End of Project Final Report

Iowa Soybean Association

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Project Title: "Stacking four plant genes to provide durable and enhanced SCN and SDS resistance in soybean"

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Progress report for the period from October 1, 2018 to December 31, 2019

Soybean is the most important legume crop that provides both protein and oil. Soybean seeds contain approximately 40% protein and 20% oil. It is an important source of animal and fish feed in addition to its major role in human nutrition. In the United States, the average annual soybean yield is valued at around $40 billion. Unfortunately, 12-15% of its yield potential is suppressed annually by pathogen attacks. Among the soybean pathogens, *Heterodera glycines*, commonly known as soybean cyst nematode (SCN), and *Fusarium virguliforme* are two of the most serious soybean pathogens. *F. virguliforme* causes sudden death syndrome (SDS). Soybean suffers average annual yield suppression valued close to $2 billions from the attacks of SCN and SDS. Our ***long-term goal*** is to alleviate soybean yield suppression from these two most serious pathogens in Iowa and as well as in the U.S. by breeding novel SCN and SDS resistant soybean cultivars.

In this project, we proposed to evaluate the joint or combined effect of four transgenes in improving the SCN and SDS resistance of a soybean line. The four genes use distinct mechanisms to confer both SCN and SDS resistance, when overexpressed in transgenic soybean plants. Of the four genes, two are from soybean and two are from *Arabidopsis thaliana*. The two soybean genes, *GmDS1* and *GmSAMT2,* encode a receptor-like protein and a salicylic acid methyl transferase, respectively. The two *Arabidopsis thaliana* genes, *PSS30* and *PSS25,* encode a folate transporter and a putative transcription factor, respectively.

We ***hypothesize*** that since the resistance mechanisms encoded by *PSS25, PSS30, GmDS1* and *GmSAMT2* are distinct, the functions of the four genes are therefore complementary to each other and together they are expected to provide soybean with stable and robust resistances against both SCN and *F. virguiforme* isolates.

The ***outcome*** of this proposed research is expected to be ***highly significant*** because it will lead to development of soybean lines with robust resistance to the two most serious soybean pathogens, SCN and *F. virguliforme*. Therefore, this project will significantly improve soybean growers' farm economy.

Goals and Objectives: The ***goal*** of this project is to significantly contribute towards developing durable resistance against both SCN and *F. virguliforme* isolates that together cause soybean yield suppression valued close to $2 billion. We propose five objectives to reach our goal in a 3-year period.

1. **Objective 1.** Map the four fusion genes, *PSS25, PSS30, GmSAMT2* and *GmDS1,* among the transgenic soybean lines.
2. **Objective 2.** Identify Williams 82 lines that carry combinations of three fusion genes: (i) *PSS25, PSS30* and *GmDS1* and (ii) *PSS30, GmDS1* and *GmSAMT2.*

3. **Objective 3.** Identify Williams 82 lines that carry all four transgenes: *PSS25, PSS30, GmDS1* and *GmSAMT2.*
4. **Objective 4.** Evaluate Williams 82 lines carrying *PSS25, PSS30, GmDS1* and *GmSAMT2* fusion genes for resistance to *F. virguliforme.*
5. **Objective 5.** Evaluate Williams 82 lines carrying *PSS25, PSS30, GmDS1* and *GmSAMT2* fusion genes for resistance to *H. glycines.*

This is a 3-year project. ***In Year 1, we planned to complete Objective 1, partially Objective 2 and initiate Objective 3.***

We report here the progresses made from October 1, 2018 to December 31, 2019 under each of the three objectives. Objectives 4 and 5 will be conducted in Year 3.

Objective 1. Map the four fusion genes, *PSS25, PSS30, GmSAMT2* and *GmDS1,* among the transgenic soybean lines.

We have mapped seven of the eight transgenes generated from four plant genes. We however failed to map the *35S-PSS30* transgene. We repeated the genome walking experiment to map this gene; but we failed again. It is unknown if the *35S-PSS30* transgene formed a complex locus to interfere with the polymerase chain-termination reaction (PCR) in two independent genome walking experiments.

Objective 2. Identify Williams 82 lines that carry combinations of three fusion genes: (i) *PSS25, PSS30* and *GmDS1* and (ii) *PSS30, GmDS1* and *GmSAMT2*.

We have conducted hybridization experiments to raise segregating populations. We have selected three greenhouses: (i) Agronomy Greenhouse, (ii) Horticulture Greenhouse and (iii) Plant Pathology Greenhouse to grow our materials and conduct hybridization experiments.

