North Central Soybean Research Program

An integrated approach to enhance durability of SCN resistance for long term strategic SCN management

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The soybean cyst nematode, *Heterodera glycine*s, (SCN)) is the most damaging pathogen of soybeans in North America. Though SCN-resistant soybean varieties frequently are available to minimize yield loss, producers are faced with limited options for rotation once virulent SCN populations develop in their fields.

The widespread lack of genetic diversity in SCN resistance in soybean has significantly increased the prevalence of virulent SCN populations and reduced the effectiveness of current sources of resistance. Thus, we have two major research challenges that, when successfully achieved, will enable us to develop more efficient management practices for this pest in the future:

(1) Plant breeders need to increase the genetic diversity of SCN resistance in commercially available SCN-resistant soybean cultivars and work with nematologists to determine the most effective rotation practices that preserve these sources,

(2) Nematologists need to complete the SCN genome (genetic blueprint of the nematode) assembly to facilitate the identification of nematode genes required for the adaptation to reproduce on resistant cultivars, use these as markers to monitor nematode population shifts in the field, and exploit this knowledge to help plant breeders identify novel sources of resistance.

Project Objectives

1. Diversify the genetic base of SCN resistance in soybean

A population segregating for Rhg1 and Rhg4 from PI 437654, the genes on chromosomes 15 and 18 from *G. soja*, and a gene on chromosome 10 from PI 567516C will be yield evaluated in two locations to test for the impact of these resistance genes on yield. Breeding will be done to incorporate new combinations of resistance genes into high yielding genetic backgrounds.

2. Identify SCN virulence genes to better understand how the nematode adapts to reproduce on resistant varieties

Create and release to collaborators draft genomes of at least five nematode HG types. Further develop SCN-Base cyberinfrastructure, GBrowse, tracks, using existing draft assembly and available SNP data/transcript data.

Further inbreed nematodes on soybean with different resistance genes and generate sufficient material for sequencing.

Extract DNA and RNA, begin genome resequencing and transcriptome analysis of virulent SCN populations selected on different resistance genes.

3. Determine what combinations of resistance genes would be beneficial in variety rotations to enhance the durability of SCN resistance in soybean.

Year 1 field trials to evaluate different rotation schemes for effectiveness at reducing population densities and the selection pressure on the nematode population.

4. Translate the results of objectives 1-3 to the SCN Coalition to increase the profitability of soybean for producers.

5. Coordinate the testing of publicly developed SCN resistant experimental lines.

<u>Reporting Period Accomplishments</u>

Objective 1: Diversify the genetic base of SCN resistance in soybean

Progress has been made in developing varieties with alternative sources of resistance.

In the 2018 SCN MG III prelim regional test, the second highest yielding line had resistance from PI 88788 combined with the chromosome 15 and 18 genes from *G. soja.* In the MG IV test, the second highest yielding line also had the same gene combination. The test of the population segregating for resistances genes Rhg1, Rhg4, the two *G. soja* genes, and the gene on chromosome 10 was completed in two locations in both Illinois and Missouri.

When the effects of the resistance genes were analyzed across the field locations, we found that only Rhg4 was associated with significantly greater yield, whereas the other genes had no detectable impact on yield or a significant negative impact on yield. One issue with these tests is that the nematode populations were low in the experimental

locations, which made it difficult to detect a positive impact for having SCN resistance.

Early generation populations segregating for Rhg1 and Rhg4 and the chromosome 11 QTL derived from cultivars in the mid-south (i.e. S11-20124C released by Shannon et al., 2019) which have sources of each of the targeted genes and QTL from PI 90763, PI 437654, and/or PI 88788 have also been developed.

Objective 2: Identify SCN virulence genes to better understand how the nematode adapts to reproduce on resistant varieties

We have successfully accomplished alignment of the first version of the SCN genome and we have published it in the journal of "BMC genomics". We have also completed and published detail analysis of "spliced leader preference in SCN" (*Heterodera glycines* utilizes promiscuous spliced leaders and demonstrates a unique preference for a species-specific spliced leader over *C. elegans* SL1. Scientific Reports 2019, 9; 1356). Currently we are working on scaffolding a second SCN assembly with Chicago and Hi-C to get closer to a pseudomolecule assembly.

