

A novel locus for soybean aphid resistance

Guorong Zhang · Cuihua Gu · Dechun Wang

Received: 26 February 2009 / Accepted: 12 December 2009 / Published online: 5 January 2010
© Springer-Verlag 2010

Abstract The soybean aphid (*Aphis glycines* Matsumura) is an important pest on soybean [*Glycine max* (L.) Merr.] in North America. Aphid resistance has recently been found on plant introduction (PI) 567543C, but little is known about its genetic control. The objectives of this study were to identify the resistance genes in PI 567543C with molecular markers and validate them in a different genetic background. A mapping population of 249 F₄ derived lines from a cross between PI 567543C and a susceptible parent was investigated for aphid resistance in both the greenhouse and the field. The broad sense heritability of aphid resistance in the field trial was over 0.95. The segregation of aphid resistance in this population suggests a major gene controlling the resistance. Bulked segregant analysis with molecular markers revealed a potential genomic region. After saturating this putative region with more markers, a genetic locus was mapped in an interval between Sat_339 and Satt414 on chromosome 16 (linkage group J) using the composite interval mapping method. This locus explained the majority of the phenotypic variation ranging from 84.7% in the field trial to 90.4% in the greenhouse trial. Therefore, the aphid resistance in PI 567543C could be mainly controlled by this gene. This aphid resistance gene was mapped on a different chromosome than the other resistance genes reported previously from other resistant germplasms. This gene appears to be additive based on the aphid resistance of the heterozygous lines at this locus.

Thus, a new symbol *Rag3* is used to designate this gene. Moreover, *Rag3* was confirmed in a validation population. This new aphid-resistance gene could be valuable in breeding aphid resistant cultivars.

Introduction

The soybean aphid (*Aphis glycines* Matsumura) has long been a pest on soybean [*Glycine max* (L.) Merr.] in many Asian countries such as China, Japan, South Korea, the Philippines, Indonesia, Malaysia, Thailand, and Vietnam (Wu et al. 2004). Recently, the soybean aphid has spread to the USA, Canada, Australia, and New Zealand (Fletcher and Desborough 2000; Hartman et al. 2001; OMAFRA 2002). Since it was first found in the upper Midwest in the USA in 2000, the soybean aphid has quickly spread to most soybean-growing states and become a major soybean pest in the USA. By 2008, only a few states (the Carolinas, Florida, and Texas) had not reported soybean aphids (Voegtlin 2008).

The soybean aphid causes damage by sucking plant sap. Heavily infested plants may show visible symptoms like curling, yellowing, or puckered leaves. Sooty mold, a charcoal-colored residue, also typically appears on heavily infested plants. Severely infested plants may be stunted or turn brown and die. Heavy aphid infestation on soybean could cause considerable yield loss, especially when aphid density peaks at the beginning of flowering. Yield loss of over 50% was reported in Minnesota (Ostlie 2002). Yield loss could be possible even at a low aphid density since the leaf photosynthetic rate could be reduced up to 50% with only 20 aphids per leaflet (Macedo et al. 2003). The soybean aphid could also reduce seed quality, which is a major concern for food grade soybean producers. Moreover, a

Communicated by D. Lightfoot.

G. Zhang · C. Gu · D. Wang (✉)
Department of Crop and Soil Sciences,
Michigan State University,
East Lansing, MI 48824, USA
e-mail: wangdech@msu.edu

potentially greater threat posed by the aphid is its ability to transmit viruses, including soybean dwarf virus, soybean mosaic virus, potato virus, alfalfa mosaic virus, and tobacco ringspot virus (Clark and Perry 2002; Davis et al. 2005).

Biological control is considered to be a long term, sustainable means for pest management. Studies have identified several native or exotic natural enemies of the soybean aphid (Fox et al. 2004; Heimpel et al. 2004; Desneux et al. 2006). However, most natural enemies can only control soybean aphids when aphids are at a low density. When the aphid density reaches an economic threshold, insecticide application might be necessary to avoid economic loss. However, chemical control may require scouting, kill beneficial insects, and cause environmental pollution. Compared to biological and chemical controls, host resistance is more effective, economical, and environmentally sound for pest control.

There are two types of host resistance to insects: antibiosis and antixenosis (Painter 1951). Antibiosis affects insect biology and causes reduced insect abundance. Antixenosis affects insect behavior and is expressed as non-preference for certain plants. Currently, commercial varieties with soybean aphid resistance are not available in the USA, since soybean aphid is a relatively new invasive pest in North America. However, several research groups have identified several aphid-resistant germplasms. In 2004, Hill et al. first reported three late maturing (maturity groups VI–VIII) aphid-resistant germplasms, of which ‘Dowling’ and ‘Jackson’ possess the antibiosis resistance while PI 71506 possesses antixenosis resistance. Mensah et al. (2005) found four early maturing (maturity group III) aphid-resistant germplasms, of which PI 567541B and PI 567598B have antibiosis resistance while PI 567543C and PI 567597C have antixenosis resistance. Most recently, Mian et al. (2008a) identified three germplasms with aphid resistance, of which PI 243540 has antibiosis resistance and the other two have antixenosis resistance.

