Interim 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:

The purpose of this proposal is to apply 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 the pre-existing technical expertise developed for rootstocks 1103P and 101-14 at UC Davis's Plant Transformation Facility to additional rootstocks germplasm important for the California wine industry. For this proposal, we are testing seven additional rootstocks for their amenability to transformation including; 110R (clone 1), 3309C (clone 05), Freedom (clone 1), Harmony, MGT 420A (clone 4), 140Ru (clone 1) and Salt Creek (clone 8). In 2016, we added rootstock genotype GRN-1 01.1 from 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), 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 cell suspension cultures and a repository of somatic embryos for rootstock and scion genotypes, which can be made available to the research community. We have made significant progress in establishing somatic embryo, suspension cultures and stored embryo germplasm bank for many of the targeted genotypes. We have now successfully established suspension and stored somatic embryo cultures for grape genotypes 1103P, 101-14, MGT 420A, 140Ru, 110R, Freedom, Harmony, GRN-1, Cabernet sauvignon, Chardonnay, Merlot and French Colombard. Based on transformation experiments using DsRed we have produce transgenic embryos for 101-14, 1103, MGT 420A, 140Ru, 110R, Freedom, Harmony, GRN-1, French Colombard and Merlot. We have now demonstrated that in addition to 101-14 and 1103, we can generate transgenic plants for rootstock genotypes 110R, Freedom, and MGT 420A along with the scion variety French Colombard.

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:

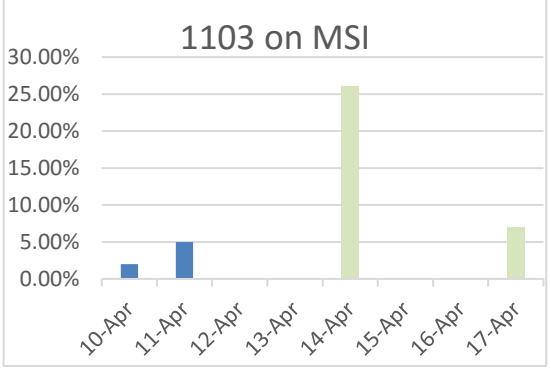
This spring (April 2017), we collected anthers from genotype for which we were not successful in generating embryos in 2015 or 2016, which include 3309C and Salt Creek. We also harvested anthers from 110R (01), 1103P and 101-14 since we needed to generate fresh somatic embryo cultures to replace our aging cultures for these genotypes. The media we used 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 (MSI) 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). This year we added Chee and Poole minimal organics medium with 30 g/l sucrose supplemented with 2.0 mg/l 2, 4-D and 0.2 mg/l BAP (AIM) to the list of media tested. Based on previous year's results we plated 1103P on MSI and MSE media, 110R on NB medium, 101-14 on PIV medium since these genotype/medium combinations resulted in the highest frequency of embryo formation in the past. Freedom and 3309C were plated on PIV and AIM media formulations and Salt Creek was plated on all five media (**Table 1**). Flowers were harvests on April 6 and April 14. We are getting very high percentage of embryos developing for 1103P this year, very soon after plating (**Figure 1, Table 2**). No embryos have been observed to date for 110R, 101-14, 3309C or Salt Creek but it is still quite early in the culture process.

Table 1. Number of flower from which anthers were extracted for each genotype and media combination tested

	Number of flowers plated for each genotype on each medium					
Grape Anther Culture	**PIV**	**MSI**	**MSE**	**NB**	**AIM**	
	2017	2017	2017	2017	2017	Totals # plated
1103P		325	325			650
110R	200			200		400
101-14	600					600
3309C	200				200	400
Salt Creek	100	100	100	100	100	500

Table 2. Number (percentage) of embryogenic callus developing for each genotype and media combination tested

