Published April 15, 2016

JOURNAL OF PLANT REGISTRATIONS

GERMPLASM

Registration of Four Near-Isogenic Soybean Lines of G00-3213 for Resistance to Asian Soybean Rust

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Abstract

Soybean rust (SBR), caused by an obligate biotrophic basidiomycete fungus, *Phakopsora pachyrhizi*, arrived in the continental United States in 2004, where it has since proven to be detrimental to southern soybean [*Glycine max* (L.) Merr.] production due to yield losses, environmental concerns, and expenses caused by reliance on fungicides for control. Resistance to SBR has been developed primarily by introgressing single, dominant resistance gene(s) into an elite soybean cultivar. Here we describe four near-isogenic lines (NILs) of G00-3213: G00-3213*Rpp1* (Reg. No. GP-400, PI 676017), G00-3213*Rpp2* (Reg. No. GP-401, PI 676018), G00-3213*Rpp3* (Reg. No. GP-402, PI 676019), and G00-3213*Rpp4* (Reg. No. GP-403, PI 676020). These NILs were developed by backcrossing rust resistance genes *Rpp1*, *Rpp2*, *Rpp3*, or *Rpp4* into G00-3213, an elite, maturity group VII soybean line. The NILs have tawny pubescence, tan pod walls, and white flowers and had the same general appearance to the recurrent parent G00-3213 in the field in 2014. Additionally, each NIL exhibits a similar level of resistance to the GA12 *P. pachyrhizi* bulk isolate as the original resistant sources of the *Rpp* genes. These NILs will be useful as parents for public and private plant breeders, as well as for extension agents, crop consultants, and plant pathologists in conducting in-field determinations for *Rpp* gene effectiveness in the southern United States.

Journal of Plant Registrations 10:189–194 (2016).
doi:10.3198/jpr2015.04.0027crg
Received 21 Apr. 2015. Accepted 20 July 2015.
Registration by CSSA.
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Soybean rust (SBR) is one of the most economically important foliar diseases of soybean [*Glycine max* (L.) Merr.] and is caused by the obligate, basidiomycete pathogen *Phakopsora pachyrhizi* (Sydow and Sydow) (Yorinori et al., 2005; Sydow and Sydow, 1914). *Phakopsora pachyrhizi* was first observed in the continental United States in 2004 (Schneider et al., 2005). Soybean rust is a threat to soybean production in much of the world and is capable of causing yield losses of 15 to 70%, resulting in significant economic losses (Hartman et al., 1991, 2011; Livingston et al., 2004; Mueller et al., 2009; Ogle et al., 1979; Sinclair, 1989; Sinclair and Hartman, 1999; Yorinori et al., 2005). Soybean plants infected with SBR can have reduced seed quality and quantity, resulting from poorly filled beans, seed abortion, and lowered oil content of seed (Ogle et al., 1979). Some soybean genotypes have shown tolerance to *P. pachyrhizi* (Hartman et al., 2005, 1991). Soybean rust can be diagnosed by the presence of small lesions that are generally tan (TAN) in appearance due to uredinia and subsequently, by the presence of uredinospores on susceptible genotypes. Most genotypes with SBR resistance react by forming reddish-brown (RB) lesions, which may give rise to uredinia with the right environmental conditions and strain of *P. pachyrhizi* (Bromfield et al., 1980; Bromfield and Hartwig, 1980; Miles et al., 2011). Some soybean genotypes show immunity to certain *P. pachyrhizi* isolates, bearing no visible signs of infection on the leaves (Bromfield and Hartwig, 1980; Bromfield et al., 1980; Miles et al., 2011; Walker et al., 2014a).

Although SBR-related yield losses have been marginal in the United States compared with losses experienced in other areas, southern states, including Georgia, South Carolina, Alabama, Mississippi, Louisiana, North Carolina, Arkansas, Oklahoma, and Texas, have been most effected. From 2005 to 2007, approximately 53.65 million metric tons of soybean yields were lost to SBR (Wrather and Koenning, 2009). In controlled studies, fungicide-treated soybean plots in Georgia and Florida yielded 15 to 55% greater than those not treated with fungicides in the presence of SBR (Mueller et al., 2009). Losses due to SBR have been mitigated through a coordinated monitoring effort of soybean

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Abbreviations: chr, chromosome; KASP, Kompetitive Allele Specific Polymerase chain reaction; NIL, near-isogenic line; RB, reddish-brown; SBR, soybean rust; SNP, single nucleotide polymorphism; SSR, simple sequence repeat; UGA, University of Georgia.

sentinel plots by public and private organizations to establish an early warning system for growers (Isard et al., 2006; Sikora et al., 2014).

