MISSISSIPPI SOYBEAN PROMOTION BOARD
PROJECT NO. 31-2015 (YEAR 2)
2015 Final Report

Title of project: Evaluation of the inheritance of resistance to Phomopsis seed decay in PI 458130 populations

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

Collaborators:
Jeff Ray, USDA-ARS, Crop Genetics Research Unit, Jeff.Ray@ARS.USDA.GOV;
James Smith, USDA-ARS, Crop Genetics Research Unit, Rusty.Smith@ARS.USDA.GOV

BACKGROUND AND OBJECTIVE

Phomopsis seed decay (PSD) can severely affect soybean seed quality and is a problem for many soybean farmers in the southern USA. Breeding for PSD resistance is the most effective long-term strategy to control PSD. The specific objective of this research is to phenotype F_2 populations of PI 458130 based on seed plating assays for the incidence of Phomopsis infection from a Phomopsis-inoculation field trial in Stoneville, Mississippi.

Our primary research goal is to increase soybean producer profitability by reducing yield losses and seed injury caused by PSD. This will be attained by 1) identifying new sources/genes of resistance to PSD; 2) breeding high-yielding cultivars and agronomically competitive breeding lines with PSD resistance. Ultimately, soybean growers will be benefit from this research because they will have higher quality seed to plant.

PI 458130 is one of the most resistant lines that we previously identified from Phomopsis-inoculation and delayed harvest field trials in Stoneville, Mississippi in a 4-year trial from 2006 to 2009 (Li et al., 2011). In collaboration with ARS and university scientists, populations with PI 458130 as one of the parents have been developed. Phenotyping F_2 populations in this proposed research will determine the inheritance of the PSD resistance in PI 458130. This will be the first step toward identifying the genomic location of the resistance associated with PI 458130 and will also allow the determination of the novelty of this source of resistance and provide markers for more rapid selection in breeding programs in the future. Incorporation of a new source of resistance into agronomically competitive breeding lines is fundamental to developing high yielding cultivars with PSD resistance.

REPORT OF RESEARCH CONDUCT AND RESULTS

Year 1 (31-2014) Research conducted

On 12 May, 2014, two F_2 (Resistant x Susceptible) populations with PI 458130 (PI 458130 x PI 399045 and 5601T x PI 458130) were planted at Stoneville, MS. Efforts were also made to test additional F_2 populations PI 399045 x PI 424324B and 5601T x PI 424324B (Susceptible x Resistant). The experimental design for the parents was a randomized complete block with 4

replications. Seeds were planted at a rate of 33/m of row in 2.74-m-long single-row plots with a 0.91-m row spacing. Each F2 population was grown in a single plot in each replication. A third F2 population (R x R) with PI 458130 (PI 458130 x PI 424324B) was grown in two rows in a single replication due to shortage of seed. In addition, multiple high-germinating breeding lines with potential PSD resistance from PI 424324B are being grown for further testing and selection.

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*. Inoculation was performed using a battery-operated backpack sprayer (30 psi) with a hand-held boom containing a single nozzle on a 20-in. boom with an adjustable orifice. Approximately 8.45 fl oz. of the spore suspension was used to inoculate each plot.

Selected single plants were manually harvested shortly after R8. By the end of November 2014, we finished the manual single-plant harvest of 363 F2 plants along with the parental plants from the field trials of three PSD populations.

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*. 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.

Year 1 Results

Results from analysis of the F_2 population 5601T x PI 458130 are shown in Table 1 and Figure 1. Phomopsis seed infection ranged from 0 to 52% among F_2 individuals. The ratio of Resistant to Susceptible plant reaction is 12:32, which fits very well an expected 1:3 ratio (X^2 =0.1212, P > 0.7277), indicating that the resistance in PI 458130 may be controlled by a single recessive gene in this population. Segregation also was found in the test of F_2 population PI 458130 x PI 3099045, but only 6 plants were available for the seed assay due to the poor emergence in the field trial.

