Theor Appl Genet (2014) 127:43–50
DOI 10.1007/s00122-013-2199-1

ORIGINAL PAPER

Inheritance of soybean aphid resistance in 21 soybean plant introductions

Carolyn M. Fox · Ki-Seung Kim · Perry B. Cregan · Curtis B. Hill · Glen L. Hartman · Brian W. Diers

Received: 17 May 2013 / Accepted: 17 September 2013 / Published online: 27 September 2013

Abstract
Key Message **The *Rag2* region was frequently identified among 21 F_2 populations evaluated for soybean aphid resistance, and dominant gene action and single-gene resistance were also commonly identified.**
Abstract The soybean aphid [*Aphis glycines* Matsumura (Hemiptera: Aphididae)] is one of the most important insect pests of soybean [*Glycine max* (L.) Merr] in the northern USA and southern Canada, and four resistance loci (*Rag1*–*rag4*) have been discovered since the pest was identified in the USA in 2000. The objective of this research was to determine whether resistance expression in recently identified soybean aphid-resistant plant introductions (PIs) was associated with the four *Rag* loci using a collection of 21 F_2 populations. The F_2 populations were phenotyped with soybean aphid biotype 1, which is avirulent on plants having any of the currently identified *Rag* genes, using choice tests in the greenhouse and were tested with genetic markers linked to the four *Rag* loci. The phenotyping results indicate that soybean aphid resistance is controlled by a single dominant gene in 14 PIs, by two genes in three PIs, and four PIs had no clear Mendelian inheritance patterns. Genetic markers flanking *Rag2* were significantly associated with aphid resistance in 20 PIs, the *Rag1* region was significantly identified in five PIs, and the *Rag3* region was identified in one PI. These results show that single dominant gene action at the *Rag2* region may be a major source for aphid resistance in the USDA soybean germplasm collection.

Abbreviations

BSA	Bulked segregant analysis
MG	Maturity group
PCR	Polymerase chain reaction
PI	Plant introduction
SNP	Single nucleotide polymorphism
SSR	Simple sequence repeats

Introduction

The soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is a common insect pest of soybean [*Glycine max* (L.) Merr] in China and other Asian countries (Wu et al. 2004). The soybean aphid was first discovered in North America in 2000 (Hartman et al. 2001) and is now considered to be one of the most important insect pests on soybean in the northern USA and Canada (Ragsdale et al. 2011). Aphid feeding is associated with plant stunting, leaf yellowing and wrinkling, reduced photosynthesis, poor pod fill, and reduced yield, seed size, and seed quality (Beckendorf et al. 2008; Kang et al. 2008; Macedo et al. 2003; Mensah et al. 2005). Under heavy aphid

Communicated by A. H. Schulman.

C. M. Fox (✉) · K.-S. Kim · C. B. Hill · G. L. Hartman · B. W. Diers
Department of Crop Sciences, University of Illinois, 1102 S. Goodwin Ave., Urbana, IL 61801, USA
e-mail: cbonin2@illinois.edu

Present Address:
K.-S. Kim
The Samuel Roberts Noble Foundation, 2510 Sam Noble Parkway, Ardmore, OK 73401, USA

P. B. Cregan
Soybean Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD 20705, USA

G. L. Hartman
USDA-ARS, Urbana, IL 61801, USA

pressure, yield losses can exceed 50 % (Ostlie 2002; Wu et al. 2004).

Initially, chemical insecticides were used to control the soybean aphid, but resistance genes have been discovered that can provide an alternative control method. Between 2001 and 2004, Hill et al. (2004) evaluated approximately 1,700 soybean cultivars for aphid resistance, including commercial cultivars, Asian cultivars, and ancestral lines. Three North American ancestral cultivars were found to exhibit resistance: Dowling, Jackson, and plant introduction (PI) 71506. Resistance in Dowling and Jackson was found to be controlled by a single dominant gene (Hill et al. 2006a, b) mapping to a region on chromosome 7 [linkage group (LG) M] (Li et al. 2007). The gene in Dowling was named *Rag1* and the gene in Jackson was named *Rag* because the genetic relationship between the two genes was unknown. Field studies indicated that *Rag1* had no negative effects on seed yield and other important agronomic traits when it was introgressed into breeding lines adapted to the Midwestern USA (Kim and Diers 2009; Mardorf et al. 2010).

