PLANT RNA BIOTECHNOLOGY

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RNA sprays - precision tools for the modulation of host genes to control viroid infections

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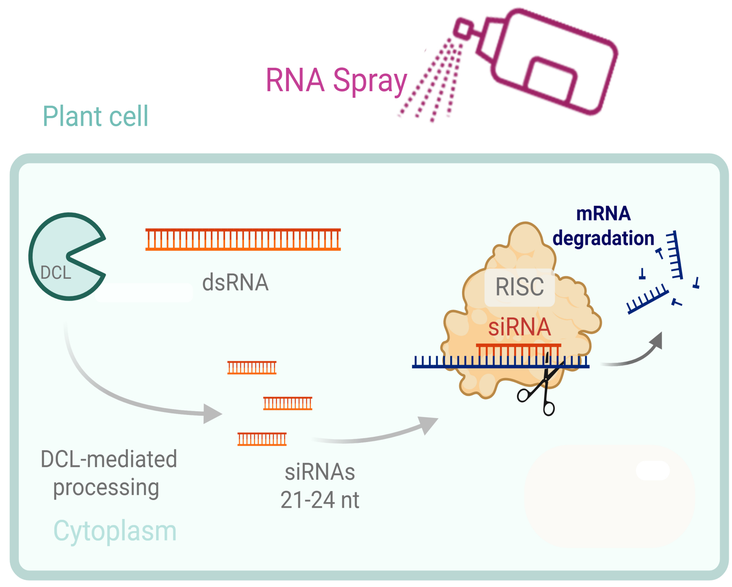
Introduction

Viroid infections, caused by the smallest known plant pathogens, pose a persistent threat to global agriculture and are becoming more frequent due to climate change and increased international trade. These non-coding circular RNAs exploit the plant host machinery, leading to severe crop yield and quality losses, with no effective chemical or biological control strategies available. The current management strategy relies on labour-intensive, unsustainable, and economically inefficient exclusion and eradication measures. To address this challenge, our research aims to explore RNA spray-induced gene modulation as a novel, non-transgenic strategy to enhance plant defense/resilience against viroid infections.

Our approach utilizes RNA interference (RNAi) and epigenetic regulation to modulate host gene expression, counteracting viroid-induced reprogramming. Our research focuses on investigating conserved host pathways that are targeted by viroids, analysing viroid-derived small RNAs (vd-sRNAs) and their impact on gene regulation. We also use RNA molecules, including double-stranded RNA (dsRNA), circular antisense RNA (caRNA), circular RNA (circRNA) and messenger RNA (mRNA), to induce targeted gene silencing or activation.

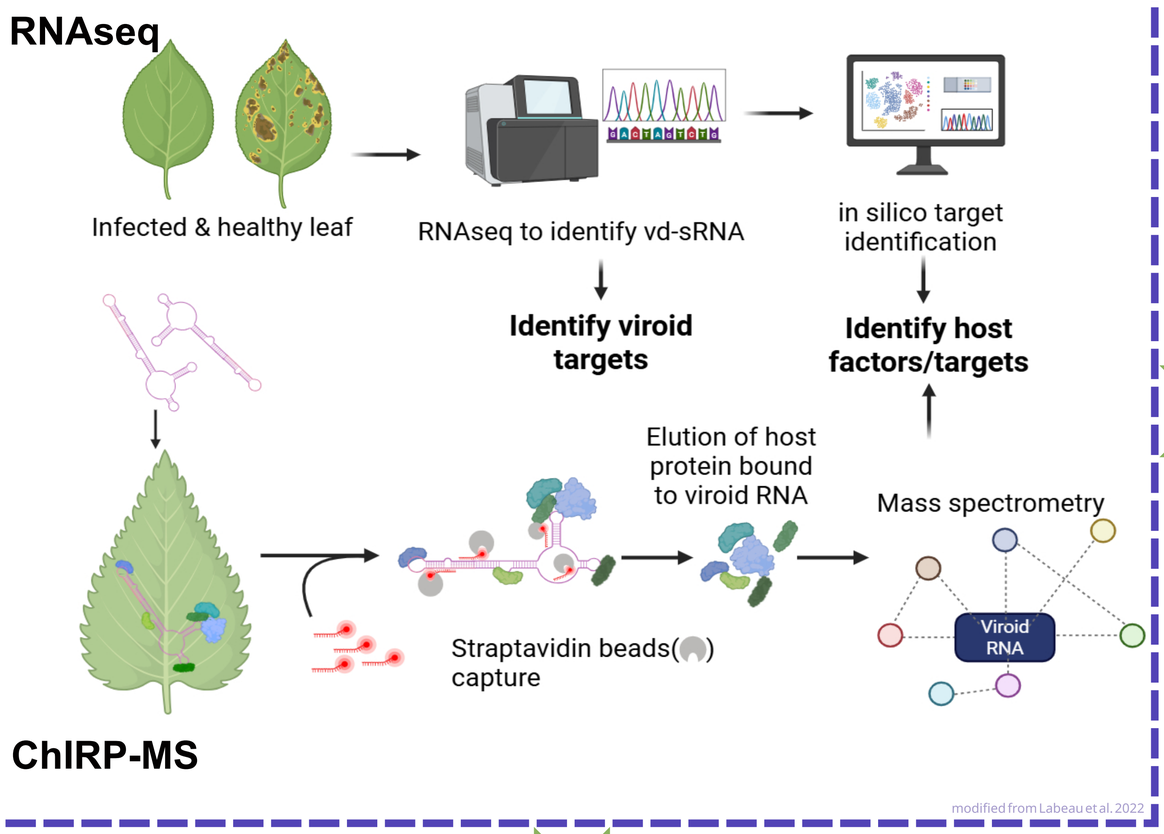
Functional validation under controlled and field conditions will assess the efficacy of these formulations in suppressing viroid replication and mitigating symptoms. Through the integration of molecular, bioinformatics, and applied agricultural approaches, this research aims to establish RNA spray technology as a sustainable, precise, and field-deployable solution for viroid management. Beyond viroids, this strategy has broader implications for plant protection, offering an environmentally friendly alternative to conventional pesticides and aligning with global efforts toward sustainable agriculture.

