Inheritance of Resistance to the Soybean Aphid in Soybean PI 200538

Curtis B. Hill,* Ki-Seung Kim, Laura Crull, Brian W. Diers, and Glen L. Hartman

ABSTRACT

The soybean aphid (*Aphis glycines* Matsumura) is a major soybean [*Glycine max* (L.) Merr.] insect pest. Soybean plant introduction (PI) 200538 has strong resistance to the aphid. The objectives of our research were to determine the inheritance of resistance and to map gene(s) controlling resistance in PI 200538. F_2 populations developed from crosses between PI 200538 and three susceptible genotypes were tested for resistance and with DNA markers. F_2 plants from the cross 'Ina' × PI 200538 segregated 114 resistant to 37 susceptible and F_2 plants from the cross 'Williams 82' × PI 200538 segregated 203 resistant to 65 susceptible when tested for resistance to soybean aphid biotype 1. F_2 plants from the cross LD02-4485 × PI 200538 segregated 167 resistant to 62 susceptible when tested for resistance to biotype 2. These populations fit a 3:1 genetic ratio (P = 0.89, 0.78, and 0.52, respectively) with resistance dominant over susceptibility. Segregation among $F_{2:3}$ families from the crosses supported the dominant resistance gene hypothesis. The gene mapped to soybean linkage group F, flanked by the simple sequence repeat marker loci Satt510, Soyhsp176, Satt114, and Sct_033, located in the same region as the aphid resistance gene *Rag2*. Since the resistance gene in PI 200538 also gave resistance to soybean aphid biotypes 1 and 2, it is possible that the gene is *Rag2* and not a new aphid resistance gene. Therefore, PI 200538 may be an additional source of *Rag2*.

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Abbreviations: LG, linkage group; PCR, polymerase chain reaction; PI, plant introduction; QTL, quantitative trait loci; SMV, *Soybean mosaic virus*; SSR, simple sequence repeat.

The first report of the soybean aphid (*Aphis glycines* Matsumura) in North America was during 2000 in the upper midwestern United States (Hartman et al., 2001). By 2004, the aphid had spread throughout the main soybean [*Glycine max* (L.) Merr.] production areas (Ragsdale et al., 2004). Dense aphid colonies on soybean plants reduce grain production directly by causing severe plant damage during feeding, including leaf distortion, stunting, and desiccation. Soybean production is indirectly affected by the growth of black sooty mold fungus on aphid honeydew that inhibits plant photosynthesis and through the vectoring of serious soybean viruses such as *Soybean mosaic virus* (SMV) (Hartman et al., 2001). The pest caused extensive economic losses in soybean in several midwestern states in 2003. Nearly 1.6 million ha of soybean were damaged in Minnesota with an estimated loss of $80 million (Associated-Press, 2003). In Illinois, about 0.5 million ha were damaged with an estimated loss of $45 million (Steffey, 2004).

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Soybean aphid resistant cultivars are currently not commercially available in North America. The application of registered insecticides is the only available means to control the pest. During the 2003 soybean aphid outbreak, nearly 3 million ha of soybeans in the United States were sprayed to control the pest (Landis et al., 2003). From $9 to 12 million was spent on insecticide applications to control soybean aphid in Illinois in 2003 (Steffey, 2004).

Plant insect resistance is a cost-effective and environmentally safe component of an integrated pest management program to control insects (Auclair, 1989; Harrewijn and Minks, 1989; Luginbill, 1969). Screening the soybean germplasm collection for aphid resistance led to the discovery of strong antibiosis-type resistance in several soybean germplasm accessions (Hill et al., 2004a; Li et al., 2004; Mensah et al., 2005; Mian et al., 2008a). Resistance to an aphid isolate collected in Illinois was controlled by single dominant genes in the ancestral cultivars Dowling (Hill et al., 2006a) and Jackson (Hill et al., 2006b). The resistance gene in Dowling was named *Rag1* (Hill et al., 2006a) and both this gene and a gene in Jackson (Hill et al., 2006b) were mapped to the same region on linkage group (LG) M (Li et al., 2006). For this reason, the Jackson resistance gene was not named because the genetic relationship with *Rag1* was unclear.