Mian et al. (2008a) identified three PIs that exhibited aphid resistance, PI 243540, PI 567301B, and PI 567324. The resistance in PI 243540 was found to be controlled by a single dominant gene named *Rag2* on chromosome 13 (LG F) that provided antibiosis-type resistance (Kang et al. 2008; Mian et al. 2008b). PI 200538 also was found to have a major soybean aphid resistance gene mapping to the same region on chromosome 13 as *Rag2* from PI 243540 (Hill et al. 2009; Kim et al. 2010b). Cultivars with *Rag2* and the combination of *Rag1* and *Rag2* were shown by Brace and Fehr (2012) to have comparable agronomic performance to susceptible cultivars. In contrast, Kim and Diers (2013) showed a negative yield association with *Rag2* in two genetic backgrounds. Two more resistance loci have since been identified including *Rag3* in PI 567543C, which was mapped to chromosome 16 (LG J) (Zhang et al. 2010) and *rag4* in PI 567541B on chromosome 13 over 65 cM away from *Rag2* (Zhang et al. 2009). Additional resistance genes continue to be identified, including genes mapping near *Rag2* (Jun et al. 2012) and *Rag3* (Zhang et al. 2013), and other soybean aphid-resistant sources have been found that could possess new genes or alleles for resistance (Diaz-Montano et al. 2006; Hesler and Dashiell 2008; Mian et al. 2008a).

Soybean aphid biotypes distinguished by differential virulence profiles on known resistance sources also have been discovered. A recent review by Hill et al. (2012) summarizes current knowledge on soybean aphid resistance in soybean and virulence in the soybean aphid. Soybean aphid biotype 1 cannot colonize plants with *Rag1* or *Rag2*. Biotype 2 is able to colonize plants with *Rag1* but not *Rag2* (Kim et al. 2008) and biotype 3 colonizes plants with *Rag2* (Hill et al. 2010). With the discovery of soybean aphid biotypes that indicate virulence diversity in North America, it has become especially critical to identify new resistance genes for the development of cultivars with resistance to emerging soybean aphid biotypes. The objective of this research was to examine the inheritance of soybean aphid resistance in 21 soybean aphid-resistant PIs and determine whether resistance in these PIs was associated with the *Rag1* to *rag4* loci.

Materials and methods

Plant material and greenhouse resistance tests

An evaluation of over 3,000 PI accessions identified 50 PIs with resistance to soybean aphid biotypes 1 and 2 in both choice and no-choice tests (unpublished data). The resistant PIs primarily originate from China and Japan, and maturity groups (MGs) range from II to X (Table 1). In 2007–2008, F_2 populations were developed by using the aphid-resistant PIs as male parents and soybean aphid-susceptible lines as female parents in crosses. The susceptible parents were high-yielding experimental lines with good agronomic traits developed from the University of Illinois soybean breeding program. The subsequent F_1 plants were selfed in the greenhouse in 2008 to produce F_2 populations for study.

From the resulting populations, 21 F_2 populations were evaluated for soybean aphid resistance between 2008 and 2011. These populations were selected to have a broad diversity of PI maturity groups and origins. Maturity group specifies the latitude (day length) and climate that the soybean genotype is adapted to, with lower MGs adapted to longer days and higher MGs adapted to shorter days. The wide range of MGs indicates that soybean PIs from a broad range of geographic areas were included in this study. Soybean aphid biotype 1 was used to infest soybean plants in the tests because it is avirulent to all known soybean aphid resistance genes. The parents, 100–300 F_2 plants from each population, and aphid-resistant and susceptible checks were tested in the greenhouse using methods previously described by Hill et al. (2006a) and briefly described below. Resistant checks were Dowling (*Rag1*) and PI 200538 (*Rag2*), and the susceptible checks were Ina, Loda, Pana, and Williams 82. The experiment was performed between late fall and spring, with supplemental greenhouse lighting set at 13 h and the temperature was controlled to 24 °C during the daytime and 18 °C at night. Each population was planted in a completely randomized design in plastic multi-pot trays containing 48 pots organized as 12 four-pot rows (Hummert Intl., Earth City, MO, USA). The susceptible and resistant check cultivars were planted in rows in random locations throughout each test tray. Susceptible check cultivars comprised about 25–30 % of the rows to create

Table 1 Soybean aphid greenhouse test results and significant *Rag* region associations for each plant introduction resistance source using soybean aphid biotype 1

