Identifying *Phytophthora sojae* pathotypes through baiting and optimizing a new molecular method

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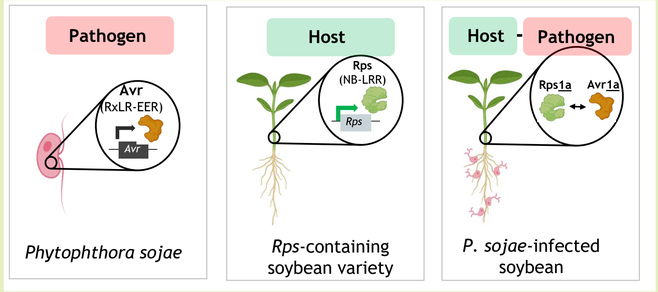
Background

Phytophthora sojae, is a soil-borne oomycete pathogen of global importance. Soybean growers in Minnesota experience **significant yield losses** and **disease management challenges**.

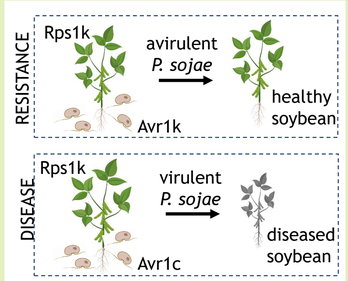
- *P. sojae* attacks plants and seedlings from the roots up, causing root and stem rot, and pre- and post-emergence damping off in poorly-drained soils[1].
- One way we can manage soybean rot caused by *P. sojae* is by planting soybeans that carry **resistance** "Rps" genes against *P. sojae*.
- Soybean Rps proteins can mount a ***strong*** defense response against *P. sojae* pathotypes that express complementary Avr proteins[2].

Our goal is to determine the current *P. sojae* pathotypes that exist in Minnesota using improved molecular methods[3]. This survey will allow us to recommend soybean varieties that could align with the *P. sojae* pathotypes present in the field so that resistance against *P. sojae* is achieved.

- Widely-used, traditional plant pathotyping methods are time-consuming, labor-intensive, and resource-intensive[3].



Plant defends itself **successfully**

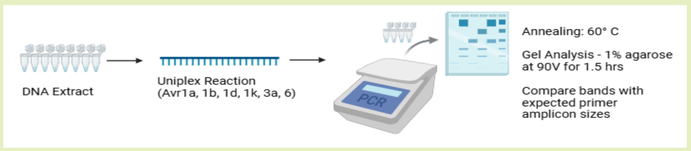


Aims and Methods

Aims:

1. Validate the efficiency of the new molecular method[3] for identifying pathotypes using *P. sojae* isolates from the Dean Malvick Lab.
2. Survey the variety of pathotypes present in Minnesota soils by soil baiting.

Molecular pathotyping via uniplex PCR:



The following *Avr* genes were screened using specific primers[3]:

1. Avr1a-indel
2. Avr1a-snp1
3. Avr1a-snp2
4. Avr1b
5. Avr1d
6. Avr1k
7. Avr3a
8. Avr6

Soil Baiting

Soil baiting workflow. To amass a diversity of pathotypes from around the state, volunteers sent us soil samples from their soybean fields. Seeds of a susceptible variety (Sloan) were surface-sterilized, germinated for 3 days, then transferred to the processed soil samples. After a period of incubation, seedlings were processed and plated on V8-agar media that was selective for *P. sojae*.

Results

Molecular pathotyping via uniplex PCR

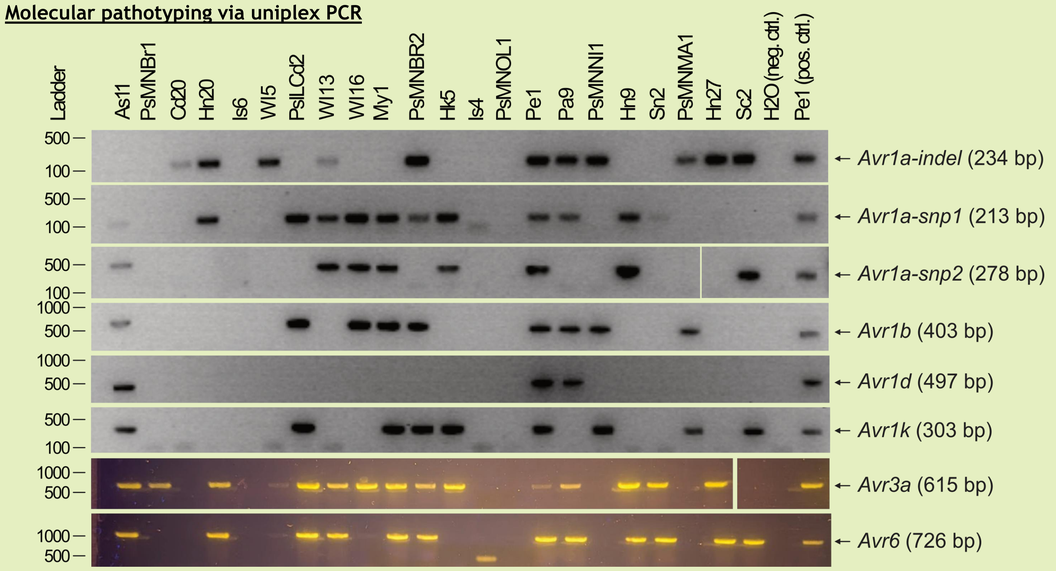


Figure 1. Agarose gel electrophoresis of uniplex PCR of 22 *P. sojae* isolates from Dean Malvick Lab. DNA from cultured isolates were extracted using a protocol developed by Zelaya-Molina et al. (2011).