The aphid resistance in Dowling and Jackson were both controlled by a single dominant gene (Hill et al. 2006a, b). The gene in Dowling was named as *Rag1* (Hill et al. 2006a). Later, *Rag1* and the resistance gene (*Rag*) in Jackson were both mapped to the same genomic region on chromosome 7 [linkage group (LG) M] (Li et al. 2007). Similarly, resistance in PI 243540 was controlled by a single dominant gene (Kang et al. 2008), which was named as *Rag2* and was mapped on chromosome 13 (LG F) (Mian et al. 2008b). In contrast, the resistance in PI 567541B and PI 567598B was each controlled by two recessive genes (Mensah et al. 2008). A genetic mapping study located two genes in PI 567541B on chromosomes 7 and 13 (LG M and F) (Zhang et al. 2009). The gene on chromosome 7 (LG M) was mapped to the same genomic region as *Rag1* and was later designated *rag1_provisional*. The gene on

chromosome 13 (LG F) was located distantly from *Rag2* and was later designated *rag4* (Zhang et al. 2009). Significant interaction was also found between those two genes identified in PI 567541B (Zhang et al. 2009).

Host resistance could be overcome by the occurrence of new insect biotypes. Most recently, a new biotype found in Ohio can overcome *Rag1* (Kim et al. 2008). Therefore, it is important to explore new resistance sources and discover new resistance genes, which can be combined with existing resistance genes to make the resistance more durable. PI 567543C might be a promising resistance source since it has shown resistance to soybean aphids from both Michigan and Ohio (Mensah et al. 2005; Mian et al. 2008a). However, little is known about the genetic basis of aphid resistance in PI 567543C, which could hinder its utilization as an alternative resistance source. Quantitative trait locus (QTL) analysis is a powerful tool to explore genetic mechanisms since it not only identifies the loci associated with quantitative traits but also determines their effects. The objectives of this study were to identify the aphid resistance loci in PI 567543C with molecular markers and further validate them in a different genetic background.

Materials and methods

Aphid resistance loci mapping

Plant materials and aphid resistance evaluation

A population of 249 F₄-derived lines was developed from the cross of PI 567543C × E00003 by single seed descent and was used for genetic mapping. PI 567543C originated from Northern China and possesses antixenosis resistance to the soybean aphid (Mensah et al. 2005), while E00003 is an advanced breeding line developed at Michigan State University (MSU) that is susceptible to the soybean aphid.

Aphid resistance was evaluated in choice tests in both greenhouse and field trials. Since aphid resistance in soybean has a high heritability (around 0.90) based on our previous greenhouse experiments (Zhang et al. 2009), replication was only applied in the field trial. In the spring of 2008, a greenhouse trial was performed in the Plant Science Greenhouse at MSU in East Lansing, Michigan. In this trial, eight seeds per line were planted in a plastic pot. The pot size was 105 mm in diameter and 125 mm deep. The population (F_{4;5}) and its parents were randomly laid out on the bench without replications. The greenhouse was maintained at 26/15°C day/night temperature, and sodium vapor lights were used to supplement light intensity during the day (14 h). In the summer of 2008, a field trial was performed on the Agronomy Farm of MSU. The population (F_{4;6}) and its parents were arranged in a randomized

complete block design with two replications in an aphid- and predator-proof polypropylene cage with 0.49-mm size mesh (Redwood Empire Awning Co., Santa Rosa, CA, USA). In each replication, eight seeds per line were planted in a single plot that was 30 cm long, with a row spacing of 60 cm.

Each plant was infested with two wingless aphids at the V1 stage (Fehr and Caviness 1977) in both greenhouse and field trials. The aphid biotype used to infest plants in the greenhouse trial was a clone from a single insect that was collected from a naturally infested field on the Agronomy Farm of MSU in 2002, and which has been maintained in the greenhouse since then. The aphids used to infest plants in the field trial were collected from the naturally infested field on the Agronomy Farm of MSU during that year. Aphid resistance was visually rated for each plant 3 and 4 weeks after infestation using a scale of 0–4 developed by Mensah et al. (2005, 2008), where 0 = no aphids; 0.5 = fewer than 10 aphids per plant, no colony formed; 1 = 11–100 aphids per plant, plants appear healthy; 1.5 = 101–150 aphids per plant, plants appear healthy; 2 = 151–300 aphids per plant, mostly on the young leaves or tender stems, plants appear healthy; 2.5 = 301–500 aphids per plant, plants appear healthy; 3 = 501–800 aphids per plant, young leaves and tender stems are covered with aphids, leaves appear slightly curly and shiny; 3.5 = more than 800 aphids per plant, plants appear stunted, leaves appear curled and slightly yellow, no sooty mold and few cast skins; 4 = more than 800 aphids per plant, plants appear stunted, leaves appear severely curled and yellow and are covered with sooty mold and cast skins. An aphid damage index (DI) for each line was calculated by the following formula: $DI = \sum(\text{scale value} \times \text{no. of plants in the category}) / (4 \times \text{total no. of plants}) \times 100$. The DI ranges between 0 for no infestation and 100 for the most severe damage (Mensah et al. 2005). The DI was used as an indicator of aphid resistance and was applied in the analysis.

