How Disrupting Viroid Biogenesis Impacts Viroid propagation

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ViroiDoc

Advanced Research on Viroid Pathogenesis and Control for Agricultural Sustainability

BACKGROUND

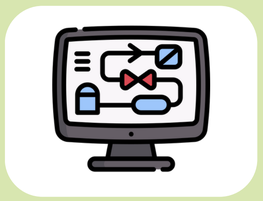
Viroids consist of small and circular RNA molecules, they are highly structured but without any coding potential. Most well known viroids infect economically important agricultural and ornamental plants. Usually transmission occurs through contaminated tools and machinery, then viroids spread throughout the plant causing stunted growth and chlorosis.

The viroid life cycle and infection is completely dependent on host plant proteins and its factors, such as the VirP1, which mediates nuclear import of viroids, replication by RNA polymerase II and circularization by DNA ligase 1. Additionally host proteins involved in the viroid biogenesis may be identified by analysing gene transcripts with significant expression changes during viroid infections of various plant species.

Main Objectives: Uncover common and unique host proteins/processes that influence viroid infection in hops or a model plant. Investigate how disruption of the identified host proteins involved in viroid replication, impacts viroid titer, disease symptoms and plant immune responses.

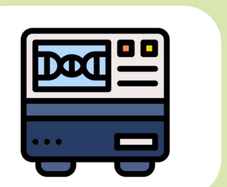
WORK PLAN / METHODS

1. **Meta-analysis of RNA-seq datasets:** Gather and analyse RNA-seq data from public databases (e.g. SRA) of various plant species infected by nuclear viroids (Pospiviroidae). Use established or create a bioinformatic workflow that integrates these datasets and determines enriched pathways during viroid infection.



2. **VIGS :** Use Virus induced gene silencing, to target up to 6 host genes in hops (*Humulus lupulus*) or a model plant (*Nicotiana benthamiana)*. Infect with CBCVd, Citrus bark cracking viroid (Cocadviroid rimocitrii), and monitor infection longitudinally compared to control plants. Secondment at UPV is planned for this task.

3. **Quantify CBCVs:** Extract RNA from viroid-infected plant tissues over time, using RT-qPCR (viroid titers) and LAMP. Evaluate disease symptoms and progression in CBCVd infected plants with impaired host proteins.



4. **Validate silencing:** Confirm impairment of host genes through RT-qPCR assays and further RNA-seq studies *in situ*.

TAKE HOME MESSAGE

Discovery of novel or known host genes that influence infection of specific viroids in diferent plant species, uncovering new plant-viroid interactions. Host proteins involved in viroid biogenesis will become targets for knock down and the further development of broad-spectrum resistance to viroids in important agricultural crops

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