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Registration of Eight Soybean Germplasm Lines Resistant to Soybean Rust

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ABSTRACT

Soybean rust (SBR; caused by *Phakopsora pachyrhizi* Sydow) is a threat to soybean [*Glycine max* (L.) Merr.] production worldwide. Although SBR has not caused widespread damage in North America, the crop is still threatened by the disease because most cultivars in production are susceptible. We backcrossed the SBR-resistance genes *Rpp1*, *Rpp1-b*, *Rpp?*(Hyuuga), and *Rpp5* into the maturity group (MG) II experimental line LD01-7323 and the MG IV cultivar LD00-3309 to develop Midwest-adapted soybean germplasm with SBR resistance. The backcross lines were tested for SBR resistance in greenhouse tests and for agronomic traits in multilocation field tests. The four MG II soybean germplasm lines LD10-30052 (Reg. No. GP-383, PI 668384), LD10-14321 (Reg. No. GP-384, PI 668385), LD10-14284 (Reg. No. GP- 385, PI 668386), and LD09-16057 (Reg. No. GP- 386, PI 668387) and the four MG IV germplasm lines LD10-14205 (Reg. No. GP- 389, PI 668390), LD10-13091 (Reg. No. GP- 387, PI 668388), LD10-14274 (Reg. No. GP- 388, PI 668389), and 08RST5-10 (Reg. No. GP- 390, PI 668391) developed through these efforts were released by the Illinois Agricultural Experiment Station in April 2012. The lines carry SBR resistance genes and are indistinguishable from the recurrent parents for morphological traits and, with only a few exceptions, are not significantly different than their recurrent parents for agronomic traits including seed yield. These lines should be useful to soybean breeders who wish to develop rust-resistant cultivars.

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Soybean rust (SBR; caused by *Phakopsora pachyrhizi* Sydow) is one of the most economically important diseases of soybean [*Glycine max* (L.) Merr.] worldwide. Although SBR was first found in the continental United States in 2004 (Schneider et al., 2005), it has not become a major disease in the midwestern USA. It has occurred each year since 2004 in the states along the Gulf of Mexico, and fungicides have been used in those states to manage the disease (IPM PIPE, 2012). Because most soybean cultivars grown in the United States are highly susceptible to SBR, epidemics could occur if weather conditions become conducive to disease development (Miles et al., 2003).

Soybean germplasm has been screened for resistance to SBR, and resistance alleles at six loci have been identified and mapped. *Rpp1* was mapped to soybean chromosome 18 (linkage group [LG] G), and two resistance alleles at this locus have been identified on the basis of differential resistance responses (Hyten et al., 2007; Chakraborty et al., 2009). *Rpp2* (Silva et al., 2008) was mapped on chromosome 16 (LG J); *Rpp3* (Hyten et al., 2009) and *Rpp?*(Hyuuga) (Monteros et al., 2007) were mapped on chromosome 6 (LG C2); and *Rpp4* (Silva et al., 2008) and *Rpp6* (Li et al., 2012) were mapped to regions on chromosome 18 (LG G) different from the one containing *Rpp1*. Resistance alleles at *Rpp5* from three plant introductions were mapped to chromosome 3 (LG N) (Garcia et al., 2008).

The *Rpp3* and *Rpp?*(Hyuuga) genes were mapped to the same position on chromosome 6. These genes were given different designations because the sources of each gene gave a different resistance reaction when inoculated with a *P. pachyrhizi* isolate from Brazil (Silva et al., 2008). Recent research showed that this differential response was caused by the cultivar Hyuuga, the source of the *Rpp?*(Hyuuga) allele, having a second resistance gene at *Rpp5* in addition to an allele in the *Rpp3* interval, suggesting that *Rpp?*(Hyuuga) and *Rpp3* (Kendrick et al., 2011) may be the same gene.

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Abbreviations: IM, immune; LG, linage group; MG, maturity group; PCR, polymerase chain reaction; RB, reddish-brown; SBR, soybean rust; SSR, simple sequence repeat.