DNA extraction and marker analysis

Six plants for each line ($F_{4,5}$) and their parents were grown in the greenhouse for DNA extraction in 2008. The non-expanded trifoliates from each line were bulk harvested for isolating the genomic DNA. The DNA was extracted with the CTAB (hexadecyltrimethyl ammonium bromide) method as described by Kisha et al. (1997), and the concentration was determined with a ND-1000 Spectrophotometer (NanoDrop Technologies, Inc., Wilmington, Delaware, USA). The PCR was performed using the genomic DNA with simple sequence repeat (SSR) markers as described by Cregan and Quigley (1997) and was run on an MJ TetradTM thermal cycler (MJ Research, Waltham, MA, USA). The SSR primer sequences were provided by

Dr. Perry Cregan of the USDA-ARS, Beltsville, Maryland. The PCR products were separated on 6% non-denaturing polyacrylamide gels using an electrophoresis unit DASG-400-50 (C.B.S. Scientific Co., Del Mar, CA, USA) as described by Wang et al. (2003). Gels were stained with ethidium bromide, visualized under UV light, and photographed.

In order to accelerate the identification of genomic regions associated with aphid resistance, the bulked segregant analysis method described by Michelmore et al. (1991) was used in this study. Twelve resistant lines with the lowest DI values and 12 susceptible lines with the highest DI values were selected to form a resistant pool and a susceptible pool, respectively. SSR markers at approximately every 10 cM on the integrated soybean map of Song et al. (2004) were selected to test the bulked DNA of each pool. The polymorphic markers between the two pools were selected to genotype the individual lines in the two pools together with the two parents. The markers that appeared to be associated with the aphid resistance were used to genotype the remaining lines of the whole mapping population. The genomic regions associated with the aphid resistance were then saturated with additional markers.

Statistical and mapping analysis

The DI data from the greenhouse and field trials were analyzed separately, as their experimental designs and infested aphids differed. Analysis of variance (ANOVA) was performed for the field data using the GLM procedure of SAS Institute (1999). The broad-sense heritability of DI in the field trial was estimated based on entry means according to Fehr (1987). Pearson correlations for the aphid resistance between trials were estimated with the CORR procedure of SAS Institute (1999). A linkage map was constructed with JoinMap 3.0 using the Kosambi function and a LOD score of 3 (Van Ooijen and Voorrips 2001). Composite interval mapping (CIM) was performed to locate aphid resistance loci using QTL Cartographer V2.5 with a standard model Zmapqtl 6 (Wang et al. 2008). The forward and backward regression method was used to select markers as cofactors to control the genetic background (Zeng 1994). The walking speed chosen for CIM was 2 cM. The empirical LOD threshold at the 5% probability level was determined by a 1,000-permutation test (Churchill and Doerge 1994). Entry means were used in the analysis for the field trial. The maps and the locus positions were drawn using MapChart (Voorrips 2002).

Resistance locus validation

A population of 96 $F_{4,5}$ lines was used to validate the resistance loci identified in the mapping population. The validation population was developed by single seed descent

from a cross between PI 567543C and ‘Skylia’, where Skylia is a cultivar developed by MSU and is aphid-susceptible (Wang et al. 2006). In the summer of 2008, aphid resistance was evaluated for the validation population together with its parents in a field trial, which was conducted in the same way as for the mapping population without replications. The fresh leaf tissues in the field trial were collected and placed into a 96-well plate. Due to the limited space in each well, only one leaflet tip was randomly sampled from each line. DNA was then extracted with the quick method as described by Bell-Johnson et al. (1998). Polymorphic markers in the region containing the

aphid resistance locus were selected to genotype the validation population. Linkage map construction and mapping analysis were performed in the same way as for the mapping population.

Results

Phenotypic analysis for mapping population

The phenotypic data of the mapping population and its parents are summarized in Table 1. In the greenhouse, susceptible parent E00003 was heavily infested by soybean aphids, while resistance parent PI 567543C had relatively lower DI than E00003 for the week-4 rating. Similarly, PI 567543C had a significantly lower DI than E00003 in the field ($P < 0.05$). Highly significant variations ($P < 0.0001$) were observed among the population lines for both week-3 and week-4 ratings in the field trial. The aphid infestation in the greenhouse was similar to that in the field. The correlation between the greenhouse and field data was strong (0.91 for both week-3 and week-4 ratings, $P < 0.0001$). The DI frequency distributions were similar between greenhouse and field trials, and both distributions appeared bimodal with a ratio of 1:1 (Fig. 1). However, there was no clear-cut separation between the resistant and susceptible ones. The broad-sense heritability for the field DI was over 0.95 (Table 1).

