iPLANTA Webinar 6:

RNAi strategy to control insect pests,

16 February 2021 4.00pm (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 *Olivier Christiaens*, postdoctoral researcher at the faculty of Bioscience Engineering at Ghent University, who conducts research on RNAi in insects, with a focus on applications in crop protection. In his introduction, Christiaens explained that the main theme of this webinar was going to be RNAi efficiency in insects and physiological and cellular barriers affecting RNAi efficacy. The implications for application of RNAi-based biocontrol and the potential for resistance emergence would be discussed

RNAi efficacy in insects

In the first presentation, an overview of all potential physiological and cellular barriers was presented by *Anastasia Cooper*, postdoctoral researcher at Kansas State University (USA). She explained how RNAi efficacy is variable between different insects and how this efficacy is affected by a wide range of factors, of which dsRNA degradation in the digestive system, cellular uptake rate and cytoplasmatic escape, are among the most important ones.

Intraspecific variability in RNAi efficacy in aphids

June-Sun Yoon, postdoctoral researcher at Oregon State University (USA) presented his previous work on aphid RNAi which he conducted at Cornell University (USA). In this study, he investigated the genetic determinants of intraspecific variability in RNAi efficacy in the pea aphid *Acyrthosiphon pisum*. He explained that different pea aphid genotypes, can vary greatly in their sensitivity to oral RNAi. In this study, two target genes were used (aquaporin and snakeskin) which showed overlap in terms of silencing efficiency between the different genotypes, but were not identical. Further investigations showed a correlation between RNAi sensitivity and SNPs in three different genes.

Cellular uptake of dsRNA in mosquitoes and the potential of 'paperclip' dsRNA

Steve Whyard, professor at the University of Manitoba (Canada) presents his work on cellular uptake of dsRNA in mosquitoes. First, he explains how experiments in mosquito cells, using chemical inhibitors of different uptake routes, showed that clathrin-mediated endocytosis (CME) is a major cellular uptake route for dsRNA in these cells. Next, he shows how so-called "paperclip dsRNA" shows an increased stability in nucleolytic environments compared to long dsRNA and how they do not require CME to enter the cells. Bioassays also showed that these paperclip dsRNAs can in some cases lead to a stronger RNAi effect than long dsRNA in mosquitoes.

Delivering long dsRNA to plant tissue for insect control

Geert Plaetinck, Director of Research at Syngenta (Ghent Innovation Center), explained different application methods, presenting advantages and disadvantages of *in planta* or foliar spray delivery. Further focusing on the foliar spray delivery route, he presented research conducted at Syngenta which looked at the potential for uptake of long dsRNA in plants, using different methods and formulations (such as physical wounding of leaf, using adjuvants/spreaders, biolistics,…) and different routes (leaves, stems, roots). Using a wide range of techniques, they could conclude that there is uptake of dsRNA possible through cut stems or cut roots, but no dsRNA uptake could be observed through intact roots or through an intact cuticle.

Improving RNAi in Lepidoptera

Subba Reddy Palli, professor at the University of Kentucky (USA) first explained that Lepidoptera are among the least sensitive insects to dietary dsRNA. He gave an overview of the research done in his lab on the physiological and cellular barriers that are at the cause of this insensitivity. These include a strong nucleolytic activity in the digestive system (midgut) of these insects and a problematic endosomal escape after endocytosis-mediated cellular uptake of the dsRNA. Finally, he also presented results on the use of different nanoparticles to improve RNAi efficacy in Lepidoptera.

Development of RNAi-resistant populations of the Colorado potato beetle

Juan Luis Jurat-Fuentes, professor at the University of Tennessee investigated the potential of emergence of resistance to RNAi in the Colorado potato beetle upon exposure to topically applied dsRNA over multiple generations. After 9 episodes of selection, he reports having obtained a colony with a 11,100-fold increase in resistance to the insecticidal dsRNA. Genetic analysis suggested that the trait(s) involved in resistance are not sex-linked, are recessive and support polygenic resistance. He further explained how their analysis suggests that the resistance may be linked with a higher expression of the target gene and/or a reduced cellular uptake efficiency.

RNAi in the stinkbug *Nezara viridula*

Rohit Sharma, a PhD student at Ghent University presented his work on RNAi in the neotropical green stinkbug *Nezara viridula*. After a nanoinjection target gene screening, the 3 best performing target genes (causing the highest/fastest mortality) were selected for oral feeding bioassays. Here, Rohit found that oral RNAi in this insect is functional, but the insect is not highly sensitive. Further investigations into physiological barriers indicated a strong nucleolytic degradation in saliva and through an RNAi-of-RNAi approach, the nuclease responsible for this lower RNAi response could be identified.

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

The webinar was attended by 119 participants.

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

https://www.iplantawebinars.com/event/5fe0cc9452a27f1f07fe2f5d

https://iplanta.univpm.it/node/84