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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 July 15, 2019.

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 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. We intend to integrate gene expression, hormone, and metabolite data to better understand how these viruses affect fruit metabolism during ripening given different rootstocks. This information will help inform future strategies to combat or resist leafroll viruses.

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.

ACTIVITIES AND RESULTS

Pre-objectives

Sampling and Sample Preparation 2017. GLRaV infection conditions were confirmed by molecular testing at Foundation Plant Services (FPS) prior to sampling. Photographs were taken and berries were collected at four 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 each of six vines at each sampling date and from each viral treatment. Berries were sampled evenly throughout the plant. Following their sampling, berries were crushed.

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. Fruits were sampled at the same four developmental stages as in 2017. As in 2017, plants were photographed to monitor the onset of leafroll symptoms. Berries were deseeded and frozen at -80°C. These samples were crushed.

Measurement of Brix 2017 and 2018. Differences in TSS were observed at each time point in the experiment that were dependent on the combination of infections and rootstock.

Objective 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; some may be associated secondary metabolism.

Selection of Samples for RNA-seq 2017 and 2018. Following the collection, crushing, and measurement of TSS in six biological replicates, four of six were selected for the preparation of RNAseq libraries.

Library Preparation and Sequencing 2017 and 2018. RNA extractions, library preparation, and sequencing are complete.

Statistical Analysis and Differential Expression 2017 and 2018. The library normalization and differential gene expression analyses are complete and we are exploring the results. This includes determining whether certain types of genes involved in particular pathways or functions are overrepresented among the differentially expressed genes and using the normalized expression data to look for similarities and differences between groups of samples **(Figure 1).** This also includes examining the reproducibility of the results between years **(Figure 2).** In addition, a gene co-expression network is being constructed to understand how possible relationships between genes change because of infection, whether they differ based on the rootstock present, and prioritizing which genes are central to changes in responses **(Figure 3).** We are approaching these questions using several tools and comparing their results.

