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ORIGINAL PAPER

Molecular mapping of soybean aphid resistance genes in PI 567541B

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Abstract The soybean aphid (*Aphis glycines* Matsumura) is an important pest of soybean [*Glycine max* (L.) Merr.] in North America since it was first reported in 2000. PI 567541B is a newly discovered aphid resistance germplasm with early maturity characteristics. The objectives of this study were to map and validate the aphid resistance genes in PI 567541B using molecular markers. A mapping population of 228 F_3 derived lines was investigated for the aphid resistance in both field and greenhouse trials. Two quantitative trait loci (QTLs) controlling the aphid resistance were found using the composite interval mapping method. These two QTLs were localized on linkage groups (LGs) F and M. PI 567541B conferred resistant alleles at both loci. An additive × additive interaction between these two QTLs was identified using the multiple interval mapping method. These two QTLs combined with their interaction explained most of the phenotypic variation in both field and greenhouse trials. In general, the QTL on LG F had less effect than the one on LG M, especially in the greenhouse trial. These two QTLs were further validated using an independent population. The effects of these two QTLs were also confirmed using 50 advanced breeding lines, which were all derived from PI 567541B and had various genetic backgrounds. Hence, these two QTLs identified and validated in this study could be useful in improving soybean aphid resistance by marker-assisted selection.

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Introduction

Soybean [*Glycine max* (L.) Merr.] is the leading oil crop in the world in terms of its acreage, production, and trade (FAO 2006). The US is the world's leading soybean producer with more than 80 million Mg produced in 2006 (FAO 2006). The soybean aphid is one of the most damaging pests of soybean (Sun et al. 2000) and can cause a considerable yield loss by feeding directly on soybean or transmitting various viruses (Wu et al. 2004). The soybean aphid originated in Asia. Since it was first detected in 2000 in the upper Midwest of the US, the soybean aphid has spread to 21 US states and three Canadian provinces and has become a major pest of soybean in North America (Chen et al. 2007).

Host resistance is the most practical, effective, and economical means of pest control. Host resistance to insects has three types: antibiosis, antixenosis, and tolerance (Painter 1951). Antibiosis resistance affects the insect biology and causes reduced insect abundance. Antixenosis resistance affects the insect behavior and is expressed as the non-preference of the insect for certain plants. Tolerance is expressed as the plant's ability to withstand or recover from the insect damage. Various studies regarding the soybean aphid have been conducted in China since the 1960s and several aphid resistance cultivars have been developed (Wu et al. 2004). However, soybean aphid research in the US is still in its early stage since the soybean aphid is a relatively new invasive pest in North America. In 2004, Hill et al. (2004) first reported seven aphid resistance lines after screening 1,542 soybean accessions of which 'Dowling' and 'Jackson' possess the antibiosis resistance. All of these resistant lines are late maturing and belong to maturity group (MG) IV–VIII. Mensah et al. (2005) identified four early maturing (MG III) soybean accessions with aphid

resistance among 2,147 accessions, of which PI 567541B and PI 567598B have the antibiosis resistance. Genetic studies suggested that 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 *Rag1* (Hill et al. 2006a). Later, *Rag1* and the resistance gene (*Rag*) in Jackson were mapped to a similar genomic region of linkage group (LG) M using micro-satellite (or simple repeat sequence, SSR) markers (Li et al. 2007). The genetic allelism between *Rag1* and *Rag* is unknown. The aphid resistance in PI 567541B and PI 567598B has been determined to be controlled by two recessive genes (Mensah et al. 2008). A genetic diversity study (Chen et al. 2007) of aphid resistance sources indicated that PI 567541B and PI 567598B were genetically very distinct from both Dowling and Jackson while Dowling and Jackson belonged to the same group. However, the molecular characterization of the aphid resistance in these PIs has not been determined. The mapping efforts involved in the new resistant germplasm could enable us to discover new genes, which could be useful for pyramiding resistance genes from various resistance sources and obtain more durable insect resistance. Therefore, the objectives of this study were to (1) characterize aphid resistance genes in PI 567541B with molecular markers, (2) validate the newly identified genes with an independent population and various advanced breeding lines.

Materials and methods

QTL mapping

Plant materials and aphid resistance evaluation

A population of 228 F_3-derived F_4 lines was developed from the cross of PI 567541B × Skylla by single seed descent and was used for QTL detection. PI 567541B possesses antibiosis resistance to the soybean aphid (Mensah et al. 2005) while 'Skylla' (Wang et al. 2006) is an aphid-susceptible cultivar.

