



Synopsis of Webinar 9 (11<sup>th</sup> March 2021)

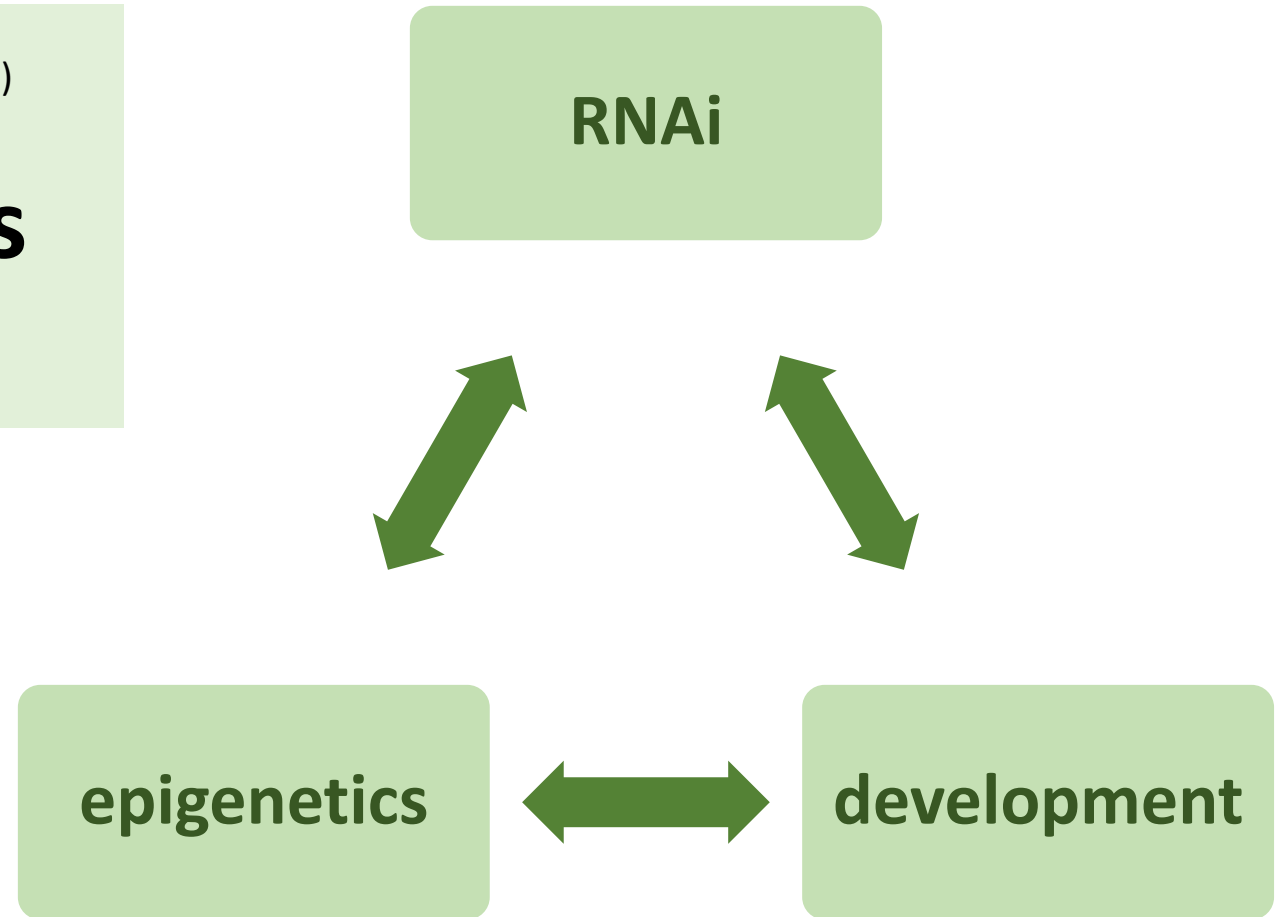
# 'RNAi, plant epigenetics and development'

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HELLENIC AGRICULTURAL  
ORGANIZATION - DEMETER





## RNA-directed DNA methylation (RdDM) mechanism:

- **De novo** establishment of DNA methylation (dsRNAs).
- **Amplification** of DNA methylation (siRNAs).
- **Maintenance** of DNA methylation (MET1, CMT3).

## RdDM tools to modify the epigenome:

- **Transgene-based RdDM:** transgenes expressing hairpin RNAs that reside in an intron.
- **Transgene-free RdDM:** spraying of dsRNAs/siRNAs.



# Epigenetic modifications upon exogenous RNA application in plants

Athanasios Dalakouras (Hellenic Agricultural Organization Demeter, Greece)

## What we know so far on sprayed dsRNAs/siRNAs:

- Sprayed siRNAs and dsRNAs trigger **mRNA degradation** in plants.
- No studies on epigenetic modifications upon RNA spraying, yet.

## Unpublished data:

- Induction of CaMV 35S promoter **DNA methylation** upon high pressure spraying of 333 bp dsRNA in *Nicotiana benthamiana*.

## Points to consider:

- RNA spraying as a **tool** to modify plant epigenome.
- Possible **epigenetic side effects and/or off-target effects** upon RNA spraying.



## Methods of RNA application (videos):

- Brush spreading, spraying, infiltration, inoculation, pipetting.

## Physiological conditions affecting *exo-RNAi*:

- Plant age, time of application, stress, moisture.

## Optimum conditions for *exo-RNAi* of *NPTII* transgene:

- **Methods:** brushing, spraying > infiltration, inoculation.
- **Conditions:** 4-week old plant, low soil moisture, late-night RNA application



# *Emerging roles for small RNAs in plant somatic embryogenesis*

Sandra Correia (University of Correia, Portugal)

## **Somatic embryogenesis (SE):**

- Importance of SE in plant biotechnology.
- Development of methods to isolate uniform populations of embryogenic cells at the very early stages of SE → RNA seq.
- Specific **miRNAs** regulate auxin-related genes involved in various phases of SE.



# Understanding the role of RNAi machinery in viroid infections

Kriton Kalantidis (University of Crete, Greece)

## Potato spindle tuber viroid (PSTVd):

- 359-nt non-coding RNA pathogen.
- Model for RNAi studies.

## DCL-processing of PSTVd in *N. benthamiana*:

- DCL2/DCL3 processing has a **negative** effect on PSTVd infectivity.
- DCL4 processing has a **positive** effect on PSTVd infectivity.
- PSTVd has evolved to preferentially attract DCL4.
- **Unpublished data:** PSTVd interacts with DCL4 to be transported into the nucleus.