

2008 Project Summary

PROJECT TITLE: Facilitating Breeding Cotton for Reniform Nematode Resistance
(Agreement No. 07-936AL)

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We produced F₃ families from individual F₂ plants, and began screening these F₃ families for reniform nematode resistance in the greenhouse using an inoculation assay. These populations were also advanced in the field this summer to increase seeds, and will begin screening of F_{2:4} lines very soon.

While the screening was ongoing, we perfected a protocol for obtaining total RNA from cotton roots, and grew plants of 4 cultivars (Delta Pearl, Suregrow 747, FiberMax 966, and Paymaster 1218) and 2 genotypes of resistant accessions (TX245 and TX1419). We infected these plants with reniform nematode, and collected samples at 0 days (uninfected), 1 day, 3 days and 5 days post infection. mRNAs have been prepared from these 6 genotypes pooled as 4 susceptible genotypes and 2 resistant genotypes at each of the 4 time points. The 0 day points are considered uninfected plants, and the 1, 3, & 5 days post-infection samples were considered infected plants. cDNA has been prepared from each of these 4 treatments (i.e. susceptible uninfected, susceptible infected, resistant uninfected, and resistant infected).

These 4 pooled samples have been submitted for ABI 454 MPSS sequencing at the University of South Carolina Environmental Genomics facility, and analysis of the output data is underway. Since we expect to get approximately 200 nucleotides of sequence for around 1,000,000 individual cDNAs (i.e. 200,000 cDNAs per pool sample[see above]), we should learn the frequency that each sequence is obtained in each of the pools. These frequencies will be compared to learn which sequences are up or down regulated by nematode infection. Once the sequences are identified, we will confirm the up and down regulated sequences, and then proceed to verify specific genotypes and time points for regulation of the sequences, using the original materials from which RNA was prepared.