Elucidating the genetic makeup of *Grapevine leafroll-associated virus 3* for managing leafroll disease in Washington State vineyards

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INTRODUCTION

Grapevine leafroll disease (GLD) is the most economically destructive virus disease of wine grapes (*Vitis vinifera*), severely affecting vine health and fruit yield and quality. It continues to threaten the sustainability of premium wine grape production in Washington State. Grapevine leafroll-associated viruses (GLRaVs) are a group of viruses documented in GLD-affected vines. Among them, *Grapevine leafroll-associated virus 3* (GLRaV-3) is the most widespread and insidious in Washington State vineyards (Rayapati et al. 2008; Naidu et al. 2015). GLRaVs have an exceptionally complex genome organization. Studies on the genetic variability of GLRaV-3 across grapevine-growing regions in the United States and abroad have reported the existence of multiple genetic variants. Based on examination of critical virus-encoded genes, ten distinct GLRaV-3 genetic variant groups, named I through X, have been reported thus far (Naidu et al. 2015; Burger et al. 2017; Diaz-Lara et al. 2018; Thompson et al. 2018).

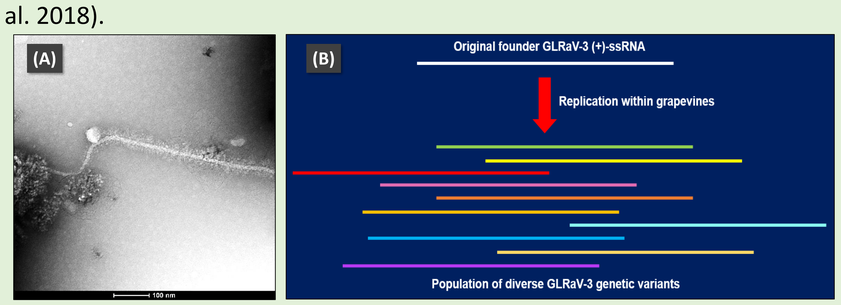
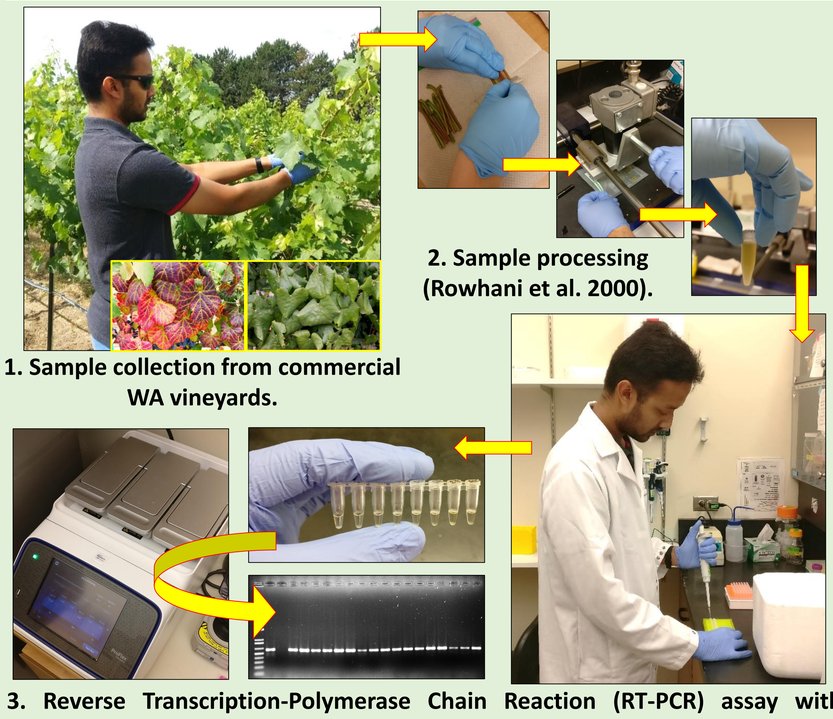


Fig. 1: (A) GLRaV-3 particle visualized under Transmission Electron Microscope (TEM), (B) Emergence of diverse GLRaV-3 genetic variants from a single founder GLRaV-3 genome molecule replicating within an infected host grapevine.

OBJECTIVE

The goal of this project was to explore the genetic diversity landscape of GLRaV-3 in Washington State vineyards, gain research-based insight into GLD epidemiology, and ultimately apply that knowledge to area-wide clean plant programs for managing grapevine leafroll disease in vineyards.

