**Summary Final 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**

**Principle Investigator:**

David Tricoli
Plant Transformation Facility
University of California
Davis, CA 95616
dmtricoli@ucdavis.edu

**Summary Reporting Period**: The summary results reported here are for work conducted from January 16, 2015-June 30, 2018

## 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 seven 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 seven rootstock genotypes and six scion genotypes.
6. Secure *in vitro* shoot cultures for seven 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 seven rootstock genotypes and six scion genotypes as an alternate to somatic embryo transformation

- Using five different media combinations, we plated anthers from over 17,500 flowers across sisteen different genotypes including 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)
- We successfully established embryogenic cultures for all of genotypes listed above with the exception of 3309C, Salt Creek and Zinfandel.
- By culturing somatic embryos in liquid medium composed of Lloyd and McCown's 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, 30 mg/l reduced glutathione, we were able to establish actively growing suspension cultures for all of the rootstock and scion genotypes for which we were able to generate somatic embryos.
- We developed an embryo storage system which allows somatic embryos to be stored for up to one year without the need to subculture or handle the material. This was accomplished by plating small aliquots (200 ul) of the suspension culture 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). These cultures could be stored at room temperature in the dark and remained viable for 9 to 12 months. Stored embryos were used for transformation studies. In addition, whole plants could be regenerated from these stored embryos by transferring them to medium lacking sorbitol.

- Using *Agrobacterium* strain EHA105 containing the nptii plant selectable marker gene and the DsRed scorable marker gene to inoculate stored embryos described above, we were able to generate transgenic DsRed expressing embryos for rootstock genotypes, 1103P, 110R, 101-14, 140Ru, Freedom, GRN1, Harmony, MGT420A and scion genotypes Chardonnay, French Colombard, and Merlot.
- From the DsRed expressing embryos, we were able to regenerate transgenic plants for 1103P, 110R, 101-14, 140R and Freedom and scion genotypes Chardonnay, and French Colombard. We produced germinating embryos of DsRed GRN-1 embryos which appear that if given more time will regenerate plants.
- Using high quality suspension cultures we develop a method for direct transformation of grape suspension cultures with the scorable marker gene DsRed. Frequency of transformation varied greatly from experiment to experiment, but with more research this could provide a faster less labor intensive alternative to the current transformation protocol.
- We explored an alternative transformation protocol using bulk meristems as the explant source. For these experiments we establish in vitro shoot cultures for seven rootstock genotypes and six scion genotypes using indexed material from Foundation Plant Services. By isolating shoot tips from these cultures and transferring them to medium with high concentrations of cytokinin, we were able to establish bulk meristem cultures for scion genotypes Chardonnay, French Colombard, Pinot noir and Zinfandel. However, rootstock genotypes did not readily produce bulk meristems in our hands. Bulk meristems 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. After co-cultivation slices were transferred to selection medium. Using this technique, we were only able to regenerate transgenic shoots from Thompson Seedless.
- 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.

See Table 1 below for a complete summary:

**Table 1. Summary Table of Accomplishment toward the Grant's Objectives**

| 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 | + | + | + | + | - | - |
| 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 |

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