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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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ABSTRACT

UC Davis Plant Transformation Facility has previously developed a method for genetically modifying 101-14 and 1103; two important grape rootstocks for the California grape industry. This technology allows researchers to introduce genes useful in combating Pierce's disease into the rootstocks of grape and allows researchers to test whether a modified rootstock is capable of conferring resistance to the grafted scion. If rootstock-mediated resistant strategies are to be successfully deployed throughout California, additional rootstocks will need to be modified in order to adequately address the rootstock requirements of the diverse wine growing regions in the State of California. To that end, we plated anthers from grape rootstocks 1103P, 110R (clone 01), 101-14, 140Ru (clone 01), 3309C (clone 05), Freedom (clone 01), GRN1, Harmony, MGT 420A (clone 04), Salt Creek (clone 08), and scion genotypes Cabernet Sauvignon (clone 07), Chardonnay (clone 04), French Colombard (clone 04), Merlot (clone 3), Pinot Noir (clone 2A), and Zinfandel (clone 01A) in order to produce somatic embryos. Embryogenic cultures were successfully generated from anther filaments for 1103P, 110R, 101-14, 140Ru, Freedom, GRN1, Harmony, MGT 420A, Cabernet sauvignon, Chardonnay, French Colombard, Merlot, and Pinot noir. In addition, we have successfully established suspension and stored embryo cultures for these grape genotypes. We were able to regenerated non-transgenic plants from embryos for nearly all the tested genotypes. The only genotypes that we were unsuccessful generating somatic embryos for were 3309c, Salt Creek and Zinfandel. Transformation experiments using DsRed were initiated using stored embryo cultures in order to access the utility of our existing transformation technologies in transforming these genotypes. We have successfully generated transgenic plants for 1103, 110R, 101-14, Freedom, MGT 420A and French Colombard. DsRed embryos have been generated at varying frequencies for 140 Ru, GRN1, Harmony and Merlot but we were unable to regenerate whole plants from these transgenic embryos. We were able to produce a very limited number of transgenic plants for Chardonnay. Acclimatization of transgenic grape plantlets to soil has been problematic in the past and we have invested considerable effort trying to rectify this problem. By modifying the soil mixture, reducing the size of the rooted plant at the time of acclimatization and modifying the plant's root architecture, survival rates of plants transferred to soil is now approximately 90 percent. To date we have generated over 650 transgenic grape lines for PD researchers.

LAYPERSONS SUMMARY

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 genotypes in addition to 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 tested our method for genetically modifying grape rootstocks on eight additional rootstock genotypes used in California wine grape production. These include 110R (clone 01), 140Ru (clone 01), 3309C (clone 05), Freedom (clone 01), GRN1, Harmony, MGT 420A (clone 04), Salt Creek (clone 08). Since it is not yet known if a rootstock-mediated disease resistance strategy will confer durable, commercially viable levels of resistance to the grafted scion, we are also tested our method for modifying grapes on a select group of scions including; Cabernet Sauvignon (clone 07), Chardonnay (clone 04), French Colombard (clone 04), Merlot (clone 3), Pinot Noir (clone 2A), Zinfandel (clone

01A). We successfully establish somatic embryos in tissue cultures for a wide range of scions and rootstocks and tested our transformation strategy for its utility in genetically modifying these additional genotypes. To date, we have demonstrated that in addition to 101-14 and 1103P; the rootstocks 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. We have produced over 650 genetically modified grape plants across four different varieties for investigators studying strategies that may be effective against Pierces Disease. Transferring grape plants from culture to soil can be difficult. We have made considerable progress improving the transfer of rooted plant from culture to soil. By modifying the soil mix, reducing the size of the transplanted plant and modifying the root architecture, survival rates of plants transferred to soil are now approximately 90%.

In addition to its utility in producing genetically modified grape plants for testing strategies to combat Pierces Disease, this work has developed methods for establishing a germplasm bank of 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.

