Minnesota Soybean Research and Promotion Council

Project Final Report

May 30, 2024

***Project Title**:* Characterizing and genetic mapping of virulence phenotypes in a unique collection of soybean cyst nematode inbred lines from Minnesota

Principal Investigator(s): Senyu Chen, Aaron Lorenz, and Cory Hirsch

Project Period: 5/1/2023 – 4/30/2024

***Summary*:** The soybean cyst nematode (SCN, *Heterodera glycines*) is the most damaging pathogen of soybean and is widespread in Minnesota and most soybean-growing regions of the world. As a species, SCN contains significant variation in virulence (ability to reproduce on different soybean lines) and morphology. For this project we study the phenotypic and genotypic diversity of SCN in Minnesota. Specifically, we have phenotyped 182 inbred lines of cyst nematodes, most of which were randomly selected from Minnesota soybean fields, for their virulence phenotypes on the SCN-resistant germplasm lines PI 88788, Peking (PI 548402), Pickett (PI 548988), PI 567516C, PI 438489B, and PI 90763. These germplasm lines contain diverse SCN resistance genes. We also study variations in SCN morphology and conduct whole-genome sequencing on these inbred SCN lines. The knowledge of SCN phenotypic and genotypic diversity will be highly useful for strategically breeding soybean cultivars resistant to soybean cyst nematode with the most effective sources of resistance. This project will advance technology to manage the most destructive pest of soybean and maintain the crop's productivity in Minnesota.

Research Question/Objectives:

Objective 1: *Characterization of virulence phenotypes of inbred SCN lines.*

In this objective, we characterize the virulence phenotypes of 182 inbred SCN lines. Specifically, the reproduction potential as measured with the female index (FI) of the nematode lines is determined on the SCN-resistance sources used or potentially used in public (e.g., UMN) and private soybean breeding programs. The data are used to analyze the diversity of SCN in Minnesota.

Objective 2: *Whole genome sequencing and morphological analysis of inbred SCN lines.*

To address this objective we have sequenced the genomes of 178 inbred SCN lines. We will compare the genomic data to virulence phenotype data to identify factors controlling virulence in the SCN genome. In addition, we will analyze morphological parameters and determine if they are associated with virulence phenotypes and regions of the genome.

Results:

Objective 1: *Characterization of virulence phenotypes of inbred SCN lines.*

A total of 182 inbred lines of the soybean cyst nematodes were used in this study. Most of the lines were developed from SCN field populations that were 'randomly' collected across Minnesota soybean growing counties in 2013 or 2007-2008. The virulence (Female Index, FI) of the SCN inbred lines were tested on SCN-resistant soybean germplasm lines PI 88788, Peking (PI 548402), Pickett (PI 548988), PI 90763, PI 567516C, and PI 438489B. Those soybean lines have various genotypes of SCN resistance.

We have finished tests of 182 SCN lines for their virulence on the six soybean lines (**Table 1**). Percentage of SCN lines, to which a soybean line is resistant (FI < 10), was 46.7% on Pickett, 50.5% on PI 88788, 76.9% on Peking, 77.5% on PI 567516C, 87.4% on PI 90763, and 87.4% on PI 834489B. A total of 8 races were found with 25.3% race 1, 4.9% race 2, 21.4% race 2, 2.2% race 4, 15.9% race 5,

14.3% race 6, 4.9% race 9, and 11.0% race 14 (**Table 2**).

The relative low number (50.5%) of SCN lines with FI <10 on PI 88788 indicates that PI 88788-derived soybean cultivars may have low level of resistance in many fields in Minnesota. Peking-derived cultivars are known to be good in rotation with PI 88788-derived cultivars for managing SCN [1]. Except for Pickett, which was derived from Peking and used for race determination, all other five lines are good alterative sources of resistance for breeding commercial SCN-resistant cultivars. PI 567516C is resistant to 80.0% of the SCN populations, to which PI 88788 is susceptible or moderately susceptible to (FI > 30) (data not shown). Therefore, PI 567516C is a good source of resistance alternative to Peking for rotation with PI 88788-derived cultivars. PI 567516C has been used in UMN breeding program as a new source of SCN resistance.