Spray-induced gene silencing



Concept of the project

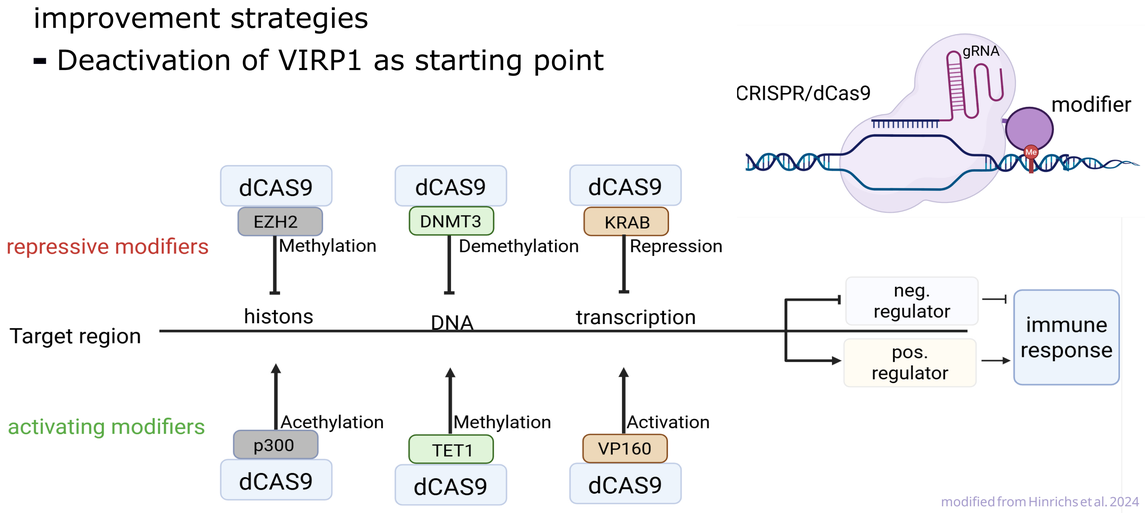
Identification of viroid and host targets



modified from Labeau et al. 2022

EpiEdit

- dCas9 fused to epigenetic modifiers
  → targeted, locus-specific regulation of before identified host genes
- Modifies DNA methylation and histone marks without altering DNA sequence
- Enables gene activation, repression, or chromatin remodeling
- Tool to study epigenetic regulation and develop non-GMO crop improvement strategies
- Deactivation of VIRP1 as starting point



