR of the

presence of Xylella in transgenic grape and grape rootstocks compared with conventional grape and grape rootstocks will provide an indication of the level of resistance to Pierce's Disease infection and the impact on the bacterial load in the respective transgenic and control plants. All these procedures have been used successfully in the ongoing greenhouse experiments for the past 5 years.

B. ***The second phase of the Solano County field planting began in 2011 with planting the untransformed commercial scions grafted onto the most resistant of the PR1 and UT456 plants as rootstocks (2010-2013).*** Transgenic rootstocks for grafting were made by removing green shoots from greenhouse-grown plants of Thompson Seedless and Freedom expressing either PR1 or UT456, surface sterilized for 30 seconds in 70% ethyl alcohol, followed by 1% sodium hypochlorite solution containing 0.2% Tween 80 for 20 min with shaking, on a rotary shaker (50 rpm). The surface sterilized shoots are cut into single node pieces and placed into solid growth media to stimulate root formation. All the grafting is conducted in sterile Magenta GA-7 Plant Culture Boxes (3 x 3 x 4") containing 50 ml media under a 16 h light, 8 h dark photoperiod at 25^{o}C. Rootstock plantlets obtained *in vitro* are allowed to grow until several leaves are produced (4-6 weeks) and divided into 3–4 explants, each containing a single node. A scion with a single node and a leaf was selected to match the size of the rootstock; cut into a wedge to match a cleft made in the rootstock and was carefully fitted on to the cleft of the rootstock on the medium. After 4 weeks incubation healing in a magenta box, the rooted plantlet is transferred to sterile soil , allowed to heal and then transferred to the greenhouse for assays. Success rate is greater than 95% using this procedure, is more space efficient relative to greenhouse grafting, can be done anytime of the year, and is as rapid as green grafting. The plants for the Solano County phase two were planted in the field May 17, 2011.

C. ***Establish a field planting in Riverside, County consisting of clonal copies of the fully transformed ungrafted PR1 and UT 456 plants that were planted in Solano County in 2010. (2011-2013)*** Field space was prepared in Riverside County and grape plants grown in our greenhouse were transported to Riverside for planting. We coordinated the movement of plants to Riverside County with Professor Steven Lindow from UC Berkeley, who also planted his materials for the first time in Riverside County. The planting occurred April 2011.

Secure patent protection as intellectual property for those genes that prove to be capable of blocking PD in grape. The grape plants containing the anti-PCD genes and the grafted rootstocks will require the use of several patented enabling technologies. Record of invention disclosures have been submitted to the UC Office of Technology Transfer. The research proposed reported herein will provide data on the activity and mechanism of action of the protective transgenes in grape relative to the presence, amount and movement of *Xylella fastidiosa* in the transformed and untransformed grape plants.

CONCLUSIONS: *Xylella fastidiosa* induces PD symptoms that result from activation of a genetically regulated process of programmed cell death. We have identified grape DNA sequences, which when constitutively expressed in transgenic grapes suppress the death-dependent symptoms of PD and reduce the bacterial titre to a level found in PD resistant wild grapes. We identified six novel anti-PCD genes from cDNA libraries of grape. Two of these grape sequences expressed as transgenes in grape, suppressed PD symptoms and dramatically reduced bacterial titer in inoculated plants in full plant transgenics. Initial data suggest that protective sequences may function across a graft union to protect an untransformed and susceptible wild type scion. This project has identified a basis for PD symptoms and a genetic mechanism to suppress symptoms and bacterial growth with an infected plant. If needed in the future, a transgenic strategy exists to address PD. The plan for the coming year is to continue the field evaluation of transgenic grapes expressing PR1 and UT456 and to test for cross-graft protection by these two sequences, also under field conditions.

LAYPERSON SUMMARY

Previously, we identified novel anti-PCD genes by a functional screen from cDNA libraries of grape. Two of these grape sequences (PR1 and UT456) expressed as transgenes in grape, suppressed Pierce's Disease (PD) symptoms and dramatically reduced bacterial titer in inoculated plants under greenhouse conditions. This project will evaluate clonal copies of these same plants under field conditions for resistance to (PD). The field evaluation will be conducted in Solano and Riverside Counties and will include mechanical inoculation with *X. fastidiosa* in Solano County and Glassy Winged Sharpshooter inoculation in Riverside County. Data sets will include visual monitoring of plant morphology, PD symptoms and bacteria titer by quantitative PCR (qPCR) assays. To date, PCR data and plating assays confirm the presence of Xf in the plants at both locations. Differential protection against defoliation was observed at the Riverside site and PCR assays confirmed a correlation between protection, transgene presence, and bacterial populations in the plants. While bacteria are present in inoculated plants at the Solano site, there is no definitive evidence of extensive symptoms or bacterial population differences. Clearly both sites will need to be monitored and assays taken over a longer period of time for meaningful results to be obtained.

INTELLECTUAL PROPERTY Record of invention disclosures have been submitted to the UC Office of Technology Transfer.

STATUS OF FUNDS: All funds were expended at the end of the agreement period

FUNDING AGENCIES: Funding for this project was provided by CDFA-Pierce's Disease Program and USDA/CSREES.

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Figure 3. (left) Inoculated grape vine canes at the Solano County site.

Figure 4. Example grape plants growing in Riverside county August 2012, subject to feeding by the Glassy-winged Sharpshooter confirmed to carry *Xylella fastidiosa*. Plant on the left is transgenic TS UT 456-6 compared with a control plant on the right expressing substantial symptoms of leaf death. Complete data set collection is in progress.