Objective 2. *Whole genome sequencing and morphological analysis of inbred SCN lines.*

The genomes of 178 inbred SCN lines have been sequenced. The sequencing output has been analyzed and confirmed to be of high quality for all samples. The sequences have been aligned to the reference genome and single nucleotide polymorphisms (SNPs) have been identified. Population structure analyses have been performed to identify clusters of SCN lines that are similar to each other. **Figure 1** shows individual lines that have the genomes from one or more of the four theoretical ancestors.

Genome-wide association studies (GWAS) to identify regions of the genome associated with different virulence phenotypes have been started. GWAS model selection is currently being performed to find a model that balances high statistical power and low error rates in this dataset.

Application/Use:

Soybean cyst nematode (SCN, *Heterodera glycines*) is the most destructive pathogen of soybean and is widespread in Minnesota and most soybean-growing regions of the world. In the U.S., annual soybean yield loss to SCN has been estimated around one billion dollars [2]. Management options for SCN include host resistance, cultural practices such as crop rotation and soil fertility management, and chemical and biological controls [3-5]. Host resistance is the most effective management strategy. However, the effectiveness of SCN resistance depends on the interaction between host (soybean) and SCN genotypes. The genetics of SCN resistance in soybean have been extensively studied, but little is known about the genetics of the virulence of SCN populations. This study will enhance our knowledge of SCN virulence diversity. In addition, we identify regions of the SCN genome associated with virulence and with morphological parameters. The technology can be used to strategically deploy effective types of SCN resistance in soybean cultivars. If virulence genes can be tagged by molecular markers, it is possible that in the future a simple lab test could be used to characterize field populations of SCN, which would help farmers choose the most effective sources of SCN resistance for their fields. The ultimate outcome will be greater availability of robust genetic tools to prevent soybean yield losses and thus minimize risk of loss for soybean farmers.

Materials and Methods:

Objective 1: *Characterization of virulence phenotypes of inbred SCN lines*

A total of 182 inbred lines of the soybean cyst nematodes were used in this study. Most of the lines were developed from SCN field populations that were 'randomly' collected across Minnesota soybean growing counties in 2013 or 2007-2008. To develop an inbred line, a single cyst was transferred to a soybean plant. After 45 days, when the first generation of females (cysts) developed, a single cyst was transferred to a new soybean plant. Each of the cysts and females were developed from fertilization of the siblings within the same parent cyst. After a number of transfers (8 to 24 transfers), the SCN lines are presumably homogenous in genetics. The 182 inbred lines may represent diversity of SCN populations in Minnesota.

The virulence (Female Index) of the inbred SCN lines were tested on SCN-resistant soybean germplasm lines PI 88788, Peking (PI 548402), Pickett (PI 548988), PI 90763, PI 567516C, and PI 438489B. PI 88788, Peking (PI 548402), Pickett (PI 548988), and PI 90763 are SCN race differential lines [6], and all of the six lines have been used for breeding SCN-resistant commercial cultivars in Minnesota and/or elsewhere. PI 88788 and Peking are two major sources of resistance in commercial

soybean cultivars.

The procedures of SCN virulence assays were modified from the previous studies [6, 7]. Briefly, soybeans were planted in 100-mL cone-tainers in the greenhouse and inoculated with 3,000 SCN eggs. Each soybean line was planted to six plants in six separate cone-tainers. After 35 days, the cysts were collected from each cone-tainer and counted. Female Index (FI) was calculated for each plant. FI = the number of cysts (females) on the SCN-resistant soybean line × 100 / the number of cysts on the standard SCN-susceptible soybean line Williams 82.

Objective 2: *Whole genome sequencing and morphological analysis of inbred SCN lines.*

To prepare samples for sequencing, the inbred lines of SCN were cultured SCN-susceptible soybean Sturdy in sterilized soil. SCN cysts were extracted from soybean roots and soil, and hand-picked to separate them from plant and soil debris. The cysts were crushed with a sterile glass crusher to release eggs that were cleaned by centrifugation. Genome DNA was prepared and sequenced using the Illumina® Nextera DNA Flex platform at the University of Minnesota Genomics Center. A total of 178 SCN lines were sequenced. The genomes of the SCN lines were compared to identify single nucleotide polymorphism (SNP) markers which were used to study the SCN genetic diversity and their associations with the phenotypic traits including SCN virulence and morphometrics.

