Registration of Asian Soybean Rust–Resistant Soybean Germplasm G01-PR16

H. Roger Boerma,* Maria J. Monteros, Bo-Keun Ha, E. Dale Wood, Daniel V. Phillips, David R. Walker, and Ali M. Missaoui

ABSTRACT

The soybean [Glycine max (L.) Merr.] germplasm line G01-PR16 (Reg No. GP-371, PI 659503) was developed and released by the Georgia agricultural experiment stations in October 2007. It was released for its combination of resistance to Asian soybean rust (ASR; caused by Phakopsora pachyrhizi Syd.), bacterial pustule [caused by Xanthomonas campestris pv. glycines (Nakano) Dye], and southern root-knot nematode [Meloidogyne incognita (Kofoid and White) Chitwood], and for its earlier maturity and higher seed yield relative to ‘Hyuuga’ (the late-maturing, ASR-resistant parent of G01-PR16). Asian soybean rust has become a consistent late-season problem in the southeastern USA, requiring soybean growers to apply fungicides to limit yield loss. G01-PR16 was selected from a cross between ‘Dillon’ and Hyuuga made in the summer of 1998. The F2–F6 generations of this population were advanced by single-seed descent to the F6 generation. Seed from a single F6 plant was compositcd and designated G01-PR16. Agronomic evaluation and ASR screening occurred in the southern USA. G01-PR16 produced the red-brown, or resistance, reaction to ASR in field plots near Attapulgus, GA with naturally occurring populations of P. pachyrhizi and in greenhouse evaluations when challenged with P. pachyrhizi isolates collected across Georgia. Over seven yield trials in Georgia and Arkansas, G01-PR16 (relative maturity of 6.4) matured an average of 2 d earlier than Dillon and had 90% of the seed yield of Dillon and 122% that of Hyuuga. Like Hyuuga, G01-PR16 contains the Rpp? (Hyuuga) resistance gene.

Soybean germplasm line G01-PR16 (Reg No. GP-371, PI 659503) was developed and released by the Georgia agricultural experiment stations in October 2007. G01-PR16 was selected from a cross between the U.S.-adapted cultivar ‘Dillon’ (PI 592756) and the Japanese cultivar ‘Hyuuga’ (PI 506764). G01-PR16 combines resistance to Asian soybean rust (ASR; caused by Phakopsora pachyrhizi Syd.), bacterial pustule [caused by Xanthomonas campestris pv. glycines (Nakano) Dye], and southern root-knot nematode [SRKN; caused by Meloidogyne incognita (Kofoid and White) Chitwood] with earlier maturity and greater seed yield than its ASR-resistant donor parent Hyuuga.

Asian soybean rust was first reported on soybean in the continental USA in November of 2004 in a field near Baton Rouge, LA (Schneider et al., 2005). Within weeks it was also found in Alabama, Georgia, and Florida. It has now been reported in all southeastern and mid-southern states, and during the 2007 growing season was reported as far north as Ontario, Canada.

Soybeans grown in the southern USA are at greater risk of ASR epidemics than those grown in the northern states because of more favorable environmental conditions, the widespread presence of alternate hosts, and the geographic proximity to overwintering sites. Since its introduction in 2004, the pathogen has been found each year overwintering on kudzu (Pueraria spp.) in Florida, Alabama, and Georgia.

Before the arrival of P. pachyrhizi in the continental United States, research conducted with Asian isolates of P. pachyrhizi in the Biosafety Level-3 Plant Pathogen Containment Facility at Fort Detrick, MD had identified four ASR resistance genes in soybean: Rpp1, identified in PI 200492; Rpp2, in PI 230970; Rpp3, in PI 462312; and Rpp4, in PI 459025 B (Hartwig and Bromfield, 1983; Hartwig, 1986). Although each of these resistance genes provides resistance to more than one isolate of the fungus, none conditioned resistance to all of the P. pachyrhizi isolates. Since the arrival
of *P. pachyrhizi* in the United States, Rpp? (Hyuuga), a dominant resistance gene from the Japanese cultivar Hyuuga, was reported by Monteros et al. (2007) and was referred to as Rpp? (Hyuuga) because of its uncertain relation to other Rpp genes. In addition, both a dominant resistance gene from PI 200526 and a recessive resistance gene from PI 200456 were identified at the newly discovered Rpp5 locus by Garcia et al. (2008), and Chakraborty et al. (2009) reported a second resistance allele (Rpp1b) at the Rpp1 locus in PI 594538A. None of the currently available U.S. soybean cultivars possesses any of these resistance genes, and North American cultivars are considered to be highly susceptible to ASR (Sinclair and Hartman, 1999).