Because of the annual threat of SBR-related yield losses in the southeastern United States and the current heavy reliance on fungicide-based control, genetic resistance to *P. pachyrhizi* would be valuable to manage this disease, as *Rpp* genes could be deployed in elite cultivars to provide a sustainable solution for farmers. The utilization of cultivars with resistance would lead to a reduction in the need for foliar fungicides and therefore contribute to a reduction in production costs in areas at risk for SBR, as well as provide a more environmentally sustainable solution for SBR management (Hartman et al., 2005; Lemos et al., 2011).

A total of 16,595 soybean accessions from the USDA Soybean Germplasm Collection were screened for resistance to soybean rust in greenhouse experiments (Miles et al., 2006). In addition, known sources of resistance have been well documented from plant introductions (PIs), with six described resistance loci on four chromosomes (chr), which have more than seven single, dominant resistance alleles, *Rpp1* (chr 18; PI 200492), *Rpp1-b* (chr 18; PI 594538A), *Rpp2* (chr 16; PI 230970), *Rpp3* (chr 6; PI 462312), *Rpp4* (chr 18; PI 459025B), *Rpp5* (chr 3; PI 200526, PI 200487, and PI 471904), and *Rpp6* (chr 18; PI 567102B), and two loci with recessive resistance alleles, *rpp2* (chr. 16; PI 224270) and *rpp5* (chr 3; PI 200456) (Bromfield and Hartwig, 1980; Chakraborty et al., 2009; Garcia et al., 2008; Hartwig, 1986; Hartwig and Bromfield, 1983; Hyten et al., 2007, 2009; Li et al., 2012; Marchetti et al., 1975; Silva et al., 2008; Yu et al., 2015). *Rpp?*(Hyuuga) (chr 6; PI 506764) was initially believed to be a novel allele at the *Rpp3* locus (Monteros et al., 2007); however, a subsequent study showed that PI 506764 actually has a natural pyramid of genes at the *Rpp3* and *Rpp5* loci (Kendrick et al., 2011). Additionally, many other resistance sources have been documented representing what appears to be many different plant introductions with resistance genes at the same loci, or novel resistance alleles (Harris et al., 2015).

L85-2378 (PI 547875), a 'Williams 82' near-isoline harboring *Rpp1* from PI 200492 (Bernard et al., 1991; Hyten et al., 2007), PI 230970 (*Rpp2*), PI 462312 (*Rpp3*), and PI 459025B (*Rpp4*) sources were demonstrated to have varying levels of resistance in multiyear field tests in the southern United States at five locations when challenged with field populations of *P. pachyrhizi* from 2009 to 2012 (Walker et al., 2014a). L85-2378 was generally highly resistant or immune in most locations and years. PI 230970 (*Rpp2*), PI 462312 (*Rpp3*), and PI 459025B (*Rpp4*) have had more varying levels of resistance, and all had moderately resistance at most locations. The exception was at the Bossier City, LA, location, where both PI 462312 (*Rpp3*), and PI 459025B (*Rpp4*) scored similarly to susceptible checks. L85-2378 (*Rpp1*) was moderately resistant, and no data were available at this location for PI 230970 (*Rpp2*), demonstrating the increased virulence of the *P. pachyrhizi* field population at this location (Walker et al., 2014a).

These PIs have shown RB resistance lesions or immunity in the greenhouse when challenged with a bulk isolate of *P. pachyrhizi* urediniospores collected in 2007, 2008, or 2012. *Phakopsora pachyrhizi* was collected from naturally infested kudzu (*Pueraria* spp.) and soybean in Georgia in 2007 and 2008, while the bulk isolate was collected from soybean only in 2012 (Walker et al., 2014b).

