Unified Grant Management for Viticulture and Enology
PRELIMINARY ANNUAL REPORT 2020-2021 FUNDING CYCLE

CALIFORNIA GRAPE ROOTSTOCK IMPROVEMENT COMMISSION (CGRIC)
CALIFORNIA GRAPE ROOTSTOCK RESEARCH FOUNDATION (CGRRF)

1. **Summary**:

Project Title: Stacking disease and pest resistance in grapevine rootstocks
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 nematodes with RNAi-mediated resistance to disease like bacterial crown gall. The 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 will target in addition to the proposed rootstock GRN1 the following additional pest resistant rootstocks: 1103P, 101-14 Mgt, 110R and Freedom. We were successful in developing somatic embryo lines for each of these rootstocks which is the first step to accomplishing objective 1 of our project. Once these somatic embryo cultures have been validated we will begin the process of introducing the RNAi-mediated resistance into these embryo lines and then select individual embryos that express the RNAi-mediated resistance and germinate them into plants. Objective 2 of this proposal will propagate these plants to testing their resistance to crown gall. We have already begun developing new and rapid methods to test the resistant rootstock root systems to evaluate their resistance to crown gall. This initiate testing will utilize embryo lines that we have just developed and that will be sensitive to crown gall so that the methods are in place when the embryo lines expressing RNAi-resistance are developed.

2. **Preliminary Annual Report**: March 31, 2020 to Dec 23 2020 for 2020-2021 funding cycle.

3. **Project Title**: Stacking disease and pest resistance in grapevine rootstocks.

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

Cooperator(s): Andrew M. Walker - Louise Rossi Endowed Chair, Department of Viticulture and Enology; University of California, Davis; 1 Shields Ave Davis CA 95616.

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

Goal: To develop grapevine rootstocks that combine existing resistance to pests with RNAi-mediated resistance to disease. We propose to accomplish this goal via the following two objectives in a period of three years.

Objective 1: Develop and introduce crown gall resistance into grapevine rootstocks resistant to *Phylloxera*.

In our proposal we had selected a single rootstock GRN-1 (*V. rupestris* x *M. rotundifolia*) based on its high resistance to *Phylloxera* and nematodes. However, after our discussion with the board on Feb 4, 2020 we have included rootstocks of their recommendation as they are of interest for California growers. These other rootstocks have a similar profile as they are resistant to different pests and environmental stress but are sensitive to crown gall. These include 1103P (*V. berlandieri* x *V. rupestris*), 101-14 Mgt. (*V. riparia* x *V. rupestris*), Freedom 1613 (*V. solonis* x. *Othello*) x Dog Ridge, 110 R (*V. berlandieri* x *V. rupestris*) x Dog Ridge. Thompson seedless somatic embryos will be generated to use as the control. In the spring 2020, we initiated embryogenic callus from anther filaments of all of the above genotypes using methods that we have previously described (Aguero et al., 2006). The embryogenic callus was induced to make embryos (Fig. 1A) that can be clonally multiplied via repetitive embryogenesis where single embryos produce clones of themselves. We have begun to germinate these embryos to make sure that they are able to be germinated into seedlings as shown (Fig. 1B). This is where we currently are confirming the ability of all of the embryogenic cultures to germinate. The next step is to transform these with the binary vector pDE00.0201 (Fig. 2A) to successfully express the RNAi-mediated resistance to crown gall as we have previously described (Escobar et al., 2001, 2002, 2003). We will begin shortly the transformation of all 5 embryo lines, GRN-1, 1103P, 101-14 Mgt, 110R, Freedom and Thompson seedless as a control. This process will take 6 to 9 months to complete.

Objective 2: Evaluate the efficacy of the combined resistance to disease and pests.