	Number (%) of embryogenic callus developing per flowers plated for each genotype on each medium				
Grape Anther Culture	**PIV**	**MSI**	**MSE**	**NB**	**AIM**
1103		39/325 (12%)	47/325 (14%)		
110R	0/200			0/200	
101-14	0/600				
3309C	0/200				0/200
Salt Creek	0/100	0/100	0/100	0/100	0/100



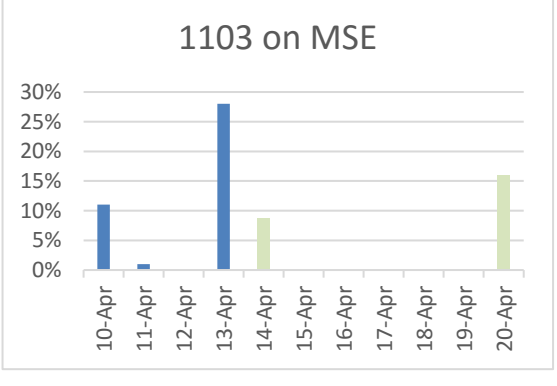


Figure 1. Percentage of anther clusters from 1103 developing embryogenic callus on MSI or MSE medium based on flower harvest date and the date anthers were plated. Blue bars represent flowers collected on 4/6/2017 and plated 4/10 or 4/11. Green bars represent flowers collected on 4/14/2017 and anthers plated from those flowers on 4/14, 4/17 or 4/20.

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:

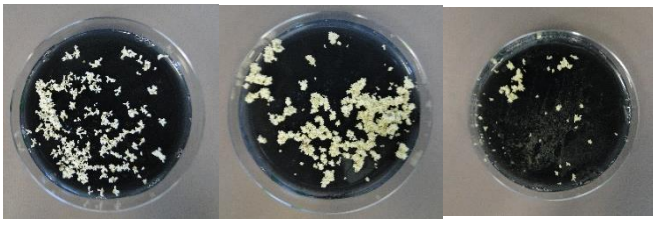
By transferring somatic embryos into liquid culture medium composed of Woody Plant Media (WPM) supplemented with 20 g/liter sucrose, 1g/liter casein hydrolysate, 500 mg/liter activated charcoal, 10 mg/liter Picloram, 2.0 mg/liter meta-topolin, we have established suspensions for rootstock genotypes 1103P, 101-14, 140Ru, Freedom, MGT 420A, 110R, GRN1 and Harmony, and scion genotypes Cabernet Sauvignon, Chardonnay, French Colombard, Merlot and Pinot noir. Occasionally the suspensions are sieved through a 520-micron screen to eliminate large embryos and cell clusters. Alternatively, the smaller fraction of the suspension is drawn up into a wide bore 10 ml pipet and transferred to a new flask leaving the larger embryos and cell aggregated behind.

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.

Progress:

We have established a germplasm bank of somatic embryos by plating aliquots of the suspension cultures onto 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 1mM MES and 14 g/l phytoagar (BN-sorb). A stored embryo germplasm bank has been established for rootstock genotypes 1103P, 101-14, 140Ru, Freedom, MGT 420A, 110R, GRN1 and Harmony as well as scion genotypes Cabernet sauvignon, Chardonnay, French Colombard and Merlot (**Figure 2**). The Harmony and GRN-1 suspension cultures have improved significantly and are producing high quality stored somatic embryos. Although we have plated aliquots of suspensions cultures of Pinot noir on this medium, unlike other genotypes, the suspensions do not form embryos.

101-14 1103 140 Ru MGT 420A 110R



GRN1 Harmony Freedom

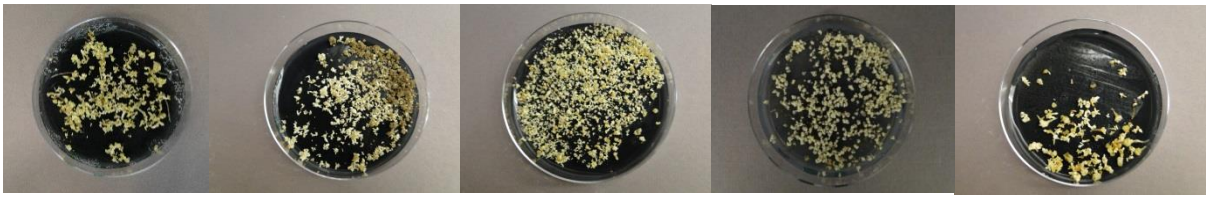


Figure 2. Germplasm bank of embryos established from grape suspension cultures plated on sorbitol containing medium.

