Review Article

Resistance to Phomopsis Seed Decay in Soybean

Shuxian Li¹ and Pengyin Chen²

¹ United States Department of Agriculture-Agricultural Research Service, Crop Genetics Research Unit, Stoneville, MS 38776, USA

² University of Arkansas, Fayetteville, AR 72701, USA

Correspondence should be addressed to Shuxian Li; shuxian.li@ars.usda.gov

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Phomopsis seed decay (PSD) of soybean is caused primarily by the fungal pathogen *Phomopsis longicolla* Hobbs along with other *Phomopsis* and *Diaporthe* spp. This disease causes poor seed quality and suppresses yield in most soybean-growing countries. Infected soybean seeds can be symptomless, but are typically shriveled, elongated, cracked, and have a chalky white appearance. Development of PSD is sensitive to environmental conditions. Hot and humid environments favor pathogen growth and disease development. Several control strategies have been used to manage PSD and reduce its impact; however, the use of resistant cultivars is the most effective method for controlling PSD. Efforts have been made to identify sources of PSD resistance in the past decades. At least 28 soybean lines were reported to have certain levels of PSD resistance in certain locations. Inheritance of resistance to PSD has been studied in several soybean lines. In this paper, general information about the disease, the causal agent, an overview of research on evaluation and identification of sources of resistance to PSD, and inheritance of resistance to PSD are presented and discussed.

1. Introduction

Phomopsis seed decay (PSD) of soybean, *Glycine max* (L.) Merrill, is caused primarily by the fungal pathogen *Phomopsis longicolla* Hobbs et al. [1] along with other *Phomopsis* and *Diaporthe* spp. This disease causes poor seed quality and suppresses yield in most soybean-growing countries, especially in the mid-southern region of the United States [2, 3]. PSD severely affects soybean seed quality due to reduction in seed viability and oil content, alteration of seed composition, and increased frequencies of moldy and/or split beans [4–7]. Development of PSD is sensitive to environmental conditions. Hot and humid environmental conditions, especially during the period from the pod fill through harvest stages, favor pathogen growth and disease development [2, 8–11].

PSD has been reported to cause significant economic losses [4, 12]. Annual yield loss caused by PSD in the United States from 1996 to 2007 ranged from 0.38 to 0.43 million metric tons (MMT) [13]. PSD has been exacerbated in recent years because of the use of the early soybean production system (ESPS), which generally involves planting of early-maturing cultivars in April to avoid the late-season droughts typical of the region in the mid-southern USA [14].

Unfortunately, seed infection by *P. longicolla* was high in some cultivars that matured in July or August, when high temperatures and high humidity were conducive to PSD development [15]. In 2009, due to the prevalence of hot and humid environments from pod fill to harvest in the southern United States, PSD caused over 0.33 MMT loss in 16 states [16]. The identification and utilization of sources of resistance to PSD for breeding programs are important, since planting resistant cultivars would be one of the most economical and effective ways to manage the disease [2, 10, 17–20].

The research on PSD including pathogen characterization, germplasm screening, and genetic resistance in the USA were summarized and presented at the World Soybean Research Conference VII in 2009 [21].

2. Disease Symptoms and the Causal Pathogens

Soybean seeds infected by *P. longicolla* can be symptomless [22] but are typically shriveled, elongated, or cracked, and often appear chalky white (Figure 1). Infected seeds either fail to germinate or germinate more slowly than healthy seeds.

FIGURE 1: *Phomopsis* seed decay caused primarily by the fungus *Phomopsis longicolla*.

Seed infection causes pre- and postemergence damping-off, and under severe conditions, stands can be reduced to the point of lowering yield [2, 3, 10, 23]. Soybean pods can be infected at any time after they form.

The culture morphology of *P. longicolla* on potato dextrose agar is floccose, dense, and white with occasional greenish yellow areas. The undersides of cultures are colorless with large, black, spreading stromata (Figure 2). *P. longicolla* has been found to account for more than 85% of all *Phomopsis* seed infection [1]. This pathogen is primarily known as a seedborne pathogen, but it can be isolated from all parts of plant and is the predominant species isolated from diseased plants [24, 25], and from both discolored and nondiscolored mature soybean stems [26]. It has been found that *P. longicolla* is more prevalent in seeds from pods at the bottom of plant than at the top [23].