Antibiosis and antixenosis-type resistance was also found in early maturing soybean germplasm accessions (Mensah et al., 2005). Results of genetic studies indicated that resistance was controlled by quantitative trait loci (QTL) in PI 567541B and by two recessive genes in PI 567598B (Zhang et al., 2009; Mensah et al., 2005). In another screen of soybean germplasm, Mian et al. (2008a, 2008b) identified antibiosis resistance in PI 243540 and identified a single, dominant gene for resistance named *Rag2* that mapped to soybean LG F.

Soybean aphid biotypes were recently identified (Kim et al., 2008). Results of choice and nonchoice experiments indicated that soybean aphid isolates collected in Ohio and Illinois were distinct biotypes distinguished by their differential virulence on plants possessing *Rag1*. The Ohio isolate developed large colonies on plants with *Rag1* and to a lesser extent on Jackson. In contrast, the Illinois isolate did not colonize plants with *Rag1*, including the soybean cultivar Dowling, as well as Jackson. Both isolates were virulent on the soybean cultivar Williams 82 and a few other lines tested that were known to be susceptible to the Illinois isolate. A few germplasm sources previously found to be resistant to the Illinois isolate (Hill et al., 2004a) were also resistant to the Ohio isolate; including the soybean germplasm accession PI 200538 (Sugao Zairai), indicating that they may have resistance genes different from *Rag1*.

Evidence of host specialization and the existence of at least two soybean aphid biotypes in North America suggest that plant resistance controlled by major genes such as *Rag1* may be vulnerable to genetic erosion. This finding is a major concern to soybean breeders engaged in developing new soybean aphid resistant cultivars. New resistance genes will need to be identified and introduced into adapted soybean cultivars and widely tested over time and different geographic locations to stay ahead of aphid populations that adapt to host resistance genes.

The objective of this study was to determine the inheritance of soybean aphid resistance in PI 200538 and map the locations of the gene or genes controlling aphid resistance from this accession.

MATERIALS AND METHODS

Aphid Culture

Two soybean aphid isolates representing two biotypes were used in this study. The Illinois isolate, originally collected in 2000, was used in previous studies (Hill et al., 2004a, 2004b, 2006a, 2006b; Li et al., 2004) and is referred to as biotype 1. The Ohio isolate was collected and established at the Ohio Agricultural Research and Development Center (OARDC), Wooster, OH, during the summer of 2005, and is distinguished from the Illinois isolate by its ability to colonize plants with *Rag1* (Kim et al., 2008). This isolate is referred to as biotype 2. Biotype 1 was maintained on a continuous supply of plants of the cultivar Williams 82 in growth chambers as described previously (Hill et al., 2004a, 2004b). Biotype 2 was maintained on the soybean breeding line LD05-16611 that possesses *Rag1*. The two biotypes were maintained in plant growth chambers located in different buildings at the University of Illinois to avoid mixing.

Population Development

Crosses were made between the soybean germplasm accession PI 200538, which is resistant to soybean aphid biotypes 1 and 2 (Kim et al., 2008), and the soybean cultivars Ina and Williams 82, which are susceptible to both biotypes. PI 200538 was the male parent in both crosses. PI 200538 is a maturity group VIII germplasm accession originating from Japan (USDA-ARS National Genetic Resources Program, 2008). In addition, a cross was made between the soybean experimental line LD02-4485, which is susceptible to biotypes 1 and 2, and the line 14257, an F_3 plant from an F_2–derived line that was breeding true for resistance to biotype 1. The line 14257 was from the Ina × PI 200538 cross. PI 200538 was also used as the male parent in crosses with the aphid resistance sources Dowling and Jackson. F_1 seeds produced from the crosses were harvested and planted in separate pots for F_2 seed production in a greenhouse maintained at 28°C with supplemental lighting provided by a mixture of 1000-W high intensity discharge and high-pressure sodium vapor lamps set to give a 14-h photoperiod. F_2 seed from each F_1 plant was harvested and kept separately. There was sufficient F_2 seed to produce two Ina × PI 200538 and three Williams 82 × PI 200538 F_2 populations along with a single LD02-4485 × 14257 F_2 population to use for genetic analysis of aphid resistance.