When soybean plants are infected with *P. pachyrhizi*, tan lesions (TAN reaction) usually form on susceptible genotypes, whereas reddish-brown lesions (RB reaction) are typically produced on incompletely resistant genotypes (Miles et al., 2011). No visible symptoms, an immune (IM) response, occur when plants carrying *Rpp1* from PI 200492 are inoculated with specific *P. pachyrhizi* isolates (Miles et al., 2011).

Although SBR has failed to become a major disease in the Midwest, there is a need to breed SBR resistance genes into soybean germplasm adapted to this region as a precaution against possible disease outbreaks. In addition, multiple genes should be bred into adapted soybean germplasm because pathogenic diversity has been observed among *P. pachyrhizi* isolates in the USA. Twizeyimana and Hartman (2012) inoculated a differential set of soybean genotypes with 72 isolates of *P. pachyrhizi* from the USA and found three pathotype and six aggressiveness groups. They found that the resistance allele *Rpp1* gave IM or RB resistance reactions to all of the isolates tested, and the *Rpp?*(Hyuuga) allele from Hyuuga gave an RB reaction to 95% of the isolates.

We report here the development of four maturity group (MG) II SBR-resistant soybean germplasm lines LD10-30052 (Reg. No. GP-383, PI 668384), LD10-14321 (Reg. No. GP-384, PI 668385), LD10-14284 (Reg. No. GP-385, PI 668386), and LD09-16057 (Reg. No. GP-386, PI 668387) and four MG IV SBR-resistant germplasm lines LD10-14205 (Reg. No. GP-389, PI 668390), LD10-13091 (Reg. No. GP-387, PI 668388), LD10-14274 (Reg. No. GP-388, PI 668389), and 08RST5-10 (Reg. No. GP-390, PI 668391). These lines have the resistance alleles *Rpp1*, *Rpp1-b*, *Rpp?*(Hyuuga), and *Rpp5* backcrossed into them and should be useful to plant breeders, plant pathologists, and other researchers. These lines will be especially useful to soybean breeders developing SBR-resistant soybean germplasm adapted to the northern USA because the resistance genes originated from MG VII to MG IX plant introductions that are not adapted to this region. Of the four resistance alleles, only *Rpp1* was previously introgressed into the genetic background of a northern USA soybean. The *Rpp1* gene from PI 200492 was backcrossed into the cultivar Williams 82 to develop the backcross line PI 547875 (Bernard et al., 1991; Hyten et al., 2007); however, this gene needs to be introgressed into more elite genetic backgrounds because Williams 82 is now more than 30 yr old. The eight SBR resistant soybean germplasm lines described here were released in 2012 by the Illinois Agricultural Experiment Station, University of Illinois, Urbana, IL.

Methods

Population Development

The SBR resistance genes *Rpp1*, *Rpp1-b*, *Rpp?*(Hyuuga), and *Rpp5* were each backcrossed four times into the backgrounds of the MG II experimental line LD01-7323 (Cary and Diers, 2006) and the MG IV cultivar LD00-3309 (Diers et al., 2006). These recurrent parents were selected on the basis of good agronomic performance when backcrossing was initiated (Cary and Diers, 2006). For both the LD01-7323 and LD00-3309 backgrounds, the donor parent for backcrossing *Rpp1* was PI 547875, the isoline of Williams 82 that carries *Rpp1* (Bernard and Cremeens, 1988; Bernard et al., 1991; Germplasm Resources Information Network, http://www.ars-grin.gov/ [accessed 7 Oct. 2012]). The pedigree of PI 547875 is Williams 82(6) × PI 200492. The *Rpp1-b* source for both backgrounds was PI 594538A (Chakraborty et al., 2009), and the source of the *Rpp?*(Hyuuga) gene was G01-PR33, an experimental line provided courtesy of H. Roger Boerma, University of Georgia. The pedigree of G01-PR33 is Dillon (Shipe et al., 1997) × Hyuuga (PI 506764), and the *Rpp?*(Hyuuga) gene was initially mapped in this population (Monteros et al., 2007). Both genetic marker analysis and resistance phenotypes indicate that G01-PR33 did not carry a resistance allele at *Rpp5* (personal communication, Roger Boerma, 2013). The source of *Rpp5* was PI 200456 in the LD01-7323 background and PI 471904 in the LD00-3309 background (Garcia et al., 2008; Germplasm Resources Information Network, http://www.ars-grin.gov/ [accessed 7 Oct. 2012]). In the LD01-7323 background, the pedigree of the line developed with *Rpp1* also included G01-PR33 because the *Rpp1* line was developed in an effort to stack *Rpp1* together with *Rpp?*(Hyuuga); however, the *Rpp?*(Hyuuga) gene was lost during this development. The lines developed in both backgrounds with *Rpp1-b* have the cultivar Loda (Nickell et al., 2001) in their background because a population developed by crossing Loda × PI 594538A was used to map *Rpp1-b* (Chakraborty et al., 2009) and a plant selected from this cross was used as a parent to start the backcrossing process.

