Using RNA interference as a biological pesticide for clubroot in canola

RNA Innovation Cohort 1
Clubroot Steering Committee Meeting
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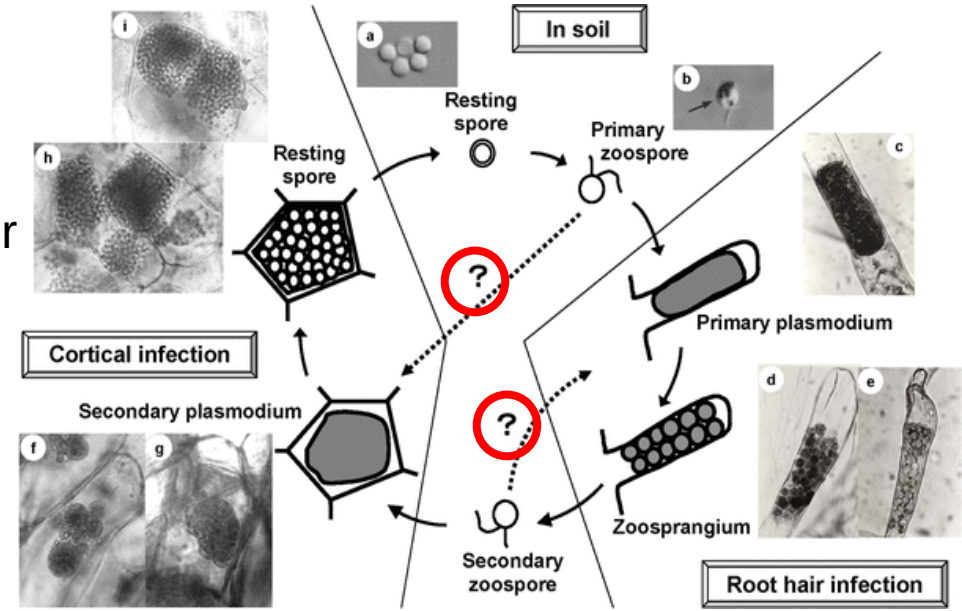
https://www.gograph.com/vector-clip-art/hand-shake.html

Current challenges in clubroot research

- Limited laboratory cultivation methods.
- Lack of annotated clubroot genome.
- Difficulties with delivery mechanisms for RNAi.
- Unsolved mechanisms in clubroot life cycle.
- Regulatory challenges in marketing GMO products.



Kageyama, K., & Asano, T. (2009)

Objectives

Using RNAi to tackle clubroot

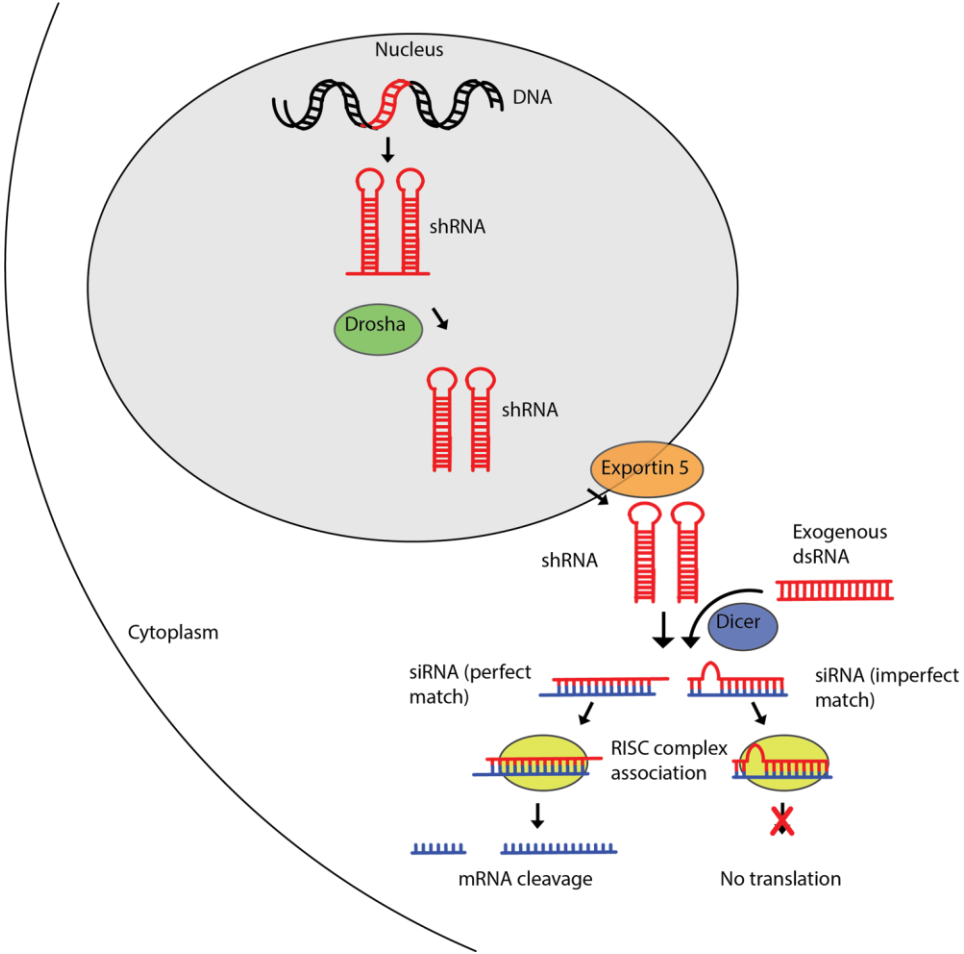
- To reduce the virulence or population of *Plasmodiophora brassicae* in the soil using RNA interference