We developed segregating populations for the *GmDS1* and *PSS30* transgenes from the support of an earlier one-year ISA grant. Molecular analyses conducted through PCR revealed an $F_{2:3}$ family of a cross between transgenic plants 107 and 480 carries progenies that do not segregate for either *Prom3-GmDS1* or *Prom2-PSS30* transgene. Therefore, this family is fixed for both transgenes and used in making the three-way crosses with each of the four transgenic plants 33, 79, 125 and 327 carrying either *PSS25* or *GmSAMT2* transgene. An additional line carrying the *Prom2-DS1* and *35S-Pss30* transgenes was used to make crosses with transgenic plants 33, 125 and 327. Several pods carrying putative F_1 seeds were formed. Seeds were harvested from forty-six pods obtained from the three-way crosses. We used PCR method to screen the plants grown from these seeds. We have identified 17 F_1 plants, each of which harbors a combination of the three transgenes: *GmDS1* and *PSS30* transgenes with either *GmSAMT2* or *PSS25* transgene (Table 1).

Table 1. Number of plants carrying three transgenes.

Cross ID	Female	Male	F_1
11	4A-4B (*Prom3-GmDS1*, *Prom2-Pss30*)	125 (*Prom3- GmSAMT2*)	5
12	4A-4B (*Prom3-GmDS1*, *Prom2-Pss30*)	33 (*Prom2-Pss25*)	3
13	4A-4B (*Prom3-GmDS1, Prom2-Pss30*)	327 (*Prom2-GmSAMT2*)	2
14	4A-4B (*Prom3-GmDS1, Prom2-Pss30*)	79 (*35S-Pss25*)	1
15	1A-1B (*Prom2-GmDS1, 35S-Pss30*)	125 (*Prom3-GmSAMT2*)	2
16	1A-1B (*Prom2-GmDS1, 35S-Pss30*)	33 (*Prom2-Pss25*)	2
17	1A-1B (*Prom2-GmDS1, 35S-Pss30*)	327 (*Prom2-GmSAMT2*)	2

Female, genotypes are shown in parentheses. Female parents of the first four crosses (Cross ID 11 through 14) are homozygous for the two transgenes. **Male**, male parents are homozygous.

Objective 3. Identify Williams 82 lines that carry all four transgenes: *PSS25, PSS30, GmDS1* and *GmSAMT2.*

We have crossed transgenic soybean plants to obtain F_1s carrying both *GmSAMT2* and *PSS25.* We have harvested the seeds of the putative hybrids between two transgenic plants, each carrying either of the two transgenes. We screened the putative F_1 plants using a PCR method and identified 45 plants carrying both genes (Table 2). We have started to cross the identified F_1 plants carrying both *PSS25* and *GmSAMT2* transgenes with the plants that are homozygous for both *PSS30* and *GmDS1*. These crosses are being made in the Agronomy greenhouse (2 rooms) and Plant Pathology greenhouse (1 room).

Table 2. Pods carrying putative F_1 seeds of the crosses made between the transgenic soybean plants carrying either *GmSAMT2* or *PSS25* fusion genes.

Cross ID	**Transgene 1**	**Transgene 2**	**F_1 (*Pss25* and *GmSAMT2*)**
9A-9B	33 (*Prom2-PSS25*)	125 (*Prom3-GmSAMT2*)	26
9C-9D	33 (*Prom2-PSS25*)	327 (*Prom2-GmSAMT2*)	19

KPIs/Performance Metrics: Self-evaluations of the progress made in Year 1 are presented below.

1. By the end of Year 1, we will complete Objectives 1.

   Self-evaluation: We already have completed Objective 1 for all eight transgenes except one. We believe that the *35S-PSS30* transgene might have integrated in multiple copes leading to a complex transgene locus, which prevented PCR amplification in our genome walking experiment. We have however necessary physical locations of the other seven transgenes, enough to reach the goal of this proposal.

2. The Objective 2 will have partially completed. We will have harvested the F_1 seeds of the two single crosses by the end of Year 1.

   Self-evaluation: Seeds of the two single crosses are harvested as proposed (Table 2).

3. In Year 2, we expect to complete Objective 2; and have the Objective 3 partially completed. We will have generated F_1 seeds of the double crosses to stack all four transgenes.

   Self-evaluation: We have completed first three months of the Year 2 and identified the F_1 seeds carrying two combinations of three transgenes as proposed (Table1).

Timelines and Milestone Deliveries: The following are the milestones to be delivered in Year 1.

1. We will have mapped all four transgenes in individual transgenic plants by December 31, 2018.

   Self-evaluation: We already have completed Objective 1 for all eight transgenes except one. We believe that the *35S-PSS30* transgene might have integrated in multiple copes leading to a complex transgene locus, which prevented PCR amplification in our genome walking experiment. We have however necessary physical locations of the other seven transgenes, which is enough for reaching the goal of this proposal.

2. We will have harvested the seeds of single crosses by September 30, 2019.

   Self-evaluation: Seeds of the two single crosses were harvested.

Overall self-evaluation: We are in the right track of progress as proposed in the proposal.