Project Report

Iowa Soybean Association

October 30, 2020

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

Investigator: Madan K. Bhattacharyya
Agronomy Hall G303
Iowa State University
Ames, IA 50011
515-294-2505
mbhattac@iastate.edu

Progress report for the period from May 1, 2020 to October 30, 2020

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; and this is the Year 3 of the project. We report here the progresses made from May 1, 2020 to October 30, 2020 under each of the five objectives.

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

This objective was completed earlier. 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. Seven transgenes are sufficient to test the hypothesis of this project and accomplish the goal.

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

Earlier we reported that we have identified two lines homozygous for *Prom3-GmDR1* and *Prom2-PSS30* which have been used in crossing with transgenic lines carrying *PSS25* and *GmSAMT2* genes.

We previously reported that we planted 16 seeds of each of the 24 F_2 plants (12 from the cross *Prom2-PSS25* X *Prom3-GmSAMT2* and 12 from the cross *Prom2-PSS25* x *Prom2-GmSAMT2*) and screened to identify the F_3 families that are homozygous for both genes, means both genes are fixed.

We have identified six putative homozygous F_3 lines, four lines (84, 86, 87, 89) carrying *Prom2-PSS25* and *Prom3-GmSAMT2* and two lines (93, 94) with *Prom2-PSS25* and *Prom2-GmSAMT2*. These lines are being harvested from the field and will used in phenotyping experiments.

We reported in the previous quarter that we identified 17 putative F_1 plants harboring a combination of the three transgenes (*GmDS1* and *PSS30* transgenes with either *GmSAMT2* or *PSS25* transgene) and that we identified four F_2 plants carrying *PSS30, GmDS1* and *GmSAMT2* (Table 1) and four F_2 plants segregating. *PSS30, GmDS1* and *PSS25* (Table 2). We have harvested F_3 seeds from these F_2 plants (Tables 1 & 2) containing two combinations of three transgenes. The F_3 seeds will be planted in the greenhouse and homozygous plants for combination of three genes, (i) *PSS30, GmDS1* and *GmSAMT2,* and (ii) *PSS30, GmDS1* and *PSS25* will be identified. F_4 seeds will be harvested from the selected plants and grown for phenotyping.

Table 1. Four F_2 plants carrying *PSS30, GmDS1* and *GmSAMT2.*

F_2 Plant	Gene		
	GmDS1	***PSS30***	***GmSAMT2***
19	yes	yes	yes
22	yes	yes	yes
23	yes	yes	yes
26	yes	yes	yes

yes, presence of a gene.

Table 2. Four F_2 plants carrying *PSS30, GmDS1* and *PSS25.*

F_2 Plant	Gene		
	GmDS1	***PSS30***	***PSS25***
3	yes	yes	yes
5	yes	yes	yes
11	yes	yes	yes
12	yes	yes	yes

yes, presence of a gene.

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

Previously, we reported hybridizing 45 F_1 plants carrying both *PSS25* and *GmSAMT2* transgenes with two transgenic soybean plants that are homozygous for both *PSS30* and *GmDS1.* PCR screening on the F_1 plants identified three F_1 plants that carry all four transgenes (Table 3). We have harvested the selected plants and F_2 seeds of these plants will be planted in greenhouse to raise the F_3 families. The F_2 plants will be screened to identify the homozygous plants for most if not all four genes. Seeds of the homozygous plants for most of the four genes will be grown in summer of 2021 in the field for phenotyping.

Table 3. F_1 plants selected (shown with colors) for harvesting seeds and further analysis of their F_2 plants.

F_1 Plant	*GmDS1*	*PSS30*	*PSS25*	*GmSAMT2*
1	yes	yes	yes	yes
7	yes	yes	yes	yes
12	yes	yes	yes	yes

yes, presence of a gene.

Objective 4. Evaluate Williams 82 lines carrying *PSS25, PSS30, GmDS1* and *GmSAMT2* fusion genes for resistance to *F. virguliforme.*

It was proposed to conduct this objective in the summer of 2021, once we have generated stacked lines with all four genes (Table 3). Since we have identified two stacked lines with two transgenes, we evaluated the lines this summer in the field to determine if there is any joint effect of the two transgenes in further enhancing SDS resistance. Results are presented below.

Phenotyping of plants carrying 2 transgenes (*GmDS1* and *PSS30*) for SDS resistance

While waiting to develop the lines with three or all four genes, we evaluated the two lines homozygous for *GmDS1* and *Pss30* for responses to *F. virguliforme* under field condition. The field trial was conducted at the ISU Horticulture Research Station. Seeds of the two homozygous transgenic soybean lines carrying *Prom2-Pss30* and *Prom3-GmDS1* genes, two parents carrying single transgenes and nontransgenic Williams 82 line were planted along with *F. virguliforme* inoculum. The plants were scored on September 14 for SDS resistance. We observed a significant enhancement in SDS resistance between the two transgene-stacked lines carrying *Prom2-Pss30* and *Prom3-GmDS1* as compared to either parent carrying *GmDS1* or *Pss30* (Figure 1).

