

BROADENING RESISTANCE OF SOYBEAN TO THE SOYBEAN CYST NEMATODE USING BIOTECHNOLOGY

Benjamin Matthews^{1,2}, Vincent Klink¹ and Nadim Alkharouf^{1,2}

¹USDA, ARS, Plant Science Institute, Soybean Genomics & Improvement Laboratory, BARC-West, Beltsville, MD 20705, USA; ² School of Computational Sciences, George Mason University, Manassas, VA 20110 USA. matthewb@ba.ars.usda.gov

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The soybean cyst nematode (SCN), *Heterodera glycines*, is the major pest of soybean and causes an estimated one-half to one billion dollars in damage each year in the US. Chemical control for soybean is not normally used because economic and environmental costs are prohibitive. Therefore, agricultural practices, such as crop rotation and the use of resistant cultivars, are used to mitigate the damage of SCN. There is usually a trade off between using the highest yielding soybean varieties, which are susceptible to SCN, and lower yielding SCN-resistant varieties. In the soil, SCN populations are diverse and are composed of numerous phenotypes or races. This variability in the SCN population complicates the effective use of cultivars resistant to SCN by farmers to control SCN in the soil and confounds rotation schemes.

My research goal is to engineer transgenic soybean using multiple approaches to provide the farmer with high yielding soybean varieties with broad, durable resistance to SCN. Durable resistance of soybean to SCN may require the use of more than one gene and strategy. Two promising approaches are (a) the use of genes expressed in roots of soybean during the resistance response and (b) silencing SCN genes *in vivo* to produce a lethal phenotype or dramatically impede SCN survival and reproduction.

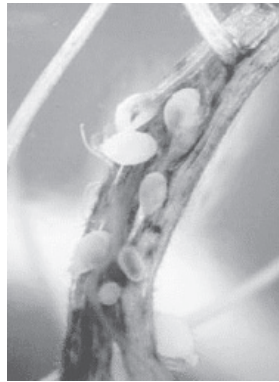


Fig. 1. Soybean cyst nematode, *Heterodera glycines*, females emerging from a susceptible soybean root approximately three weeks after inoculation.

For the first approach, my laboratory constructed several cDNA libraries for expressed sequence tag (EST) analysis. cDNA libraries were constructed from soybean cv. Peking at 12 h, 2 to 4 days and 6 to 8 days post invasion and showing the resistant reaction. Over 3400 inserts were sequenced, analyzed, and compared with ESTs from uninfected roots (Alkharouf et al. 2004).

The ESTs and other clones were used to construct microarrays to identify soybean genes expressed in soybean cv. Kent roots inoculated with SCN race 3 (susceptible response; Matthews 2004; Khan et al. 2004; Alkharouf et al. 2006, in press). Roots were trimmed and harvested from two independent biological samples of Kent at 6, 12h, 1, 2, 4, 6, and 8 days after inoculation. We used RT-PCR to confirm that WRKY6 transcription factor, trehalose phosphate synthase, EIF4a, Skp1 and CLB1 were induced across most time-points. Lipoxigenase, calmodulin, phospholipase C, metallothionein-like protein, and

chalcone reductase were also induced across most time-points. Transcripts encoding Kunitz trypsin inhibitor and sucrose synthase were also at higher levels during the first 12 hours of infection as indicated by RT-PCR, as were the stress-related gene, SAM-22, phospholipase D and 12-oxophytodienoate reductase. Also, there was an abundance of transcripts encoding genes involved in transcription and protein synthesis at 6 and 8 dpi. Some of those genes included ribosomal proteins, and initiation and elongation factors. Several genes involved in carbon metabolism and transport including glyceraldehydes 3-phosphate dehydrogenase, fructose-bisphosphate aldolase and sucrose synthase were more abundant also.

For the same time points we also examined Peking inoculated with SCN race 3 (isolate NL1-RHp; resistant reaction) and from roots inoculated with SCN race 14 (isolate TN8; susceptible reaction) at the same time points (Klink et al, in preparation). This provided data using the same soybean genotype, but using different SCN isolates. Although this data is still being analyzed, a number of genes appear to be expressed higher in resistant roots as compared to susceptible roots. Several genes related to plant defense were identified as more strongly expressed in the resistant roots as compared to susceptible roots.

