Molecular Biology of Grapevine leafroll-associated viruses

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WineVit 2021

Grapevine leafroll disease (GLD)

Naidu et al., 2014. *Plant Disease*. 98(9): 1172-1185.

Symptoms in cv. Malbec

Reduced vine vigor

Reduced berry yield and quality

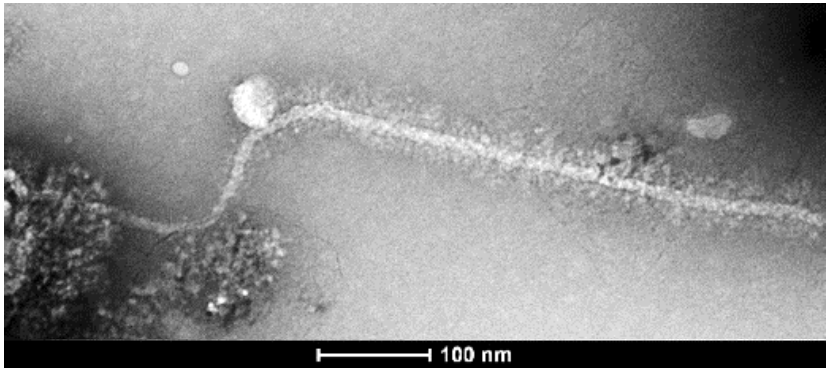
Symptoms in cv. Chardonnay

Grapevine leafroll-associated viruses (GLRaVs)

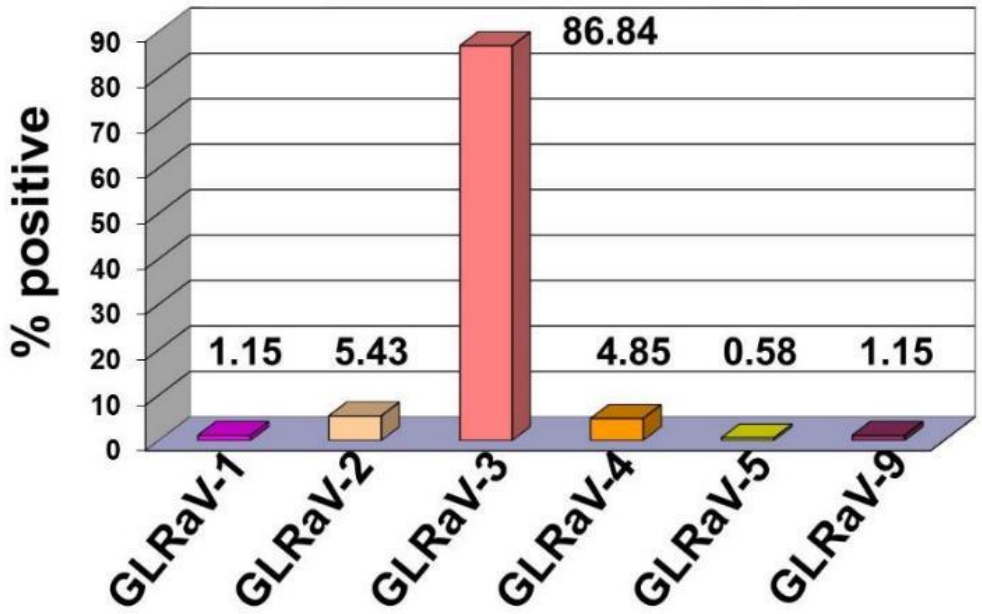
- Six species: GLRaV-1, GLRaV-2, **GLRaV-3**, GLRaV-4, GLRaV-7, and GLRaV-13.

- GLRaV-1, GLRaV-2, GLRaV-3, and GLRaV-4 reported from WA vineyards.

- GLRaV-3 predominant compared to other GLRaVs in WA vineyards.



TEM image of a GLRaV-3 virus particle



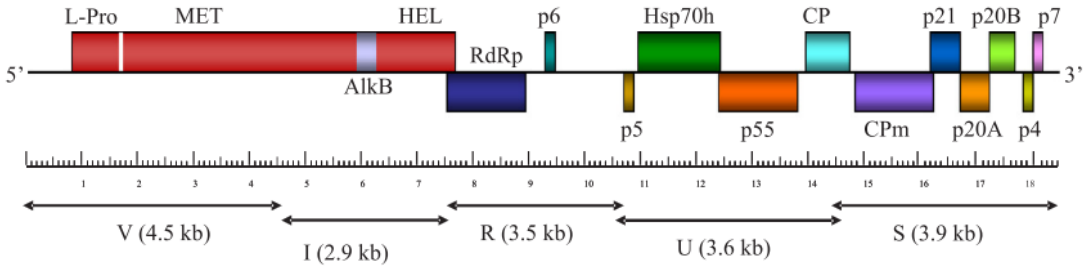
Naidu, 2011. *Viticulture and Enology Extension News*. Fall 2011: 6-7.

