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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 February 15, 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 metatabolites 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. 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.

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. TSS in berries from nearly each sampling date were significantly higher in plants grafted to Kober 5BB with GLRaV-1 + GLRaV-2 dual infections than in healthy, single infection, and GLRaV-1 + GLRaV-3 dual-infection plants on the same rootstock (**Figure 1**). In plants with the GLRaV-1 + GLRaV-2 dual infection, TSS were significantly higher in plants grafted on Kober 5BB than on MGT 101-14 (Tukey HSD test, *p-value* < 0.05). Though surprising, this might be associated with visibly poorer fruitset on these plants (**Figure 2**). Furthermore, the fruits on these plants were visibly beginning to desiccate by harvest. Berries from plants grafted on Kober 5BB and with GLRaV-1, GLRaV-3, and GLRaV-1+GLRaV-3 dual infections appeared to have lower TSS than healthy plants on the same rootstock, though these differences were not significant. Differences in TSS relative to healthy plants and between treatments were not observed among berries from plants grafted to MGT 101-14 rootstock. These results suggest that rootstock may impact the severity of disease symptoms, though how is unclear. Further, it appears that different leafroll viruses or different combinations of viruses disparately impact ripening.

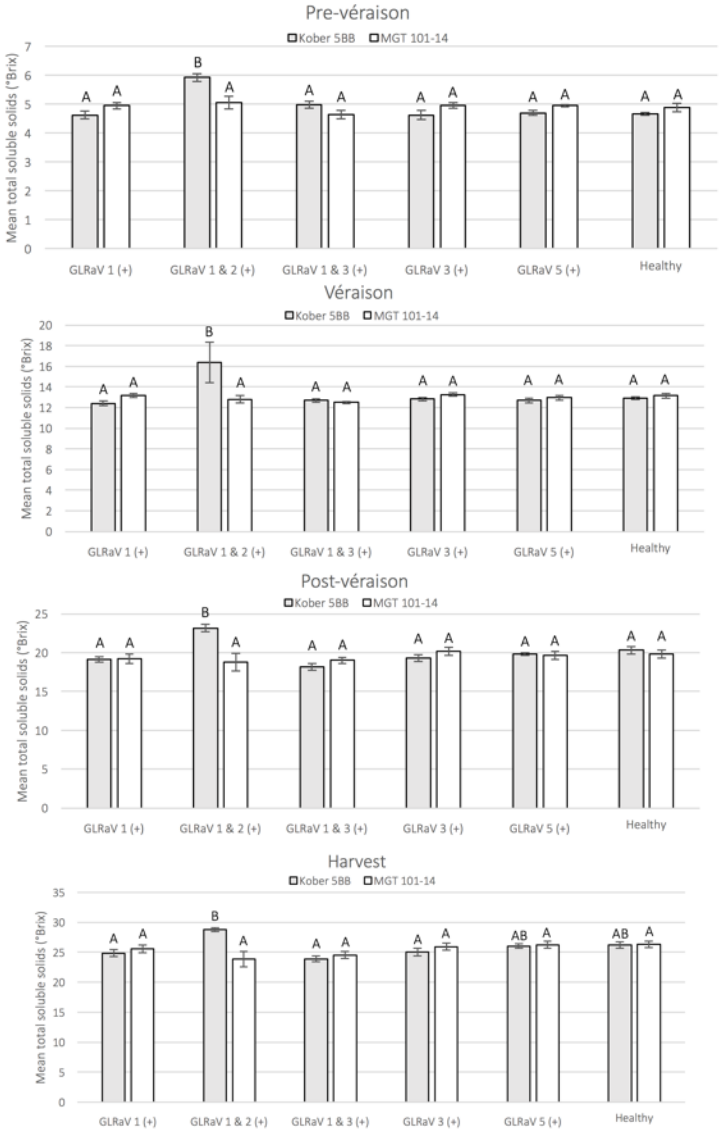

Figure 1. Mean total soluble solids observed at each time point of this study in plants grafted to different rootstocks and with no, single, and dual infections. ±SEM shown. Statistical comparisons were made for

each time point separately, among infections in plants grafted to the same rootstock, and between plants on different rootstocks with identical disease status. Given these comparisons, significant differences are denoted with different letters. Tukey honest significant difference, *p-value* < 0.05.

Figure 2. Photographs of grapevines and berries. Plants are grafted to Kober 5BB. Top two, Healthy; Bottom two, GLRaV 1 & GLRaV 2 (+). The top-most photo is rotated counterclockwise.

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. RNA extractions are complete and RNA sequencing libraries are currently being prepared.

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.

   Library preparation. The preparation of RNA sequencing libraries upon which this objective depends is underway.

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 are currently being used by the Ebeler group to identify the correct signatures of the hormones of interest. The same samples used for RNA sequencing will be also used for the measurement of hormones and other metabolites.

Layperson summary of progress

The last sampling date was in September 1, 2017. Following that date, all the samples were crushed and TSS were measured for all sampling dates. From those samples, a subset were selected for RNA extractions necessary for the pursuit of Objectives 1 and 2. These extractions were recently completed and the preparation of RNA sequencing libraries is currently underway. Towards the completion of Objective 3, we are finalizing the list of hormones capturable by LC-MS/MS using existing methods employed by the Ebeler lab.

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