Project 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 April 1, 2021 to October 31, 2021

Overview: 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 the Year 3 of the 3-year project, end on September 30, 2021. ISA recently approved additional six months until March 31, 2022 to continue this project under a no-cost extension.

In our previous report submitted on April 30, 2021, we reported the progresses made from November 1, 2020 to March 31, 2021, mostly under the Objectives 2 and 3.

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 the generation of the segregating lines for various combinations of transgenes. To identify the segregating lines carrying expected combinations of transgenes, we conducted polymerase chain termination reactions (PCR) using synthesized oligo-nucleotide primers, specific to the transgenes. In the previous reports, we mentioned the identification of 335 plants carrying various combinations of transgenes. We also reported that among these plants, 37 contained four transgenes and 74 carried combinations of two or three genes. We harvested seeds from these plants in the greenhouse between April and July 2021. Seeds of 40 transgenic lines, harvested before the first week of June, 2021, were planted along with control nontransgenic cultivars in the field this summer, 2021 to determine the responses of the transgenic soybean lines carrying combinations of transgenes to *F. virguliforme* (Table 1).

Table 1. Randomization of transgenic and nontransgenic lines planted in three blocks of a field located at the ISU Horticulture Research Station during the summer of 2021. *F. virguliforme* inoculum was added to the rows during planting to determine the responses of the transgenic soybean lines against the SDS pathogen. The numbers under three blocks are plot numbers, in which individual lines were grown.

Serial No.	Fusion Gene(s)	Line Name	Block 1	Block 2	Block 3
Transgenic Lines					
1	Prom1-PSS25	ST306-3-2-186	13	125	221
2	Prom1-PSS25	ST306-14-6-131	16	111	216
3	Prom2-PSS25	ST307-12-1-312	75	146	223
4	Prom2-PSS25	ST307-17-6-308	32	149	182
5	Prom2-PSS25	ST307-14-2-35	39	116	195
6	Prom2-PSS25	ST307-12-6-9	41	83	151
7	Prom2-DS1	Prom2-DS1-24	53	89	225
8	Prom3-DS1	Prom3-DS1-4	51	97	160
9	Prom3-DS1	Prom3-DS1-12	61	101	163
10	Prom1-SAMT2	Prom1-SAMT2-5	28	88	220
11	Prom3-SAMT2	Prom3-SAMT2-19	27	147	171
12	35S-Pss30	35S-Pss30-16-1	74	82	152
13	Prom2-Pss30	Prom2-Pss30-5-7	70	93	174
14	Prom2-Pss30	Prom2-Pss30-5-480	47	102	207
15	Prom2-Pss25	Prom2-Pss25-33	73	119	164
16	Prom3-DS1/Prom2-Pss30	Prom3-DS1/Prom2-Pss30-HM37	65	144	167
17	Prom3-DS1/Prom2-Pss30	Prom3-DS1/Prom2-Pss30-HM47	58	132	183
18	Prom3-DS1/Prom2-Pss30	Prom3-DS1/Prom2-Pss30-HT54	15	108	198
19	Prom3-SAMT2/Prom2-Pss25	Prom3-SAMT2/Prom2-Pss25-84	72	122	179
20	Prom3-SAMT2/Prom2-Pss25	Prom3-SAMT2/Prom2-Pss25-86	33	76	217