One field and one greenhouse trial were conducted for aphid resistance evaluation. In the summer of 2007, a field trial was performed on the Agronomy Farm of Michigan State University (MSU). A polypropylene cage with the 0.49-mm size mesh (Redwood Empire Awning Co., Santa Rosa, CA), which was aphid- and predator- proof, was constructed over the field experiment to create conditions for an artificial aphid infestation. The whole population ($F_{3:4}$ generation) and its parents were randomly arranged in the field plots without replication. Depending on the seed availability, two to twenty seeds per line were seeded in a single row plot, 60 cm long with a row spacing of 60 cm. The average number of plants per line was about 11 with most plots having at least ten plants. In the spring of 2008, a greenhouse trial was performed in the Plant Science Greenhouse on the MSU campus. In this trial, the whole population ($F_{3:5}$ generation) and its parents were arranged in a randomized complete block design with two replications. In each replication, six seeds per line were seeded in a plastic pot. The pot size was 105 mm wide × 105 mm long × 125 mm deep. The greenhouse was maintained at a temperature of 26°C by day, 15°C by night, and sodium vapor lights were used to supplement light intensity during the day (14 h).

Both trials were choice tests for aphid resistance evaluation, which identifies resistance genotypes with either antibiosis or antixenosis. Each plant was inoculated with two wingless aphids at the V1 stage. The aphids inoculated in the field trial were collected from the naturally infested field on the Agronomy Farm of MSU during that year. The aphid inoculated in the greenhouse trial was a single clone, which was collected from the naturally infested field on the Agronomy Farm of MSU in 2002 and has been maintained in the greenhouse ever since. Aphid resistance was visually rated for each plant 3 and 4 weeks after inoculation using a scale of 0–4 developed by Mensah et al. (2005, 2008), where 0 = no aphids; 0.5 = less than 10 aphids per plant, no colony formed; 1 = 11–100 aphids per plant, plant appears healthy; 1.5 = 101–150 aphids per plant, plant appears healthy; 2 = 151–300 aphids per plant, mostly on the young leaves or tender stems, plant appears healthy; 2.5 = 301–500 aphids per plant, plant appears healthy; 3 = 501–800 aphids per plant, young leaves and tender stems covered with aphids, leaves slightly curly and shiny; 3.5 = more than 800 aphids per plant, plants stunted, leaves curled and slightly yellow, no sooty mold and few cast skins; 4 = more than 800 aphids per plant, plant stunted, leaves severely curled and yellow, covered with sooty mold and cast skins. A damage index (DI) for each line was calculated by the following formula (Mensah et al. 2005): DI = (scale value × no. of plants in the category)/(4 × total no. of plants) × 100. The DI ranges between 0 for no infestation and 100 for the most severe damage. The DI was used as an indicator of aphid resistance and was applied in the following analysis.

DNA extraction and marker analysis

About ten plants for each line ($F_{3:4}$ generation) and their parents were grown in the greenhouse for DNA extraction in 2007. 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). The PCR was performed using the genomic DNA with SSR markers as described by Cregan and Quigley (1997) and run on a MJ TetradTM thermal cycler (MJ Research, Waltham, MA). 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) as described by Wang et al. (2003). Gels were stained with ethidium bromide, visualized under UV light, and photographed.

A total of 1,056 SSR markers were screened for the parental polymorphism, of which 329 markers showed polymorphism. Based on the soybean consensus map (Song et al. 2004); these polymorphic markers covered most of the genomic regions. The SSR primer sequences were provided by Dr. Perry Cregan at USDA-ARS, Beltsville, MD. In order to accelerate the identification of genomic regions for aphid resistance, markers at an approximate distance of 20 cM were used to first genotype a subset of 94 lines, which were randomly selected from the whole population. The markers in regions potentially associated with aphid resistance were genotyped on the remaining lines and these regions were further saturated with more markers.

Statistical and QTL analysis

The DI data from the field and greenhouse trials were analyzed separately since their experimental designs and inoculums differed. Analysis of variance (ANOVA) was performed for the greenhouse data using the GLM procedure of SAS (1999). The broad sense heritability of DI in the greenhouse trial was calculated based on entry means according to Fehr (1987). Pearson correlation for the aphid resistance between trials was calculated with the CORR procedure of SAS (1999). Linkage map was constructed with the Kosambi function and a LOD score of 3 or lower (to force some distantly located markers to be linked) using Map Manager QTXb20 (Manly et al. 2001). The maps were drawn using MapChart (Voorrips 2002). Assignment of linkage groups to the specific linkage groups was based on the soybean consensus map (Song et al. 2004). Composite interval mapping (CIM) was performed to detect aphid resistance QTLs using QTL Cartographer V2.5 with the standard model Zmapqtl 6 (Wang et al. 2008). Entry means were used in the analysis for the greenhouse trial data. The CIM analysis uses markers other than the interval being tested as cofactors to control the genetic background (Zeng 1994). The forward and backward regression method was used to select markers as cofactors. The walking speed chosen for CIM was 2 cM. The empirical LOD threshold at 5% probability level was determined by a 1,000-permutation test (Churchill and Doerge 1994). The QTL × QTL interaction was further determined using the multiple interval mapping (MIM) method of QTL Cartographer. The whole genome scan was only conducted on the subset of 94 lines.