Aphid Resistance Tests

Three choice tests were conducted in the greenhouse under environmental conditions previously described (Hill et al.,

2006a). The first choice test was a test for resistance to biotype 1 with 168 F_2 seeds from the Ina × PI 200538 cross and 340 F_2 seeds from the Williams 82 × PI 200538 cross sown along with the parents. The second choice test was a test for resistance to biotype 2 with 248 F_2 seeds from the cross LD02-4485 × 14257 (Ina × PI 200538) sown along with susceptible and resistant checks. The third choice test was a genetic allelism test with 48 Dowling × PI 200538 F_2 seeds and 80 Jackson × PI 200538 F_2 seeds sown with parental and susceptible checks. Emerging plants in this test were inoculated with biotype 1 to determine if the resistance genes in Dowling or Jackson were allelic with a gene PI 200538.

Methods for plant culture, aphid infestation, and experimental design were previously described (Hill et al., 2006a). Seeds were planted at a rate of one seed per pot in soil-less media (Sunshine Mix, LC1, Sun Gro Horticulture Inc., Bellevue, WA) in 48-pot plastic inserts, with 12 rows of four pots (Hummert International, Earth City, MO, no. 1204) contained in flats without drainage holes (Hummert International, no. F1020). F_2 populations, parents, and resistant and susceptible checks were planted in four-pot rows that were randomized throughout all of the flats in each of the choice tests. Experimental units were each individual plant. In the first choice test with biotype 1, 42 rows of Ina × PI 200538 F_2 plants and 85 rows of Williams 82 × PI 200538 F2 plants were randomized with 20 rows of each of the parents Ina, Williams 82, and PI 200538, 20 rows of each of the susceptible checks 'Dwight', 'Loda', and 'Pana', along with the biotype 1 resistant breeding line LD05-16611. In the second choice test with biotype 2, 62 rows of LD02-4485 × 14257 F_2 plants were randomized with 14 rows each of the susceptible parent LD02-4485 and susceptible checks Dwight and Ina, plus three rows each of PI 200538 and biotype 1 resistant LD05-16611.

Individual plants were visually rated for the level of aphid colonization 3 wk after aphid infestation. A 1 to 4 nonparametric, ordinal rating scale was used with 1 = few solitary live aphids, often with dead aphids; 2 = several transient aphids present along with some viviparous aptera surrounded by a few nymphs, but without established colonies; 3 = dense aphid colonies; and 4 = dense colonies accompanied by plant damage, including leaf distortion and stunting (Hill et al., 2006a, 2006b). Earlier tests indicated that PI 200538 resistance was qualitative in expression (Li et al., 2004). F_2 and F_3 plants were considered resistant when they had the phenotype ratings of 1 or 2, which were within the range observed for the resistant parent PI 200538 and susceptible when they had ratings 3 or 4, which were the ratings for the susceptible parents Ina, Williams 82, LD02-4485, and other susceptible soybean genotypes.

After the completion of the aphid resistance tests of F_2 plants in the Ina × PI 200538, Williams 82 × PI 200538, and LD02-4485 × 14257 populations, the plants were transplanted to produce $F_{2:3}$ seed (F_2–derived F_3 lines) following treatment with the systemic insecticide imidacloprid {1-[(6-chloro-3-pyridinyl)methyl]-*N*-nitro-2-imidazolidinimine} for progeny testing and genotyping using previously described methods (Hill et al., 2006a, 2006b). $F_{2:3}$ lines that had at least 12 seeds, regardless of F_2 soybean aphid resistance phenotype, were used in progeny tests of aphid resistance. At least 12 and a maximum of 20 F_3 seeds were planted for each $F_{2:3}$ family in four-pot rows that were randomized with four-pot rows of the parents and checks throughout the test. Each individual plant was an experimental unit as described above. Ina × PI 200538 and Williams 82 × PI 200538 F_3 plants were tested for resistance to soybean aphid biotype 1. Initially, all of the LD02-4485 × 14257 $F_{2:3}$ families from F_2 plants that produced at least 12 seeds were tested for resistance to biotype 2. Then, $F_{2:3}$ families that had at least 12 seeds remaining were subsequently tested for resistance to biotype 1. A minimum family size of 11 $F_{2:3}$ plants was evaluated for resistance to determine the genotype of an F_2 plant, to have 95% confidence that at least one homozygous recessive susceptible plant would be found in lines derived from a heterozygous plants, and to distinguish between lines derived from homozygous resistant or heterozygous F_2 plants (Fehr, 1987).

