Liposome nanoparticles as Trojan horses transporting dsRNA into plants

M2 internship to be continued by a Ph.D. thesis

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Introduction, scientific context

In the current context of world population growth and climate change, we need smarter ways to enhance plant performance while reducing environmental impacts. Anticipation of biotic and abiotic stresses is possible thanks to reliable weather predictions coupled to environmental and ecological sciences. But rather than follow guidelines recommending applying pesticides or other chemicals to face an upcoming stress, our strategy is to warn and prepare plants so that they engage their own defenses, a form of *priming* and *acclimation* (see Fig. 1). This can be done without resorting to GMOs (genetically modified organisms) through application of RNAs since RNAs do not modify the genome. Their action typically operates *via* RNA interference (RNAi) which is a regulatory mechanism able to modify the expression of a given gene using double-stranded RNA (dsRNA). Indeed, the natural cellular processing of dsRNA into small RNA molecules of 21 to 24nt leads to the silencing of targeted genes through complementary sequences. First discovered in plants showing resistance to virus infection, the technology has so far mainly been used as a genetic tool. Recently a proof of concept of the approach has been provided *via* controled application of dsRNA that induced a silencing response (Fletcher et al. 2020). A key challenge now is to find practical ways to deliver such RNAs into plant cells in a cheap and reliable way so as to remodel at will plant-wide genome expression.

Fig. 1

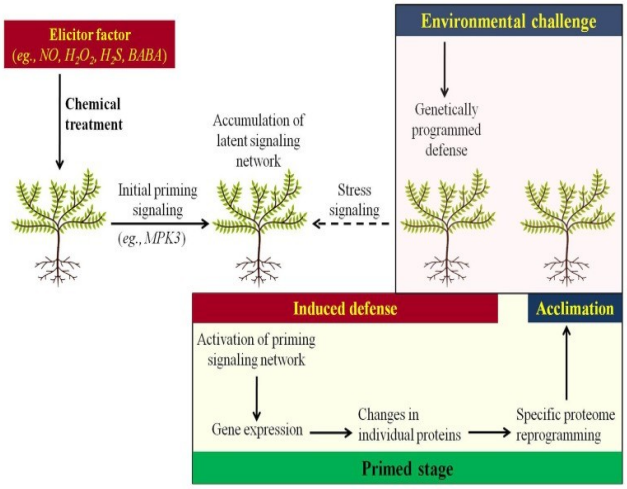
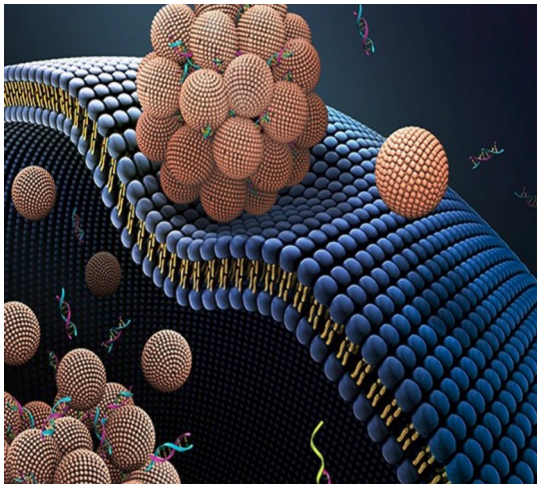


Fig. 2



Research proposal

Recent advances in nanotechnologies allow the use of various nanoparticles as carriers of nucleic acids to protect and deliver them into plant cells (see Fig. 2). For example, the use of non-toxic layered double hydroxide nanoparticles loaded with dsRNA against Cauliflower Mosaic Virus (CMV) has successfully provided virus resistance on tobacco leaves. Also, the use of carbon nanotubes allowed the delivery of DNA and dsRNA in a number of other plant species (Sanzari et al. 2019). Hence, nanoparticles are emerging as a new technology for RNAi. In this project, we propose to develop a technology using cationic liposome nanoparticles as well as cationic dendrimers, successfully used so far in mammalian cells, for the delivery of RNA into plants. The objective is to ensure that the functionalisation of these nanovectors allows them to act as *Trojan horses*, overcoming the cell wall and cell membrane barriers by inducing the cells to engulf them (endocytosis) and release their active ingredients (dsRNA). In collaboration with the group of Nicolas Tsapis (Institut Galien Paris-Saclay), liposome and dendrimer nanoparticles containing different dsRNA constructs synthesized *in vitro* will be generated and their efficiency for gene silencing will be measured in plant tissues. To detect the silencing, we will use plants expressing a GFP reporter gene. The intern will study different protocols for applying combinations of dsRNA/ nanoparticles to different parts of the plant (protoplasts, root, leaves) and he/she will monitor GFP silencing as a proof of activity. We aim also to obtain mechanistic insights into the dynamics of nanovector action as this will provide ways to optimise the technology and likely turn it into a large scale tool in agriculture.

Methodologies

- RNA transcription (data acquisition and analysis)
- liposome nanoparticles formulation, synthesis and characterization
- fluorescence microscopy and tracking
- *in-vitro* plant growth and preparation of protoplasts
- gene expression quantification.

References

- Fletcher, SJ et al. *A Perspective on RNAi-Based Biopesticides*. Front Plant Sci. 2020 12;11:51. doi: 10.3389/fpls.2020.00051
- Sanzari, I et al. *Nanotechnology in Plant Science: To Make a Long Story Short*. Front. Bioeng. Biotechnol. 2019 29;7:120. doi: 10.3389/fbioe.2019.00120