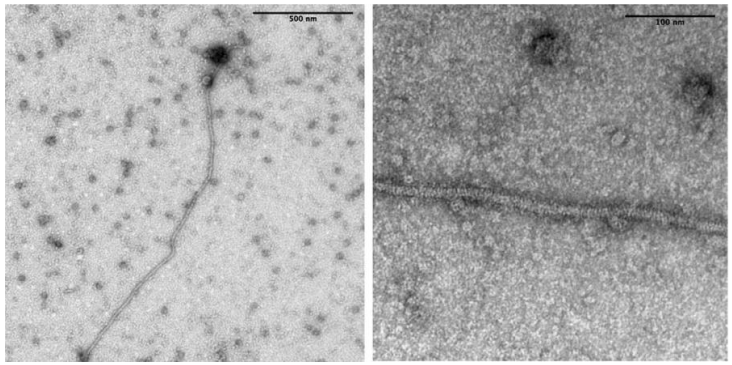
GLD Epidemiology

- Limited understanding.
- Elucidating the role of different GLRaVs in disease development and impact on grapevines.
- Innovative molecular approaches needed.

GLRaV-3

- Full-length complementary DNA (cDNA) clones constructed.
- Replicate and form virus particles in *Nicotiana benthamiana*.



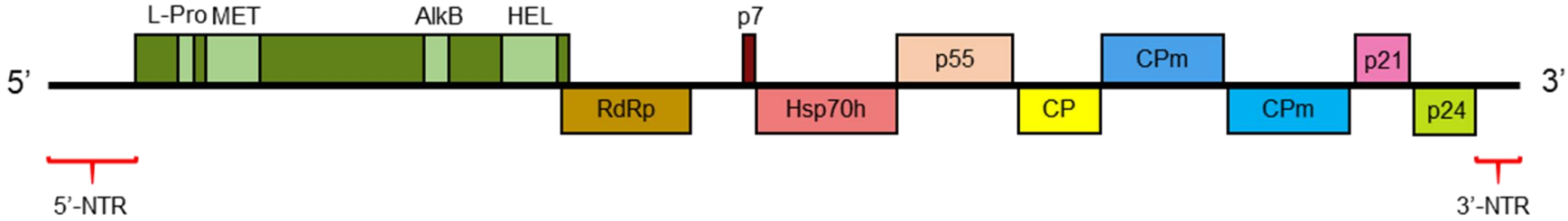


Jarugula et al., 2018. *Virology*, *523* (89-99).

Applications – gene function, gene expression, cultivar-specific effects, etc.

GLRaV-1

- Second most prevalent after GLRaV-3.
- Single and mixed infections.
- Similar to GLRaV-3 – symptomatology, virus particle structure, genome organization.



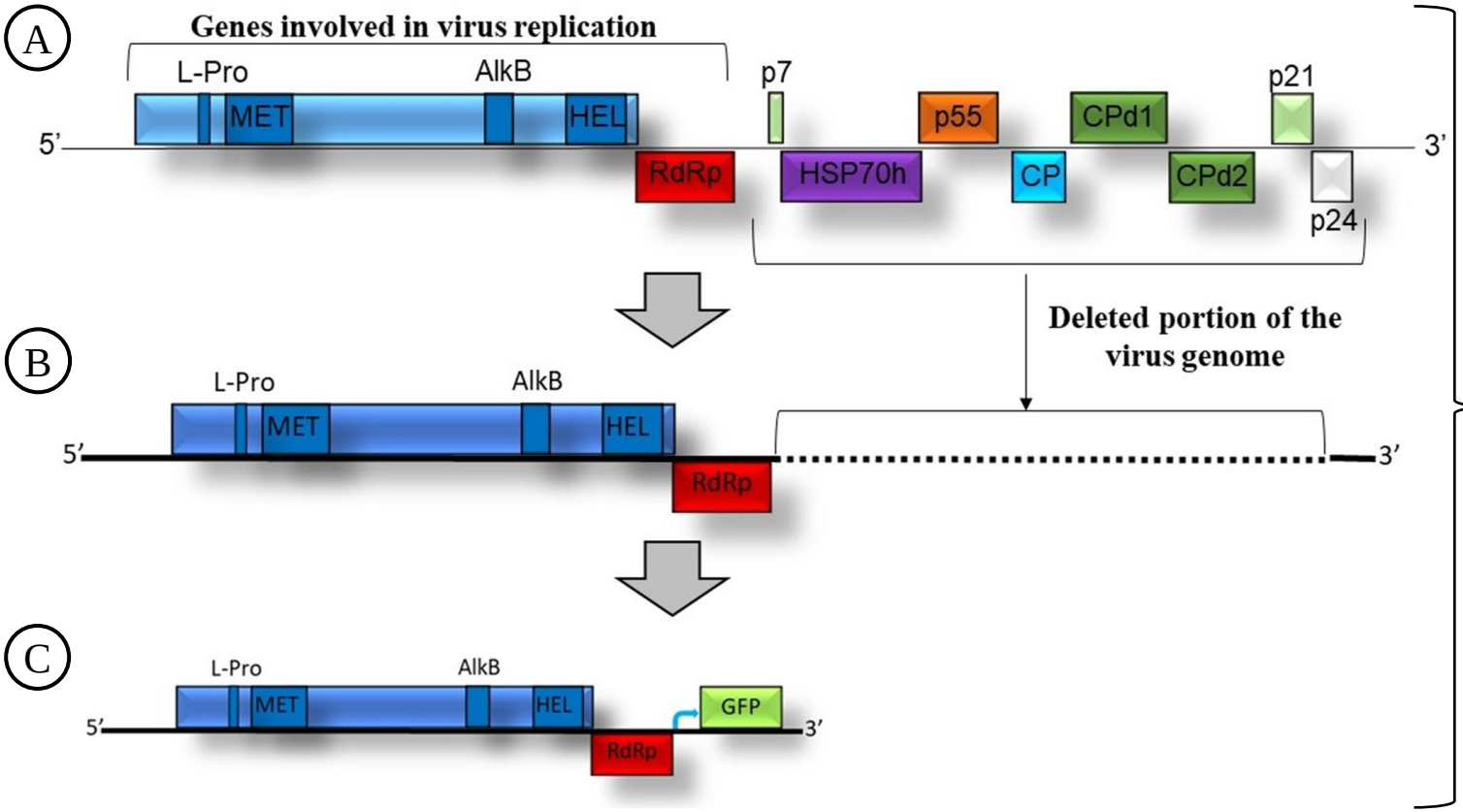
GLRaV-1 genome organization
(adapted from Naidu et al., 2015 and Dolja et al., 2017)