Design siRNAs against target genes

- Designing and characterization of siRNAs against potential genes which are identified in the Clubroot genome.

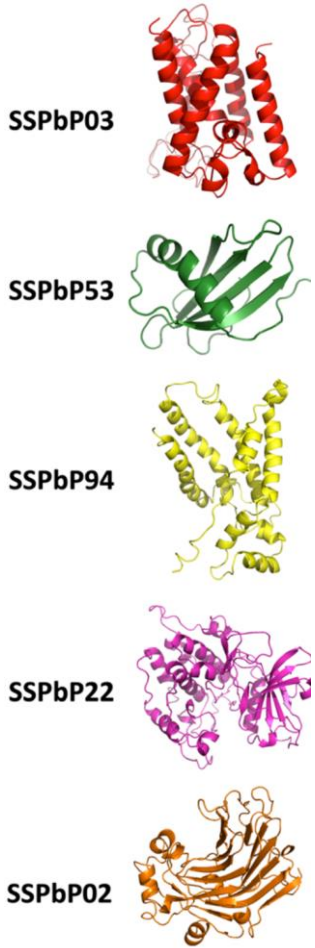
Overview of RNAi

- Process used to inhibit gene expression
- Commercially available SmartStax® Pro seeds contain RNAi
- Minimal work reported on RNAi use for clubroot
- RISC complex is present in clubroot genome
    - Based on bioinformatics work conducted by our group



Screening of target genes using bioinformatics

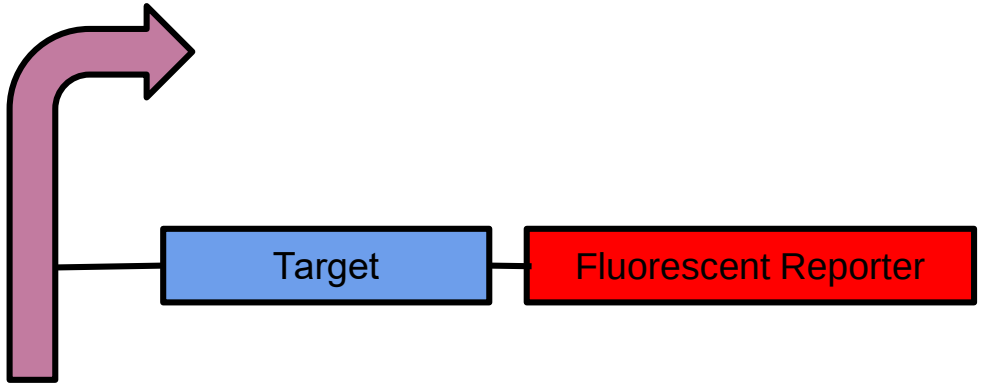
- Selected 7 genes from *P. brassicae* as potential targets
- 2 genes are related to zoospore primary infection (Fei *et al*., 2016).
- 5 genes are related to secondary infection of clubroot (Pérez-López *et al.* 2020).