Statistical Analyses

Chi-square tests were performed to test the goodness-of-fit with different genetic ratios for the observed segregation among F_2 plants for resistance or susceptibility. F_2 populations from different F_1 parents were analyzed separately. Heterogeneity between the different F_2 populations was tested. Segregation among $F_{2:3}$ families with a minimum of 11 plants was analyzed after classifying each family as homozygous resistant (all plants had a rating of 1 to 2), homozygous susceptible (all plants had a rating 3 to 4), and segregating, if both resistant and susceptible plants were identified within the family.

Simple Sequence Repeat (SSR) Marker Genotyping

Genomic DNA was extracted from the tips of young, expanding trifoliolates on each plant using the CTAB method described by Honeycutt et al. (1992) with modifications. DNA concentration was quantified by ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE) and diluted to 25 ng μL^{-1} for SSR genotyping.

Bulk segregant analysis (Michelmore et al., 1991) was conducted on the Ina × PI 200538 F_2 population to identify SSR markers that were polymorphic between the parents of the cross and potentially associated with the soybean aphid resistance gene. Genomic DNA was extracted from 10 randomly selected susceptible F_2 plants and the parents. DNA from the susceptible F_2 plants was bulked into one sample for analysis. The bulked susceptible and parental DNA samples were screened with SSR markers from soybean LGs E and F (Song et al., 2004) that were linked to resistance to peanut root-knot nematode [*Meloidogyne arenaria* (Neal) Chitwood], found in PI 200538 (Tamulonis et al., 1997).

Mapping populations were made from 69 Ina × PI 200538 and 95 LD02-4485 × 14257 $F_{2:3}$ lines that were progeny tested. Genomic DNA extracted from each F_2 plant was screened with SSR markers potentially linked to the aphid resistance gene in the bulk segregant analysis. DNA samples from Williams 82 × PI 200538 F_2 plants were not used to map the soybean aphid resistance gene in PI 200538.

Polymerase chain reaction (PCR) and evaluation of PCR products was performed as previously described (Wang et al., 2003). Reactions were done in 15-μL volumes with 50 to 250 ng of template DNA, 2 μM of each primer, 30 mM $MgCl_2$, 3 mM of each dNTP, 2.5 U of *Taq* polymerase, and 1× PCR buffer. Reactions were run for 36 cycles of denaturation at 94°C for 25 s, annealing at 46°C for 25 s, and extension at 68°C for 25 s with a PTC100 Programmable Thermal Controller (MJ Research INC., Watertown, MA). The PCR products were evaluated by electrophoresis in 3% agarose and 3% metaphor-agarose gels.

Genetic Mapping

The segregation of aphid resistance and the SSR markers in the two mapping populations was analyzed with the program Joinmap 3.0 (Van Ooijen and Voorrips, 2001) using the Kosambi mapping function. A logarithm of the odds threshold of 3.0 was used to declare linkage between loci. A χ^2 goodness-of-fit test was performed to analyze the segregation of alleles at each locus using Joinmap 3.0. Aphid resistance in the F_2 populations was scored as incompletely dominant (homozygous resistant, heterozygous, or homozygous susceptible) after confirmation from the F_3 progeny tests.

RESULTS

Inheritance of Soybean Aphid Resistance in PI 200538

Expression of resistance in the F_2 and $F_{2:3}$ populations appeared to be qualitative because there were only two main classes of reaction, resistant or susceptible, distinguished by highly visible differences in aphid colonization and plant damage. Resistant plants had few live aphids and lacked established colonies, whereas susceptible plants had dense colonies often accompanied by plant damage such as distorted, crinkled leaves and plant stunting. All of the PI 200538 plants were classified as resistant (rating 1 or 2) and all of the plants of the susceptible parents Ina, Williams 82, and LD02-4485 were classified as susceptible (ratings 3 or 4). Observed phenotypes in the F_2 followed the parental phenotypes and fell into either the resistant or susceptible phenotype categories. The numbers of plants present in Tables 1 through 3 were the actual numbers of plants that emerged and were available for resistance evaluation in the tests.