Resistance source	MG[a]	Origin	Susceptible parent	F_2 plants tested	Observed R:S[b]	Expected R:S[c]	χ^2 test *p* value	Significant *Rag* region	Significant marker[d]	Marker *p* value[e]	R^2 [f]	GG region[g]
PI 71506	IV	China	LD03-6566	266	130:136	9:7	0.02	*Rag1*	Satt540	0.0003	0.06	*Rag1*
								Rag3	Satt414	0.02	0.03	
								Interaction		0.0002	0.02	
PI 88508	II	China	LD03-6566	149	94:55	3:1	0.001	*Rag2*	SNP #20	<0.0001	0.55	–
PI 437696	V	China	LD03-6566	276	265:11	15:1	0.12	*Rag1*	Satt540	0.02	0.03	*Rag2*
								Rag2	Satt114	<0.0001	0.24	
								Interaction		<0.0001	0.06	
PI 499955	VII	China	LD02-4485	160	122:38	3:1	0.72	*Rag2*	SNP #20	<0.0001	0.82	–
PI 507298	VI	Japan	LD03-6566	126	97:29	3:1	0.61	*Rag2*	SNP #20	<0.0001	0.41	*Rag2*
PI 548237	VII	USA	LD02-4485	160	126:34	3:1	0.27	*Rag2*	SNP #20	<0.0001	0.72	–
PI 567391	VII	China	LD02-4485	143	114:29	3:1	0.19	*Rag2*	Satt114	<0.0001	0.66	*Rag2*
PI 587656	VII	China	LD03-6566	97	57:40	3:1	0.00	*Rag2*	SNP #20	<0.0001	0.63	–
PI 587669	VI	China	LD02-5320	130	80:50	3:1	0.00	*Rag2*	SNP #20	0.003	0.11	–
PI 587775	VII	China	LD03-6566	151	109:42	3:1	0.42	*Rag2*	SNP #20	<0.0001	0.66	–
PI 587870	VII	China	LD03-6566	159	124:35	13:3	0.29	*Rag1*	SNP 65906.2	<0.003	0.13	–
								Rag2	SNP #20	<0.0001	0.51	
								Interaction		<0.0001	0.08	
PI 587871	VII	China	LD02-5320	127	93:34	3:1	0.64	*Rag2*	SNP #20	<0.0001	0.52	–
PI 587899	VII	China	LD03-10504	140	111:29	3:1	0.24	*Rag2*	SNP #20	<0.0001	0.44	*Rag2*
PI 588000	X	China	LD02-4485	150	125:25	13:3	0.52	*Rag1*	Satt540	0.04	0.07	
								Rag2	SNP #20	<0.0001	0.39	–
								Interaction		<0.0001	0.02	
PI 588040	VII	China	LD02-5320	130	90:40	3:1	0.13	*Rag2*	SNP #20	<0.0001	0.28	–
PI 594431	V	China	LD02-5320	124	93:31	3:1	1.00	*Rag2*	SNP #20	<0.0001	0.55	–
PI 594499	VIII	China	LD03-6566	127	89:38	3:1	0.20	*Rag2*	SNP #20	<0.0001	0.67	–
PI 594573	VI	China	LD03-6566	136	93:43	3:1	0.07	*Rag1*	Satt463	0.01	0.07	–
								Rag2	SNP #20	<0.0001	0.54	
								Interaction		–		
PI 594707	VII	China	LD02-5320	155	113:42	3:1	0.55	*Rag2*	SNP #20	<0.0001	0.84	–
PI 594822	IX	China	LD02-5320	145	108:37	3:1	0.89	*Rag2*	SNP #20	<0.0001	0.54	–
PI 594879	VII	China	LD03-6566	107	75:32	3:1	0.24	*Rag2*	Satt114	<0.0001	0.33	*Rag2*

[a] Maturity group of the resistant parent

[b] Observed number of plants exhibiting resistant and susceptible responses (R:S)

[c] The expected Mendelian ratio predicted from the observed R:S responses

[d] The SSR (simple sequence repeats) or SNP (single nucleotide polymorphism) marker that was significantly associated with aphid resistance in each *Rag* region, identified by the non-parametric Kruskal–Wallis test

[e] Significance level of resistance-associated marker calculated by the non-parametric Kruskal–Wallis test

[f] Total phenotypic variation explained by the identified marker estimated using a parametric ANOVA analysis

[g] The significant *Rag* region identified by the GoldenGate (GG) assay using the bulked segregant analysis

aphid refuges and maintain high aphid pressure after the plants were infested.

Soybean seedlings were infested with aphids when the seedlings had completely expanded unifoliolates and expanding first trifoliolates, at the VC to V1 growth stage (Fehr and Caviness 1977) by distributing aphid-infested leaves and stems of Williams 82 evenly on top of the seedlings. The plants were rated when there was maximum aphid colonization on the susceptible check cultivars, usually between the V4 and V6 growth stage, when the plants had 3–5 fully expanded trifoliolates, about 3 weeks after inoculation. For 13 populations, aphid colonization on each plant was visually assessed on a 1–4 scale to estimate the degree of aphid colonization and plant damage

caused by aphid feeding (Hill et al. 2006a, b, 2010). A score of 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 such as leaf distortion and stunting. A score of 1 or 2 was considered a resistant response and a score of 3 or 4 was considered a susceptible response, and phenotypic ratios of resistant:susceptible were used to test different Mendelian models to estimate the number of major genes controlling resistance in each PI resistance source.

Eight F_2 populations, developed using PI 88508, PI 499955, PI 548237, PI 587669, PI 587656, PI 587775, PI 587870, and PI 594707, were scored on a 1–5 scale for the resistance evaluations, where 1 = no aphids present; 2 = few solitary live aphids, often with dead aphids; 3 = several transient aphids present, but without established colonies; 4 = dense aphid colonies; and 5 = dense colonies accompanied by plant damage (Kim et al. 2008). The five-point rating scale was used to differentiate plants that had zero aphids on them from plants that had aphids present at very low levels. The combination of ratings 1 and 2 under the five-point scale is equivalent to a rating of 1 under the four-point scale. The ratings 3, 4, and 5 under the five-point scale correspond with the ratings 2, 3, and 4 under the four-point scale. Using the five-point scale, scores of 1, 2, and 3 were considered resistant responses and scores of 4 and 5 were considered susceptible responses.