Aphid resistance loci mapping

A total of 223 SSR markers, which were distributed throughout the soybean genome based on the consensus

Table 1 Aphid damage index (DI) in the greenhouse in spring 2008 and in the field cage in summer 2008 for the parents, PI 567543C and E00003, and 249 F_4 derived lines of the mapping population

Trials	Parents		F_4 -derived lines			
	PI 567543C	E00003	Mean	Range	SE	H^{2a}
Greenhouse						
Week-3 rating	NA	NA	53.9	25.0–87.5	26.4	NA
Week-4 rating	50.0	100.0	65.1	25.0–100.0	29.1	NA
Field cage						
Week-3 rating	32.3a	76.0b	51.3	22.9–80.0	19.2	0.96
Week-4 rating	37.7a	97.4b	62.1	25.0–100.0	26.0	0.95

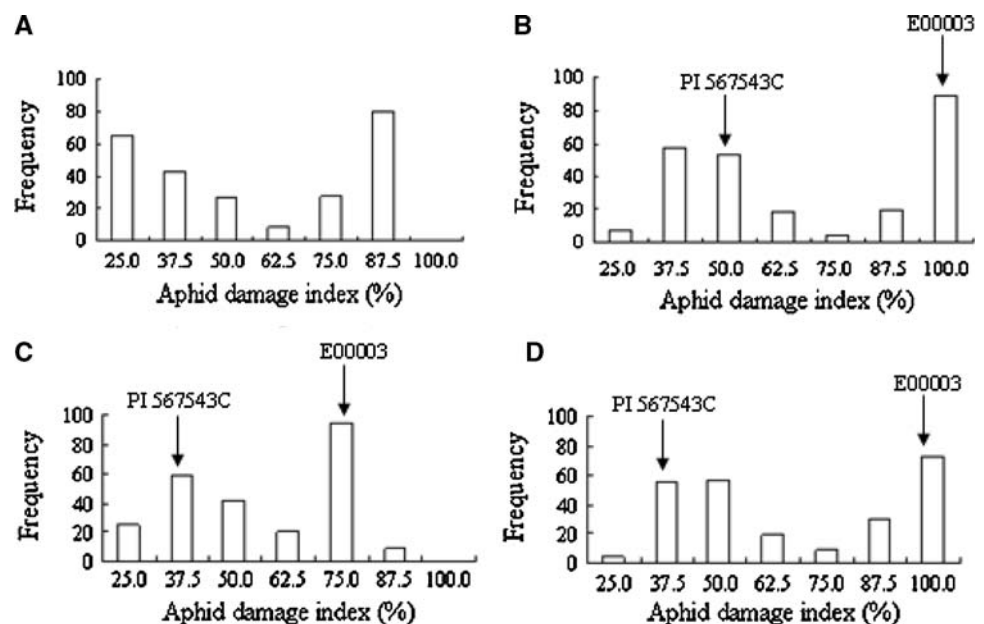
DI = $\sum(\text{scale value} \times \text{no. of plants in the category}) / (4 \times \text{total no. of plants}) \times 100$, ranging between 0 for no infestation and 100 for the most severe damage (Mensah et al. 2005)

Mean followed by different letters within the same row are significantly different at $P < 0.05$

SE standard error, NA data unavailable

^a Broad sense heritability

Fig. 1 Frequency distribution of soybean aphid damage index for 249 lines derived from the PI 567541B \times E00003 cross. Parents are shown by arrows. **a** Week-3 rating in the greenhouse trial; **b** week-4 rating in the greenhouse trial; **c** week-3 rating in the field trial; **d** week-4 rating in the field trial



map (Song et al. 2004), were selected to test the two DNA pools. Several markers on chromosomes 1, 6, 9, 13, 16, and 19 (LGs D1a, K, C2, F, J, and L) showed polymorphism between the two DNA pools. However, only Satt686 on chromosome 16 (LG J) appeared to be associated with the aphid resistance when the individual lines from the DNA pools were genotyped and compared to the parents' genotypes. Therefore, Satt686 was further genotyped on the rest of the population, which confirmed its association with aphid resistance. Then, all SSR markers within ± 20 cM of this marker were screened for parental polymorphism. Seven additional polymorphic markers in that region were further genotyped on the whole population. All markers fit a 7:2:7 (homozygous female:heterozygotes:homozygous male) segregation ratio ($P > 0.001$) except Satt596 ($P = 0.0009$), Satt622 ($P = 0.0003$), and Sat_339 ($P = 0.0000002$). The distorted ratios of these three markers were due to fewer heterozygotes than expected. These eight markers formed one linkage group, and the linkage map spanned a total distance of 36.6 cM (Fig. 2a), which was about 12 cM larger than the corresponding map distance of 24.8 cM in the consensus map of Song et al. (2004) (Fig. 2b). The main inflated interval on the map was between Sat_339 and Satt414 (10 cM larger than expected). However, the marker order in this study was highly comparable with the consensus map (Song et al. 2004) except that the order of Satt686 and Satt596 was inverted (Fig. 2a). The linkage map was then used in QTL analysis with the CIM method. One QTL was consistently identified in the interval between Sat_339 and Satt414 for

