The Spread of Genetic Variants of Grapevine Leafroll-Associated Virus 3 by Mealybug Vectors

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INTRODUCTION

The Pacific Northwest (PNW) is one of the leading producers of premium wine grapes (*Vitis vinifera*). Viral diseases are one of the major constraints to vineyard health and productivity affecting economic returns to growers in the region. Among the viral diseases, grapevine leafroll (GLD) is by far the most prevalent and economically devastating disease. *Grapevine leafroll-associated virus 3* (GLRaV-3), the main etiological agent of GLD, is ubiquitous in the PNW. The virus is known to be present as genetically distinct variants in different wine regions around the world. Our studies in Washington vineyards revealed the presence of unusually diverse and novel genetic variants of GLRaV-3 compared to reports from other wine regions. Preventing the spread of genetic variants of GLRaV-3 is critical for certification and clean plant programs and managing GLD in vineyards.

Since genetic diversity of GLRaV-3 has practical implications, such as symptoms, spread by mealybug vectors, diagnosis, and disease management, this project was initiated for a better understanding of genetic variability of the virus in commercial vineyards. Building on the results from year 1, studies were conducted during this year to examine the role of grape mealybugs (*Pseudococcus maritimus*) in the spread of genetic variants of GLRaV-3 to fill knowledge gaps in the epidemiology of leafroll disease. The results showed that natural populations of mealybug nymphs, the actively feeding and easily dispersible life stages of mealybugs, dwelling on infected vines in commercial vineyards can acquire and transmit acquire and transmit a single variant of GLRaV-3 or simultaneously acquire and transmit distinct variants of the virus.

MATERIALS AND METHODS

A commercial vineyard planted with cv. Malbec and known to be infected with leafroll disease was selected for this study. During 2022 season, 1st and 2nd instar mealybug nymphs were collected at weekly intervals in May from five individual vines known to be infected with distinct variants of GLRaV-3 based on high-throughput sequencing.

We optimized methods for the detection of GLRaV-3 in individual nymphs by RT-qPCR using virus-specific primers. Concurrently, we collected 1st/2nd instar nymphs from GLRaV-3-infected source vines and released them on young grapevine seedlings (cv. Chardonnay) @1 insect/plant. Plants were covered with perforated plastic bags and maintained under greenhouse conditions to allow transmission of the virus during feeding.

Three days post-inoculation (DPI), seedlings were sprayed with a contact insecticide to kill mealybugs. Leaf samples were collected from Chardonnay seedlings inoculated with individual mealybug nymphs at approximately 30- and 90-DPI. Extracts made from each sample was tested by RT-qPCR for the presence of GLRaV-3. The approximately 144-base pair (bp) DNA fragments amplified in RT-qPCR were cloned and sequenced. The nucleotide sequences were compared with GLRaV-3 sequences available in GenBank to identify genetic variants present in virus-positive plants.

RESULTS

Detection of GLRaV-3 in individual mealybug nymphs.

We have collected mealybug nymphs from source vines during May 2022 and tested by RT-qPCR for the presence of GLRaV-3 (Fig. 1). In RT-qPCR, the expected 144 bp DNA fragment was amplified in 161 of the 363 nymphs tested, indicating that 44.35% of nymphs have acquired the virus. The PCR product obtained from 13 mealybug nymphs was cloned and sequenced. The derived nucleotide sequences were compared with GLRaV-3 genome sequences available in GenBank. The sequence data confirmed RT-qPCR results for the presence of GLRaV-3 in individual mealybug nymphs. Further analysis of nucleotide sequences indicated that 11 of the 13 virus-positive nymphs had multiple variants of GLRaV-3, while the other two nymphs had sequences specific to a single variant. These results showed that individual mealybug nymphs dwelling on infected vines in vineyards can acquire either a single variant or multiple variants of GLRaV-3.