METHODOLOGY



1. Sample collection from commercial WA vineyards.

2. Sample processing (Rowhani et al. 2000).

3. Reverse Transcription-Polymerase Chain Reaction (RT-PCR) assay with Hsp70h-based primers (Donda et al. 2016).

4. Cloning and Sanger sequencing of RT-PCR amplicons.

5. Sequence alignment with globally reported GLRaV-3 sequences and phylogenetic analysis (MEGA7 software package).

RESULTS

Sampling details (2016 to 2018):

- Total samples collected : 1260
- Red-fruited cultivars sampled : 11
- White-fruited cultivars sampled : 11
- Juice grape cultivars sampled : 2
- Number of vineyards : 13
- GLRaV-3-positive samples : **1146**

Screening samples for the presence of GLRaV-3 by RT-PCR:

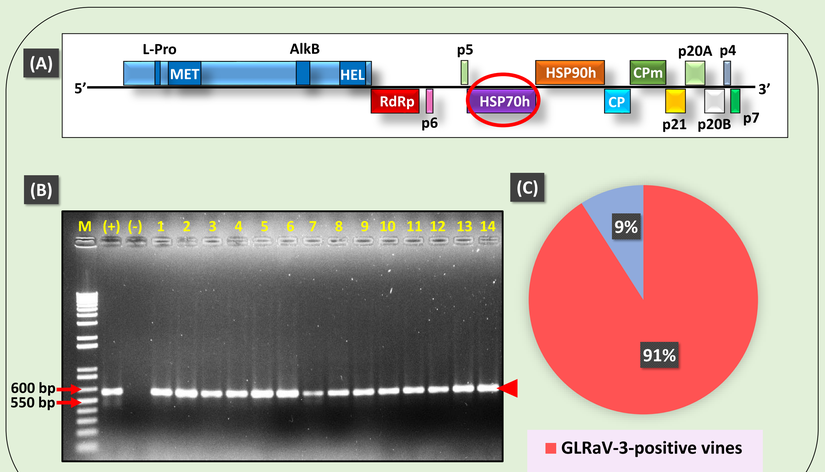


Fig. 2: (A) Genome organization of GLRaV-3 (adapted from Naidu et al. 2015). The red circle highlights the Hsp70h gene targeted for RT-PCR amplification with gene-specific primers, (B) Agarose gel electrophoresis image of the ~580 base pair (bp) RT-PCR amplicons (indicated by red arrow-head on the right) generated from the RT-PCR diagnostic assay. M: 1 Kb+ DNA molecular size marker, (+): GLRaV-3 positive control, (-): GLRaV-3 negative control, 1-14: samples tested, (C) Summary of RT-PCR results of the samples tested for GLRaV-3 in this study.

Overall, GLRaV-3 isolates belonging to five reported genetic variant groups were identified. 52% of the GLRaV-3 isolates identified in this study clustered with GLRaV-3 variant group I, 20% aligned with variant group III, 16% aligned with variant group VI, 5% aligned with variant group V, and 3% aligned with group II. This suggests that GLRaV-3 isolates belonging to variant group I are predominant compared to virus isolates belonging to other variant groups in Washington vineyards. In addition, 4% of the GLRaV-3 isolates did not align with any of the reported variant groups and appeared to be divergent (Fig. 4A).

Further analysis showed that majority (91%) of GLRaV-3-positive samples from individual vines contained sequences belonging to a single variant group and 9% of the samples were found to have sequences belonging to two or more variant groups of GLRaV-3 (Fig. 4B).

The partial Hsp70h gene sequences of GLRaV-3 isolates found in Washington vineyards were compared with corresponding virus sequences reported from other grapevine-growing regions worldwide to profile genetic diversity of the virus. Phylogenetic analysis of Hsp70h gene sequences showed that GLRaV-3 isolates from Washington State fell into 5 reported variant groups of GLRaV-3: groups I, II, III, V, and VI, and the majority of GLRaV-3 isolates belonged to variant group I (Fig. 3).