A molecular approach similar to GLRaV-3 was desired for comparative studies with both viruses.

Objective

To build a "mini-genome" cDNA clone of GLRaV-1 and use it as a simplified experimental system to examine the role of the 5' non-translated region (5'-NTR) in viral replication in *N. benthamiana*.

Methodology



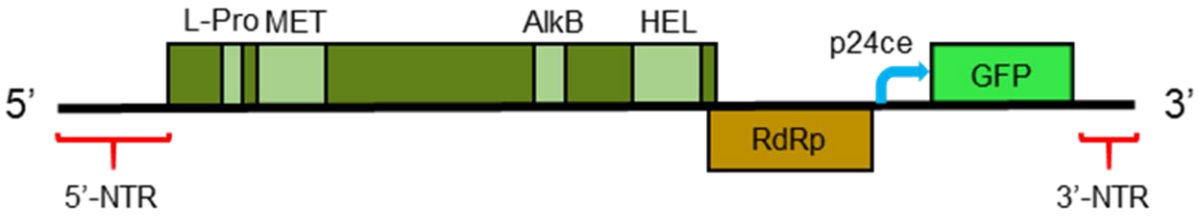
GLRaV-1 (isolate WA-CH) mini-genome construction

D

GLRaV-1 mini-genome functional validation by agro-coinfiltrations

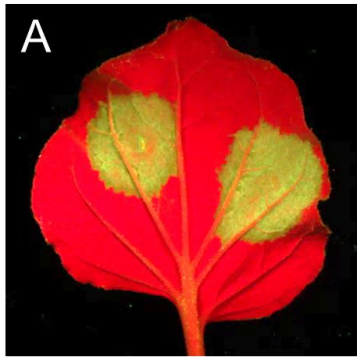
Results

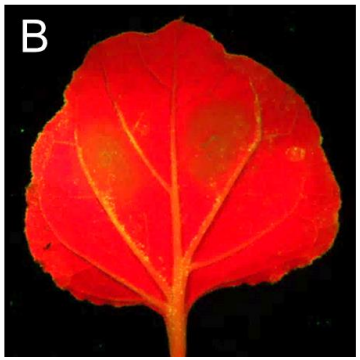
1: Validation of GLRaV-1 mini-genome functionality.