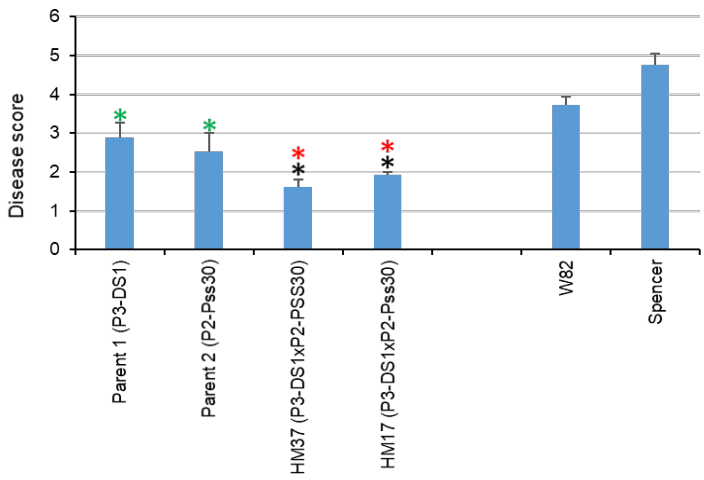

Figure 1. Enhanced SDS resistance among the two stacked lines carrying *GmDS1* and *Pss30*.
Disease scores of two homozygous lines carrying *Prom2-Pss30* and *Prom3-GmDS1* genes were compared with those of Parent 1 and Parent 2 during the SDS field trial in the summer of 2020. Black * indicates the significant difference in SDS disease score of a stacked line with that of Parent 1. Red * indicates significant difference in SDS disease score of a stacked line with that of Parent 2. Green * indicates significant difference in SDS disease score of a parental line with that of non-transgenic Williams 82 line, used to generate the transgenic lines.

Objective 5. Evaluate Williams 82 lines carrying *PSS25, PSS30, GmDS1* and *GmSAMT2* fusion genes for resistance to *H. glycines*

It was proposed to conduct this objective in Year 3 of the project period, once we have generated stacked lines with all three or four genes, considered in this study. Since we have identified two stacked lines with two transgenes, we evaluated the lines earlier for possible further enhancement in SCN resistance and data were presented in the last semi-annual report. Results were similar to the ones shown in Figure 1 for SDS resistance.

We have harvested three F_1s that carry all four transgenes (Table 3). Progenies of these three lines will segregate for four genes. We will grow the progenies in greenhouse; and by conducting PCR, we will identify the lines that are homozygous for most of the four transgenes. Seeds of those homozygous plants will be harvested and evaluated for SCN resistance in 2021.

KPIs/Performance Metrics: We expect to accomplish the followings by years:

1. By the end of Year 1, we will complete Objectives 1.
   Self-review: We already have completed Objective 1.
2. We will have harvested the F_1 seeds of the two single crosses by the end of Year 1.
   Self-review: We have completed.
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-review: We already have generated pods that are expected to carry all four transgenes (Table 3). We have evaluated two stacked lines with two transgenes and showed that the lines are better than their either parent.
4. In Year 3 (Starting October 1):
   a. we expect to have evaluated the F_2 segregating population generated to segregate all four transgenes. We will have done phenotyping and genotyping of individuals to determine the association between the number of transgenes and levels of SDS or SCN resistance. Genotypes carrying all four transgenes will be identified.
   b. A manuscript describing the SCN resistance will be published in a peer reviewed journal by the end of Year 3.
   c. The manuscript describing the SDS resistance will be ready only after completion of this project since the foliar SDS data will most likely be collected by the September of Year 3 and we need to carry on field trial at least one more year under the support of a renewal proposal.

   Self-review: We have harvested seeds three F_1s (double cross) segregating for all four genes. We will be growing the seeds in greenhouse in greenhouse in next month and identified lines carrying most of the four genes in homozygous condition. In 2021, we will evaluate the lines carrying all four genes for SDS and SCN resistances. We are in the right track.

Economic Impact/Significance

In the U.S., the total annual soybean yield suppression from SDS and SCN is approximately $1.8 billion. Even if we can reduce the SDS and SCN incidence by 20% through cultivation of novel SDS and SCN resistant cultivars to be generated from the outcomes of this project, we can expect to have significant increase in the annual soybean yield values close to $360 million in U.S. and approximately $50 million in Iowa.

Timelines and Milestone Deliveries: The following are our milestones and deliverables.

1. We will have mapped all four transgenes in individual transgenic plants by December 31, 2018. – **Delivered** (earlier report).
2. We will have harvested the seeds of single crosses by September 30, 2019. – **Delivered** (earlier report).
3. We will have harvested F_2 seeds carrying three transgenes in greenhouse during the winter of 2019-2020. – **Delivered** (earlier report).
4. We will have harvested the F_1 seeds carrying all four transgenes by October 31, 2020. We have harvested the seeds on October 29, 2020. **Delivered** (Table 3).

5. We will have accomplished initial evaluation of homozygous plants carrying at least three transgenes for responses to both *F. virguliforme* and *H. glycines* by the end of Year 3. We have evaluated two lines carrying two transgenes and showed that stacking of two transgenes increases SDS (Figure 1) and SCN resistance (last report) as compared to their parental lines carrying either of the two transgenes. **Delivered** partially ahead of the deadline.
6. It will be established if we observe complementary effects among the transgenes that use distinct genetic mechanisms to confer SDS and SCN resistance.

   Preliminary data indicate that SDS and SCN resistances are enhanced further among the two stacked lines carrying two transgenes as compared to the two parental lines carrying single transgenes.

7. A peer reviewed journal article describing the responses of lines with different combinations of four transgenes (at most three genes in one genotype) to *H. glycines* will be published.
   We **expect** to publish the results by the end 2021.
8. We will have identified homozygous lines for all four transgenes and made available to private seed industries by October of 2021.
   We **expect** to get lines carrying all fours transgenes in homozygous condition by our deadline.

Overall self-evaluation: We are making progresses as proposed in the proposal.