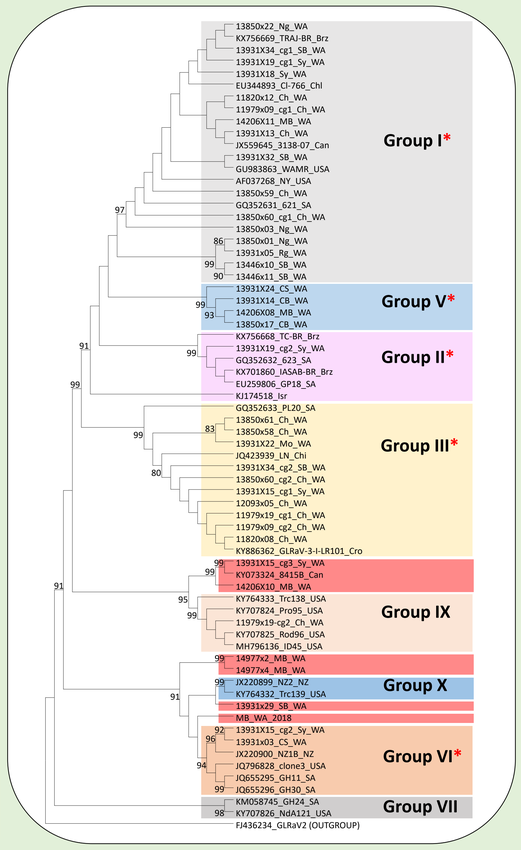


Fig. 3: Maximum likelihood phylogenetic tree of partial Hsp70h sequences (~580 bp) of representative GenBank-sourced GLRaV-3 isolates and corresponding GLRaV-3 sequences obtained in this study (adapted from Diaz-Lara et al. 2018). The bootstrap numbers at the branches are >80% confidence values derived from 1,000 replications of phylogenetic reconstruction of the tree. The partial Hsp70h sequence from GLRaV-2 was used as an outgroup. Horizontal branch length is proportional to genetic distance.
Red boxes indicate divergent groups of GLRaV-3 isolates identified in this study in Washington vineyards.
Red asterisks indicate the five reported GLRaV-3 variant groups which were also identified to be present in Washington vineyards from this study.

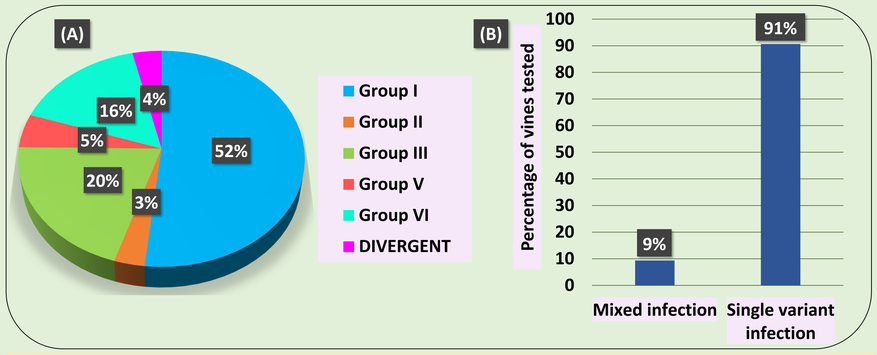


Fig. 4: (A) Proportion of GLRaV-3 isolates belonging to different reported genetic variant groups across Washington State vineyards, (B) Proportion of GLRaV-3-infected grapevines harboring a single GLRaV-3 variant group or a mixture of variant groups.

CONCLUSIONS

This is the first comprehensive report demonstrating the presence of several distinct genetic variant groups of GLRaV-3 in Washington State vineyards.

GLRaV-3 isolates belonging to five reported variant groups were identified in this study. Isolates belonging to variant group I were predominant compared to isolates belonging to other reported GLRaV-3 variant groups.

Although majority of the GLRaV-3 isolate sequences aligned with reported variant groups, ~4% of the isolates were 'divergent' and did not align with the established classification system of GLRaV-3 genetic variants. Occurrence of these distinct variants warrant further research to understand their overall diversity and spread across vineyards in the state.

The data from this study will be used in improving the currently used laboratory-based diagnostic methods for detecting all variants of GLRaV-3 in planting materials. Therefore, knowledge of the genetic diversity of GLRaV-3 will provide opportunities to resolve the complex epidemiology of GLD for implementing disease management strategies and improving grapevine planting material supply chain for healthy vineyards.

ACKNOWLEDGEMENTS

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