**Development of dsRNA to control fungal pathogens of grape, tomato and strawberry**

Small RNAs (sRNAs) that mediate RNAi in plant hosts represent a novel class of pathogen effectors that inhibit host immunity for successful infection. The use of RNAi technology, bidirectional cross-kingdom

RNAi and two-way sRNA trafficking between pests/pathogens and hosts offers new options in plant protection with the generation of new RNAi-based products that can be applied on plants, by spray (SIGS)

or injection, with a targeted specific effect on pathogens

Therefore, such pathogen-gene-targeting RNAs represent a tool to protect plants through both endogenous expression or topical application (Spray-Induced Gene Silencing - SIGS) of specific

siRNA, in the latter case as new-generation environmentally friendly fungicides

Recent studies are demonstrating the possibility of creating new commercial products based on stable

and effective RNAi for plant defence. However, no products on the market are available and no legislation for their possible classification at European level has been developed yet.

Currently, it is known that any new RNAi-based product will not be classified under the GMOs legislation, but it will be subjected to that concerning pesticides, most likely along with resistance inducers.

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If this classification will be confirmed, RNAi-based products will be easier to be spread once problems concerning production, ease of application, and persistence will be solved. Results on this approach are needed to demonstrate the potential benefits with absence of risks for the consumer and the environment.

Aim of this this project is to identify and produce dsRNA molecules against major pathogens of tomato/potato, strawberry and grapevine, namely, Phytophthora infestans, Botrytis cinerea and Plasmopara viticola. Gene targets for each pathogen will be diverse consisting of 2-3 gene target for each pathogen.

Some of these constructs are already available in the lab. The dsRNA molecules will be produced in different ways, such as E.coli, Nicotiana tabacum, in vitro transcription and chemical synthesis.

Once the molecules will be available, their ability to limit growth of pathogens will be tested in vitro or in vivo (in greenhouse potted plants) depending on the pathogen. Spray assay and drop assays will be performed.

Besides these activities, the selected candidate will be involved also in the production of plasmid constructs for agrobacterium transformation of plants. This will allow to produce tomato, strawberry and grapevine that will express hairpin RNA sequences. These through activation of the RNAi machinery in plants and fungi, will drive silencing of key pathogen genes and control its growth.

**Activity plan**

The candidate will be firstly involved in the design of the ds RNA molecules, through the use of suitable software. Specificity and off target effect will be checked out. Subsequently the cloning procedures necessary to insert the hairpin gene constructs in the appropriate plasmids will be set up. PCR and ligation will allow recombinant molecules to be produced in order to proceed with the heterologous expression of the dsRNA or the in vitro transcription. Different targets for the different pathogens will be identified and relative construct generated.

Once the molecules will be produced and purified they will be aliquoted and stored in -20°C for using on plants potted in greenhouse, or in vitro assays. Different concentration and different experimental design will be pursued in order to explore the most effective dosage and timings and the preventive and/or curative applicative potential of the molecules.