Phenotyping of F_2 populations with PI 424324B is summarized in Table 2. The ratio of Resistant to Susceptible plant reaction in the population PI 399045x PI 424324B was 66:18, which fits very well an expected 3:1 ratio (X^2 =0.5714, P > 0.44970, while the ratio of Resistant to Susceptible plant reaction in the population 5601T x PI 424324B was 73:26, which also fits very well an expected 3:1 ratio (X^2 =0.842, P > 0.7717). These results indicate that the Resistance in PI 424324B may be controlled by a single dominant gene.

Seed assays of the PI 458130 x PI 424324B F_2 population (Resistant x Resistant) revealed that, of 24 plants tested, 7 (29%) did not have any Phomopsis seed infection, while 17 plants (71%) had different levels of Phomopsis seed infection (Fig. 2). Segregation in this R x R cross indicates that PI 458130 and PI 424324B likely contain different resistance genes to PSD.

Seed plating assays of breeding lines that were from the crosses between PSD-resistant and high germination lines were performed and analyzed. The Phomopsis seed infection of 50 breeding lines, for which we have finished seed assays, ranged from 0 to 52%, with 24 lines free of Phomopsis infection (Table 3). In 2014, 147 breeding lines were planted in the field in Stoneville, MS for further testing and selection for breeding for resistance to PSD. In addition, we field-tested two other PSD resistant populations (NC-Miller PG x PI 567381B, and N08-147 PT x PI 567381B) that were not listed in the original grant proposal.

Year 2 (31-2015) Research conducted

In the first quarter of FY2015, we finished seed plating assay for phenotyping F_2 populations that were planted in Stoneville, MS in 2014. All results were summarized in Year 1 (31-2014) report (see above).

An F_2 population NC-Miller X PI 458130 (Susceptible x Resistant) was planted in greenhouses and in growth chambers for evaluating the inheritance of resistance to PSD. Efforts were also made to test additional F_2 population PI 424324B x 5601T (Resistant x Susceptible) that was planted at Stoneville, MS on 5 May, 2015. The experimental design for the parents was a randomized complete block design with 8 replications along with parent seeds (2 reps). Seeds were planted at a rate of 33/m of row, in 2.74 m-long single-row plots with a 0.91-m row spacing. The F_2 population was grown in a single plot in each replication.

To develop soybean lines with resistance to PSD, multiple breeding populations were developed from crosses between PSD-resistant and high-germination lines, as well as between PSD-resistant lines. Soybean breeding lines derived from the above crosses were planted at Stoneville, MS for evaluation of resistance to PSD. Breeding lines were derived from five pedigrees and utilized four sources of PSD-resistance. Sources of resistance were compared for percent seed infection by *P. longicolla* in a completely randomized design, where individual lines within each source served as replications for each source. As such, each source was represented by a different number of replications. A total of 145 breeding lines were selected to plant and test in 2014 in Stoneville. Plants were inoculated at the R5 to R6 growth stages on 6 August, 20 August, and 3 September with a spore suspension (10^{-6}/ml) that was prepared using a Mississippi isolate of *P. longicolla*. The inoculation procedure, inoculum preparation, seed assay protocol were the same as described in above year 1 report.

Year 2 Results

Results from preliminary analysis of the F_2 population NC-Miller X PI 458130 showed that the Phomopsis seed infection ranged from 0 to 72% among F_2 individuals. Of 352 plants tested, 256 were free of PSD, while 96 plants were susceptible to PSD. The ratio of Susceptible to Resistant reactions fits an expected 1:3 ratio (X^2 =0.9697, $P > 0.3248$), indicating that the resistance in PI 458130 may be controlled by a single dominant gene in this population.

Results from analysis of the F_2 population PI 424324B x 5601T are summarized in Table 4. A total of 76 lines have been assayed. The Phomopsis seed infection ranged from 0 to 72% among F_2 individuals. The ratio of Resistant to Susceptible plant reaction is 59:17, which fits very well an expected 1:3 ratio (X^2 =0.2807, $P > 0.5962$), indicating that the resistance in PI 424324B may be controlled by a single dominant gene. This second year results confirm our finding in 2014.

