MSPB Project 31-2018 Final Report

Title of project: Evaluation of soybean breeding lines for resistance to Phomopsis seed decay and for high seed germinability

Principal investigator: Shuxian Li, Research Plant Pathologist, USDA-ARS, Crop Genetics Research Unit (CGRU), Stoneville, MS 38776. Phone: 662-686-3061. Email: shuxian.li@usda.gov.

Collaborator: James Smith, USDA-ARS, Crop Genetics Research Unit, Stoneville, MS 38776. rusty.smith@usda.gov

Background and Objectives

Phomopsis seed decay (PSD) of soybean is a major cause of poor seed quality in most soybean production areas, especially in the mid-southern region of the United States. PSD is caused by the seed-borne fungal pathogen *Phomopsis longicolla* (syn. *Diaporthe longicolla*). Breeding for PSD-resistance is the most cost-effective long-term strategy to control this disease. In recent years, new sources of resistance to PSD have been identified (Li et al., 2011, 2015). Lines with high germinability have also been identified (Smith et al., 2008). Crosses were made between these new sources, resulting in the development of multiple heterogeneous breeding lines with the potential for having both high germinability and resistance to PSD. The main goal of this research project was to develop and select soybean breeding lines with both PSD resistance and high germinability under Phomopsis-inoculated field conditions at Stoneville, MS. Seed quality assays, including seed plating for percentage of Phomopsis seed infection and standard germination tests were conducted following the harvest of plants selected for improved agronomic traits. Results of this research will provide to scientists and the seed industry, and may lead to the release of new soybean lines with both PSD resistance and high seed germinability. The new lines could both reduce elevator dockage due to damaged seed and be suitable for seed bean production.

The objectives of this research were to:

1. Evaluate over 200 selected heterogeneous soybean breeding lines for resistance to Phomopsis seed decay (PSD) under Phomopsis field-inoculated conditions in 2016.
2. Test PSD-resistant homogeneous breeding lines with high seed quality in multi-year trials (beyond 2016).
3. Identify agronomically-improved PSD-resistant homogeneous lines with high germinability by the end of the project in 2019.
4. Provide information to soybean breeders, growers, and others in the seed industry interested in disease resistance and seed quality.

APPROACH AND EXPERIMENT CONDUCT:

A total of 221 heterogeneous breeding lines derived from crosses between PSD-resistant and high-germination lines, as well as between PSD-resistant lines, were planted at Stoneville, MS on April 26, 2016 for evaluation of resistance to PSD and seed germinability. These breeding lines were derived from six pedigrees and utilized five sources of PSD-resistance. Sources of resistance were compared for percent seed infection by *Phomopsis longicolla* in a completely randomized design, where individual plant selections within each source serve as replications. Eight seeds/ft of row were planted in 10-ft long single-row plots with a row spacing of 36 in. Plants were inoculated at the R5 to R6 growth stages with a spore suspension (10^{-6}/ml) that was prepared using a Mississippi isolate of *P. longicolla*. From each harvested plant, a total of 25 seeds were randomly selected from each selected plant for the plating assay. Seeds were surface-disinfected in 0.5% sodium hypochlorite for 3 min, rinsed in sterile distilled water, and then placed on potato dextrose agar (Difco Laboratories, Detroit, MI) that was acidified (pH 4.8) with 25% lactic acid (APDA). Five seeds per Petri dish and five Petri dishes per sample were used. After 4 d of incubation at 24°C, the incidence of *P. longicolla* growing on the APDA was recorded and calculated as percent seed infected by *P. longicolla*. Germination tests were conducted using the standard protocol as described (Association of Official Seed Analysts, 2001). Data were analyzed using SAS (version 9.4, SAS Institute, Cary, NC.). Analysis of variance was used to estimate error and Fisher's least significant difference (LSD) at $P \leq 0.05$ was used to determine differences among sources of resistance.

The above protocol was followed multiple years before the project started in 2016 with the goal of developing homogeneous lines (pure lines). A two-week delayed harvest protocol (two weeks after R8) was followed to avoid pathogen escapes from the inoculation treatment. After harvest, the above lab assays were conducted. Homogeneous lines that had resistance to PSD and that had high germinability were evaluated for agronomic traits (yield, maturity, lodging, height, etc.) in standard replicated trials. Selected lines also were tested in regional trials, where the best lines were identified for future release.

