



Using RNA interference as a biological pesticide for clubroot in canola

RNA Innovation Cohort 1
Clubroot Steering Committee Meeting
April 30, 2020



RNA  **Innovation**

The logo for RNA Innovation. The word "RNA" is in red, and "Innovation" is in black. A stylized black icon of a keyhole or a vertical bar with a circular top and a dashed vertical line inside connects the two words. A thick black horizontal line underlines the entire text.

UNIVERSITÉ DE
SHERBROOKE



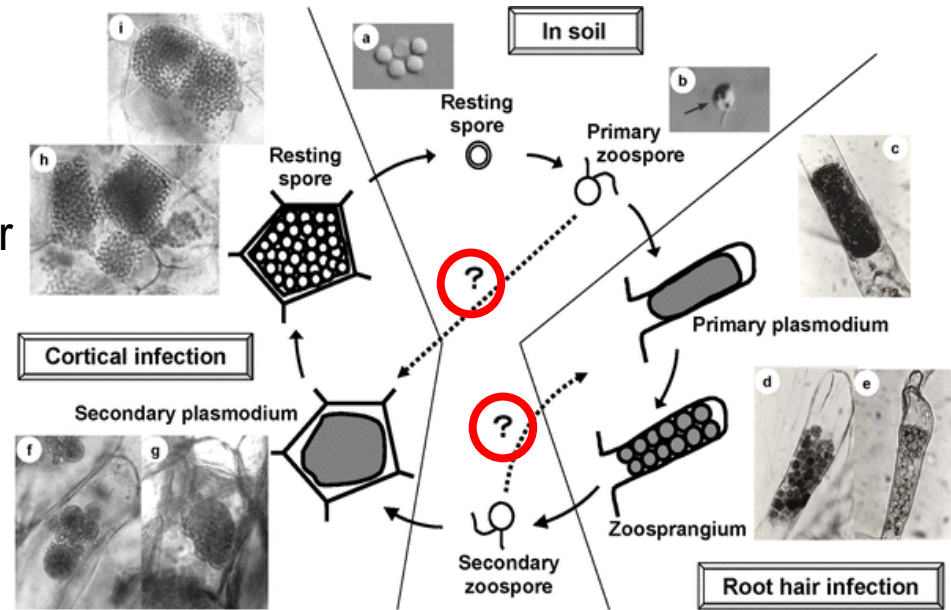
University of
Lethbridge

Bridging the Gap between Academia and Industry



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

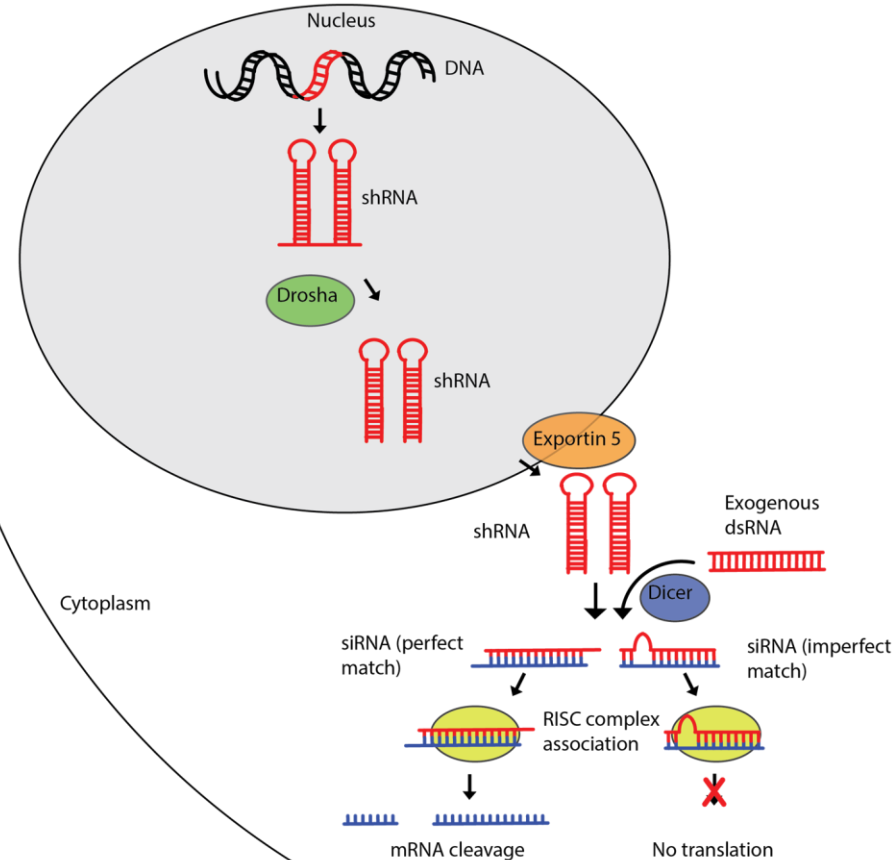
- To reduce the virulence or population of *Plasmodiophora brassicae* in the soil using RNA interference

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



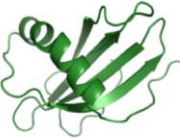
Screening of target genes using bioinformatics

- 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).

