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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 22, 2019.

INTRODUCTION
This ongoing study used RNA sequencing and metabolite profiling to explore the effects of individual and mixed infections of Grapevine Leafroll-associated Viruses (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 consists of Cabernet Franc grapevines grafted to Kober 5BB or MGT 101-14 rootstocks that carry 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 may be used 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.

ACTIVITIES

Pre-objectives

Sampling, Sample Preparation, and TSS measurement: 2017 and 2018. The presence and absence of specific GLRaV infections were confirmed 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. Berries were picked from each of six vines at each sampling date and from each virus x rootstock condition. Berries were sampled evenly throughout the plant. Following their sampling, berries were deseeded, crushed and their total soluble solids (TSS) were measured.

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 associated with primary and secondary berry 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. RNA extractions, library preparation, and sequencing for the 192 samples collected in 2017 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.

Library Preparation and Sequencing 2018. RNA extractions, library preparation, and sequencing for the 192 samples collected in 2018 are complete. Analyses of these libraries have begun.

Statistical Analysis and Differential Expression 2017 and 2018. The 2017 library normalization and initial differential gene expression analysis is complete and we are exploring the results. Among these results were as many as approximately 5,000 genes differentially expressed in berries given identical virus infections but from plants grafted to different rootstocks. Now, we are normalizing the sequencing data from 2018, and will rerun the differential analysis while accounting for year-to-year effects. Ultimately, we will look to see which specific pathways are affected and relate the expression data to hormone and metabolite data.

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 2017 and 2018. To summarize the disparate impact of the viruses and rootstocks on gene expression during ripening, an overrepresentation test was used to do an initial identification of overrepresented groups among differentially expressed genes, as well as disparately affected metabolite pathways. This step was done for the 2017 data and will be repeated using the 2017 and 2018 data, accounting first for year-to-year effects in doing differential expression analyses.

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 by Liquid Chromatography – Mass Spectrometry (LC-MS) 2017: Pre-existing datasets were used by the Ebeler group to identify the correct signatures of several hormones of interest. The same 2017 samples used for RNA sequencing are being used for the measurement of hormones and other metabolites. We optimized our extraction method, completed the extractions, and used LC-MS to analyze the samples. The extractions were performed in technical duplicate. The preparation of extracts of commercially important phenolic metabolites, including anthocyanins and other flavonoids, is ongoing. A preliminary analysis of the hormone data was completed. An excerpt of this data is shown in Figure 1. Abscisic acid (ABA), auxin, saliylic acid, and jasmonic acid were measured. Among these, ABA is notably important because of its relationship with the ripening process and abiotic stress. We observed significant differences in ABA between treatments during ripening (Tukey HSD $p\text{-}value < 0.05$; Figure 1).

Pre-véraison, significant differences were observed in ABA between rootstocks in vines infected with both dual infections. The level of abscisic acid was also significantly higher in plants on Kober 5BB and infected with GLRaVs 1 and 2 than healthy vines grafted to the same rootstock and vines with any other infection.

At véraison, ABA was significantly higher in healthy and GLRaV-5(+) plants grafted to MGT 101-14 than Kober 5BB. The opposite was true for plants infected with GLRaV 1 and 3; ABA was significantly higher in plants grafted to Kober 5BB than to MGT 101-14. In addition, GLRaV 1 and 3(+) plants grafted to Kober 5BB had significantly higher levels of ABA than healthy plants grafted to the same rootstock.

Mid-ripening, ABA levels were significantly higher for Kober 5BB grafted plants with both GLRaV dual infections than healthy plants and GLRaV5(+) grafted to the same rootstock. The levels of ABA were also significantly different between GLRaV3(+) and GLRaV 1 and 3 (+) for plants grafted to Kober 5BB. For plants infected with GLRaVs 3, 5, or 1 and 3, different rootstocks were associated with significantly different levels of ABA (Figure 1).