QTL validation

A population of 51 F_3-derived lines was developed by single-seed descent from a cross between PI 567541B and E00003, where E00003 is an elite advanced breeding line and is susceptible to the soybean aphid. This population and another 50 advanced breeding lines were used for the QTL validation. The 50 advanced breeding lines (F_4 generation in the 2007 field trial and F_5 in the 2008 greenhouse trial) pre-selected for agronomic traits were derived from five different crosses, where the male parent is PI 567541B or F_1 progeny derived from PI 567541B, and the female parent is an aphid-susceptible cultivar or breeding line (Table 1). The same type of trials conducted for the mapping population were performed for the validation population and the advanced breeding lines in the summer of 2007 and spring of 2008. However, there was no replication in the greenhouse trial and the aphid resistance was only rated 4 weeks after inoculation in the field trial. The DNA was extracted with a quick-extraction method (Bell-Johnson et al. 1998). Several markers in the QTL associated regions were genotyped for the validation population. Linkage maps were constructed and QTL analysis was performed in the same way as in the mapping population. For the advanced breeding lines, only markers closely linked to the identified QTLs were genotyped and their allele effects were calculated and compared using a *t* test ($P = 0.05$).

Results

Phenotypic analysis

The phenotypic values of the mapping population and its parents are summarized in Table 2. In the field cage, susceptible parent Skylla was severely damaged by the aphid infestation while resistant parent PI 567541B had

Table 1 The crosses used for deriving the 50 advanced breeding lines

Cross ID[a]	Male	Female	Line no.
050016	PI 567541B	E00003	2
050023	(PI 567541B × SDx00R-39-42) F_1	E00003	9
050027	PI 567541B	E01260	7
050098	PI 567541B	SDx00R-39-42	9
050105	PI 567541B	Skylla	23

[a] Crosses 050016 and 050105 have the same parents as in the mapping and validation population, but they were made independently in a different year

Table 2 Phenotypic summary of the mapping population and its parents PI 567541B and Skylla for the soybean aphid damage index investigated in the field cage in summer 2007 and in the greenhouse in spring 2008

Trials	Parents[a]		RILs population			
	PI 567541B	Skylla	Mean	Range	SE	H^{2b}
Field Cage						
3-week rating	60.0	95.8	82.2	32.5 ~ 100.0	16.3	–
4-week rating	75.0	100.0	91.4	50.0 ~ 100.0	13.2	–
Greenhouse						
3-week rating	26.8a	56.1b	45.9	18.5 ~ 77.9	17.5	0.89
4-week rating	25.0a	81.3b	55.7	22.3 ~ 87.9	24.1	0.93

[a] Means followed by different letters within the same row are significantly different at $P < 0.05$

[b] Broad sense heritability. Unavailable heritability is marked with '–'

relatively lower DI than Skylla for both 3- and 4-week ratings. Similarly, PI 567541B in the greenhouse trial had a significantly ($P < 0.05$) lower DI than Skylla. Highly significant variation ($P < 0.0001$) was observed among the population lines for both 3- and 4-week ratings in the greenhouse trial. The aphid infestation in the field cage was generally more severe than in the greenhouse, which might be because the field environment was more favorable for the aphid development. However, correlation coefficients between the field and greenhouse data were significant (0.68 and 0.66 for the 3- and 4–week ratings, respectively, $P < 0.0001$). The frequency distributions of the field DI were continuous, but not normal and skewed to the susceptible parent (Fig. 1a, b), indicating that more than one recessive gene might control the aphid resistance. However, the frequency distributions of the greenhouse DI appeared more bimodal with a ratio of 1:1 (Fig. 1c, d). Additionally, the broad sense heritability for the greenhouse DI was high (0.89 and 0.93 for the 3- and 4-week ratings, respectively) (Table 2). These might indicate that only one gene controls the aphid resistance in the greenhouse trial.

QTL mapping using CIM

A total of 123 SSR markers, which distribute throughout the soybean genome based on the consensus map (Song et al. 2004), were genotyped on the subset of 94 lines. These markers generated 131 loci, of which 124 loci were mapped into 25 linkage groups that were segments of the 20 linkage groups on the consensus map. The linkage map spanned 1,703 cM with an average interval length of 13.7 cM. This map was used to conduct the whole genome scan to identify aphid resistance QTLs with the subset of 94 lines. Two QTLs were detected, which were located on LGs F and M