both ratings in each trial and was located closer to Satt414 (Table 2; Fig. 2a). The PI 567543C allele at this locus conferred aphid resistance. This QTL explained the majority of the phenotypic variation ranging from 84.7% for the week-3 rating in the field trial to 90.4% in the greenhouse trial. Considering the high percentage of phenotypic variation explained by this QTL and the high heritability of this trait, it is very possible that a single gene controls the aphid resistance in PI 567543C. To determine this resistance gene's action, the DI value for each genotype class of Satt414 was estimated (Table 3) and compared statistically. The DI average for the heterozygous class was significantly ($P < 0.05$) lower than the susceptible class, higher than the resistant class, and not significantly different from the average of the two homozygous classes, indicating that this resistance might be additive. This gene's location is totally different from those of genes that were previously identified for the other three resistant germplasms, including *Rag1*, *Rag2*, and *rag4* (Li et al. 2007; Mian et al. 2008b; Zhang et al. 2009). Therefore, we named this gene as *Rag3* according to the conventions of Soybean Genetics Committee.

Aphid resistance locus validation

A total of eight polymorphic SSR markers in the region containing *Rag3* were chosen to genotype the validation population. All eight markers formed one linkage group. A distorted segregation ratio (7:2:7) was only observed for marker Satt674 ($P = 0.0000007$), which had a higher

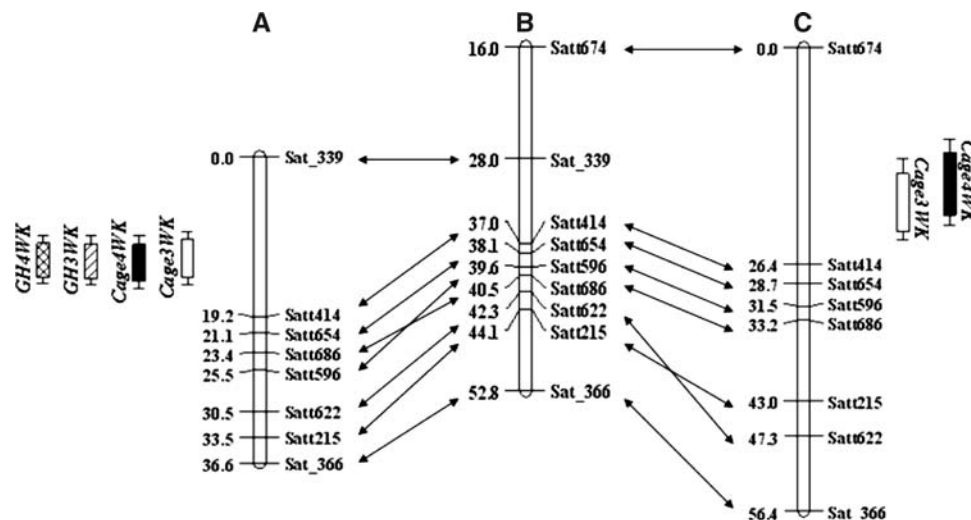


Fig. 2 Locations of soybean aphid resistance loci as determined using the composite interval mapping method. 1-LOD and 2-LOD support intervals of each locus are marked by *thick* and *thin bars*, respectively. *Bars filled with hatch lines* represent loci for the week-3 rating in the greenhouse trial (*GH3WK*). *Bars filled with cross lines* represent loci for the week-4 rating in the greenhouse trial (*GH4WK*). *Unfilled bars* represent loci for the week-3 rating in the field cage trial

(*Cage3WK*). *Black bars* represent loci for the week-4 rating in the field cage trial (*Cage4WK*). **a** Map of chromosome 16 (linkage group J) in mapping population PI 567543C \times E00003 with the aphid resistance locus shown on the *left*; **b** map of chromosome 16 (linkage group J) on the consensus map (Song et al. 2004); **c** map of chromosome 16 (linkage group J) in validation population PI 567543C \times Skylla with the aphid resistance locus shown on the *right*

Table 2 Summary for aphid resistance locus detected in the mapping population PI 567543C × E00003 and in the validation population PI 567543C × Skylla with aphid damage index data using the composite interval mapping method

Population	Trials	Chr/LG ^a	Peak Pos. ^b	Flanking markers	LOD	R ^{2c}	a ^d
PI 567543C × E00003	Field cage						
	Week-3 rating	16/J	12.0	Sat_339–Satt414	69.8	84.7	17.6
	Week-4 rating	16/J	12.0	Sat_339–Satt414	81.0	88.1	24.4
	Greenhouse						
PI 567543C × Skylla	Field Cage						
	Week-3 rating	16/J	20.0	Satt674–Satt414	11.3	74.3	16.2
	Week-4 rating	16/J	16.0	Satt674–Satt414	18.1	85.2	23.5

^a Chromosome/linkage group. The chromosome number and linkage group name are according to the SoyBase (Grant et al. 2009)