Economic Benefit to a Typical 500 Acre Soybean Enterprise:

The soybean cyst nematode is widely spread in soybean fields in Minnesota and it is the most important biological factor limiting soybean yield [2]. Use of SCN-resistant soybean cultivars is the most common and effective strategy to manage SCN. This study advances the knowledge of SCN virulence diversity and genetics that is useful for soybean breeders to select appropriate sources of resistance and evaluate SCN resistance in breeding commercial soybean cultivars. The knowledge is also useful for soyban growers to use the SCN-resistant soybean cultivars correctly and effectively. There is a high possibility that in future molecular markers of SCN virulence could be developed and used to characterize field populations of SCN, which would help farmers choose the most effective sources of SCN resistance for their fields. With the effective SCN management, soybean enterprise will have economic benefit of improved soybean yield.

References:

1. Chen, S. Y. 2020. Dynamics of population density and virulence phenotype of the soybean cyst nematode as influenced by resistance source sequence and tillage. Plant Disease 104:2111-2122.
2. Allen, T. W., Bradley, C. A., Sisson, J. A., Byamukama, E., Chilvers, M. I., Coker, C. M., Collins, A. A., Damicone, J. P., Dorrance, A. E., Dufault, N. S., Esker, P. D., Faske, T. R., LFaske, T. R., Giesler, L. J., Grybauskas, A. P., Hershman, D. E., Hollier, C. A., Isakeit, T., Jardine, D. J., Kelly, H. M., Kemerait, R. C., Kleczewski, N. M., Koenning, S. R., Kurle, J. E., Malvick, D. K., Markell, S. G., Mehl, H. L., Mueller, D. S., Mueller, D. S., Mulrooney, R. P., Nelson, MNelson, B. D., Newman, M. A., Osborne, L., Overstreet, C., Padgett, G. B., Phipps, P. M., Price, P. P., Sikora, E. J., Smith, D. L., Spurlock, T. N., Tande, C. A., Tenuta, A. U., Wise, and Wrather, J. A. 2017. Soybean yield loss estimates due to diseases in the United States and Ontario, Canada, from 2010 to 2014. Plant Health Progress 18:19-27.
3. Chen, S., J. Kurle, D. Malvick, B. Potter, and J. Orf .2011. Soybean cyst nematode management guide. University of Minnesota Extension. Web/URL: https://extension.umn.edu/soybean-pest-management/soybean-cyst-nematode-management-guide. Access date: 10/31/2023.
4. Niblack, T. L., and Chen, S. Y. 2004. Cropping systems. Pp. 181-206 *in* D. P. Schmitt, J. A. Wrather, and R. D. Riggs, eds. Biology and management of the soybean cyst nematode. Marceline, MO: Schmitt & Associates of Marceline.
5. Niblack, T. and G. Tylka .2008. Soybean cyst nematode management guide 5th edition. Web/URL: https://scn.plantpath.iastate.edu/files/inline-files/SCN_Management_1.pdf. Access date: 10/31/2023.
6. Riggs, R. D., and Schmitt, D. P. 1988. Complete characterization of the race scheme for *Heterodera glycines*. Journal of Nematology 20:392-395.
7. Niblack, T. L., Arelli, P. R., Noel, G. R., Opperman, C. H., Orf, J. H., Schmitt, D. P., Shannon, J. G., and Tylka, G. L. 2002. A revised classification scheme for genetically diverse populations of *Heterodera glycines*. Journal of Nematology 34:279-288.

Publications:

Abstracts and poster presentations:

Docherty, L., Lorenz, A., and Chen, S. 2023. Virulence diversity of soybean cyst nematode in Minnesota. Society of Nematologists Annual Meeting, July 9-14, Columbus, Ohio.

Docherty, L., Lorenz, A., and Chen, S. 2022. Long term storage of *Heterodera glycines* cysts. 2022 Soybean Nematode Conference.

Poster presentations:

Docherty, L., Lorenz, A., and Chen, S. Virulence diversity of soybean cyst nematode in Minnesota. Minnesota Ag Expo. January 19, 2023.

Docherty, L., Lorenz, A., and Chen, S. Virulence diversity of soybean cyst nematode in Minnesota. University of Minnesota College of Food, Agricultural, and Natural Resource Sciences Symposium. March 14, 2023.