RESULTS AND DISCUSSION:

Results from the seed plating assays of breeding lines from the 2016 field trail showed that percentage of Phomopsis seed infection ranged from 0 to 100% (Table 1). Since rain and humid conditions prevailed late into the harvest season, the overall PSD incidence was higher than that in 2014 and 2015 (Table 1).

Mean percentages of Phomopsis seed infection of F_4, F_5, F_6, and F_7-derived progenies developed by pedigree selection in field-inoculated tests at Stoneville, MS in 2014, 2015, 2016, and 2017 are shown in Table 1.

Mean percentages of seed germination of putative Phomopsis-resistant soybean breeding lines from field trials in 2015, 2016, 2017, and 2018 are shown in Table 2. Overall, breeding lines had their highest germinations in 2015 and 2016, while in 2018 the germinations were the lowest among the tests from 2015 to 2018 (Table 2).

Mean percentages of Phomopsis seed infection, seed germination, and total seed damage for six soybean cultivars and seven soybean lines that were transferred to the soybean community for breeding programs under Material Transfer Agreements are shown in Table 3. The years 2017 and 2018 featured warm wet weather throughout harvest that was highly conducive to seed infection. 2018 was especially severe. Cultivar checks ranged in maturity from late III to late IV were highly infected. Some of the improved breeding lines had substantially less damage than cultivar check of comparable maturity.

Due to the excessive rains during the harvest season in southern states in 2018, the overall PSD incidence was very high. Results from the seed plating assays of breeding lines from the 2018 trials indicated that mean percentages of Phomopsis seed infection ranged from 24 to 100%. Of

158 seed samples tested, 142 had higher than 50% Phomopsis infection, and 18 of them had higher than 90% Phomopsis infection (data no show).

RESEARCH CONCLUSION:

In this project, over 200 selected heterogeneous soybean breeding lines were tested for resistance to Phomopsis seed decay (PSD) under Phomopsis field-inoculated conditions. Twenty-seven PSD-resistant homogeneous breeding lines with resistance to PSD and high seed quality were identified and tested in multi-year trials. Seven soybean lines, 11043-225-72, 11043-224-91, 11030-541-28, 10061-236-11, 10076-121-11, DS65-1, and DS31-243, have been transferred to the soybean community for breeding programs under Material Transfer Agreements.

PUBLICATIONS RELATED TO THE PROJECT:

1. Li. S. and J. Smith. Evaluation of soybean breeding lines for resistance to Phomopsis seed decay in Stoneville, Mississippi, 2014. Plant Disease Management Report. 10:FC045. 2016. Online publication.
2. Li, S. and Smith J. Evaluating soybean breeding lines developed from different sources of resistance to Phomopsis seed decay. http://www.apsnet.org/meetings/annual/abstracts/pages/abstractdetail.aspx?MID=563. 2016.
3. Li, S. and Smith J. Assessment of soybean breeding lines for resistance to Phomopsis seed decay from field trials in Stoneville, Mississippi. Phytopathology 107(12S): S5.73.
4. Li, S. and Smith, J. Rusty. Evaluation of soybean breeding lines for resistance to Phomopsis seed decay: Results from field trials of 2014, 2015, and 2016 in Stoneville, Mississippi http://www.apsnet.org/meetings/annual/abstracts/pages/abstractdetail.aspx?. 2018.
5. Smith, J. R., Gillen, M. A., Nelson, R. L., Bruns, A., Mengistu, A., Li, S., and Bellaloui, N. Notice of release of LG03-4561-14, Soybean. Docket Number 0057.18. Released 9-26-2018. (Germplasm Release)
6. Smith, J. R., Gillen, M. A., Nelson, R. L., Bruns, A., Mengistu, A. Li, S., and Bellaloui, N. Registration of high-yielding exotically-derived soybean germplasm line LG03-4561-14. Journal of Plant Registrations. 13: 237-244. 2019. doi:10.3198/jpr2018.09.0061crg.

