**Final report**

**Project Title:** Development of a rapid SCN virulence test

**Lead PI:** Kris Lambert**,** knlamber@uiuc.edu

**Co-PI:** Khalid Meksem**,** meksemk@siu.edu

**Co-PI:** Silvia Cianzio, scianzio@iastate.edu

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**Issue and objectives:**

*Heterodera glycines*, commonly called the soybean cyst nematode (SCN), is a chronic disease of soybean that lowers yields throughout the North Central United States, costing soybean producers over 1 billion dollars a year. There is a critical need for effective management approaches to lower SCN populations and prevent nematode mediated soybean yield loss. Soybean cyst nematode (SCN) is currently managed with rotations of non-host crops and the use of SCN resistant soybean cultivars. SCN resistant soybean can be effective, but since SCN is genetically diverse and adaptable, virulent nematodes (SCN that grow and reproduce on a resistant plant) represent a problem. This is also due to the over reliance upon one source of SCN resistance from soybean PI88788. However, if virulent SCN accumulation could be monitored in a cost effective way, then the most effective source of SCN resistance could be planted, eliminating much of the SCN damage. Currently SCN virulence is measured via the use of the Hg Type test. This greenhouse procedure tests a SCN population for virulence based upon growth on a set of known SCN resistant soybean plants. The Hg Type does work, but since it is a bioassay conducted in a greenhouse it takes one to two months, or longer, to complete. This project has the aim of developing an inexpensive quantitative virulence test, based upon the use of nematode virulence genes, that will take only hours to complete.

A critical feature of this management strategy is the need for different sources of resistance. In the past, most soybean contained resistance derived from PI88788. While other sources of SCN resistance existed, they were less frequently bred into high yielding soybean varieties. However, therecent isolation of SCN resistance genes from the Peking source of resistance (the Rhg4 resistance gene), suggests soon other sources of resistance will be available. The Rhg1 resistance gene from PI88788 was also recently isolated, suggesting different forms PI88788 resistance may also soon be on the market. However, even if new sources of SCN resistance are deployed, it will still be critical to match these varieties to a given field population of SCN. For example, if a soybean grower planted a PI88788 source of SCN resistance, but their SCN population on the farm was Hg Type 2 (2 refers to SCN that are able to grow on PI88788), then this would mean that the nematodes would grow and damage the plants. In this case, a Peking derived resistance would be the most effective and would prevent nematode reproduction. Such information will allow soybean producers to rotate resistance to suppress virulent SCN populations. This type of active “SCN management practice” will minimize SCN damage in a cost effective way and will also preserve valuable SCN resistant germplasm, which in turn will lead to a sustainable SCN management strategy.

This project has two main goals:

1) Identify new SCN virulence genes that help the nematode suppress or evade soybean resistance conferred by the Rhg4 resistance gene and other novel resistance gene combinations.

2) Test SCN virulence gene candidates for their ability to predict virulence in field SCN populations.

3) Test different combinations of SCN resistance genes to identify differential selection of virulent SCN

Accomplishments:

1. Using a genomic method to associate SNPs in the SCN genome to the virulence phenotype (allelic imbalance analysis) three putative SCN virulence genes were identified. These genes appear to regulate SCN reproduction on PI88788 type resistant plants. The method used to identify the genes utilizes a custom SNP array that was generated using past funding from NCSRP. Since this method of SCN virulence gene identification was successful for PI88788-type resistance, it was deemed important to expand its use to different types of SCN resistant plants and combinations of SCN resistance genes. This will determine if different SCN virulence genes are utilized on different types of resistant plants. The Co-PIs on this project, the plant resistance gene experts Khalid Meksem and Silvia Cianzio, assembled a set of soybean plants that have different combinations of SCN resistance genes. During this reporting period, we initiated the allelic imbalance analysis of this larger set of resistant plants to measure SCN virulence gene frequency changes on different SCN resistant backgrounds. In our previous study on PI88788-type resistance, we did observe SCN growth on PI88788-type resistant soybean. In fact, growth of SCN on resistant plants is expected since the nematode population used in the allelic imbalance analysis is derived from a cross of virulent SCN (HgType 1, 2, 3, 4, 5, 6, 7) to non-virulent SCN (HgType 0). This means that both virulent and non-virulent SCN should be in the population used for allelic imbalance analysis. However, when the soybean plants containing the new combinations of resistant genes were tested in the allelic imbalance analysis, several resistant plant types were much more resistant to the SCN than others. Since the virulent SCN parent used forthe allelic imbalance analysis can grow on all the resistant plants, it suggests certain combinations of SCN resistance genes act synergistically to prevent SCN reproduction. This is an exciting result since it suggests existing SCN resistant germplasm, different from PI88788, is very effective at suppressing virulent SCN. The genotyping of the SCN selected in the allelic imbalance analysis indicated the main virulence gene that differed in this experiment was the SCN BioB gene. This nematode enzyme is responsible for converting dethiobiotin to biotin, an essential vitamin for the nematode. This data suggests a rotational plan, involving PI88788 and Peking resistance, should suppress the growth of virulent SCN.
2. Once a SCN virulence gene candidate has been identified, the next step is to identify DNA sequence variation between virulent and avirulent SCN and the useof this information to devise a rapid DNA-based assay to predict SCN virulence. In our initial allelic imbalance screen, three potential virulence genes that mapped to two loci in the SCN genetic map were identified. One of these genes seemed to encode a new type of SCN effector protein, a SCN SNARE mimic protein we named HgSLP-1, so it was selected for initial conversioninto a rapid virulence assay. This HgSLP-1 gene was unusual in that the gene appeared to be absent in virulent nematodes, but present in non-virulent SCN. The observation that HgSLP-1 binds to the PI88788 alpha-SNAP resistance gene, suggests that losing the gene may be a mechanism that allows the nematode to avoid a soybean resistance mechanism. However, this extreme DNA deletion made it easier to detect the polymorphism. The HgSLP-1 virulence assay consists of two quantitative PCR (QPCR) tests, one to the target HgSLP-1 gene and another to a control gene that is not deleted in SCN. The ratio of the copy number of the two genes reflects their frequency in a given nematode population. In this assay, if the ratio of the two genes is nearly equal then the HgSLP-1 gene is frequent in the population and the nematode cannot grow on resistant plants. However, if the ratio is low, approaching zero, then the HgSLP-1 gene is low in the SCN population and the nematode can grow on resistant plants. This assay was first validated using well-characterized virulent and avirulent laboratory populations of SCN. Figure 1 contains the results of the assay and shows that low signals perfectly correlate with the virulence phenotype on three unrelated virulent SCN populations, while higher signals are obtained for two avirulent SCN strains. The QPCR assay currently takes less than 2 hours to complete and is capable of assaying less than a single SCN cyst worth of DNA.

Since the rapid virulence test provided excellent results for laboratory populations of SCN, the assay was extended to testing SCN isolated from fields in Illinois and Iowa. In the field tests, SCN cysts were extracted from 100 cc of soil and then DNA was purified from the cysts (1-20 cysts/soil sample). The DNA was subjected to the rapid QPCR-based virulence test. The results for 14 different locations (Figure 2) look similar to the laboratory strain tests suggesting the virulence gene used in the assay is highly variable in field populations. In this field test, samples 1, 3, 7, 9, 10 would be predicted to be the most virulent nematodes, but importantly samples 2, 4, 6, 12 gave an avirulent signal and thus should be easy to control via the use of PI88788 type SCN resistance. The other sampleswere predicted to be more mixed virulent and avirulent SCN. We grew SCN from the 14 sample locations on susceptible and PI88788 resistant soybean. As expected, the SCN populations with the lowest HgSLP-1 gene levels, grew the best on the PI88788 type resistance. As other SCN virulence genes are identified, this type of analysis will be conducted on them as well to validate the rapid virulence test. We expect all three virulence genes, in different combinations, will be required to predict all HgTypes of virulent SCN in field populations of SCN.

3. We also received 5121 packets of soybean seed with different combinations of SCN resistance genes from of Silvia Cianzio’s laboratory. These seeds were grown and genotyped for known SCN resistance genes. The plan was to incorporate these different combinations of SCN resistance genes into our SCN virulence gene identification/selection studies, but since the grant was not funded for a second year (as originally proposed), this final goal was not completed. We hope to complete this phase of the project using funds from the United Soybean Board.

Figure 1 Relative QPCR of DNA extracted from laboratory populations of SCN. The QPCR assay measures the frequency of a SCN virulence gene in each nematode population. The smaller the bars on the graph are predicted to be the more virulent SCN populations. TN10 and OP25 are non-virulent, while TN20, OP20 and OP50 are highly virulent. The Y-axis shows the delta-delta Ct values, while the x-axis shows the sample name.

Figure 2 Relative QPCR of DNA extracted from field isolates of SCN from 14 different locations. The QPCR assay measures the frequency of a SCN virulence gene in each nematode population. The smaller the bars on the graph are predicted to be the more virulent SCN populations. The Y-axis shows the delta-delta Ct values, while the x-axis shows the sample name.