MISSISSIPPI SOYBEAN PROMOTION BOARD
PROJECT 31-2016 (YEAR 1)
2016 ANNUAL REPORT

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

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BACKGROUND AND OBJECTIVE

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). Also recently, lines with high germinability have 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 of having both high germinability and resistance to PSD.

The objective of this proposed research is to identify 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, will be conducted following the harvest of plants selected for improved agronomic traits. Results of this research will be provided 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 from damaged seed and be suitable for seed bean production.

The objectives of this research are 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 resistance to PSD and 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.

REPORT OF PROGRESS/ACTIVITY

During this reporting year, we planted 221 breeding lines in Stoneville, MS on April 26, 2016 for evaluation of resistance to PSD and seed germinability. Those breeding lines were derived from six pedigrees and utilized five sources of PSD-resistance. Sources of resistance will be 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 is prepared using a Mississippi isolate of *P. longicolla*. Selected single plants were manually harvested shortly after R8.

From each harvested plant, a total of 25 seeds were randomly selected from each selected plant for the plating assay. Seeds are 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 is 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 were 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.).

Results from the seed plating assays of 80 selected breeding lines from the 2016 field trail show that percentage of Phomopsis seed infection ranged from 0 to 100%. Because of rain and humid conditions late in the season, the overall PSD incidence was higher than that in 2014 and 2015. The mean percentage of Phomopsis seed infection of F2, F3, and F4-derived progenies developed by pedigree selection from the field tests at Stoneville, MS in 2014, 2015, and 2016 is show in **Table 1**. Fourteen breeding 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. Results of germination tests of those resistant lines are shown in **Table 2**.

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. This year,
we have submitted an abstract entitled “Assessment of soybean breeding lines for resistance to Phomopsis seed decay from field trials in Stoneville, Mississippi” and will present at the APS Annual Meeting at San Antonia, Texas in August 2017.

Table 1. Mean percentage of Phomopsis seed infection of F2, F3, and F4-derived progenies developed by pedigree selection from the field tests at Stoneville, MS in 2014, 2015, and 2016.

Pedigree	Selection number				2014		2015		2016		2015 and 2016
	F1	F2	F3	F4	No. Obs.	PSD (%)	No. Obs.	PSD (%)	No. Obs.	PSD (%)	PSD Mean (95% C.I.)
(DT98-9102/PI 587982A)/PI 424324B	2	2	4	2	1	0.0	2	0.0	5	0.8	0.4
(DT98-9102/PI 587982A)/PI 424324B	2	2	4	4	1	0.0	3	1.3	5	0.0	0.7
(DT98-9102/PI 587982A)/PI 424324B	4	4	2	2	1	0.0	1	0.0	2	2.0	1.0
(DT98-9102/PI 587982A)/PI 424324B	4	4	2	6	1	0.0	1	0.0	1	0.0	0.0
(DT98-9102/PI 587982A)/PI 424324B	4	4	2	7	1	0.0	1	0.0	1	0.0	0.0
(DT98-9102/PI 587982A)/PI 424324B	5	2	8	1	1	0.0	1	0.0	4	10.0	5.0
(DT98-9102/PI 587982A)/PI 424324B	5	2	8	2	1	52.0	2	0.0	12	15.0	7.5
(DT98-9102/PI 587982A)/PI 424324B	5	2	8	3	1	0.0	1	0.0	3	2.7	1.3
(DT98-9102/PI 587982A)/PI 424324B	5	2	8	5	1	0.0	1	0.0	2	2.0	1.0
(DT98-9102/PI 587982A)/PI 424324B	5	2	8	6	1	4.0	1	4.0	1	12.0	8.0
(DT98-9102/PI 587982A)/PI 424324B	5	2	8	9	1	0.0	1	4.0	1	28.0	16.0
(DT98-9102/PI 587982A)/PI 424324B	5	4	1	2	1	4.0	10	0.0	10	14.4	7.2
(DT98-9102/PI 587982A)/PI 424324B	5	4	5	2	1	4.0	1	0.0	1	12.0	6.0
(DT98-9102/PI 587982A)/PI 424324B	5	4	7	3	1	8.0	1	0.0	1	16.0	8.0
(DT98-9102/PI 587982A)/PI 417050	1	1	3	3	1	0.0	1	0.0	1	24.0	12.0
(DT98-9102/PI 587982A)/PI 417050	1	1	3	6	1	0.0	2	0.0	3	10.7	5.3
(DT98-9102/PI 587982A)/PI 417050	1	1	3	10	1	0.0	1	0.0	1	100.0	50.0
(DT98-9102/PI 587982A)/PI 417050	1	3	1	2	1	0.0	1	0.0	1	28.0	14.0
(DT98-9102/PI 587982A)/PI 417050	2	2	2	2	1	12.0	1	0.0	1	28.0	14.0
(DT98-9102/PI 587982A)/PI 417050	2	2	3	4	1	36.0	1	0.0	1	20.0	10.0
(DT98-9102/PI 587982A)/PI 417050	2	2	4	8	1	0.0	1	0.0	1	0.0	0.0
(DT98-9102/PI 587982A)/PI 417050	2	2	4	9	1	16.0	1	0.0	1	8.0	4.0
(DT98-9102/PI 587982A)/PI 417050	2	2	5	7	1	0.0	2	0.0	2	28.0	14.0
(DT98-9102/PI 587982A)/PI 417274	1	2	1	1	1	0.0	1	0.0	1	16.0	8.0
(5601T/PI 587982A)/PI 424324B	5	1	1	1	1	0.0	1	0.0	1	36.0	18.0
(5601T/PI 587982A)/PI 424324B	6	1	1	1	1	0.0	1	0.0	1	72.0	36.0
PI 80837/SS93-6181	1	2	1	3	2	4.0	2	0.0	4	7.0	3.5