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

Progress:

Transformation experiments were initiated using somatic embryos for rootstock genotypes 1103P, 101-14, 140Ru, Freedom, MGT 420A, 110R, GRN1 and Harmony and scion genotypes Cabernet sauvignon, Chardonnay, French Colombard and Merlot using a construct containing the DsRed florescent scorable marker. Thompson Seedless is being included as a positive control. DsRed expression was scored 3 months post inoculation (**Table 3**) and has shown that significant numbers of transgenic somatic embryos can be generated for 1103P, 101-14, 110R, 140Ru, MGT 420A, Harmony, GRN-1 and French Colombard. However, very little DsRed expression was seen in Chardonnay somatic embryos. The relative transformation efficiency based on recovery of whole plants is higher for 110R than that seen for 1103P and equal to or greater than that seen for 101-14. We have also demonstrated that we can generate transgenic plants for French Colombard and MGT420A (**Figure 3**)**.** We are in the process of determining if we can regenerate whole plants from transgenic embryos of Ru140, Merlot, Harmony and GRN1. The percentage of embryos expressing DsRed for each genotype is provided in **Table 3**. Images of DsRed expressing Freedom, GRN1, Harmony and Merlot are shown in **Figure 4**.

Table 3. 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	**# of Experiments**	**% of tissue expressing DsRed**
110R	5	59%
101-14	2	25%
140 Ru	5	21%
MGT 40A	5	15%
1103	2	8%
TS-14	4	36%
Colombard	5	22%
Chardonnay	4	<1%
Freedom	3	25%
GRN1	3	40%
Harmony	3	20%
Merlot	3	10%*

* Data on first of three experiments only- waiting for data on experiments 2 and 3

Figure 3. Transgenic plantlets from left to right MGT 420, French Colombard, 101-14 and 1103P

Figure 4. Transgenic embryos from left to right of Freedom, GRN1, Harmony and Merlot expressing DsRed.

Using the stored somatic embryo-based transformation system, to date we have produced 485 genetically modified grape plants across five different genotypes for principle investigators studying strategies to combat Pierces Disease (**figure 5**).

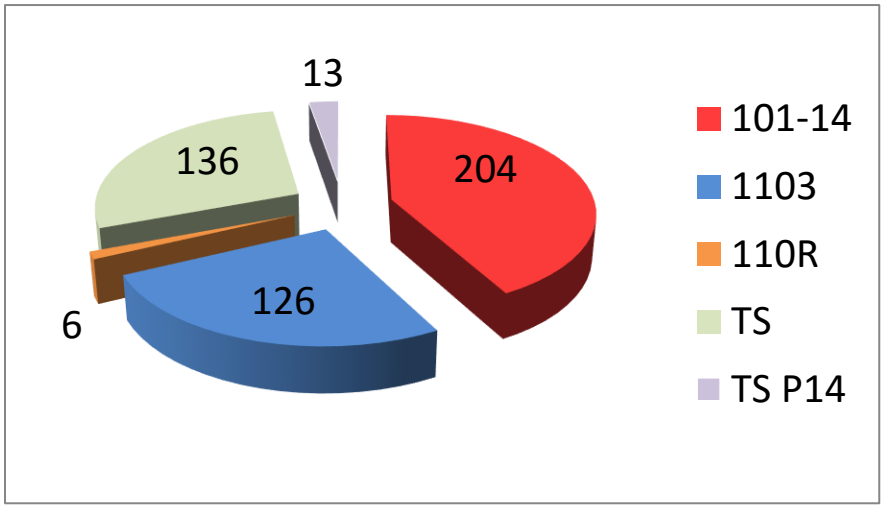


Figure 5. The number of transgenic plant lines produced to date for PIs testing various strategies to combat Pierces Disease.