Pérez-López *et al.* (2020)

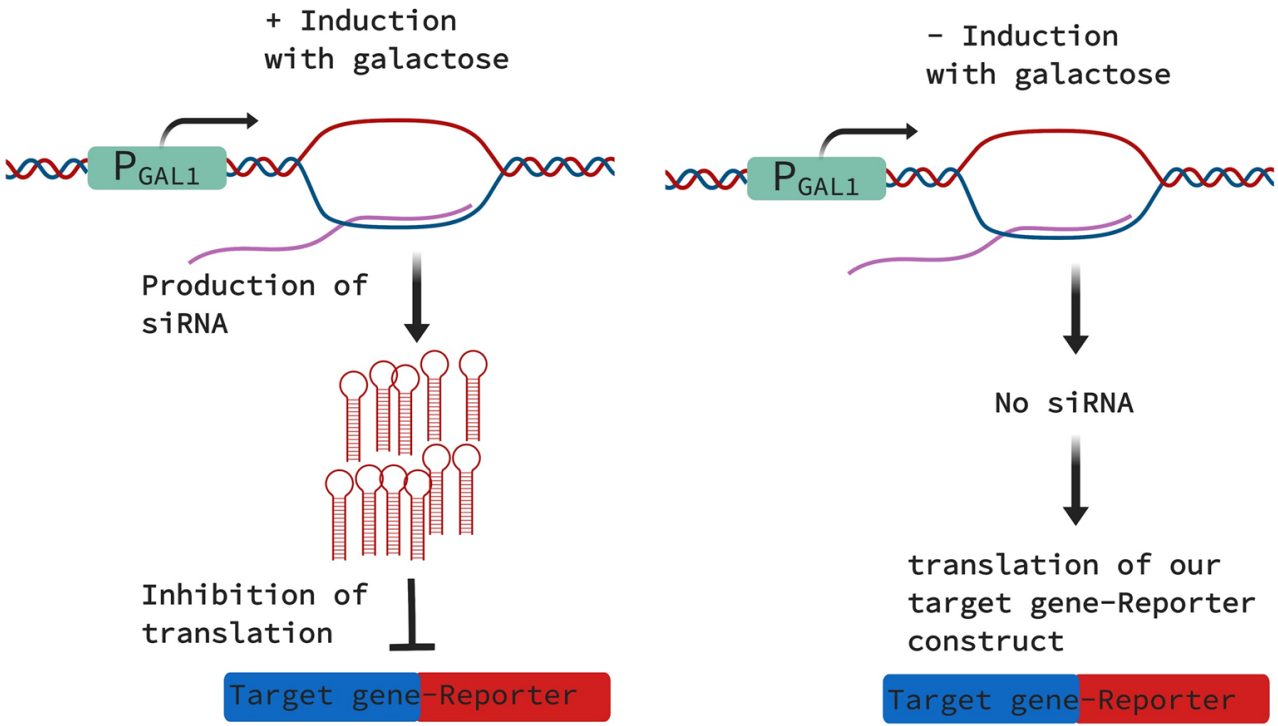
S. cerevisiae as a model organism

- Single celled eukaryotic organism and easy to manipulate yeast genetics.
- Obtained engineered yeast strains which contain the RISC complex.



- Towards this project we are creating new yeast strain with *P. brassicae* gene in its genome by using homologous recombination.
- For that we constructed the target gene with fluorescent reporter protein.
- Upon successful construction of yeast strain, we would like to use it for our future experiments.

Work plan



- Conditional expression of siRNA in yeast
- Screen for potential siRNA molecule against our target gene

Future Directions

- Insertion of target genes in yeast genome.
- Screening of potential siRNAs agonistic in *P. brassicae.*
- Upon successful knockdown in yeast, expand the study to *P. brassicae*

https://www.canolacouncil.org/canola-encyclopedia/diseases/clubroot/about-clubroot/

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Team Members (left to right)
Kristi Turton
Gayatri Namala
Sydnee Calhoun
Preethi Seelam Prabhakar
Matthew Stuart-Edwards
Keith Aubrey
Prakash Chukka

RNA Innovation Program Coordinator
Dr. Laura Keffer-Wilkes,
Justin Vigar, M.Sc.

Industry Coordinator

Dr. Rory Degenhardt,
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References

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