Development of a GMO-free RNA Interference Approach to mitigate the negative effects of Red Blotch Disease on Grapevine

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Introduction

Red Blotch Disease is a grapevine viral disease that was first observed in California in 2008 and that in the last decade has spread across the U.S.. The etiological agent of Red Blotch is a monopartite single-stranded circular DNA *geminivirus*, termed as **Grapevine Red Blotch Virus (GRBV)**.
Infected plants present symptoms on both leaves, which develop red blotches or chlorosis, and berries that are characterized by a delayed ripening and an alteration of metabolites accumulation.
During the early phases of a viral infection the plant activates one defense mechanism known as RNAi.

Source: Impact of Grapevine Red Blotch Disease on Grape Composition of Vitis vinifera Cabernet Sauvignon, Merlot, and Chardonnay (R. C. Girardello, et al. 2019)

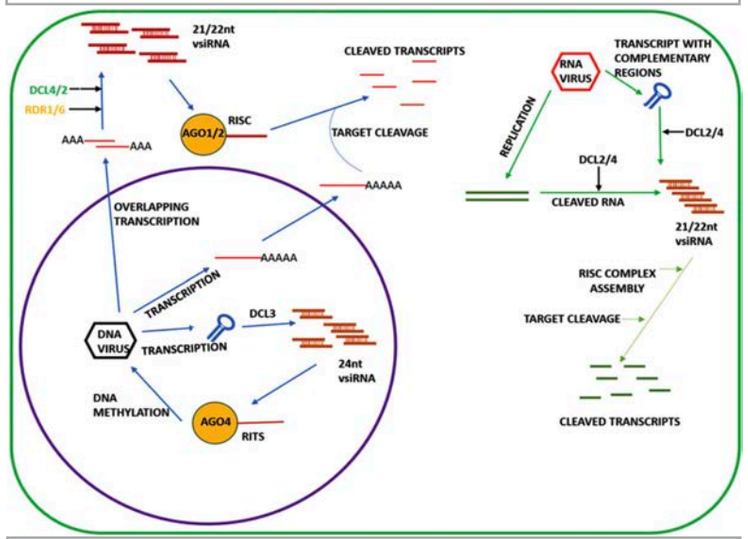


Figure 1 - small-RNAs-mediated plant-virus interaction. In DNA viruses (as GRBV), overlapping transcription and transcripts with self-complementary regions are sources of double-stranded-RNAs (dsRNAs) that can act as substrates for Dicer Like Enzymes (DCLs). (A. Prasad *et al.*, 2019)

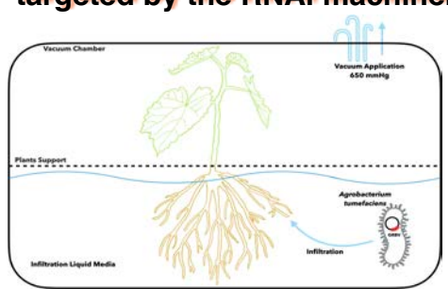
In **DNA Viruses:**

- **Overlapping Transcripts** are recognized by **DCL4/2** to produce 21/22 nt vsiRNAs, which can guide the **RISC** complex across the viral transcriptome for **transcript cleavage** (**Post-Transcriptional Gene Silencing**).
- **Self-Complementary Transcripts** are recognized by **DCL3** to produce 24 nt vsiRNAs. These dsRNAs act as a guide for the **RITS complex** across the viral genome, for the generation of **Cytosine Methylation** (**Transcriptional Gene Silencing**).

Objectives

AIM 1

Identification of viral transcripts and genomic regions targeted by the RNAi machinery.



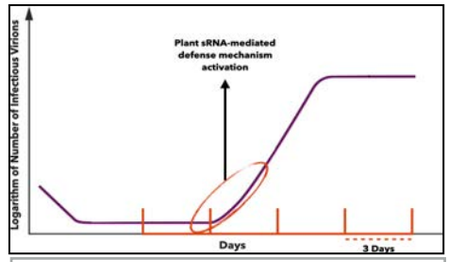
Vacuum-Assisted Agro-Infiltration

1

2

RT-PCR

3



Detection of Viral Early Phase Infection

Hotspots Identification

4

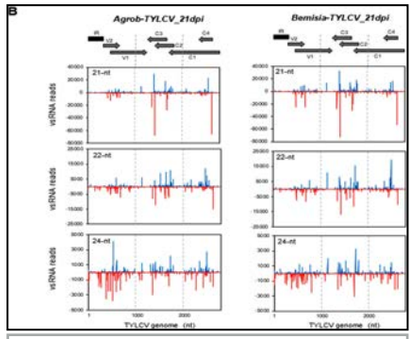


Figure 2 - Maps of geminivirus-derived siRNAs in tomato plants between 7 and 21 days-post-inoculation (dpi). (A. Piedra-Aguilera *et al.*, 2019)

AIM 2

Validation of the processing and the silencing effect of the identified dsRNAs.