^b Peak position is expressed in cM

^c R², percentage of phenotypic variation explained by the locus

^d Additive effect: the positive value implies that the PI 567543C allele decreased the phenotypic value

Table 3 Average aphid damage index for different genotypes of marker Satt414 in the mapping population PI 567543C × E00003

Numbers followed by different letters within the same row are significantly different at $P < 0.05$

Trials	PI 567543C type	Heterozygous type	E00003 type	Average of PI 567543C and E00003 type
Greenhouse				
Week-3 rating	35.1a	58.3b	75.2c	55.2b
Week-4 rating	44.0a	71.1b	88.6c	66.3b
Field cage				
Week-3 rating	37.9a	54.6b	65.9c	51.9b
Week-4 rating	43.9a	64.4b	83.0c	63.5b

number of homozygous PI 567543C genotypes (67 lines) than expected (41 lines). The order of these eight markers was similar to that on the consensus map (Song et al. 2004) except that the order of Satt622 and Satt215 was inverted (Fig. 2c). However, the map of the validation population was expanded about 19 cM more compared with the consensus map (Song et al. 2004) (Fig. 2b, c). In the QTL analysis with the CIM method, one QTL was detected in the interval between Satt674 and Satt414 at a position of about 6 or 10 cM above Satt414 (Fig. 2c), which is the same as the QTL detected in the mapping population. Similarly, the QTL identified in the validation population explained the majority of the phenotypic variation (75.3 and 85.4% for week-3 and week-4 ratings, respectively) in the field trial (Table 2). Hence, the results from the validation population confirmed the location of the *Rag3* locus identified in the mapping population.

Discussion

Both maps in this study were inflated compared with the consensus map (Song et al. 2004). Similar inflation was

observed in the study of Mian et al. (2008b). The severe segregation distortion of markers Sat_339 in the mapping population and Satt674 in the confirmation population might have contributed partially to the inflated maps in this study. Additionally, a number of chromosome factors such as deletions, insertions, and translocations or other chromosome modifications might also contribute to the varied map distances among populations (Williams et al. 1995). Heterochromatin is known to suppress the recombination of the chromosome segments in its vicinity. The differences in heterochromatin distribution in certain regions due to the additions or deletions could be the cause for differences in recombination rates and therefore for the differences in map distances estimated among different populations (John and King 1985).

The aphid resistance gene *Rag3* mapped in this study is located in the interval between Sat_339 and Satt414 in the mapping population, or in the interval between Satt674 and Satt414 in the validation population. This locus is located at the same position in both maps and is more closely linked to Satt414. There are only a few polymorphic SSR markers in the *Rag3* region, which resulted in a relatively large interval for this resistance locus. In order to narrow

down this genomic region and/or fine map this locus, additional polymorphic markers, especially single nucleotide polymorphism markers, need to be explored or developed for this region.

The aphid resistance in this study was evaluated with choice tests, which could not determine if the resistance is antixenosis or antibiosis. However, PI 567543C was evaluated with both choice and no choice tests in the study of Mensah et al. (2005) and its aphid resistance has been determined to be antixenosis. The aphids used in the greenhouse trial of this study originated from the same aphid isolate as in the Mensah et al. (2005) greenhouse trial. Dowling and Jackson, the common resistant checks in the greenhouse, also showed resistance to the aphids used in the greenhouse trial of this study, indicating that the aphid isolate we used is the same one as in Mensah et al. (2005). Thus, it is very possible that the resistance gene discovered in this study is responsible for antixenosis. Antixenosis resistance is the non-preference of host plants, which usually discourages insect feeding and/or oviposition. The underlying mechanism could be morphological (e.g., sharp or dense pubescence) or biochemical (the presence of deterrent compounds or absence of attractants). In soybean, the sharp pubescence gene located on LG E has been found related to the antixenosis resistance to beet armyworm (*Spodoptera exigua* Hubner), corn earworm (*Helicoverpa zea* Boddie), and soybean looper (*Pseudoplusia includens* Walker) (Parrott et al. 2008). However, the pubescence of PI 567543C is blunt and semi-sparse, similar to the susceptible parents. Therefore, the antixenosis resistance in PI 567543C might be associated with chemical compounds instead of its morphological traits. The *Tasty* locus discovered in Arabidopsis has antixenosis resistance to cabbage looper, and was found to be related to the formation of isothiocyanates which deter the feeding of insects (Jander et al. 2001; Lambrix et al. 2001; Zhang et al. 2006). It would be interesting to determine if *Rag3* affects the production of certain chemical compounds that cause the non-preference of soybean aphids on PI 567543C in future research.