Soybean rust resistance has been controlled by single *Rpp* genes that are capable of providing effective resistance against certain populations or strains of *P. pachyrhizi*. The goal of this work was to integrate the resistance genes from each of four resistance sources, PI 547875 (*Rpp1*; chr 18), PI 230970 (*Rpp2*, chr 16), PI 462312 (*Rpp3*, chr 6), and PI 459025B (*Rpp4*, chr 18), into a high-yielding soybean line, G00-3213 (Table 1). The most recent DNA markers available to flank each *Rpp* gene interval are Sct_187 and Sat_064 (*Rpp1*), BARCSOYSSR_16_0902 and BARCSOYSSR_16_0908 (*Rpp2*), Satt460 and Sat_263 (*Rpp3*), and Satt288 and AF162283 (*Rpp4*) (Hyten et al., 2007, 2009; Meyer et al., 2009; Yu et al., 2015). G00-3213 is a MG VII soybean that was developed at the University of Georgia from the cross of 'N7001' (Carter et al., 2003) and 'Boggs' (Boerma et al., 2000), and it is well adapted to the southeastern United States. N7001 was derived from crossing N77-114 × PI 416937; N77-114 was a derivative from a cross of 'Essex' × N70-2173; and N70-2173 was selected from a 'Hampton' × 'Ransom' cross (Smith and Camper, 1973; Webb and Hicks, 1965; Brim and Elledge, 1973). G00-3213 has tawny pubescence, tan pod walls, black hila, and white flowers, and is highly susceptible to SBR.

Table 1. Soybean rust resistance genes, original sources, and reaction types.

Resistant germplasm source	Gene designation	*Phakopsora pachyrhizi* reaction†	Mapped interval defined by SNPs‡	Physical positions of flanking markers§	KASP markers developed for each gene, ssID (GSM#)¶
PI 547875 (L85-2378)††	*Rpp1*	Immune, no sporulation	ss715632302, ss715632319	Chr. 18 60,460,936–60,616,971	ss715632302 (GSM0419), ss715632313 (GSM0422)
PI 230970	*Rpp2*	Reddish-brown, some sporulation	ss715624066, ss715624108	Chr. 16 28,882,177– 29,084,869	ss715624122 (GSM0425), ss715624131 (GSM0426)
PI 462312	*Rpp3*	Reddish-brown or immune, no sporulation if RB	ss715594464, ss715594493	Chr. 6 43,324,763– 44,307,623	ss715594485 (GSM0412), ss715594488 (GSM0415)
PI 459025B	*Rpp4*	Reddish-brown, some sporulation	ss715631693, ss715631715	Chr. 18 55,715,639– 55,913,511	ss715631686 (GSM0416), ss715631693 (GSM0417), ss715631723 (GSM0418)

† A bulk isolate of *P. pachyrhizi* collected from field-grown kudzu and soybean in 2012 (Walker et al., 2014b).

‡ Flanking SNP markers were identified by Harris et al. (2015) for *Rpp1*, *Rpp3,* and *Rpp4*; and Yu et al. (2015) identified the flanking markers used for *Rpp2.*

§ Physical genomic locations correspond to the Wm82.a1 sequence and indicate the dbSNP location.

¶ KASP (Kompetitive Allele Specific Polymerase chain reaction) markers were developed for each gene. Sequences of primers are listed in Table 3.

†† PI 200492 was used to derive L85-2378 (PI 547875), which is a 'Williams 82' isogenic line containing the *Rpp1* resistance allele (Bernard et al., 1991; Hyten et al., 2007).

Methods

Development of Near-Isogenic Lines of G00-3213

The four near-isogenic lines (NILs)—G00-3213*Rpp1* (Reg. No. GP-400, PI 676017), G00-3213*Rpp2* (Reg. No. GP-401, PI 676018), G00-3213*Rpp3* (Reg. No. GP-402, PI 676019), and G00-3213*Rpp4* (Reg. No. GP-403, PI 676020)—were developed by making five backcrosses to G00-3213 directly using a PI with soybean rust resistance, or indirectly using a PI-derived soybean elite line. For the development of the NILs containing *Rpp1* and *Rpp3*, soybean progeny derived from crosses between a PI with rust resistance and a Roundup Ready (Monsanto Co.) soybean cultivar, P97M50, were used as resistance gene donors (Table 2). P97M50 was derived from backcrossing a glyphosate-resistant donor plant to G93-2225. The glyphosate-resistant donor plant was an F_1 plant of the cross of 'Benning' (4) × ('Resnik'(2)-RR) (McBlain et al., 1990; Boerma et al., 1992). G93-2225 is a MG VIII breeding line developed from the cross of 'Cook' × 'Coker 6727' (Boerma et al., 1997).

