MISSISSIPPI SOYBEAN PROMOTION BOARD
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2017 ANNUAL REPORT

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

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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 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 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 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 2017.
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 finished seed assays and data analysis of 158 seed samples of soybean breeding lines that were harvested in the trials with Phomopsis field-inoculation in Stoneville, MS in 2017. Those seed samples were harvested from four field tests including yield, weathered, and "May Plant Rows (MPR bulks, and single plants)". Germination tests of selected breeding lines harvested in 2017 have also been finished. Further selections have been made based on the data of seed assays. Below are the current active Phomopsis breeding populations from which the 52 lines were developed:

PI 80837 x SS93-6181 (1 F14-derived and 1 F12-derived lines); total of 2.
DS25-1 x PI 424324B (10 F6-derived, 5 F5-derived, and 31 F7-derived lines): total of 46.
DS25-1 x PI 417050 (1 F6-derived and 3 F5-derived lines): total of 4.

The most advanced lines have been advanced in replicated field tests. Phomopsis inoculation treatment with delayed harvest will be conducted 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 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 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 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.).

Mean percentages of Phomopsis seed infection of F2, F3, and F4-derived progenies developed by pedigree selection from the field tests at Stoneville, Miss. are show in Table 1. Fourteen breeding lines were completely free of Phomopsis seed decay based on seed plating assays in 2017 using F5, F6, and F7 seed, respectively. Results of germination tests of those resistant lines are shown in Table 2.

Results of our research entitled "Assessment of soybean breeding lines for resistance to Phomopsis seed decay from field trials in Stoneville, Mississippi" was presented at the American Phytopathological Society Annual Meeting in August 2017. An abstract entitled "Evaluation of soybean breeding lines for resistance to Phomopsis seed decay: Results of 2014, 2015, and 2016 field trials in Stoneville, Mississippi" has been submitted and accepted for presentation at the International Congress of Plant Pathology (ICPP) 2018/American Phytopathological Society Annual Meeting in Boston, August 2018.

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Besides research and presentation activities, we hosted four scientists from Dupont Pioneer for a field site visit on August 21, 2017. 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 about research on Phomopsis and seed quality issues in Stoneville 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 Breeder Meeting on January 10, 2018 in Memphis.

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 2017.

	Selection number [b]				2017		
Pedigree [a]	**F1**	**F2**	**F3**	**F4**	**No. Lines**	**PSD (%)**	**Test [c]**
(DT98-9102/PI 587982A)/PI 424324B	2	2	4	2	5	15.2	BH
(DT98-9102/PI 587982A)/PI 424324B	2	2	4	4	4	2.0	BH
(DT98-9102/PI 587982A)/PI 424324B	4	4	2	2	1	0.0	BH
(DT98-9102/PI 587982A)/PI 424324B	4	4	2	6	NT	NT	NT
(DT98-9102/PI 587982A)/PI 424324B	4	4	2	7	NT	NT	NT
(DT98-9102/PI 587982A)/PI 424324B	5	2	8	1	3	4.0	SP
(DT98-9102/PI 587982A)/PI 424324B	5	2	8	2	3	12.0	SP
(DT98-9102/PI 587982A)/PI 424324B	5	2	8	3	11	9.5	SP
(DT98-9102/PI 587982A)/PI 424324B	5	2	8	5	13	6.5	SP
(DT98-9102/PI 587982A)/PI 424324B	5	2	8	6	NT	NT	NT
(DT98-9102/PI 587982A)/PI 424324B	5	2	8	9	NT	NT	NT
(DT98-9102/PI 587982A)/PI 424324B	5	4	1	2	5	28.5	RP
(DT98-9102/PI 587982A)/PI 424324B	5	4	5	2	NT	NT	NT
(DT98-9102/PI 587982A)/PI 424324B	5	4	7	3	NT	NT	NT
(DT98-9102/PI 587982A)/PI 417050	1	1	3	3	NT	NT	NT
(DT98-9102/PI 587982A)/PI 417050	1	1	3	6	1	80.0	BH
(DT98-9102/PI 587982A)/PI 417050	1	1	3	10	NT	NT	NT
(DT98-9102/PI 587982A)/PI 417050	1	3	1	2	NT	NT	NT
(DT98-9102/PI 587982A)/PI 417050	2	2	2	2	NT	NT	NT
(DT98-9102/PI 587982A)/PI 417050	2	2	3	4	NT	NT	NT
(DT98-9102/PI 587982A)/PI 417050	2	2	4	8	1	33.8	RP
(DT98-9102/PI 587982A)/PI 417050	2	2	4	9	1	37.3	RP
(DT98-9102/PI 587982A)/PI 417050	2	2	5	7	1	8.4	RP
(DT98-9102/PI 587982A)/PI 417274	1	2	1	1	NT	NT	NT
(5601T/PI 587982A)/PI 424324B	5	1	1	1	NT	NT	NT
(5601T/PI 587982A)/PI 424324B	6	1	1	1	NT	NT	NT
PI 80837/SS93-6181	1	2	1	3	1	52	BH

[a] Parentage of a given F2, F3, or F4 plant and its derived progenies.
[b] Percentage of seed infection by *Phomopsis longicolla* determined by the seed plating assay.
[c] NT = not test; RP = replicated plots (3); mean of multiple trials; BH = bulk harvest of 1 row; SP = single plant.

Table 2. Percentage of seed germination of putative Phomopsis-resistant soybean breeding lines from field trials in 2017.

Entry[b]	Generation	Cross	Germination (%)[c]
11030-2-2-4-2-2-1	F6:7	DS25-1 x PI424324B[d]	35.0
11030-2-2-4-2-2-2	F6:7	DS25-1 x PI424324B	87.0
11030-2-2-4-2-2-4	F6:7	DS25-1 x PI424324B	87.0
11030-2-2-4-2-5-1	F6:7	DS25-1 x PI424324B	91.0
11030-2-2-4-4-1-2	F6:7	DS25-1 x PI424324B	96.0
11030-2-2-4-4-4-1	F6:7	DS25-1 x PI424324B	86.0
11030-4-4-2-2-1-1	F6:7	DS25-1 x PI424324B	82.0
11030-5-2-8-3-3-3-6	F6:7	DS25-1 x PI424324B	90.0
11043-1-1-3-6-5-2	F6:7	DS25-1 x PI417050	23.0
11030-5-2-8-5-1-1-1	F6:7	DS25-1 x PI424324B	90.0
11030-5-2-8-5-1-1-2	F6:7	DS25-1 x PI424324B	88.0
11030-5-2-8-5-1-1-3	F6:7	DS25-1 x PI424324B	88.0
11030-5-2-8-5-1-1-4	F6:7	DS25-1 x PI424324B	96.0
11030-5-2-8-5-1-1-5	F6:7	DS25-1 x PI424324B	90.0
11043-225-72	F6:7	DS25-1 x PI417050	75.0
11043-224-91	F6:7	DS25-1 x PI417050	46.3
11030-541-24	F6:7	DS25-1 x PI424324B	74.3
11030-224-81	F6:7	DS25-1 x PI424324B	64.7
11030-541-28	F6:7	DS25-1 x PI424324B	73.3
11030-541-26	F6:7	DS25-1 x PI424324B	57.3
11030-541-29	F6:7	DS25-1 x PI424324B	62.7
11030-541-210	F6:7	DS25-1 x PI424324B	84.3
Mean ± S.E.			75.8 ± 4.3

[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. 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.