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ORIGINAL PAPER

Molecular mapping of two genes conferring resistance to *Phytophthora sojae* in a soybean landrace PI 567139B

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Abstract Phytophthora root and stem rot (PRR), caused by the soil-borne oomycete pathogen *Phytophthora sojae*, is one of the most destructive diseases of soybean. PRR can be effectively controlled by race-specific genes conferring resistance to *P. sojae* (*Rps*). However, the *Rps* genes are usually non-durable, as populations of *P. sojae* are highly diverse and quick to adapt, and can be overcome 8–15 years after deployment. Thus, it is important to identify novel *Rps* genes for development of resistant soybean cultivars. PI 567139B is a soybean landrace carrying excellent resistance to nearly all predominant *P. sojae* races in Indiana. A mapping population consisting of 245 F_2 individuals and 403 $F_{2:3}$ families was developed from a cross between PI 567139B and the susceptible cultivar 'Williams', and used to dissect the resistance carried by PI 567139B. We found that the resistance in PI 567139B was conferred by two independent *Rps* genes, designated *RpsUN1* and *RpsUN2*. The former was mapped to a 6.5 cM region between SSR markers Satt159 and BARCSOYSSR_03_0250 that spans the *Rps1* locus on chromosome 3, while the latter was mapped to a 3.0 cM region between BARCSOYSSR_16_1275 and Sat_144, approximately 3.0–3.4 cM upstream of *Rps2* on chromosome 16. According to the 'Williams 82' reference genome sequence, both regions are highly enriched with NBS-LRR genes. Marker assisted resistance spectrum analyses of these genes with 16 isolates of *P. sojae*, in combination with the mapping results, suggested that *RpsUN1* was likely to be a novel allele at the *Rps1* locus, while *RpsUN2* was more likely to be a novel *Rps* gene.

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Introduction

Phytophthora root and stem rot (PRR), caused by the soil-borne semi-biotrophic oomycete pathogen *Phytophthora sojae*, has become one of the most economically important diseases of soybean (*Glycine max* (L.) Merr.), since it was first reported in Indiana in 1948 (Schmitthenner 1985). The annual yield loss caused by this disease exceeds 250 million dollars in the US (Wrather and Koenning 2006). Several methods have been described to control this disease, such as fungicide seed treatments and cultural practices that reduce excess soil moisture (Dorrance et al. 2007). However, the most effective and economical way to manage PRR is the deployment of race-specific resistance genes, designated *Rps* genes (**R**esistance to ***Phytophthora sojae***).

Currently, there are 16 *Rps* genes/alleles that have been designated and mapped to ten loci on four chromosomes (molecular linkage groups-MLG). *Rps1* contains five

alleles (*1-a*, *1-b*, *1-c*, *1-d* and *1-k*) and has been mapped on the short arm of chromosome 3 (MLG N), and is linked with *Rps7* (Demirbas et al. 2001; Gao et al. 2005; Weng et al. 2001). *Rps2* is located in a resistance gene-rich region of chromosome 16 (MLG J), and is linked with nodulation response gene *Rj2*, powdery mildew resistance gene *Rmd* and brown stem rot resistance genes *Rbs1* and *Rbs2* (Demirbas et al. 2001; Hanson et al. 1988; Polzin et al. 1994). *Rps3*, containing three alleles (*3-a*, *3-b* and *3-c*), has been mapped to chromosome 13 (MLG F), and is either linked to or allelic with *Rps8* (Demirbas et al. 2001; Gordon et al. 2006; Sandhu et al. 2005). *Rps4*, *5* and 6 are linked and located on chromosome 18 (MLG G) (Demirbas et al. 2001; Sandhu et al. 2004). More recently, three novel *Rps* genes/alleles have been reported from Asian soybean varieties. *RpsYu25* and *Rps9* were characterized from two Chinese cultivars 'Yudou 25' and 'Ludou 4', respectively (Sun et al. 2011; Wu et al. 2011), and an unnamed *Rps* gene was identified in a Japanese cultivar 'Waseshiroge' (Sugimoto et al. 2011). Interestingly, all these three newly identified *Rps* genes were located on the short arm of chromosome 3 (MLG N) and are either allelic with or linked to *Rps1*.

Although the resistance contributed by a single *Rps* gene remains the most effective way to control PRR, it is usually non-durable, due to the rapid shift in populations of *P. sojae*, which limits the effectiveness of a gene to 8–15 years (Schmitthenner 1985). For example, *Rps1-k* has been the most widely deployed *Rps* gene for breeding commercial soybean cultivars since 1982 because of its excellent resistance to known races of *P. sojae* (Gordon et al. 2006). However, there has been a steady increase in the number of isolates of *P. sojae* that have been identified in subsequent years that are virulent to *Rps1-k* due to the strong selection pressure (Abney et al. 1997). Of the 55 classified races of *P. sojae*, 20 are virulent to *Rps1-k* (Sugimoto et al. 2012). In addition, none of the 16 known *Rps* genes alone can confer resistance to contemporary isolates of *P. sojae* (Hughes et al. unpublished data). As such, soybean breeding programs are in urgent need of novel *Rps* genes that can be effectively used to manage PRR.

