Unified Grant Management for Viticulture and Enology
ANNUAL REPORT 2024-2025 FUNDING CYCLE

CALIFORNIA GRAPE ROOTSTOCK RESEARCH FOUNDATION (CGRRF)

1. **Summary**:

Project Title: Development and Testing Grapevine Rootstocks with Stacked Traits for Resistance to Disease and Pests
Principle Investigator: Abhaya M. Dandekar, Plant Sciences Department, UC Davis

The goal of this project is to develop grapevine rootstocks that combine their existing resistance to pests like Phylloxera and/or to nematodes with RNAi-mediated resistance to bacterial crown gall disease. The first project was initiated on March 31, 2020, with input from members of the commission during their meeting on Feb. 4, 2020. Based on this input we targeted 5 commercially significant grapevine rootstocks, GRN1, 1103P, 101-14 Mgt, 110R and Freedom. We were successful in developing somatic embryo lines for 3 of the five target rootstocks from the get-go. 110R and Freedom were delayed by 2 years as fresh embryo cultures had to be initiated. A new round of this project with the abovementioned title was initiated after a research committee meeting and lab tour on July 22, 2024, to complete the development and testing of all 5 rootstocks. We have successfully completed objective 1 which was the development of embryogenic callus cultures, concluded all transformation experiments and have grapevine plants generated for all the 5 grapevine rootstock genotypes. Objective 2 has also been completed with identification of elite lines for all 5 modified rootstock genotypes. We have initiated objective 3 which is to conduct analysis of the elite modified lines from all 5 genotypes to meet federal regulatory requirements for field testing.

2. **Final Progress Report**: May 1, 2024, to April 30, 2025, for 2024-2025 funding cycle.

3. **Project Title and proposal number**: Development and Testing Grapevine Rootstocks with Stacked Traits for Resistance to Disease and Pests – A24-0799-001.

4. **Principal Investigator**: Abhaya M. Dandekar, Department of Plant Sciences, University of California, Davis; 1 Shields Ave; Davis CA 95616.

Cooperator(s): David M. Tricoli - Plant Transformation Facility; 192 Robbins Hall, University of California, Davis; 1 Shields Ave; Davis CA 95616.

5. **Objective(s) and Experiments Conducted to Meet Stated Objective(s)**:

Goal: To develop grapevine rootstocks that combine crown gall suppression with existing resistance to pests and environmental stress.

Objective 1: Develop embryogenic callus for different pest and environmental stress resistant rootstock genotypes.
Objective 2: Identify elite grapevine rootstock genotype lines that display strong suppression of bacterial crown gall disease.
Objective 3: Conduct analysis of elite grapevine rootstock genotypes so that they meet federal regulatory requirements for field testing.

Objective 1: Develop embryogenic callus for different pest and environmental stress resistant rootstock genotypes. Embryogenic callus lines were successfully developed for the following 5 grapevine rootstocks, that include 1103P (*V. berlandieri* x *V. rupestris*), 101-14 Mgt. (*V. riparia* x *V. rupestris*), Freedom 1613 (*V. solonis* x. *Othello*) x Dog Ridge, 110R (*V. berlandieri* x *V. rupestris*) x Dog Ridge and GRN-1 (*V. rupestris* x *M. rotundifolia*). Thompson Seedless somatic embryos were also included as a control. The next step was also successfully completed, to transform these embryos with the binary vector pDE00.0201 (Fig. 1) to successfully express the RNAi-mediated resistance to crown gall as we have previously described (Escobar et al., 2001, 2002, 2003). Table 1 below summarizes the progress that we have made with these 5 rootstocks.

Table 1: Availability of somatic embryos and regenerated plants in the lab and greenhouse

Rootstock	Somatic embryos generated	Germinated to obtain wildtype plants	Embryos transformed to stack crown gall resistance	Transgenic plants in tissue culture	Wildtype plants in the green house	Transgenic plants in the green house
TS	Yes	Yes	Yes	Yes	Yes	Yes
GRN-1	Yes	Yes	Yes	Yes	Yes	Yes
1103 P	Yes	Yes	Yes	Yes	Yes	Yes
101-14 Mgt	Yes	Yes	Yes	Yes	Yes	Yes
110 R	Yes	Yes	Yes	Yes	Yes	Yes
Freedom	Yes	Yes	Yes	Yes	Yes	Yes