Isolates were screened for *Avr1b*, *Avr1d*, *Avr1k*, *Avr3a*, *Avr6*, and *Avr1a* (Avr1a-indel, Avr1a-snp1, Avr-snp2 primers). The presence of a band indicates the gene was detected. Experiment was performed once. More biological replicates will be performed.

Molecular Pathotyping - Preliminary Pathotypes

Isolate	Avr1a-indel	Avr1a-snp1	Avr1a-snp2	Avr1b	Avr1d	Avr1k	Avr3a	Avr6	Pathotype
As11		+	+	+	+	+	+	+	1a
PsMNBr1							+		1a, 1b, 1d, 1k, 6
Cd20	+								1a, 1b, 1d, 1k, 3a, 6
Hn20	+	+					+	+	1a, 1b, 1d, 1k
Is6									1a, 1b, 1d, 1k, 3a, 6
Wi5	+								1a, 1b, 1d, 1k, 3a, 6
PsILCd2		+		+		+	+	+	1a, 1d
Wi13	+	+	+				+	+	1b, 1d, 1k
WI16		+	+	+			+		1a, 1d, 1k, 6
My1		+	+	+		+	+	+	1a, 1d
PsMNBr2	+	+		+		+	+	+	1a, 1d
Hk5		+	+			+			1b, 1d, 3a, 6
Is4									1a, 1b, 1d, 1k, 3a, 6
PsMNOL1									1a, 1b, 1d, 1k, 3a, 6
Pe1	+	+	+	+	+	+	+	+	
Pa9	+	+		+	+		+	+	1a, 1k
PsMNNi1	+			+		+			1a, 1d, 3a, 6
Hn9		+	+				+	+	1b, 1d, 1k
Sn2							+	+	1a, 1b, 1d, 1k
PsMNMa1	+			+		+			1a, 1d, 3a, 6
Hn27	+						+	+	1a, 1b, 1d, 1k
Sc2	+		+			+		+	1a, 1b, 1d, 3a

***P. sojae* identified from soil baiting**

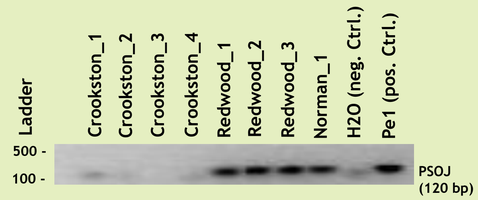


Figure 2. Agarose gel electrophoresis of uniplex PCR of unknown isolates baited from soil sampled from Crookston, Redwood, and Norman counties. Each lane is a separate isolate that was baited from the county. A specific *P. sojae* primer, PSOJ, was used to detect *P. sojae*[6].

Table 1: *Preliminary pathotypes for Dean Malvick isolates.* Plus sign denotes that the *Avr* gene was detected. Blank denotes that the *Avr* gene was not detected. Pathotypes are determined by which *Avr* genes were not detected.

Discussion

I was able to successfully elicit *P. sojae* pathogen from soils around MN. I also was able to complete one round of uniplex PCR pathotyping of our known isolates from the Malvick Lab using the new molecular method. Using this method, we can help farmers decide on what varieties of soybean to plant in their fields in order to combat disease and minimize losses.

Future Directions

1. Perform molecular pathotyping on more biological replicates of the Dean Malvick P. sojae isolates.
2. Continue baiting and pathotyping *P. sojae* from more counties across Minnesota.
3. Test and optimize a multiplex PCR that amplifies multiple *Avr* genes simultaneously.

1. Dorrance, A. E., et al. "Pathotype diversity of Phytophthora sojae in eleven states in the United States." Plant Disease, vol. 100, no. 7, 4 Apr. 2016, pp. 1429-1437, https://doi.org/10.1094/pdis-08-15-0879-re.
2. Tremblay, Vanessa, et al. "Molecular assessment of pathotype diversity of Phytophthora sojae in Canada highlights declining sources of resistance in soybean." Plant Disease, vol. 105, no. 12, 1 Dec. 2021, pp. 4006-4013, https://doi.org/10.1094/pdis-04-21-0762-re.
3. Dussault-Benoit, Chloé, et al. "Discriminant haplotypes of avirulence genes of Phytophthora sojae lead to a molecular assay to predict phenotypes." Molecular Plant Pathology, vol. 21, no. 3, 7 Jan. 2020, pp. 318-329, https://doi.org/10.1111/mpp.12898.
4. Matthiesen, Rashelle L., et al. "Comparison of baiting techniques for recovering Phytophthora sojae from soybean fields in Iowa." Plant Health Progress, vol. 22, no. 3, 1 Jan. 2021, pp. 316-322, https://doi.org/10.1094/php-02-21-0040-fi
5. Zelaya-Molina, L. X., Ortega, M. A., & Dorrance, A. E. (2011). Easy and efficient protocol for oomycete DNA extraction suitable for population genetic analysis. Biotechnology letters, 33(4), 715-720. https://doi.org/10.1007/s10529-010-0478-3
6. Bienapfl, John C., et al. "Specific molecular detection of phytophthora sojae using conventional and real-time PCR." Fungal Biology, vol. 115, no. 8, Aug. 2011, pp. 733-740, https://doi.org/10.1016/j.funbio.2011.05.007.