Backcrosses of PIs with rust resistance were conducted in the field at the University of Georgia (UGA) Plant Sciences Farm near Bogart, GA, or in the greenhouse at UGA located in Athens, GA. Upon each backcross, the heterozygous F_1 plants for each respective rust gene were identified with a simple sequence repeat (SSR) marker tightly linked to the resistance gene (data not shown). The BC_1F_1 to BC_5F_1 generations were produced in the same manner by marker-assisted backcrossing of the heterozygous plants carrying the respective resistance allele. No phenotypic selection was performed during the backcrossing process.

During summer 2013, BC_5F_2 plants were selfed at the UGA Plant Sciences Farm, and individual plants were selected for good agronomic appearance and were single plant threshed. In 2014, $BC_5F_{2:3}$ lines were grown at the UGA Plant Sciences Farm, and superior individual lines were selected and bulk harvested.

SNP Marker Assays for NILs

Leaf tissue was harvested in a bulk from at least 12 plants of each NIL. Leaf issue was lyophilized and DNA was extracted using a CTAB (hexadecyltrimethylammonium bromide) method (Keim et al., 1988). At the end of the backcrossing process, single nucleotide polymorphism (SNP) assays were developed to confirm the presence of SNP alleles that are tightly linked to *Rpp1* (PI 200492), *Rpp2* (PI 230970), *Rpp3* (PI 462312), or *Rpp4* (PI 459025B) in these NILs.

Based on the haplotype windows within or closely linked to those established by Harris et al. (2015) for *Rpp1, Rpp3*, and *Rpp4,* and Yu et al. (2015) for *Rpp2*, as well as 50K SoySNP Infinium Chip data (Song et al., 2013), polymorphic SNP markers were identified for each of the *Rpp* loci. Kompetitive Allele Specific Polymerase chain reaction (KASP) SNP assays were developed from the selected polymorphic SNPs for these regions using the protocol reported by Pham et al. (2013) (Table 3). The SNP marker assays were performed per the KASP manual (LGC Genomics, 2013), as well as the protocol from Pham et al. (2013). The PCR products were read using a Tecan M1000 Pro Infinite Reader (Tecan Group Ltd.), whereby allele calls were made using KlusterCaller software (LGC Genomics). Only validated markers with clear clustering were selected and reported here (Table 3). In the case of G00-3213*Rpp1* and G00-3213*Rpp3*, the donor pedigrees contain P97M50, which carries the Roundup Ready gene. To ensure that the Roundup Ready gene was not present, all NILs were screened using a proprietary DNA marker assay provided by Monsanto Company (Table 2).

Soybean Rust Resistance Screening

In March 2014, each $BC_5F_{2:3}$ family was screened for SBR resistance reaction in the UGA Griffin Campus Greenhouse as described by Harris et al. (2015) with a bulk soybean rust isolate known as GA12. GA12 was collected from SBR-infected soybean plants around the state of Georgia in 2012 and maintained on susceptible soybean plants in the greenhouse (Walker et al., 2014b). Twelve plants were rated per $BC_5F_{2:3}$ NIL family approximately 14 d post-infection. Only the families in which

Table 2. Pedigrees and phenotypes of G00-3213 near-isogenic lines that were challenged with *Phakopsora pachyrhizi*.

Line name	Pedigree	Generation of release	Reaction to *P. pachyrhizi*†	Presence of Roundup Ready gene‡	Lesion reaction	Sporulation§
G00-3213 (recurrent parent)	N7001 × Boggs	Breeding line	Susceptible	No	TAN¶	Highly sporulating
G00-3213*Rpp1*	G00-3213(6) × [P97M50(3) × L85-2378(*Rpp1*)]F2	$BC_5F_{3:5}$	Resistant	No	Immune#	No sporulation, like L85-2378
G00-3213*Rpp2*	G00-3213(6) × PI 230970(*Rpp2*)	$BC_5F_{3:5}$	Resistant	No	RB††	Some sporulation, like PI 230970
G00-3213*Rpp3*	G00-3213(6) × [P97M50 × PI 462312(*Rpp3*)]	$BC_5F_{3:5}$	Resistant	No	RB††	No sporulation, like PI 462312
G00-3213*Rpp4*	G00-3213(6) × PI 459025B(*Rpp4*)	$BC_5F_{2:4}$	Resistant	No	RB††	Some sporulation, like PI 459025B

† A bulk isolate of *P. pachyrhizi* collected from field grown kudzu and soybean in 2012 (GA12).