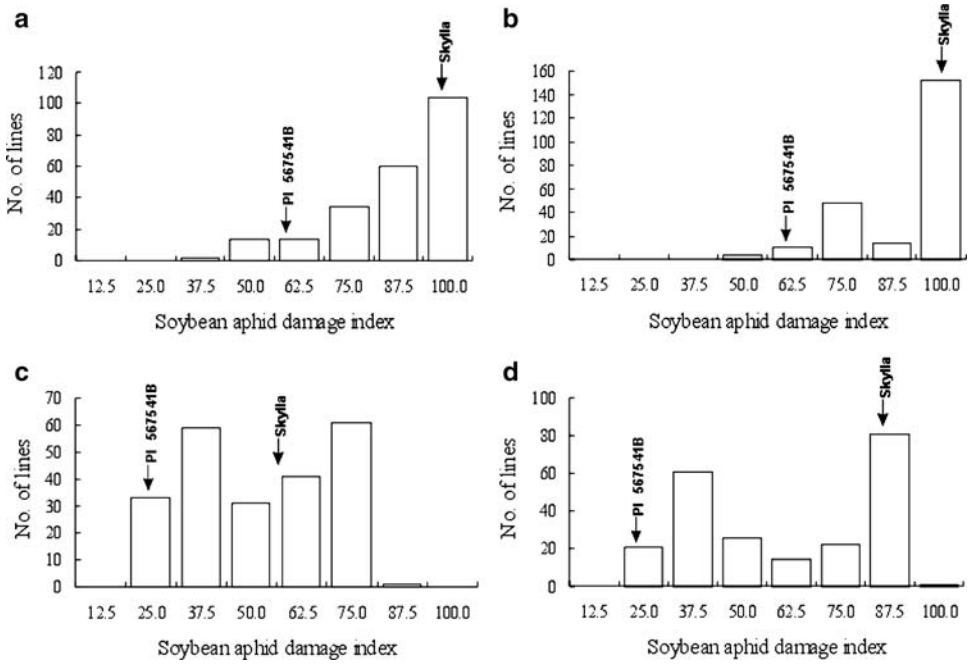

Fig. 1 Frequency distribution of soybean aphid damage index for 228 lines derived from the PI 567541B × Skylla cross. Parents are shown by *arrows*. **a** 3-week rating in the field trial, **b** 4-week rating in the field trial, **c** 3-week rating in the greenhouse trial, **d** 4-week rating in the greenhouse trial

Table 3 Summary of QTLs for soybean aphid resistance detected in the mapping population derived from the cross of PI 567541B × Skylla using the composite interval mapping method

Trials	LG[a]	Peak pos.[b]	Flanking markers[c]	94 lines[d]			228 lines		
				LOD	R^{2e}	a^f	LOD	R^2	a
Field cage									
3-week rating	M	33.4	Satt299	15.4	43.6	12.4	30.4	44.7	10.9
	F	5.1	Satt649	4.4	9.1	5.6	8.0	9.2	4.9
4-week rating	M	23.5	Satt150-Satt435	26.1	76.8	13.4	35.0	79.6	12.8
	F	5.1	Satt649	–	–	–	5.2	6.3	3.3
Greenhouse									
3-week rating	M	35.4	Satt299-Sat_244	36.2	82.9	16.3	76.2	83.2	16.1
	F	7.1	Satt649-Satt348	–	–	–	2.1	1.5	2.1
4-week rating	M	35.4	Satt299-Sat_244	40.8	85.2	22.4	87.9	87.7	22.5
	F	7.1	Satt649-Satt348	–	–	–	1.8	0.9	2.3

[a] Linkage group

[b] QTL peak position is expressed in cM and based on the analysis from the whole population (228 lines)

[c] Markers flanking the peak position or the marker at the peak position are based on the analysis from the whole population (228 lines)

[d] A subset of 94 lines. The LOD threshold for this subset of lines is 2.8. The LOD threshold for the whole population (228 lines) is 1.8. QTL not significant in the subset is marked with '–'

[e] Percentage of phenotypic variation explained by a QTL

[f] Additive effect. The positive value implies that the PI 567541B allele decreases the phenotypic value

(Table 3). The PI 567541B allele conferred aphid resistance at both loci. The QTL on LG M was consistently detected for both 3- and 4-week ratings in each trial and explained a large portion of phenotypic variations ranging from 43.6 to 85.2%. The QTL on LG F was only associated with the 3-week rating in the field trial and had much less effect (explained 9.1% of the phenotypic variation) than the one on LG M. However, using the whole population of 228 lines, these two QTLs were both consistently detected for both 3- and 4-week ratings in each trial (Table 3, Fig. 2). The QTL on LG M was closely linked to marker Satt299 or Satt435, which was only 3.6 cM away from Satt299. Its peak position was located at Satt299 or 2 cM below in most cases, but it shifted about 10 cM above for the 4-week rating in the field trial, which might be due to the limited marker saturation in the region above Satt229. However, the genomic region around Satt299 could be conservatively declared as a major QTL region. The QTL on LG F was closely linked to marker Satt649 or Satt343, which was only 1.8 cM away from Satt649. Its peak position was located at Satt649 or 2 cM below. Although the QTL on LG F was significant in the greenhouse trial, it only explained very little phenotypic variation (1.5 and 0.9% for the 3- and 4-week ratings, respectively) (Table 3).