OTHER ACTIVITIES RELATED TO THE PROJECT:

In August 2016, we presented a paper entitled “Evaluating soybean breeding lines developed from different sources of resistance to Phomopsis seed decay” at the American Phytopathological Society (APS) Annual Meeting in Tampa, Florida.

In August 2017, a poster entitled “Assessment of soybean breeding lines for resistance to Phomopsis seed decay from field trials in Stoneville, Mississippi” was presented at the APS Annual Meeting at San Antonia, Texas.

On August 21, 2017, we hosted four scientists from Dupont Pioneer for a field site visit. Pioneer was interested in obtaining resistant lines with genetic markers for use in selection. In addition, we had a meeting with scientists from Dupont Pioneer and discussed our research on Phomopsis and seed quality issues at the Pioneer facility, Elizabeth, MS on March 15, 2018. Pioneer is interested in evaluating soybean lines for resistance to Phomopsis seed decay (PSD) and obtaining inoculum from us for field screening for PSD resistance. Data on seed damage was also presented at the Mid-South Soybean Board Meeting on January 10, 2018 in Memphis.

On June 20, 2018, an oral presentation was made based upon an invitation for giving a seminar on soybean diseases at the Department of Biological Sciences at Fayetteville State University (FSU), North Carolina. As part of this seminar, there was participation in a local field trip during which there was diagnosis of soybean diseases on site. FSU is a historically black public regional university established in 1937.

In August 2018, a poster, related to this research project, entitled “Evaluation of soybean breeding lines for resistance to Phomopsis seed decay: Results of 2014, 2015, and 2016 field trials in Stoneville, Mississippi” was presented at the International Congress of Plant Pathology (ICPP) 2018/American Phytopathological Society Annual Meeting in Boston.

On August 19, 2019, Dr. Dennis B. Reginelli, Executive Director, Mississippi Soybean Promotion Board, was shown our Stoneville experimental plots and ongoing research.

In addition, a total of 6,000 infested toothpicks of the soybean stem canker inoculum and 5 gallons of Phomopsis inoculum were prepared and provided to ARS breeders for USDA-ARS Uniform Soybean Tests-Southern States each year, and the collaborative research. In conjunction with the proper required paper work, soybean pathogen inocula were prepared and have been provided to the following private and public soybean breeders:

1. University of Missouri (Drs. Grover Shannon and Pengyin Chen) received: 15,000 infested toothpicks of soybean stem canker inoculum and *P. longicolla* spore stock suspension for 10 gallons of working inoculum (2016-2019).
2. Pioneer (Mr. Bob Sutter) received: *P. longicolla* spore stock suspension for 50 gallons of working inoculum (inoculation of 510 field plots) in 2018.
3. Mississippi State University (Dr. Tessie Wikerson) received: *P. longicolla* spore stock suspension for over10 gallons of working inoculum (2016- 2018).

Table 1. Mean percentages of Phomopsis seed infection of F_4, F_5, F_6, and F_7-derived progenies developed by pedigree selection from field inoculated tests at Stoneville, MS in 2014, 2015, 2016, and 2017.

