Renewal Progress Report for CDFA Agreement Number 14-0486-SA

EXPANDING THE RANGE OF GRAPE ROOTSTOCK AND SCION GENOTYPES THAT CAN BE GENETICALLY MODIFIED FOR USE IN RESEARCH AND PRODUCT DEVELOPMENT

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Introduction:

This proposal is aimed at applying the progress that has been made in grape cell biology and transformation technology of rootstock genotypes 1103P and 101-14 to additional grape rootstock genotypes in order to expand the range of genotypes amenable to transformation. The research will apply pre-existing expertise and technical know-how developed for rootstocks 1103P and 101-14 at UC Davis's Plant Transformation Facility to additional rootstock germplasms important for the California wine industry. For this proposal, we are testing seven additional rootstocks for their amenability to transformation including; Richter 110 (clone 1), 3309C (clone 05), Freedom (clone 1), Harmony, MGT 420A (clone 4), 140Ru (clone 1) and Salt Creek (clone 8). In 2016 we will add rootstock genotype GRN-1 01.1from Andy Walker's program. This work will expand the range of rootstocks that can be effectively transformed which will allow rootstock-mediated disease resistance technology to be employed across the major wine growing regions in California. Although a rootstock-mediated resistance strategy is the preferred mechanism for achieving resistance to Pierce's Disease in grape, investing in the development of transformation technology for scions will serve an important fallback position should rootstock-mediated resistance fail to confer adequate levels of resistance to the scion and direct transformation of scion varieties be required. Therefore, in addition to testing the utility of our tissue culture and transformation protocols on eight additional grape rootstocks, we will also screen six important California scion genotypes for their amenability to transformation including Cabernet Sauvignon (clone 07/08), Chardonnay (clone 04), Pinot Noir (2A), Merlot (clone 03), Zinfandel (clone 01A), and French Colombard (clone 02). Although it is unlikely that all eight rootstock and six scion genotypes will be amenable to transformation using our established protocols, we believe that a significant number will respond positively. The results of this work will allow for the establishment grape tissue culture and transformation technologies that can be utilized by the PD/GWSS Research Community. It will also establish a germplasm bank of grape suspension cultures and a repository of somatic embryos for rootstock and scion genotypes used in California, which can be made available to the research community. To date, we have made significant progress in establishing somatic embryo, in vitro shoot cultures and bulk meristem cultures, cell suspension cultures and stored embryo germplasm bank for many of the targeted genotypes. We have now demonstrated that in addition to rootstocks 101-14 and 1103, that Richter MGT 420A and Freedom can be included in the list of rootstock genotypes we can successfully transform. Next year we will focus on establishing somatic embryos, cell suspension, stored somatic embryos and bulk meristems from the remaining genotypes, while continuing to evaluate the transformation efficiency of this material with the target of adding 3309c 140 Ru, GRN-1, Harmony, and Salt Creek to our transformation portfolio.

OBJECTIVES

1. Develop embryogenic cultures from anthers of seven rootstock genotypes and six scion genotypes for use in establishing embryogenic suspension cultures.

2. Develop embryogenic suspension cultures for seven rootstock genotypes and six scion genotypes, which will provide a continuous supply of somatic embryos for use transformation experiments.

3. Establish a germplasm bank of somatic embryos for seven rootstock genotypes and six scion genotypes by plating aliquots of the cell suspension culture on high osmotic medium.

4. Test transformation efficiencies of seven rootstock genotypes and six scion genotypes using our established somatic embryo transformation protocols.

5. Test direct cell suspension transformation technology on seven rootstock genotypes and six scion genotypes.

6. Establish in vitro shoot cultures for seven rootstock genotypes and six scion genotypes using indexed material from Foundation Plant Services (FPS) or field material from FPS and establish bulk meristem cultures for all 13 genotypes for use in transformation.

7. Test Mezzetti et al., 2002, bulk meristem transformation methodology for seven rootstock genotypes and six scion genotypes as an alternate to somatic embryo transformation.

Description of activities conducted to accomplish each objective and a summary of accomplishments and results for each objective.

Objective 1. Develop embryogenic cultures from anthers of seven rootstock genotypes and six scion genotypes for use in establishing embryogenic suspension cultures.