QTL mapping using MIM

The data were also subjected to MIM analysis and the MIM results using the whole mapping population are presented in Table 4. The two QTLs identified with the CIM method were also found using the MIM method. Additionally, a significant additive × additive interaction between these two QTLs was detected using the MIM method. For the 3-week rating in the field trial, the LOD score of the QTL interaction was 10.3 and it explained 6.5% of the phenotypic variation. The two QTLs combined with their interaction explained 67.4% of the phenotypic variation. For the 4-week rating in the field trial, the two QTLs combined with their interaction explained 87.2% of the phenotypic variation, of which the interaction explained 24.7%. The QTL position on LG M was refined to 38.4 cM. For the 3-week rating in the greenhouse trial, the two QTLs combined with their interaction explained 85.6% of the phenotypic variation, of which the major QTL on LG M explained 83.8% of the phenotypic variation while the QTL on LG F and the interaction only explained a very small portion (1.6 and 0.2%). For the 4-week rating in the greenhouse trial, both QTLs were detected, but their interaction was not significant. These two QTLs explained 88.7% of the phenotypic variation, of which the major QTL on LG M explained 88.0%. Given the broad sense heritability of 0.89 and 0.93 for the 3- and 4-week ratings in the greenhouse trial, the major QTL on LG M accounted for 94.2 and 94.6% of the genetic variation, respectively.

QTL validation

Four and five markers around the QTLs identified on LGs F and M using the mapping population were genotyped on the validation population, respectively. The linkage maps for

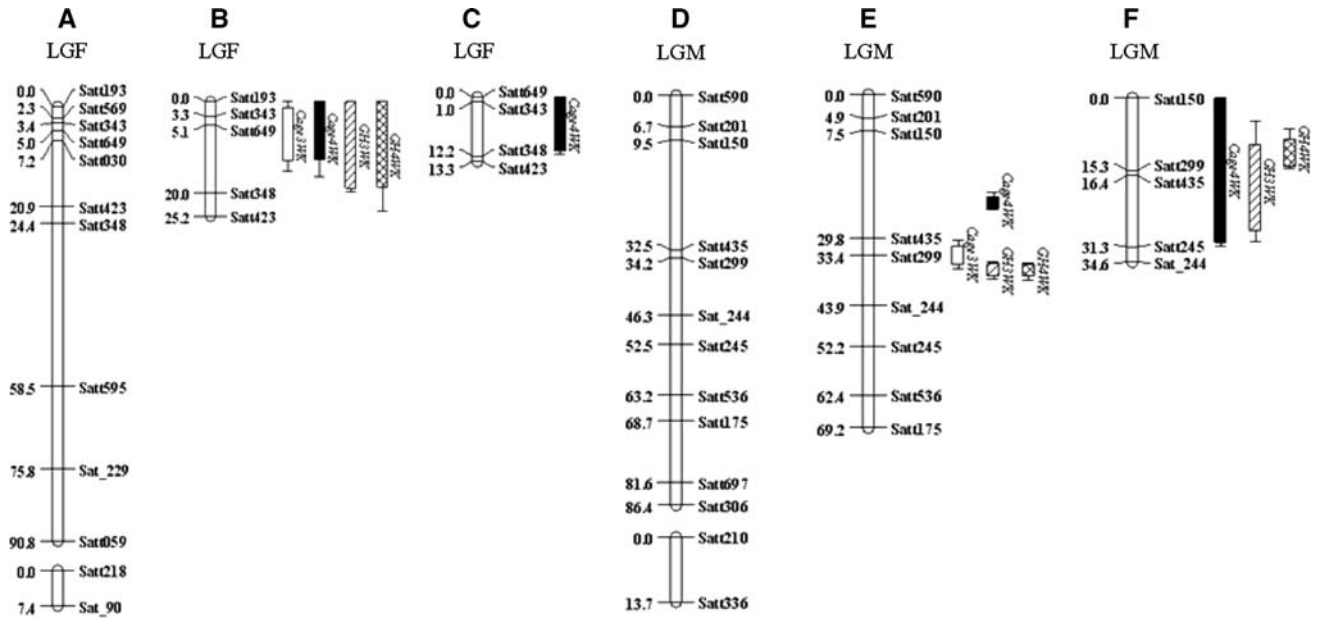

Fig. 2 Locations of soybean aphid resistance QTLs using composite interval mapping method. 1-LOD and 2-LOD support intervals of each QTL are marked by *thick* and *thin bars*, respectively. *Unfilled bars* represent QTLs for the 3-week rating in the field cage trial (*Cage3WK*). *Black bars* represent QTLs for the 4-week rating in the field cage trial (*Cage4WK*). *Bars filled with hatch lines* represent QTLs for the 3-week rating in the greenhouse trial (*GH3WK*). *Bars filled with cross lines* represent QTLs for the 4-week rating in the greenhouse trial (*GH4WK*). **a** and **d** Maps for linkage groups F and M using a subset of 94 lines selected from the mapping population PI 567541B × Skylla, the QTLs positions are not listed. **b** and **e** maps for linkage groups F and M using the whole mapping population PI 567541B × Skylla. The QTL positions based on this map are listed at its *right side*. **c** and **f** Maps for linkage groups F and M using the validation population PI 567541B × E00003. The QTL positions based on this map are listed at its *right side*

