

Summary Final Report for Cdfa Agreement Number SA-16-0559

Project Title: Transgenic rootstock-mediated protection of grapevine scion by single and stacked DNA constructs

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Time Period of Project: This final report covers the period July 1, 2016 to December 31, 2018 to complete development and analysis of the stacked gene transgenics and prepare selected individuals for moving to the field in the summer of 2018 for Pierce's Disease and pathogen analysis. The research described herein is now continuing under Cdfa Agreement Number SA-18-0307.

Introduction: This continuing research project focused the goal of identifying plant genes, which when transformed into cultivated wine grape (*Vitis vinifera*) would depress symptoms of Pierce's Disease (PD) caused by the bacteria *Xylella fastidiosa* (Xf). Previously, a team of researchers (Lindow, Dandekar, and Gilchrist identified five novel DNA constructs, that, when engineered individually into grapevines, suppressed symptoms of Pierce's Disease (PD) under field conditions. Furthermore, these constructs appeared to function by several different mechanism: a) reducing the titer of *Xylella fastidiosa* (Xf) in the plant, b) reducing its systemic spread in the plant, or c) by blocking Xf's ability to trigger tissue death symptoms of PD. The current project was designed to prepare transgenic rootstocks to begin a second field trial evaluating whether pairs of these genes introduced into adapted rootstocks (gene stacking) would suppress symptoms of PD across a graft union to protect an untransformed susceptible Chardonnay scion.

Objectives of Proposed Research Completed Successfully

The following objectives were addressed successfully in the timelines proposed initially and initial planting of the grafted plans was begun in the summer of 2018.

- Complete introduction pairs of protective paired constructs via the dual insert binary vector into adapted grapevine rootstocks 1103 and 101-14 for a total of 20 independent transgenic lines to be evaluated with at least 10 paired combinations from each rootstock line delivered by the transformation facility.
- Conduct extensive analysis, both by Northern analysis and PCR and RTqPCR experiments of each transgenic plant to verify the presence of the two stacked genes in the genome, the full RNA sequence and the expression level of each of the mRNAs expected to be produced by the inserted genes before they are subjected to grafting and greenhouse assays for transgene movement and resistance to PD.
- Production of the clonal ramets of each plant line to enable two cane growth development of the rootstocks and grafting of the Chardonnay scions
- Evaluate the resulting lines for efficacy by inoculation with Xf in a preliminary greenhouse experiment to identify the most protective lines from each combination of genes. A total of 5 independent transgenic

lines of each dual construct in each rootstock was selected and bulked up to 6 copies of each for field planting at the APHIS approved site in Solano County. **Note: the greenhouse inoculation step was eliminated after several attempts since it did not provide a reliable indicator of disease symptoms in response to the bacterial numbers. PCR confirmation of dual transformation was successful and was carried forward as the selection criteria**

- Following verification of the genotypic integrity of the transgenic rootstock plants, clonal copies of each plant line were made to enable two cane growth development for production of rootstocks and grafting with Chardonnay scions.
- Grafted plants began moving to the field in August 2018 with final planting in 2019.

Relevance of the Research

This translational research conducted herein will test for potential cross-graft protection of a PD susceptible Chardonnay 04 scion against the development of Pierce's Disease symptoms by expression of dual combinations of five PD suppressive transgenes in two adapted rootstocks. The protocol includes planting, training, inoculating to evaluate both disease and yield components specifically in the PD susceptible scions. It also will enable assessing both potential cross-graft protection of a non-transformed scion and the effect of the transgenes to protect the rootstocks against bacterial movement and death compared to equivalent combinations of untransformed rootstock/scion control combinations.

Research Capacity and Accomplishment of Objectives within the Timelines

The anticipated timeline was completed per expectations and is continuing under CDFA Agreement Number SA-18-0307 with an end date of June 23, 2020. The transgenic rootstocks were delivered as anticipated which confirmed that our capacity to achieve all the objectives was assured based on prior accomplishments. All techniques and resources available in the lab were proven reliable, informative, and reproducible. This project brought together a full time research commitment for this team of experienced scientists to Pierce's Disease. Each of the senior personnel, including Dr. Lincoln have been with this project since 2007 and have different skills and training that complement changing needs of this project in the areas of molecular biology, plant transformation and analysis of transgenic rootstocks for suppression of Pierce's Disease symptoms under field conditions.



Planting of the dual constructs. This image illustrates the new planting of the dual construct transformed rootstocks grafted with an untransformed clone of Chardonnay. This first phase of the planting was completed August 1, 2018.