Genotyping and marker associations

Leaf tissue was collected from young trifoliolates of all F_2 plants and the parents in each population and genomic DNA was extracted using a CTAB (cetyltrimethylammonium bromide) extraction procedure described by Keim and Shoemaker (1988). The parents of the populations were first screened to identify polymorphic genetic markers in the *Rag1* to *4* regions using single nucleotide polymorphism (SNP) and simple sequence repeat (SSR) markers, and information for the SSR markers can be found at the SoyBase website (http://soybase.org). In the *Rag1* region, the SSR marker Satt540 was frequently used to genotype the populations; SNP 65906.2 (Kim et al. 2010a) and SSR markers Satt435 and Satt463 were also used for some of the populations when Satt540 was not polymorphic. The SNP marker #20 (Kim et al. 2010b) was used to genotype the *Rag2* region for 16 of the 21 populations; the 5 remaining populations were genotyped with either SSR marker Satt114 or Satt510. The *Rag3* and r*ag4* regions did not have consistently polymorphic markers across the majority of the populations, and a few markers were required to genotype the two regions. SSR markers Satt654 and Satt406 were most commonly used for genotyping the *Rag3* region, with Satt285, Satt693, and Satt596 also used for some populations. In the r*ag4* region, SSR markers Satt586, Satt649, and Satt569 were commonly used to genotype the populations. One or two polymorphic markers in each *Rag* region were selected to genotype each F_2 population. Polymerase chain reactions (PCR) for the SSR markers were performed (Cregan and Quigley 1997) and the resulting PCR products were analyzed using non-denatured polyacrylamide gel electrophoresis (Wang et al. 2003). SNP marker analyses were performed using TaqMan assays conducted with a Roche LightCycler 480 System (Roche Diagnostics, Indianapolis, IN) as described by Kaczorowski et al. (2008).

To identify potential genetic regions associated with soybean aphid resistance, six F_2 populations developed using PI 71506, PI 437696, PI 507298, PI 567391, PI 587899, and PI 594879 were further tested by bulked segregant analysis (BSA) (Michelmore et al. 1991) using the Illumina GoldenGate 1,536 Universal Soy Linkage Panel 1.0 (USLP 1.0) as previously described (Hyten et al. 2008, 2010). For each population, DNA from ten F_2 plants with resistant phenotypes and ten plants with susceptible phenotypes were selected and bulked separately. Each bulk was further sub-divided into two samples containing DNA of five plants each. The SNP genotypes of the resistant and susceptible bulks were compared with the SNP genotypes of the population parents to determine probable genetic regions associated with soybean aphid resistance across the entire genome.

Statistical analyses

Reactions of each plant to soybean aphid feeding were classified as resistant or susceptible using the aphid colonization ratings as previously described. The ratio between resistant and susceptible plants segregating in each F_2 population was analyzed using the Chi-square procedure to predict Mendelian inheritance patterns and the number of genes involved in aphid resistance. For each population, a small number of basic Mendelian ratios were considered, and the most probable ratio (i.e., the theoretical ratio closest to the observed ratio) was selected for the Chi-square test.

Significant associations between genetic markers and resistance were identified using the NPAR1WAY procedure of SAS v9.2 (SAS Institute 2008) to perform the non-parametric Kruskal–Wallis test. The Kruskal–Wallis test was chosen because it tests the distribution of an ordinally scaled response variable (aphid rating) using a Chi-square distribution (Stokes et al. 2000). Markers identified as significantly associated with resistance ratings were further tested with the GLM procedure of SAS to identify

interactions between significant markers and to estimate the proportion of phenotypic variation for soybean aphid colonization ratings explained by the genotypes of the significant marker (R^2), providing information about the proportion of the overall resistance the identified marker is explaining. Fisher's least significant difference (LSD) was used to rank the genotypes for aphid resistance.

Results

In many crops, aphid resistance originating from a single resistance source is controlled by one or two genes (Mian et al. 2008a; Smith and Boyko 2007). With the assumption that this trend continues to hold true for the soybean aphid, Mendelian segregation ratios for one or two genes (such as 3:1 or 15:1) were tested in each F_2 population. The segregation of resistant and susceptible phenotypes in the 21 F_2 populations fit models suggesting that soybean aphid resistance was controlled by a single dominant gene in 14 PIs and two genes in three PIs, while four F_2 populations had no clear Mendelian segregation ratio between resistant and susceptible plants in the tests (Table 1).