AIM 3

Evaluation of the long-term protection of Clay Nanoparticles on dsRNAs for the development of a Spray-Induced Gene Silencing (SIGS) system.

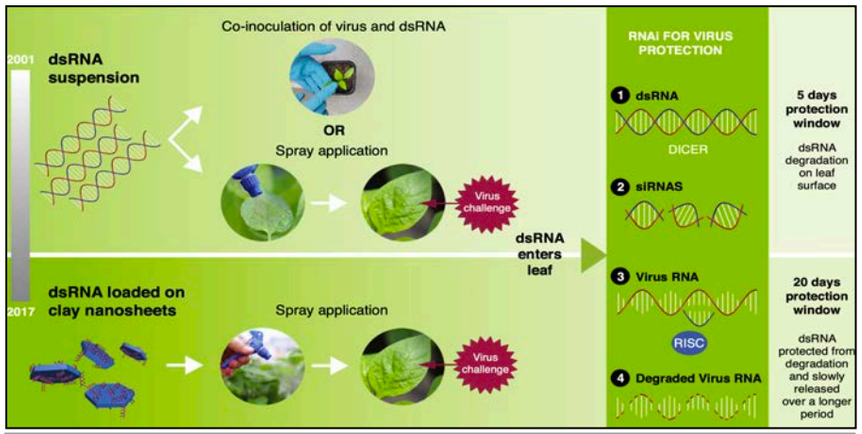


Figure 3 - Exogenous application of naked-dsRNA (**Aim 2**) and clay-nanosheet-loaded-dsRNA (**Aim 3**). In this paper naked-dsRNA has provided RNAi-mediated protection to homologous viruses for up to 5 days post-spray, while clay-nanosheets increased the stability and the release efficiency of dsRNA until 20 days post-spray. (N. Mitter *et al.*, 2017)

Future Perspectives

- The SIGS system could represent a **non-GMO Treatment** since there are no sequence modification in both plant and pathogen.
- It could contribute to the foundational knowledge for the future **application of RNAi on other crops** and biotic stressors.
- The reduction of chemicals use in Horticulture could lead to an increase of **environmental sustainability** related to this sector.

Current Progress

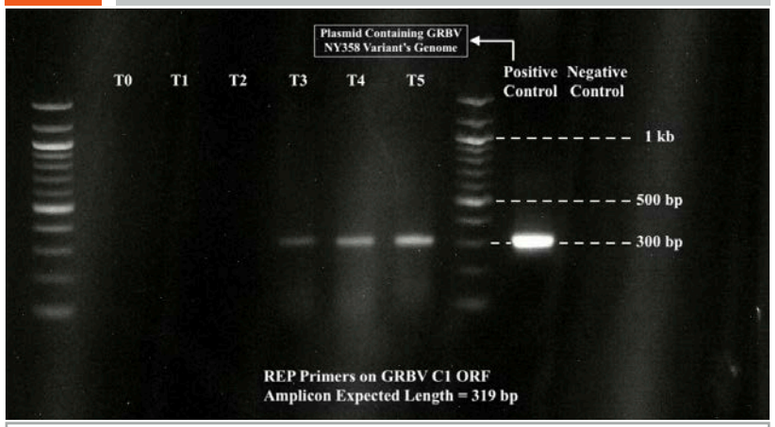


Figure 4 - 1.5% Agarose gel run. Total RNA has been extracted at each sampling date and used as template for reverse-transcription PCR (RT-PCR). The expected amplicon length was 319 bp. A plasmid containing the GRBV genome (variant NY358) has been used as positive control in the RT-PCR reaction, while the negative control is represented by water. The plasmid containing the GRBV genome has been provided by Keith Lloyd Perry (Cornell University).

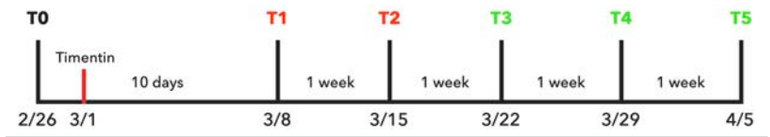


Figure 5 - Timeline for the total RNA extraction performed during the first Agro-infiltration experiment

After **24 days post-infiltration** (2/26 to 3/22) we detected the presence of the viral transcript encoding for the Replicase (REP) in the total RNA samples extracted from the treated plants.
Another infiltration experiment will be performed in order to increase the resolution across the week in **between T2 and T3** and highlight the very **early phase of GRBV infection** in Grapevine plants.