The backcrosses were completed at the Crop Sciences Research and Extension Center in Urbana, IL and in greenhouses on the University of Illinois campus. During the backcrossing process, F_1 plants that were heterozygous for SBR resistance genes were selected with linked genetic markers and were used as the male parent in the next backcross. No background selection was done with markers or plant phenotype during the backcrossing. The backcrossing continued until BC_4F_1 plants heterozygous for the resistance gene were identified with genetic markers. The progeny from the selected heterozygous plants were then tested with genetic markers, and BC_4F_2 plants homozygous for the resistance genes were selected. The selected plants were allowed to self-pollinate, forming BC_4F_2–derived lines.

Genetic Marker Testing

Plants were tested with simple sequence repeat (SSR) markers to identify those that are homozygous or heterozygous for the SBR resistance genes. Genomic DNA was isolated from young trifoliate leaf tissue with the CTAB method described by Keim et al. (1988) or the quick extraction method of Bell-Johnson et al. (1998). Primer sequences of the SSR markers were obtained from SoyBase (http://soybase.org/resources/ssr.php; accessed 7 Oct, 2012) and Song et al. (2010). The markers were selected on the basis of map positions of each gene in Hyten et al. (2007), Chakraborty et al. (2009), Monteros et al. (2007), and Garcia et al. (2008). Polymerase chain reactions (PCR) and evaluation of PCR products were performed as previously described by Wang et al. (2003). PCR consisted of 36 cycles of denaturation at 94°C for 25 to 30 s, annealing at 46 to 62°C for 25 to 30 s, and extension at 68°C for 25 to 30 s with a PTC 100 Programmable Thermal Controller (MJ Research). The PCR products were analyzed by electrophoresis in both 3% agarose gels (BMA) and 6% nondenaturing polyacrylamide gels (Wang et al., 2003). The

markers used to select each gene in each background are listed in Table 1.

After the fourth backcross of the *Rpp?*(Hyuuga) gene, it was noticed that BC_4F_2 plants homozygous for the resistance allele matured later than the recurrent parent and plants homozygous for the susceptible allele. This occurrence was probably the result of coupling linkage between the allele at *E1* that confers delayed flowering and maturity (Molnar et al., 2003) and the resistance allele at *Rpp?*(Hyuuga). On the soybean GmComposite 2003 map of chromosome 6, *E1* maps to cM position 113 and *Rpp?*(Hyuuga) is located at cM position 118 (http://soybase.org/resources/ssr.php; accessed 7 Oct, 2012). To recover plants with recombination between the two genes, BC_4F_2 plants were screened for recombination between the markers Satt460 and Satt100, and recombinant plants were selected with the resistance allele at *Rpp?*(Hyuuga) and the early allele at *E1*.

Table 1. Markers used to select soybean rust resistance genes during backcrossing in each recurrent background.