Although *P. longicolla* is the primary cause of PSD, other fungal members in the *Diaporthe-Phomopsis* complex may also be associated with PSD [23]. The *Diaporthe-Phomopsis* complex consists of *P. longicolla* and three varieties of *Diaporthe phaseolorum* (Cooke and Ellis) Sacc. (anamorph *P. phaseoli* (Desmaz) Sacc.), in which *D. phaseolorum* var. *caulivora* K. L. Athow and R. M. Caldwell and *D. phaseolorum* var. *meridionalis* Fernández cause stem canker of soybean while *D. phaseolorum* var. *sojae* (S. G. Lehman) Wehmeyer causes pod and stem blight [2, 27, 28]. *P. longicolla* differs from others in the *Diaporthe-Phomopsis* complex in its morphology. It also does not have a known teleomorph [1].

Understanding the nature and the variability of the pathogen is essential for understanding its population diversity, and such information will also be important for selecting isolates to develop broad-based disease resistance in soybean lines. However, there are only a few publications on the variability of aggressiveness on soybean among *P. longicolla* isolates. The term “pathogen aggressiveness,” as used in this paper, is based on colonization of and damage to soybean [29, 30].

In a greenhouse study, Li et al. [32] evaluated 48 isolates from the National Soybean Pathogen Collection Center at the

University of Illinois at Urbana-Champaign using the cut-stem inoculation method. Isolates used in this study included 35 *P. longicolla* isolates from soybean in eight states in the U.S., along with the type culture of *P. longicolla* (Fau 600, ATCC 64802) from soybean in Ohio [1], two *P. longicolla* isolates from velvetleaf in Illinois [33], and 11 other *Phomopsis* spp. isolates from other hosts in four states in the USA, as well as from Canada and Costa Rica. Besides morphological identification, the identities of the isolates were also verified by sequence analysis of the ITS regions and the mitochondrial small subunit rRNA genes ([33–35] (<http://nt.ars-grin.gov/fungalbases/specimens/specimens.cfm>)).

There were significant ($P \leq 0.0001$) differences among isolates based on stem length and stem lesion length. The *P. longicolla* soybean isolate PL16, from Mississippi, caused the shortest stem length while the nonsoybean isolate P9, from Illinois, caused the greatest stem lesion length. The type isolate of *P. longicolla*, PL31 (Fau 600), was one of the three most aggressive isolates among the 48 isolates evaluated. The velvetleaf isolate P9 from Illinois was the most aggressive among 13 isolates from nonsoybean hosts. This study provided the first evaluation of aggressiveness of *P. longicolla* isolates from different geographic origins and the first demonstration that *Phomopsis* spp. isolated from cantaloupe, eggplant, and watermelon were able to infect soybean [32]. This study provided important information that is very useful for selecting isolates for screening in breeding broad-based resistance in soybean lines to PSD.

3. Evaluation and Identification of Sources for Resistance to PSD

Screening soybean lines for resistance to PSD is the first step toward developing PSD-resistant cultivars. Efforts have been made to identify sources of PSD resistance in the past decades. In a 4-year project carried out by the USDA-ARS scientists, seeds of 208 representative maturity group V soybean plant introductions (PIs) obtained from the USDA Soybean Germplasm Collection in 2006 were plated on cultural medium and assayed for the percentage of *Phomopsis* seed infection [31]. The general procedure for seed plating is shown in Figure 3. Briefly, the seeds were first surface-disinfected in 0.5% sodium hypochlorite for 3 min, rinsed in sterile distilled water, and then placed on potato dextrose agar 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 days of incubation at 24°C, the incidence of *P. longicolla* growing on the APDA was recorded.