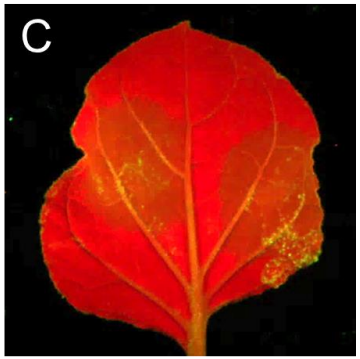


Agro-coinfiltration

Expose to UV light at 5 days post-infiltration (dpi)





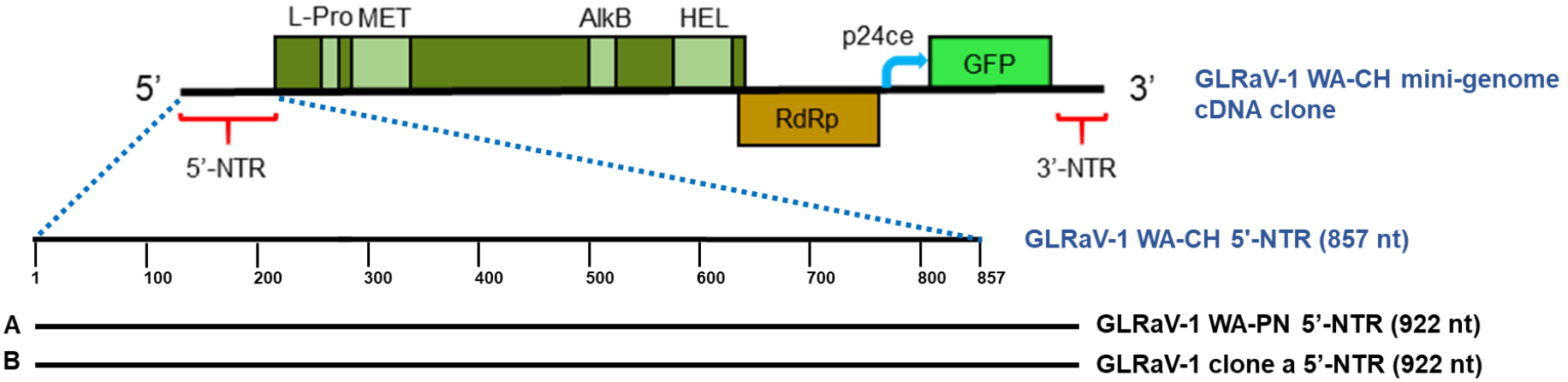


A: Leaf infiltrated with the GLRaV-1 mini-genome + RNA silencing suppressor
B: Leaf infiltrated with the GLRaV-1 mini-genome alone
C: Leaf infiltrated with empty binary vector (not carrying the mini-genome)

This shows that the GLRaV-1 mini-genome was functional!

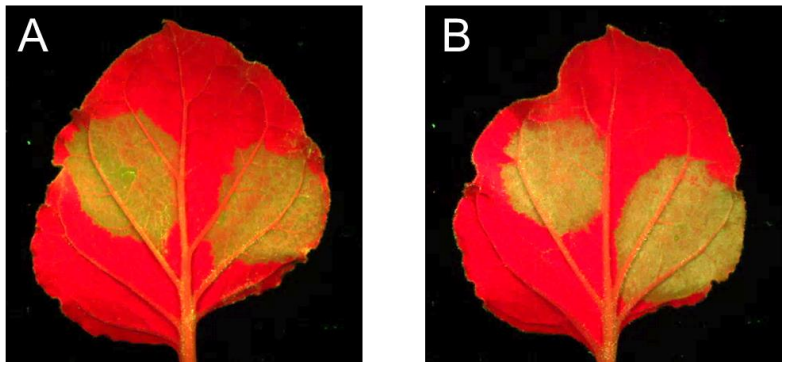
Results

2: The 5’-NTR sequence can be exchanged between GLRaV-1 genetic variants.



Agro-coinfiltration

Expose to UV light at 5 days post-infiltration (dpi)

