

## iPLANTA Webinar 9:

### RNAi, plant epigenetics and development,

11 March 2021 10:00 am (CET)

#### Summary notes

iPlanta Chair *Bruno Mezzetti* opened the webinar and introduced the iPlanta webinar series to the participants.

#### **Introduction:**

The webinar was chaired by *Athanasios Dalakouras*, researcher at Hellenic Agricultural Organization 'Demeter', who conducts research on RNAi and epigenetics in plants. In his introduction, Athanasios Dalakouras explained the main theme of this webinar which focused on the tripartite interaction between RNAi (emphasis on siRNAs and miRNAs), epigenetic modifications (DNA methylation and histone modifications) and various aspects of plant development.

#### **RNA-directed DNA methylation as tool to modify plant epigenetics**

In the first presentation, *Michael Wassenegger* (professor at Heidelberg University and principal investigator in RLP AgroScience GmbH, Germany) explained the mechanistic details of RNA-directed DNA methylation (RdDM) in plants, in terms of its de novo establishment, amplification and maintenance. In addition, he highlighted methods to modify the plant epigenome with the use of intron-based RdDM systems, which result in extremely efficient and precise RdDM in plants, but also upon high-pressure spraying of carbon dots-conjugated siRNAs, the latter approach being still in progress.

#### **Epigenetic modifications: an unexplored facet of exogenous RNA application in plants**

In the second presentation, *Athanasios Dalakouras* (researcher at Hellenic Agricultural Organization 'Demeter') provided an outline of the current knowledge on exogenous application of RNA molecules in plants (21-nt siRNAs, 22-nt siRNAs, 24-

nt siRNAs, long dsRNAs), comparing their efficiency at the onset of mRNA degradation and DNA methylation. He stressed the fact that most studies employing exogenous RNA application focused so far on mRNA degradation but not on DNA methylation, and presented unpublished data demonstrating that exogenous application of dsRNA in *Nicotiana benthamiana* can trigger DNA methylation of the CaMV 35S promoter. The implications of these data for epi-breeding but also for shaping the corresponding regulatory framework were discussed.

### **Application strategies for exo-RNAi induction in *Arabidopsis thaliana***

In the third presentation, *Alexandra Dubrovina* (researcher at the Federal Scientific Center of the East Asia Terrestrial Biodiversity, Russia), presented videos describing the exact methodologies of exogenous application of RNA molecules in *Arabidopsis thaliana* (brush spreading, spraying, infiltration, inoculation, needle injection, pipetting). Moreover, she described that the physiological condition of plants (presence or absence of stress, plant age, photoperiod, soil moisture) greatly influence the uptake of the applied dsRNA/siRNA molecules and thus affect the onset of the NPTII transgene RNAi in *Arabidopsis thaliana*.

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

In the fourth presentation, *Sandra Correia* (researcher at the University of Coimbra, Portugal) explained the importance of somatic embryogenesis in plant biotechnology and provided data linking the fine-tuning of several aspects of somatic embryogenesis (from dedifferentiation to totipotency and differentiation) with the expression of specific miRNAs targeting specific genes.

### **Understanding the role of RNAi machinery in viroid and viral satellite infections**

In the fifth (and final) presentation, *Kriton Kalantidis* (professor in University of Crete, Greece), proposed that viroids, which are approximately 350 nt non-coding RNA pathogens, can serve as a model system for the elucidation of various aspects of RNAi in plants. He presented data showing that in *Nicotiana benthamiana* all four DICER-LIKE proteins (DCL1-DCL4) are involved in viroid infection in a highly complexed and hierarchical manner. Moreover, he shared unpublished data suggesting that a nucleus-replicating viroid, PSTVd, is transported to the nucleus through its interaction with DCL4.

The webinar ended with an extended section of responses to the many questions brought forward by the participants.

The webinar was attended by 140 participants.

The video of the meeting, the presentations and the report can be found at the following links:

<https://www.iplantawebinars.com/event/5fe1f20a52a27f1f072c9f12>

<https://iplanta.univpm.it/node/87>