From the seed plating data in 2006, 122 PIs without seed infection were selected and field screened under natural infection in Stoneville, MS, USA, in 2007. On the basis of assays of naturally infected seeds from 2006 and 2007, 14 PIs were selected for further evaluation with *Phomopsis*-inoculated and non-inoculated treatments in 2008 and 2009 (Table 1). PI 424324B was identified as the most PSD-resistant line. It had no *Phomopsis* infection in the seed plating assays from 2006, 2007, and 2008. In 2009, frequent rainfall during seed maturation led to high levels of seed infection by

FIGURE 2: *Phomopsis longicolla* isolate MS10-6 collected from Mississippi Delta in 2010 and grown on acidified potato dextrose agar (pH 4.5) at 24°C for 50 days.

FIGURE 3: General procedure of seed plating.

Phomopsis (up to 80%) and other fungal pathogens on most of the soybean lines tested in Stoneville, MS, USA. However, only 1% and 2% of the seeds from PI 424324B were infected by *P. longicolla* in the non-inoculated and inoculated treatments, respectively [31]. In addition, PI 458130 was also resistant to PSD, with no seed infection from the naturally infected trials in 2006 and 2007, and less than 3% *Phomopsis* seed infection in the 2008 and 2009 inoculated trails [31]. Eight of 14 accessions selected for resistance (PI 424324B, PI 458130, PI 506647, PI 567270C, PI 567381B, PI 567521, PI 567635, and PI 594858A) had an average percent seed infection of less than 5% in 2008 and 2009, and this level was significantly less than that of 5601T, a well-adapted cultivar in Mississippi (Table 1). Some of these resistant accessions have been used to develop populations for genetics studies and breeding for resistance to PSD.

A 6-year project on “Screening germplasm and breeding for resistance to *Phomopsis* seed decay in soybean” has been funded by the United Soybean Board (USB), a group of farmer directors that administers the organization’s soybean checkoff program, which seeks to strengthen soybean marketing, production technology, and research on new, value-added uses. In this project, hundreds of soybean germplasm accessions representing 28 regions or origins and from MG III to V, breeding lines, and commercial cultivars collected around the world were screened for resistance to PSD in three states (Arkansas, Mississippi, and Missouri) of the USA starting in 2009. The seeds, 33 seeds m⁻¹ of row, were planted in 3.04 m long single-row plots with a 0.96 m row spacing. The experimental design was a randomized complete block with four replications. The seeds were harvested from each plot when the plants were mature. The seeds from each plot were

TABLE 1: Means of percent seed infected by *Phomopsis longicolla* of 16 soybean lines in replicated field tests with inoculated and non-inoculated treatments at Stoneville, Mississippi, in 2008 and 2009 [31].

Soybean line	2008		2009		2008 and 2009		Mean
	Non	Inoc.	Non	Inoc.	Non	Inoc.	
5601T	3.0	9.0	6.7	9.8	4.8	9.4	7.1
PI 304217	7.0	12.0	7.8	8.3	7.4	10.2	8.8
PI 398745	8.0	9.0	4.0	6.0	6.0	7.5	6.8
PI 399045	18.0	18.0	11.8	12.4	14.9	15.2	15.1
PI 408305	6.0	7.0	5.0	5.6	5.5	6.3	5.9
PI 424324B	0.0	0.0	1.0	2.0	0.5	1.0	0.8
PI 458130	0.0	3.0	2.0	2.7	1.0	2.8	1.9
PI 506647	2.0	4.0	3.0	3.0	2.5	3.5	3.0
PI 507290	0.0	1.0	5.8	6.2	2.9	3.6	3.3
PI 549020	2.0	7.0	18.2	18.3	10.1	12.6	11.4
PI 549021B	1.0	3.0	1.0	10.8	1.0	6.9	4.0
PI 567270C	3.0	7.0	1.0	2.0	2.0	4.5	3.3
PI 567381B	0.0	1.0	3.7	5.0	1.8	3.0	2.4
PI 567521	1.0	2.0	4.0	5.7	2.5	3.8	3.2
PI 567635	2.0	3.0	3.0	4.5	2.5	3.8	3.1
PI 594858A	3.0	3.0	2.0	4.3	2.5	3.7	3.1
Mean	3.5	5.6	5.0	6.7	4.2	6.1	5.2
LSD ($P \leq 0.05$)	7.5	10.5	2.9	3.2	4.5	5.5	3.3