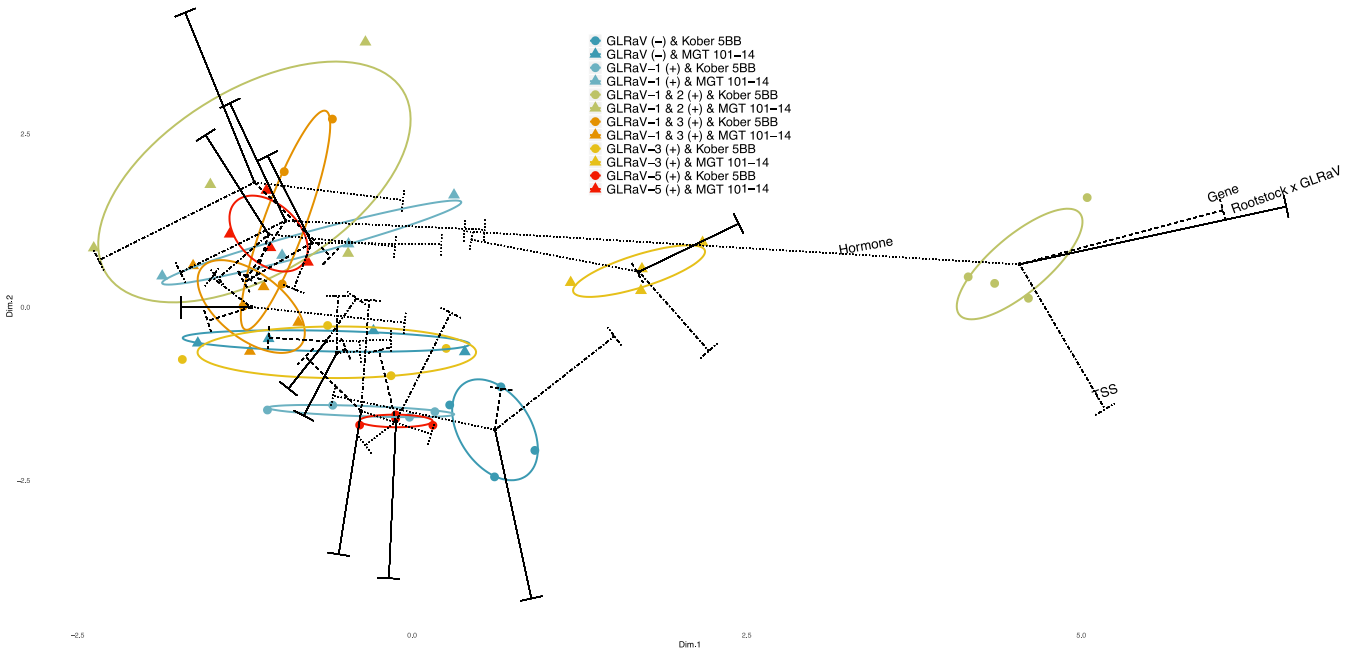

Figure 1. Integration of 2017 RNAseq, LC-MS, and TSS data. Multiple factor analysis (MFA) of berries from plants with different GLRaV status and two different rootstocks mid-ripening. Normalized gene expression level, total soluble solids, and hormone abundance were used as quantitative variables. Rootstock and GLRaV status were used as qualitative variables. These data show significant differences between some groups based on these variables. All variables are shown as vectors. 95% Confidence ellipses for groups are shown. Each point is one biological replicate. Different GLRaV conditions indicated with different colors. Different rootstock indicated by point shape. x-axis, Dimension 1, 13.4%; y-axis, Dimension 2, 8.2%. This analysis was repeated for each of the other 3 stages sampled as well (not shown). Genes significantly and highly correlated ($R^2 > 0.7$ and *p-value* < 0.01) with either dimension were extracted and subjected to an overrepresentation test. Genes associated with photosynthesis, NAD binding, glucose metabolism, and GTPase activity were overrepresented (*p-value* < 0.01) among these genes.

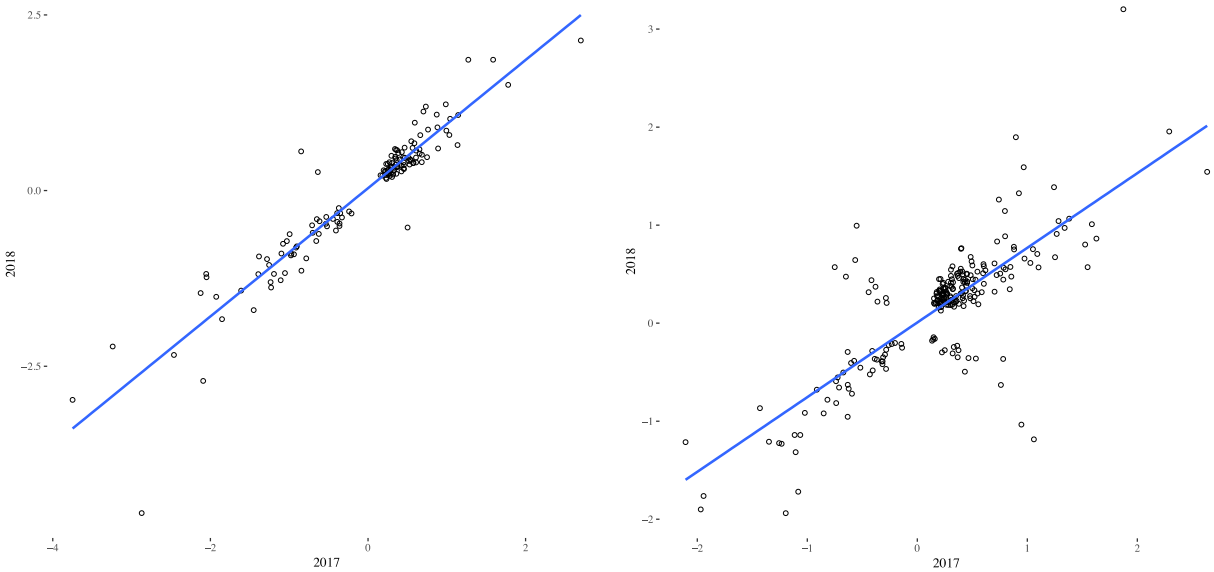

Figure 2. Reproducibility of RNAseq between 2017 and 2018. Each point is one gene and only genes differentially expressed in both years are plotted. Line of best fit is shown. (Left) The Log2 fold-change in gene expression at harvest between healthy versus GLRaV-1 & 2 (+) plants grafted to Kober5BB in both years. This result indicates that the effect of GLRaV-1 & 2 on these genes was of similar magnitude and directionally (upregulated / downregulated) similar in both years. (Right) Log2 fold-change in gene expression for Kober 5BB versus MGT 101-14-grafted plants that are GLRaV-1 & 2 (+). Although these genes were differentially expressed in both years, some genes did not change in directionally the same way in both years. All other combinations of comparisons (healthy vs. infected, MGT 101-14 vs. Kober 5BB) were also made (not shown). The normalized expression values for genes differentially expressed in only one year were also examined to see if those genes behaved similarly in both years (not shown). Overall, we observed considerable consistency in the behavior of genes in both years.