[a]Parentage of a given F2, F3, or F4 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 F4 plant was derived from the fourth F3 plant, which was derived from the second F2 plant, which was derived from the second F1 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 F4-derived F5 in 2014, F4-derived F6 in 2015, and F4-derived F7 in 2016. Seed assayed from (5601T/PI 587982A)/PI 424324G were F2-derived F3 in 2014, F3-derived F4 in 2015, and F4-derived F5 in 2016. Seed assayed from PI 80837/SS93-6181 were F4-derived F13 in 2014, F4-derived F14 in 2015, and F4-derived F15 in 2016.

[c]Number of Observations; indicates the number of single plants 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. In cases where multiple plants were used to sample a given genotype in a given year, those plants all trace back to a specific common F4 plant and are therefore listed as F4-derived because they are from the same F4 plant.

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

Table 2. Percentage of seed germination of 14 Phomopsis-resistant soybean breeding lines from field trials in 2015 and 2016[a]

	2017		Germination (%)[c]	
Entry[b]	Generation	Cross	2015	2016
11030-2-2-4-2-2-1	F6:7	DS25-1 x PI424324B[d]	96	96
11030-2-2-4-2-2-2	F6:7	DS25-1 x PI424324B	96	98
11030-2-2-4-2-2-4	F6:7	DS25-1 x PI424324B	96	96
11030-2-2-4-2-5-1	F6:7	DS25-1 x PI424324B	92	96
11030-2-2-4-4-1-1	F6:7	DS25-1 x PI424324B	96	84
11030-2-2-4-4-1-2	F6:7	DS25-1 x PI424324B	N/A	94
11030-2-2-4-4-4-1	F6:7	DS25-1 x PI424324B	96	96
11030-4-4-2-2-1-1	F6:7	DS25-1 x PI424324B	96	94
11030-4-4-2-6-5-1	F6:7	DS25-1 x PI424324B	94	88
11030-4-4-2-7-2-1	F6:7	DS25-1 x PI424324B	96	86
11030-5-2-8-3-3-3	F6:7	DS25-1 x PI424324B	96	98
11030-5-2-8-5-1-1	F6:7	DS25-1 x PI424324B	98	96
11043-1-1-3-6-5-2	F6:7	DS25-1 x PI417050	96	88
11043-2-2-4-8-1	F5:7	DS25-1 x PI417050	94	90
Mean ± S.E.			95.5±0.4	92.9±1.3

[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]Entry: 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 F1, F2, F3, F4, F5, and F6, respectively. 11043-2-2-4-8-1 has no F6 plant number because the row was bulked as an F5-derived row. The sequence of numbers for each line can be used to indicate relatedness among lines. For example, the first two lines were derived from the same F1, F2, F3, F4, and F5 plant, but different F6 plants. These two lines are highly related.

[c]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 F6-derived lines and 200 seeds were assayed for the F5:7 line.

[d] Phomopsis resistance source; either PI 424324B or PI 417050.