INTRODUCTION

The purpose of this proposal was 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 tested 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 tested eight additional rootstocks for their amenability to transformation including; 110R (clone 01), 140Ru (clone 01), 3309C (clone 05), Freedom (clone 01), GRN1, Harmony, MGT 420A (clone 04), Salt Creek (clone 08). This work expanded the range of rootstocks that can be effectively transformed. 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 as 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 also screened six important California scion genotypes for their amenability to transformation including, Cabernet Sauvignon (clone 07), Chardonnay (clone 04), French Colombard (clone 04), Merlot (clone 3), Pinot Noir (clone 2A), Zinfandel (clone 01A). The results of this work established grape tissue culture and transformation technologies that can be utilized by the PD/GWSS Research Community. It also established a methodology for creating a germplasm bank of suspension cultures and a repository of somatic embryos for rootstock and scion genotypes, which can be made available to the research community. We successfully established suspension and stored somatic embryo cultures for grape rootstock genotypes 1103P, 110R, 101-14, 140Ru, Freedom, GRN1, Harmony, MGT 420A and scion genotypes Cabernet Sauvignon, Chardonnay, French Colombard, Merlot, and Pinot Noir. Based on transformation experiments using a gene that fluoresces in transgenic plant tissue called DsRed, we have produced transgenic embryos for rootstock genotypes 1103P, 110R, 101-14, 140Ru, Freedom, GRN1, Harmony, MGT 420A and scion genotypes Cabernet sauvignon, Chardonnay, French Colombard, Merlot, Pinot Noir. We have now demonstrated that in addition to 101-14 and 1103, we can generate transgenic plants for rootstock genotypes 110R, Freedom, MGT 420A, along with the scion variety French Colombard. Overall, scion genotypes have been difficult to transform using our protocol, possibly due to low rates of gene transfer into stored scion somatic embryos compared to rootstock somatic embryos as observable in the amount of expression of DsRed.

OBJECTIVES

1. Develop embryogenic cultures from anther filaments of eight rootstock genotypes and six scion genotypes for use in establishing embryogenic suspension cultures.
2. Develop embryogenic suspension cultures for eight 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 eight rootstock genotypes and six scion genotypes by plating aliquots of the suspension culture on high osmotic medium.
4. Test transformation efficiencies of eight rootstock genotypes and six scion genotypes using our established somatic embryo transformation protocols.
5. Test direct suspension transformation technology on eight rootstock genotypes and six scion genotypes.

6. Secure *in vitro* shoot cultures for eight rootstock genotypes and six scion genotypes using indexed material from Foundation Plant Services or field material from FPS and establish bulk meristem cultures for all thirteen genotypes for use in transformation.
7. Test Mezzetti et al., 2002 bulk meristem transformation system for eight rootstock genotypes and six scion genotypes as an alternate to somatic embryo transformation.

RESULTS AND DISCUSSION

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

Progress: During the spring of 2015 through 2018, we collected anthers from genotype 1103P, 110R (clone 01), 101-14, 140Ru (clone 01), 3309C (clone 05), Freedom (clone 01), GRN1, Harmony, MGT 420A (clone 04), Salt Creek (clone 08), and scion genotypes Cabernet Sauvignon (clone 07), Chardonnay (clone 04), Merlot (clone 3), Pinot Noir (clone 2A), Zinfandel (clone 01A), and French Colombard (clone 04). 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). In 2017, 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. We plated over 17,500 flowers from 2015-2018. We generated embryogenic callus and somatic embryos for all genotypes tested except for 3309C, Salt Creek and Zinfandel. The response of each genotype is given in **Table 1**.

Table 1. Total number of flowers plated per genotype across media from 2015 through 2018 and the number and percent that generated somatic embryos.