21	Prom3-SAMT2/Prom2-Pss25	Prom3-SAMT2/Prom2-Pss25-87	4	143	181
22	Prom3-SAMT2/Prom2-Pss25	Prom3-SAMT2/Prom2-Pss25-89	36	86	165
23	Prom1-SAMT2/Prom2-Pss25	Prom1-SAMT2/Prom2-Pss25-93	21	98	209
24	Prom1-SAMT2/Prom2-Pss25	Prom1-SAMT2/Prom2-Pss25-94	1	85	224
25	Prom3-DS1/Prom2-Pss30/Prom2-Pss25	Prom3-DS1/Prom2-Pss30/Prom2-Pss25-126	56	131	212
26	Prom3-DS1/Prom2-Pss30/Prom2-Pss25	Prom3-DS1/Prom2-Pss30/Prom2-Pss25-115	24	145	204
27	Prom3-DS1/Prom2-Pss25/Prom3-SAMT2	Prom3-DS1/Prom2-Pss25/Prom3-SAMT2-41	20	126	190
28	Prom3-DS1/Prom2-Pss25/Prom3-SAMT2	Prom3-DS1/Prom2-Pss25/Prom3-SAMT2-118	67	80	154
29	Prom3-DS1/Prom2-Pss25/Prom3-SAMT2	Prom3-DS1/Prom2-Pss25/Prom3-SAMT2-106	9	118	210
30	Prom3-DS1/Prom2-Pss30/Prom3-SAMT2	Prom3-DS1/Prom2-Pss30/Prom3-SAMT2-221	57	81	173
31	Prom3-DS1/Prom2-Pss30/Prom3-SAMT2	Prom3-DS1/Prom2-Pss30/Prom3-SAMT2-39	35	112	153
32	Prom2-Pss30/Prom2-Pss25/Prom3-SAMT2	Prom2-Pss30/Prom2-Pss25/Prom3-SAMT2-150	52	103	178
33	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2 -109	60	95	187
34	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2 -34	8	130	194
35	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2 -36	44	84	155
36	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2 -40	25	148	176
37	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2 -42	29	113	168
38	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2 -108	71	78	189
39	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2-120	54	133	175
40	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2 -128	17	120	192
Non-transgenic Lines					
41	None	Williams 82-1	26	137	172
42	None	Williams 82-2	30	134	205
43	None	Williams 82-3	62	127	177
44	None	Williams 82-4	31	96	170

45	None	Williams 82-5	63	100	200
46	None	Spencer-1	14	90	188
47	None	Spencer-2	69	92	166
48	None	Spencer-3	38	104	186
49	None	MN1606-1	10	114	197
50	None	MN1606-2	43	150	191
51	None	MN1606-3	45	105	203

Williams 82 and Spencer are SDS susceptible cultivars; MN1606 is an SDS resistant cultivar.

Stacked Lines Carrying Two Transgenes:

Previously, we harvested seeds from at least eight individual F_3 plants from each of the F_3 homozygous families, viz., 84, 86, 87, and 89 carrying *Prom2-PSS25* and *Prom3-GmSAMT2*, and 93 and 94 carrying *Prom2-PSS25* x *Prom1-GmSAMT2*. The F_4 plants homozygous for both *PSS25* and *GmSAMT2* genes were grown during this summer for SDS resistance, along with the three F_4 lines carrying the two transgenes *Prom3-DS1* and *Prom2-Pss30* identified and analyzed in the summer of 2020 (Table 2). The plot numbers for these lines are presented in Table 1.

Table 2. Nine F_4 lines carrying a combination of two transgenes, *Prom3-DS1*/*Prom2-Pss30*, *Prom2-PSS25/Prom3-SAMT2,* and *Prom2-PSS25/Prom1-SAMT2* transgenes, grown in the field during summer 2021.

Serial No.	Stacked Fusion Genes	Line Name
1	Prom3-DS1/Prom2-Pss30	Prom3-DS1/Prom2-Pss30-HM37
2	Prom3-DS1/Prom2-Pss30	Prom3-DS1/Prom2-Pss30-HM47
3	Prom3-DS1/Prom2-Pss30	Prom3-DS1/Prom2-Pss30-HT54
4	Prom3-SAMT2/Prom2-Pss25	Prom3-SAMT2/Prom2-Pss25-84
5	Prom3-SAMT2/Prom2-Pss25	Prom3-SAMT2/Prom2-Pss25-86
6	Prom3-SAMT2/Prom2-Pss25	Prom3-SAMT2/Prom2-Pss25-87
7	Prom3-SAMT2/Prom2-Pss25	Prom3-SAMT2/Prom2-Pss25-89
8	Prom1-SAMT2/Prom2-Pss25	Prom1-SAMT2/Prom2-Pss25-93
9	Prom1-SAMT2/Prom2-Pss25	Prom1-SAMT2/Prom2-Pss25-94

Stacked Lines Carrying Three Transgenes:

Earlier we identified F_3 plants carrying a combination of three transgenes. Eighteen of those lines including five carrying *Prom3-GmDS1, Prom2-PSS30* and *Prom2-PSS25* transgenes; 11 carrying *Prom3-GmDS1, Prom2-PSS30* and *Prom3-GmSAMT2*; 19 plants harboring *Prom2-PSS25, Prom3-GmDS1* and *Prom3-GmSAMT2*; and two carrying *Prom2-PSS30, Prom2-PSS25* and *Prom3-GmSAMT2* transgenes were planted in the field to evaluate their responses to *F. virguliforme* (Table 3). The plot numbers for these lines with stacked transgenes are presented in Table 1.