Progress:

In 2015, we collected anthers of rootstock genotypes 3309C (clone 05), Freedom (clone 01), Richter 110 (clone 01), MGT 420A (clone 04), 140Ru (clone 01), Salt Creek (clone 08) 11-03P, 101-14, and scion genotypes Cabernet Sauvignon (clone 07), Chardonnay (clone 04), Pinot Noir (clone 2A), Zinfandel (clone 01A), and Colombard (clone 04). This spring (2016), we collected anthers from genotypes which did not produce embryogenic cultures in 2015 which included; 3309C, Salt Creek, and Zinfandel. We also collected anthers from three additional genotypes not included in the original project; GRN-1, Harmony and Merlot. As in 2015, anthers were plated on four different embryogenic callus inducing media. The media included; Nitsch and Nitsch minimal organics medium (1969) supplemented with 60 g/l sucrose, 1.0 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) and 2.0 mg/l benzylaminopurine (BAP) (PIV), MS minimal organics medium supplemented with 20 g/l sucrose 1.0 mg/l 2,4-D and 0.2 mg/l BAP (MSE), MS minimal organics medium supplemented with 30 g/l sucrose 1.0 mg/l 2,4-D and 1.0 mg/l BAP (MS1) or one half strength MS minimal organics medium supplemented with 15 g/l sucrose 1.0 mg/l NOA and 0.2 mg/l BAP (NB). The number of anther clusters plated for each genotype is given and the number of putative embryogenic calli or embryos developing to date is provided in **Table 1**. We have successfully established somatic embryo cultures for rootstock genotypes Freedom, Richter 110R, MGT 420A, 140Ru 01, 1103P and 101-14 and scion genotypes; Cabernet sauvignon, Chardonnay, Colombard and Merlot.

Other genotypes such as Salt Creek, 3309C, Pinot noir and Zinfandel have produced callus, but this callus failed to generate somatic embryos. In the spring of 2016 we concentrated on establishing embryogenic cultures of rootstock genotype for which we were unsuccessful in establishing somatic embryos in 2015 which include; 3309C, GRN-1, Harmony and Salt Creek as well as scion genotypes Merlot, and Zinfandel. Merlot has been especially responsive in producing embryogenic callus and we should have enough embryos to initiate suspension cultures by the end of August. Salt Creek again produced significant amounts of callus but to date; it does not appear to be embryogenic in morphology.

Table 1. Number of anther clusters planted and forming putative embryogenic callus or embryos in 2015 and 2016

Grape Anther Culture	PIV		MSI		MSE		NB	
	2015	2016	2015	2016	2015	2016	2015	2016
Rootstocks								
140Ru 01	0/245		7/196		0/98		1/196	
110R	4/438 *		0/49		nt		2/49	
101-14	2/539 *		0/49		nt		0/49	
1103	0/49		9/49		8/49		0/49	
3309C 05	1/196	0/150	0/196	0/100	0/196		0/196	0/150
MGT 420A	0/147		5/196		1/98		0/196	
Freedom 01	1/294		0/147		0/49		0/245	
Harmony		0/150		0/150		0/100		0/150
GRN-1		0/150		0/100		0/100		0/100
Richter 110 (01)	0/49				0/49		2/49	
Scions								
Cabernet sauvignon	5/539	0/50	1/147	0/150	4/147	0/150	1/196	0/200
Chardonnay								
Colombard 04	7/172		16/123		0/49		2/123	
Merlot		7/200		10/250		0/150		2/250
Pinot Noir 02A	4/196		0/96		0/49		6/147	
Salt Creek 08	5/196	0/100	4/147	0/100	0	0/100	1/147	0/150
Zinfandel 01A	147	0/150	196	2/150	49	0/50	196	0/150

* 2014

Objective 2. Develop embryogenic suspension cultures for seven rootstock genotypes and six scion genotypes, which will provide a continuous supply of somatic embryos for use transformation experiments.

Progress:

To date, we have established suspension cultures for rootstock genotypes Richter 110, 1103, 101-14, MGT 420A, 140Ru, Freedom and scion genotypes Cabernet sauvignon (clone 8), Chardonnay and Colombard. In addition, we are in the process of bulking embryogenic callus cultures for Merlot.

anticipate having adequate amounts of embryogenic tissue to initiate suspension cultures by the end of summer.

