North Central Soybean Research Program

Engineered resistance to soybean cyst nematode via induced gene silencing (RNAi)

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Soybean cyst nematode (SCN) continues to be the top biotic stress causing losses in excess of 100 million bushels annually. Incorporation of natural genetic resistance has been only partially effective because it is apparent that the variations within the nematode populations adapt to the new cultivars and overcome resistance genes within a few years.

The primary goal of this research project is to establish a new set of biotech traits that have durable resistance to soybean cyst nematode (SCN). Turning off genes by a process known as RNA interference (RNAi) has tremendous potential as a new strategy to increase nematode resistance. Past research with other nematode species has demonstrated the scientific merit of the technique. This project will investigate opportunities to silence specific SCN gene sequences using RNAi in transgenic soybean providing a durable genetic material that will be lethal to SCN populations.

Project Objectives

- Engineer stable transgenic soybean plants with traits that can silence specific nematode genes;
- Complete the production of stable transgenic soybean plants with traits that can silence specific nematode genes;
- Perform bioassays with the engineered plants to confirm the effectiveness of the level of SCN resistance; and
- Examine the durability of the transgenic traits on single and diverse populations of SCN.

Reporting Period Accomplishments

We have selected four transgenic soybean lines that have shown consistent improved resistance to SCN. Two lines that target a specific nematode gene were able to reduce the number of SCN cysts by 50-60% and the number of SCN eggs by 55-70%, compared to the control. Two lines targeting a second gene were able to reduce both cyst and egg densities by 50 to 70% compared to the control. These four transgenic lines have been increased for more seeds for future studies.
Three bioassays have been conducted over the past year to confirm the resistance of transgenic lines targeting these two genes. The results from these independent

experiments confirmed that stable transgenic lines significantly reduce the survival rate of SCN Race 3 on host soybeans.

We also measured the expression levels of target genes within eggs that were isolated from cyst feeding on our transgenic lines. RNA was isolated from eggs derived from two separate transgenic soybean lines and we found that the expression levels of the two targeted SCN parasitism genes were significantly suppressed compared to the controls (SCN eggs recovered from non-transgenic plants). The reduction rates ranged from 3.5 to 4.8-fold for one gene and 4.2 to 5.5- fold for the second, compared to controls. This finding is additional evidence that the host-induced RNA interference can regulate the gene expression of the soybean cyst nematode and reduce the survival rate of the SCN population.

From the results of RNA-sequencing analysis, it appears that the resistance resulting from siRNAs expression is dependent upon the amount of siRNA molecules in the host plant and that there is a certain threshold expression needed to elicit a resistant phenotype. We have sequenced two of our target genes from a number of HG types and have demonstrated that the genes are highly conserved, which tells us we are working with genes that are unique and essential, and not likely to change.

We are preparing a manuscript on these latest results and will post a link on this website when available. An earlier research publication, *Host-derived suppression of nematode reproductive and fitness genes decreases fecundity of Heterodera glycines,* has been published in *Planta*