The Effect of Foliar Salicylic Acid Applications on Clubroot Disease in Canola

Keegan Kirchen, Emilee Storfie, Dr. Stephen Strelkov
Department of Agriculture, Food, and Nutrition Sciences, University of Alberta

Introduction

- *Plasmodiophora brassicae* is a plant pathogen that causes clubroot disease on canola (*Brassica napus*), among other species of the Brassicae family.
- Clubroot is a common plant disease in Alberta and is difficult to manage resulting in decreased crop yield of canola.
- The pathogen causes gall formation in the plant roots, which contain spores. Water and nutrient uptake are also disrupted causing plant wilting and stunting.
- Salicylic acid (SA) is a major plant hormone that regulates plant defense systems.
- Lemarié et al. (2015) and other studies have found the SA pathway to be upregulated in resistant plants experiencing *P. brassicae* infection.
- Lemarié et al. (2015) also determined that clubroot disease severity decreased when infected plants were treated by dipping their roots in SA solution.
- The purpose of this project is to determine if a foliar spray of SA on canola, which may be more feasible for farmers than a root dip, would be effective against clubroot disease.

Figure 1. Clubroot disease visible in the roots of *B. napus* (www.canolacouncil.org/canola-encyclopedia/diseases/clubroot/)

Methods

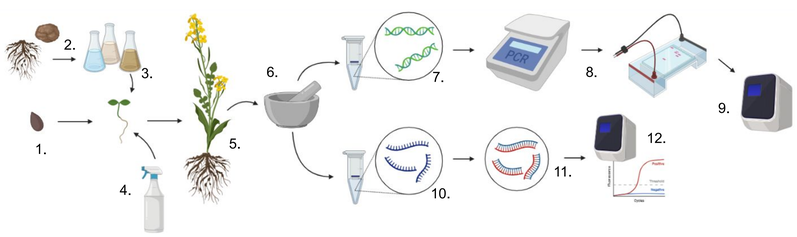

Figure 2. Pathotype 3A inoculation of canola cultivars and downstream methods used to quantify clubroot and assess gene expression. Created with Biorender.com

1. Seeds of three canola cultivars, Granaat (universally susceptible), Laurentian (susceptible to pathotype 3A), and Wilhelmsburger (partially resistant to pathotype 3A), were planted in soil and on filter paper in petri dishes. Seed germination was monitored.

2. Pathotype 3A spores, harvested from galls, were counted using a hemocytometer and diluted to concentrations of 10^7 and 10^5 spores per mL of water (Figure 3).

3. Seven-day-old plants were inoculated with 1 mL of spore solution. The control cultivars were inoculated with 1 mL of water.

4. SA solutions were prepared at concentrations of 1, 5 and 10 millimolar (mM). Plants were sprayed one day post inoculation (dpi), and again at 8, 15, and 22 dpi.

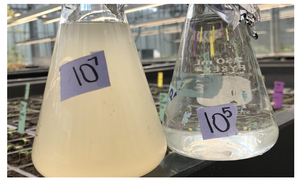

Figure 3. Inoculum concentrations were made at 10^7 spores/mL and 10^5 spores/mL.

5. Roots and galls were harvested from mature plants at 6 weeks and stored at -80° C until use.

6. Roots and galls were ground into a fine powder using baked mortar and pestles and liquid nitrogen.

7. Genomic DNA (gDNA) was separately extracted from ground samples using the Qiagen DNeasy Plant Pro Kit and a CTAB organic method. DNA quantity and quality were measured using a Nanodrop 2000C. The CTAB-extracted samples were used in downstream applications.

8. To preliminarily quantify clubroot, a polymerase chain reaction (PCR) using clubroot-specific primers was conducted on the gDNA samples. The PCR results were visualized on an agarose gel.

9. A quantitative PCR (qPCR) determined the amount of clubroot using clubroot-specific primers, compared to primers targeting a plant housekeeping gene. DNA amplification was measured with StepOnePlus Real-Time PCR System.

10. RNA was extracted using a hybrid TRIzol organic method and Qiagen RNeasy Mini Kit. Sample quality and quantity were measured using a NanoDrop 2000C.

11. Single-stranded RNA was reverse transcribed into double-stranded complementary DNA (cDNA).

12. Expression of the SA-dependent defense response gene, *PR1*, was measured using qPCR. The cDNA was separately amplified with PR1-specific primers and primers designed to amplify a plant housekeeping gene. Relative expression values were calculated using the $2-\Delta\Delta Ct$ method of Livak and Schmittgen (2001).

Results

- Seeds in the greenhouse germinated slower and less successfully than those in the petri dishes (Figures 4a and 4b).
- Granaat germinated slower and with less success than the other cultivars.