A summary table of our transformation progress with all the rootstock and scion genotypes is presented at the end of this report in **Table 5**.

Acclimation of Plants to Soil

We have improved our protocol for acclimatizing transgenic grape plants to soil. Historically we have allowed transgenic embryos to germinate on the primary root that develops from the embryos. However, this often results in the production of callus at the shoot-root interface and we speculated that this might be detrimental to survival of the plants during acclimatization to soil. We are now removing the shoot from the germinating embryo and re-initiate roots on the excised shoot. This has resulted in the development of a stronger root system with no associated callus tissue as well as a healthier plant, which acclimates better to soil (**Figure 6**).

Figure 6. Transgenic grape plant from somatic embryos germinated on its own root (left). Note callus at the shoot/root interface. Transgenic grape shoot re-rooted as an in vitro cutting (right)

Rootstock genotypes 101-14 and especially 1103P have been difficult to acclimate to soil from tissue culture. Significant leaf necrosis develops rapidly as relative humidity is reduced from culture conditions to soil. To avoid this, plants must be maintained at 100% relative humidity for a minimum of one week upon transfer to soil. Despite washing plants prior to transplanting into soil, this extended period at high humidity often results in plant loss due to fungal contamination orleaf collapse. To improve drainage, we have modified the soil mix to include one part supersoil to two parts vermiculite. We are also now using distilled water containing 4 ml/l PTC3 a broad-spectrum biostat/fungistat to moisten the soil mix and to water the plants when under high humidity in order to reduce microbial and fungal contamination.

We have also employed an additional culture step prior to transplanting the plantlet to soil. We are aseptically removing the shoot tips from each transgenic plant before transfer to so and culturing the shoot tip in fresh rooting medium in order to establish a backup clone for each transgenic plant. These backup clones can be used should the original plantlet die upon transfer to soil.

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

Progress:

We have tried to leverage the progress we have made in developing high quality cell suspensions that can 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, 1mM MES, 500 mg/l activated charcoal, 0.1 mg/l BAP, 400 mg/l carbenicillin, 150 mg/l timentin, 200 mg/l kanamycin, and 8 g/l agar for germination. We tested this protocol on 110R, 1103P, 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 1103P and 101-14 at very low frequency. For example, only two of the twenty–one putatively transformed embryos that formed from one experiment with 101-14 germinated into plants after transfer to medium lacking sorbitol. We are observing germinating embryos of MGT 420A **(Figure 7)**. However, currently the transformation frequency using this protocol is too low to be practical for routine transformations and we will not pursue this approach in the future. A summary of the experiments and the transformation frequency is given in **Table 4**.

Figure 7. Twenty-one embryos from transformation of cell suspension cultures of 101-14 (left) cultured on WPM supplemented with 20 g/l sucrose, 1g/l casein, 1mM MES, 500 mg/l activated charcoal, 0.5 mg/l BAP, 0.1 mg/l NAA 50 g/l sorbitol and 14 g/l agar and transfer to WPM supplemented with 20 g/l sucrose, 1g/l casein, 1mM MES, 500 mg/l activated charcoal, 0.1 mg/l BAP, 8 g/l agar for plant regeneration. Only two of the twenty–one putatively transformed embryos on this plate, germinated after transfer to medium lacking sorbitol. DsRed expressing embryos of MGT 420A (middle and right).

Table 4. 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
110R	5	1	0
140 RU	2	0	0
MGT 420a	2	7	4
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.