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Objective 3. Establish a germplasm bank of somatic embryos for seven rootstock genotypes and six scion genotypes by plating aliquots of the cell suspension culture on high osmotic medium.

We have established a germplasm bank of somatic embryos on agar solidified Woody Plant Media (WPM) supplemented with 20 g/liter sucrose, 1g/liter casein hydrolysate, 500 mg/liter activated charcoal, 0.5 mg/liter BAP, 0.1 mg/liter NAA, 5% sorbitol and 14 g/l phytoagar (BN-sorb) for rootstock genotypes Freedom, Richter 110, 1103, 101-14, MGT 420A, 140Ru and scion genotypes Cabernet sauvignon, Chardonnay and Colombard (**Figure 1**). These cultures provide a reliable source of somatic embryos for use in transformation studies.

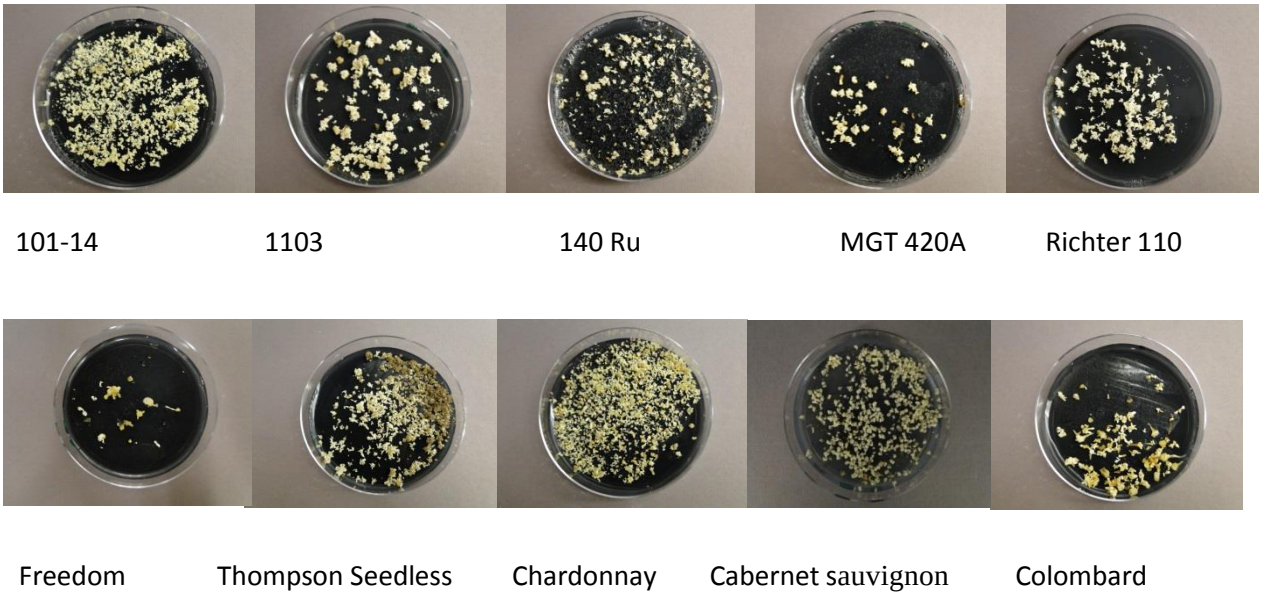

Figure 1. Germplasm bank of embryos established from grape suspension cultures plated on sorbitol containing medium

Objective 4. Test transformation efficiencies of seven rootstock genotypes and six scion genotypes using our established somatic embryo transformation protocols.

Transformation experiments are ongoing using known amounts for somatic embryos as determined by fresh weight for rootstock genotypes Richter 110, 1103, 101-14, MGT 420A, 140Ru and scion genotypes Chardonnay and Colombard using a construct containing the DsRed florescent scorable marker gene which allows us to monitor the progress of transformation in real time (**Table 2**). Thompson Seedless is being included as a positive control. DsRed expression is being evaluated at various intervals post inoculation. We have demonstrated that in addition to rootstock genotypes 101-14 and 1103, rootstock genotypes Freedom and Richter are also amenable to transformation. The relative transformation efficiency of Richter is higher than that seen for 1103 and comparable to 101-14. We have also demonstrated that we can transform French Colombard. Based on DsRed expression we have also generated transgenic embryos for MGT 420A and Ru 140 and we are in the process of determining if we can regenerate whole plants from the embryos (**Figure 2**). Once germplasm banks of somatic embryos are established for Merlot, we will begin testing our transformation system on somatic embryos.