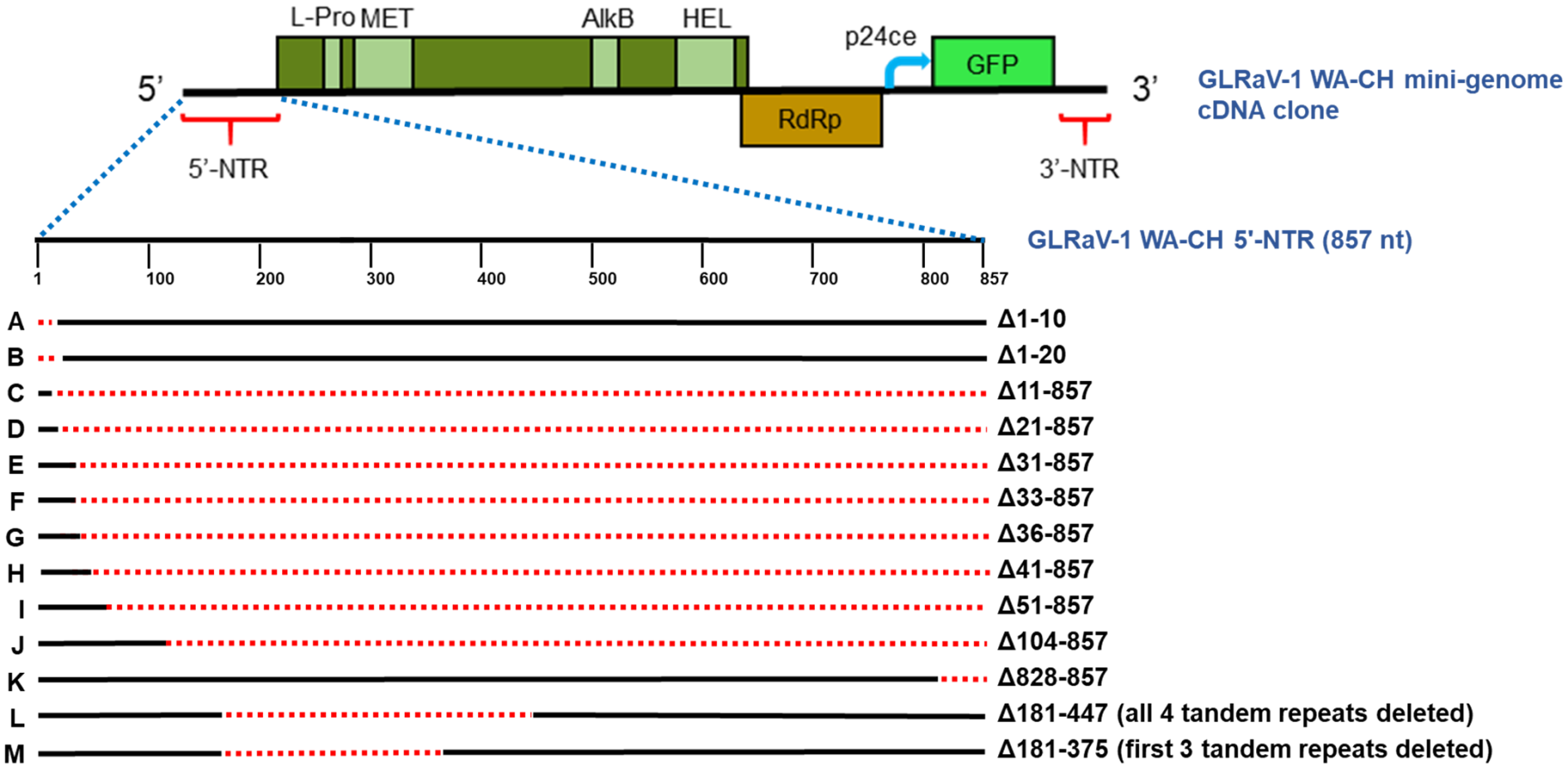


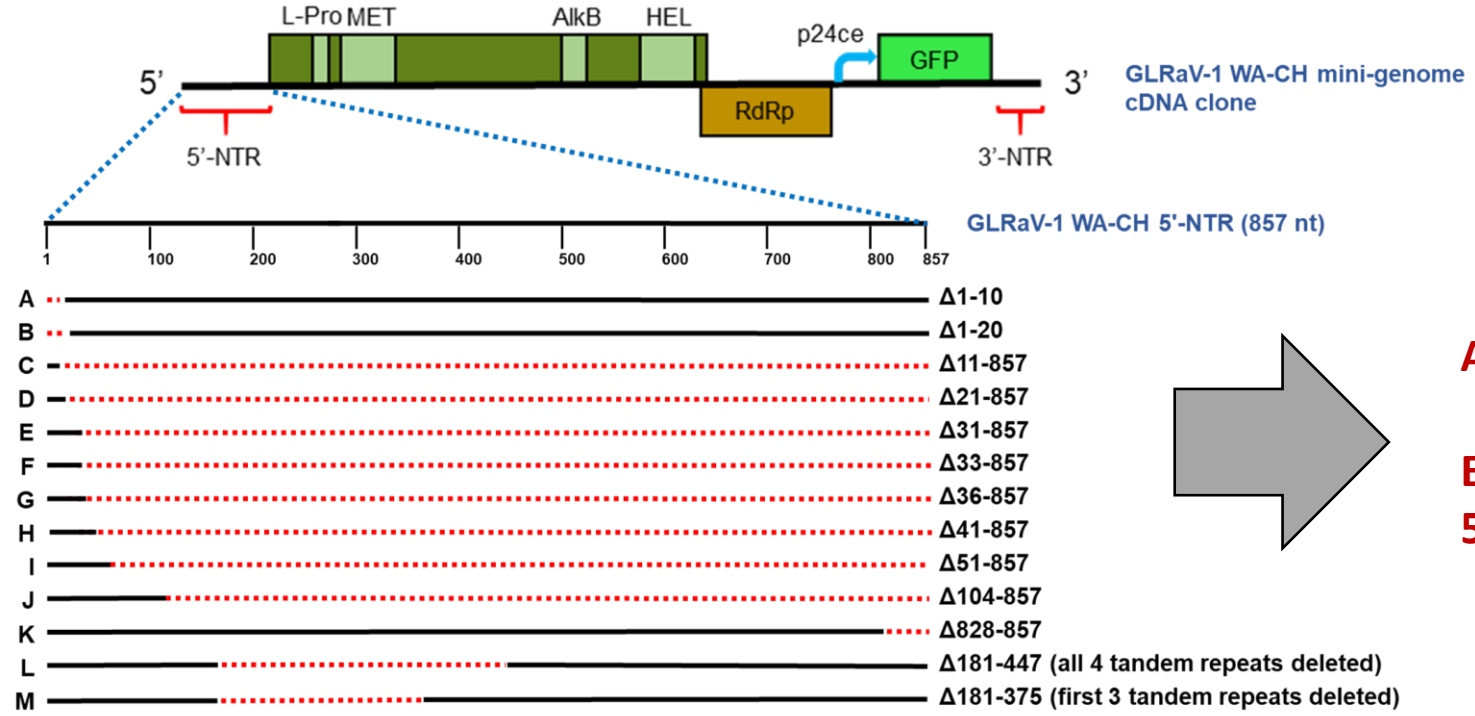
A: Leaf infiltrated with the GLRaV-1 isolate ‘WA-PN’