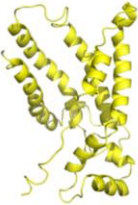
SSPbP03



SSPbP53



SSPbP94



SSPbP22



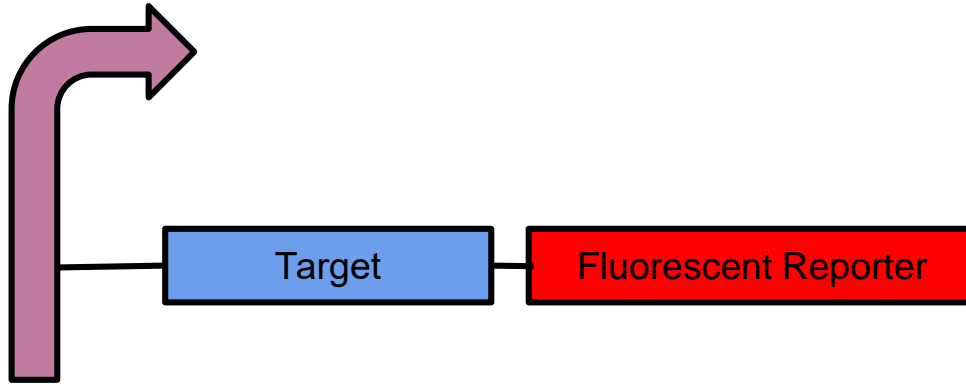
SSPbP02



Pérez-López *et al.* (2020)

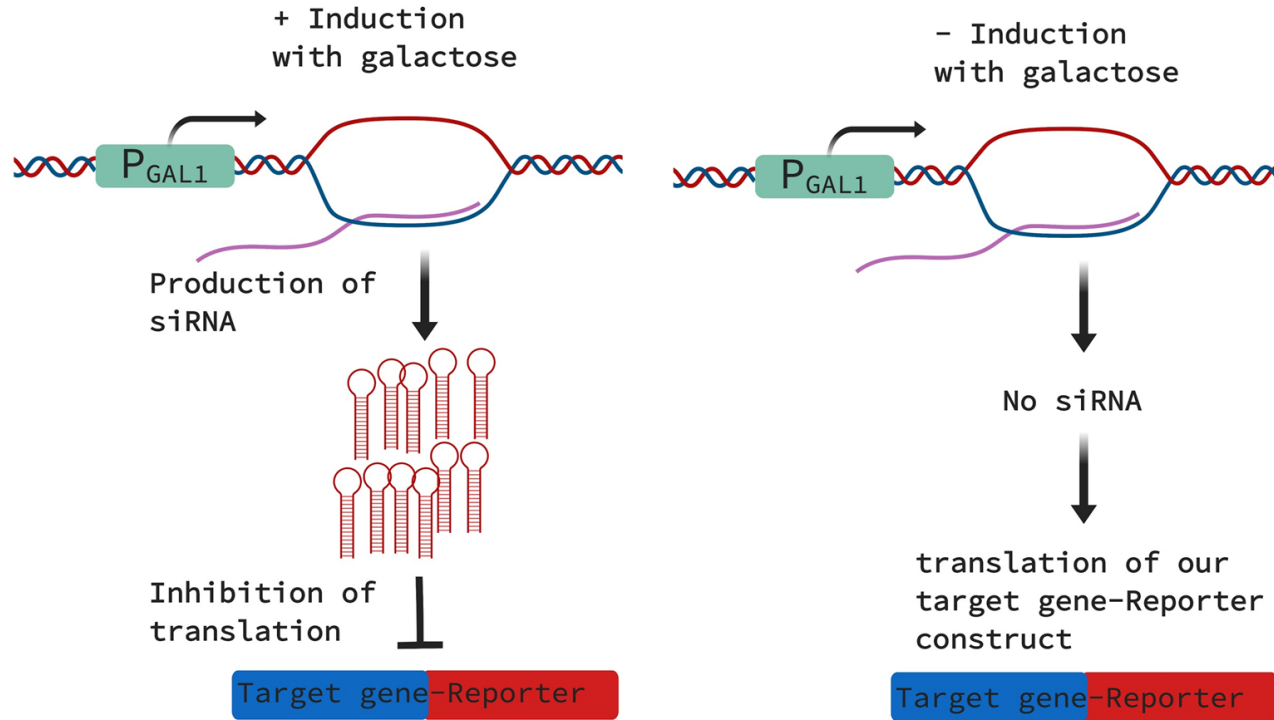
S. cerevisiae as a model organism

- 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/>



Team Members (left to right)

Kristi Turton
Gayatri Namala
Sydnee Calhoun
Preethi Seelam Prabhakar
Matthew Stuart-Edwards
Keith Aubrey
Prakash Chukka



Industry Coordinator

Dr. Rory Degenhardt,
Corteva agriscience

**RNA Innovation
Program Coordinator**

Dr. Laura Keffer-Wilkes,
Justin Vigar, M.Sc.



CORTEVATM
agriscience

References

- Drinnenberg, I., Wienberg, D., Xie, K., Mower, J., Wolfe, K., Fink, G., and Bartel, D. (2009) RNAi in budding yeast. *Science*. 326(5952):544-550. doi: 10.1126/science.1176945.
- Fei, W., Feng, J., Rong, S., Strelkov, S., Gao, Z., and Hwang, S. 2016. Infection and gene expression of the clubroot pathogen *plasmodiophora brassicae* in resistant and susceptible canola cultivars. *American pathological society*. doi: 10.1094/PDIS-11-15-1255-RE
- E Pérez-López, M Waldner, M Hossain, AJ Kusalik, Y Wei, PC Bonham-Smith & CD Todd* (2018) Identification of *Plasmodiophora brassicae* effectors – a challenging goal, *Virulence* 9:1 1344-1353. <https://doi.org/10.1080/21505594.2018.1504560>
- Kageyama, K., & Asano, T. (2009). Life cycle of *Plasmodiophora brassicae*. *Journal of Plant Growth Regulation*, 28(3), 203.