Resistance gene	Recurrent parent	Markers used
Rpp1	LD00-3309, LD01-7323	Sct_187, Sat_064
Rpp1-b	LD00-3309, LD01-7323	Sat_117, Sat_064, Sat_372
Rpp?(Hyuuga)	LD00-3309, LD01-7323	Satt307, Satt460, Satt100
Rpp5	LD00-3309	Sat_166
Rpp5	LD01-7323	Satt485, BARCSOYSSR_03_939

SBR-Resistance Evaluations

BC_4F_2–derived lines selected with genetic markers were evaluated along with the recurrent parents and resistant and susceptible checks to confirm SBR resistance in the lines. Selected lines with *Rpp1* and *Rpp?*(Hyuuga) were evaluated with the *P. pachyrhizi* isolate FL-07, which was collected at Quincy, FL during 2007. Plants carrying *Rpp1-b* and *Rpp5* were tested using the *P. pachyrhizi* isolate ZM01-1, which was collected in Zimbabwe during 2001. These *P. pachyrhizi* isolates were used because they were previously shown to provide clear differential resistance reactions for the backcrossed resistance genes. Plants were rated based on their reaction types: TAN, RB, and IM (Miles et al., 2011). The evaluations with FL-07 were conducted at the USDA-ARS Plant Pathogen Containment Facility at Urbana, IL. Seeds from each entry were sown in 13-cm-diameter pots, which were thinned to three plants per pot after germination. Each pot was considered a separate replication. Plants were inoculated with *P. pachyrhizi* 14 d after sowing to coincide with the full expansion of the first trifoliolate leaf. The plants were inoculated with a suspension of urediniospores (approximately 1×10^6 spores mL^{-1}) using a hand-held sprayer until runoff. Inoculated plants were then maintained at 100% relative humidity for 24 h.

The tests with ZM01-1 were conducted at the USDA-ARS Foreign Disease-Weed Science Research Unit (FDWSRU) Plant Pathogen Containment Facility at Fort Detrick, MD (Melching et al., 1983) under the appropriate permits from the USDA Animal and Plant Health Inspection Service. At least 12 plants of each genotype were tested for SBR resistance using the methods outlined by Kim et al. (2012). The plants were inoculated approximately 14 to 21 d after sowing with a spore suspension of 60,000 spores mL^{-1}. After inoculation, plants were incubated approximately 16 h in a dew chamber, placed in a greenhouse for 15 d, and then rated for their resistance reactions.

Field Evaluations

All field trials were conducted in 2011 and were arranged in a randomized complete block design with two replications at each location except for the DeKalb location of Test 1, which was not replicated. The plots were two or four rows wide, with a 76-cm row spacing, a length of 3.6 m, and a seeding rate of 30 seeds m^{-1} of row. For two-row plots, both rows were harvested to estimate yield, and the middle two rows of the four-row plots were harvested. Tests 1 and 2 included LD01-7323 and SBR-resistant backcross lines with this genetic background, and Tests 3 and 4 included LD00-3309 and SBR-resistant backcross lines developed with this background. Test 1 was grown in two-row plots near Urbana, Pontiac, and DeKalb, IL. Test 2 was grown in four-row plots near DeKalb and Gibson City, IL and in two fields, approximately 3 km apart, near Urbana, IL. Test 3 was grown in two-row plots near Urbana and Arthur, IL and Test 4 was grown in four-row plots near Brownstown and Carbondale, IL, as well as at two locations near Urbana, IL. Conventional tillage and herbicide practices were followed at all locations to maintain weed-free environments, and recommended fertilizer amounts were applied. Plots were rated for maturity date, plant height, and lodging. Maturity date was recorded as the day when approximately 95% of the pods had reached mature pod color (R8; Fehr et al., 1971). Plant height (cm) was measured at maturity as the average distance from the soil surface to the apex of the main stem. Lodging was scored at maturity on a scale of 1 to 5, with 1 designated as all plants standing erect and 5 as all plants prostrate. Plots were harvested to measure seed yield (kg ha^{-1}), and yield values were adjusted to 130 g kg^{-1} moisture.

Statistical Analysis

Data were analyzed with the PROC GLM function of SAS 9.2 (SAS Institute, 2002) with genotypes as fixed effects and environments and the environment × location interaction as random effects. LSD values were calculated from the ANOVAs according to Snedecor and Cochran (1980).

Characteristics

Eight lines, each with one of the four genes in one of the two soybean backgrounds were released. These lines were developed through four backcrosses and were selected for their resistance to SBR and their similarity to the recurrent parent. The backcross lines share morphological traits with their recurrent parents. The lines with LD01-7323 as a recurrent parent have purple flowers, gray pubescence, tan pods, and yellow seeds with yellow hila. The lines with LD00-3309 as a recurrent parent have purple flowers, tawny pubescence, brown pods, and yellow seeds with black hila. The lines in both backgrounds have the indeterminate growth habit.