	Number (%) of embryogenic callus developing per # of flowers plated for each genotype/medium combination					
Grape Anther Culture	**PIV**	**MSI**	**MSE**	**NB**	**AIM**	
Genotype						Total # plated
1103P	0/98 (0)	60/717 (8.4)	79/725 (10.9)	8/98 (8.2)		147/1638 (8.9)
110R	5/883 (0.5)	0/98 (0)	-	2/298 (0.6)		7/1279 (0.5)
101-14	3/1629 (0.2)	0/98 (0)	-	0/98 (0)		3/1825 (0.1)
3309	0/788 (0)	0/292 (0)	0/292 (0)	0/292 (0)	0/196 (0)	0/1860 (0)
Freedom	1/294 (0.3)	0/147 (0)	0/49 (0)	0/245 (0)		1/735 (0.1)
GRN1	0/147 (0)	2/98 (2.0)	0/98 (0)	0/98 (0)	-	
Harmony	0/147 (0)	1/147 (0.6)	0/98 (0)	0/147 (0)		1/539 (0.2)
MGT 420A	1/294 (0.3)	10/392 (2.5)	2/196 (1)	1/196 (0.5)		14/1078 (1.3)
Ru140	0/294 (0)	7/245 (2.8)	0/98 (0)	1/245 (0.4)		8/882 (0.9)
Salt Creek	0/490 (0)	0/490 (0)	0/198 (0)	0/392 (0)	0/98 (0)	0/1670 (0)
Cabernet Sauvignon	5/1078 (0.41)	2/441 (0.45)	8/441 (1.8)	2/539 (0.4)		17/2499 (0.7)
Chardonnay	6/882 (0.7)					6/882 (0.7)
French Colombard	7/172 (4.1)	16/123 (13)	0/49 (0)	2/123 (1.6)		25/467 (5.3)
Merlot	20/441 (4.9)	9/443 (2)	0/147 (0)	5/245 (2)		34/1276 (2.7)
Pinot noir	4/196 (2)	0/98 (0)	0/49 (0)	6/147 (4.1)		10/490 (2)
Zinfandel	0/294 (0)	0/343 (0)	0/294 (0)	0/466 (0)		0/1397 (0)

Objective 2. Develop embryogenic suspension cultures for eight rootstock genotypes and six scion genotypes, which 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, 2g/liter activated charcoal, 10 mg/liter Picloram, 2.0 mg/liter meta-topolin, 100 mg/l ascorbic acid and 30 mg/l reduced glutathione, we were able to establish suspension cultures for all of the rootstock and scion genotypes for which we were able to generate somatic embryos. These include; 1103P, 101-14, 140Ru, Freedom, MGT 420A, 110R, GRN1, Harmony, Cabernet Sauvignon, Chardonnay, French Colombard, Merlot and Pinot noir. If cell/embryos aggregates became too large, the suspensions were sieved through a 520-micron screen to eliminate large embryos and cell clusters. Alternatively, the smaller fraction of the suspension was 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 established a germplasm bank of somatic embryos by plating a 200 ul 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). We established stored embryo 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 1**). Although we have plated aliquots of suspensions cultures of Pinot noir on this medium, unlike other genotypes, the suspensions do not develop into embryos.