Three different soybean reactions to *P. pachyrhizi* infection were described by Bromfield and Hartwig (1980) and Bromfield (1984): (i) tan lesions with many uredinia and abundant sporulation, (ii) red-brown (RB) lesions with few uredinia and limited sporulation, and (iii) an immune reaction with a lack of visible infection. The tan-lesion reaction indicates a susceptible reaction, whereas the RB and immune responses are considered resistant reactions (Sinclair and Hartman, 1999; Miles et al., 2003). The RB reaction was associated with ASR resistance conditioned by three of the original four known resistance genes (Hartman et al., 2004).

The Rpp? (Hyuuga) gene condition resistance to the current isolates of *P. pachyrhizi* in Georgia. Hyuuga produced the RB-lesion reaction when infected with *P. pachyrhizi* field isolates for 2 yr at Attapulgus, GA, and when inoculated in the greenhouse with a mixture of *P. pachyrhizi* isolates collected from across Georgia (Monteros et al., 2007). The Rpp? (Hyuuga) resistance gene mapped to a 3.2-cM region of chromosome Gm_06 (linkage group C2) flanked by SSR markers Satt460 and Satt307 (http://soybeanbreederstoolbox.org/) [verified 23 Aug. 2010]; Monteros et al., 2007; Song et al., 2004). This is the same genomic region to which Hyten et al. (2009) mapped the Rpp3 gene. Garcia et al. (2008) reported that Rpp3 and Rpp? (Hyuuga) are probably different alleles at the Rpp3 locus or tightly linked resistance loci, since Hyuuga developed an RB (resistant) reaction and PI 462312 a tan (susceptible) reaction when challenged with Brazilian *P. pachyrhizi* isolates in Londrina, Paraná, Brazil. When 117 recombinant inbred lines (RILs) from a Dillon × Hyuuga population were evaluated for ASR canopy damage in the field and for ASR lesion number and sporulation in the greenhouse, the RILs homozygous for the Rpp? (Hyuuga) allele averaged significantly less canopy damage, fewer lesions, and reduced sporulation in comparison with RILs homozygous for the rpp? (Hyuuga) allele (Monteros et al., 2007).

A search of the Genetic Resources Information Network database identified seven improved germplasm accessions that were developed from populations with one of the known sources of resistance to ASR. Three of these (PI 368037, PI 368038, and PI 368039) were developed at the Taiwan Agricultural Research Institute from the cross of ‘Nungshih H-11’ × PI 200492 and likely possess the Rpp1 allele for ASR resistance. The other four accessions (PI 547875, PI 547878, PI 547879, and PI 518772) were lines derived from backcrosses to either ‘Williams 82’ (PI 518671; Bernard and Cremeens, 1988) or ‘Forrest’ (PI 548655; Hartwig and Epps, 1973). PI 547875 was derived from PI 200492 (source of Rpp1), PI 547879 from PI 459025 B (source of Rpp4), and PI 547878 and PI 518772 from PI 230970 (source of Rpp2). Our search did not identify any improved germplasm accessions with either the Rpp3 or the Rpp? (Hyuuga) ASR resistance genes. The rationale for releasing G01-PR16 is its resistance to ASR, along with its earlier maturity and higher seed yield than its ASR-resistant donor parent Hyuuga.

**Methods**

G01-PR16 originated from the cross of Dillon × Hyuuga, which was made in the summer of 1998 at the Univ. of Georgia Plant Sciences Farm near Athens, GA. Dillon (PI 592756; Shipe et al., 1997) is a maturity group (MG) VI cultivar derived from an F1 plant selection from the cross ‘Centennial’ (PI 548975; Hartwig and Epps, 1977) × ‘Young’ (PI 508266; Burton et al., 1987). Dillon is susceptible to ASR but is resistant to the foliar disease bacterial pustule and to SRKN. Hyuuga (PI 506764) is a late MG VII or early MG VIII Japanese cultivar from the southern island of Kyūshū that has partial resistance to bacterial pustule and pod dehiscence (Manjarrez-Sandoval et al., 1998). Hyuuga was released in 1969 for use in tofu manufacture (Zhou et al., 2002) and was derived from the cross of ‘Akasaya’ × ‘Ako Musume’. Akasaya is a landrace, and Aso Musume is derived from the cross of two other landraces, ‘Tamanishiki’ and ‘Iyo’. Hyuuga was found to produce the RB resistance reaction when it was inoculated with a combination of Brazilian *P. pachyrhizi* isolates in a greenhouse screening conducted in 2005 at Londrina, Brazil (Monteros et al., 2007).