‡ Near-isogenic lines were screened using a proprietary DNA marker assay provided by Monsanto Company to ensure the Roundup Ready gene was not present.

§ Indicates whether or not the lesions produced uredinia and subsequently the presence of sporulation and was compared to the source of the resistance in the same test.

¶ Lesion reactions are TAN (susceptible and highly sporulating).

Immune response (Immune), a resistant reaction whereby no lesions are visible.

†† Reddish-brown (RB) lesions (resistance reaction).

Table 3. Validated Kompetitive Allele Specific Polymerase chain reaction (KASP) markers developed for marker-assisted selection of *Rpp1, Rpp2, Rpp3,* or *Rpp4*.

Assay ID	dbSNP ID†	SNP ID‡	Gene	PI SNP allele§	Gene source	Forward primer 1 5′–3′ (FAM)	Forward primer 2 5′–3′ (HEX)	Reverse primer 5′-3′
GSM0419	ss715632302	Gm18_60460936_T_C	*Rpp1*	T	PI200492 (*Rpp1*)	GAAGGTGACCAAGTTCATGCTCAGC TCATTATAACTCGGGACCc	GAAGGTCGGAGTCAACGGATTCCA GCTCATTATAACTCGGGACCt	GGCAAAACTAGGTATCCATCCCA
GSM0422	ss715632313	Gm18_60590718_C_T	*Rpp1*	T	PI200492 (*Rpp1*)	GAAGGTGACCAAGTTCATGCTCATT GGAGAGACTTCATTATGCCAc	GAAGGTCGGAGTCAACGGATTCAT TGGAGAGACTTCATTATGCCAt	GCTCATGTACCTTGTAAGACACCG
GSM0425	ss715624122	Gm16_29128926_T_G	*Rpp2*	T	PI230970 (*Rpp2*)	GAAGGTGACCAAGTTCATGCTAGTC ATCTTGTTTTTCAGCAAGCg	GAAGGTCGGAGTCAACGGATTGAG TAGTCATCTTGTTTTTCAGCAAGCt	TGTCATCTCAGCAACCACTTTTG
GSM0426	ss715624131	Gm16_29153474_C_T	*Rpp2*	C	PI230970 (*Rpp2*)	GAAGGTGACCAAGTTCATGCTACAG TGTTTGAACAGTACAGATCTCc	GAAGGTCGGAGTCAACGGATTCAC AGTGTTTGAACAGTACAGATCTCt	CGTGTGGTTTGAATCGGAAC
GSM0412	ss715594485	Gm06_44068533_T_C	*Rpp3*	C	PI462312 (*Rpp3*)	GAAGGTGACCAAGTTCATGCTTGAC CGACAAGATGGCTTCAAc	GAAGGTCGGAGTCAACGGATTTGA CCGACAAGATGGCTTCAAt	GGCCTTCACACCCTCCACT
GSM0415	ss715594488	Gm06_44185370_G_T	*Rpp3*	A	PI462312 (*Rpp3*)	GAAGGTGACCAAGTTCATGCTTATG GACCAAAGACCCTTCCa	GAAGGTCGGAGTCAACGGATTTAT GGACCAAAGACCCTTCCc	CCCTCTGTAGTTCCACACGCT
GSM0416	ss715631686	Gm18_55672211_A_G	*Rpp4*	G	PI459025B (*Rpp4*)	GAAGGTGACCAAGTTCATGCTGGCA CGAGAACTCGCTGCTa	GAAGGTCGGAGTCAACGGATTGCA CGAGAACTCGCTGCTg	CGATCATAGCAGGACCTCCA
GSM0417	ss715631693	Gm18_55715639_C_T	*Rpp4*	C	PI459025B (*Rpp4*)	GAAGGTGACCAAGTTCATGCTAGAT GCTTTGGCCGAGGc	GAAGGTCGGAGTCAACGGATTTTA AGATGCTTTGGCCGAGGt	GTCAGAGTAATCGCACCTTGGC
GSM0418	ss715631723	Gm18_55986655_A_C	*Rpp4*	A	PI459025B (*Rpp4*)	GAAGGTGACCAAGTTCATGCTAGTC AAGTGTACTTTATAAACACCCTCa	GAAGGTCGGAGTCAACGGATTGTC AAGTGTACTTTATAAACACCCTCc	CACTTTGCCAGGCTAACAGATAA

† SNP = single nucleotide polymorphism. dbSNP ID from www.soybase.org/dlpages/index.php#snp50k.