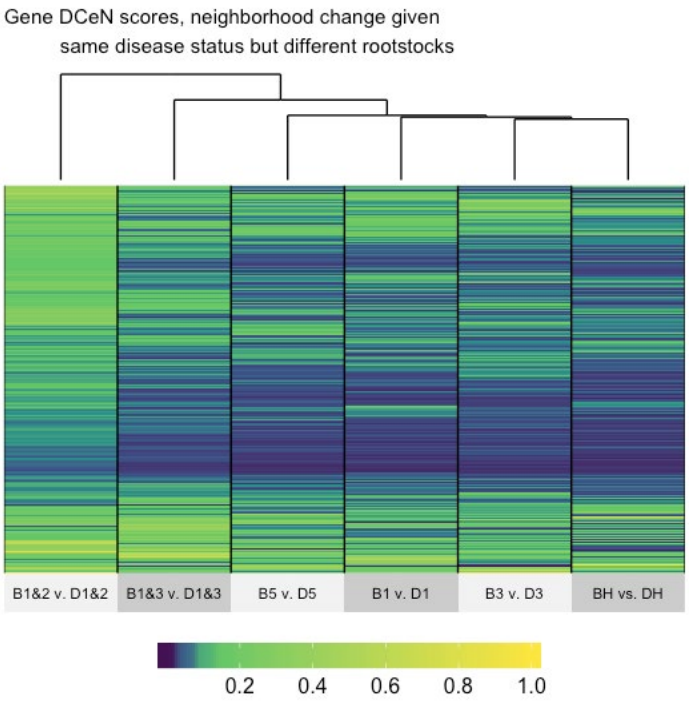

Figure 3. Dynamically Co-expressed Neighborhoods (DCeN) analyses. DCeN scores indicate the proportion of the co-expression neighborhood of a single gene that differs between two conditions and high DCeN genes tend to have significant regulatory roles (Elo and Schwikowski, 2013). Key: B = Kober 5BB; D = MGT 101-14; H= Healthy; GLRaV infections indicated by 1, 3, 5, 1 & 2, 1 & 3. Color scale indicates DCeN score. DCeN scores of 0 indicate no difference in neighborhood between the groups compared for a gene. DCeN scores of 1 indicate 100% difference in neighborhood between the groups compared for a gene. In this figure, each row is for a single gene and each column is a distinct comparison made. This result suggests somewhat consistent differences between rootstocks across infection conditions. There are the fewest rootstock-associated differences in neighborhood when plants are healthy (far right), and the greatest number of rootstock-associated differences when plants are infected with GLRaV-1 and 2 (far left). This result shows some gene neighborhoods that consistently differ between rootstocks regardless of infection status, and some gene neighborhoods for which the difference between rootstocks will depend upon the infection present.

Objective 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.

Ripening-related metabolite analysis. This is ongoing and we intend to intersect the differential expression analyses with ripening-related metabolite data once generated.

Objective 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 and quantitation by Liquid Chromatography – Mass Spectrometry (LC-MS). 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 were used for the measurement of hormones and other metabolites. We optimized our extraction method, completed the hormone analysis of 2017 samples. These results indicate significant effects of GLRaVs on abscisic acid and salicylic acid. We are weighing the 2018 samples to repeat the analysis using a second year of data.

PRESENTATIONS

Our preliminary results were presented at the Pierce's Disease Research Symposium in San Diego, California, in December 2018.

RELEVANCE

This ongoing study used RNA sequencing and metabolite profiling to explore the effects of individual and mixed infections of GLRaVs on ripening and to identify and better understand the pathways 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 consists of Cabernet Franc grapevines grafted to Kober 5BB or MGT 101-14 rootstocks and carrying 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 because infections with one or more of these viruses are associated with a range of symptoms of varying severities. The data generated will improve our understanding of the basis of symptoms and to develop strategies to mitigate the detrimental effects of these viruses on ripening in the future.

LAYPERSON SUMMARY

The purpose of this study is to understand the impact of individual and combinations of Grapevine Leafroll-associated Viruses on ripening in Cabernet Franc grapevines grafted to two different rootstocks. Different virus combinations and different rootstocks were chosen because of their association with varying levels of symptoms given virus infection. This study has thus far included data collection and analyses in two consecutive years.

In each year, the first steps towards generating data include monitoring the infection status of the vines, sampling consistently each year, deseeding berries, and crushing tissue. Our previous reports stated that these steps were completed for 2017 and 2018 samples.

The next steps towards data generation include measuring total soluble solids (TSS, °Brix), choosing samples for sequencing, preparing RNA sequencing libraries, and sequencing those libraries. Our previous reports stated that these steps were complete for 2017 and 2018 samples.

In addition, hormone and metabolite extractions were undertaken. This first involves optimizing a detection method for specific hormones and ripening-associated metabolites. Then, generating the data involves weighing crushed tissue for each sample in duplicate, performing the extractions, and subjecting the sample extracts to liquid chromatography and mass spectrometry (LC-MS). Our previous reports stated that the hormone analyses were completed for the 2017 samples. The ripening-related metabolite extractions for 2017 samples are still ongoing. Weighing 2018 tissue for hormone and ripening-related metabolite extractions are currently underway and the LC-MS analyses of all remaining samples is scheduled to begin shortly.

Finally, as described in the previous report, analyses of the 2017 RNA sequencing analyses are ongoing. The 2018 RNA sequencing analysis is also ongoing. We are comparing years to evaluate year-to-year effects of the viruses and rootstocks on berry response during ripening, looking closely at the affected genes under the various experimental conditions, and are examining condition-dependent changes in the relationships between genes. We have also begun to integrate the hormone data that was generated and intend to integrate the forthcoming metabolite data into our analyses.

STATUS OF FUNDS

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

INTELLECUAL PROPERTY

None to report in this period.

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