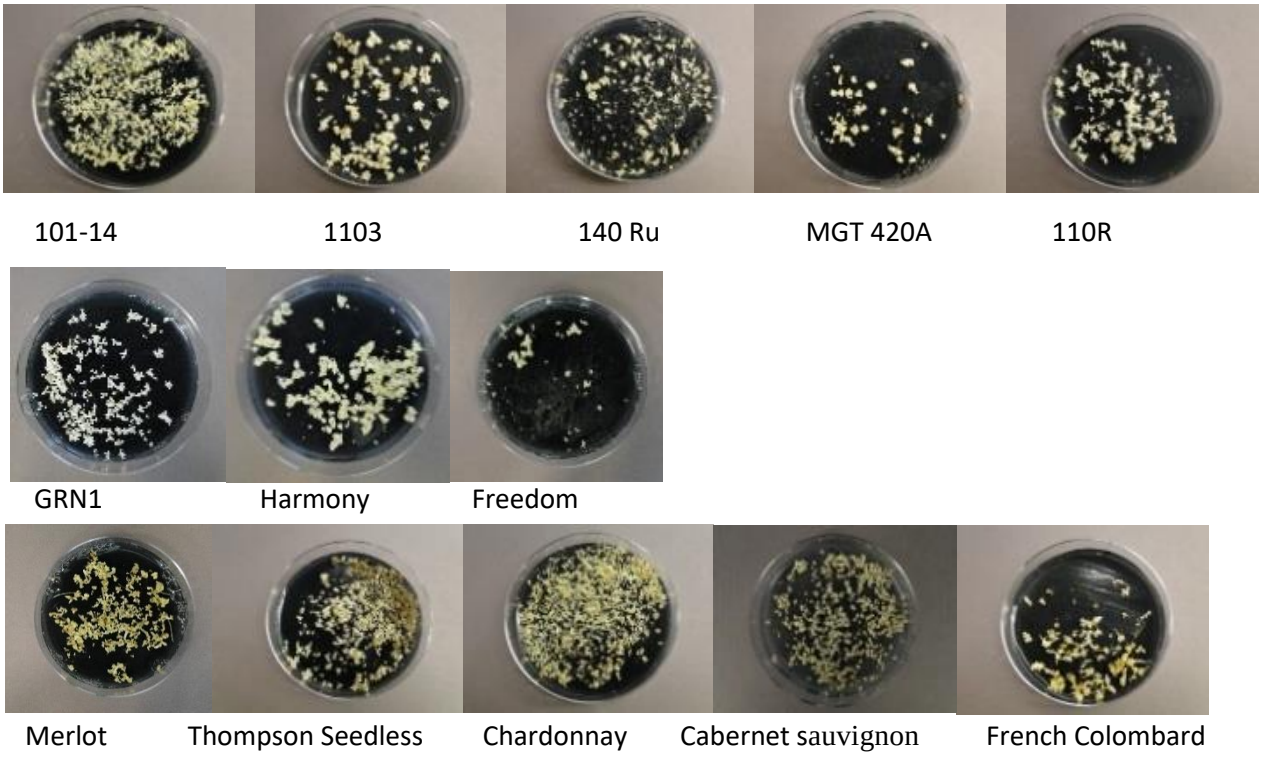

Figure 1. Germplasm bank of embryos established from grape suspension cultures plated on sorbitol containing medium.

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

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 gene. Thompson Seedless was 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 produced for 1103P, 101-14, 110R, 140Ru, MGT 420A, Harmony, GRN-1, French Colombard. However very little DsRed expression was seen in Chardonnay or Merlot somatic embryos. We demonstrated that along with Freedom, 1103 and 101-14 we can regenerate transgenic plants for 110R, MGT 420 and French Colombard. (**Figure 2**). We also were able to regenerate a few Chardonnay transgenic plants but only at an extremely low frequency. We were unable to regenerate whole transgenic plants from Harmony, 140Ru, or GRN1although we still have partially germinated embryos of GRN1 which we believe given more time will convert into plants **(Figure 3).** The percentage of embryos expressing DsRed for each genotype is provided in **Table 3**. Transformation efficiency based on DsRed expression are very low for both Chardonnay and Merlot. Images of DsRed expressing Freedom, GRN1, Harmony and Merlot are shown in **Figure 3**. There appears to be a correlation between the percentage of tissue expressing Dsred and our ability to generate transgenic plants. Based on Dsred expression results, with the exception of French Colombard, it appears that Agrobacterium-mediated transformation rates are significantly lower for most scion genotypes than for rootstock genotypes using our transformation protocol. This may explain the difficulty we had producing transgenic plants for scion genotypes. Biolistic-mediated transformation of scion genotypes should be explored if transgenic scions are required.

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	Number of Experiments	Estimate of the % of embryos/tissue expressing DsRed
110R	5	60%
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	5	1.0%

Figure 2. Transgenic plantlets from left to right MGT 420, French Colombard, 101-14 and 1103P with germinating embryos of putatively transgenic GRN1 (far right)

Figure 3. Transgenic embryos from left to right of Freedom, GRN1, Harmony, 140 Ru, MGT420 and Merlot expressing DsRed.

Using the stored somatic embryo-based transformation system, to date we have produced over 650 genetically modified grape plants across five different genotypes using 90 constructs for principle investigators studying strategies to combat Pierces Disease (**Figure 4**).