this validation population were similar as the mapping population except that the orders of a few tightly linked markers were switched (Fig. 2), which might be due to the small size of the validation population. Using both CIM and MIM methods, two QTLs were detected at similar regions as in the mapping population (Tables 4, 5; Fig. 2). Both QTLs were detected in the field trial while only the major QTL on LG M was significant in the greenhouse trial. The interaction between these two QTLs was also significant in the field trial (Table 4). The two QTLs combined with their interaction explained 95.2% of the phenotypic variation in the field trial. Hence, the results from the validation population further confirmed the QTLs identified in the mapping population.

The 50 advanced breeding lines were genotyped with the markers closely linked to the QTLs identified in the mapping population, which were Satt299, Satt435, Satt649, and Satt343. Table 6 summarized these allele effects for the aphid resistance. The DI for the breeding lines with the resistant allele (PI 567541B allele) from either Satt435 or Satt299 was significantly ($P < 0.05$) lower than the ones with the corresponding susceptible allele in either field or greenhouse trials. However, the resistant allele from Satt649 or Satt343 only had effects in the field trial and for the 3-week rating in the greenhouse trial. This is consistent with our mapping results, where the QTL on LG F explained the least phenotypic variation for the 4-week rating in the greenhouse trial. Therefore, the allele effects in these advanced breeding lines further validated the QTLs identified in the mapping population.

Band pattern analysis of markers linked to QTLs for aphid resistance

Three parental lines, PI 567541B, Skylla, and E00003, together with two other aphid resistance germplasms, Dowling and Jackson, were genotyped using markers Satt299, Satt435, Satt649, and Satt343 that were tightly linked with the QTLs identified in this study. The band patterns of the PCR products from PI 567541B were different from those in Dowling and Jackson for all the four markers (Fig. 3), indicating that PI 567541B is a different resistance source than Dowling and Jackson. However, the band patterns between Dowling and Jackson were the same for each of the four markers. None of the band patterns in the susceptible genotypes Skylla and E00003 were the same as the ones in the three resistant genotypes.

Discussion

Using 123 SSR markers and a subset of 94 lines, a linkage map of 25 linkage groups was constructed, which covered

Table 4 Summary of QTLs and their interactions for soybean aphid resistance detected in the whole mapping population derived from PI 567541B × Skylla and in the validation population derived from PI 567541B × E00003 using the multiple interval mapping method

Population	Trials	LG[a]	Peak pos.[b]	Flanking markers[c]	Genetic effect		
					LOD[d]	R^{2e}	a^f
PI 567541B × Skylla	Field cage						
	3-week rating	M	33.4	Satt299	39.6	50.3	12.0
		F	8.1	Satt649-Satt348	12.2	10.6	5.7
		Interaction			10.3	6.5	5.3
		Total				67.4	
	4-week rating	M	38.4	Satt299-Sat_244	43.8	36.7	7.5
		F	10.1	Satt649-Satt348	22.2	25.8	6.4
		Interaction			21.3	24.7	6.4
		Total				87.2	
	Greenhouse						
	3-week rating	M	35.4	Satt299-Sat_244	78.2	83.8	16.2
		F	5.2	Satt649-Satt348	5.7	1.6	2.6
		Interaction			1.8	0.2	1.4
		Total				85.6	
	4-week rating	M	35.4	Satt299-Sat_244	91.8	88.0	22.6
		F	6.1	Satt649-Satt348	3.8	0.7	2.6
PI 567541B × E00003	Field cage						
	4-week rating	M	8.1	Satt150-Satt299	21.2	30.9	6.8
		F	4.1	Satt343	21.5	29.5	7.0
		Interaction			20.4	34.7	6.8
		Total				95.2	
	Greenhouse						
	3-week rating	M	20.4	Satt435-Satt245	4.9	43.7	9.9
	4-week rating	M	13.0	Satt150-Satt299	14.4	81.4	17.0

[a] Linkage group

[b] QTL peak position is expressed in cM

[c] Markers flanking the peak position or the marker at the peak position

[d] Using the same LOD threshold as in the composite interval mapping method. The LOD threshold for the mapping population is 1.8. The LOD threshold for the validation population is 1.7

[e] Percentage of phenotypic variation explained by a QTL

[f] Additive effect. The positive value implies that the PI 567541B allele decreases the phenotypic value