Progress:

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, 110R, MGT 420A, 140Ru, Salt Creek 1103P, and scion genotypes Cabernet Sauvignon, Pinot Noir, Zinfandel, and French Colombard. We have collected shoot tips for three additional genotypes, Merlot, Harmony and MGT 420A that we were not successful in establishing shoot cultures last season. Four-inch shoot tips were collected and 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. Aseptic shoot cultures have been established and maintained on Chee and Poole minimal organics medium supplemented with 0.01 mg/l IBA. Shoot tips were collected and plated onto Mezzetti medium with increasing levels of BAP in order to establish bulk meristem cultures (**Figure 8**). We have produced good quality bulk meristem cultures for scion genotypes Chardonnay, French Colombard, Pinot noir and Zinfandel. However, rootstock genotypes do not readily produce bulk meristems in our hands, but produce elongated shoots with a significant amount of non-organized callus making it unsuitable for bulk meristem transformation (**Figure 9**). Based on the difficulty of generating bulk meristems for rootstock genotypes and the limited success we have had with transforming thin slices of bulk meristems compared to our standard somatic embryo-based transformation (see below), we do not plan to pursue this strategy in the future.

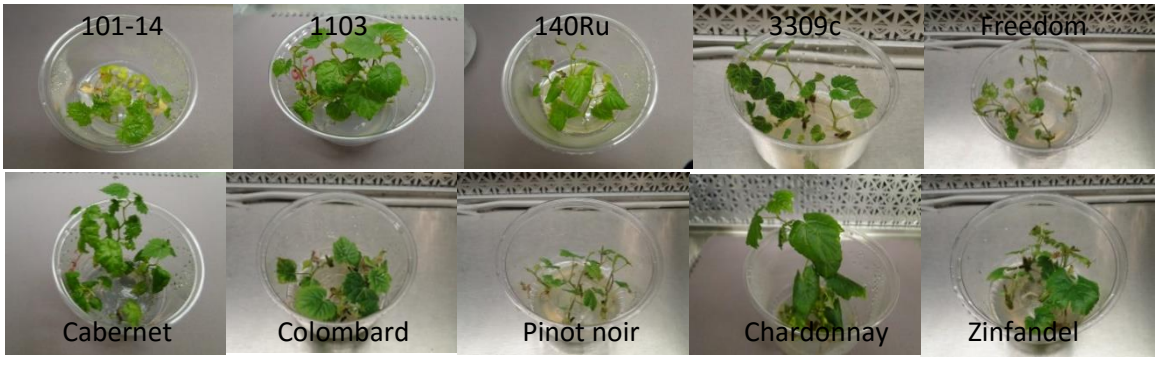


Figure 8. Shoot cultures established for rootstock and scion genotypes

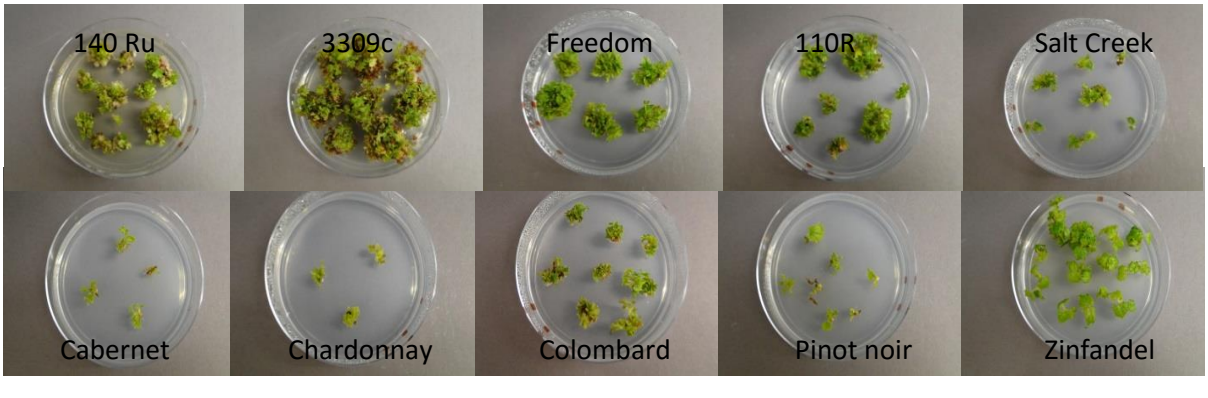


Figure 9. Initiation of bulk meristem cultures for rootstock and scion germplasm

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.