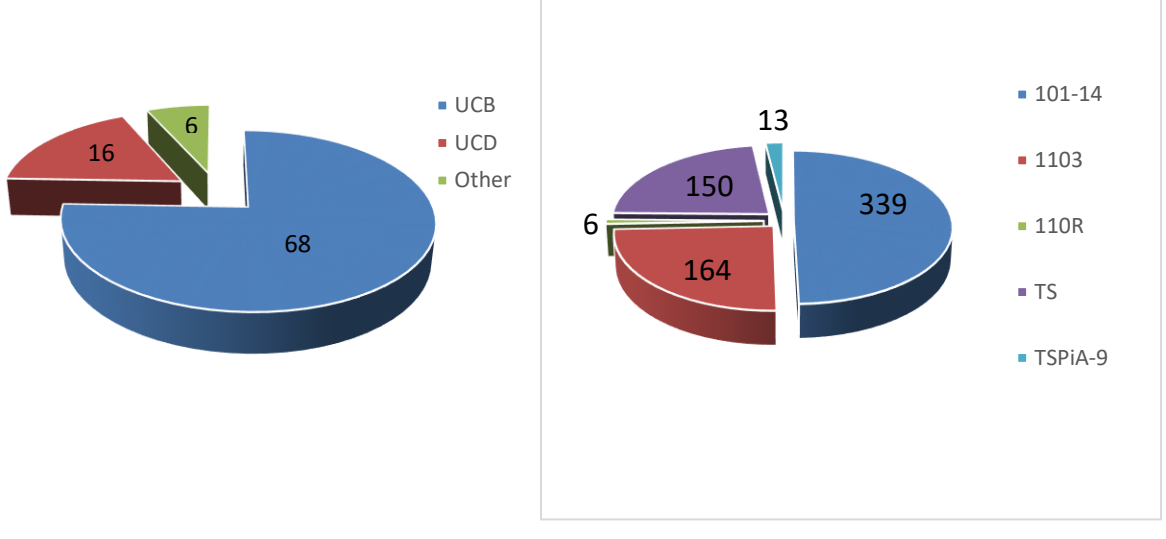

Figure 4. The number of constructs transformed into grape (left) and the number of transgenic grape plants produced to date to evaluate various strategies for control of Pierces Disease (right)

A summary table of our transformation progress with all the rootstock and scion genotypes is presented at the end of this report in **Table 6**.

Acclimation of Plants to Soil

We developed a new procedure for improving the survival rate for acclimatizing transgenic grape plants to soil. Previously, survival rates of rooted plantlets transferred from culture to soil have been as low as 50%. In the past, we have allowed transgenic embryos to germinate on the primary root that develops from the germinating somatic embryos. However, this often results in the production of weak roots as well as callus at the shoot-root interface and we believe this is detrimental to survival of the plants during acclimatization to soil. We found that are excising the shoot from the germinating embryo and re-initiating roots on the excised shoot results in a healthier rooted in vitro plant, which acclimates better to soil. We also significantly reduced the size of the rooted plantlet that we transfer to soil. Previously we transferred large (6 cm or larger) rooted shoots to soil, but the leaves on these larger plants often turn necrotic even under conditions of high relative humidity. Smaller plantlets exhibit less transpiration and acclimate quickly to soil conditions (**Figure** 5) with little to no leaf drop. We have also modified our protocol by transferring plants to soil just as the root are visible (1-2 mm) and before they have elongated which results in rapid acclimatization during transplantation, possibly due to less root damage upon transfer to soil. We also improved drainage by modifying the soil mix to include one part sunshine mix to two parts vermiculite. Using the modifications, survival rates of grape plants transferred from culture to soil is approximately 90%.

Figure 5. Transgenic grape plant from somatic embryos germinated on its own roots verse transgenic shoot re-rooted as an in vitro cutting. Note callus at the shoot/root interface (left). In vitro rooted grape plant with root initials-note shoot-tip has been removed to generate a backup clone (middle), transgenic grape plant acclimated to soil (right).

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

Progress:

We tried to leverage the progress we have made in developing high quality suspensions that can rapidly regenerate whole plants when plated onto agar-solidified medium by directly transforming our grape suspension cultures with Agrobacterium tumefaciens containing a selectable marker gene and the scorable marker gene DsRed. Ten ml of a grape suspensions grown in liquid Pic/MT medium and containing pre-embryogenic masses or small globular embryos were collected in a 15 ml conical centrifuge tube and pelleted by centrifugation at 1000 x G for 3 minutes. The suspensions were subjected to heat shock by placing the conical tube in a 45-degree water bath for 5 minutes. After heat shock, the supernatant was removed and replaced with 5 ml liquid BN medium containing 200 uM acetosyringone and the *Agrobacterium* strain containing the appropriate vector diluted to an OD_{600} of 01.- 0.2. The suspension was centrifuged at 1000 x G for five minutes and allowed to incubate for 25 minutes at room temperature. After 25 minutes, all but 0.5 ml of the supernatant was removed. The grape and *Agrobacterium* cells were 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 were 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, 1mM 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 was transferred to fresh medium every 2 weeks. Within eight weeks resistant embryos developed. Developing embryos were 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, 101-14 and MGT 420A at very low frequency **(Figure 6)**. 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. However 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 4**.