Plant Introduction (PI) 567139B is a soybean line from Indonesia that carries excellent resistance to *P. sojae* race1 (*Rps7* virulence), race7 (*Rps 1-a*, *3-a*, *6* and *7* virulence), race17 (*Rps1-b*, *1-d*, *3-a*, *6* and *7* virulence) and race25 (*Rps1-a*, *1-b*, *1-c*, *1-k* and *7* virulence) (Smith et al. 2007). Further evaluation of PI 567139B with more isolates of *P. sojae* confirmed the excellence of resistance. The objectives of this study were to (1) dissect the inheritance pattern of resistance by crossing PI 567139B with the susceptible soybean line 'Williams' (no known *Rps* genes) to develop an $F_{2:3}$ mapping population, (2) determine the location of the resistance gene(s) on soybean chromosomes using molecular markers, and (3) characterize the resistance pattern of each identified *Rps* gene in PI 567139B against multiple pathotypes of *P. sojae* using marker assisted resistance spectrum (MARS) analyses.

Materials and methods

Plant materials and mapping population

A mapping population of 245 F_2 individuals and 403 $F_{2:3}$ families was obtained from a cross between 'Williams' and PI 567139B. The F_1 plant from this cross was self-pollinated to produce F_2 seeds in the greenhouse in spring 2010. A total of 245 individual F_2 were used for initial testing while the remaining 410 F_2 were self-pollinated and the seeds were collected for $F_{2:3}$ families under field conditions in 2010.

Initial mapping of the 'Williams' × PI 567139B population leads to the identification of a gene near *Rps1*, which was designated *RpsUN1*. To differentiate *RpsUN1* from *Rps1*, a population with 97 F_2 individuals and 71 $F_{2:3}$ families was derived from a cross between PI 567139B × 'Williams 82' in the summer of 2012 in the greenhouse. 'Williams 82' is a near isogenic line of 'Williams' containing the *Rps1-k* gene.

Isolates of *P. sojae*

For genetic mapping, two isolates of *P. sojae*, *pmg(17)-1* and *pmg(25)-1*, with pathotypes corresponding to races 17 and 25, respectively, were used to evaluate each of the 403 $F_{2:3}$ families. An additional 14 isolates were used to characterize the resistance identified among the $F_{2:3}$ families. Isolates of *P. sojae*, *pmg(1)-3* (race 1), *94-14-432(2)* (race 3), *94-13p-197* (race4) and *95-11-117(4)* (race7), were tested with two replicates. Isolates *pmg(5)-3* (race 5), *pmg(8)-3* (race8), *pmg(10)-1* (race 10), *pmg(13)-1* (race 13), *95-15-15* (race 24) and *96-13S-106A.1* (race 28), along with four newly obtained isolates *ISA 19A-1*, *ISA19B-2*, *ISA 71D-1* and *ISA 33O-8*, whose pathotypes do not fit any known race designation, were tested with one replicate. Isolates were collected in Indiana and Ohio and were maintained on lima bean agar-LBA (150 g Fordhook Lima beans, 20 g agar, and 1 l deionized water).

Disease evaluation

A standard hypocotyl inoculation method was deployed for disease evaluation in all the experiments as described by Abney et al. (1997). Briefly, inoculum was prepared by transferring an agar plug from an actively growing isolate on LBA to a 60 × 15 mm petri dish containing oat meal

agar-OA (60 g old-fashion rolled oats, 20 g agar, 1 l deionized water). Plates were incubated at room temperature, out of direct light, for 2 weeks. The mycelial mat was removed from the agar and sectioned into 2 × 2 mm pieces immediately before inoculation. A randomly selected piece was mounted on a glass slide and observed with a compound microscope to estimate the abundance of oospores. Seven-day-old soybean seedlings were inoculated by making a small incision approximately 1 cm below cotyledons and inserting a section of mycelium into the wound. The wound was then covered by petrolatum jelly to prevent desiccation. Plants were maintained in the greenhouse at 24–27 °C and disease reactions were evaluated 7 days post inoculation.

Standard soybean differentials (Dorrance et al. 2004) were included as checks in all experiments. These differentials were Williams (*rps*), Union (*Rps1-a*), Harosoy 13xx (*Rps1-b*), Williams 79 (*Rps1-c*), PI103091 (*Rps1-d*), Williams 82 (*Rps1-k*), L76-1988 (*Rps2*), L83-570 (*Rps3-a*), PRx146-36 (*Rps3-b*), PRx145-48 (*Rps3-c*), L85-2352 (*Rps4*), L85-3059 (*Rps5*), Harosoy 62xx (*Rps6*), Harosoy (*Rps7*) and PI399073 (*Rps8*). The phenotype of an $F_{2:3}$ family was evaluated using the percentage of resistant progenies (survival-no expanding lesion). An $F_{2:3}$ family was considered homozygous resistant (R) if more than 70 % of the progenies survived, segregating (Rs) if 25–70 % survived and susceptible (S) if fewer than 25 % survived (Dorrance et al. 2008; Sun et al. 2011).

