Pospiviroids are plant pathogens, composed only of infectious RNA molecules. They are both latent on many ornamentals and harmful for cultivated plant species like tomato, potato, sweet pepper, citrus or chrysanthemum. Throughout the world, these pathogens cause sporadic outbreaks on susceptible crops, while they often remain undetected although widespread on ornamentals.

In the EU, no official detection methods have yet been recognized for the detection of pospiviroids. There is also a lack of publicly available comparisons of existing methods. A new generic detection method based on reverse transcription polymerase chain reaction has been developed and validated according to the international standard ISO/IEC 17025. To assess this new method with already existing ones, an inter-laboratory comparison was organized at the EU level.

In a second step, High-Throughput Sequencing, which arises as the next generation technology in plant disease diagnosis, was used to characterize the pospiviroid *Citrus exocortis viroid*-tomato pathosystem. In-depth analysis of generated sequences coupled with innovative bioinformatic analyses allowed for gaining insights into the molecular interactions between pospiviroids and their hosts. This new perspective provides a coherent answer to questions regarding viroid degradation, cell-to-cell trafficking, symptom expression and, consequently, to pending questions concerning viroid phytosanitary risk management. Moreover, from these analyses, emerging hypotheses allowing understanding the activation of plant innate immunity and systemic acquired resistance by these non-coding RNAs are proposed. Together with the possible interaction of RNAi in these processes, such hypotheses offer both new paths of research regarding these key phenomena of plant defense as well as new hope for the sustainable management of viruses and viroids in crops.