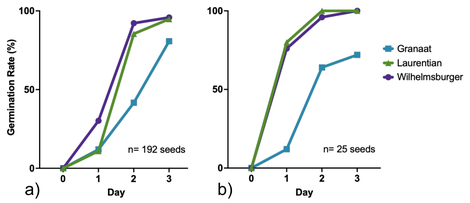

Figure 4. Seed germination rate in a) soil under greenhouse conditions b) petri dish with wet filter paper.

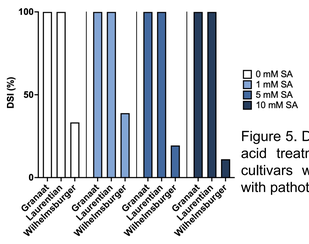

Figure 5. DSI of salicylic acid treatments across cultivars when infected with pathotype 3A.

- The Disease Severity Index (DSI), a way of quantifying the severity of visual clubroot symptoms, was calculated as detailed in Strelkov et al. (2006).
- Only Wilhelmsburger inoculated with pathotype 3A had reduced DSI when treated with 5 and 10 mM SA.

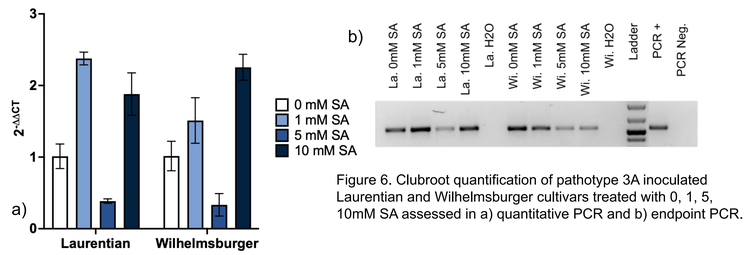

Figure 6. Clubroot quantification of pathotype 3A inoculated Laurentian and Wilhelmsburger cultivars treated with 0, 1, 5, 10mM SA assessed in a) quantitative PCR and b) endpoint PCR.

- In both the qPCR and endpoint PCR, a significant decrease in amount of clubroot was found in both cultivars when treated with 5 mM SA (Figures 6a and 6b).
- The higher amount of clubroot in the 1 mM SA was consistent with the DSI whereas 10 mM SA had reduced DSI but increased clubroot amount.
- Across all SA treatments and cultivars, *PR1* was highly expressed in the pathotype 3A inoculated cultivars relative to the water inoculated controls.
- *PR1* was highly expressed in pathotype 3A infected Laurentian treated with 5 mM SA compared to 0, 1, and 10 mM SA.
- Wilhelmsburger infected with pathotype 3A had a steady increase in *PR1* expression with increasing SA concentrations.

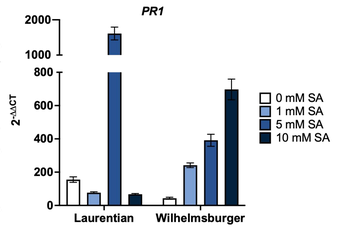

Figure 7. *PR1* expression in pathotype 3A inoculated cultivars in reference to water inoculated cultivars when treated with 0, 1, 5, 10mM SA.

Conclusions

- Laurentian and Wilhelmsburger seeds germinated with similar speed and success rates. Granaat was much slower and had a reduced ability to germinate.
- The SA treatments significantly decreased disease severity in the resistant Wilhelmsburger but did not in the susceptible cultivars.
- The 5 mM SA treatment showed the most promise in reducing the amount and severity of clubroot in canola.
- Upregulated expression of the SA-dependent defense response gene (*PR1*) indicated that the plant responded to the different treatments when inoculated with clubroot.
- Future research includes further biological replicates, the examination of more SA-related genes, and testing of the impact of SA on mature canola plants.

Acknowledgements

A special thanks to WISEST and Dr. Strelkov for their support and sponsorship throughout this project.

Thank you to Tracey Hurtubise, my biology teacher at Archbishop Jordan School, for fostering my love of science and plants and encouraging me in all that I do.

Literature Cited

Lemarié, S. et al. (2015). Both the Jasmonic Acid and the Salicylic Acid Pathways Contribute to Resistance to the Biotrophic Clubroot Agent *Plasmodiophora brassicae* in Arabidopsis. *Plant and Cell Physiology*, *56*(11), 2158–2168. https://doi.org/10.1093/pcp/pcv127

Livak, K.J. et al. (2001). Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method. *Methods, 25*(4), 402-408. https://doi.org/10.1006/meth.2001.1262

Strelkov, S.E. et al. (2006). Characterization of *Plasmodiophora brassicae* populations from Alberta, Canada. *Canadian Journal of Plant Pathology*, *28*(3), 467-474. https://doi.org/10.1080/07060660609507321