Objective 2: Identify elite grapevine rootstock genotype lines that display strong suppression of bacterial crown gall disease. We accomplished this objective through two activities. We have completed the transformation of grapevine embryo cultures by Agrobacterium-mediated transformation using the binary vector pDE00.0201 (Fig. 1; Escobar et al., 2001). The T-DNA in this vector is designed to expresses two transgenes, one that targets the *iaaM* gene and the other that targets the *ipt* gene present in the T-DNA of infecting wild type *Agrobacterium tumefaciens* (At). Crown gall formation is mediated by 3 highly conserved genes, *iaaM*, *iaaH* and *ipt*, the first two convert the amino acid tryptophan into indole acetic acid (IAA), a powerful auxin, while the third is responsible for the synthesis of zeatin, a powerful cytokinin (Escobar and Dandekar 2003b; Britton et al., 2008). The RNAi-expressing transgenes in pDE00.0201 incorporate the coding regions of these genes in a sense-antisense orientation such that when expressed, the sense and antisense segments within the expressed mRNA folds on itself creating a double

stranded RNA (dsRNA). The dsRNA is a trigger for the expression of the RNAi-mechanism present in all plants (Escobar and Dandekar 2003a).

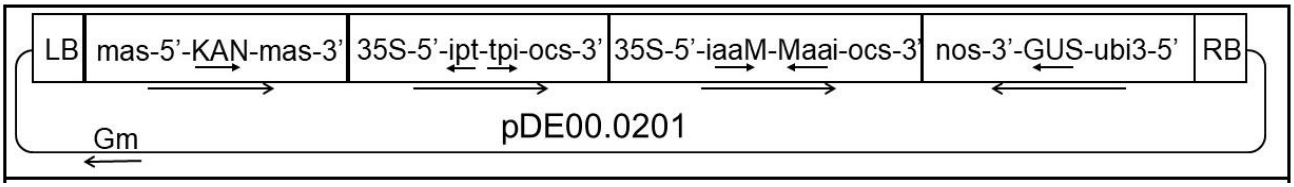

Figure 1: Binary vector pDE00.0201 used to develop RNAi-mediated resistance to crown gall

Independent transgenic lines have been obtained for all five genotypes, GRN-1, 1103P, 101-14 Mgt, 110R and Freedom. The transformation to create these lines was carried out by David Tricoli at the UC Davis, Plant Transformation Facility using a procedure previously described by us (Aguero et al., 2006). Transgenic lines for 110R and Freedom were obtained more recently in 2024. To evaluate the degree of suppression to crown gall development, we inoculated shoots and leaves of tissue culture grown plants. The assay we developed is very sensitive and quick, giving results within 3 weeks after challenging with *Agrobacterium* strains. For this experiment we used three different tumor forming *Agrobacterium* strains: C58, 20W-5A and A281. Liquid *Agrobacterium* cultures were prepared and at least eight plants or leaf petioles were inoculated from each genotype. Once inoculated, the plants or leaves were transferred to co-cultivation media for 48 hours and then transferred to a media with an antibiotic to control the growth of the infecting bacteria. Tumor formation was observed starting three weeks after inoculation. For this analysis the size of the gall was considered and a scale of 1-4 was used. No galls = 0, 25% = 1, 50% = 2, 75% = 3 and 100% = 4. Average scores from eight replicates were considered when deciding the degree of resistance. Galls were observed after 2 ½ to 3 weeks on inoculated shoots and leaves. Results from these tests were used to identify the elite lines that make no galls shown below in Table 2.

Table 2: Transgenic events of desired rootstock genotypes available in the lab and greenhouse
*Indicates no galls formed *in vitro* rapid test compared to wild type.

Transgenic Events					
Thompson Seedless	GRN-1	101-14 Mgt.	1103P	Freedom	110R
19121906 (TS-06)	*20106601 (GRN-1-01)	20106701 (101-14-01)	*20106801 (1103 P-01)	*29946801 (Freedom-01)	29946901 (110R-01)
*19121907 (TS-07)	20106602 (GRN-1-02)	*20106702 (101-14-02)	20106802 (1103 P-02)	*29946802 (Fredom-02)	29946902 (110R-02)
*19121908 (TS-08)	*20106604 (GRN-1-04)	20106703 (101-14-03)	20106803 (1103 P-03)	29946803 (Fredom-03)	*29946903 (110R-03)