Previous studies on three antibiosis resistant germplasm have identified several resistance loci on chromosomes 7 and 13 (LGs M and F) (Li et al. 2007; Mian et al. 2008b; Zhang et al. 2009). This study found a novel aphid-resistant locus, *Rag3*, on chromosome 16 (LG J). Two QTLs (CEW6-2 and CEW7-4) for corn earworm resistance have been positioned in a genomic region (Grant et al. 2009) close to *Rag3*. However, *Rag3* and these corn earworm QTLs are about 15 cM apart and the corn earworm resistance parents, Minsoy (James Orf, personal communication) and PI 229358 (Parrott et al. 2008), are aphid susceptible. Therefore, *Rag3* and the corn earworm QTLs on chromosome 16 (LG J) may not be the same

locus. Interestingly, a large cluster of disease resistance gene analogs [RGAs, encoding the nucleotide binding site-leucine rich repeat (NBS-LRR) resistance proteins] representing five different classes have been mapped on chromosome 16 (LG J), one of which (RGA6) was close to *Rag3* and located a few cM down from Sat_339 (Kanazin et al. 1996). Coincidentally, *Rag1* was found close to RGA5b and RGA2b on chromosome 7 (LG M) (Li et al. 2007), and *Rag2* was located in a genomic region on chromosome 13 (LG F) where a cluster of *R*-genes resides (Mian et al. 2008b). Additionally, Klingler et al. (2005) mapped an antixenosis aphid resistance gene to a region flanked by RGAs in barrel medic (*Medicago truncatula* Gaert.). The cloned genes *Mi-1* in tomato (*Lycopersicon esculentum*) and *vat* in melon (*Cucumis melo* L.) encoded specific NBS-LRR proteins conferring aphid resistance (Gregg and Jander 2008). Thus, it is possible that the molecular basis for the three aphid resistance loci, *Rag1*, *Rag2*, and *Rag3* may be similar to *R*-gene mediated disease resistance. However, more experiments, such as fine mapping and cloning, are warranted to further understand their resistance mechanisms.

Plants with the first reported aphid resistance gene, *Rag1*, have been found susceptible to the Ohio aphid biotype (Kim et al. 2008) and the aphids from Michigan in 2007 (unpublished data). The second reported gene, *Rag2*, also did not provide resistance to plants infested with the aphids from Michigan in 2008 (unpublished data). In our study, two different isolates of aphids were used for infestations in the greenhouse and field experiments, respectively. These two isolates of aphids might belong to different biotypes (Zhang et al. 2009). However, PI 567543C showed resistance to both isolates of aphids. Moreover, PI 567543C has also shown resistance to the Ohio aphid biotype (Mian et al. 2008a). These facts indicate that PI 567543C has a broad resistance to soybean aphids. Therefore, this novel aphid-resistant locus and the linked molecular markers found in this study could be valuable in breeding aphid-resistant soybean cultivars.

References

- Bell-Johnson B, Garvey G, Johnson J, Lightfoot D, Meksem K (1998) Biotechnology approaches to improving resistance to SCN and SDS: methods for high throughput marker assisted selection. Soybean Genet Newsl 25:115–117
- Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971
- Clark AJ, Perry KL (2002) Transmissibility of field isolates of soybean viruses by *Aphis glycines*. Plant Dis 86:1219–1222
- Cregan PB, Quigley CV (1997) Simple sequence repeat DNA marker analysis. In: Caetano-Anolles G, Gresshoff PM (eds) DNA markers: protocols, applications and overviews. Wiley, New York, pp 173–185