Progress:

Bulk meristems of Thompson Seedless, Chardonnay and Cabernet Sauvignon were sliced into thin, 2mm slices and inoculated with Agrobacterium strain EHA105 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 increase to 75 mg/liter. Subsequently tissue was subcultures 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 (**Figure 10**) and three of these sectors 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 that 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. The application of this technique in our hands appears limited compared to the somatic embryo-based transformation protocol and we do not intend on continuing this approach.

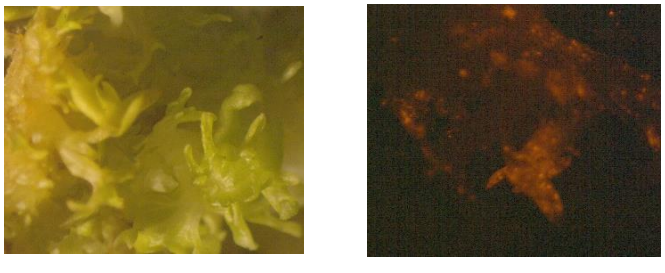


Figure 10. DsRed expressing shoot developing from inoculates thin slice of a Thompson Seedless bulk meristem culture -bright field (left), fluorescence (right)

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

Genotype	Somatic embryos established from anthers	Suspensions established from somatic embryos	Establishment of stored somatic embryo cultures	Production of transgenic somatic embryos +	Production of transgenic plants	Relative Transform-ation efficiency*
Rootstocks						
1103	+	+	+	+	+	3
101-14	+	+	+	+	+	5
110 Richter	+	+	+	+	+	5
140 Ru	+	+	+	+	-	ND**
3309C	-	-	-	-	-	ND
GRN-1	+	+	+	+	-	ND
MGT 420A	+	+	+	+	+	ND***
Freedom	+	+	+	+	+	5
Harmony	+	+	+	+	-	ND
Salt Creek	-	-	-	-	-	ND
Scions						
Cabernet sauvignon	+	+	+	-	-	0
Chardonnay	+	+	+	+	+	<1
French Colombard	+	+	+	+	+	4
Merlot	+	+	+	+	-	ND
Pinot noir	+	+	-	-	-	ND
Thompson seedless	+	+	+	+	+	10
Zinfandel	-	-	-	-	-	ND

+ based on DsRed expression

* Relative transformation efficiency on a scale of zero worst, 10 best with 10 reflecting the transformation efficiency for Thompson Seedless

**ND- not determine

***- not enough data has been accumulated yet to compare the relative transformation efficiencies compared to Thompson Seedless

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

Tricoli D. M. 2016. 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.

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 eight additional rootstock genotypes including 110R, 3309C, GRN-1, Harmony, Freedom, MGT 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, GRN1, 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 embryos in tissue culture for a wide range of scions and rootstocks including genotypes MGT 420A, 140Ru, 110R, Freedom, Harmony, GRN-1, Cabernet sauvignon, Chardonnay, Merlot and French Colombard. We are testing our transformation strategy for its utility in genetically modify these additional genotypes. To date, we have demonstrated that in addition to 101-14 and 1103P; the rootstock Freedom, 110R and MGT 420A and the scion genotype French Colombard can be included in the list of grape genotypes that we can successfully transform. To date we have produced over 480 genetically modified grape plants across four different varieties to test strategies for investigators studying strategies that may be effective against Pierces Disease.

In addition to its utility in producing genetically modified grape plants for testing strategies to combat Pierces disease, this work has 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 for a wide variety of research purposes.

K. Status of funds.

We anticipate that all funds allocated for fiscal year FY2017-2018 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.