Here we have initiated two activities, the first is to develop an efficient micropropagation system to multiply seedlings obtained from individual embryo lines that will be used for testing the RNAi-mediated resistance to bacterial crown gall delivered by the binary vector pDE00.0201 shown in Fig 2A. The second activity that we have initiated is to develop efficient root systems that can be used for testing not only crown gall but also to confirm the pest resistance status of the combined resistance present in the individual rootstocks. To successfully accomplish this activity, we have cloned the two-root inducing (RolB and RolC) genes from *Agrobacterium rhizogenes* strain A4 (ArA4) (Britton et al., 2008). Using the known DNA sequence of this strain we were able to design appropriate primers to successfully PCR amplify these two genes on a single fragment of DNA. This fragment of DNA also contains the natural regulatory regions that are competent to express these two genes simultaneously in plant tissues. This piece of DNA that contains these two genes was incorporated to create two binary vectors shown in Fig 2 B and C. The first (Fig 2B) also contains a gene that gives a red florescence and will be useful to identify the induced roots. The second binary (Fig 2C) contains a gene that improves the resilience of the root system. We plan to initially test these on wild type plants germinated from the different embryo lines that we now have in culture to validate their sensitivity to crown gall and to develop the protocols for efficacy testing with the two vectors, all of the different rootstock genotypes.

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

The current roster of grapevine rootstocks used in California are typically resistant to an individual disease or pest. Therefore, combining resistance traits in a single rootstock could make for a more sustainable and durable solution. The objective 1 of this project is to stack resistance traits in grapevine rootstocks and develop a single rootstock that has resistance to multiple pathogens. To achieve that objective, first we selected the rootstocks of interest to CA growers that are naturally resistant to *Phylloxera* and nematodes like GRN-1 (*V. rupestris* x *M. rotundifolia*) or others with similar profile of combined resistance to different pests and environmental stress like 1103P (*V. berlandieri* x *V. rupestris*), 101-14 Mgt. (*V. riparia* x *V. rupestris*), Freedom 1613 (*V. solonis* x. *Othello*) x Dog Ridge, 110 R (*V. berlandieri* x *V. rupestris*) x Dog Ridge. In spring 2020, we initiated embryogenic callus from anther filaments of all of above genotypes as we have previously described (Aguero et al., 2006). The embryogenic callus was induced to make embryos that can be clonally multiplied via repetitive embryogenesis where single embryos produce clones of themselves. For accomplishments with Objective 2 we have begun germinating these embryos to develop seedling that will be multiplied by micropropagation. Grape shoots are now ready for *in vitro* crown gall assay to find out the level of basal resistance or susceptibility to crown gall disease of each genotype prior to transformation with the binary vector pDE00.0201. We have also successfully cloned the root-inducing RolB and RolC genes from *Agrobacterium rhizogenes* A4 and build two binary vectors

(Fig 2B,C) that will be used to develop root systems for efficacy testing of the RNAi-mediated resistance to crown gall more efficiently.

7. **Outside Presentations of Research**:

Given the current COVID-19 situation there has been no opportunity to present this work. Also, we are at very early stages in our proposed work, just 9 months have elapsed. We look forward to communicating our research results to end-users and stakeholders at the appropriate time.

8. **Research Success Statements**:

This project in 3 years will establish a pathway to stack disease and pest resistance in grapevine rootstocks. We are currently 9 months into this 3 year project. 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 nematodes and fungal pathogens.

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

We have expended 75% of the funds the remaining 25% will be expended in the next 3 months. We have submitted a new proposal to continue the work beyond March 31 2021 and this proposal is under consideration currently.