The lines LD10-30052, LD10-14321, LD10-14284, and LD09-16057, which have LD01-7323 as a recurrent parent, are not significantly different from LD01-7323 in yield, lodging, and height based on the field tests (Tables 2 and 3). LD10-14321 matured significantly later than LD01-7323, but none of the other three lines in this background had significantly different maturity than the recurrent parent (Tables 2 and 3). Among the backcross lines developed in the LD00-3309 background, the line LD10-14205 had a significantly greater yield than LD00-3309, whereas none of the other lines differed significantly from the recurrent parent (Tables 4 and 5). LD10-14205 matured significantly later than LD00-3309, whereas the other three backcross lines in the LD00-3309 background (LD10-13091, LD10-14274, and 08RST5-10) matured significantly earlier than LD00-3309 (Tables 4 and 5). None of the LD00-3309 backcross lines differed significantly from LD00-3309 in lodging, and only LD10-13091 was significantly different from LD00-3309 for plant height, and this line was shorter than LD00-3309. The significant differences between the lines and recurrent parents are probably the result of the effect of either genes linked to the backcrossed regions or other unlinked introgressed regions. The cause of these significant effects was not investigated and are therefore not known.

The eight backcross lines were predicted to be homozygous for SBR resistance genes on the basis of test results with genetic markers linked to the resistance genes. These marker predictions were confirmed by testing the lines for resistance to SBR. Those lines with *Rpp1* and *Rpp?*(Hyuuga) were uniformly resistant to the *P. pachyrhizi* isolate FL-07, and lines with *Rpp1-b* and *Rpp5* were uniformly resistant to the *P. pachyrhizi* isolate ZM01-1 (Tables 2–5). The test with FL-07 and ZM01-1 included William 82 as a susceptible check and both recurrent parents. These genotypes gave uniform TAN reactions to both isolates. PI 547875, the source of the *Rpp1* in the backcrossing, was included in the test with FL-07, and all plants of this genotype gave an IM reaction. Tests with ZM01-1 included the resistance sources PI 594538A, PI 471904, and PI 200456; all plants of PI 594538A and PI 471904 gave RB reactions, and PI 200456 gave a mixture of seven plants with TAN and four plants with RB reactions. These backcross lines represent the first available sources of the SBR resistance alleles *Rpp?*(Hyuuga), *Rpp1-b*, and *Rpp5* available in midwestern USA backgrounds and should be useful to soybean breeders developing SBR-resistant cultivars and germplasm adapted to this region.

Availability

Small quantities of seed of the released backcross lines and the recurrent parents will be available from the corresponding author for 5 yr. This seed came from field plots that were inspected at flowering and maturity and variant plants were rogued. Seed had been deposited in the National Plant Germplasm System, where it will be available for distribution immediately. We

Table 2. Lines in Test 1 developed with the maturity group II cultivar LD01-7323 as a recurrent parent.

Strain	Yield	Maturity	Lodging	Height	Gene	Resistance reaction†	*P. pachyrhizi* isolate	Pedigree
	kg ha^{-1}	date‡	1–5§	cm¶				
LD10-30052	4022	13 Sept.	1.6	74	*Rpp1-b*	RB	ZM01-1	LD01-7323(5) × [LD00-4970 × (Loda × PI594538A)]
LD10-14321	4015	18 Sept.	1.6	72	*Rpp?*(Hyuuga)	RB	FL-07	LD01-7323(5) × (G01-PR33)
LD10-14284	4096	14 Sept.	1.6	75	*Rpp5*	RB	ZM01-1	LD01-7323(5) × PI 200456
LD01-7323	3813	13 Sept.	1.7	71		TAN	ZM01-1, FL-07	
No. of environments#	3	3	3	3				
LSD (0.05)	377	2.0	0.4	5				

† RB, reddish-brown colored lesions (resistant reaction); TAN, tan colored lesions (susceptible reaction).