Table 2. Transformation experiments to access the amenability of transformation of stored grape embryos for a range of rootstock and scion genotypes using the scorable fluorescent marker gene DsRed.

Genotype	Date	Experiment #	Tissue Weight	Percentage of tissue expressing DsRed
	5/29/2015	159030	NA	15%
TS-14	6/26/2015	159050	0.53	40%
	7/24/2015	159070	0.52	50%
	8/26/2015	159096	0.92	40%
Chardonnay	6/26/2015	159048	2.72	0%
	7/10/2015	159064	1.12	0%
	7/17/2015	159068	1.12	<1%
	7/24/2015	159071	0.57	0%
Richter	6/26/2016	159049	0.49	60%
	7/10/2015	159065	1.65	60%
	7/17/2015	159069	1.83	75%
	7/24/2015	159072	0.42	80%
	8/26/2015	159095	0.89	20%
1103	7/24/2015	159073	1.11	10%
	8/26/2015	159093	1.09	5%
Colombard	12/16/2015	159150	1.96	30%
	1/15/2016	169007	0.55	20%
	2/5/2016	169029	0.76	10%
	3/2/2016	169042	0.53	25%
	5/6/2016	169049		25%
140 Ru	12/16/2015	159151	1.49	20%
	1/15/2016	169008	0.92	25%
	2/5/2016	169030	1.91	25%
	3/4/2016	169043	1.44	15%
	5/6/2016	169050		20%
MGT 40A	12/16/2015	159152	0.53	20%
	1/15/2016	169009	0.21	
	2/5/2016	169031		25%
	3/4/2016	169044		15%
	5/6/2016	169051		15%
101-14	7/24/2015	159074	0.86	30
	8/26/2015	159094	0.97	20

Figure 2 Transgenic Colombard plant (left) and transgenic embryos of MGT 40A (middle) and Ru 140 (right) expressing dsRed

Objective 5. Test direct cell suspension transformation technology on seven rootstock genotypes and six scion genotypes.

We are trying to leverage the progress we have made in developing high quality cell suspensions that have the ability to rapidly regenerate whole plants when plated onto agar-solidified medium by directly transforming our grape cell suspension cultures with the scorable marker gene DsRed. Ten ml of a grape cell suspensions grown in liquid Pic/MT medium and containing pre-embryogenic masses or small globular embryos are collected in a 15 ml conical centrifuge tube and pelleted by centrifugation at 1000 x G for 3 minutes. The cells are subjected to heat shock by placing the conical tube in a 45 degree water bath for 5 minutes. After heat shock the supernatant is removed and replaced with 5 ml liquid BN medium containing 200 uM acetosyringone and the *Agrobacterium* strain and appropriate vector at an OD_{600} of 01.-0.2. The suspension is centrifuged at 1000 x G for 5 minutes and allowed to incubate for 25 minutes at room temperature. After 25 minutes all but 0.5 ml of the supernatant is removed. The grape and *Agrobacterium* cells are then re-suspended and transferred to sterile Whatman filter paper in an empty 100 x 20 mm petri dish. Any excess fluid was carefully blotted up with a second sterile filter paper. The plates are co-cultured in the dark for 2-3 days at 23 degrees and then transferred to selection medium consisting of WPM supplemented with 20 g/l sucrose, 1g/l casein, 1M MES, 500 mg/l activated charcoal, 0.5 mg/l BAP, 0.1 mg/l NAA, 400 mg/l carbenicillin, 150 mg/l timentin, 200 mg/l kanamycin, 50 g/ sorbitol and 14 g/l agar. The filter paper is transferred to fresh medium every 2 weeks. Within eight weeks resistant embryos develop. Developing embryos are transferred to WPM supplemented with 20 g/l sucrose, 1g/l casein, 1M MES, 500 mg/l activated charcoal, 0.1 mg/l BAP, 400 mg/l carbenicillin, 150 mg/l timentin, 200 mg/l kanamycin, 0 g/ sorbitol and 8 g/l agar for germination. We are currently testing this protocol on Richter 110, 1103, 101-14 Ru 140 MGT 420A Colombard and Chardonnay using a construct containing the DsRed transgene. We have been able to recover transgenic plants using this protocol for 1103 and 101-14. We are also observing germinating embryos of MGT 420A, **(Figure 3)**. However, currently the transformation frequency using this protocol is too low to be practical for routine transformations. A summary of the experiments and the transformation frequency is given in **Table 3**.