Using genetic marker analyses, the *Rag2* region was found to be significantly associated with resistance in 20 of the 21 populations evaluated (Table 1). The *Rag1* region was significantly associated with resistance in five F_2 populations and the *Rag3* region was associated with aphid resistance in one F_2 population. There was no association between markers flanking *rag4* and resistance in any of the populations evaluated. The phenotypic variance explained by the most significant marker associated with resistance (R^2) ranged from 0.03 to 0.84 in the F_2 populations, with an average value of 0.43. There did not appear to be any relationship between the MG of the PI parent of the populations and the identified resistance regions. PIs with the *Rag1* region significantly identified originated from MG IV, V, VII VII, and X. Five F_2 populations, developed using the resistance sources PI 71506, PI 437696, PI 587870, PI 588000, and PI 594573, were found to have significant associations with markers for two different *Rag* regions. No significant interaction between the two identified *Rag* regions was identified in the PI 594573 population, while significant interactions were found in the remaining four populations. Across the four populations with interactions between the two significant *Rag* regions, the average percentage of phenotypic variance explained by the interaction (R^2) was 5 % (Table 1).

Among the five populations that were identified as having two significant regions associated with resistance, four of the populations, developed from PI 437696, PI 587870, PI 588000, and PI 594573, were identified as having a significant association at the *Rag1* and *Rag2* regions. For all of these populations, plants homozygous for the susceptible parent allele at both *Rag* regions were significantly more susceptible to the soybean aphid than plants with two resistant parent alleles at both regions. When a test of Fisher's LSD was used to group the genotypes, genotypes with resistant PI alleles at the *Rag2* region scored as slightly more resistant than genotypes with PI alleles at the *Rag1* region. Plants having one or both of the resistant parent alleles in the *Rag2* region, regardless of the alleles at the *Rag1* region, did not significantly differ from the most resistant class in both populations. In the PI 71506 population, the *Rag1* and *Rag3* regions were both significantly associated with resistance although the R^2 value associated with each marker was low. Plants homozygous for PI alleles at the *Rag1* and *Rag3* regions were significantly more resistant to soybean aphid than plants with two susceptible parent alleles in these regions. In this population, the separation between genotypic classes was less distinct compared with the previous four populations. In general, plant resistance increased with the total number of resistant PI alleles at the *Rag1* and *Rag3* regions in the genotype.

For all of the populations, the phenotypic aphid response average for each genotypic class was examined using the identified significant markers. In 10 of the 21 populations there was no significant difference between the phenotypic response of the plants homozygous for the PI allele and the heterozygous plants at the major resistance locus, indicating the inheritance of the resistance gene was completely dominant. In the 11 remaining populations, the plants homozygous for the PI allele at the *Rag* region had a significantly greater resistant response than heterozygous plants. However, the plants with heterozygous alleles at the locus scored numerically closer to the resistance score of plants homozygous for the PI allele than plants homozygous for the susceptible parent allele. In no case did the single-marker analysis find recessive resistance among the 21 populations, which is consistent with the greenhouse Mendelian segregation ratios that were observed.

With the GoldenGate BSA assay, SNP markers across the entire genome were tested for association with aphid resistance. The GoldenGate BSA tests resulted in the identification of a single major genetic region in each of the six F_2 populations that were evaluated using this method (Table 1) and these regions were consistent with those identified with the single-marker analysis. The SNP markers flanking the *Rag2* region were identified as potentially associated with resistance in five populations and the markers near *Rag1* were identified in one population with the GoldenGate BSA tests. These tests revealed no other regions of the soybean genome that were associated with aphid resistance in any of the six populations. In two populations, developed using PI 437696 and PI 71506 as the resistant source parents, single-marker analysis identified a

second significant genetic region in addition to the region identified by the BSA using the GoldenGate assay. In these cases, the second genetic region detected by single-marker analysis had a lower R^2 value than the region identified by both the single-marker analysis and the GoldenGate assay.

Discussion

Among the PI accessions evaluated, the *Rag2* region was commonly identified as associated with resistance. The results also showed that soybean aphid resistance was controlled by one or two genes, which is consistent with previous studies (Hill et al. 2006a, b, 2009; Kang et al. 2008; Mensah et al. 2008). The greenhouse phenotypic and genetic marker results both indicated that resistance in most of the PIs is dominant. For the populations where resistance was not completely dominant, the aphid resistance rating of the heterozygous class was numerically closer to the homozygous resistant genotype class rating, suggesting that possession of just one resistant PI allele still can contribute a large component of resistance, and this resistance may be augmented in some genotypes when two resistant alleles are present in a homozygous state.

For 17 of the populations, the phenotypic variation explained by the major significant markers was moderate to high ($R^2 = 0.30$–0.72). This is an acceptable range assuming a single major resistance gene and considering that the phenotyping was done on individual F_2 plants in populations which would have lower heritabilities compared to replicated testing of more advanced generations of experimental material. For four populations, the phenotypic variation explained by the significant marker was low ($R^2 < 0.30$). These low R^2 values could be attributed to phenotyping error, which may be strongly affected by the evaluation environment. Accurate phenotyping for soybean aphid resistance is influenced by the aphid pressure on the soybean population and pressure that is too high or low may result in inaccurate phenotypic reactions that could hinder the identification of resistance gene(s). Aphid pressure can vary based on the percentage of susceptible plants in a population, the number of aphids initially applied, timing of aphid infestation, and the greenhouse environment.

