

Renewal Progress Report for CDFR Agreement Number 17-0417-000-SA

A study on the impact of individual and mixed leafroll infections on the metabolism of ripening wine grape berries

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Reporting Period

The results reported here are from work conducted July 1, 2017 to June 30, 2018

Introduction

Grapevine leafroll-associated viruses (GLRaVs) are the most widespread and economically damaging viruses affecting viticulture (Goheen *et al.*, 1959; Maree *et al.*, 2013; Naidu *et al.*, 2015; Atallah *et al.* 2012). Plants' responses to viruses generally include a multitude of changes in metabolism, gene expression, and gene regulation (Alazem & Lin, 2014; Bester *et al.*, 2016; Blanco-Ulate *et al.*, 2017; Moon & Park, 2016). However, there is a gap in knowledge concerning the specific regulation of the response to GLRaVs and which pathways determine GLRaV symptoms and their severity. The effects of GLRaVs can include poor color development in red grapes, non-uniform or delayed ripening, reduced sugar content in berries, altered tannins, pigments, and acids, curling leaves, reddening or chlorotic interveinal areas, and high crop loss (Atallah *et al.*, 2012; Guidoni *et al.*, 2000; Vega *et al.*, 2011; Alabi *et al.*, 2016; Lee & Martin, 2009; Lee & Schreiner, 2010). The severity of GLRaV symptoms is influenced by host genotype (Guidoni *et al.*, 2000), which virus or combination of viruses is present, scion-rootstock pairings (Fuchs *et al.*, 2009; Prosser *et al.*, 2007; Golino *et al.*, 2003; Lee & Martin, 2009), and environmental factors (Cui *et al.*, 2017). The experiments proposed will test our hypotheses that (1) GLRaVs disrupt berry development and the accumulation of flavor and aroma metabolites by altering hormone networks and (2) the differences in symptoms associated with different GLRaVs are due to non-uniform impacts on some metabolite and gene regulatory pathways.

Research relevance

This study is using RNA sequencing and metabolite profiling to explore the effects of individual and mixed infections of GLRaVs on ripening and to identify which pathways are involved in responses and symptoms. The rootstocks, scions, and infections used in this study were selected to improve the likelihood of generating commercially transferable knowledge. The vineyard used for this study consists of Cabernet Franc grapevines grafted to different rootstocks and carrying commercially consequential GLRaVs. Cabernet Franc was used because it produces clear symptoms to GLRaVs. Among the treatments established in the vineyard, vines carrying GLRaV-1, GLRaV-3, GLRaV-5, GLRaV-1 + GLRaV-2, and GLRaV-1 + GLRaV-3 were included in this study because these infections are associated with a range of symptoms and symptom severities. Among the rootstock-scion pairings planted in the experimental vineyard, Cabernet Franc grafted to Kober 5BB and MGT 101-14 rootstocks were used because these rootstocks are commonly used in California. The data generated may be used in the future to develop strategies to mitigate the detrimental effects of these viruses on ripening.

Objectives

1. Profile genome-wide transcriptional changes as a result of individual and combinations of GLRaV infections during grape berry development.
2. Identify secondary metabolic pathways that underlie the altered biochemical composition of GLRaV infected berries.
3. Determine changes in plant hormone biosynthesis, accumulation and signaling that are associated with the abnormal ripening of GLRaV-infected berries.

Intellectual property

None to report in this period.

Publications and presentations

None to report in this period.

Status of funds

Funds have supported supplies and salaries for the activities described in this report.

Progress

Pre-objectives

Sampling and sample preparation 2017. GLRaV infections (or their lack of in control vines) as well as the specific strains involved were confirmed by molecular testing at FPS prior to sampling. Berries were collected at 4 distinct developmental stages (pre-véraison, véraison, post-véraison, and harvest) from Cabernet Franc grapevines grafted to MGT 101-14 and Kober 5BB rootstocks. Twenty berries were picked from from each of 6 vines at each sampling date and from each viral treatment. Berries were sampled evenly throughout the plant. Following their sampling, berries were crushed and their total soluble solid (TSS) were measured.

Sampling and sample preparation 2018. The Golino group oversaw re-testing of the experimental vines for viruses to ensure the same conditions in 2018 as in 2017. The grapevines were monitored throughout June in order to best estimate the beginning of samplings in 2018.

Measurement of Brix. Differences in total soluble solids (TSS) were observed at each time point in the experiment that were dependent on the combination of infections and rootstock. These results were reported previously.

1. Profile genome-wide transcriptional changes as a result of individual and combinations of GLRaV infections during grape berry development.

Justification. The RNA-sequencing data to be generated will provide a quantitative, comprehensive view of the changes in gene expression due to GLRaVs associated with primary and secondary berry metabolism.

Selection of samples for RNA-seq. Following the collection, crushing and measurement of total soluble solids in six biological replicates, four of six were selected for the preparation of RNAseq libraries.

Library preparation and sequencing. RNA extractions, library preparation, and sequencing are complete. Libraries with fewer than 12 million reads were re-sequenced. Following resequencing, the median number of reads sequenced for the 192 libraries was 17,256,960. The minimum and maximum number of reads sequenced among the 192 libraries were 12,007,531 and 34,591,412, respectively.

Statistical analysis and differential expression. The library normalization and differential gene expression analysis is complete and we are exploring the results.

2. Identify secondary metabolic pathways that underlie the altered biochemical composition of GLRaV infected berries.

Justification. Changes in the expression of secondary metabolism-associated genes can reveal mechanisms that underlie impaired berry metabolism and accumulation of commercially significant metabolites.

Overrepresented gene ontological categories. To summarize the disparate impact of the viruses and rootstocks on gene expression during ripening, an overrepresentation test was used to identify overrepresented groups among differentially expressed genes, as well as disparately affected metabolite pathways.

3. Determine changes in plant hormone biosynthesis, accumulation and signaling that are associated with the abnormal ripening of GLRaV-infected berries.

Justification. Hormones play a major role in regulating ripening, disease responses and the metabolic changes associated with both. Changes in the abundance of hormones will show which hormone pathways regulate GLRaV responses.

Hormone identification by LC-MS/MS using an in-house dataset. Pre-existing datasets were used by the Ebeler group to identify the correct signatures of several hormones of interest. The same samples used for RNA sequencing are being used for the measurement of hormones and other metabolites. We optimized our extraction method and these extractions are ongoing. We are also preparing extracts for the measurement of commercially important phenolic metabolites, including anthocyanins and other flavonoids.

Layperson summary of progress

Our previous report described that the samples had been crushed, TSS measured, RNA extractions completed, and hormone detection and quantification methods in development at the time of its submission. Since then, the RNA sequencing libraries were constructed and sequenced, the data were prepared for analysis, and initial statistics were generated to determine which genes were impacted by each individual or dual infection and how rootstock might influence these effects. We confirmed the infection status of the experimental vines for the upcoming sampling season and will use the same vines for sampling as in 2017. More detailed analyses of the RNA sequencing data are ongoing, as are the hormone and metabolite extractions.

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