*19121909 (TS-09)	20106606 (GRN-1-06)	*20106704 (101-14-04	20106804 (1103 P-04)	29946804 (Freedom-04)	29946904 (110R-04)
191219010 (TS-10)			*20106805 (1103 P-05)	29947002 (Freedom-02A)	*29946905 (110R-05)
191219012 (TS-12)				29947003 (Freedom-03A)	

Objective 3: Conduct analysis of elite grapevine rootstock genotypes so that they meet federal regulatory requirements for field testing. We have just initiated work on this objective that will focus on DNA analysis of the individual transgenic lines to validate the presence of the 4 genes present on the T-DNA and the absence of binary plasmid DNA. Preliminary data that shows the presence of the GUS gene (Fig. 2) and Kan genes (Fig 3) are shown below.

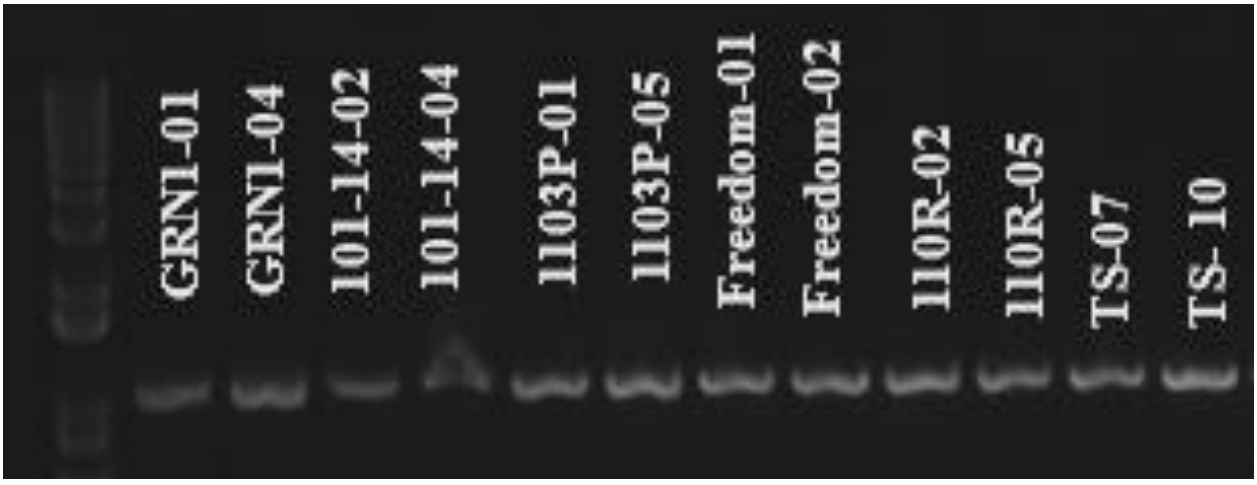

Figure 2: Confirmation of presence of **β-glucuronidase** (GUS) a visual marker gene present in the DNA obtained from different transgenic lines.