‡ Date when >95% of the pods on the main stem had reached their mature pod color (R8; Fehr et al., 1971).

§ 1 = all plants erect; 5 = all plants prostrate.

¶ Average length from soil surface to the apex of the main stem.

Number of environments in which experiment was conducted; conducted near Urbana, Pontiac, and DeKalb, IL.

Table 3. Lines in Test 2 developed with the maturity group II cultivar LD01-7323 as a recurrent parent.

Strain	Yield	Maturity	Lodging	Height	Gene	Resistance reaction†	*P. pachyrhizi* isolate	Pedigree
	kg ha^{-1}	date‡	1–5§	cm¶				
LD09-16057	3571	12 Sept.	1.7	76.2	*Rpp1*	IM	FL-07	LD01-7323(5) × [(LD01-7323(2) × PI 547875) × (LD01-7323(2) × G01-PR33)]
LD01-7323	3625	12 Sept.	1.8	76.2		TAN	ZM01-1, FL-07	
No. of environments#	4	4	4	4				
LSD (0.05)	471	3.3	0.5	6.6				

† IM, immune response (no visible symptoms; resistant reaction); TAN, tan-colored lesions (susceptible reaction).

‡ Date when >95% of the pods on the main stem had reached their mature pod color (R8; Fehr et al., 1971).

§ 1 = all plants erect; 5 = all plants prostrate.

¶ Average length from soil surface to the apex of the main stem.

Number of environments in which experiment was conducted; conducted near DeKalb, Gibson City, and two locations near Urbana, IL.

Table 4. Lines developed with the maturity group IV cultivar LD00-3309 as a recurrent parent.

Strain	Yield	Maturity	Lodging	Height	Gene	Resistance reaction†	*P. pachyrhizi* isolate	Pedigree
	kg ha^{-1}	date‡	1–5§	cm¶				
LD10-13091	3867	15 Sept.	1.4	72	*Rpp1-b*	RB	ZM01-1	LD00-3309(5) × [LD00-4970 × (Loda × PI594538A)]
LD10-14274	3685	18 Sept.	1.6	85	*Rpp5*	RB	ZM01-1	LD00-3309(5) × PI 471904
LD10-14205	4048	24 Sept.	1.8	89	*Rpp?*(Hyuuga)	RB	FL-07	LD00-3309(5) × G01-PR33
LD00-3309	3510	21 Sept.	1.5	88		TAN	ZM01-1, FL-07	
No. of environments#	2	2	2	2				
LSD (0.05)	457	2.6	0.3	7.3				

† RB, reddish-brown colored lesions (resistant reaction); TAN, tan-colored lesions (susceptible reaction).

‡ Date when > 95% of the pods on the main stem had reached their mature pod color (R8; Fehr et al., 1971).

§ 1 = all plants erect; 5 = all plants prostrate.

¶ Average length from soil surface to the apex of the main stem.

Number of environments in which experiment was conducted; conducted near Urbana and Arthur, IL.

Table 5. Lines developed with the maturity group IV cultivar LD00-3309 as a recurrent parent.

Strain	Yield	Maturity	Lodging	Height	Gene	Resistance reaction†	*P. pachyrhizi* isolate	Pedigree
	kg ha^{-1}	date‡	1–5§	cm¶				
08RST5-10	3275	21 Sept.	1.2	89	*Rpp1*	IM	FL-07	LD00-3309 (5) × PI 547875
LD00-3309	3288	25 Sept.	1.1	89		TAN	ZM01-1, FL-07	
No. of environments#	5	5	5	5				
LSD (0.05)	316	2.3	0.3	11.1				

† IM, immune response (no visible symptoms; resistant reaction); TAN, tan colored lesions (susceptible reaction).

‡ Date when > 95% of the pods on the main stem had reached their mature pod color (R8; Fehr et al., 1971).

§ 1 = all plants erect; 5 = all plants prostrate.

¶ Average length from soil surface to the apex of the main stem.

Number of environments in which experiment was conducted; conducted near Brownstown, Carbondale, and two locations near Urbana, IL.

request that recognition be given when these lines are used in research contributing to publications or in the development of cultivars or germplasm.

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