Segregation of resistant to susceptible plants in each of the F_2 populations from different F_1 plants and from different crosses was approximately 3:1 resistant/susceptible (Table 1) and χ^2 analyses failed to reject the 3:1 ratio in all of the F_2 populations tested ($P = 0.05$), indicating that resistance in PI 200538 was controlled by a single, dominant gene. Heterogeneity for segregation of resistance to biotype 1 between Ina × PI 200538 F_2 populations from different F_1 plants ($P = 0.69$) and between Williams 82 × PI 200538 F_2 populations from different F_1 plants ($P = 0.85$) was nonsignificant. Analysis of pooled F_2 data supported the 3:1 ratio for the Ina × PI 200538 ($P_{3:1} = 0.89$) and the Williams 82 × PI 200538 ($P_{3:1} = 0.78$) F_2 populations. Analysis of segregation for resistance to biotype 2 in the LD02-4485 × 14257 F_2 population also failed to reject the 3:1 single, dominant gene model ($P_{3:1} = 0.52$). LD02-4485 × 14257 $F_{2:3}$ families that were also tested for resistance to biotype 1 had identical segregation patterns as initially found when tested with biotype 2, indicating that the gene in PI 200538 controlled resistance to both biotypes. Analyses of the segregation of $F_{2:3}$ families in all of the progeny tests failed to reject the 1:2:1 ratio of resistant to segregating to susceptible $F_{2:3}$ families (Table 2), supporting the 3:1 F_2 phenotype ratio. Based on the progeny test results, 7 out of 345 F_2 plants that were progeny tested were mis-scored for soybean aphid resistance in the F_2 generation, giving a 2% F_2 phenotyping error rate in this study.

Analyses of segregation for resistance to soybean aphid biotype 1 in the Dowling × PI 200538 ($P = 0.81$) and Jackson × PI 200538 ($P = 0.58$) F_2 populations failed to reject a 15:1 ratio of resistant to susceptible plants (Table 3). This result indicated that the resistance gene in PI 200538 was nonallelic and segregated independently from *Rag1* in Dowling and the resistance gene in Jackson.

Table 1. Genetic analysis of the segregation of soybean aphid resistance in two Ina × PI 200538 and three Williams 82 × PI 200538 F_2 populations, tested with soybean aphid biotype 1, and a LD02-4485 × 14257 (Ina × PI 200538 F_2) F_2 population, tested with soybean aphid biotype 2.

Cross†	F_2 population	Observed F_2 segregation		$\chi^2_{3:1}$	$P_{3:1}$
		Resistant	Susceptible		
Ina × PI 200538	4401	39	14	0.06	0.81
	4741	75	23	0.12	0.73
	Totals			0.18	0.91
	Pooled	114	37	0.02	0.89
	Heterogeneity			0.16	0.69
Williams 82 × PI 200538	4791	88	30	0.01	0.92
	4792	67	19	0.39	0.53
	4793	48	16	0.00	1.00
	Totals			0.40	0.94
	Pooled	203	65	0.08	0.78
	Heterogeneity			0.32	0.85
LD02-4485 × 14257	4991	167	62	0.42	0.52

†The Ina × PI 200538 and Williams × PI 200538 populations were tested with the soybean aphid biotype 1, which was collected in Illinois, and the LD002-4485 × 14257 population was tested with the soybean aphid biotype 2, which was collected in Ohio. Kim et al. (2008) previously reported these biotypes.

Location of the Resistance Gene in the Soybean Genetic Map

Analysis of the bulk of susceptible F_2 plants from the Ina × PI 200538 population indicated that the soybean SSR markers Sat_234, Soyhsp176, and Satt510 on soybean LG F (Song et al., 2004) were associated with resistance. Ina and the bulked susceptible plant sample had identical SSR alleles for those markers, whereas PI 200538 had different alleles. No LG E markers were identified as associated with aphid resistance on the basis of the bulked segregant analysis.