Table 3. Eighteen F_4 plants carrying combinations of three transgenes were planted in the field to determine their responses to *F. virguliforme* infection SDS infection.

Serial No.	Sacked Fusion Genes	Line Name
1	Prom3-DS1/Prom2-Pss30/Prom2-Pss25	Prom3-DS1/Prom2-Pss30/Prom2-Pss25-115
2	Prom3-DS1/Prom2-Pss30/Prom2-Pss25	Prom3-DS1/Prom2-Pss30/Prom2-Pss25-124
3	Prom3-DS1/Prom2-Pss30/Prom2-Pss25	Prom3-DS1/Prom2-Pss30/Prom2-Pss25-126
4	Prom3-DS1/Prom2-Pss30/Prom2-Pss25	Prom3-DS1/Prom2-Pss30/Prom2-Pss25-132
5	Prom3-DS1/Prom2-Pss30/Prom2-Pss25	Prom3-DS1/Prom2-Pss30/Prom2-Pss25-37
6	Prom3-DS1/Prom2-Pss30/Prom3-SAMT2	Prom3-DS1/Prom2-Pss30/Prom3-SAMT2-176
7	Prom3-DS1/Prom2-Pss30/Prom3-SAMT2	Prom3-DS1/Prom2-Pss30/Prom3-SAMT2-214
8	Prom3-DS1/Prom2-Pss30/Prom3-SAMT2	Prom3-DS1/Prom2-Pss30/Prom3-SAMT2-221
9	Prom3-DS1/Prom2-Pss30/Prom3-SAMT2	Prom3-DS1/Prom2-Pss30/Prom3-SAMT2-221
10	Prom3-DS1/Prom2-Pss30/Prom3-SAMT2	Prom3-DS1/Prom2-Pss30/Prom3-SAMT2-223
11	Prom3-DS1/Prom2-Pss30/Prom3-SAMT2	Prom3-DS1/Prom2-Pss30/Prom3-SAMT2-238
12	Prom3-DS1/Prom2-Pss30/Prom3-SAMT2	Prom3-DS1/Prom2-Pss30/Prom3-SAMT2-39
13	GmDS1/Prom2-PSS25/Prom3-SAMT2	Prom3-DS1/Prom2-PSS25/Prom3-SAMT2 -100
14	Prom3-DS1/Prom2-Pss25/Prom3-SAMT2	Prom3-DS1/Prom2-Pss25/Prom3-SAMT2-106

15	Prom3-DS1/Prom2-Pss25/Prom3-SAMT2	Prom3-DS1/Prom2-Pss25/Prom3-SAMT2-118
16	Prom3-DS1/Prom2-Pss25/Prom3-SAMT2	Prom3-DS1/Prom2-Pss25/Prom3-SAMT2-41
17	Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2	Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2 -149
18	Prom2-Pss30/Prom2-Pss25/Prom3-SAMT2	Prom2-Pss30/Prom2-Pss25/Prom3-SAMT2-150

Objective 3. Identify Williams 82 lines that carry all four fusion transgenes: Prom2-*PSS25, Prom2-PSS30, GmDS1* and *GmSAMT2.*

Earlier we reported three F_1 plants that carry all four transgenes: *PSS25, PSS30, GmDS1* and *GmSAMT2* developed by crossing two F_1s. F_2 seeds of these plants were planted in two greenhouses to raise the F_2s. The F_2 plants were evaluated by conducting PCR to identify plants carrying all four transgenes. Thirty-eight plants carrying all four transgenes were identified. From April to July 2021, seeds from these lines were harvested. Seeds of eight lines were randomly planted in three blocks of the field (Table 1). At least 30 seeds of each line were planted in individual rows of each block. For the rest five lines, the number of seeds were low and were only grown with other eight lines in the fourth block just for seed increase.

