**Kansas Soybean Commission Final report for FY2016**

**Principle investigators:**

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

**Amount of funding:** $ ***76,014***

# **Department heads:** Erick DeWolf (interim) 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**.

**Progress:**

**A. Nematode research summary:**

The T3 generation of soybean events expressing DNA vectors targeting the nematode Y25 and Prp17 genes for down-regulation were used in the bioassay with 20 plants of JackX as controls. DNA and RNA for each plant was collected and extracted for confirmation of transgene expression. Six weeks post germination, each tube was broken down and the SCN cysts and eggs were counted. The reduction of SCN population fed on transgenic plants compared to non-transgenic control was consistently ranged from 40% to70%, values consistent with previous results.

The eggs collected from each transgenic line were randomly mixed to sampled for RNA extraction. RT-qPCR was performed to measure the expression level of target gene (Prp17 or Y25) in SCN egg. Significant suppression of these genes were observed ranging from 3.5 fold to 6 fold compared to controls. The leaf tissue of selected plants were flash-frozen and sent out for RNA-seq, along with tissues from last bioassay. A manuscript is undergoing for this project.

Ten additional plants from each line were also grown for seed increasing. From PCR detection of DNA, all plants were positive for GOI. As the homozygous seeds become available, additional bioassays will test the durability of the resistance on diverse populations.

A population of Root Knot Nematodes (RKN) was increased in the greenhouse earlier in the funding cycle. PRP, Y25 transgenic line planted in RKN infested soil (3-4 seeds per pot for each pot with 10 pots for each transgenic line) along with JackX as susceptible control, and KS3406 as resistance control. All seedlings were tested for PCR to confirm positive GOI. After 8 weeks, the experiment was broken down, plant symptoms recorded, and roots were incubated in flask for 1 week. This experiment is still ongoing and we intend to count the RKN Juveniles in each line and compare to the values found in the control.

In a second approach for nematode resistance we are attempting to modify a biochemical pathway in soybean to produce compound that will affect nematode reproduction. As a preliminary experiment we evaluated the exogenously application on seedlings at various concentrations (1nM to 1uM). Experiments using this compound exogenously on soybean seedlings indicated that there is no deleterious effect on soybean root growth. The experiment showed no significant difference between the root mass and total length between treatment and control groups. Despite positive results from a previous lab assay using this compound against SCN, two similar assays using the exogenously applied compound on soybean seedlings showed no significant difference between treated and control plants. Because the compound is volatile it is possible the solutions used in the bioassays did not have the proper concentrations. Further experiments are underway with higher concentrations of the compounds to confirm that this compound has no adverse effects on soybean development but can reduce soybean cysts. We have identified two genes needed to construct a new biochemical pathway in soybean to produce this compound.

Two genes for shunting a biochemical pathway in soybean towards the production of this compound were codon optimized for soybean, synthesized and inserted into vectors suitable for expression in soybean. These vectors were then purified and used for transforming soybean tissues. Transformed cultures are currently under selection.

As part of this project, another related goal is to produce the chemical compound in plant growth promoting microbes, such as *Rhizobium*. We received a plasmid for gene expression in bacteria from a colleague at a different university. Before cloning our genes into the plasmid we inserted the green fluorescent reporter gene to confirm bacterial expression. Unfortunately there was no expression in our bacteria so we need to either repeat our cloning steps or find alternate bacterial expression elements to get successful expression in bacteria.

**B. Antimicrobial peptide and SDS resistance research summary:**

Three antimicrobial peptide genes have been independently cloned into pGmubi vector: pGmubi-euRug, pGmubi-euHep, pGmubi-euGae and a number of transformation experiments have been performed. Based on PCR analysis of putative transgenic soybean cultures, several lines have been confirmed for the antimicrobial peptide genes of interest including positive putative plants for Rug, Hep, and Gae genes. These cultures are being regenerated into plants and we have harvested nine positive T0 and two T1 events of pGmubi-euRug. the other events are still being regenerated or waiting to produce seeds in the greenhouse. The next stage of this research will be to use seeds in bioassays to challenge with different pathogens such as *Fasurium virguliforme* (SDS), and/or *Pseudomonas* bacterial pathogens.

In the second antifungal project, an RNAi vector pANDA35HK FvTox381 has been constructed and sequenced. This construction targets the toxin gene of *Fusarium virguliforme*, which causes the foliar symptoms of SDS. This DNA vector was transformed into *Agrobacterium rhizogenes* K599 to generate transgenic roots for bioassay test. Since 3-4 week-old seedlings were used for inoculation, we proposed the better way could be using the slightly trimmed roots dipping into high concentrated inoculum, however, the time length for dipping, concentration of inoculum, and trimming and planting method need to test for optimal results. We are using KS4607 cultivar for this protocol establishment.

For SDS bioassay, we have established an evaluation system in collaboration with Chris Little. Plants in which hairy root were initiated are transferred and grown 50ml tubes with a *Fusarium virguliform* spore inoculum. Symptoms of SDS are observed about 2 weeks after transfer and the symptom scale range from 2 to 5. There was no significant difference between trimmed roots and intact roots. We plan to use this hairy roots assay to evaluate the FvTox RNAi vector expressed in hairy roots.