The Ina × PI 200538 and the LD02-4485 × 14257 populations were subsequently tested with the markers identified in the bulked segregant analysis and other markers on LG F. The segregation of markers failed to reject 1:2:1 or 3:1 segregation ratios with the exception of Sat_375 SSR (Table 4).

The SSR and soybean aphid resistance data from two mapping populations were used to map the location of resistance gene in PI 200538 in the soybean genetic map. Simple sequence repeat markers Soyhsp176 and Satt510 were closely linked and flanked the resistance gene in the Ina × PI 200538 F_2 mapping population (Fig. 1). Satt114, Satt510, and Sct_033 flanked the resistance gene in the LD02-4485 × 14257 F_2 mapping population. Satt510 was 4 cM from the resistance gene in each map made from the two mapping populations and in a composite map made from the combined genotypic data of both mapping populations. The order of SSR markers in the maps was in agreement with the soybean composite map (Song et al., 2004), except for the reversed positions of Sat_234 and Satt114 in the LD02-4485 × 14257 map.

Table 2. Genetic analysis of the segregation of $F_{2:3}$ families for soybean aphid resistance, derived from plants in two Ina × PI 200538 F_2 populations and three Williams 82 × PI 200538 F_2 populations, tested with soybean aphid biotype 1, and from an LD02-4485 × 14257 (Ina × PI 200538 F_2) F_2 population, tested with biotype 2.

Cross†	F_2 plant phenotype	F_2 plant genotype	No. of $F_{2:3}$ families	$\chi^2_{1:2:1}$	$P_{1:2:1}$
Ina × PI 200538	Resistant	RR (all $F_{2:3}$ plants resistant)	22		
		Rr (resistant and susceptible $F_{2:3}$ plants)	29		
		rr (all $F_{2:3}$ plants susceptible)	0		
	Susceptible	RR (all $F_{2:3}$ plants resistant)	0		
		Rr (resistant and susceptible $F_{2:3}$ plants)	2		
		rr (all $F_{2:3}$ plants susceptible)	16		
				1.75	0.42
Williams 82 × PI 200538	Resistant	RR (all $F_{2:3}$ plants resistant)	41		
		Rr (resistant and susceptible $F_{2:3}$ plants)	101		
		rr (all $F_{2:3}$ plants susceptible)	1		
	Susceptible	RR (all $F_{2:3}$ plants resistant)	1		
		Rr (resistant and susceptible $F_{2:3}$ plants)	3		
		rr (all $F_{2:3}$ plants susceptible)	34		
				4.57	0.10
LD02-4485 × 14257 (Ina × PI 200538 F_2)	Resistant	RR (all $F_{2:3}$ plants resistant)	20		
		Rr (resistant and susceptible $F_{2:3}$ plants)	57		
		rr (all $F_{2:3}$ plants susceptible)	0		
	Susceptible	RR (all $F_{2:3}$ plants resistant)	0		
		Rr (resistant and susceptible $F_{2:3}$ plants)	0		
		rr (all $F_{2:3}$ plants susceptible)	18		
				3.88	0.14

†The Ina × PI 200538 and Williams × PI 200538 populations were tested with the soybean aphid biotype 1, which was collected in Illinois, and the LD002-4485 × 14257 population was tested with the soybean aphid biotype 2, which was collected in Ohio. Kim et al. (2008) previously reported these biotypes.

DISCUSSION

A single, dominant soybean aphid resistance gene controlled resistance to soybean aphid biotypes 1 and 2 in the soybean germplasm accession PI 200538 was mapped. Because this gene was mapped in the same region on LG F and gave identical resistance reactions to different soybean aphid biotypes as *Rag2* (Mian et al., 2008b), the gene in PI 200538 may be *Rag2* and not a new aphid resistance gene. Results of genetic allelism tests with Dowling and Jackson confirmed that the resistance gene in PI 200538 was not *Rag1* either.