In addition, we used laser capture microdissection (LCM) to collect syncytial cells (feeding cells) formed by SCN (Klink et al. 2005). Roots of soybean cv. Kent were inoculated with SCN race 3 (susceptible response) and allowed to develop feeding sites (syncytia). Syncytial cells were isolated and collected using LCM. RNA was extracted from the isolated syncytia, linearly amplified, and used to make a cDNA library. Expressed sequence tags (ESTs) were produced and analyzed. RT-PCR was used to measure transcript levels of several genes expressed in the syncytia. RT-PCR analysis indicated enhanced expression of GmTubA1, GmTubB4, GmPIP2,2, aquaporin and several other genes including a pathogen resistance gene in syncytium-enriched samples as compared to samples extracted from whole roots. Antibody to tubulin was used to demonstrate increased tubulin present in syncytial cells.

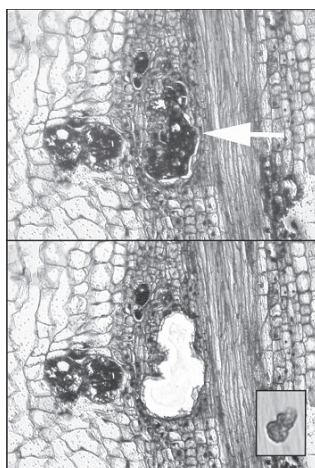


Figure 2. Micrograph of a longitudinal section of a soybean cv. Kent root infected with SCN (susceptible reaction). Arrow in top panel indicates the location of the syncytium. Lower panel shows the precise removal of the syncytium while leaving other root cells behind. The inset in lower panel shows the captured syncytial cells that are now in the cap of a microfuge tube.

Gene and microarray databases were constructed (Alkharouf and Matthews 2004) and posted on-line. Although raw microarray data is available at this web site, we also provide on-line analytical processing capabilities (OLAP), so scientists can mine the data without importing the data into third-party software (Alkharouf et al. 2005). Analysis of these data identified a number of genes we are pursuing further to determine their role in the defense

response and if they can be used to broaden resistance of soybean to SCN. See our web site at <http://bldg6.arsusda.gov/benlab/> for further information.

For my laboratory's second approach, we used bioinformatics to identify SCN genes that may be lethal if silenced or mutated. We then genetically engineered soybean roots to produce RNA interference (RNAi) targeted to these SCN genes, and are currently challenging the transformed soybean roots with SCN to determine if SCN is no longer able to complete its life cycle (Alkharouf et al. submitted). The *Caenorhabditis elegans* gene database was compared with the SCN EST database to identify sequences of high similarity. SCN homologs of *C. elegans* genes, that cause paralysis or lethality when mutated or silenced, were identified and used to design gene silencing constructs for transformation of soybean roots with *Agrobacterium rhizogenes* (hairy root; Fig. 2).

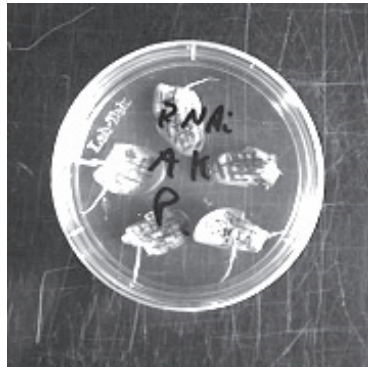


Figure 3. Soybean cotyledons transformed with *A. rhizogenes* strain K599 harboring plasmid pKYLX-GUS.

To further our studies with soybean, we developed a “model” soybean plant that is smaller and has a faster generation time than soybean cultivars planted by farmers. This soybean, MiniMax, is an F9 derived line, is approximately 22 cm tall, and matures in the field or greenhouse in 73 to 85 days. We believe it will be useful to scientists interested in mutant screening, transformation, and other procedures that require high throughput of soybean in restricted space and rapid generation time.

In conclusion, we identified soybean genes that are potential candidates for over-expression in soybean roots and we identified SCN genes that are potential targets for RNAi gene silencing to broaden resistance of soybean to SCN. We are testing DNA constructs in soybean root and challenge those roots with SCN. In the future, several constructs providing resistance to SCN may be combined to generate a composite transgenic soybean plant containing pyramided genes to make it more difficult for SCN to overcome resistance.

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