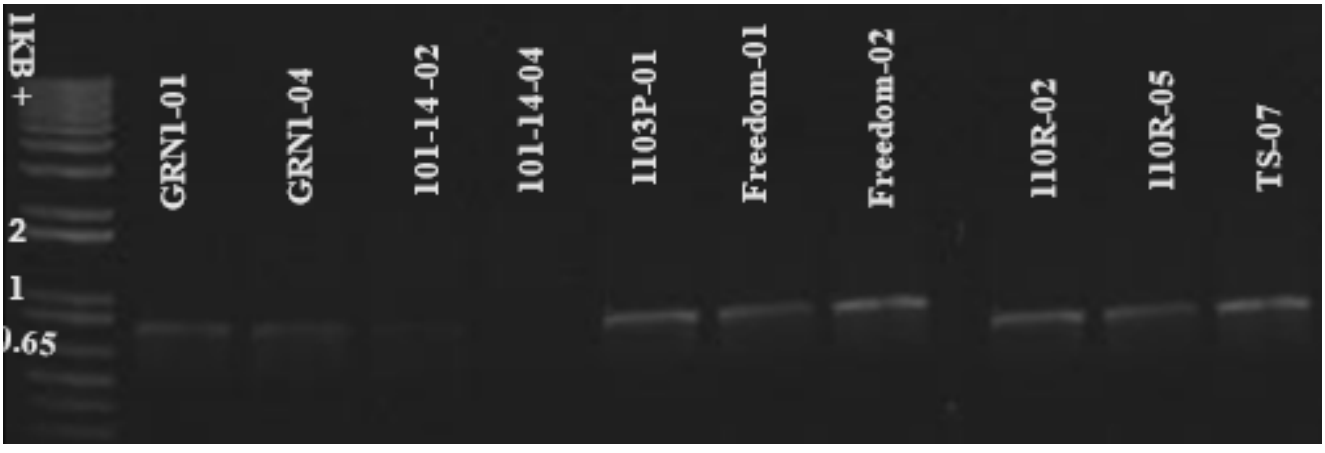

Figure 3: Confirmation of presence of **the *nptII* gene** (neomycin phosphotransferase II) that encodes resistance to the antibiotic **kanamycin present** in the DNA extracted from different transgenic lines.

We have developed PCR primers to test for the presence of the ipt and iaaM gene sequences to validate the presence of both RNAi triggers. We will develop and test PCR primers to demonstrate that no plasmid sequences adjacent to the T-DNA have been incorporated into the transgenic lines. This information will be used to petition USDA APHIS under their 'Regulatory Status Review' process to enable the field evaluation of these rootstocks without the need to obtain a permit.

6. **Summary of Major Research Accomplishments and Results by Objective**

Objective 1: Completed.

Embryogenic callus lines were successfully developed for the following 5 grapevine rootstocks, that include 1103P (*V. berlandieri* x *V. rupestris*), 101-14 Mgt. (*V. riparia* x *V. rupestris*), Freedom 1613 (*V. solonis* x. *Othello*) x Dog Ridge, 110R (*V. berlandieri* x *V. rupestris*) x Dog Ridge and GRN-1 (*V. rupestris* x *M. rotundifolia*).

Objective 2: Completed.

Transgenic lines were successfully developed for the following 5 grapevine rootstocks, that include 1103P (*V. berlandieri* x *V. rupestris*), 101-14 Mgt. (*V. riparia* x *V. rupestris*), Freedom 1613 (*V. solonis* x. *Othello*) x Dog Ridge, 110R (*V. berlandieri* x *V. rupestris*) x Dog Ridge and GRN-1 (*V. rupestris* x *M. rotundifolia*).

Transgenic lines that suppress crown gall development have been successfully identified for 3 grapevine genotypes GRN-1, 1103P, 101-14 Mgt, Freedom and 110R.

Objective 3: Initiated

DNA sequence analysis of the elite genotypes has been initiated to develop USDA APHIS RSR package. To determine the site of integration of the transgenic DNA in all the elite lines, genome sequence will be performed. To obtain high quality genome assemblies, high molecular weight DNA will be extracted and C-HiFi sequencing libraries for the PacBio Revio system will be built. Enough sequencing will be performed to obtain at least 30x coverage of each genome, providing enough information to locate the T-DNA in each genome. This procedure will be done at the UC Davis DNA Core. Sequencing fragments will be analyzed to remove low quality reads and assemble the chromosome pseudomolecules. Complete reference genome sequences of the relevant rootstocks previously generated by the Cantu Lab at UC Davis will be used to map and assemble the new transgenic genomes (https://grapegenomics.com/pages/all.hybrids.php). A final analysis will be done to determine if the insertion of the T-DNA disrupted any native gene,

as part of the requirements for the USDA APHIS RSR package. The bioinformatic pipeline will be done by one of our collaborators to save project funds without compromising on quality.

7. **Outside Presentations of Research**:

We had a great opportunity of presenting our progress on this work to members of the CGRRF at a meeting and lab tour and demonstration held on July 22, 2024 in Robbins Hall on the UC Davis campus.

8. **Research Success Statements**:

This project will establish a pathway to stack disease and pest resistance in grapevine rootstocks. The project output will be elite rootstock lines that are resistant to both crown gall and *Phylloxera*. The elite rootstocks developed by this project will be candidates for commercialization after field-testing to evaluate horticultural attributes and further validation of disease and pest resistance under field conditions. The validation of the stacking strategy developed by this project can be used to stack additional sources of resistance using RNAi against other pathogens.

9. **Funds Status**: Include a general summary of how funds were spent.

We have spent 100% of the funds as of April 30, 2025. We have a continuing proposal that was submitted Jan 31, 2025, and that will fund the work through to its conclusion in 2027.

10. **Literature Cited**:

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