Based on the seed plating assay of the 145 breeding lines, there were significant differences in the reaction to PSD among lines within the same source, as well as among lines within the same source, and among lines among sources (Table 5). More important for making future populations, the mean PSD score of the PI 424324B source (5.1) was significantly lower than that of the PI 80837/SS93-6181 source (12.7). The PSD value of the PI 424324B source was numerically lower than that of the PI 417050 source (9.7), although the difference was not significant. The PI 417050 source was not different from the PI 80837/SS93-6181 source. These data indicate an apparent superiority for using the PI 424324B source for breeding improved lines. The range of percent seed infected by *P. longicolla* among all lines was from 0 to 52%, with an overall line mean of 7.5%. In the pedigree of PI 80837 x SS93-6181 (Resistant x Resistant line), seven out of 11 lines had Phomopsis seed infection over 10%, but one line was free from PSD. In the pedigrees of DS25-1 x PI 424324B and DS25-1 x PI 417050, 33 and 26 lines, respectively, had zero percent Phomopsis seed infection. Some of these soybean breeding lines may have good potential to be released in the future as resistant to PSD.

SUMMARY AND FUTURE DIRECTION

Phomopsis seed decay (PSD) can severely affect soybean seed quality and is a problem for many soybean farmers in the southern USA. Breeding for PSD-resistance is the most effective long-term strategy to control PSD. Phenotyping F_2 populations developed from sources of PSD resistance will be the first step toward identifying the genomic location of the resistance. It will also allow the determination of the novelty of sources of resistance and provide markers for more rapid selection in breeding programs in the future study.

In this MSPB-funded research, experiments were conducted to phenotype five populations derived from the new PSD resistance sources of PI 458130 and PI 424324B in 2014 and 2015. Results from preliminary analysis of the F_2 population of 5601T x PI 458130 indicated that the resistance in PI 458130 may be controlled by a single recessive gene. However, results from analysis of the F_2 population NC-Miller x PI 458130 showed that the resistance in PI 458130 may be controlled by a single dominant gene. Different results obtained from the tests of two different populations with the same resistance source could be explained by the theory that parental combination can effect gene action, which has been reported in soybean rust resistance study.

Results from analysis of the F_2 populations of 5601T x PI 424324B (Susceptible x Resistant) and PI 424324B x 5601T (Resistant x Susceptible) showed that the resistance in PI 458130 is controlled by a single dominant gene. Segregation in PI 458130 x PI 424324B (Resistant x Resistant) cross indicated that PI 458130 and PI 424324B likely contain different resistance genes to PSD. To confirm this result, DNA was isolated from the F_2 tissue samples of the above S x R cross, and will be evaluated by molecular markers (SSR and SNP) near known PSD-resistant loci to determine if the resistance in PI 458130 and PI 424324B is a known or new gene. If it is a new PSD-resistance gene, then we will begin the process of mapping the new gene. Either way (new or old gene), additional plants will be assayed to confirm the above preliminary data. In addition, the breeding/selection process will continue with the goal of developing improved soybean germplasm with PSD resistance. Possibly, the resistance may be quantitative, in which case the segregating population will be used to develop a recombinant inbred population for future genetic mapping efforts.

For the effort of breeding for resistance to PSD, a total of 145 breeding lines were tested, among which 60 lines had zero percent Phomopsis seed infection in the 2014 tests. Some of these soybean breeding lines may have good potential to be released in the future as resistant to PSD.

Table 1. Phenotyping F_2 population 5601T X PI 458130 for resistance to Phomopsis seed decay from PI 458130 in 2014.

Parent/Population	No. of plants tested	No. of seed tested	No. of culture plates for seed assay	No. of plants with Phomopsis	Percentage of plants infected	Range of Phomopsis (%)
5601T*	30	750	150	28	93.3	0 to 64
5601T**	15	375	75	15	100	0 to 64
PI 458130*	4	100	4	0	0	0
PI 458130**	15	375	75	0	0	0
5601T x PI 458130	44	1100	220	32	72.7	0 to 52

* Seeds were harvested from field trial.
** Seeds were harvested from greenhouse trial.