For genetic mapping, a total of 20–30 F_3 seedlings from each of the 403 $F_{2:3}$ families were evaluated for their reaction to races 17 and 25 of *P. sojae* in replicated experiments. Twenty to twenty-four seedlings from each selected $F_{2:3}$ family were challenged with races 1, 3, 4 and 7, while an additional 10–12 seedlings were evaluated against the remaining 10 isolates used in this study. For allelism tests, one to eight seedlings were tested in each family with isolate *pmg*(*17*)-*1* (race 17), depending on the availability and vitality of the progeny seeds.

Sample collection and DNA isolation

Leaf samples from the F_2 individual plants for the $F_{2:3}$ mapping population were collected in the field at soybean growth stage R1–R5 and maintained on ice until they were brought to the lab. Leaf samples were then placed in liquid nitrogen and lyophilized immediately or stored at −80 °C until lyophilized. Genomic DNA was extracted using a standard Cetyl Trimethyl Ammonium Bromide (CTAB) method and the DNA pellet was dissolved in 200 µl TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0). The DNA samples were quantified using a Smart Spec™ Plus spectrophotometer (Bio-Rad Lab, Hercules, CA, USA) and diluted to a final concentration of 50 ng/µl for PCR.

Linkage analysis and genetic mapping

The 16 known *Rps* genes have been mapped to 10 loci on four chromosomes and linkage of these genes to simple sequence repeat (SSR) markers has been reported (Cregan 2003). Four SSR markers, Satt159 (linked to *Rps1*, *7*, *9* and *Yu25*), Satt114 (linked to *Rps3* and *8*), Satt431 (linked to *Rps2*) and Satt472 (linked to *Rps4*, *5* and *6*) were initially selected for single marker linkage analysis using 93 randomly selected $F_{2:3}$ families. The four SSR markers all showed polymorphism between 'Williams' and PI 567139B.

Initial marker analysis indicated that Satt159 (chromosome 3) and Satt431 (chromosome 16) were significantly linked to resistance against *P. sojae* races 17 and 25, respectively. Resistance linked to Satt159 was designated *RpsUN1* and the resistance linked to Satt431 was designated *RpsUN2*. Based on the predicted position of SSR markers (Song et al. 2010), five additional polymorphic markers on chromosome 3 were selected (BARCSOYSSR_03_0018, BARCSOYSSR_03_0112, BARCSOYSSR_03_0250, Satt675 and Satt683). These five markers together with Satt159 were used to screen all 403 $F_{2:3}$ families and to map *RpsUN1*. Six polymorphic SSR markers on chromosome 16 (Satt215, Sat_366, Satt244, BARCSOYSSR_16_1275, Sat_144 and Satt712), together with Satt431, were used to screen the 403 $F_{2:3}$ families and to map *RpsUN2* (Online Resource 1).

PCR amplifications were performed on MyCycler thermo cycler (Bio-Rad Lab, Hercules, CA, USA) in 20 µl reaction volumes containing 100 ng of template DNA, 1× PCR buffer, 2.5 mM Mg^{2+}, 200 µM dNTP, 100 nM forward and reverse primers, and 1 U of *Taq* DNA polymerase. The PCR cycles consisted of 1 cycle of denaturation at 95 °C for 2 min, followed by 35 cycles of 95 °C for 40 s, 55 °C for 40 s and 72 °C for 40 s, with a final extension for 10 min at 72 °C. The PCR products were resolved on 3 % agarose gel (DOT Scientific Inc., Burton, MI, USA), stained with ethidium bromide and analyzed on Molecular Imager Gel Doc XR system (Bio-Rad Lab, Hercules, CA, USA).

Marker assisted resistance spectrum (MARS) analyses of *RpsUN1* and *RpsUN2*

To characterize the resistance of *RpsUN1* and *RpsUN2*, and to distinguish them from previously identified *Rps* genes, MARS analysis was performed. Eight and nine homozygous resistance lines, respectively, were selected from the 403 $F_{2:3}$ families on the basis of the most tightly linked markers (BARCSOYSSR_03_0250 for *RpsUN1* and sat_144 for *RpsUN2*). Another 11 and 10 segregating lines for *RpsUN1* and *RpsUN2*, respectively, were also used in

supporting tests. Fifteen more $F_{2:3}$ families, containing neither *RpsUN1* nor *RpsUN2* were used as controls (Online Resource 2). These selected families were screened with the 16 isolates of *P. sojae* used in this study.

Statistical and linkage analysis

A Chi square test was performed to test the phenotypic data for a goodness-of-fit to the ratio of 1:2:1 for races 17 and 25. Because two dominant genes were indicated, *RpsUN1* and *RpsUN2*, then a 9:3:3:1 ratio was carried out to infer the linkage of the two genes. The calculations were performed using SAS 9.2 software (SAS Institute Inc. Cary, NC) with a significance threshold of $P = 0.05$. Single marker linkage analysis of the four SSR markers was conducted using Quantitative Trait Loci (QTL) cartographer software version 2.5 (North Carolina University, Raleigh, NC). Linkage maps for each of the genes were then constructed using Joinmap 3.0 software (Van Ooijen and Voorrips 2001). Linkage groups were determined using a logarithm of the odds (LOD) score of 3.0, and Kosambi's mapping function was deployed.

