Molecular approaches for building a comprehensive knowledge base on grapevine responses to virus infection

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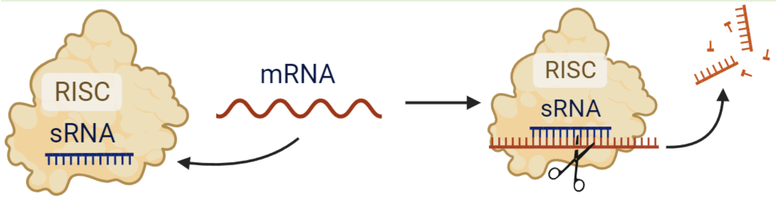
Background

Red blotch disease, caused by Grapevine red blotch virus (GRBV; genus *Grablovirus*; family *Geminiviridae*; Yepes et al., 2018), is an economically important viral disease infecting grapevine (Ricketts et al., 2017). Infection by GRBV results in lowered plant vigor, fruit yield, and fruit quality, leading to significant losses to growers (Girardello et al., 2020 and cited references). The visual symptoms of red blotch disease, which include the formation of characteristic red "blotches" in red-fruited cultivars, do not appear until after veraison.

A single *Vitis vinifera* cv. 'Merlot' vine infected with GRBV at pre- and post-veraison.

Small (s)RNAs are regulatory molecules which target and silence mRNA transcripts via RNA-induced silencing complexes (RISCs).



RISCs use sRNAs to bind and degrade mRNAs with complementary sequences.

Types of sRNA include micro- (mi)RNAs and small-interfering (si)RNAs. miRNAs are key regulators for many plant physiological and developmental processes. Their expression has been shown to be impacted by biotic and abiotic stressors, including viral infections (Alabi et al., 2012; Bester et al., 2017). Contrastingly, siRNAs derived from viruses (vsiRNAs) are used by plants to silence viral transcripts.

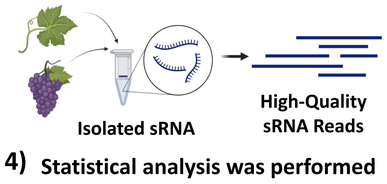
Objective

While GRBV epidemiology is well-understood, much needs to be learned at the fundamental level. This study was undertaken as a first step towards building comprehensive knowledge on grapevine molecular responses to GRBV infection, especially with regard to the role of RNA silencing as a defense strategy against viral infection.

Materials and Methods

1) **Leaves (L) and berries (B) were collected from adjacent healthy (H) and diseased (D) vines (cv. Merlot) at pre- (P) and post- (PO) veraison stages in July and September, respectively.**

2) **Small RNA sequencing libraries were prepared from leaf and berry samples. Subsequently, Illumina sequencing of sRNAs was performed at BGI Genomics.**

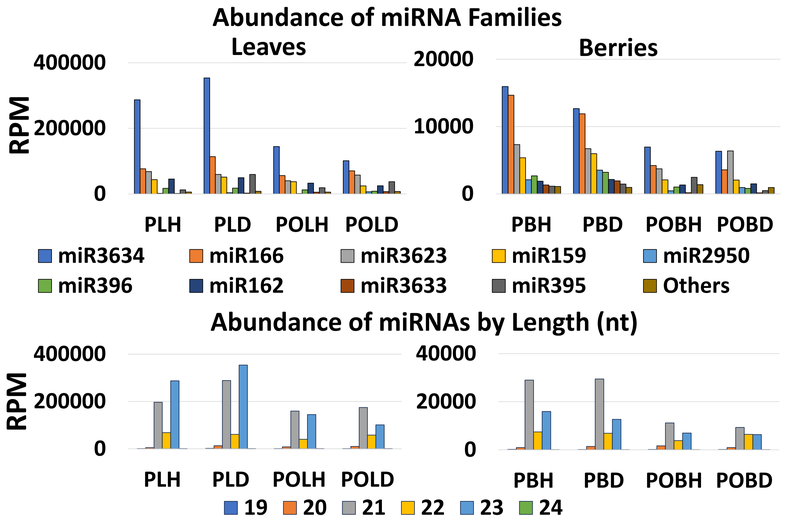


3) **Grapevine miRNAs and GRBV vsiRNAs were identified by mapping high-quality sRNA reads to a grapevine reference genome (12X.v2), the Genbank viral database, and other sequence databases.**