Non: non-inoculated control sprayed with distilled water, Inoc.: inoculated with spore suspension of *Phomopsis longicolla* (2×10^5) at the R5 stage twice with a 10-day interval between inoculations, Mean: overall mean of non-inoculated and inoculated treatments across both 2008 and 2009, LSD: Fisher's protected least significant difference at $P \leq 0.05$.

tested for percent seed infected by *Phomopsis* spp., percent seed germination [42], and visual quality using a scale of 1 to 5 where 1 = excellent (no bad/infected seed); 2 = good (less than 10% bad/infected seed); 3 = fair (11–30% bad/infected seed); 4 = poor (31–50% bad/infected seed); and 5 = very poor (more than 50% bad/infected seed). Factors considered in estimating seed quality were seed wrinkling, molding, mottling, and discoloration. Frequent rainfall during seed maturation led to high levels of seed infection by a number of fungi. Significant differences in seed infection by *Phomopsis* spp. were observed among soybean lines with some lines having no infection, while others had infection levels as high as 90% [43–45]. These differences between lines were also reflected in visual seed quality and seed germination [43–45]. Soybean lines with low infection incidences, good visual quality, and high germination rates at all locations have potential to be resistant to PSD. Further tests of those lines are under progress.

Besides evaluating germplasm lines, 50 soybean cultivars were planted at Stoneville, Mississippi, to determine their reaction to PSD in 2007 [46]. Two lines, SS93-6012 and SS93-6181, previously reported to be PSD-resistant in Missouri were included [19], and the cultivars Hill and Williams 82 were used as susceptible checks. The seeds of soybean lines selected for planting were generally healthy. Of 50 lines tested, six lines had 100% germination, 30 lines had germination rates ranging from 80% to 97%, and 12 lines had rates ranging from 63% to 77%. Two lines had germination rates of 50% and 53%, respectively. In the seed plating assay of non-inoculated seed, 37 lines had no *P. longicolla*-infected seed and 10

and three lines had *P. longicolla* incidences of 3% and 7%, respectively. Incidence of *P. longicolla* in seed from inoculated field plots differed significantly ($P \leq 0.05$) ranging from 6% to 50% among soybean lines. Several soybean cultivars with lower disease incidence than the PSD resistant lines SS93-6181 and SS93-6013 were identified [46].

To date, at least 28 soybean lines have been reported to have certain levels of PSD resistance (Table 2). However, some resistant lines identified in other regions were susceptible in Arkansas, Mississippi, or Missouri, USA (Li et al., unpublished). It is not known if there is the possibility of diversity between populations of the pathogen in different locations, but there is a need to identify new sources of resistance to PSD.

4. Inheritance of Resistance to *Phomopsis* Seed Decay

Inheritance of resistance to PSD has been studied in several soybean lines including PI 417479, MO/PSD-0259, PI 80837, and PI 360841 (Table 1). In all cases, PSD resistance was characterized as qualitative traits and conditioned by major dominant genes [17, 18, 47–49]. However, allelism of PSD resistance is still not clear; therefore, no gene symbol has been assigned for PSD resistance yet.

Soybean PI 417479 was identified as a source of resistance to PSD from screening approximately 3,000 soybean introductions in MGs III and IV from 1983 through 1985 at the

TABLE 2: A list of reported sources of resistance to *Phomopsis* seed decay.

