**Kansas Soybean Commission Final Report for FY2017**

**Principle investigators:**

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**Title:** “Enhancement of Soybean through Genetic Engineering”

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# **Department heads:** Marty Draper and Gary Pierzynski

**Objectives:**

**1.** Enhance **Soybean Cyst Nematode (SCN)** resistance in transgenic soybean by modifying current silencing strategies.

**2. Test the effectiveness of gene silencing constructions for root knot nematode resistance** using RKN genes homologous to effective SCN genes.

**3.** Transgenic approaches for increased fungal resistance with **emphasis on SDS resistance**.

In our first approach for increased SCN resistance transgenic events transformed with the hpRNAi-Y25 and -Prp17 vectors continue to show about a 60-80% reduction in cysts and eggs compared to controls in greenhouse bioassays. We also looked at SCN development on these plants at different time points at 3, 7, 14, 20, and 30 days post inoculation by Acid Fuchsin staining. The preliminary results observed of that Prp17 transgenic plants were likely disturbed the establishment of J2 entry into roots, and/or development of juvenile nematodes because much less juveniles were observed in Prp17 transgenic plants at all stages compared to the susceptible JackX cultivar. The Y25 transgenic plants had no obviously different numbers of juveniles compared to the control but overall less cysts. This may suggest that the suppressed Y25 gene might have affected on reproduction stages of SCN.

We have begun backcrossing transgenic soybean with hpRNAi\_Y25 and Prp17, which were transformed to JackX cultivar as background into Kansas adapted lines. Both transgenic lines were crossed with cultivar K11-2363B (mild resistance to SCN HG type7) and K12-2333 (mild tolerance) separately. In addition, transgenic soybean with hpRNAi\_Y25 and Prp17 were crossed with each other to stack two RNAi constructs together. All hpRNAi\_Y25 or Prp17 transgenic plants were tested by PCR to confirm GOI. These two homozygous lines identified previously were all positive in the test too. Currently, a total of approximately 50 plants were used for crossing and are in the greenhouse growing to maturity. hpRNAi\_Y25 events with high expression of GOI (tested by qRT-PCR) were also increased for seeds and at the end of the quarter we have begun to harvest these seeds. We plan use these lines to test the durability of SCN resistance along with testing their effectiveness with different SCN populations.

In a second approach for nematode resistance we are attempting to modify a biochemical pathway in soybean to produce a compound that will affect nematode reproduction. Two genes for shunting a biochemical pathway in soybean towards the production of this compound were placed in vector constructs to express these genes independently and together and were also used to transform soybean cultures. We have identified seven positive events from one gene and 1 event that contains both transgenes. These cultures were regenerated and plants have been transferred to the green house for further testing and seed production.

The root knot nematode (RKN) bioassays with Prp17 and Y25 transgenic lines were performed. The number of galls was recorded by two methods, one quick evaluation scale used in the Todd lab and one developed by Bridge et al. (Bridge and Page, Tropical Pest Management 26, 296-298, 1980). No visual differences were observed among the galls from the transgenic lines and susceptible controls. After one-week incubation in flask, RKN juveniles were counted for each sample and the number was normalized by root weight. The mean number of RKN Juveniles on Prp17 transgenic line was reduced only by about 24% compared to the susceptible controls, and Y25 transgenic lines obtained similar densities compared to control. These results may be due to the gene similarity between the SCN and RKN, or some gene silenced transgenic plants merged to bias the mean density in the transgenic lines. We will to perform additional assays and include other lines to better evaluate RKN resistance.

To increase SDS tolerance, the hpRNAi vector for the FvTox gene has been completed and used to transform soybean cultures. The host derived RNAi constructs for FvTox1 gene were used for stable transformation in soybean, 5 positive putative plants were identified in tissue culture. These plants have been hardened off and are beginning to be transferred to the greenhouse for seed production.