‡ Physical genomic locations correspond to the Wm82.a1 sequence of the dbSNP location (www.soybase.org/dlpages/index.php#snp50k) (Song et al., 2013).

§ Indicates the SNP allele linked to the given *Rpp* gene resistance and PI source.

all 12 plants were resistant were selected, indicating that no segregation was observed in the progeny. Remnant seed of the selected $BC_5F_{2:3}$ families with resistance was used to produce the $BC_5F_{2:4}$ lines in summer 2014. These lines were again confirmed to have rust resistance using the GA12 isolate in January 2015 in the UGA Griffin Campus Greenhouse in the same manner as described above (Table 2; Fig. 1).

Field Evaluations of NILs

Based on the SBR resistance, 10 to 12 NILs for each resistance gene that were uniformly resistant in the BC_5F_2 stage were selected and planted in Athens, GA, during the 2014 growing season in a single replication. The soil type in this location is an Appling coarse sandy loam (fine, kaolinitic, thermic Typic Kanhapludult). Rows were 3.65 m long and 70.2 cm apart. The elite recurrent parent, G00-3213, was placed in each NIL set and used for agronomic comparison to the NILs. Soybean rust was not detected during the growing season, and no notes were taken on the presence of any diseases. Data were collected on flower color when at least 75% of the plants in a row were flowering. Maturity date, plant height, lodging, pubescence color, and pod wall color were recorded at the R8 stage as described by Fehr et al. (1971) when 95% of pods had reached their mature color. Plant height and lodging score were recorded as per Diers et al. (2014). Post-harvest observations of seed weight, seed quality, hilum color, and 100-seed weight were determined using 100 randomly selected seeds and inspecting all seeds sampled to ensure uniformity.

Characteristics

The final NILs selected are designated as G00-3213*Rpp1*, G00-3213*Rpp2*, G00-3213*Rpp3*, and G00-3213*Rpp4*. The NILs developed in this work had the expected level of resistance to the GA12 isolate compared with the corresponding PI parent in greenhouse assays: G00-3213*Rpp1* was immune, G00-3213*Rpp2* had RB lesions with some sporulation, G00-3213*Rpp3* had RB lesions with no sporulation, and G00-3213*Rpp4* had RB lesions with some sporulation (Table 1 and 2). The recurrent parent, G00-3213, showed TAN, highly sporulating lesions, when challenged with GA12, which was as expected (Table 2).

Each NIL has the same haplotype as defined by the SNP markers in the region of the resistance locus as the PI used in the backcrossing process (Table 1 and 3). This confirms the integration of the resistance locus in each NIL in relation to the parent with SBR resistance. These NILs have tawny pubescence, tan pod walls, white flowers, comparable height, maturity, lodging, seed characteristics, and general appearance to G00-3213 in the field (Table 4).

Discussion

The release of G00-3213*Rpp1*, G00-3213*Rpp2*, G00-3213*Rpp3*, and G00-3213*Rpp4* was approved by the UGA College of Agricultural and Environmental Sciences Plant Cultivar and Germplasm Release Committee in February 2015. These germplasm lines are all resistant to the *P. pachyrhizi* bulk isolate GA12 and have agronomic