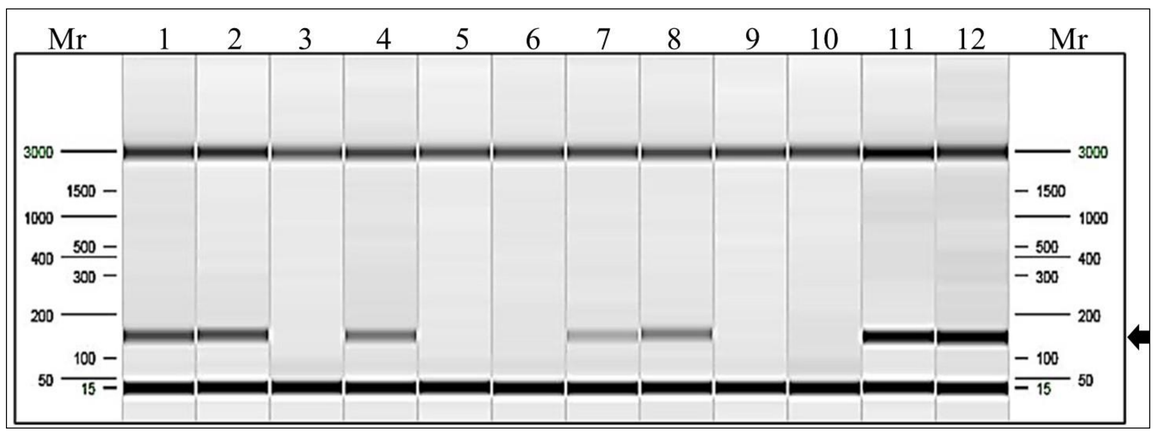

Fig. 1. Detection of GLRaV-3 in mealybugs**.** Individual nymphs (1st and 2nd instar nymphs) collected from virus-infected vines were tested by RT-qPCR using primers specific to GLRaV-3. The figure shows PCR products resolved in 12 individual wells of a 96-well plate using QIAxcel Advanced System. Virus-specific DNA fragment of about 144 bp amplified from individual mealybugs is shown by arrow on the right side. Among the eight nymphs tested (Lanes 1 to 8), virus-specific DNA was amplified from five nymphs (Lane 1, 2, 4, 7, and 8). Lane 'Mr' shows the size of DNA molecular weight markers used to estimate the size of virus-specific DNA fragments amplified from nymphs. Lanes 9 and 10 are negative controls and lanes 11 and 12 are positive controls to validate RT-qPCR assay.

Transmission of GLRaV-3 using mealybug nymphs collected from virus-infected vines.

A total of 261 Chardonnay seedlings were inoculated with individual mealybug nymphs collected from virus-infected source vines. These seedlings were tested by RT-qPCR at approximately 30- and 90-DPI for the presence of GLRaV-3. The combined results showed 6.5% (17/261) of mealybug-inoculated plants tested positive for GLRaV-3 (Table 1). RT-qPCR products (144 bp DNA fragment) from virus-positive Chardonnay plants were cloned into TOPO TA cloning vector and plasmid DNA isolated from eight independent colonies derived from each amplicon was subjected to Sanger sequencing. A comparison of nucleotide sequences obtained from this study with GLRaV-3 sequences available in GenBank further confirmed the presence of virus in mealybug-inoculated plants. In addition, the sequence analysis indicated that 47% (8/17) of virus-positive plants had a single variant of GLRaV-3 and 53% (9/17) of virus-positive plants contained multiple variants of the virus.

Table 1. Transmission of GLRaV-3 by individual mealybug nymphs.

Inoculation date	Seedlings inoculated	GLRaV-3 positive seedlings (30 DPI)	GLRaV-3 positive seedlings (90 DPI)
5/09/2022	72	3/72 (4.17 %)	3/72 (4.17 %)
5/16/2022	72	1/72 (1.39 %)	5/72 (6.9 %)
5/20/2022	72	5/72 (6.94 %)	6/72 (8.3 %)
5/31/2022	45	3/45 (6.67 %)	3/45 (6.67 %)
Total	261	12/261 (4.60 %)	17/261 (6.5%)

SUMMARY/CONCLUSIONS

Overall, the above results confirm that natural populations of mealybug nymphs dwelling on GLRaV-3-infected vines in vineyards can transmit the virus. Further, individual mealybug nymphs can acquire and transmit a single variant or simultaneously acquire and transmit distinct variants of the virus. We are continuing this study during 2023 season to build comprehensive data on the role of mealybug vectors in spreading genetic variants of GLRaV-3 in vineyards.

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