Results

Inheritance pattern of *P. sojae* resistance in PI567139B

Three isolates of *P. sojae*, *pmg(1)-3* (race 1), *pmg(17)-1* (race 17), and *pmg(25)-1* (race 25) were tested using F_2 individuals. The F_2 population segregated with a ratio of 113:27 and 41:19 for races 17 and 25, respectively, both fitting a 3:1 ratio (Table 1). Against race 1, the F_2 population segregated fitting a 15:1 ratio that suggested two independently inherited dominant resistance genes (Table 1).

The inheritance pattern of resistance detected among the F_2 population was further confirmed by the evaluation of the 403 $F_{2:3}$ families against races 17 and 25. The ratio of R (homozygous resistant): Rs (segregating): S (homozygous susceptible) observed for race17 was 94: 212: 97 and 101:209:93 for race25. The two ratios both fit the expected 1:2:1 ratio (Table 1). Moreover, a ratio of 239:67:71:26 was observed to fit the ratio of 9:3:3:1 (Table 2) for linkage detection, which confirmed that two independently inherited dominant resistance genes, *RpsUN1* and *RpsUN2*, were present in PI 567139B.

Linkage analysis and genetic mapping

The linkage of *RpsUN1* and *RpsUN2* to the 16 known *Rps* genes was evaluated using four SSR markers. Satt159 (linked to *Rps1*, *7*, *9* and *Yu25*) was significantly linked with *RpsUN1* but not linked to *RpsUN2*. Satt431 (linked to *Rps2*) was linked to *RpsUN2* but not linked to *RpsUN1*. Satt114 (linked to *Rps3* and *8*) and Satt472 (linked to *Rps4*, *5* and *6*) did not show linkage with either of the two genes (Table 3). These results anchored *RpsUN1* on chromosome 3 (MLG N) and *RpsUN2* on chromosome 16 (MLG J).

The genetic location of *RpsUN1* and *RpsUN2* was further confirmed by genetic mapping using all 403 $F_{2:3}$ mapping populations and five and six additional SSR markers for *RpsUN1* and *RpsUN2*, respectively. *RpsUN1* was mapped between Satt159 and BARCSOYSSR_03_0250, which were located a distance of 6.0 and 0.5 cM, respectively, from the gene (Fig. 1b). *RpsUN2* was mapped between BARCSOYSSR_16_1275 and Sat_144 with a distance of 1.8 and 1.2 cM, respectively, from *RpsUN2* (Fig. 2b). Each SSR marker evaluated on chromosomes 3 and 16 was in good agreement with the marker order

Table 2 Goodness-of-fit test of the two resistance genes to 9:3:3:1

P. sojae isolate		No. of $F_{2:3}$ families	
pmg(17)-1	*pmg(25)-1*	Observed	Expected
Res.	Res.	239	226.69
Res.	Sus.	67	75.56
Sus.	Res.	71	75.56
Sus.	Sus.	26	25.19
Total		403	403
$\chi^2_{9:3:3:1} = 1.94$, $P = 0.59$			

Res. resistant, *Sus.* susceptible

Table 1 Reaction of F_2 individuals and $F_{2:3}$ families derived from a cross between 'Williams' and PI567139B to *P. sojae* races

P. sojae		F_2 population			$F_{2:3}$ progeny test		
Race	Isolate	Res.:Sus.	χ^2	P	R:Rs:S	$\chi^2_{1:2:1}$	P
1	*pmg(1)-3*	41:4	$\chi^2_{15:1} = 0.53$	0.46	–	–	
17	*pmg(17)-1*	113:27	$\chi^2_{3:1} = 2.44$	0.12	94:212:97	1.14	0.57
25	*pmg(25)-1*	41:19	$\chi^2_{3:1} = 1.42$	0.23	101:209:93	0.88	0.65

Res. resistant, *Sus.* susceptible

R homozygous resistant, *Rs* heterozygous resistant, *S* susceptible

Table 3 Single marker linkage analysis of *RpsUN1* and *RpsUN2* using 93 randomly selected $F_{2:3}$ families

SSR marker	Chromosome Location (MLG)	Linkage to known *Rps* genes	Linkage to *RpsUN1*	Linkage to *RpsUN2*
Satt159	Gm03 (N)	*Rps1*, *7*, *9*, *Yu25*	$P = 0.000$*	$P = 0.525$
Satt431	Gm16 (J)	*Rps2*	$P = 0.829$	$P = 0.000$*
Satt114	Gm13 (F)	*Rps3*, *8*	$P = 0.389$	$P = 0.480$
Satt472	Gm18 (G)	*Rps4* ,*5*, *6*	$P = 0.486$	$P = 0.744$

MLG molecular linkage group

* Significant if $P < 0.05$

annotated on the 'Williams 82' reference genome sequence (Schmutz et al. 2010; Song et al. 2010).

MARS analyses and allelism test

Of the 16 isolates of *P. sojae* used in this study, *RpsUN1* conferred resistance to eight and intermediate resistance to four, and susceptibility to four (Table 4). *RpsUN2* was resistant to six isolates, with intermediate resistance to seven, and susceptibility to three (Table 4). The combination of *RpsUN1* and *RpsUN2* conferred resistance or intermediate resistance to all 16 isolates of *P. sojae*, and explained the resistance observed in the donor line PI 567139B. The 15 $F_{2:3}$ families that did not contain *RpsUN1* or *RpsUN2*, along with recessive parent 'Williams' were susceptible to all 16 isolates tested. These results suggested that *RpsUN1* and *RpsUN2* are the only two *Rps* genes present in PI 567139B (Table 4).

