Renewal Progress Report for CDFA Agreement Number 09-0780

Project Title. Field evaluation of grape plants expressing PR1 and UT 456 transgenic DNA sequences for protection against Pierce's Disease

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Reporting Period: The results reported here are from work conducted July 1, 2011 to March 3, 2012

I. OBJECTIVES

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 starting 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 inoculated sets and uninoculated control plants. All plants to be inoculated will be infected by stem puncture with ~20,000 *Xf* bacterial cells per inoculation site. Timing of inoculation will depend on growth of the plants but is expected to occur late spring of 2011.

C. The second phase of the Solano County field planting will begin 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 to be planted in Riverside County will begin in the Spring of 2011. The planting will consist of clonal copies of the fully transformed ungrafted plants expressing PR1 or UT 456 that were planted in 2010 in Solano County. These plants will not be inoculated with *X. fastidiosa* but will be exposed to infection via the glassy winged sharpshooter vector of the bacteria.

II. Summary of major research accomplishments for each objective

Each of the objectives has moved at the rate projected and interim goals for this fiscal year will be met. Details of the progress to date is presented in the following section.

A. *The first phase of the field experiment starting 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 (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 will begin 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 as rapid as green grafting. The plants for the Solano County phase two planting have been successfully grafted and 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 on July 12, 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.

III. Publications: None

IV. Presentations. None

V. Research relevance

The objective is to evaluate transgenic grape plants and grape rootstocks expressing two DNA constructs designated PR1 and UT456 genes in a field site at UC Davis for resistance to *Xylella fastidiosa* (Pierce's Disease strain) following mechanical inoculation and in a field site at UC Riverside which has endemic sharpshooters carrying Xf. In four inoculation experiments in a controlled greenhouse over a two year period, involving more than 300 transgenic plants of PR1 and UT456, the suppression of PD symptoms and reduction in bacterial titer has been consistent. A positive correlation between the PR1 and UT456 message level, suppression of bacterial titer and absence of PD symptoms was established using qPCR to measure both the message and the bacteria titer. The Solano field experiment will be conducted in two phases. The first phase starting in 2010 will evaluate clonal copies of the fully transformed ungrafted plants that exhibited suppressed PD symptoms and low bacterial titers. The second phase will begin with planting the untransformed commercial scions grafted onto the most resistant of the PR1 and UT456 plants as rootstocks. Over the course of the 3 year field evaluation, 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 to be compared with equivalent combinations expressing the test genes grafted to untransformed PD susceptible scions.

VI. 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.

VII. Status of funds. We anticipate that all funds allocated for fiscal year 2011-2012 will be expended by June 30, 2012.