We have sequenced the genomes of five virulent SCN inbred populations that differ in their ability to reproduce on resistant varieties (i.e., different HG types). All primary raw sequencing data from all strains and technologies has been deposited under embargo into NCBI SRA repository with required BioProject and BioSample accessions.

The adapted populations under Obj 2.2 have also been further selected, typed, and are currently being reared for collection of nematode material for sequencing.

Simultaneously, we continue to work on improving our SCNBase website. We have added tutorials to SCNBase describing how to upload different data types to the web repository. We have also generated a Github repository for all the scripts needed to create files for SCNBase. The SCNBase paper has been written and is currently under review by collaborators before submission to the "Database" journal.

In support of Objective 2, we have been working on the development of transcriptomic resources from an avirulent and a highly virulent population of SCN. We have isolated esophageal gland cells from each of these populations and have constructed RNA-seq libraries from pools of 100 gland cells from each population. Our goal is to have three independent biological replications of each population. Once we complete our independent biological replications for each population, we can conduct more in-depth

studies of transcript differences between these populations and how that may relate to virulence.

We have performed sequencing of some of these libraries and have confirmed their utility in allowing us to validate the candidacy of recently discovered novel effector candidates from our prior work (Novel global effector mining from the transcriptome of early life stages of the soybean cyst nematode *Heterodera glycines*. Scientific Reports 2018; 8:2505). Several novel candidate effector candidates have been cloned utilizing the SCN genome sequence. Additional sequence analysis and confirmation of gland expression is underway.

The successful SCN parasitism relies on syncytium establishment and its continued maintenance via long-term host defense suppression. These complex molecular tasks are accomplished by the SCN by injecting a suite of effectors into the host tissue. As a part of this project, while we continue to identify novel and population specific effectors or effector variants, we are conducting in-depth molecular characterizations of the previously identified effectors. We have published our results so far in a high impact, peer-reviewed journal (An Effector from the Cyst Nematode *Heterodera schachtii* Derepresses Host rRNA Genes by Altering Histone Acetylation. The Plant Cell 2018, 30 (11) 2795-2812).

Objective 3. Determine what combinations of resistance genes would be beneficial in variety rotations to enhance the durability of SCN resistance in soybean.

The project investigators have established the details of the field experiments to be conducted in Illinois, Missouri, and Iowa in 2019 in order to address this objective.

Objective 4. Translate the results of objectives 1-3 to the SCN Coalition to increase the profitability of soybean for producers.

The SCN Coalition is a national educational campaign funded by NCSRP, USB, and private industry to educate farmers and agribusiness personnel who advise farmers about the current situation with SCN resistance as well as about new research developments, including new and genetically divers sources of SCN resistance.

A news conference was organized by the SCN Coalition at Commodity Classic in Orlando on February 28, 2019. At the press conference, project PIs presented the six goals of the recently released "National Soybean Nematode Strategic Plan".

Goal 2 of the plan is to "Discover, leverage and enhance native nematode resistance in soybean." Our current NCSRP-funded research project was mentioned at the press conference as an example of work under goal 2 of the strategic plan. See Soybean checkoff organizations team up to tackle nematodes via National Soybean Nematode Strategic Plan (press release February 2019).

Objective 5. Coordinate the testing of publicly developed SCN resistant experimental lines.

During the past 6 months, results from the 2018 trials were analyzed, summarized into a report and provided to cooperators and other interested parties. Because of the need for breeders to make quick decisions on advancing experimental lines, we made the preliminary analysis available as soon as possible after harvest.

In 2018, 182 experimental lines and checks were grown in 39 locations in 11 states and one Canadian province. In addition, soil samples from the test locations were analyzed for SCN egg number and HG type and the lines in the test were evaluated for resistance to two SCN isolates.

All soil samples had an HG type of 2.5.7 except one that had an HG type 1.2.5.7. This means that at all test locations the nematodes can overcome resistance from PI 88788. The egg counts from locations ranged from 40 to 8160 eggs / 100 cc of soil.

Progress has been made in developing varieties with resistance other than from PI 88788. In the MG II test, the highest and second highest yielding lines have high levels of resistance from PI 437654 to an HG 2.5.7 population. As mentioned previously, the second highest yielding line in the MG III prelim test and the MG IV test had resistance from PI 88788 combined with the chromosome 15 and 18 genes from *G. soja*. The 2019 test has been planned and seed of lines in the test will be distributed to test cooperators soon.