Table 2. Phenotyping F_2 populations for resistance to Phomopsis seed decay from PI 424324B in 2014.

Parent/Population	Generation	No. of plant tested	No. of seed tested	No. of culture plates for seed assay	No. of plants with Phomopsis	Percentage of plant infected	Rang of Phomopsis (%)
5601T	(Parent 1)	27	675	135	7	25.9	0 to 16
PI 399045	(Parent 2)	35	875	175	10	28.6	0 to 20
PI 424324B	(Parent 3)	23	575	115	0	0	0
5601T x PI 424324B	F1	1	25	5	0	0	0
PI 399045 x PI424324B	F1	21	525	105	10	47.6	0 to 24
PI 399045 x PI424324B	F2	84	2100	420	18	21.4	0 to 20
5601T x PI 424324B	F2	99	2475	495	26	26.3	0 to 24
Total		290	7250	1450	71	24.5	0 to 24

Table 3. Means of percent seed infection by *Phomopsis longicolla* of 50 soybean breeding lines in replicated field tests with inoculation treatments in Stoneville, Mississippi in 2013.

Plant ID	Generation	Pedigree	PSD (%)
14P5	F12:13	PI 80837 x SN93-6181	0.0
14P12	F4:5	DS25-1 x PI 424324B	0.0
14P13	F4:5	DS25-1 x PI 424324B	0.0
14P14	F4:5	DS25-1 x PI 424324B	0.0
14P15	F4:5	DS25-1 x PI 424324B	0.0
14P16	F4:5	DS25-1 x PI 424324B	0.0
14P17	F4:5	DS25-1 x PI 424324B	0.0
14P18	F4:5	DS25-1 x PI 424324B	0.0
14P19	F4:5	DS25-1 x PI 424324B	0.0
14P22	F4:5	DS25-1 x PI 424324B	0.0
14P23	F4:5	DS25-1 x PI 424324B	0.0
14P25	F4:5	DS25-1 x PI 424324B	0.0
14P26	F4:5	DS25-1 x PI 424324B	0.0
14P27	F4:5	DS25-1 x PI 424324B	0.0
14P28	F4:5	DS25-1 x PI 424324B	0.0
14P30	F4:5	DS25-1 x PI 424324B	0.0
14P31	F4:5	DS25-1 x PI 424324B	0.0
14P32	F4:5	DS25-1 x PI 424324B	0.0
14P33	F4:5	DS25-1 x PI 424324B	0.0
14P40	F4:5	DS25-1 x PI 424324B	0.0
14P42	F4:5	DS25-1 x PI 424324B	0.0
14P44	F4:5	DS25-1 x PI 424324B	0.0
14P47	F4:5	DS25-1 x PI 424324B	0.0
14P48	F4:5	DS25-1 x PI 424324B	0.0
14P8	F12:13	PI 80837 x SN93-6181	4.0
14P36	F4:5	DS25-1 x PI 424324B	4.0
14P45	F4:5	DS25-1 x PI 424324B	4.0

14P1	F12:13	PI 80837 x SN93-6181	8.0
14P4	F12:13	PI 80837 x SN93-6181	8.0
14P20	F4:5	DS25-1 x PI 424324B	8.0
14P24	F4:5	DS25-1 x PI 424324B	8.0
14P34	F4:5	DS25-1 x PI 424324B	8.0
14P35	F4:5	DS25-1 x PI 424324B	8.0
14P39	F4:5	DS25-1 x PI 424324B	8.0
14P49	F4:5	DS25-1 x PI 424324B	8.0
14P2	F12:13	PI 80837 x SN93-6181	12.0
14P21	F4:5	DS25-1 x PI 424324B	12.0
14P29	F4:5	DS25-1 x PI 424324B	12.0
14P43	F4:5	DS25-1 x PI 424324B	12.0
14P50	F4:5	DS25-1 x PI 424324B	12.0
14P3	F12:13	PI 80837 x SN93-6181	16.0
14P7	F12:13	PI 80837 x SN93-6181	16.0
14P11	F12:13	PI 80837 x SN93-6181	16.0
14P37	F4:5	DS25-1 x PI 424324B	16.0
14P46	F4:5	DS25-1 x PI 424324B	16.0
14P6	F12:13	PI 80837 x SN93-6181	20.0
14P9	F12:13	PI 80837 x SN93-6181	20.0
14P10	F12:13	PI 80837 x SN93-6181	20.0
14P38	F4:5	DS25-1 x PI 424324B	24.0
14P41	F4:5	DS25-1 x PI 424324B	52.0