	Soybean line	Reference
1	PI 548438 (Arksoy)	[17]
2	PI 80837	[36], [37]
3	PI 82264	[38]
4	PI 88264*	[17]
5	PI 181550	[39]
6	PI 200501	[40]
7	PI 200510	[17]
8	PI 204331	http://www.soydiseases.illinois.edu/
9	PI 205089	http://www.soydiseases.illinois.edu/
10	PI 205907	http://www.soydiseases.illinois.edu/
11	PI 205908	http://www.soydiseases.illinois.edu/
12	PI 205912	http://www.soydiseases.illinois.edu/
13	PI 209908	[17]
14	PI 219635*	http://www.soydiseases.illinois.edu/
15	PI 227687	[17]
16	PI 229358	[17]
17	PI 259539	http://www.soydiseases.illinois.edu/
18	PI 279088	http://www.soydiseases.illinois.edu/
19	PI 341249	http://www.soydiseases.illinois.edu/
20	PI 360835	http://www.soydiseases.illinois.edu/
21	PI 360841	[36]
22	PI 385942	http://www.soydiseases.illinois.edu/
23	PI 417419	[36]
24	PI 423903**	http://www.soydiseases.illinois.edu/
25	PI 562694	[41]
26	PI 424324B	[31]
27	PI 458130	[31]
28	PI 567381B	[31]

*Named/assigned by the authors (see reference), not the accession number of the USDA Soybean Germplasm Collection.

**Equal to PI 385942 (personal communication with Dr. Randy Nelson).

Agronomy Research Center of the University of Missouri, Columbia, MO, USA, and at the Isabela Substation of the University of Puerto Rico at Mayaguez [36]. To study the inheritance of PSD resistance in PI 417479, crosses were made between PI 417419 and two PSD-susceptible genotypes [49]. By analyzing PSD incidence on plants from five generations (F_1 , F_2 , F_3 , BC_1 , and BC_2 , in which BC_1 represented a backcross between the F_1 and the resistant parent and BC_2 represented a backcross between the F_1 and the susceptible parents), it was concluded that the PSD resistance in PI 417479 was controlled by two complementary dominant nuclear genes. The two resistance genes can thus be transferred using a backcross procedure in a breeding program [49].

It was reported that the PSD-resistant line MO/PSD-0259 derived its resistance from PI 417479 [18]. PI 80837 had low

levels of PSD infection in field trials [37] and also exhibited resistance to soybean mosaic virus and purple seed stain [50–52]. To characterize the inheritance of PSD resistance in PI 80837 and to determine if it differs from resistance in MO/PSD-0259, PI 80837 was crossed with two PSD-susceptible lines Agripro 350 (AP350) and PI 9113, and with MO/PSD-0259 [18] (Table 3). The derived genetic populations were screened in the field and *Phomopsis* infection was assayed by plating seed. Results showed that seed infection of reciprocal F_1 plants of AP350 $\times$ PI 80837 was not different from that of PI 80837. Data from F_2 populations of AP350 $\times$ PI 80837 and PI 9113 $\times$ PI 80837 and $F_{2,3}$ lines from AP350 $\times$ PI 80837 fit the models for a single dominant gene in PI 80837 that confers PSD resistance. Likewise, F_2 population data from AP350 $\times$ MO/PSD-0259 fit a model for single dominant gene resistance in MO/PSD-0259. However, data from an F_2 population and $F_{2,3}$ lines of PI 80837 $\times$ MO/PSD-0259 fit a model for two different dominant genes. Based on those results, Jackson et al. [18] concluded that PSD resistance in PI 80837 is conferred by a single dominant gene under nuclear control that is different from the gene in MO/PSD-0259.

Studies on the inheritance of PSD resistance in soybean PI 360841 were conducted to test populations developed from the crosses between PI 360841 and two PSD-susceptible (S) genotypes, Agripro 350 (AP350) and PI 9113 (Figure 4), to determine the number of genes for PSD resistance [48]. Other crosses were made between PI 360841 and the PSD-resistant (R) parents MO/PSD-0259 and PI 80837 to test the allelic relationships of the resistance genes [48]. Seeds from the parents and the F_2 population were assayed for *Phomopsis* infection. Chi-square analysis of F_2 data from the resistant $\times$ susceptible crosses indicated a good fit to a 9R:7S model for two complementary dominant genes conferring PSD resistance in PI 360841. F_2 data from MO/PSD-0259 $\times$ PI 360841 showed a good fit to a 57R:7S model for two complementary dominant genes from PI 360841 and a different dominant gene from MO/PSD-0259. Since there was no apparent segregation for resistance in the F_2 population derived from PI 360841 $\times$ PI 80837, except for one suspicious susceptible plant, it suggested that one of the genes in PI 360841 is allelic to a PSD resistance gene in PI 80837 [48]. This was the first report of a new gene in PI 360841 for PSD resistance. This gene, along with other different PSD-resistance genes in MO/PSD-0259 and PI 80837, is useful to breeders for developing lines with a high level of resistance to PSD. Further studies have identified simple sequence repeat (SSR) markers linked to genes for PSD resistance in PI 80837 and MO/PSD-0259. Jackson et al. (2009) reported that PSD resistance in MO/PSD-0259 and PI 80837 is controlled by two different single dominant genes [47]. The gene in PI 80837 is located in the vicinity of Sat_177 (4.3 cM) and Sat_342 (15.8 cM) on molecular linkage group (MLG) B2 (chromosome 14). The gene that conditions resistance in MO/PSD-0259 is linked to Sat_317 (5.9 cM) and Sat_120 (12.7 cM) on MLG F (chromosome 13). Identification of these loci and markers linked to them will facilitate marker-assisted selection in breeding programs.