modified from Hinrichs et al. 2024

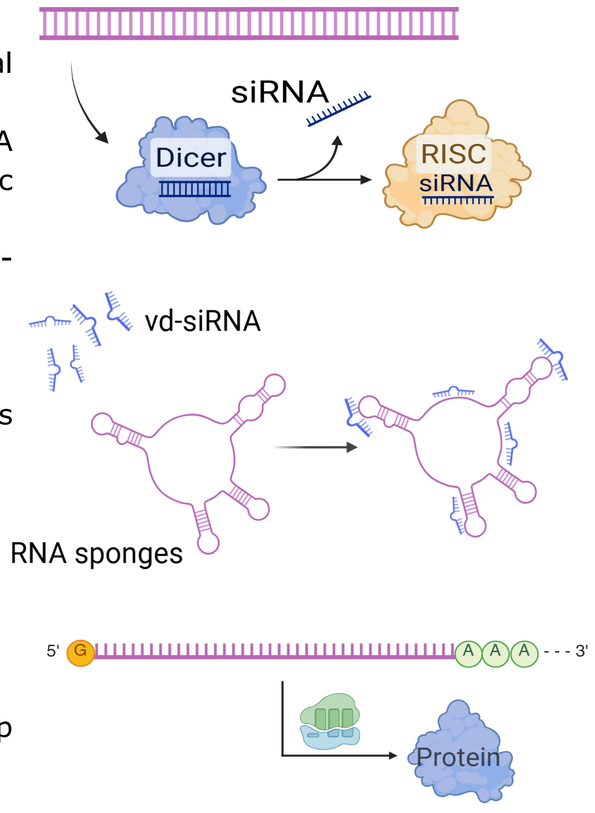
RNA-sprays

dsRNA
- Targeting binding site of ribosomal protein L5 (RPL5)
- Silencing Transcription factor IIIA splicing variant with seven zinc fingers (TFIIIA-7ZF)
- Silencing of bromodomain-containing protein 1 (VIRP1)

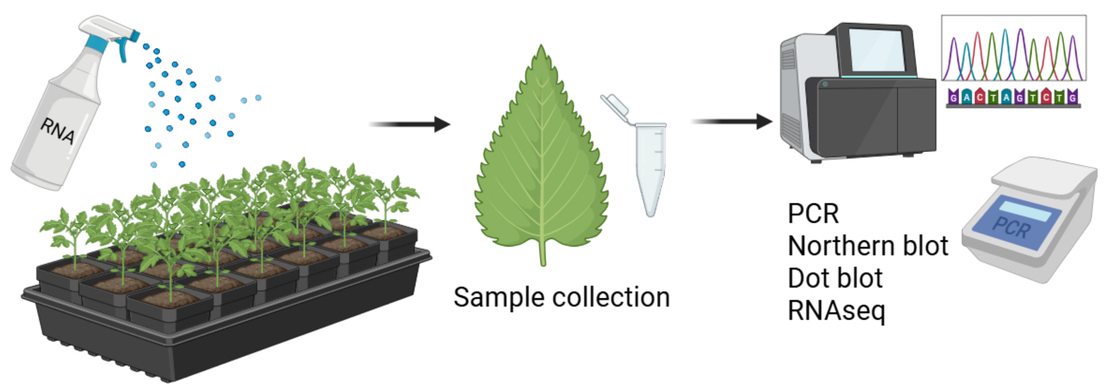
sRNA
- Targeting viroid-derived small RNAs (vd-sRNAs)

Circular RNA
- RNA sponges binding to vd-sRNA
- circular antisense RNAs

mRNA
- to express nucleases like e.g. hop pollen nuclease 1 (HBN1)



Evaluating RNA-spray efficiency



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Refernces:

Hinrichs, A.-K., Koch, A., Richter, A.M., 2024. Dual-Luciferase Reporter Assay for Prescreening CRISPR (d)Cas9-Mediated Epigenetic Editing on a Plant Promoter Using Human Cells, in: Maghuly, F. (Ed.), Plant Functional Genomics, Methods in Molecular Biology. Springer US, New York, NY, pp. 273–285. https://doi.org/10.1007/978-1-0716-3782-1_16

Labeau, A., Fery-Simonian, L., Lefevre-Utile, A., Pourcelot, M., Bonnet-Madin, L., Soumelis, V., Lotteau, V., Vidalain, P.-O., Amara, A., Meertens, L., 2022. Characterization and functional interrogation of the SARS-CoV-2 RNA interactome. Cell Rep. 39, 110744. https://doi.org/10.1016/j.celrep.2022.110744

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