**Population Development**

Eleven F1 plants from the cross of Dillon × Hyuuga were grown during the winter of 1999 at the USDA-ARS Soybean Nursery near Isabela, Puerto Rico. Leaf tissue was sampled from each F1 plant, and the DNA extracted and assayed with four SSR markers (Satt364, Satt329, Satt520, and Satt372) that were polymorphic between Dillon and Hyuuga. All 11 plants contained the Dillon and the Hyuuga band for these four SSR markers, indicating that they resulted from cross pollinations. From spring 1999 to winter 2000, the F1, F2, F3, and F4 generations were advanced by the single-seed descent method (Brim, 1966) in the Monsanto Puerto Rican Nursery located near Isabela, Puerto Rico. In the summer of 2000, approximately 780 plants in the F6 generation were grown at the Univ. of Georgia Plant Sciences Farm near Watkinsville, GA. In October of that year, 275 F6 plants with similar maturity as Dillon were selected and single-plant threshed. In the summer of 2001, seed of each of these 275 plants was planted in a progeny row at the Univ. of Georgia Plant Sciences Farm. In October a total of 117 progeny rows were selected based on having similar maturity as Dillon, resistance to pod dehiscence, and overall agronomic desirability. These selection criteria were applied to allow reliable yield comparisons among the selected lines. Each selected row
was harvested in bulk to create a F$_2$ RIL. In the winter of 2002, the RILs were grown for a seed increase in the lighted area of the USDA-ARS Tropical Agriculture Research Station winter soybean nursery near Isabela, Puerto Rico.

**Field Screening for ASR Reaction**

The 117 RILs and both parents were planted on 3 Sept. 2005 and 17 Aug. 2006 at the Univ. of Georgia Attapulgus Research and Education Center near Attapulgus, GA. Each plot consisted of a single row that was 2.44 m in length, and each entry was planted in two replications of a randomized complete block design. Mobile lighting towers were used to extend the photoperiod in the experimental area to 24 h for 30 d after planting. The ASR screening nursery was planted late in the summer to maximize the opportunity for an ASR epidemic to develop in surrounding commercial soybean fields and soybean research plots and to serve as an inoculum source for the experiments. To reduce the probability of bacterial foliar diseases like bacterial pustule and bacterial blight [caused by *Pseudomonas syringae* pv. *glycinea* (Coerper) Young, Dye, & Wilke] from becoming established, an application of Bac-Master agricultural streptomycin (21.3% streptomycin sulfate; Amvac Chemical Corporation, Los Angeles, CA) was applied at a rate of 0.6 g L$^{-1}$ (100 ppm) to all plots on a weekly basis starting 3 wk after planting.

Beginning 5 wk after planting, suspensions of *P. pachyrhizi* urediniospores were applied along with Bac-Master to promote uniform infection. The urediniospores were obtained from ASR-infected soybean plants collected at the Attapulgus Research and Education Center by submerging the infected plants in a 40-L barrel containing 30 L of water. The solution containing the urediniospores was poured through a triple layer of #50 cheesecloth to remove plant debris. Plants in the experimental area were inoculated with unknown but uniform concentrations of *P. pachyrhizi* urediniospores for six consecutive weeks. On 17 Nov. 2005, a single leaflet was sampled from the middle- to lower-canopy position of 10 individual plants within each plot, placed in plastic bags, and transported to the laboratory on ice. Leaflets were observed under 10× magnification and scored based on the type of lesion: tan, RB, or mixed. Approximately 12 wk after planting in 2005 and 2006, ASR canopy-severity ratings were recorded on a plot basis using the following scale: 1 = dark green canopy, 2 = some yellowing on the bottom leaves, 3 = significant yellowing on the bottom leaves, 4 = significant yellowing at the bottom of the canopy and some yellowing in the middle of the canopy, and 5 = significant yellowing at the bottom and middle of the canopy, and some yellowing in the top of the canopy.

**Greenhouse Screening for ASR and Nematode Reaction**

During the winter of 2006, the 117 RILs from the Dillon × Hyuuga population and both parents were planted in three replications of a randomized complete block design in a greenhouse located at the Griffin campus of the Univ. of Georgia. Six seeds of each genotype were planted in a 10.2-cm pot (an experimental unit), and the pots were arranged in a multiple-pot tray. Each tray contained 14 pots of the RILs and one pot of the ASR-susceptible cultivar ‘Cobb’ (PI 548644; not registered).