TABLE 3: Genetics of resistance to *Phomopsis* seed decay in soybean.

Crosses	Type	Generation	Ratio	Reference
AP350 × PI 80837	S × R	F ₂	3R : 1S	[18, 47]
PI 91113 × PI 80837	S × R	F ₂	3R : 1S	[18]
AP 350 × MO/PSD-0259	S × R	F ₂	3R : 1S	[18]
AP350 × PI 80837	S × R	F _{2:3}	1R : 2H : 1S	[18]
PI 80837 × MO/PSD-0259	R × R	F ₂	15R : 1S	[18]
PI 80837 × MO/PSD-0259	R × R	F _{2:3}	7R : 8H : 1S	[18]
PI 360841 × AP350	R × S	F ₂	9R : 7S	[48]
PI 91113 × PI 360841	S × R	F ₂	9R : 7S	[48]
MO/PSD-0259 × PI 360841	R × R	F ₂	57R : 7S	[48]
PI 360841 × PI 80837	R × R	F ₂	63 : 1S	[48]
PI 417479 × AP350	R × S	F ₂	3R : 1S	[49]
PI 417479 × AP350	R × S	F ₃	5R : 3S	[49]
(PI 417479 × AP350) × AP 350	(R × S) × S	BC ₁	1R : 1S	[49]
PI 417479 × PI 91113	R × S	F ₂	3R : 1S	[49]
PI 417479 × PI 91113	R × S	F ₃	5R : 3S	[49]
(PI 417479 × PI 91113) × PI 91113	(R × S) × S	BC ₁	1R : 1S	[49]

R: resistant, S: susceptible, H: heterozygous and segregating for PSD reaction, BC: backcross.

(a)

(b)

FIGURE 4: Susceptible parent PI 91113 and resistant parent PI 360841 with and without *Phomopsis* spp. infection, respectively.

5. Conclusions

Phomopsis seed decay (PSD) of soybean causes poor seed quality and suppresses yield in most soybean-growing countries. The disease is caused primarily by the fungal pathogen *Phomopsis longicolla* along with other *Phomopsis* and *Diaporthe* spp. Infected seeds range from symptomless to shriveled, elongated, cracked, and often appear chalky white. Seed quality is poor due to reduction in seed viability and oil content, alteration of seed composition, and increased frequencies of moldy and/or split beans. Hot and humid environments, especially during the period from pod fill through harvest, favor pathogen growth and disease development. The

use of resistant cultivars is the most effective method for controlling PSD. Extensive screening for PSD resistance has resulted in the identification of resistant sources. MO/PSD-0259 carries a single dominant gene for PSD resistance derived from PI 417479, whereas resistance in PI 80837 is conferred by a different gene. PI 360841 carries two complementary dominant genes for PSD resistance; both are different from the gene in MO/PSD-0259 but one of them maps to the same region as the gene in PI 80837. Simple sequence repeat (SSR) markers linked to PSD resistance genes in PI 80837 and MO/PSD-0259 have been identified. These SSR markers should be useful in selection for resistant genotypes in breeding programs.

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