- Davis JA, Radcliffe EB, Ragsdale DW (2005) Soybean aphid, *Aphis glycines* Matsumura, a new vector of potato virus Y in potato. *Am J Potato Res* 82:197–201
- Desneux N, O'Neil RJ, Yoo HJS (2006) Suppression of population growth of the soybean aphid, *Aphis glycines* Matsumura, by predators: the identification of a key predator and the effects of prey dispersion, predator abundance, and temperature. *Env Entomol* 35:1342–1349
- Fehr WR (1987) Principles of cultivar development: theory and technique. MacMillan, New York
- Fehr WR, Caviness CE (1977) Stages of soybean development. Special Report, Agriculture and Home Economics Experiment Station, Iowa State University, 1977, issue 80, p 11
- Fletcher MJ, Desborough P (2000) The soybean aphid, *Aphis glycines*, present in Australia. <http://www.agric.nsw.gov.au/hort/ascu/insects/aglycin.htm>. Accessed 2 Jan 2009
- Fox TB, Landis DA, Cardoso FF, Difonzo CD (2004) Predators suppress *Aphis glycines* Matsumura population growth in soybean. *Env Entomol* 33:608–618
- Grant D, Imsande MI, Shoemaker RC (2009) SoyBase, The USDA-ARS soybean genome database. <http://soybase.agron.iastate.edu>. Accessed 24 Feb 2009
- Gregg AW, Jander G (2008) Plant immunity to insect herbivores. *Annu Rev Plant Biol* 59:41–66
- 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. <http://www.plantmanagementnetwork.org/pub/php/brief/aphisglycines>. Accessed 2 Jan 2009
- Heimpel GE, Ragsdale DW, Venette R, Hopper KR, O'Neil RJ, Rutledge CE, Wu ZS (2004) Prospects for importation biological control of the soybean aphid: anticipating potential costs and benefits. *Ann Entomol Soc Amer* 97:249–258
- 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
- Jander G, Cui JP, Nhan B, Pierce NE, Ausubel FM (2001) The *TASTY* locus on chromosome 1 of *Arabidopsis* affects feeding of the insect herbivore *Trichoplusia ni*. *Plant Physiol* 126:890–898
- John B, King M (1985) Interrelationship between heterochromatin distribution and chiasma distribution. *Genetica* 66:183–194
- Kanazin V, Marek LF, Shoemaker RC (1996) Resistance gene analogs are conserved and clustered in soybean. *Proc Natl Acad Sci* 93:11746–11750
- Kang ST, Mian MAR, Hammond RB (2008) Soybean aphid resistance in PI 243540 is controlled by a single dominant Gene. *Crop Sci* 48:1744–1748
- Kim K, Hill CB, Hartman GL, Mian MR, Diers BW (2008) Discovery of soybean aphid biotypes. *Crop Sci* 48:923–928
- Kisha T, Sneller CH, Diers BW (1997) Relationship between genetic distance among parents and genetic variance in populations of soybean. *Crop Sci* 37:1317–1325
- Klingler J, Creasy R, Gao L, Nair RM, Calix AS, Jacob HS, Edwards OR, Singh KB (2005) Aphid resistance in *Medicago truncatula* involves antixenosis and phloem-specific, inducible antibiosis, and maps to a single locus flanked by NBS-LRR resistance gene analogs. *Plant Physiol* 137:1445–1455
- Lambrix V, Reichelt M, Mitchell-Olds T, Kliebenstein DJ, Gershenzon J (2001) The *Arabidopsis* epithiospecifier protein promotes the hydrolysis of glucosinolates to nitriles and influences *Trichoplusia ni* herbivory. *Plant Cell* 13:2793–2807
- 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
- Mensah C, Difonzo C, Nelson RL, Wang D (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, St Martin SK (2008a) New plant introductions with resistance to the soybean aphid. *Crop Sci* 48:1055–1061
- Mian MAR, Kang ST, 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 J, 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
- OMAFRA (2002) Soybeans: soybean aphid. Agronomy guide for field crops, Publication 811. <http://www.omafra.gov.on.ca/english/crops/pub811/4aphid.htm>. Accessed 2 Jan 2009
- 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 2 Jan 2009
- Painter RH (1951) Insect resistance in crop plants. Macmillan, New York
- Parrott W, Walker D, Zhu S, Boerma HR, All J (2008) Genomics of insect-soybean interactions. In: Stacey G (ed) Genetics and genomics of soybean. Springer, New York
- SAS Institute (1999) SAS/SAT users guide, version 8.0. SAS Institute, Cary
- Song QJ, Marek LF, Shoemaker RC, Lark KG, Concibido VC, Delannay X, Specht JE, Cregan PB (2004) A new integrated genetic linkage map of the soybean. *Theor Appl Genet* 109:122–128
- Van Ooijen JW, Voorrips RE (2001) JoinMap 3.0. Software for the calculation of genetic linkage maps in experimental populations. Kyazma BV, Wageningen
- Voegtlin D (2008) United States soybean aphid commentary. http://sba.ipmpipe.org/cgi-bin/sbr/public.cgi?host=All%20Legumes/Kudzu&pest=soybean_aphid. Accessed 2 Jan 2009
- Voorrips RE (2002) MapChart: Software for the graphical presentation of linkage maps and QTLs. *J Heredity* 93:77–78
- Wang D, Shi J, Carlson SR, Cregan PB, Ward RW, Diers BW (2003) A low-cost and high-throughput system for high-resolution genotyping with microsatellite DNA markers. *Crop Sci* 43:1828–1832
- Wang D, Boyse J, Diers BW (2006) Registration of 'Skylia' soybean. *Crop Sci* 46:974–975
- Wang S, Basten CJ, Zeng ZB (2008) Windows QTL Cartographer 2.5. Dept of Statistics, North Carolina State University, Raleigh. <http://statgen.ncsu.edu/qtlcart/WQTLCart.htm>. Accessed 2 Jan 2009
- Williams CG, Goodman MM, Stuber CW (1995) Comparative recombination distances among *Zea mays* L. inbreds, wide crosses and interspecific hybrids. *Genetics* 141:1573–1581
- Wu Z, Schenk-Hamlin D, Zhan W, Ragsdale DW, Heimpel GE (2004) The soybean aphid in China: a historical review. *Ann Entomol Soc Am* 97:209–218

- Zeng ZB (1994) Precision mapping of quantitative trait loci. *Genetics* 136:1457–1468
- Zhang Z, Ober JA, Kliebenstein DJ (2006) The gene controlling the quantitative trait locus *EPITHIOSPECIFIER MODIFIER1* alters glucosinolate hydrolysis and insect resistance in *Arabidopsis*. *Plant Cell* 18:1524–1536
- Zhang G, Gu C, Wang D (2009) Molecular mapping of soybean aphid resistance in PI 567541B. *Theor Appl Genet* 118:473–482