PI 200538 was selected to be screened for soybean aphid resistance in an earlier study (Hill et al., 2004a) because it had resistance to the peanut root-knot nematode, *M. arenaria* race 2 (Luzzi et al., 1995). Sources of root-knot nematode resistance were selected to be screened for aphid resistance because of the analogy of the *Mi* gene in tomato (*Solanum lycopersicum* var. *lycopersicum* L.), which controls resistance to the potato aphid [*Macrosiphum euphorbiae* (Thomas)], the root-knot nematode [*Meloidogyne incognita* (Kofoid and White) Chitwood] (Rossi et al., 1998), and other insect pests (Casteel et al., 2006; Nombela et al., 2003). A locus in PI 200538 responsible for most of the nematode resistance is linked to the restriction fragment length polymorphism locus B212_1, which also maps within the interval between the SSR loci Satt510 and Soyhsp176 (Tamulonis et al., 1997). The genetic

Table 3. Genetic analysis of the segregation of F_2 progeny from crosses between Dowling and PI 200538 and Jackson × PI 200538 for resistance to soybean aphid biotype 1.

Cross	Observed F_2 segregation		$\chi^2_{15:1}$	$P_{15:1}$
	Resistant	Susceptible		
Dowling × PI 200538	39	3	0.06	0.81
Jackson × PI 200538	71	6	0.31	0.58

Table 4. Chi-square analysis of the segregation of the aphid resistance gene in PI 200538 and the linked soybean linkage group F simple sequence repeat (SSR) markers in 69 F_2 plants from the cross Ina × PI 200538 tested for resistance to soybean aphid biotype 1 and in 95 F_2 plants from the cross of LD02-4485 × 14257 tested for resistance to soybean aphid biotype 2.

Cross	Locus	No. of F_2 plants for each genotype†				Genotype unresolved	$\chi^2_{1:2:1}$	*P*	$\chi^2_{3:1}$	*P*
		a	h	b	d					
Ina × PI 200538	Aphid resistance	22	31	16	0	0	1.75	0.42	0.24	0.89
	Sat_120	29	21	16	0	3	13.38	< 0.01	0.06	0.80
	Sat_234	22	28	12	0	7	4.13	0.04	1.34	0.25
	Sat_297	14	32	13	0	10	1.84	0.17	1.35	0.25
	Sat_375	24	20	9	0	16	12.68	< 0.01	4.50	0.03
	Satt510	23	31	14	0	1	2.88	0.09	0.58	0.44
	Soyhsp176	20	30	14	0	5	1.64	0.20	0.47	0.49
LD02-4485 × 14257	Aphid resistance	20	57	18	0	0	3.88	0.14	1.96	0.38
	Sat_120	23	55	17	0	0	3.13	0.08	2.38	0.12
	Sat_234	0	0	24	70	1	–	–	0.03	0.85
	Sat_297	16	55	19	0	5	4.66	0.03	0.76	0.38
	Sct_033	21	57	17	0	0	4.14	0.04	2.38	0.12
	Satt114	26	49	20	0	0	0.85	0.36	0.70	0.40
	Satt510	21	57	17	0	0	4.14	0.04	2.38	0.12

†Codominant SSR markers were scored as 'a' = the SSR allele of the resistant parent, 'h' = the SSR alleles from both resistant and susceptible parents, and 'b' = the SSR allele of the susceptible parent. Dominant SSR markers were scored as 'd' = 'a + h' or 'c' = 'b + h'. Genotypes of the aphid resistance genes in the F_2 populations were scored as codominant (homozygous resistant, heterozygous, or homozygous susceptible) after confirmation from the F_3 progeny tests.