B: Leaf infiltrated with the GLRaV-1 isolate ‘clone a’

Results

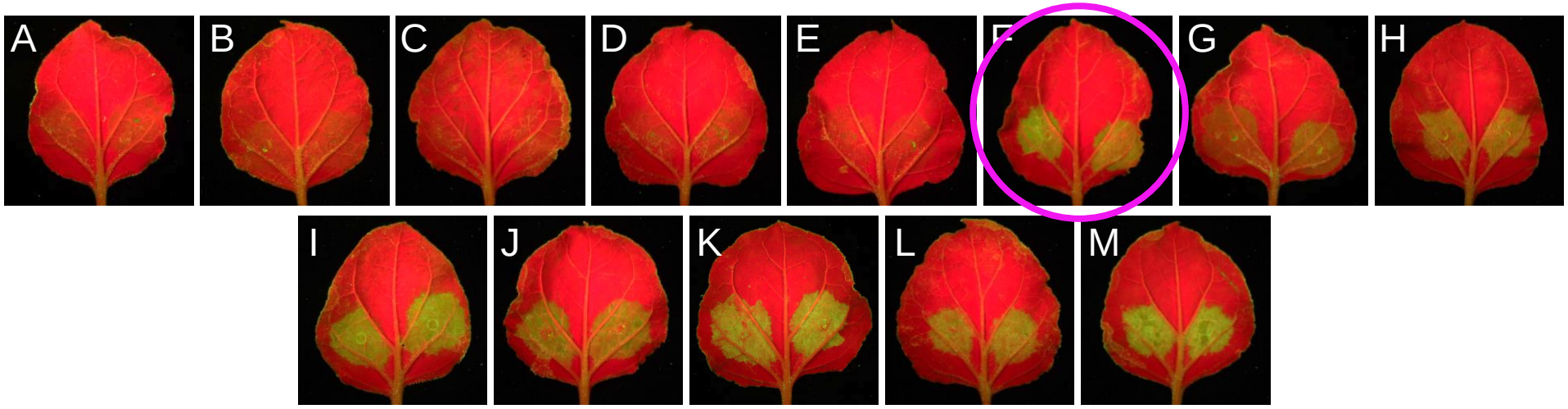
3: The first 32 nucleotides at the 5’-terminus of GLRaV-1 genome are essential for mini-genome replication.





Agro-coinfiltration

Expose to UV light at 5 dpi



Conclusions

- GLRaV-1 (isolate WA-CH) mini-genome cDNA clone successfully constructed, and functionality validated.
- 5′-NTR of GLRaV-1 of one isolate can be exchanged with 5′-NTRs from other genetic variants – WA-PN and clone a.
- First 32 nt of the 5′-NTR are adequate for GLRaV-1 replication. Any deletions within this region resulted in no GFP expression.

Acknowledgements

Funding sources:

1. Washington State University
2. Auction of Washington Wines
3. Washington Wine Commission

Check out my WineVit 2021 poster too!

Molecular Biology of Grapevine leafroll-associated viruses

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INTRODUCTION

Grapevine leafroll disease (GLD) continues to be the most economically important viral disease in Washington vineyards, besides in other grapevine-growing regions worldwide. Currently six distinct species of Grapevine leafroll-associated viruses (GLRaVs), designated GLRaV-1, -2, -3, -4, -7, and -13 have been identified in grapevines (Naidu et al. 2015, Dolja et al. 2017).