Pedigree [a]	**Selection number** [b]				**2014**		**2015**		**2016**		**2017**		
	F_1	**F_2**	**F_3**	**F_4**	**No. Obs.** [c]	**PSD** [d] **(%)**	**No. Obs.**	**PSD (%)**	**No. Obs.**	**PSD (%)**	**No. Obs.**	**PSD (%)**	**Test**
(DT98-9102/PI 587982A)/PI 424324B	2	2	4	2	1	0.0	2	0.0	5	0.8	5	15.2	BH [e]
(DT98-9102/PI 587982A)/PI 424324B	2	2	4	4	1	0.0	3	1.3	5	0.0	4	2	BH
(DT98-9102/PI 587982A)/PI 424324B	4	4	2	2	1	0.0	1	0.0	2	2.0	1	0	BH
(DT98-9102/PI 587982A)/PI 424324B	4	4	2	6	1	0.0	1	0.0	1	0.0	NT [f]	NT	NT
(DT98-9102/PI 587982A)/PI 424324B	4	4	2	7	1	0.0	1	0.0	1	0.0	NT	NT	NT
(DT98-9102/PI 587982A)/PI 424324B	5	2	8	1	1	0.0	1	0.0	4	10.0	3	4	SP [g]
(DT98-9102/PI 587982A)/PI 424324B	5	2	8	2	1	52.0	2	0.0	12	15.0	3	12	SP
(DT98-9102/PI 587982A)/PI 424324B	5	2	8	3	1	0.0	1	0.0	3	2.7	11	9.5	SP
(DT98-9102/PI 587982A)/PI 424324B	5	2	8	5	1	0.0	1	0.0	2	2.0	13	6.5	SP
(DT98-9102/PI 587982A)/PI 424324B	5	2	8	6	1	4.0	1	4.0	1	12.0	NT	NT	NT
(DT98-9102/PI 587982A)/PI 424324B	5	2	8	9	1	0.0	1	4.0	1	28.0	NT	NT	NT
(DT98-9102/PI 587982A)/PI 424324B	5	4	1	2	1	4.0	10	0.0	10	14.4	5	28.5	RP [h]
(DT98-9102/PI 587982A)/PI 424324B	5	4	5	2	1	4.0	1	0.0	1	12.0	NT	NT	NT
(DT98-9102/PI 587982A)/PI 424324B	5	4	7	3	1	8.0	1	0.0	1	16.0	NT	NT	NT
(DT98-9102/PI 587982A)/PI 417050	1	1	3	3	1	0.0	1	0.0	1	24.0	NT	NT	NT
(DT98-9102/PI 587982A)/PI 417050	1	1	3	6	1	0.0	2	0.0	3	10.7	1	80	BH
(DT98-9102/PI 587982A)/PI 417050	1	1	3	10	1	0.0	1	0.0	1	100.0	NT	NT	NT
(DT98-9102/PI 587982A)/PI 417050	1	3	1	2	1	0.0	1	0.0	1	28.0	NT	NT	NT
(DT98-9102/PI 587982A)/PI 417050	2	2	2	2	1	12.0	1	0.0	1	28.0	NT	NT	NT
(DT98-9102/PI 587982A)/PI 417050	2	2	3	4	1	36.0	1	0.0	1	20.0	NT	NT	NT
(DT98-9102/PI 587982A)/PI 417050	2	2	4	8	1	0.0	1	0.0	1	0.0	1	33.8	RP
(DT98-9102/PI 587982A)/PI 417050	2	2	4	9	1	16.0	1	0.0	1	8.0	3	37.3	RP
(DT98-9102/PI 587982A)/PI 417050	2	2	5	7	1	0.0	2	0.0	2	28.0	3	8.4	RP
(DT98-9102/PI 587982A)/PI 417274	1	2	1	1	1	0.0	1	0.0	1	16.0	NT	NT	NT
(5601T/PI 587982A)/PI 424324B	5	1	1	1	1	0.0	1	0.0	1	36.0	NT	NT	NT
(5601T/PI 587982A)/PI 424324B	6	1	1	1	1	0.0	1	0.0	1	72.0	NT	NT	NT
PI 80837/SS93-6181	1	2	1	3	2	4.0	2	0.0	4	7.0	1	52	BH

[a] Parentage of a given F_2, F_3, F_4, or F_5 plant and its derived progenies.

[b] Numbers in each column refer to the individual plant selection in a given generation of inbreeding. For example, for the first genotype, the second F_4 plant was derived from the fourth F_3 plant, which was derived from the second F_2 plant, which was derived from the second F_1 plant. Seed assayed from the crosses (DT98-9102/PI 587982A)/PI 424324B, (DT98-9102/PI

587982A)/PI 417050, and (DT98-9102/PI 587982A)/PI 417274 were F_4-derived F_5 in 2014, F_5-derived F_6 in 2015, F_6-derived F_7 or F_5-derived F_7 in 2016, and F_7-derived F_8 or F_6-derived F_8 in 2017. Seed assayed from (5601T/PI 587982A)/PI 424324B were F_2-derived F_3 in 2014, F_3-derived F_4 in 2015, and F_4-derived F_5 in 2016. Seed assayed from PI 80837/SS93-6181 were F_{12}-derived F_{13} in 2014, F_{13}-derived F_{14} in 2015, and F_{14}-derived F_{15} in 2016.