Rps1-a, *1-b*, *1-c*, *1-d* and *1-k* have been mapped to the same region of chromosome 3 as *RpsUN1*. However, based on MARS analysis, none of the previously described *Rps1* alleles showed a pattern identical to *RpsUN1* (Table 4), suggesting that *RpsUN1* was either a novel *Rps* gene or a novel *Rps1* allele. To clarify which, a population of 97 F_2 individuals and 71 $F_{2:3}$ families obtained from a cross between PI 567139B × 'Williams 82' (*Rps1-k*) were evaluated against *pmg*(*17*)*-1* (race 17). This isolate was selected because it was avirulent to both *RpsUN1* and *Rps1-k*, yet virulent to *RpsUN2*, so that the influence of this gene could be prevented. Of the 97 F_2 individuals evaluated, only one was susceptible. In addition, all 71 $F_{2:3}$ families were resistant (Online Resource 3). Taking into account the influence of environment on the reaction of *Rps* genes, the one susceptible individual did not provide enough evidence to support *RpsUN1* as a novel *Rps* locus. Instead, it was concluded that *RpsUN1* is likely to be a novel allele of *Rps1*.

RpsUN2 was different from *Rps2* based on both genetic and MARS analysis. *Rps2* has been placed on chromosome 16 (MLG J) using RFLP markers (Devine et al. 1991; Diers et al. 1992; Lohnes et al. 1993; Polzin et al. 1994), yet has not been ordered in relation to closely linked SSR markers (Demirbas et al. 2001). Instead, we found that the *Rj2* gene can be used as a marker to estimate the genetic distance of *RpsUN2* and *Rps2*. *Rj2* has been confirmed to be Glyma16g33780 (Yang et al. 2010), which is located between 36,560,752 and 36,565,287 bp of chromosome 16, according to the soybean reference genome sequence (Schmutz et al. 2010), 3.8–4.2 cM upstream of *Rps2* (Lohnes et al. 1993; Polzin et al. 1994). Based on our genetic mapping data and the physical positions of SSR markers in the reference genome sequence, we were able to estimate that *RpsUN2* is located approximately 0.8 cM downstream of *Rj2*, and the genetic distance between *RpsUN2* and *Rps2* is between 3.0 and 3.4 cM. Thus, it is most likely that *RpsUN2* is a distinct *Rps* locus rather than an allele of *Rps2*. In addition, MARS analysis indicated that *RpsUN2* had a much broader resistance spectrum than *Rps2* (Table 4). Of the 16 isolates tested, *Rps2* gave a susceptible reaction to nine, while *RpsUN2* was susceptible to three (Table 4). Moreover, the resistance conferred by *Rps2* is usually incomplete (Mideros et al. 2007), whereas *RpsUN2* completely arrests the growth of *P. sojae*. Together these data suggested that *RpsUN2* is distinct from any other previously identified *Rps* genes.

Discussion

In this study, two novel *P. sojae* resistance genes, *RpsUN1* and *RpsUN2* were identified from the soybean line PI 567139B. The two genes were both mapped to resistance gene rich regions on soybean chromosomes 3 and 16. On the short arm of chromosome 3, previously identified *Rps* genes, *Rps1* and *7*, have been mapped (Fig. 1a, f, g) (Anderson and Buzzell 1992; Demirbas et al. 2001; Gardner et al. 2001; Sugimoto et al. 2008; Weng et al. 2001). *Rps1-a*, *1-b*, *1-c*, *1-d* and *1-k* are believed to be different alleles of *Rps1*. However, this locus may not consist of a single gene but a cluster of genes encoding nucleotide binding site-leucine rich repeats (NBS-LRR) proteins. Map-based cloning approach identified four coiled-coil (CC)-NBS-LRR genes following fine mapping of the *Rps1-k* region, two of which were identical in sequence (Gao et al. 2005; Kasuga et al. 1997). The four *Rps1-k* gene family members each expressed the same resistance pattern, suggesting that they are independently functional against *P. sojae* (Gao et al. 2005). Further identification of abundant repetitive sequences and nonfunctional genes by sequencing also suggests that *Rps1-k* is located in or near a heterochromatic region (Gao and