Due to the complex biology of GLD, molecular approaches are needed to better understand the role of individual GLRaVs in different aspects of GLD for developing disease control strategies. Towards this objective, we have constructed full-length complementary DNA (cDNA) clones of GLRaV-3 and demonstrated that these cDNA clones can faithfully replicate and form virus particles when introduced via Agrobacterium-mediated infiltration into leaves of an experimental host plant, *Nicotiana benthamiana* (Jarugula et al. 2018). We are currently adopting a similar strategy to build full-length, infectious cDNA clones for GLRaV-1, the second most prevalent virus globally after GLRaV-3, for studying comparative molecular biology of GLRaVs.

Previously, we have determined the complete genome sequence of GLRaV-1 isolates from Washington vineyards (Donda et al. 2017). The virus has a large genome ranging in size between 18,731-18,946 nucleotides (nt) with an unusually long 5' non-translated region (5'-NTR) ranging in length between 857-922 nt (Fig.1).

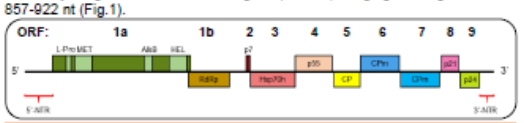


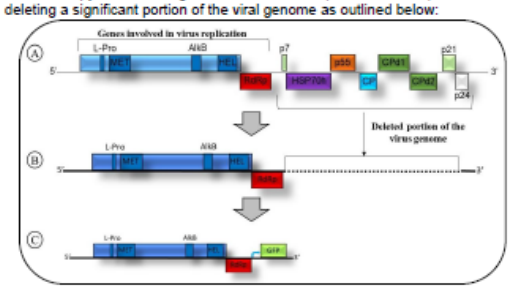
Fig. 1: Genome organization of GLRaV-1 illustrating 9 open reading frames (ORFs) or genes (shown as colored boxes) encoded by the virus. ORFs 1a and 1b encode replication-associated proteins made up of domains for leader proteinase (L-Pro), AlkB, methyltransferase (MET), RNA helicase (HEL), and RNA dependent RNA polymerase (RdRp). ORFs 2-9 encode, respectively, a 7-kDa protein (p7), a Hsp70-homologue (Hsp70h), a 55-kDa protein (p55), the coat protein (CP), two divergent copies of the CP (CPm), a 21-kDa protein (p21), and a 24-kDa protein (p24). The non-translated sequences on either end of the genome are designated as 5'-NTR (left end) and 3'-NTR (right end). Adapted from Naidu et al. 2015 and Dolja et al. 2017.

OBJECTIVE

In this study, a mini-genome cDNA clone of GLRaV-1 was built as a first step to study molecular biology of the virus. This mini-genome cDNA clone was subsequently used as a simplified experimental system to examine the role of the 5' non-translated region (5'-NTR) in viral replication in *N. benthamiana*.

METHODOLOGY

A cDNA copy of the mini-genome of GLRaV-1 (isolate WA-CH) was built by deleting a significant portion of the viral genome as outlined below:



(A) Schematic representation of GLRaV-1 (isolate WA-CH) genome of 18,731 nt in size. The replication gene module, consisting of genes critical for virus replication, is indicated (see Fig. 1 above for details).

(B) All genes of GLRaV-1 between the replication gene module and the non-translated region at the 3'-terminus were deleted (shown with a broken line) for building the mini-genome cDNA clone.

(C) A Green Fluorescent Protein (GFP) gene was inserted between the RdRp gene and 3'-NTR as a marker for studying replication function of the mini-genome. The final construct of the mini-genome is significantly smaller than the original size of the GLRaV-1 genome and contains the 5'-NTR, replication gene module, GFP gene, and the 3'-NTR.

RESULTS

I. Validation of GLRaV-1 mini-genome functionality.

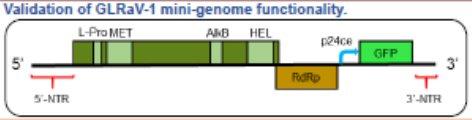


Fig. 2: Schematic representation of the GLRaV-1 isolate WA-CH mini-genome outlining the genes present (5'-NTR, replication gene module, GFP, 3'-NTR). 'p24ce' is a controller element sequence from p24 gene of GLRaV-1 (see Fig. 1). This controller element sequence helps to drive the expression of GFP by the viral replication genes in *N. benthamiana* leaves.

Fig. 3: Images of *N. benthamiana* leaves. Patches of green florescence due to the expression of GFP can be observed when leaves were exposed to UV light at 5 days post-infiltration (dpi). Expression of GFP seen as green patches indicates functionality of the mini-genome in *N. benthamiana*. A: Leaf infiltrated with the GLRaV-1 mini-genome and RNA silencing suppressor together; B: Leaf infiltrated with the GLRaV-1 mini-genome alone; C: Leaf infiltrated with empty binary vector (not carrying the mini-genome construct).

The above results show that we have successfully built a functional mini-genome of GLRaV-1.