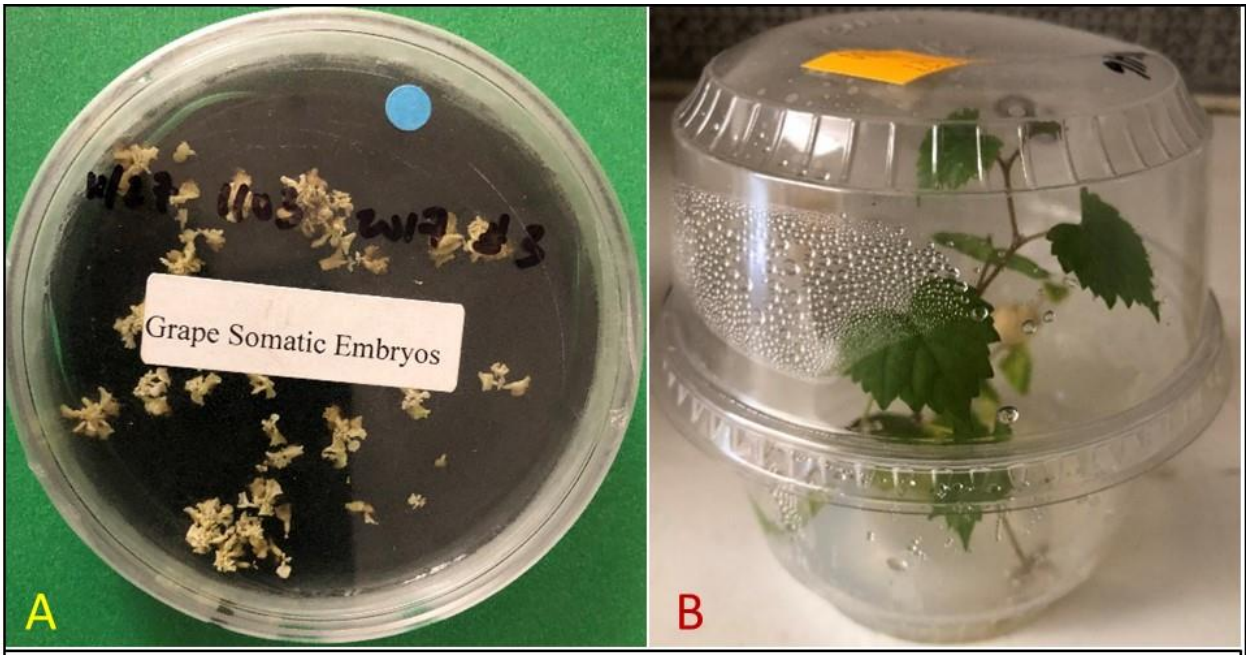

Figure 1: (A) Somatic embryo cultures developed from grapevine rootstocks for introduction of RNAi-mediated resistance crown gall. B) Grapevine rootstock seedling germinated from a somatic embryo culture ready for propagation and testing.

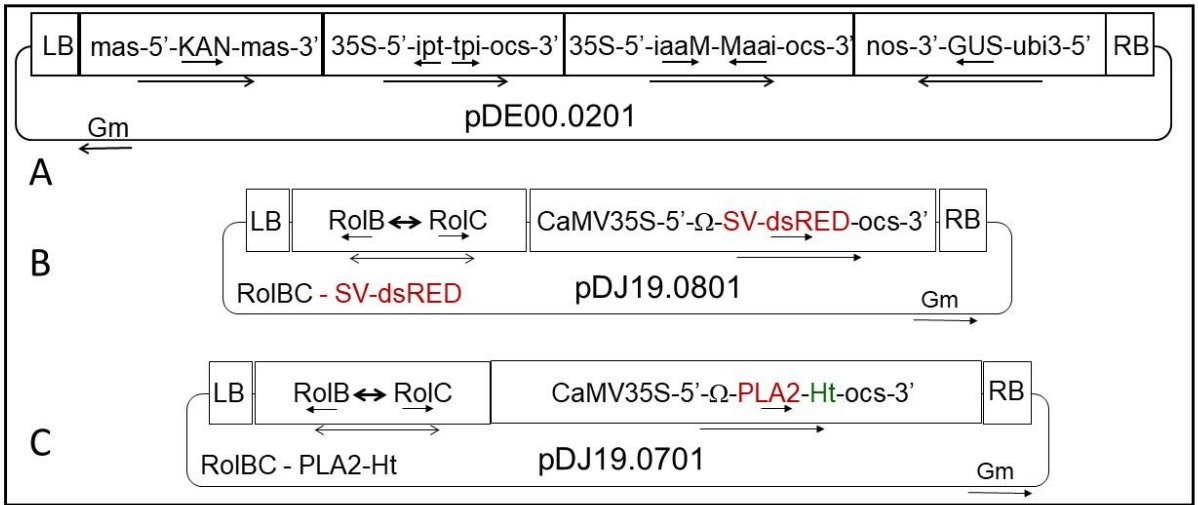

Figure 2: Binary vectors constructed to confer and to test for resistance to crown gall. A) Binary vector pDE00.0201 will be used to develop RNAi-mediated resistance to crown gall. Binary vector pDJ19.0801 (B) and pDJ19.0701 will be used to induce test for efficacy of RNAi-mediated resistance to crown gall.

10. **Literature Cited**:

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