Low R^2 values also may have occurred if the identified resistance region was not the major gene controlling resistance, leaving the primary resistance gene unidentified after genotyping. In this case, there is the possibility that unknown major genes may still be involved in resistance in some of the populations that were examined, particularly the PI 71506 resistance source which had very low R^2 values associated with markers in two genetic regions identified in the population developed from this PI. A potential reason for the low R^2 value is that aphid pressure was moderately heavy during the greenhouse evaluation for the PI 71506 population, which may have confounded the clear identification of aphid resistance genes. Heavier aphid pressure skews the aphid response ratings towards the susceptible end, resulting in a narrower range of phenotypic responses which may have hindered the strong identification of resistance regions. Rescreening the populations with low R^2 values or using $F_{2:3}$ progeny from these populations for a second evaluation may help improving the phenotyping accuracy and the marker R^2 values, increasing our ability to predict the number of major genes involved in resistance and could aid in identifying other genetic regions significantly associated with resistance.

PI 71506 was previously studied by other researchers in a population developed using this PI as a parent and segregation for resistance in this population was consistent with a single dominant gene (Nurden et al. 2010). In this previous study, the resistance from PI 71506 was weaker than the resistance provided by Dowling, which carries *Rag1*, indicating that resistance from PI 71506 is potentially different from *Rag1* in Dowling.

Researchers are currently attempting to fine map and discover the genes associated with identified *Rag* loci. The *Rag2* locus is located in a region that contains genes controlling resistance to biotic stresses including *Pseudomonas syringae* pv. *glycinea* (Coerper) Young et al. (*Rpg1*), *Phytophthora sojae* (Kaufmann and Gerdemann) (*Rps3*), soybean mosaic virus (*Rsv1*), root-knot nematode [*Meloidogyne incognita* (Kofoid and White, 1919) Chitwood, 1949], and corn ear worm [*Helicoverpa zea* (Boddie)] (Mian et al. 2008b). The *Rag2* locus in PI 200538 has been fine mapped to a 54-kb interval on the Williams 82 assembly that contains one possible nucleotide binding leucine-rich repeat (NBS-LRR) candidate gene, Glyma13g26000 (Kim et al. 2010b). The *Rag1* region was associated with resistance in three populations, and the region *Rag1* maps to encompasses genes for resistance to corn ear worm and soybean cyst nematode (*Heterodera glycines* Ichinohe) (Li et al. 2007), indicating that these regions could contain complexes of pest-resistance genes or that there may be a single locus that commonly acts in the resistance pathway against a number of pests. The region *Rag1* is located in also has been fine mapped and contains two NBS-LRR genes that may be candidates for *Rag1* (Kim et al. 2010a). The candidate genes for both *Rag1* and *Rag2* were identified from the genome sequence of the soybean aphid-susceptible cultivar Williams 82, requiring *Rag1* and *Rag2* to be cloned from resistant genotypes before the genes can be definitively identified.

This study showed that soybean aphid resistance is often controlled by one or two major genes, acting in a dominant manner. Single-gene-dominant aphid resistance has been found among many crop species including resistance against the pea aphid in *Medicago truncatula* (Gao et al.

2008), the Russian wheat aphid in wheat (*Triticum aestivum*) (Dong and Quick 1995), the lettuce root aphid in lettuce (*Latuca satvia*) (Ellis et al. 1994), and the cotton-melon aphid in melon (*Cucumis melo*) (Klingler et al. 2001). Single-gene aphid resistance is not universal, however, and corn leaf aphid resistance in maize (*Zea mays*) may be controlled by multiple genes (Carena and Glogoza 2004). For breeders, the simple inheritance of soybean aphid resistance implies that incorporating new resistance genes into existing cultivars may be relatively straightforward and easily accomplished. It also seems likely that additional secondary or modifying genes could be present in some of the resistant PIs, and that these genes may be useful in strengthening or broadening the resistance provided by a major gene. Even though the *Rag2* region was frequently associated with resistance, it is not known whether all of the PIs evaluated possess the same allele at the *Rag2* locus or if the PIs contain different resistance genes closely linked to *Rag2* that could be effective against emerging soybean aphid biotypes.

Additional research still needs to be performed to discern if any of the PIs with soybean aphid resistance at the *Rag2* region have novel alleles or new genes that could be used to breed soybean varieties with resistance against a broader spectrum of soybean aphid biotypes. With the current results, it is impossible to tell which, if any, of the resistant PI parents possess new and valuable alleles at or close to *Rag2*. One route to identify new resistance alleles or genes is through the high-density genotyping, or sequencing and cloning, of the *Rag2* region. Over the last few years, genotyping using molecular markers and genome sequencing has become more efficient and less expensive, making this technique a viable option for allele discovery that could help identify soybean aphid resistance sources as having unique alleles at the *Rag2* locus. As soybean aphid biotypes continue to be discovered that can overcome the known *Rag* genes, it will become increasingly important to focus on identifying new *Rag* genes and alleles that can protect soybean yield from the damage resulting from the soybean aphid.