II. The 5'-NTR sequence can be exchanged between GLRaV-1 genetic variants.

Since GLRaV-1 occurs as genetically distinct variants with variable sizes of the 5'-NTR, we asked the question whether this region can be exchanged between genetically distinct variants of the virus. As shown below, the mini-genome of GLRaV-1 isolate WA-CH can remain functional (Fig. 4) when its 5'-NTR sequence was replaced with the corresponding sequence from isolate 'WA-PN' and isolate 'clone a'.

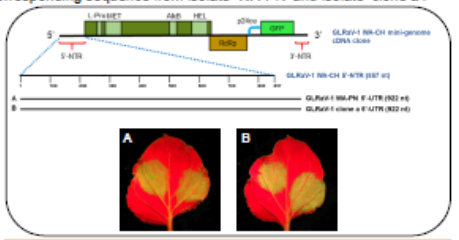


Fig. 4: 5'-NTR sequence in the mini-genome of GLRaV-1 isolate WA-CH was replaced with corresponding 5'-NTR sequences from virus variants 'WA-PN' and 'clone a'. The size of the 5'-NTR sequence of these two variants are shown as solid lines. Leaves of *N. benthamiana* agro-coinfiltrated with 'WA-PN' (A) and 'clone a' (B) showed GFP expression when exposed to UV light at 5 dpi.

III. The first 32 nucleotides at the 5'-terminus of GLRaV-1 genome are essential for mini-genome replication.

The mini-genome was subsequently used to examine the role of 5'-NTR sequences in virus RNA replication. Testing mini-replicon clones with different portions of the 5'-NTR deleted showed that the first 32 nucleotides at the 5'-terminus of the non-translated region are sufficient for replication and GFP expression in *N. benthamiana* leaves (Fig. 5).

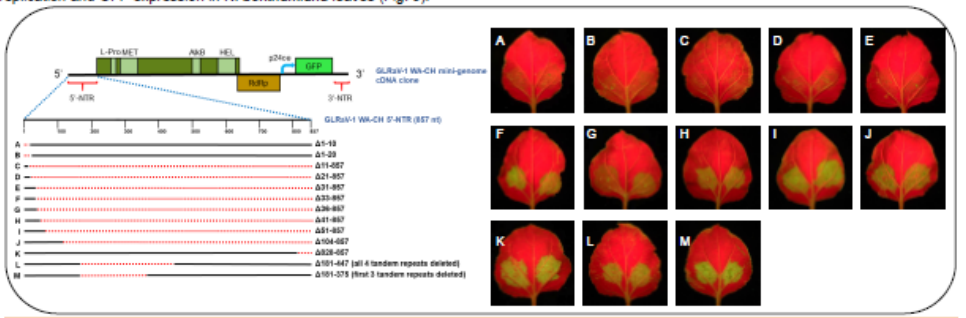


Fig. 5: The effect of sequence deletions in the 5'-NTR on replication of the GLRaV-1 mini-genome. The diagram above on the left-side shows schematic representation of GLRaV-1 mini-genome constructs with different portions of the 5'-NTR deleted. The 'Δ' symbol represents coordinates of the deleted portion and broken line represents the deleted portion in each construct. Pictures **A** to **M** above on the right-side show *N. benthamiana* leaves agro-coinfiltrated with the corresponding GLRaV-1 mini-genome constructs with different portions of the 5'-NTR sequence deleted. Pictures taken at 5 dpi under UV light.

CONCLUSIONS

Based on our work thus far, we conclude that:

- The GLRaV-1 (isolate WA-CH) mini-genome cDNA clone is able to successfully replicate in *N. benthamiana* leaves as shown by the expression of GFP fluorescence in infiltrated leaves (Fig. 3).
- In this study, we observed that the 5'-NTR of GLRaV-1 isolate WA-CH can be replaced with corresponding 5'-NTRs from isolates 'WA-PN' and 'clone a', without affecting functionality of the mini-genome (Fig. 4). This result shows that the 5'-NTR of GLRaV-1 of one isolate can be exchanged with 5'-NTRs from other variants of the virus.
- Our studies also indicate that the first 32 nt of the 5'-NTR are adequate for GLRaV-1 replication (Fig. 5). Any deletions within this 32 nt region resulted in no GFP expression, suggesting that the first 32 nt at the 5'-terminus of the virus genome could play a critical role in virus replication.

These results will help to design future experiments for studying comparative replication strategies between GLRaV-1 and -3.

ACKNOWLEDGEMENTS

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REFERENCES

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- Jarugula, S., Gowda, S., Dawson, W.O. and Naidu, R.A., 2018. Development of infectious cDNA clones of Grapevine leafroll-associated virus 3 and analyses of the 5' non-translated region for replication and virion formation. *Virology*, *523*, pp.89-99.
- Naidu, R.A., Maree, H.J. and Burger, J.T., 2015. Grapevine leafroll disease and associated viruses: A unique pathosystem. *Annual Review of Phytopathology*, *53*, pp.613-634.


Thank you!