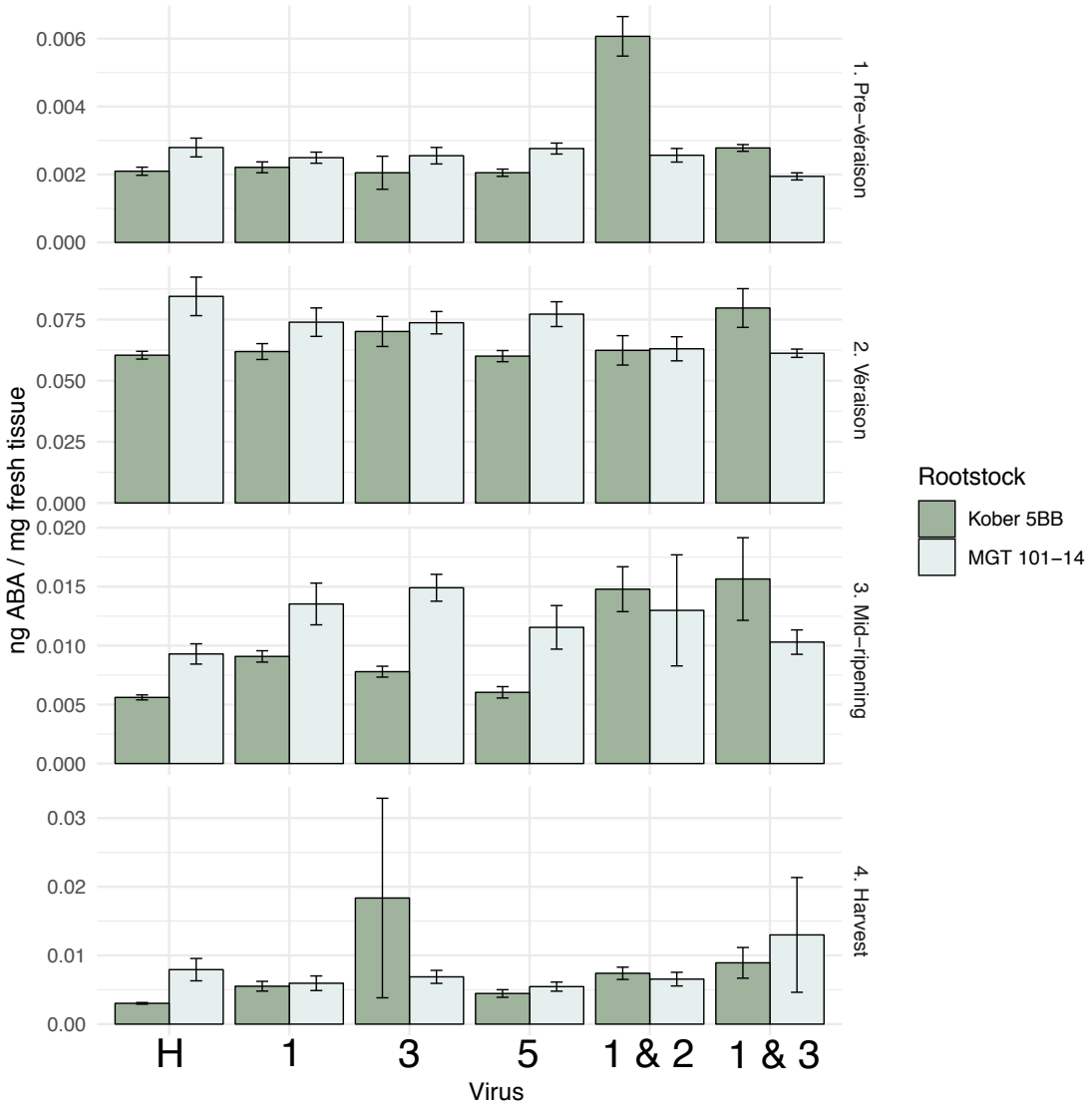

Figure 1. Abscisic acid levels during ripening in Cabernet Franc grapevines grafted to two rootstocks and infected with zero, one or two GLRaVs.

PRESENTATIONS

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

RESEARCH RELEVANCE

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. 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 that combat or resist leafroll viruses.

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 samples, that sampling was completed for 2018, and that crushing was underway for the 2018 samples. The 2018 samples have now been crushed as well.

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 samples, and that TSS measurements were underway for the 2018 samples. To date, all of these steps were completed for the 2018 samples as well.

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 chromatography and mass spectrometry. Our previous reports stated that the detection methods were optimized, and the extractions were underway for the 2017 samples. As of now, the hormone data from 2017 samples were generated completely and are currently being analyzed. The ripening-related metabolite extractions for 2017 samples are underway.

Finally, as described in the previous report, analyses of the 2017 RNA sequencing analyses are ongoing. We intend to integrate the 2018 RNA sequencing data to evaluate year-to-year effects of the viruses and rootstocks on berry response during ripening, as well the hormone data that was generated and the forthcoming metabolite data.

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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