[c] Number of Observations. Indicates the number of single plants, bulked single rows, or replicated means of bulked lines providing seed that were assayed from a given genotype in a given year. For example, in 2014 seed was assayed from one plant for all genotypes except for seed derived from PI 80837/SS93-6181, which was represented by two plants. The total number of plants providing seed for assays increased from 2014 to 2015 to 2016 to 2017. In cases where multiple plants were used to sample a given genotype in a given year, those plants all traced back to a specific common F4 plant and are therefore listed as F_4-derived because they are from the same F_4 plant.

[d] Percentages of seed infection by *Phomopsis longicolla* determined by the seed plating assay.

[e] BH: bulk harvest of 1 row.

[f] NT: not tested. It was dropped from the program due to poor seed quality.

[g] SP: single plant.

[h] RP: mean of 3 replicated plots.

Table. 2. Mean percentages of seed germination of putative Phomopsis-resistant soybean breeding lines[a] from field trials in 2015, 2016, 2017, and 2018.

Entry[b]	**Pedigree**	**Generation**[c]	**Germination (%)**[d]			
			2015	**2016**	**2017**	**2018**
11030-2-2-4-2-2-1	DS25-1 x PI424324B[e]	F6	96.0	96.0	35.0	NT[f]
11030-2-2-4-2-2-2	DS25-1 x PI424324B	F6	96.0	98.0	87.0	87.0
11030-2-2-4-2-2-3	DS25-1 x PI424324B	F6	96.0	96.0	82.0	76.0
11030-2-2-4-2-2-4	DS25-1 x PI424324B	F6	96.0	96.0	87.0	77.7
11030-2-2-4-2-5-1	DS25-1 x PI424324B	F6	92.0	96.0	91.0	72.7
11030-2-2-4-4-1-1	DS25-1 x PI424324B	F6	96.0	84.0	NT	NT
11030-2-2-4-4-1-2	DS25-1 x PI424324B	F6	96.0	94.0	96.0	77.0
11030-2-2-4-4-4-1	DS25-1 x PI424324B	F6	96.0	96.0	86.0	81.0
11030-2-2-4-4-5-1	DS25-1 x PI424324B	F6	98.0	94.0	90.0	83.0
11030-2-2-4-4-5-2	DS25-1 x PI424324B	F6	98.0	98.0	81.0	65.3
11030-4-4-2-2-1-1	DS25-1 x PI424324B	F6	96.0	94.0	82.0	84.7
11030-4-4-2-6-5-1	DS25-1 x PI424324B	F6	94.0	88.0	NT	NT
11030-4-4-2-7-2-1	DS25-1 x PI424324B	F6	96.0	86.0	NT	NT
11030-5-2-8-3-3-3-6	DS25-1 x PI424324B	F7	96.0	98.0	90.0	NT
11030-5-2-8-5-1-1-1	DS25-1 x PI424324B	F7	98.0	88.0	90.0	NT
11030-5-2-8-5-1-1-2	DS25-1 x PI424324B	F7	98.0	96.0	88.0	NT
11030-5-2-8-5-1-1-3	DS25-1 x PI424324B	F7	98.0	96.0	88.0	NT
11030-5-2-8-5-1-1-4	DS25-1 x PI424324B	F7	98.0	96.0	96.0	NT
11030-5-2-8-5-1-1-5	DS25-1 x PI424324B	F7	98.0	96.0	90.0	NT
11043-1-1-3-6-5-2	DS25-1 x PI417050	F6	96.0	96.0	23.0	NT
11043-225-72	DS25-1 x PI417050	F5	98.0	96.0	75.0	59.3
11043-224-91	DS25-1 x PI417050	F5	96.0	92.0	46.3	60.0
11030-541-24	DS25-1 x PI424324B	F5	94.0	96.0	74.3	64.0
11043-224-81	DS25-1 x PI417050	F5	94.0	93.0	64.7	10.3
11030-541-28	DS25-1 x PI424324B	F5	98.0	93.0	73.3	61.3
11030-541-26	DS25-1 x PI424324B	F5	96.0	93.0	57.3	NT
11030-541-29	DS25-1 x PI424324B	F5	94.0	89.0	62.7	NT
11030-541-210	DS25-1 x PI424324B	F5	96.0	94.0	84.3	NT
Mean ± S.E.			96.2 ± 0.4	93.9 ± 1.0	76.8± 5.7	68.5± 6.4