4) **Statistical analysis was performed using the R Statistical Coding Language (v. 3.6.3; R Core Team, 2020). Differential expression (DE) was analyzed using "edgeR" package from Bioconductor (Robinson et al., 2010) with a generalized likelihood ratio model (P-value < .05; Log(Fold Change) > |1.0|). Additional analyses were made using two-way ANOVAs and Tukey's post-hoc tests.**

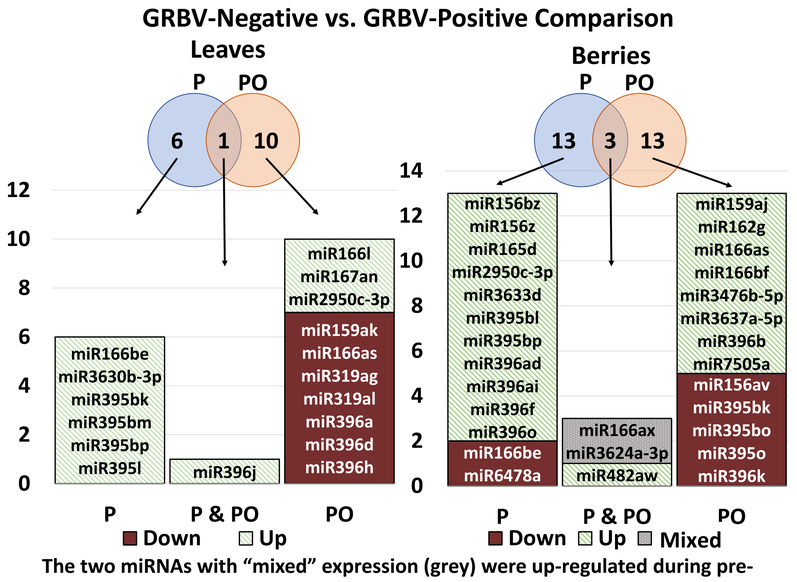
Results

- 140 distinct miRNA sequences belonging to 42 miRNA families were identified in both leaf and berry samples.

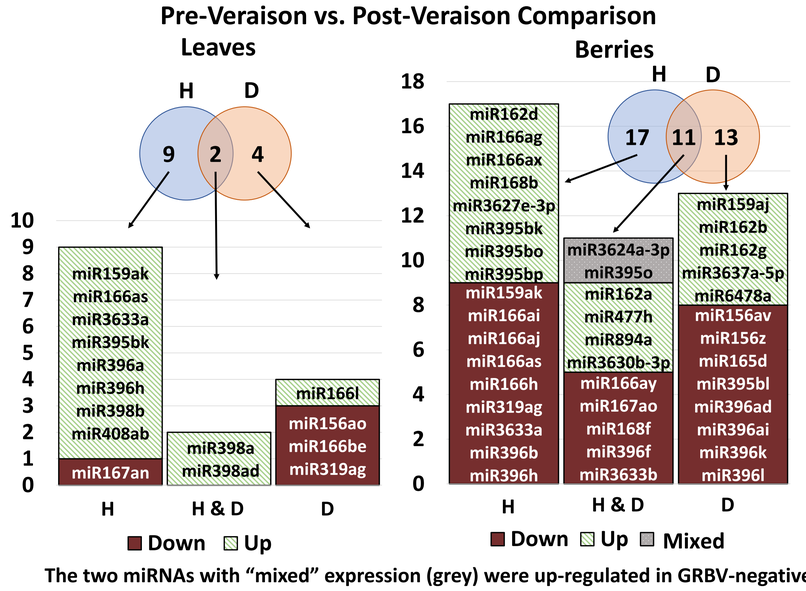


Abundances of sRNA reads mapped to miRNA sequences were normalized to RPM (reads per million mapped reads). The overabundance of the miR3634 family was a major contributor to the observed high abundance of 23nt reads in leaves.

- 41 miRNAs were differentially expressed in leaf and berry samples from GRBV-infected vines relative to uninfected vines.
- 50 miRNAs were differentially expressed between samples from pre- and post-veraison stages, independent of virus infection.