Figure 3. Germinating embryos of MGT 420A produced by direct transformation of suspension cultures

Table 3. Number of embryogenic colonies forming after inoculating approximately 1-2 ml of cell suspension with Agrobacterium and plating onto selection medium.

Genotype	Number of Experiments	# Of putative transgenic embryos/ml of plated suspension	# of putative transgenic plants produced
101-14	17	54	2
1103	20	30	2
Richter 110	5	1	0
140 RU	2	0	0
MGT 420a	2	7	In progress
Colombard	2	0	0
Chardonnay	2	0	0

Objective 6. Establish in vitro shoot cultures for seven rootstock genotypes and six scion genotypes using indexed material from Foundation Plant Services (FPS) or field material from FPS and establish bulk meristem cultures for all 13 genotypes for use in transformation.

We are maintaining disease free *in vitro* stock plants of 101-14, Chardonnay and Cabernet Sauvignon that we received as *in vitro* cultures from Foundation Plant Services (FPS). For material that was not available through FPS we have collected shoot tips from field material grown at FPS. This includes genotypes 3309C, Freedom, Richter 110, MGT 420A, 140Ru, Salt Creek 11-03, and scion genotypes Cabernet Sauvignon, Pinot Noir, Zinfandel, and Colombard. We are collecting shoot tips for three additional genotypes, Merlot, Harmony and MGT 420A which we were not successful in establishing shoot cultures last season. Four inch shoot tips were collected, cut into 3 inch sections, transferred to 50 ml centrifuge tubes and surface sterilized in 0.526% sodium hypochlorite for 15 minutes followed by three rinses in sterile distilled water. The shoot tip was cut into nodal sections and any tissue damaged by sterilization was removed. The nodal sections were transferred onto agar solidified Chee and Poole C2d Vitis medium containing 5mg/l chlorophenol red or agar solidified MS minimal organics medium supplemented with 1.0 mg/l BAP, 0.1 mg/l IBA, 0.1 mg/l GA3 and 5 mg/l chlorophenol red. Established aseptic shoot cultures have been established and have been plated onto Mezzetti medium with increasing levels of BAP in order to establish bulk meristem cultures. We are finding differences in our ability to produce bulk meristem cultures depending on the genotype. We have good quality bulk meristem cultures for scion genotypes Chardonnay, Colombard, Pinot noir, Zinfandel; however rootstock genotypes do not readily produce bulk meristems in our hands. The rootstock genotypes produce elongated shoots with a significant amount of non-organized callus making it unsuitable for bulk meristem transformation (**Figure 4**).

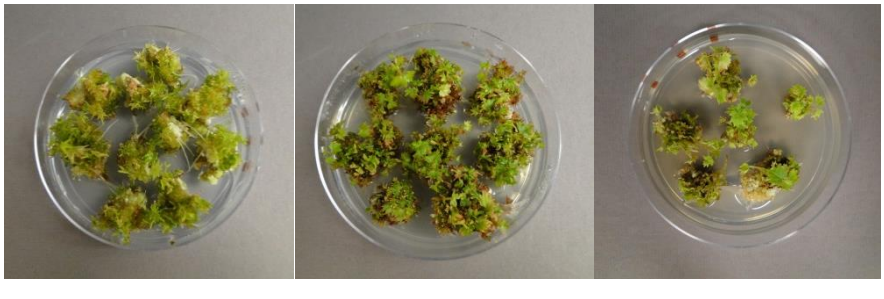

Figure 4. Examples of bulk meristem cultures of scion genotypes Thompson Seedless (left) and Chardonnay (middle) verses bulk meristems of rootstocks Freedom, (right).

Objective 7. Test Mezzetti et al., 2002, bulk meristem transformation methodology for seven rootstock genotypes and six scion genotypes as an alternate to somatic embryo transformation.