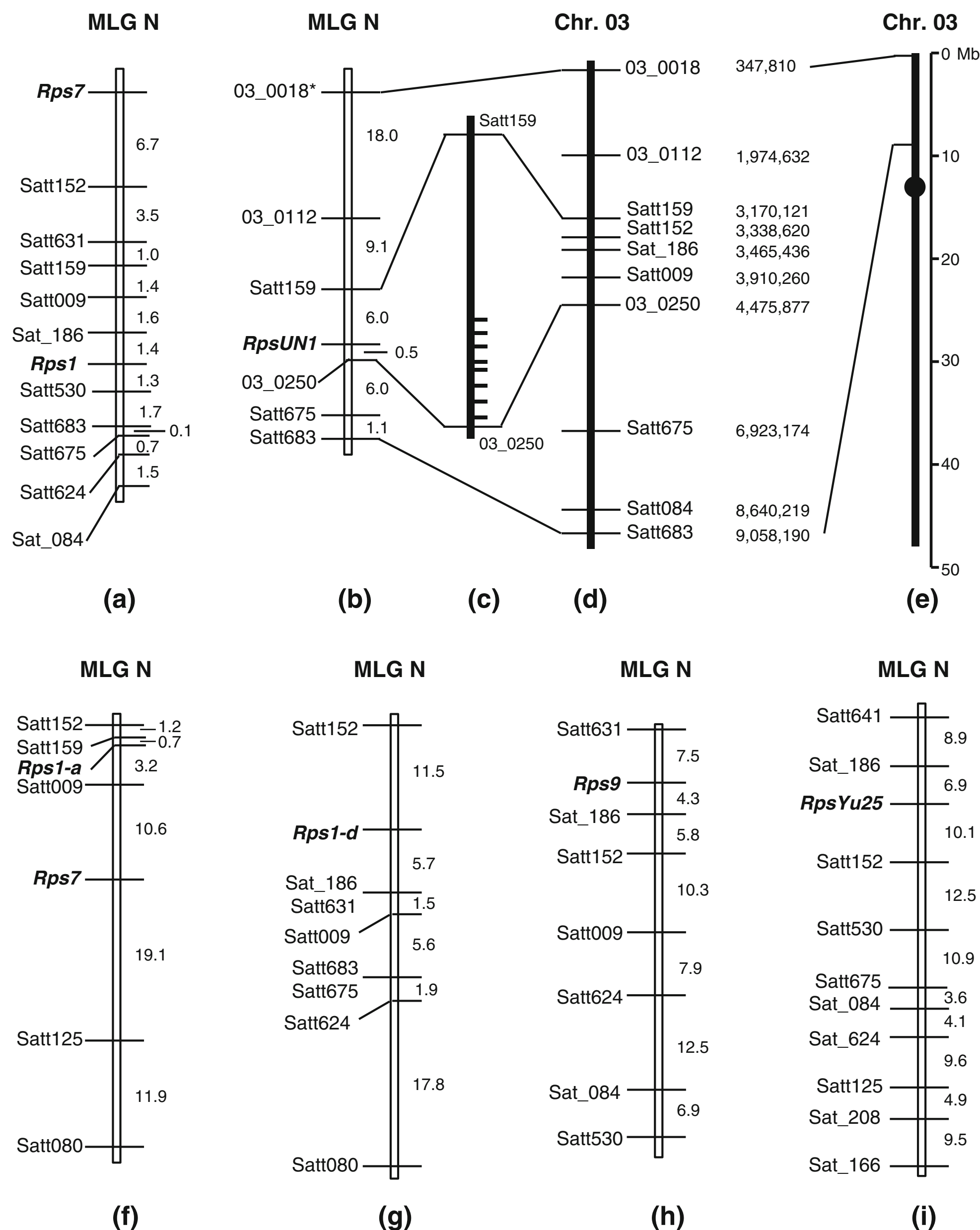

Fig. 1 Genetic and physical map of *RpsUN1* and linked *Rps* genes on chromosome 3. For genetic maps (**a**, **b**, **f**, **g**, **h**, **i**), loci names are shown on the *left side* of the map. Genetic distances on the right hand of each map are in cM. For physical maps (**c**, **d**), loci names are on the *right side*. *abbreviation for 'BARCSOYSSR_03_0018'. **a** Composite genetic map of *Rps1* and *Rps7* region reported by Cregan (2003). **b** Linkage map of *RpsUN1* from this study. **c** Distribution of annotated NBS-LRR genes within mapped region of *RpsUN1*. Each *bar* represents the position of a NBS-LRR gene. **d** Physical position of SSR markers on chromosome 3 reported by Song et al. (2010). The start position of *marker* is shown on the *right side* of each locus. **e** Physical position of the mapped region of *RpsUN1* on chromosome 3 (Schmutz et al. 2010). The *round circle* indicates the position of centromeric region. **f** Linkage map of *Rps1-a* and *7* reported by Weng et al. (2001). **g** Linkage map of *Rps1-d* reported by Sugimoto et al. (2008). **h** Linkage map of *Rps9* reported by Wu et al. (2011). **i** Linkage map of *RpsYu25* reported by Sun et al. (2011)

Bhattacharyya 2008). Unfortunately, none of the four previously isolated *Rps1-k* gene sequences were identified in the reference genome sequence of 'Williams 82' (Schmutz et al. 2010), which carries *Rps1-k*, so the physical location of this gene corresponding to the 'Williams 82' reference genome remains unknown.