Acknowledgments This work was supported by soybean check-off funding from the Illinois Soybean Association to B.D. and funding from the United Soybean Board to C.F. We also thank Laura Crull and Sarah Schultz for experiment assistance and support.

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The authors state that the experiments comply with the current laws of the country in which they were performed (USA).

References

Beckendorf EA, Catangui MA, Riedell WE (2008) Soybean aphid feeding injury and soybean yield, yield components, and seed composition. Agron J 100:237–246

Brace RC, Fehr WR (2012) Impact of combining the *Rag1* and *Rag2* alleles for aphid resistance on agronomic and seed traits of soybean. Crop Sci 52:2070–2074

Carena M, Glogoza P (2004) Resistance of maize to the corn leaf aphid: a review. Maydica 49:241–254

Cregan PB, Quigley CV (1997) Simple sequence repeat DNA marker analysis. In: Caetano-Anolles G, Gresshoff PM (eds) DNA markers: protocols, applications and overview. Wiley, New York, pp 173–185

Diaz-Montano J, Reese JC, Schapaugh WT, Campbell LR (2006) Characterization of antibiosis and antixenosis to the soybean aphid (Hemiptera: Aphididae) in several soybean genotypes. J Econ Entomol 99:1884–1889

Dong H, Quick J (1995) Inheritance and allelism of resistances to the Russian wheat aphid in 7 wheat lines. Euphytica 81:299–303

Ellis P, Pink D, Ramsey A (1994) Inheritance of resistance to lettuce root aphid in the lettuce cultivars Avoncrisp and Lakeland. Ann Appl Biol 124:141–151

Fehr WR, Caviness CE (1977) Stages of soybean development. Cooperative Extension Service, Agriculture and Home Economics Experiment Station, Iowa State University, Ames

Gao L, Klingler JP, Anderson JP, Edwards OR, Singh KB (2008) Characterization of pea aphid resistance in *Medicago truncatula*. Plant Physiol 146:996–1009

Hartman GL, Domier LL, Wax LM, Helm CG, Onstad DW, Shaw JT, Solter LF, Voegtlin DJ, D'Arcy CJ, Gray ME, Steffey KL, Isard SA, Orwick PL (2001) Occurrence and distribution of *Aphis glycines* on soybeans in Illinois in 2000 and its potential control. Plant Health Progess. doi:10.1094/PHP-2001-0205-01-HN

Hesler LS, Dashiell KE (2008) Identification and characterization of new sources of resistance to *Aphis glycines* Matsumura (Hemiptera: Aphididae) in soybean lines. Appl Entomol Zool 43:197–206

Hill CB, Li Y, Hartman GL (2004) Resistance to the soybean aphid in soybean germplasm. Crop Sci 44:98–106

Hill CB, Li Y, Hartman GL (2006a) A single dominant gene for resistance to the soybean aphid in the soybean cultivar Dowling. Crop Sci 46:1601–1605

Hill CB, Li Y, Hartman GL (2006b) Soybean aphid resistance in soybean Jackson is controlled by a single dominant gene. Crop Sci 46:1606–1608

Hill CB, Kim K, Crull L, Diers BW, Hartman GL (2009) Inheritance of resistance to the soybean aphid in soybean PI 200538. Crop Sci 49:1193–1200

Hill CB, Crull L, Herman TK, Voegtlin DJ, Hartman GL (2010) A new soybean aphid (Hemiptera: Aphididae) biotype identified. J Econ Entomol 103:509–515

Hill CB, Chirumamilla A, Hartman GL (2012) Resistance and virulence in the soybean–*Aphis glycines* interaction. Euphytica 186:635–646

Hyten DL, Song Q, Choi I, Yoon M, Specht JE, Matukumalli LK, Nelson RL, Shoemaker RC, Young ND, Cregan PB (2008) High-throughput genotyping with the GoldenGate assay in the complex genome of soybean. Theor Appl Genet 116:945–952

Hyten DL, Choi I, Song Q, Specht JE, Carter TE Jr, Shoemaker RC, Hwang E, Matukumalli LK, Cregan PB (2010) A high density integrated genetic linkage map of soybean and the development of a 1536 universal soy linkage panel for quantitative trait locus mapping. Crop Sci 50:960–968

Jun T, Rouf Mian MA, Michel AP (2012) Genetic mapping revealed two loci for soybean aphid resistance in PI 567301B. Theor Appl Genet 124:13–22