We have focused our efforts on studying bulk meristems transformation in scion genotypes since we have not observed good bulk meristem development on rootstock genotypes. Bulk meristems of Thompson Seedless, Chardonnay and Cabernet Sauvignon were sliced into thin, 2mm slices and inoculated with Agrobacterium strain EHA105 containing the DsRed gene and the plant selectable marker gene nptii and co-cultures on Mezzetti medium supplemented with 3 mg/l BAP in the dark at 23 degrees centigrade. After three days, the thin slices were transferred to Mezzetti medium supplemented with 3 mg/l BAP, 400 mg/l carbenicillin, 150 mg/l timentin and 25 mg/l kanamycin sulfate. After three weeks tissue was transferred to the same medium formulation, but the kanamycin level was increased to 50 mg/l. After an additional three weeks the tissue was transferred to medium of the same formulation but the kanamycin level was increased to 75 mg/liter. Subsequently tissue was subcultured every three weeks on medium containing 75 mg/l kanamycin. Since the construct used to transform the bulk meristems contained DsRed gene, we were able to monitor transformation efficiencies in real time. To date, we have only been successful producing transgenic shoots from bulk meristems of Thompson Seedless. Twenty four of the 75 thin slices sections of Thompson seedless produced DsRed sectors and three of these regenerated into shoots. We were able to produce DsRed expressing callus on Cabernet sauvignon and Chardonnay, but none of this tissue regenerated into shoots. In our hands the use of kanamycin at 75mg/l appears to be suboptimal for selection. Although we did identify a limited number of DsRed shoots for Thompson Seedless, many additional shoots which developed on selection medium containing 75 mg/l kanamycin were non-transgenic based on DsRed expression. If not for the use of the scorable marker DsRed, we would not be able to distinguish the true transgenic shoots from the non-transgenic escape shoots until they were transferred to rooting medium with kanamycin. We repeated bulk meristem transformation experiments using higher levels of kanamycin, starting at 75mg/l for three subcultures and then increasing the kanamycin concentration to 150 mg/l but we still observed the regeneration of many non-transgenic shoots and chimeric shoots especially in Thompson Seedless. Low frequency of transformation was confirmed in Thompson Seedless; however no transgenic shoots were recovered from Cabernet sauvignon and Chardonnay. A summary of the bulk meristem transformation experiments initiated to date is given in **Table 4.** A summary table providing an update of the progress for all objectives for each genotype is provided in **Table 5.**

Table 4. Summary of bulk meristem transformation experiments

Experiment	Date	Genotype	Plant Selection	Explant	Results
169022	1/22/2016	Thompson Seedless	75mg/l kanamycin	Bulk Meristems	Low frequency transgenic shoot
169024	1/22/2016	Cabernet sauvignon	75mg/l kanamycin	Bulk Meristems	No transgenic shoots
169025	1/22/2016	Chardonnay	75mg/l kanamycin	Bulk Meristems	No transgenic shoots
169045	3/09/2016	Thompson Seedless	150mg/l kanamycin	Bulk Meristems	
169047	3/09/2016	Chardonnay	150mg/l kanamycin	Bulk Meristems	No transgenic shoots
169048	3/09/2016	Cabernet sauvignon	150mg/l kanamycin	Bulk Meristems	No transgenic shoots

Table 5. Summary table providing the progress for each objective for each of the grape rootstock and scion genotypes

Genotype	Somatic embryos from anthers	Suspensions established	Stored somatic embryos	Shoot tip culture	Bulk meristems	Transgenic embryos	Transgenic plants	Relative Transformation efficiency *
Thompson seedless	+	+	+	+	+	+	+	10
1103	+	+	+	+	Excess callus	+	+	3
101-14	+	+	+	+	Callus few shoots	+	+	5
110 Richter	+	+	+	+	Callus few shoots	+	+	5
140 Ru	+	+	+	+	Callus few shoots	+	-	ND**
3309C	-	-	-	+	Callus few shoots	-	-	ND
MGT 420A	+	+	+	+	Callus few shoots	+	+	ND
Freedom	+	+	+	+	Callus some shoots	+	+	5
Harmony	-	-	-	-	ND	-	-	ND
Salt Creek	-	-	-	-	Callus w/ some shoots	-		ND
Cabernet sauvignon	+	+	+	+	+	-	-	ND
Chardonnay	+	+	+	+	+	+	+	1
Colombard	+	+	+	+	+	+	+	3
Merlot	+	-	-	-	-	-	-	ND
Pinot noir	+	-	-	+	Poor quality	-	-	ND
Zinfandel	-	-	-	+	+	-	-	ND

* Relative transformation efficiency on a scale of 0 worst, 10 best with 10 reflecting the transformation efficiency for Thompson Seedless
**ND- not determine

H. Publications produced and presentations made that relate to the funded project.

Tricoli D. M. 2015. Expanding the range of grape rootstocks and scion genotypes that can be genetically modified for use in research and product development. Pierce's Disease Symposium Report pp 223-230.