The spores used for inoculation in the greenhouse were collected from field-grown soybean plants and surrounding kudzu plants near Athens, Attapulgus, Griffin, and Eatonton, GA during the summer 2005. Additionally, soybean leaves with *P. pachyrhizi* spores from susceptible cobb plants grown in the greenhouse were collected and stored in plastic bags overnight. The inoculum was prepared from a combination of field- and greenhouse-collected urediniospores suspended in 1.2 L of sterile water and 0.04% Tween 20 (Roche, Mannheim, Germany). A funnel with #50 cheesecloth was used to filter out debris. The concentration of spores used for inoculation was approximately 7.5 $\times 10^4$ mL$^{-1}$. Approximately 3 wk after seeding, the plants were inoculated with the spore suspension using an atomizer. After inoculation, the plants were placed in a darkened humidity chamber for 24 h (RH near 100%; temperature, 25–30°C). Upon removal from the humidity chamber, the plants were returned to the greenhouse bench.

Three weeks after inoculation the plants were rated for the development of ASR. For each pot, the leaflet with the most ASR lesions from each of two of the most severely ASR-infected plants from each pot was harvested and inspected using 10× magnification to determine lesion type. The presence or absence of *P. pachyrhizi* spores was also recorded.

**Seed Yield and Agronomic-Trait Evaluation**

A total of 117 of the Dillon × Hyuuga RILs and both parents were yield-tested from 2002 to 2004 in a total of seven environments (three in Georgia: Athens in 2002, Plains in 2003, and Athens in 2004; and four in Arkansas: West Memphis in 2002, 2003, and 2004 and Bay in 2004). The plots at each location were consider “standard” yield plots for the specific site. For example, the yield plots were end-trimmed at the three Georgia locations in early September but were not end-trimmed at any of the Arkansas locations. In each environment the 117 RILs and parents were grown

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in multiple-row plots in two replications of a randomized complete block design. *P. pachyrhizi* was not present in any of these seven environments.

Data were recorded for maturity (date at which 95% of pods had reached their mature color), plant lodging (scored on a scale of 1–5, where 1 = all plants upright, and 5 = all plants prostrate), and plant height (average distance from soil surface to apical meristem of three plants per plot). Once the plants in all plots had reached maturity, the plots were mechanically harvested with a plot combine. To determine protein and oil content, a 50-g seed sample from each plot was sent to the USDA-ARS National Center for Agricultural Utilization Research in Peoria, IL, where an 18- to 20-g seed sample was evaluated for protein and oil composition with a model 1255 Infratec NIR Food and Feed Grain Analyzer (Ultra Tec Manufacturing, Inc., Santa Ana, CA). Seed-quality scores were based on visual observations (scored on a scale of 1–5, where 1 = very good, and 5 = very poor). Seed weight was measured from a 100-seed sample from each plot.

### Statistical Analysis

Statistical analyses were performed using SAS (SAS Institute, Cary, NC). Individual plot data were subjected to analysis of variance for each environment and across environments assuming lines, blocks, and environments as random effects by ANOVA using Agrobase software (Agronomix Software Inc., Winnipeg, Canada). Fisher’s Restricted LSD test ($\alpha = 0.05$) was used to detect significant differences between the means of each genotype for the various traits.

### Seed Purification and Increase

In 2007 twelve 6.1-m rows of G01-PR16 were grown at the Plant Sciences Farm near Athens, GA for seed purification. The planting seed originated from 2004 seed produced in Athens. Individual plants in each row were checked for flower, pubescence, and pod-wall color, and any “off-type” plants were removed from the row. As the plants matured, any individual plant that matured 3 d earlier or later than the majority of the plants was removed from the row. After maturity, the remaining plants were harvested in bulk. In addition, 48 plants from this seed source were screened for ASR in the Griffin, GA greenhouse as described above. All 48 plants produced RB lesions that did not sporulate and so were classified as resistant to ASR.

### Characteristics

G01-PR16 possesses the *Rpp*?(Hyuuga) gene conditioning resistance to ASR. This conclusion is based on its RB reaction when inoculated with Georgia isolates of *P. pachyrhizi* in the greenhouse and in the field (Table 1) and on its possession of the Hyuuga marker alleles at the SSR markers Satt460 and Satt307 (Monteros et al., 2007). In 2 yr of field testing in the ASR screening nursery at Attapulgus, GA, G01-PR16 averaged significantly less ASR canopy damage (a mean canopy severity score of 1.9) than the ASR-susceptible cultivar Dillon (mean canopy severity score of 3.3) and had a similar canopy rating as its resistant parent, Hyuuga. In a greenhouse screening, G01-PR16 produced RB lesions that did not sporulate when inoculated with a mixture of isolates collected from Georgia, whereas Dillon produced tan sporulating lesions (Table 1).