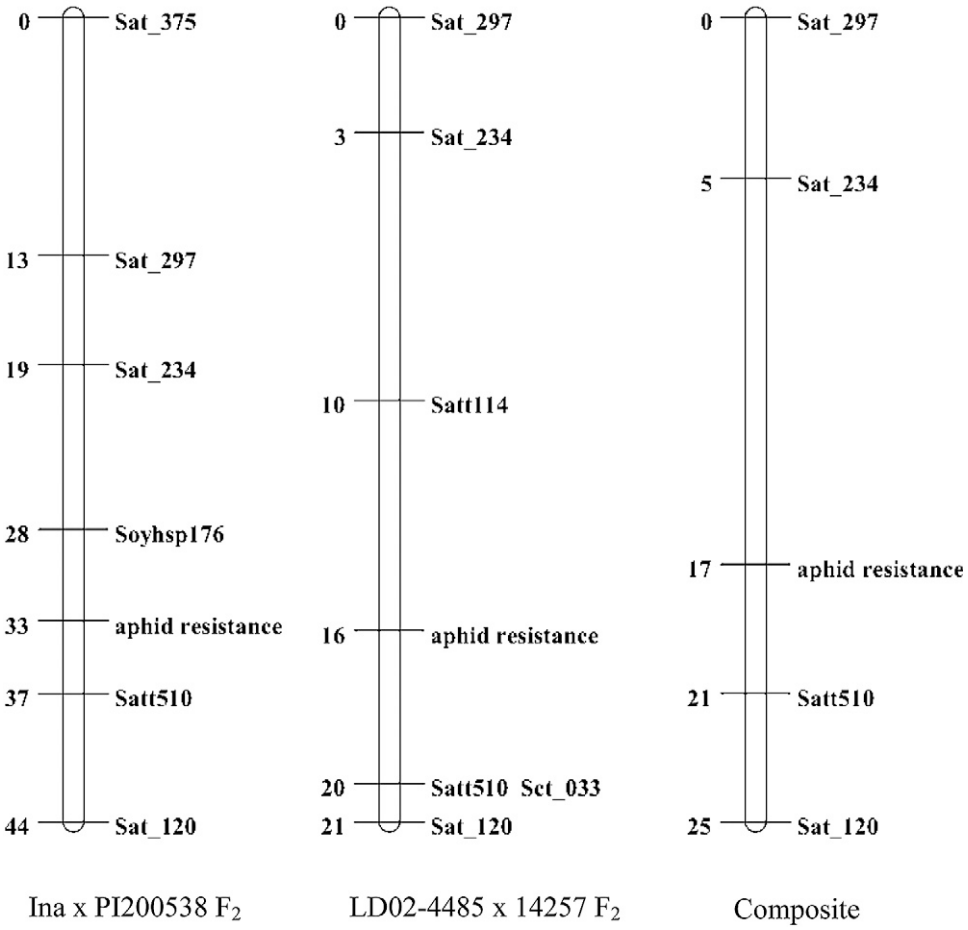

Figure 1. Maps showing the location of the aphid resistance gene in PI 200538 on soybean linkage group F constructed with genotype data from Ina × PI 200538 and LD02-4485 × 14257 F_2 mapping populations, and a composite map constructed from the combined data of both mapping populations.

relationship between peanut root-knot nematode and soybean aphid resistance is unknown. It is possible that the aphid resistance gene in PI 200538, which may be *Rag2*, controls resistance to both the peanut root-knot nematode and the soybean aphid, analogous to the *Mi* gene in tomato (Rossi et al., 1998); however, further testing is required to study this possibility. For instance, soybean lines derived from plants with recombination events within the interval between DNA marker loci flanking the resistance gene in PI 200538, possibly *Rag2*, and the QTL controlling resistance to peanut root-knot nematode race 2 could be tested for resistance to both organisms to obtain additional evidence for whether the same gene confers resistance to both pests. Work underway to produce a high-resolution genetic map with single nucleotide polymorphism markers surrounding the resistance gene in PI 200538 could also be useful to help determine the genetic relationship between the nematode and aphid resistance in PI 200538.

A gene giving resistance to several strains of SMV also maps in the

same interval as the resistance gene in PI 200538 (Yu et al., 1994). Resistance to SMV in PI 200538 has not been reported. Other resistance genes that map in the same region of soybean LG F include the Phytophthora root and stem rot (caused by *Phytophthora sojae* Kaufmann and Gerdemann) resistance gene *Rps3* (Diers et al., 1992), the *Pseudomonas syringae* pv. *glycinea* (Coerper) Young, Dye, and Wilkie resistance gene *Rpg1* (Ashfield et al., 1998), and the *Peanut mottle virus* resistance gene *Rpv1* (Gore et al., 2002).

Results in this study indicated that PI 200538 may be an additional source of *Rag2* as PI 243540 (Mian et al., 2008a). This gene will be useful to soybean breeders especially in the development of soybean aphid resistant soybean cultivars where the aphid has adapted to *Rag1* or other resistant genes. It can also be stacked with other resistance genes to potentially provide a broader spectrum of resistance to multiple soybean aphid biotypes and make adaptation to resistance genes more complicated for aphid populations.

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