## **Strengthening A Billion-Dollar Gene In Soybeans**

## URBANA, ILL.

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Diers and Hudson, with researchers at Wisconsin and the University of Nebraska, have been studying an area on chromosome 18 called Rhg1 (Resistance to H. glycines) that is known to be the location of the main source of SCN resistance. Rhg1 disrupts the formation and maintenance of potential nematode-feeding sites on plant roots.

Most SCN-resistant soybeans in the Midwest are bred to contain Rhg1, but no one knew the DNA sequence of the gene that was responsible for the resistance. Diers wanted to find it.

"You could say it's a billion-dollar gene because it's in many varieties, it's widely used, and it's protecting varieties against these nematodes," he explained.

Using fine mapping, which is a technique that involves mapping genes in a very constrained area, Diers narrowed the search down to a few gene candidates. At that point, Hudson and Bent got involved in the analysis.

By then, the soybean genome sequence had been completed, greatly facilitating their research. "It became possible to know which genes were within the genetic intervals that people had historically used to confer traits like nematode resistance," Hudson said.

"When we had the genome sequenced, most people were shocked by how many genes there were in regions that people considered to be one gene," he continued. "By doing these fine-mapping experiments, you could get it down to a smaller number of possible genes."

There was, however, one big problem: the soybean that had been sequenced was not nematode-resistant.

"So, however many genes there were in the Rhg1 interval, we knew that the gene that actually makes the plants nematode-resistant wasn't there," Hudson said.

They went back to the nematode-resistant line and sequenced the genome in the interval. When they finished, they saw something very unusual. Rather than finding a gene in the resistant line that was not present in the susceptible line or changes in a gene that was present in both, they saw that a group of four genes had been replicated several times.

With further work, they found that nearly

every soybean variety that is known to be SCN resistant has more than one set of these genes. The Peking variety has three copies of this group, and the Fayette variety has 10. The susceptible variety, Williams, has only one copy.

The Wisconsin researchers used a technique called Fiber-FISH to show that the genes make soybeans nematode-resistant. It allowed them to look into the DNA molecule and count the number of genes in a row. They also found that levels of expression of these genes were higher where there were more copies of the genes.

They artificially increased the expression rates of three of the genes together on soybean roots and were able to replicate the resistance effect. They were not able to replicate the effect using any of the genes on its own.

The results are interesting from a scientific point of view because having several genes next to each other that control the same trait is unusual in multicellular organisms. So is having an effect that is clearly due to multiple repeats of a stretch of DNA.

"We think we've found a new mechanism for plant resistance," Hudson said. "It's not a question of the presence versus the absence of a resistance gene, it's a question of the level of expression of these genes."

The practical implication of this study is that it suggests a way to engineer artificial resistance that is stronger than natural resistance. The researchers have received a grant from the United Soybean Board to pursue this.

The Soybean Disease Biotech Research Center at the U of I provided funding for this project.  $\Delta$ 



A plant infested with SCN in the field. The little white dots on the roots are female nematodes that have emerged from the roots.