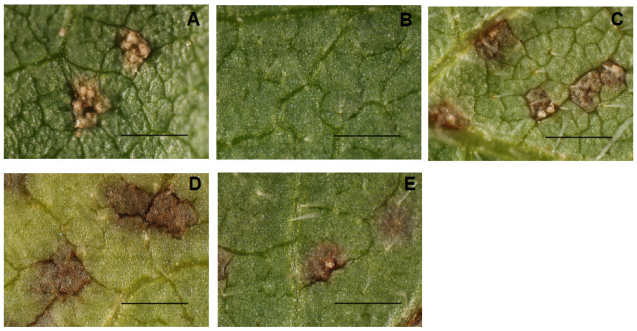

Fig. 1. The reactions of the recurrent parent, (A) G00-3213 and near-isogenic lines (B) G00-3213*Rpp1*, (C) G00-3213*Rpp2*, (D) G00-3213*Rpp3*, and (E) G00-3213*Rpp4* to the GA12 *Phakopsora pachyrhizi* bulk isolate. G00-3213 developed TAN, highly sporulating lesions, while the near-isogenic lines were immune (B) or developed red-dish-brown (RB) lesions associated with resistance (C, D, and E). Bar = 1 mm.

equivalency to the recurrent parent G00-3213 in the field (Table 2 and 4). These MG VII germplasm lines will allow private and public breeders to make crosses readily without the negative agronomic traits associated with the respective donor plant introductions and to develop new soybean cultivars for geographic regions where the soybean crop is at the highest level of risk. Similar work was completed by Diers et al. (2014) in early maturity soybean germplasm, whereby *Rpp1, Rpp1-b, Rpp?*(Hyuuga), and *Rpp5* were backcrossed into the MG II and MG IV breeding lines LD01-7323 and LD00-3309, respectively. Our work complements theirs, as our germplasm will perform well agronomically in the southern United States.

G00-3213*Rpp1*, G00-3213*Rpp2*, G00-3213*Rpp3*, and G00-3213*Rpp4* will also will be useful resources for geneticists and plant pathologists studying the underlying genes causing resistance and will help them elucidate the various races of *P. pachyrhizi*. Having each *Rpp* gene in the same genetic background allows researchers to evaluate and compare the robustness of each gene without the confounding of different genetic backgrounds; it also provides a tool for extension agents and crop consultants to conduct in-field determinations for *Rpp* gene effectiveness. For this reason, determining races of *P. pachyrhizi* can be most accurately achieved by putting different *Rpp* genes in the same background, as is the case presented here.

Availability

Seed for the USDA–ARS National Plant Germplasm System (NPGS) was developed by growing the selected NILs in a well-maintained field in Athens, GA, in 2015. Research plots were inspected for purity, and the final lines were completed at the $BC_5F_{3:4}$ generation. Seed in the NPGS is available on request from the date of this publication. Seed of G00-3213*Rpp1*, G00-3213*Rpp2*, G00-3213*Rpp3*, or G00-3213*Rpp4* can be requested from the corresponding author for up to five years. If G00-3213*Rpp1*, G00-3213*Rpp2*, G00-3213*Rpp3*, or G00-3213*Rpp4* contribute to research or the development of germplasm lines and cultivars, we request an appropriate acknowledgment.

Acknowledgments

We thank the United Soybean Board for funding support to develop this germplasm and to support Zachary King as a United Soybean Board Fellow. We also thank David Spradlin and Brian Vermeer for technical assistance in rust phenotyping.

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Table 4. Phenotypes of G00-3213 and near-isogenic lines in Athens, GA, in 2014.

Germplasm line	Maturity date†	Plant height	Lodging score	Seed weight	Seed quality	Flower color	Hilum color	Pubescence color	Pod wall color
		cm	1–5‡	mg seed^{-1}	1–5§				
G00-3213	25 Oct.¶	53–74#	1	163	1	White	Black	Tawny	Tan
G00-3213*Rpp1*	28 Oct.	71	1	170	1	White	Black	Tawny	Tan
G00-3213*Rpp2*	25 Oct.	69	1	179	1	White	Black	Tawny	Tan
G00-3213*Rpp3*	24 Oct.	66	1	170	1	White	Black	Tawny	Tan
G00-3213*Rpp4*	28 Oct.	69	1	178	1	White	Black	Tawny	Tan

† Date of the R8 stage when 95% of the pods reached their mature color (Fehr et al., 1971).

‡ Lodging score, where 1 = erect and 5 = prostrate.

§ Seed quality rating, where 1 = very good and 5 = very poor.

¶ G00-3213 maturity based on the average of six, single-row plots within the experiment in Athens during the 2014 growing season.

G00-3213 height range based on the average of six, single-row plots within the experiment in Athens during the 2014 growing season.

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