Tricoli, D.M. 2016 American Vineyard Foundation (AVF) meeting.

I. Research relevance statement, indicating how this research will contribute towards finding solutions to Pierce's disease in California.

UC Davis Plant Transformation Facility has previously developed grape transformation technology for 101-14 and 1103P; two important grape rootstocks for the California grape industry for use in rootstock-mediated Pierce's disease resistance strategy. However, if rootstock-mediated resistant strategies are to be successful deployed, additional rootstock genotypes will need to be transformed in order to adequately cover the major wine growing regions in California. We therefore are testing our grape rootstock transformation technology on seven additional rootstock genotypes including 110R, 3309C, Harmony, Freedom, 420A, 140Ru and Salt Creek. Additionally, it is not yet known if a rootstock-mediated Pierce's disease resistance strategy will prove to confer durable, commercially viable levels of resistance to the grafted scion. If rootstock-mediates resistance proves unsuccessful, direct transformation of scion clones with disease resistant transgenes may be required. Therefore as a fallback strategy, in addition to testing the range of rootstocks that we can transform, we are also testing our existing transformation technology on a select group of scions. For this proposal we are testing our transformation protocol to six scion varieties including Cabernet Sauvignon, Chardonnay, Pinot Noir, Zinfandel, Merlot and French Colombard.

J. Layperson summary of project accomplishments.

UC Davis Plant Transformation Facility has previously developed a method for genetically modifying 101-14 and 1103P; two important grape rootstocks for the California grape industry. This technology will allow us to introduce genes useful in combating Pierce's disease into the rootstocks of grape and allow us to test whether a modified rootstock is capable of conferring resistance to the grafted scion. This strategy is commonly referred to as rootstock-mediated resistance. If rootstock-mediated resistant strategies are to be successful deployed throughout California, additional rootstock genotypes besides 1103P and 101-14 will need to be modified in order to adequately address the rootstock requirements of the diverse wine growing regions in the State of California. We therefore are currently testing if our method for genetically modifying grape rootstocks can be used successfully on eight additional rootstock genotypes used in California wine grape production. These include; 110R, 3309C, Harmony, Freedom, GRN-1, MGT 420A, 140Ru and Salt Creek. Since it is not yet known if a rootstock-mediated disease resistance strategy will prove to confer durable, commercially viable levels of resistance to the grafted scion we are also testing our method for modifying grapes on a select group of scions including; Cabernet Sauvignon, Chardonnay, Merlot, Pinot Noir, Zinfandel, and French Colombard. We have made significant progress in establishing the tissue culture materials needed to test our transformation strategies in these additional genotypes. To date, we have demonstrated that in addition to rootstock genotypes 101-14 and 1103, Richter and Freedom can be included in the list of rootstock genotypes that we can successfully transform. We have also successfully produced transgenic embryos for MGT 420A and Ru 140 and we are in the process of trying to determine if we can recover whole plants from these embryos. Merlot has been especially productive in producing embryogenic tissues from anthers. The results of this work will allow for the establishment of a self-sustaining grape tissue culture and transformation service for a range of rootstock genotypes that can be utilized by the PD/GWSS Research Community in their research efforts. It will also establish a germ bank of cell suspension cultures and a repository of somatic embryos for rootstock and scion genotypes used in California, which can be made available to the grape research community. Suspension cultures offer the potential of exploring gene editing technology such as CRISPRs for grape improvement.

K. Status of funds.
We anticipate that all funds allocated for fiscal year FY2016-2017 will be expended.

L. Summary and status of intellectual property associated with the project
Methods developed under this proposal will be employed as part of a, cost-effective grape tissue culture and transformation service that can be utilized by the PD/GWSS Research Community.