Besides *Rps1* and *7*, more recently reported *Rps* genes, *Rps9* (Fig. 1h); (Wu et al. 2011), *RpsYu25* (Fig. 1i) (Sun et al. 2011), and an unnamed *Rps* gene/allele (Sugimoto et al. 2011), have also been mapped to this region. Whole genome sequencing identified a cluster of more than 30 NBS-LRR genes in a 6 Mb region between Satt159 and

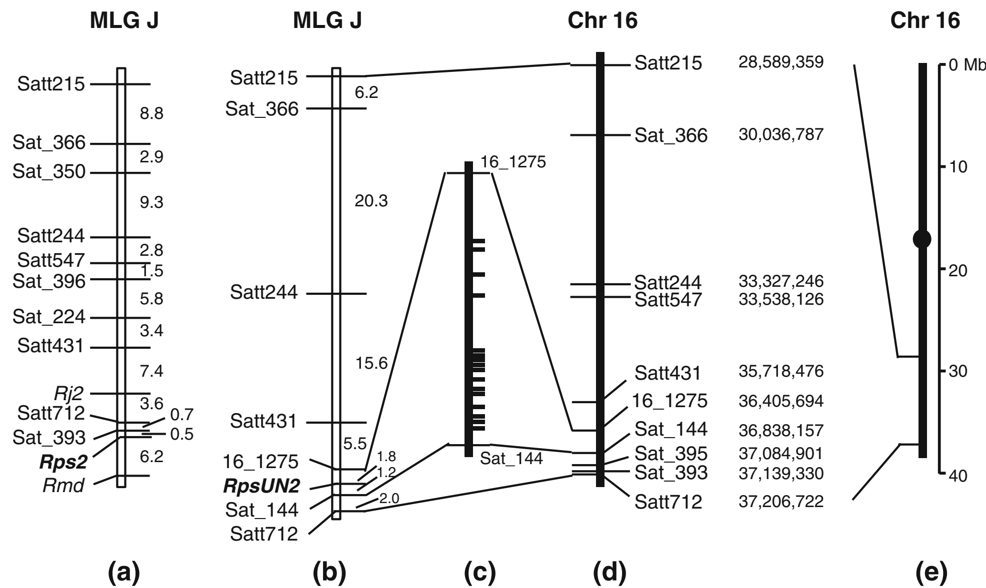

Fig. 2 Linkage and physical map of *RpsUN2* on chromosome 16. For genetic maps (**a**, **b**), loci names are shown on the *left side* of the map. Genetic distances on the right hand of each map are in cM. For physical maps, loci names are on the *right side*. **a** Composite genetic map of *Rps2* region reported by Cregan (2003). **b** Linkage map of *RpsUN2* from this study. **c** Distribution of annotated NBS-LRR genes within the mapped region of *RpsUN2*. Each *bar* represents the position of a NBS-LRR gene. **d** Physical position of SSR markers on chromosome 16 reported by Song et al. (2010). The start position of *marker* is shown on the right side of the loci. **e** Physical position of mapped region of *RpsUN2* on chromosome 16 (Schmutz et al. 2010). The *round circle* indicates the position of centromeric region

Satt683 (Schmutz et al. 2010). Eight of these genes are distributed between Satt159 and BARCSOYSSR_03_0250, where *RpsUN1* was mapped (Fig. 1b–e). Although NBS-LRR genes can evolve fast by unequal crossing over or other mechanisms (Bhattacharyya et al. 2005; Leister 2004; Michelmore and Meyers 1998), the frequency of occurrence of *Rps* genes appears to be unevenly distributed on the 20 soybean chromosomes, especially when taking into account that nearly half of the described *Rps* genes (9 out of 19) are located on the short arm of chromosome 3. One possible explanation for this is that this region may fit the birth-and-death model and undergo a stronger divergent selection against *P. sojae* than other NBS-LRR gene rich regions (Michelmore and Meyers 1998). With the unusually high appearance of *Rps* genes/alleles on the short arm of chromosome 3, a preliminary examination of this region might expedite the identification of novel *Rps* genes in future studies.

Although *RpsUN2* is most likely to be distinct from *Rps2*, the possibility that *RpsUN2* is a novel allele at the *Rps2* locus may not be fully excluded. A contig from the Bacterial Artificial Chromosome (BAC) clones of 'Williams 82' (*rps2*) spanning or adjacent to the *Rps2* region was sequenced in which a cluster of resistance gene analogs were identified (Graham et al. 2002a, b). This contig shares 99.99 % identity with the region of chromosome 16 between 36,657,381 and 36,776,150 in *G. max* genome sequence (v1.01), where 11 high-confidence NBS-LRR genes were annotated (Schmutz et al. 2010). Intriguingly, this 118 kb contig is also included in the 432 kb mapped region of *RpsUN2*, between BARCSOYSSR_16_1275 and Sat_144, where five additional high-confidence NBS-LRR genes were identified (Fig. 2c). Among these five genes, Glyma16g33780, has been cloned and functionally confirmed to be the *Rj2* gene controlling host specificity in the legume-rhizobia symbiosis (Yang et al. 2010). Thus, *Rps2* and *RpsUN2* appear to be both located in the same cluster of NBS-LRR genes.