VIII. Intellectual property.

plant	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Row 10	FD#3	TS P14 #9	TS UT456 #10	FD P14 #13	FD UT456 #3	TS02A	TS UT456 #8	TS P14 #14	TS P14 #14	FD P14 #13	TS UT456 #10	TS02A	TS P14 #9	FD UT456 #3	FD#3	TSO2A	FD#3	TS P14 #14	FD UT456 #3	TS UT456 #10	TS P14 #9	TSO2A	FD P14 #13	TS UT456 #8	TSO2A
Row 11	FD UT456 #3	TS02A	TS UT456 #8	TS UT456 #10	TS P14 #9	FD#3	TS P14 #15	FD P14 #13	Δ FD#3	FD UT456 #3	TS02A	FD P14 #13	TS P14 #15	Δ¤ TS P14 #9	TS UT456 #10	TS UT456 #8	TS UT456 #10	TS P14 #9	FD P14 #13	TS UT456 #8	TS P14 #15	Δ¤ TS02A	FD#3	FD UT456 #3	Δ TS UT456 #10
Row 12	FD UT456 #3	TS02A	TS UT456 #8	FD P14 #13	TS P14 #9	TS P14 #16	TS UT456 #10	Δ FD#3	FD UT456 #3	TS UT456 #8	TS UT456 #10	TS02A	TS P14 #9	FD#3	FD P14 #13	TS P14 #16	TS UT456 #8	FD P14 #13	Δ TS02A	TS UT456 #10	FD UT456 #3	TS P14 #16	TS P14 #9	FD#3	TS P14 #9
Row 13	GT/FD3	GT/TSP14-9	GT/UT456-10	GT/FDP14-13	GT/FD456-3	GT/TS02A	GT/UT456-8	GT/TSP14-15	GT/TSP14-15	GT/FDP14-13	GT/UT456-10	GT/UT456-8	GT/TSP14-9	GT/FD456-3	GT/FD3	GT/TSO2A	GT/FD3	GT/UT456-10	GT/FD456-3	GT/UT456-10	GT/TSP14-9	GT/TSO2A	GT/FDP14-13	GT/UT456-8	GT/TSO2A
Row 14	GT/FD-3	GT/TSO2A	GT/UT456-8	GT/UT456-10	GT/TSP14-9	GT/FD3	GT/UT456-10	GT/FDP14-13	GT/FD3	GT/FD-3	GT/TSO2A	GT/FDP14-13	GT/TSP14-15	GT/TSP14-9	GT/UT456-10	GT/UT456-8	GT/UT456-10	GT/TSP14-9	GT/FD P14-13	GT/UT456-8	GT/TS P14-9	GT/TSO2A	GT/FD3	GT/FD456-3	GT/UT456-10
Row 15	GT/FD-3	GT/TSO2A	GT/UT456-8	GT/FDP14-13	GT/TSP14-9	GT/TS 02A	GT/UT456-10	GT/TSO2A	GT/FD-3	GT/UT456-6	GT/UT456-8	GT/TSO2A	GT/TSP14-9	GT/TSO2A	GT/FDP14-13	GT/FD456-15	GT/UT456-6	GT/FDP14-13	GT/TSO2A	GT/UT456-10	GT/FDP14-13	GT/UTP14-9	GT/TSP14-15	GT/TSO2A	GT/TS P14-9

North

are inoculated with 20ul of "temecula" Xf @ 1000 cells/ul on 7/13/2011

are grafted and will be inoculated spring 2012

None envisioned at this point. All IP is being handled from the previous greenhouse studies under tightly controlled conditions with mechanical inoculation.

Figure 2. (left) Inoculated grape vine canes marked with orange tags at the Solano County site.

Figure 4. Transgenic grape growing in Riverside county September 2011.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Row DGG5	TS UT456-6	TS02A	TS UT456 #8	FD P14 #13	TS P14 #9	TS P14 #16	TS UT456 #10	FD#3	TS UT456-5	TS UT456 #8	TS UT456 #10	TS02A	TS P14-9	FD#3	TS UT456-6	TS P14 #16	TS UT456 #8	TS UT456-5	TS02A	TS UT456 #10	TS P14-9	TS P14 #16	TS P14 #9	FD#3	TS P14 #9
Row DGG6	TS UT456-10	TS02A	TS P14-9	TS UT456-10	TS P14 #9	FD#3	TS P14-16	FD P14 #13	FD#3	TS P14-9	TS02A	TS UT456-5	TS P14-16	TS P14 #9	TS UT456 #10	TS UT456 #8	TS UT456 #10	TS P14 #9	TS UT456-6	TS UT456 #8	TS P14-16	TS02A	FD#3	TS P14-9	TS UT456 #10
Row DGG6	TS UT456-6	TS P14 #9	TS UT456 #10	FD P14 #13	FD UT456 #3	TS02A	TS UT456-8	TS UT456-5	FD P14 #13	TS P14-9	TS UT456 #10	TS UT456 #8	TS P14 #9	TS P14 #9	FD#3	TSO2A	FD#3	TS P14-9	TS P14-9	TS UT456 #10	TS P14 #9	TSO2A	TS UT456-6	TS UT456 #8	TS P14-9
Creek	Creek	Creek	Creek	Creek	Creek	Creek	Creek	Creek	Creek	Creek	Creek	Creek	Creek	Creek	Creek	Creek	Creek	Creek	Creek	Creek	Creek	Creek	Creek	Creek	Creek

Figure 3. Gilchrist Riverside Field Grape Map. Plants are planted on a sloping river bank with a creek at the bottom. Xylella spreading sharpshooters are common to this site.