Based on field observations in three Georgia field environments (2002–2004) and in the 2007 purification increase, G01-PR16 was consistently rated resistant to bacterial pustule (no pustules on leaves), whereas other RILs were consistently rated as susceptible (a few to many pustules on leaves). The results from the SRKN screening experiment indicated that G01-PR16 was resistant to SRKN. G01-PR16 had an average gall score of 1.0 compared with 1.0, 1.4, 1.4, 5.0, and 5.0 for G93–9009, Haskell, Hartwig, GaSoy 17, and Bossier, respectively (LSD 0.05 = 0.4).

G01-PR16 has a determinate growth habit, purple flowers, gray pubescence, tan pod walls, and yellow seed coats with buff hilia. G01-PR16 is a Maturity Group VI line (relative maturity 6.4) that matures 2 d earlier than Dillon and 19 d earlier than Hyuuga (Table 1). Earlier soybean maturity groups are better adapted to the primary soybean growing regions of the mid-South and Midwest. G01-PR16 was an average of 10 cm shorter than Dillon and was similar to Dillon with regard to lodging. G01-PR16 produced seed that averaged 35 mg seed$^{-1}$ heavier than Dillon seed but

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**Table 1. Asian soybean rust (ASR) reactions in the field and greenhouse, mean canopy severity scores from two ASR-infected field environments, and mean agronomic performance and seed composition in ASR-free environments (2002–2004) for G01-PR16 and its parents.**

<table>
<thead>
<tr>
<th>Line</th>
<th>ASR lesion type (1 F; 1 GH)$^{1}$</th>
<th><em>P. pachyrhizi</em> spores present</th>
<th>ASR severity rating$^{2}$ (2 F)</th>
<th>Seed yield (7 F) kg ha$^{-1}$</th>
<th>Maturity date (4 F)</th>
<th>Plant height (4 F) cm</th>
<th>Plant lodging$^{3}$ (4 F)</th>
<th>Seed weight (4 F) g kg$^{-1}$</th>
<th>Seed protein (4 F) g kg$^{-1}$</th>
<th>Seed oil (4 F) g kg$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>G01-PR16</td>
<td>red-brown</td>
<td>no</td>
<td>1.9a$^{1}$</td>
<td>2950b</td>
<td>8 Oct. a</td>
<td>74b</td>
<td>2.0a</td>
<td>195b</td>
<td>406a</td>
<td>203a</td>
</tr>
<tr>
<td>Dillon</td>
<td>tan</td>
<td>yes</td>
<td>3.3b</td>
<td>3265a</td>
<td>10 Oct. b</td>
<td>84a</td>
<td>2.1a</td>
<td>160a</td>
<td>401a</td>
<td>197a</td>
</tr>
<tr>
<td>Hyuuga</td>
<td>red-brown</td>
<td>no</td>
<td>2.2a</td>
<td>2426c</td>
<td>27 Oct. c</td>
<td>81a</td>
<td>2.3a</td>
<td>210c</td>
<td>404a</td>
<td>185b</td>
</tr>
</tbody>
</table>

$^{1}$Number and type of environments in which the trait was evaluated; F, field environment; GH, greenhouse environment.

$^{2}$ASR canopy severity rating: 1 = dark green canopy; 2 = some yellowing on the bottom leaves; 3 = significant yellowing on the bottom leaves; 4 = significant yellowing at the bottom of the canopy and some yellowing in the middle of the canopy; and 5 = significant yellowing at the bottom and middle of the canopy and some yellowing in the top of the canopy.

$^{3}$Rating: 1 = all plants upright, to 5 = all plants prostrate.

$^{4}$Means with the same letter within columns are not significantly different ($P > 0.05$).
had similar protein and oil concentrations as Dillon. When tested in seven ASR-free environments, G01-PR16 had an average 19% higher seed yield (524 kg ha\(^{-1}\)) than Hyuuga and 10% less than Dillon (Table 1).

**Availability**

A seed sample has been deposited in the USDA-ARS National Center for Genetic Resources Preservation (NCGRP), where it will be available for distribution. Small quantities of seed for research purposes may be obtained from the corresponding author for at least 5 yr from the date of this publication. It is requested that proper recognition of the source be given when G01-PR16 contributes to the development of an improved germplasm or cultivar.

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**References**