Table 5 Summary of QTLs for soybean aphid resistance detected in the validation population PI 567541B × E00003 using the composite interval mapping method

Trials	LG[a]	Peak pos.[b]	Flanking markers[c]	Genetic effect		
				LOD[d]	R^{2e}	a[f]
Field cage						
4-week rating	M	16.4	Satt435	2.5	16.6	4.6
	F	1.0	Satt343	2.3	15.1	4.4
Greenhouse						
3-week rating	M	20.4	Satt435-Satt245	5.1	40.0	9.6
4-week rating	M	12.0	Satt150-Satt299	15.6	80.4	17.1

[a] Linkage group

[b] QTL peak position is expressed in cM

[c] Markers flanking the peak position or the marker at the peak position

[d] The LOD threshold is 1.7

[e] Percentage of phenotypic variation explained by a QTL

[f] Additive effect. The positive value implies that the PI 567541B allele decreases the phenotypic value

the majority of the soybean genome except for some regions where no polymorphic markers were found in this study. For LGs A2, B2, F, L, M, each LG was consisting of two unlinked segments. Based on the consensus map (Song et al. 2004); the intervals between two unlinked segments were about 30 cM or more. These intervals could be exaggerated due to the small size of the subset. Since there were no polymorphic SSR loci in these intervals for this study, it was difficult to connect these unlinked segments. Three markers, Satt194 (LG C1), Sat_130 (LG C2), and Satt353 (LG H), were unlinked because of the lack of polymorphic loci around them. These three markers are about 46 cM, 59 cM, and 56 cM away from the rest of mapped markers in this study based on the soybean consensus map (Song et al. 2004), respectively. Additionally, the top half of the LG E had no polymorphic markers (about 40 cM). In this study, a subset of lines was used to perform the whole genome scan first, which saved us a large amount of time and resources. The maps for LGs F and M constructed using the whole population were similar to the maps using the subset of 94 lines (Fig. 2), which indicates the effectiveness of the subset of lines used in this study.

In this study, two QTLs for controlling the aphid resistance in PI 567541B were identified using a mapping population that was inoculated with either the natural mixed

Table 6 Effects of alternative alleles at four soybean aphid resistance associated markers among the 50 advanced breeding lines, which were all derived from PI 567541B

LG[a]	Marker	Allele[b]	Average phenotypic value[c]		
			Field cage	GH3WK	GH4WK
M	Satt299	R (26)	54.5a	52.1a	51.4a
		S (17)	69.1b	64.7b	75.0b
	Satt435	R (33)	49.1a	51.7a	50.3a
		S (12)	75.0b	69.0b	82.2b
F	Satt649	R (27)	46.8a	52.7a	55.3a
		S (23)	68.8b	59.7b	64.2a
	Satt343	R (23)	46.8a	52.1a	55.7a
		S (24)	67.3b	59.7b	63.3a

[a] Linkage group

[b] Alternate alleles for each marker. *R* implies allele from resistant parent PI 567541B. *S* implies susceptible allele from another parent. The number in the parentheses indicates the number of the lines that had the allele

[c] Average soybean aphid damage index for the lines with the same allele. Field cage: 4-week rating in the field cage trial. GH3WK: 3-week rating in the greenhouse trial. GH4WK: 4-week rating in the greenhouse trial. Means from each pair of alleles followed by the same letter are not significant at $P = 0.05$

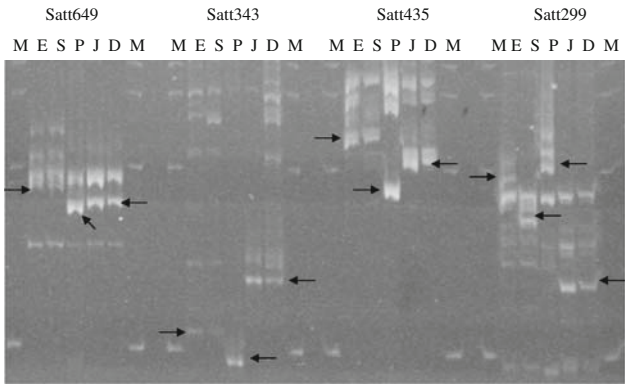

Fig. 3 PCR products amplified by SSR markers Satt299, Satt435, Satt649 and Satt343 for E00003 (E), Skylla (S), PI567541B (P), Jackson (J), and Dowling (D). *M* = 123 bp PCR marker. *Arrow* in the figure points to the particular band for differentiating among the cultivars (lines)