Oral presentations:

Docherty, L. Diversity among inbred lines of soybean cyst nematode collected in Minnesota. October 24, 2022, University of Minnesota Department of Agronomy and Plant Genetics Seminar.

Docherty, L. (*presenter*), and Chen, S. Virulence diversity of soybean cyst nematode in Minnesota. North Central Nematology Research Committee Annual Report Meeting, July 14-15, 2023, Columbus, Ohio.

Table 1. Female Index (FI) of the soybean cyst nematode (SCN) inbred lines on the SCN race differential lines and breeding soybean lines.

	Soybean lines					
	Pickett	Peking	PI 88788	PI 90763	PI 438489B	PI 567516C
Total populations	182	182	182	182	182	182
Number of populations with FI < 10	85	140	92	159	159	141
Number of populations with FI < 30	123	159	137	171	176	158
% of populations with FI < 10	46.7	76.9	50.5	87.4	87.4	77.5
% of population with FI < 30	67.6	87.4	75.3	94.0	96.7	86.8
Minimum FI	0.0	0.0	0.0	0.0	0.0	0.0
Maximum FI	107.8	128.1	105.8	71.7	108.7	117.9
Average FI	23.4	9.5	18.6	4.8	4.4	9.9
Median FI	10.6	0.2	9.2	0.0	0.1	0.5

Table 2. Resistance of the soybean cyst nematode race differential and breeding soybean lines to inbred SCN lines of different races. R is resistant to SCN at FI < 10, and MR is moderately resistant to SCN at 10 < FI < 30.

Race	Number of lines	Percent of total lines	Number lines of the race		Percentage lines of the race	
			MR	R	MR	R
			Pickett			
1	46	25.3	0	46	0	100
2	9	4.9	2	0	22.2	0
3	39	21.4	0	39	0	100
4	4	2.2	0	0	0	0
5	29	15.9	16	0	55.2	0
6	26	14.3	15	0	57.7	0
9	9	4.9	2	0	22.2	0
14	20	11.0	3	0	15	0
Total	182	100	38	85	20.9	46.7
			Peking			
1	46	25.3	0	46	0	100
2	9	4.9	7	0	77.8	0
3	39	21.4	0	39	0	100
4	4	2.2	0	0	0	0
5	29	15.9	0	29	0	100
6	26	14.3	0	26	0	100
9	9	4.9	4	0	44.4	0
14	20	11.0	7	1	35	5
Total	182	100	18	142	9.9	78.0

			PI 88788			
1	46	25.3	24	0	52.2	0
2	9	4.9	4	0	44.4	0
3	39	21.4	0	39	0	100
4	4	2.2	4	0	100	0
5	29	15.9	12	0	41.4	0
6	26	14.3	0	26	0	100
9	9	4.9	0	9	0	100
14	20	11.0	1	16	5	80
Total	182	100	46	93	25.3	51.1
			PI 90763			
1	46	25.3	0	46	0	100
2	9	4.9	0	9	0	100
3	39	21.4	0	38	0	100
4	4	2.2	0	0	0	0
5	29	15.9	0	29	0	100
6	26	14.3	0	26	0	100
9	9	4.9	0	9	0	100
14	20	11.0	13	0	65	0
Total	182	100	15	158	8.2	86.8
			PI 438489B			
1	46	25.3	2	44	4.3	95.7
2	9	4.9	1	7	11.1	77.8
3	39	21.4	2	37	5.1	94.9
4	4	2.2	2	0	50.0	0.0
5	29	15.9	0	29	0.0	100.0
6	26	14.3	0	26	0.0	100.0
9	9	4.9	3	6	33.3	66.7
14	20	11.0	7	9	35.0	45.0
Total	182	100	19	159	10.4	87.4
			PI 567516C			
1	46	25.3	2	37	4.3	80.4
2	9	4.9	3	4	33.3	44.4
3	39	21.4	4	32	10.3	82.1
4	4	2.2	1	2	25.0	50.0
5	29	15.9	2	25	6.9	86.2
6	26	14.3	2	24	7.7	92.3
9	9	4.9	1	3	11.1	33.3
14	20	11.0	1	13	5.0	65.0
Total	182	100	16	142	8.8	78.0