Table 4. Phenotyping F_2 population (PI 424324B x 5601T) for resistance to Phomopsis seed decay from PI 424324B in 2015.

Parent/Population	Generation	No. of plants tested	No. of seed tested	No. of culture plates for seed assay	No. of plants with Phomopsis	Percentage of plants infected	Range of Phomopsis (%)
5601T	(Parent S)	13	325	65	6	46	0 to 12
PI424324B	(Parent R)	9	225	115	3	33	0 to 3.2
PI 424324B x 5601T	F_2	76	1900	380	17	22.4	0 to 3.2
Total		98	2450	560	26	26.5	0 to 3.2

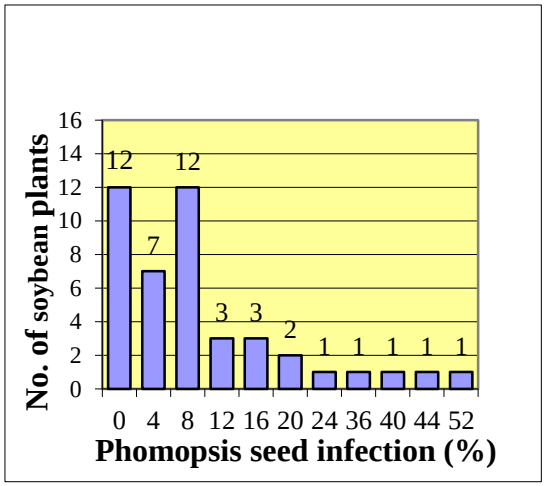
Table 5. Means of percent seed infection by *Phomopsis longicolla* of 145 soybean breeding lines and their resistance sources, pedigrees and generations that were field-tested with inoculation treatments in Stoneville, Mississippi in 2014.

Resistance source	Pedigree	Generation	No. Lines [z]	PSD (%)[y]
PI 80837 and SS93-6181	PI 80837 x SS93-6181[x]	F12:13	1	0.0
PI 80837 and SS93-6181	PI 80837 x SS93-6181	F12:13	1	4.0
PI 80837 and SS93-6181	PI 80837 x SS93-6181	F12:13	2	8.0
PI 80837 and SS93-6181	PI 80837 x SS93-6181	F12:13	1	12.0
PI 80837 and SS93-6181	PI 80837 x SS93-6181	F12:13	3	16.0
PI 80837 and SS93-6181	PI 80837 x SS93-6181	F12:13	3	20.0
Total No. Lines tested			11	
PSD source mean and SE				12.7±2.1
PI 424324B	DS25-1 x PI 424324B[w]	F4:5	33	0.0
PI 424324B	DS25-1 x PI 424324B	F4:5	9	4.0
PI 424324B	DS25-1 x PI 424324B	F4:5	9	8.0
PI 424324B	DS25-1 x PI 424324B	F4:5	6	12.0
PI 424324B	DS25-1 x PI 424324B	F4:5	4	16.0
PI 424324B	DS25-1 x PI 424324B	F4:5	1	20.0
PI 424324B	DS25-1 x PI 424324B	F4:5	1	24.0
PI 424324B	DS25-1 x PI 424324B	F4:5	1	44.0
PI 424324B	DS25-1 x PI 424324B	F4:5	1	52.0
PI 424324B	DS25-1 x PI 424324B	F3:4	5	0.0
PI 424324B	DS30-1 x PI 424324B[w]	F2:3	4	0.0
PI 424324B	DS30-1 x PI 424324B	F2:3	1	4.0
PI 424324B	DS34-1 x PI 424324B[w]	F2:3	1	0.0
Total No. Lines tested			76	
PSD source mean and SE				5.1±1.0
PI 417050	DS25-1 x PI 417050[w]	F4:5	26	0.0
PI 417050	DS25-1 x PI 417050	F4:5	3	4.0
PI 417050	DS25-1 x PI 417050	F4:5	10	8.0
PI 417050	DS25-1 x PI 417050	F4:5	2	12.0
PI 417050	DS25-1 x PI 417050	F4:5	4	16.0
PI 417050	DS25-1 x PI 417050	F4:5	3	20.0
PI 417050	DS25-1 x PI 417050	F4:5	4	24.0
PI 417050	DS25-1 x PI 417050	F4:5	1	28.0
PI 417050	DS25-1 x PI 417050	F4:5	3	36.0
PI 417050	DS25-1 x PI 417050	F4:5	1	44.0
PI 417050	DS25-1 x PI 417050	F4:5	1	48.0