[a] These lines were completely free of Phomopsis seed decay based on seed plating assays in 2014, 2015, and 2016, using F5, F6, and F7 seed, respectively.

[b] Specific soybean breeding line designation is based on pedigree selection. Each successive number after the first hyphen (moving left to right) indicates the plant number selected in the F_1, F_2, F_3, F_4, F_5, F_6, and F_7, respectively. 11043-2-2-4-8-1 has no F_6 plant number because the row was bulked as an F_5-derived row. The sequence of numbers for each line can be used to indicate relatedness among lines. For example, the first four lines were derived from the same F_1, F_2, F_3, F_4, and F_5 plant, but different F_6 plants. These four lines are highly related.

[c] Generation that the lines were bulked.

[d] Germination assays were conducted by the Mississippi Bureau of Plant Industry State Seed Lab per the official protocol for standard germination tests. Due to seed availability, fifty seeds were assayed per line for single plant-derived lines and 200 seeds were assayed for bulked lines.

[e] Phomopsis resistance source, either PI 424324B, PI 417050, or PI587982A. DS25-1 is derived from PI587982A.

[f] Not tested. It was dropped from the program due to poor seed quality.

Table 3. Mean percentages of Phomopsis seed infection, seed germination, and total seed damage for six soybean cultivars and seven soybean lines that have been transferred to the soybean community for breeding programs under Material Transfer Agreements.

Entry	PSD (%)				Germination (%)				DKT (%)[b]	
	2015	2016	2017[a]	2018[a]	2015	2016	2017	2018	2017	2018
Breeding lines										
11043-225-72	0.0	28.0	17.3	76.0	98.0	96.0	75.0	59.3	3.3	4.6
11043-224-91	0.0	8.0	33.3	78.7	96.0	92.0	46.3	60.0	3.5	9.6
11030-541-28	0.0	14.4	33.3	54.7	98.0	93.0	73.3	61.3	3.9	8.4
10061-236-11	NA[c]	NA	NA	62.7	95.0	93.0	85.0	67.3	3.6	13.1
10076-121-11	NA	NA	NA	56.0	93.0	92.0	58.0	56.0	4.6	13.8
DS65-1	NA	NA	34.7	57.3	98.0	96.0	86.0	71.2	4.6	4.0
DS31-243	NA	NA	NA	70.7	90.0	94.0	NT[d]	72.0	NT	5.9
Cultivar checks										
SS93-6181 (Early MG IV, Resistant check)	NT	NT	40.0	65.3	NT	NT	NT	71.3	5.5	4.6
CZ3841 LL (Late MG III)	NT	NT	57.3	85.3	NT	51.5	NT	36.3	8.9	12.9
LG03-4561-14 (Late MG III)	NT	NT	54.7	80.0	47.0	46.0	NT	NT	13.3	15.1
LD06-7620 (Early IV)	NT	NT	44.0	76.0	31.0	34.0	NT	NT	14.1	48.9
P46T59R (Mid MG IV)	NT	NT	NT	85.3	NT	NT	NT	NT	NT	14.3
P48A32X (Late MG IV)	NT	NT	NT	89.3	NT	NT	NT	NT	NT	11.0

[a] The years 2017 and 2018 featured warm wet weather throughout harvest that was highly conducive to seed infection. 2018 was especially severe. Note that cultivar checks ranged in maturity from late III to late IV and V were highly infected throughout.

[b] "Damage Kernel Total" was determined by Federal Grain Inspection Services (FGIS) standards for the levels of seed damage.

[c] Seeds were not available because the line had not yet been developed.

[d] Not tested due to limited seed.