aphids in the field trial or the single-clone aphid in the greenhouse trial. These two QTLs were further confirmed using another independent population and some advanced breeding lines, which were derived from the same resistance source, but under various genetic backgrounds. Our results showed that these two QTLs explained most of the phenotypic variations, indicating that the aphid resistance in PI567541B was mainly controlled by these two genes. This finding is consistent with the conclusion of Mensah et al. (2008), who conducted a genetic study and suggested a two-gene model for the aphid resistance in PI 567541B. One QTL was detected on LG M in this study and explained a large portion of the phenotypic variation. This major QTL was tightly linked to marker Satt299, which has not been mapped before. In this study, Satt299 was only about 3 cM away from Satt435, which was the closely linked marker for the aphid resistance genes identified in Dowling and Jackson (Li et al. 2007). Thus, the major QTL identified in this study is coincidently located in a similar genomic region as the resistance genes in Dowling and Jackson, which indicates that they are either allelic at the same locus or different genes, but tightly linked to each other. Kim et al. (2008) recently found that PI 567541B was resistant to the aphids from Ohio while both Dowling and Jackson were susceptible. Our results suggest that the better resistance of PI 567541B compared with Dowling and Jackson could be due to one or more of the following three factors: (1) the existence of the other resistance gene, (2) a different resistance allele conferring better resistance at the same locus on LG M as the resistance genes in Dowling and Jackson, and (3) a new resistance locus conferring better resistance on LG M that is closely linked to the resistance genes in Dowling and Jackson. Understanding the allelic relationship between the major QTL in PI 567541B and the resistance genes found in Dowling and Jackson could be important for soybean breeders to determine if these genes from different resistance sources can be pyramided. However, an allelic test using progenies from the cross of PI 567541B by Dowling or Jackson might not resolve the question because of the confounding effects of the additional resistance gene in PI 567541B and its interaction with the major gene. Therefore, fine mapping or gene cloning might be necessary to determine their allelic relationship.

The other QTL was identified on LG F in this study and had a smaller effect than the one on LG M. Aphid or other insect resistance genes have not been reported in that region yet, but a QTL for leaf phosphorus content was reported at a similar region (Li et al. 2005). The relationship between leaf phosphorus content and soybean aphid resistance is unknown. However, potassium content in leaf has been related to the soybean aphid resistance in several studies (Myers et al. 2005; Myers and Gratton 2006; Walter and Difonzo 2007), which concluded that the deficiency of potassium could increase the reproduction of the soybean aphid. Phosphorus is an essential element for all the living cells; therefore, it is possible that the deficiency of phosphorus in leaf tissues might also affect the soybean aphid.

Interestingly, in the greenhouse trial, the QTL on LG F only explained very little phenotypic variation while the major QTL on LG M explained the majority of the genetic variation (over 94%). Most likely the aphid resistance in PI

567541B in the greenhouse trial is mainly controlled by a single gene, the major QTL on LG M. Although the QTL on LG F had very little effect in the greenhouse trial, it was detected and validated in the field trials. This QTL even explained a relatively large portion of the phenotypic variation in the field trial, over 25% for the 4-week rating in either mapping or validation population. This indicates that the QTL on LG F might be critical in the field resistance. The two QTLs combined with their interaction explained the majority of the phenotypic variation in the field trial. Thus, the aphid resistance in PI 567541B might be mainly controlled by these two genes under the field conditions. Therefore, this study demonstrates that the two resistance genes in PI 567541B were expressed differently in the field and greenhouse trials. Only the major gene on LG M was needed for providing aphid resistance in the greenhouse trial while both genes were required in the field trial. This difference might be due to the different inoculum used in the trials. The aphids used in the greenhouse trial were a single clone aphid, which was collected from the field in 2002 and maintained in the greenhouse thereafter. In contrast, the aphids used in the field trial were a mixture of the natural aphids collected from the infested fields during the year of the field trial. It is possible that the aphids used in the field trials had a different biotype, which caused different reactions of the resistance genes in PI 567541B. Recently, Kim et al. (2008) reported a new soybean aphid biotype in Ohio, which has overcome the resistance genes in Dowling and Jackson. In 2006, Dowling was also found susceptible to aphids in the field while PI 567541B was resistant (Mensah et al. 2007). This might be evidence of a new soybean aphid biotype occurrence in the fields of Michigan. This study was the first to map genes conferring resistance to mixed natural aphids using a field trial. Our results indicate that the QTL on LG F might have played an important role in providing resistance to an unknown new aphid biotype in Michigan. However, further investigation is warranted to determine the role of the QTL on LG F in the field aphid resistance.

Evaluation of aphid resistance usually requires artificial infestation, which is laborious and time consuming. The infestation of aphid might also be complicated by the environmental conditions, such as heavy rainfalls and strong winds. Marker-assisted selection (MAS) can be a useful and powerful tool for breeders to select aphid resistance lines even without the aphid infestation. The two aphid resistance QTLs identified in PI 567541B could be ready for MAS since they have been validated in different genetic backgrounds in this study. Moreover, the resistance genes found in PI 567541B may confer broader resistance to various biotypes of aphids than the ones in Dowling and Jackson because PI 567541B provides resistance to some new aphid biotypes that have overcome the resistance in Dowling and Jackson (Kim et al. 2008; Mensah et al. 2007).

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