Kaczorowski KA, Kim K, Diers BW, Hudson ME (2008) Microarray-based genetic mapping using soybean near-isogenic lines and generation of SNP markers in the *Rag1* aphid-resistance interval. Plant Gen 1:89–98

Kang S, Mian MAR, Hammond RB (2008) Soybean aphid resistance in PI 243540 is controlled by a single dominant gene. Crop Sci 48:1744–1748

Keim P, Shoemaker RC (1988) A rapid protocol for isolating soybean DNA. Soybean Genet Newsl 15:150–152

Kim KS, Diers BW (2009) The associated effects of the soybean aphid resistance locus *Rag1* on soybean yield and other agronomic traits. Crop Sci 49:1726–1732

Kim KS, Diers BW (2013) The associated effects of the soybean aphid resistance gene *Rag2* from PI 200538 on agronomic traits in soybean. Crop Sci 53:1326–1334

Kim KS, Hill CB, Hartman GL, Mian MAR, Diers BW (2008) Discovery of soybean aphid biotypes. Crop Sci 48:923–928

Kim K, Bellendir S, Hudson KA, Hill CB, Hartman GL, Hyten DL, Hudson ME, Diers BW (2010a) Fine mapping the soybean aphid resistance gene *Rag1* in soybean. Theor Appl Genet 120:1063–1071

Kim KS, Hill CB, Hartman GL, Hyten DL, Hudson ME, Diers BW (2010b) Fine mapping of the soybean aphid-resistance gene *Rag2* in soybean PI 200538. Theor Appl Genet 121:599–610

Klingler J, Kovalski I, Silberstein L, Thompson G, Perl-Treves R (2001) Mapping of cotton-melon aphid resistance in melon. J Am Soc Hortic Sci 126:56–63

Li Y, Hill CB, Carlson SR, Diers BW, Hartman GL (2007) Soybean aphid resistance genes in the soybean cultivars Dowling and Jackson map to linkage group M. Mol Breed 19:25–34

Macedo TB, Bastos CS, Higley LG, Ostlie KR, Madhavan S (2003) Photosynthetic responses of soybean to soybean aphid (Homoptera: Aphididae) injury. J Econ Entomol 96:188–193

Mardorf JL, Fehr WR, O'Neal ME (2010) Agronomic and seed traits of soybean lines with the *Rag1* gene for aphid resistance. Crop Sci 50:1891–1895

Mensah C, DiFonzo C, Nelson RL, Wang DC (2005) Resistance to soybean aphid in early maturing soybean germplasm. Crop Sci 45:2228–2233

Mensah C, DiFonzo C, Wang D (2008) Inheritance of soybean aphid resistance in PI 567541B and PI 567598B. Crop Sci 48:1759–1763

Mian MAR, Hammond RB, Martin SKS (2008a) New plant introductions with resistance to the soybean aphid. Crop Sci 48:1055–1061

Mian MAR, Kang S, Beil SE, Hammond RB (2008b) Genetic linkage mapping of the soybean aphid resistance gene in PI 243540. Theor Appl Genet 117:955–962

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis—a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sci 88:9828–9832

Nurden Av, Scott RA, Hesler L, Tilmon K, Glover K, Carter C (2010) Inheritance of soybean aphid resistance from PI 71506. J Crop Improv 24:400–416

Ostlie K (2002) Managing soybean aphid, University of Minnesota Extension Service, St Paul. http://www.soybeans.umn.edu/crop/insects/aphid/aphid_publication_managingsba.htm. Accessed on 09 April 2013

Ragsdale DW, Landis DA, Brodeur J, Heimpel GE, Desneux N (2011) Ecology and management of the soybean aphid in North America. Annu Rev Entomol 56:375–399

SAS Institute (2008) The SAS system for Microsoft Windows. Release 9.2. SAS Inst., Cary, NC

Smith CM, Boyko EV (2007) The molecular bases of plant resistance and defense responses to aphid feeding: current status. Entomol Exp Appl 122:1–16

Stokes ME, Davis CS, Koch GG (2000) Categorical data analysis using the SAS system, 2nd edn. SAS Institute Inc, Cary

Wang D, Shi J, Carlson SR, Cregan PB, Ward RW, Diers BW (2003) A low-cost, high-throughput polyacrylamide gel electrophoresis system for genotyping with microsatellite DNA markers. Crop Sci 43:1828–1832

Wu ZS, Schenk-Hamlin D, Zhan WY, Ragsdale DW, Heimpel GE (2004) The soybean aphid in China: a historical review. Ann Entomol Soc Am 97:209–218

Zhang G, Gu C, Wang D (2009) Molecular mapping of soybean aphid resistance genes in PI 567541B. Theor Appl Genet 118:473–482

Zhang G, Gu C, Wang D (2010) A novel locus for soybean aphid resistance. Theor Appl Genet 120:1183–1191

Zhang G, Gu C, Wang D (2013) Mapping and validation of a gene for soybean aphid resistance in PI 567537. Mol Bio. doi:10.1007/s11032-013-9857-5