To be certain that *RpsUN2* is distinct from *Rps2*, two different allelism tests (Gordon et al. 2006) could be performed. Both approaches will require the construction of a population of $F_{2:3}$ families by crossing a line homozygous for *Rps2* with PI 567139B or a line homozygous for *RpsUN2* and the *rpsUN1* loci. The first approach would use a common isolate of *P. sojae* that is avirulent to *Rps2* and *RpsUN2*, yet virulent to *RpsUN1* (e.g. *pmg*(*25*)-*1*) (if PI 567139B is used), to detect susceptible $F_{2:3}$ families. This method will require a very large population (e.g. >1,000 individuals) given that *Rps2* and *RpsUN2*, if different, are closely linked. The second approach would use two isolates of *P. sojae*: one that is avirulent to *Rps2* but virulent to *RpsUN2*, and a second that is avirulent to *RpsUN2* but virulent to *Rps2*, which would allow both genes to be mapped in an identical population. Unfortunately, none of the 16 isolates used in this study or in the current collection of isolates is avirulent to *Rps2* and virulent to *RpsUN2*.

The application of the two novel *Rps* genes identified from PI 567139B in soybean breeding may be more

Table 4 Marker assisted resistance spectrum analysis (MARS) of *RpsUN1* and *RpsUN2* to isolates of *P. sojae*

P. sojae		$F_{2:3}$ families selected[a]			Parental lines		*Rps* genes on Gm03 and Gm16					
Isolate	Race	*RpsUN1*	*RpsUN2*	*rpsUN1*, *rpsUN2*	PI 567139B	Williams	*1-a*	*1-b*	*1-c*	*1-d*	*1-k*	*2*
pmg(1)-3	1	R[b]	R	S	R	S	R[c]	R	R	R	R	I
94-14-432(2)	3	I	R	S	R	S	S	R	R	R	R	I
94-13p-197	4	S	I	S	R	S	S	R	S	S	R	S
pmg(5)-3	5	I	I	S	R	S	S	R	S	R	R	S
95-11-117 (4)	7	R	S	S	R	S	S	R	R	R	R	S
pmg(8)-3	8	I	S	S	R	S	S	R	R	S	R	S
pmg(10)-1	10	R	R	S	R	S	R	S	R	I	R	S
pmg(13)-1	13	R	I	S	R	S	R	R	R	I	R	S
pmg(17)-1	17	R	S	S	R	S	R	S	R	S	R	S
95-15-15	24	I	I	S	R	S	S	S	S	I	S	S
pmg(25)-1	25	S	R	S	R	S	S	S	S	R	S	R
96-13S-106A.1	28	R	R	S	R	S	S	S	R	R	S	R
ISA 19A-1	N/A	R	I	S	R	S	S	S	R	R	S	R
ISA 19B-2	N/A	R	I	S	R	S	S	S	R	I	S	R
ISA 71D-1	N/A	S	I	S	I	S	S	R	S	R	R	R
ISA 33O-8	N/A	S	R	S	I	S	S	S	S	S	S	S

[a] The molecular markers for selections are BARCSOYSSR_03_0250 for *RpsUN1* and Sat_144 for *RpsUN2* loci, respectively. The resistance pattern of *RpsUN1* against a *P. sojae* isolate was evaluated by the average proportion of resistance progenies of all the selected 8 homozygous and 11 heterozygous $F_{2:3}$ families. Similarly, the resistance pattern of *RpsUN2* was evaluated by 9 homozygous families and 10 heterozygous $F_{2:3}$ families. The reaction of *rpsUN1rpsUN2* was calculated from 15 double recessive $F_{2:3}$ families

[b] The reaction of a novel *Rps* gene was considered resistant (R) if the average resistance proportion of homozygous lines was >70 % and that of heterozygous lines was >25 %; intermediate resistant (I) if average resistance proportion of homozygous lines was between 25 and 70 %; and susceptible if average resistance proportion of both homozygous lines and heterozygous lines were <25 %. For more detailed data and statistical analysis, see Online Resource Table 2a

[c] A differential was recorded as resistant (R) if >70 % of seedlings were survival, intermediate resistant (I) between 25 and 70 %, and susceptible if <25 %. For reaction of whole set of differentials, see Online Resource Table 2b

complicated than that of a single *Rps* gene. In addition, PI 567139B is a late maturity group (MG) IX line that does not possess currently acceptable agronomic traits, which could affect the efficiency and successfulness of its use in breeding. As such, a pure breeding line with a shorter maturity that contains these unique *Rps* genes will be needed in research and breeding efforts. The successful application of molecular markers for the selection of a single gene provided us with powerful tools to help achieve this goal. Four and three homozygous resistant 'Williams' × PI 567139B $F_{2:3}$ families have been selected for *RpsUN1* and *RpsUN2*, respectively, based on their reaction to *P. sojae*, and have been advanced to F_5 generation. In each generation, the lines with the earliest maturity were selected and advanced in the greenhouse. The selected F_5 lines will be increased in the field in 2013 and the progenies will be re-tested to examine their reactions. The best lines for *RpsUN1* and *RpsUN2* will be released for public acquisition.

Nineteen *Rps* genes/alleles have now been identified that confer a broad spectrum of resistance to PRR. However, no single *Rps* gene is able to defeat all known isolates of *P. sojae*. Nevertheless, the identification of two novel *Rps* genes/alleles will provide breeders with more options in their breeding programs. For example, *RpsUN1* and *RpsUN2* together provide resistance to a greater diversity of isolates of *P. sojae* than either one alone. By stacking these genes into elite varieties, breeders will be able to provide growers with effective resistance to contemporary populations of *P. sojae*. Meanwhile, continued efforts are needed in the search for novel *Rps* genes.

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