Figure 6. 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 transferred 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.

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:
For experiments exploring bulk meristem transformation described below, we established and maintained 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 collected shoot tips from field material grown at FPS. This includes genotypes 1103P, 110R, 140Ru, 3309C, Freedom, GRN1, Harmony, MGT 420A, Salt Creek, and scion genotypes French Colombard, Merlot, Pinot Noir, and Zinfandel. 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 shoots were 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 GA_3 and 5 mg/l chlorophenol red. Once established, aseptic shoot cultures were maintained on Chee and Poole minimal organics medium supplemented with 0.01 mg/l IBA **(Figure 7).**

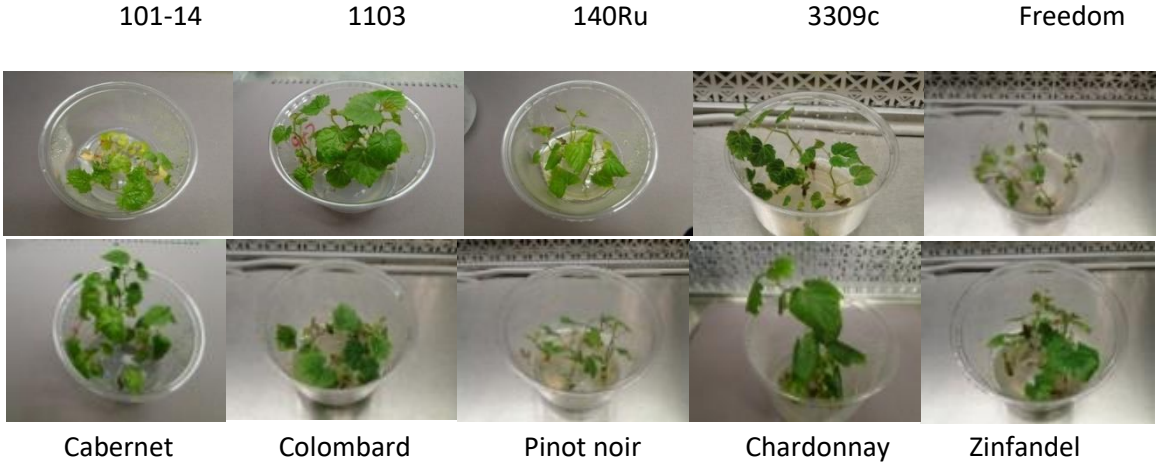

Figure 7. Shoot cultures established for rootstock and scion genotypes

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:

Shoot-tips were collected and plated onto Mezzetti medium with increasing levels of BAP in order to establish bulk meristem cultures. We produced good quality bulk meristem cultures for scion genotypes Chardonnay, French Colombard, Pinot noir Thompson seedless and Zinfandel. However, rootstock genotypes did not readily produce bulk meristems in our hands, but produced elongated shoots with a significant amount of non-organized callus making them unsuitable for bulk meristem transformation (**Figure 8**). Bulk meristems of Thompson Seedless, Chardonnay and Cabernet Sauvignon were sliced into thin, 2mm slices and inoculated with *Agrobacterium* strain EHA105 containing the nptii plant selectable marker gene and the DsRed scorable marker gene 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. We have only been successful in producing transgenic shoots from bulk meristems of Thompson Seedless. Twenty-four of the 75 thin slices sections of Thompson seedless produced DsRed sectors (**Figure 9 and Table 5**) 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. 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 are no longer pursuing this strategy. This technique may have utility for scion genotypes if somatic embryo-based transformations are unsuccessful especially Pinot noir.