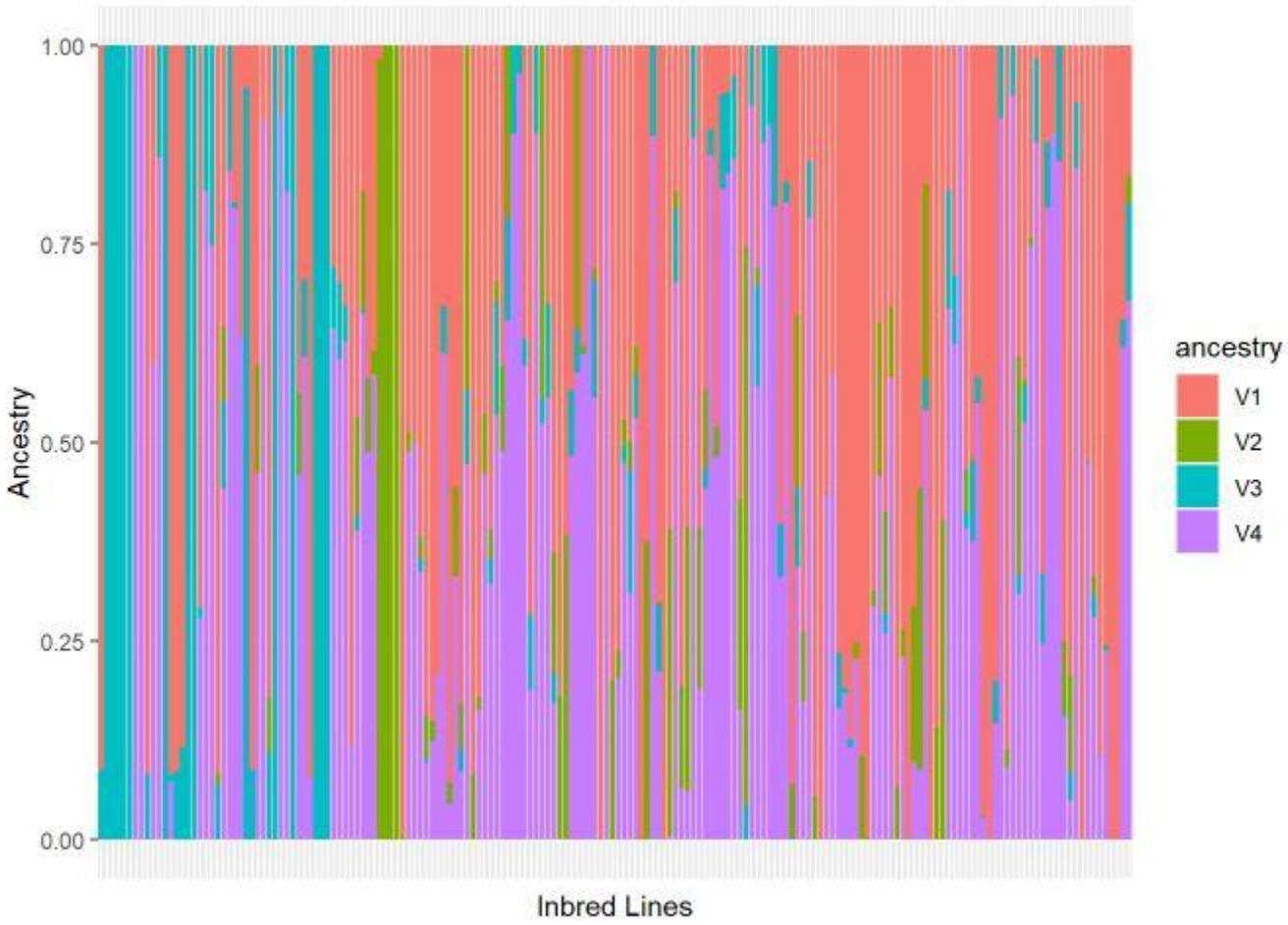

Figure 1. Plot created using ADMIXTURE software. This software estimates the proportion of each sample that is descended from a number of theoretical ancestral populations (in this case, 4 theoretical ancestral populations were used). Each vertical line in the figure represents an inbred line from the study. Each of the colors in a line represents the proportion of the genome that is from each theoretical ancestor.