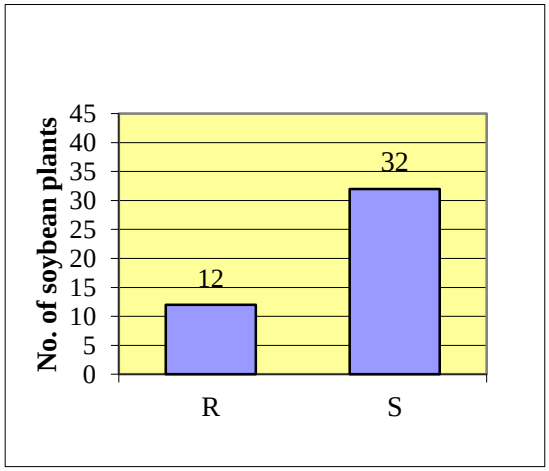
Total No. Lines tested	58	
PSD source mean and SE		9.7±1.6

Total No. Lines tested over all sources	145	
PSD mean over all sources		7.5
LSD ($P \leq 0.05$)[v]		5.9

[z] This column indicates the number of lines with a given percent Phomopsis seed infection for a given source.
[y] Percent *Phomopsis longicolla* infection of seed from the test of 25 seeds from each line.
[x] Resistant line crossed with resistant line (R x R).
[w] High-germination/susceptible line crossed with resistant line (S x R).
[v] Fisher's protected least significant difference (LSD) test was used to determine differences among sources of resistance.



(A)



(B)

Fig. 1. Analysis of 5601T x PI 458130 (Susceptible x Resistance) F_2 population for reaction to Phomopsis seed decay. (A). Frequency distribution of soybean plants with Phomopsis seed infection. (B). The ratio of resistant and susceptible plant reactions to Phomopsis seed decay.

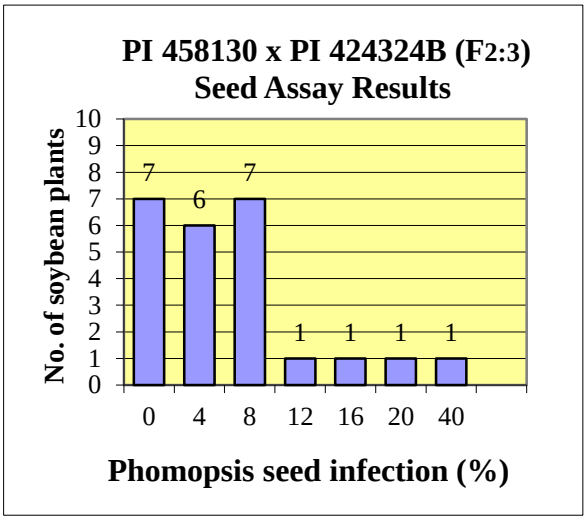


Fig. 2. Frequency distribution of soybean plants with Phomopsis seed infection in PI 458130 x PI 424324B (Resistant x Resistant) F_2 population.