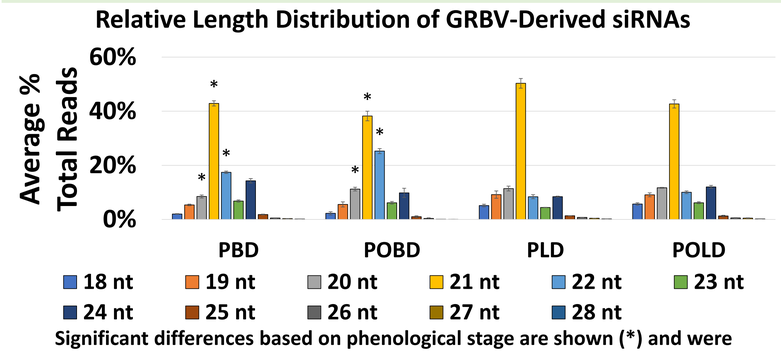


The two miRNAs with "mixed" expression (grey) were up-regulated during pre-veraison and down-regulated during post-veraison. Notably, miR159, miR166, miR395, miR396, and vvi-miR2950 families show differences in both tissue types.



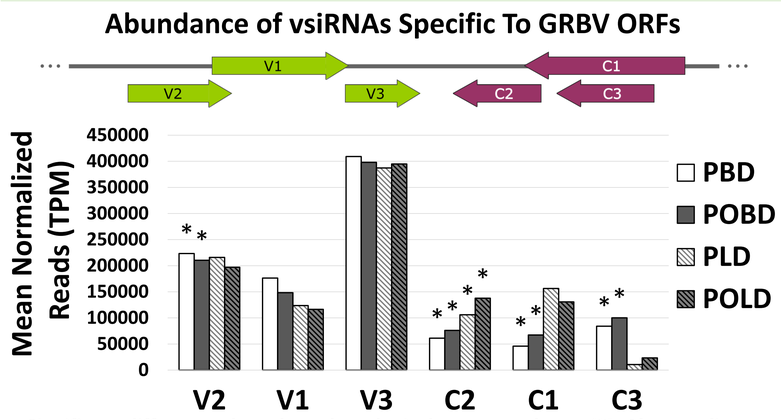
The two miRNAs with "mixed" expression (grey) were up-regulated in GRBV-negative samples and down-regulated in GRBV-positive samples.

- The presence of GRBV in symptomatic vines and its absence in asymptomatic vines were confirmed in both RNA and sRNA sequencing data.
- No other viruses were present in samples from either symptomatic and asymptomatic vines.



Significant differences based on phenological stage are shown (*) and were calculated based on normalized counts, not percentage.

- GRBV-derived vsiRNAs were mapped to six open reading frames (ORFs) encoded by the virus genome.
- There was significantly higher abundance of vsiRNAs specific to the V3 ORF compared to vsiRNAs mapped to other ORFs.



Significant differences based on phenological stage are shown (*). Above, a linear map of the single-stranded DNA genome of GRBV from 5' to 3' is shown (not drawn to scale). This map was created using SnapGene software (www.snapgene.com).

Summary and Conclusions

- miRNAs belonging to the miR166, miR2950, and miR3634 families were the most abundant in all samples at both pre- and post-veraison stages, independent of virus infection.
- More miRNAs were down-regulated in leaf and berry samples from GRBV-positive relative to GRBV-negative samples taken at post-veraison than at pre-veraison. This indicates a negative impact of virus infection on miRNAs during post-veraison when symptoms are apparent in Merlot grapevines. Such a dramatic negative impact on miRNAs was not observed during the asymptomatic pre-veraison stage.
- Both 21nt- and 23nt-long miRNAs were the most abundant in all samples from healthy and virus-infected vines. However, their relative abundance is higher in pre-veraison than post-veraison samples independent of infection.
- In contrast, 21nt-long vsiRNAs were the most abundant in leaf and berry samples from GRBV-infected vines during both pre- and post-veraison stages.
- The largest number of vsiRNAs were mapped to the V3 ORF relative to other ORFs. The functional significance of these differences is not yet determined.
- Overall results from this study aid in better understanding the sRNA dynamics in GRBV-grapevine interactions and in designing future studies to elucidate grapevine defenses against GRBV.

Acknowledgements

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- Illustrations and figures under Background and Materials and Methods were created with BioRender.com.