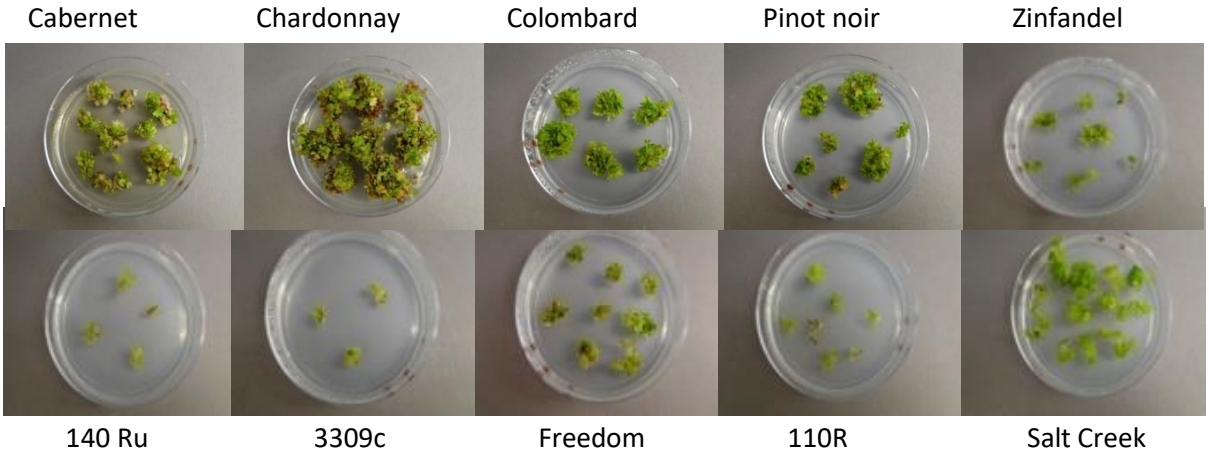

Figure 8. Initiation of bulk meristem cultures for rootstock and scion germplasm

Figure 9. DsRed expressing shoot developing from inoculates thin slice of a Thompson Seedless bulk meristem culture-bright field (left), fluorescence (right)

Table 5. Results of bulk meristem transformation using the scorable marker gene DsRed

Genotype	Number of experiments	Number (%) explants generated DsRed callus	Number (%) explants generated DsRed shoots
Cabernet sauvignon	2	1/36 (3)	0/36 (0)
Chardonnay	2	2/38 (5)	0/38 (0)
Thompson Seedless	2	24/75 (32)	3/75 (4)

Table 6. 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
110R	+	+	+	+	+	5
140 Ru	+	+	+	+	-	-
3309C	-	-	-	-	-	0
GRN-1	+	+	+	+	-	-
MGT 420A	+	+	+	+	+	-
Freedom	+	+	+	+	+	5
Harmony	+	+	+	+	-	-
Salt Creek	-	-	-	-	-	0
Scions						
Cabernet sauvignon	+	+	+	-	-	0
Chardonnay	+	+	+	+	+	<1
French Colombard	+	+	+	+	+	4
Merlot	+	+	+	+	-	-
Pinot noir	+	+	-	-	-	0
Thompson seedless	+	+	+	+	+	10
Zinfandel	-	-	-	-	-	0

+ based on DsRed expression

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

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

Tricoli D. M. 2017. 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 109-118.

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. This technology can be used to introduce genes into grape for studying 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 for adequate coverage of the major wine growing regions in California. We therefore tested our grape rootstock transformation technology on eight additional rootstock genotypes including 110R, 140Ru, 3309C, Freedom, Harmony, MGT 420A, Salt Creek. If rootstock-mediates resistance proves unsuccessful in conferring durable, commercially viable levels of resistance to the grafted scion, 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 also tested our existing transformation technology on a select group of scions including Cabernet Sauvignon, Chardonnay, French Colombard, Merlot, Pinot Noir, and Zinfandel. Overall the advances achieved in grape cell biology during this research (somatic embryo formation, suspension establishment and maintenance, long-term storage of somatic embryos, transgene delivery) all are valuable technologies which

can have utility for germplasm storage, protoplast isolation and transfection, and gene editing and nanoparticle-mediated delivery of DNA.