To identify the individual plants carrying all four transgenes in homozygous condition, leaf samples were collected from randomly selected over 800 single plants. The pods of these selected plants have been harvested. The seeds from these lines will be shortly threshed. DNA is being extracted from these 800 samples and will be used to conduct molecular analysis for identifying the homozygous plants containing all four fusion transgenes. In four-gene segregation with independent assortment (genes are not linked, segregate independently), screening of a large segregating population (256 plants) is required for identifying a single individual with all four genes in homozygous condition. From screening 800 F_2 plants, we expect to obtain only three homozygous F_2 plants. The homozygous individual plant for all four genes will be investigated this winter for responses to *F. virguliforme* and SCN in growth chambers and greenhouse conditions, respectively.

Table 4. Thirteen F_3 plants carrying a combination of three transgenes from *Prom2-PSS30, Prom3-GmDS1*, *Prom3-SAMT2*, and *Prom2-PSS25* were grown in the field for SDS infection and/or seed increase.

Serial No.	Fusion Gene	Line
1	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2 -102
2	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2 -108
3	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2 -109
4	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2 -128
5	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2 -15
6	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2 -28
7	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2 -30
8	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2 -31
9	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2 -34
10	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2 -36
11	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2 -40
12	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2 -42
13	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2	Prom3-GmDS1/Prom2-PSS30/Prom2-PSS25/Prom3-SAMT2-120

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

In our original proposal, we planned to generate lines containing only three fusion genes. We expanded the scope of the project and generated lines that carry all four fusion transgenes. Lines carrying two or three

fusion genes along with population segregating all four fusion genes were planted this summer in the field to evaluate their responses against *F. virguliforme* infection (Table 1). Unfortunately, acceptable SDS symptoms were not developed this summer in our experimental plot.

This year, the soybean growing season was very dry. We continuously irrigated the plot from the 4th week of August when the soybean lines just reached the R3 stage (starting to form pods). We irrigated twice a day, ¼ inch water in the mid-day and 1/4th inch water at dusk. The soil was muddy and moist with high humidity. Despite our effort of maintaining the ideal condition for SDS foliar disease development, the disease appeared only in a limited number of plants of some of the rows. The scored data are not meaningful to report. This is the first time we failed to observe any meaningful SDS foliar disease development since 2013, the year we started to conduct field trial for SDS resistance screening.

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

We have harvested seeds from most of the plants that carry combinations of two, three and all four transgenes considered for this study (Tables 2,3,4). The lines that are homozygous for the fusion gene combinations will be identified and evaluated for responses to *F. virguliforme* and SCN infection under growth chamber and greenhouse conditions, respectively, by March 31, 2022, the new extended last date for this project.

Key Performance Indicators/Performance Metrics: We expect to accomplish in Year 3 of the project the following.

1. The F_1 and F_2 populations will be evaluated and lines with either two, three or four transgenes will be generated.
   Self-evaluation: We have identified soybean genotypes carrying either two, three or four fusion gene combinations. Seeds of these plants have been harvested.

2. Levels of SCN and SDS resistances of lines carrying 2, 3 or 4 fusion gene combinations will be known.
   Self-evaluation: Responses of lines carrying two fusion genes to *F. virguliforme* and SCN have been reported earlier. We failed to observe the foliar SDS symptoms among the transgenic lines this summer. We however plan to identify the homozygous lines for 2, 3 and 4 gene combinations and then evaluated them for their responses against *F. virguliforme* and SCN by March 31, 2022 (under the no-cost extension).

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 for Deliverables: Timelines and milestones for deliverables are:

1. We will deliver the seeds of genotypes carrying all four transgenes by May, 2021.
   Self-evaluation: Seeds of line genotypes carrying all four transgenes or fusion genes were obtained and grown in the field this summer. Seeds of these lines have been harvested.
2. SCN and SDS resistances of lines carrying 2, 3 or 4 fusion gene combinations will be known by September, 2021.
   Self-evaluation: We have shown earlier that two genes further enhance SDS and SCN resistance due to complementary effects between the two fusion or transgenes. We failed to get the foliar SDS symptoms this year despite we irrigated routinely during the reproductive

phase starting R3 stage. The season was very dry and we observe only sporadic foliar symptoms among the transgenic lines.
We expect to identify homozygous lines for 3 and 4 gene combinations and evaluate the lines carrying 2, 3 and 4 genes for SDS and SCN resistance by March 31, 2022.