

## ORIGINAL ARTICLE

# Genetic mapping of three quantitative trait loci for soybean aphid resistance in PI 567324

T-H Jun<sup>1</sup>, MA Rouf Mian<sup>2</sup> and AP Michel<sup>1</sup>

Host-plant resistance is an effective method for controlling soybean aphid (*Aphis glycines* Matsumura), the most damaging insect pest of soybean (*Glycine max* (L.) Merr.) in North America. Recently, resistant soybean lines have been discovered and at least four aphid resistance genes (*Rag1*, *Rag2*, *Rag3* and *rag4*) have been mapped on different soybean chromosomes. However, the evolution of new soybean aphid biotypes capable of defeating host-plant resistance conferred by most single genes demonstrates the need for finding germplasm with multigenic resistance to the aphid. This study was conducted to map quantitative trait loci (QTL) for aphid resistance in PI 567324. We identified two major QTL (QTL\_13\_1 and QTL\_13\_2) for aphid resistance on soybean chromosome 13 using 184 recombinant inbred lines from a 'Wyandot' × PI 567324 cross. QTL\_13\_1 was located close to the previously reported *Rag2* gene locus, and QTL\_13\_2 was close to the *rag4* locus. A minor QTL (QTL\_6\_1) was also detected on chromosome 6, where no gene for soybean aphid resistance has been reported so far. These results indicate that PI 567324 possesses oligogenic resistance to the soybean aphid. The molecular markers closely linked to the QTL reported here will be useful for development of cultivars with oligogenic resistance that are expected to provide broader and more durable resistance against soybean aphids compared with cultivars with monogenic resistance. *Heredity* (2013) **111**, 16–22; doi:10.1038/hdy.2013.10; published online 13 March 2013

**Keywords:** soybean; aphid resistance; quantitative trait loci; antixenosis; antibiosis

## INTRODUCTION

The soybean aphid (*Aphis glycines* Matsumura) is the most serious insect pest affecting soybean yields in the north-central United States, and it can be found in 30 US states and three Canadian provinces (Ragsdale *et al.*, 2011). Severe damage by heavy infestations of soybean aphid can result in reductions in seed yield as high as 50% and seed quality deterioration caused by infection with viruses vectored by aphids (Hill *et al.*, 2001; Clark and Perry, 2002).

Host-plant resistance is an effective method for controlling insect pests and reducing the use of chemical insecticides. Identification of soybean varieties and plant introductions (PIs) that show resistance to soybean aphids and characterization of the genes from the resistant sources are important early steps in the development of cultivars with resistance to soybean aphid. At least four soybean aphid resistance genes (*Rag1*, *Rag2*, *Rag3* and *rag4*) have been reported in different genomic regions on soybean chromosomes 7, 13 and 16 (Li *et al.*, 2007; Mian *et al.*, 2008a; Zhang *et al.*, 2009; Kim *et al.*, 2010; Zhang *et al.*, 2010). Soybean cultivars with a single-aphid resistance gene (for example, *Rag1* and *Rag2*) have been developed, and both genetically modified organism (GMO) and non-GMO cultivars with the *Rag1* gene are commercially available (Wiarda *et al.*, 2012). However, biotypes of soybean aphid have been discovered in North America that can overcome host-plant resistance provided by the *Rag1* and *Rag2* genes. Biotype 2, which is capable of defeating the *Rag1* gene, has been found in Ohio (Kim *et al.*, 2008), and biotype 3, which is able to colonize plants with the *Rag2* resistance gene, was discovered from the overwintering host *Frangula alnus* in Indiana (Hill *et al.*,

2010). The presence of such soybean aphid biotypes can decrease the effectiveness and sustainability of host-plant resistance provided by most single genes (R-genes). Therefore, it is necessary to find new aphid resistance sources with multigenic resistance, which can provide more durable resistance than single genes (Palloix *et al.*, 2009).

The *Rag2* gene controlling aphid resistance in PI 243540 was mapped to chromosome 13 and the aphid resistance gene in PI 200538 was also located close to the *Rag2* locus, although it is not known whether these two aphid resistance genes are identical (Mian *et al.*, 2008a; Hill *et al.*, 2009; Kim *et al.*, 2010). Furthermore, Jun *et al.* (2012) reported a major gene-controlling aphid resistance in PI 567301B, which was also mapped to a position near the *Rag2* gene. Recently, Kim *et al.* (2010) conducted fine mapping of the aphid resistance gene in PI 200538 to a 54-kb interval, and the fine mapping of the *Rag2* gene in PI 243540 and the resistance gene in PI 567301B are currently in progress (unpublished data). Identification of the exact location for each resistance gene can provide information useful for determining genetic relationships. Both PI 243540 and PI 200538 showed strong antibiosis resistance, whereas PI 567301B was found to have antixenosis resistance (Kim *et al.*, 2008; Mian *et al.*, 2008b). Interestingly, a recent study revealed that detached leaves of PI 243540 maintained their resistance against biotype 2, whereas detached leaves from PI 567301B lost their aphid resistance in the same study (Michel *et al.*, 2010).

Aphid resistance in soybean can also be controlled by minor genes or quantitative trait loci (QTL). Zhang *et al.* (2009) reported that the aphid resistance in PI 567541B was controlled by two QTL located on

<sup>1</sup>Department of Entomology, The Ohio State University/OARDC, Wooster, OH, USA and <sup>2</sup>USDA-ARS, Department of Horticulture and Crop Science, The Ohio State University, Wooster, OH, USA  
Correspondence: Dr MA Rouf Mian, USDA-ARS, Department of Horticulture and Crop Science, The Ohio State University, 1680 Madison Avenue, Wooster, OH 44691, USA.  
E-mail: mian.3@osu.edu

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chromosomes 7 and 13. In a previous study, PI 567324 showed resistance to both biotypes 1 and 2 of the soybean aphid (Mian *et al.*, 2008b). The objective of this study was to map aphid resistance QTL in PI 567324.

## MATERIALS AND METHODS

### Mapping population and aphid resistance evaluation

One hundred and eighty-four F<sub>7</sub>-derived recombinant inbred lines (RILs) from a cross of 'Wyandot' × PI 567324 and the two parental lines were used in the study. Wyandot is a maturity group II soybean cultivar developed in Ohio and it is susceptible to the soybean aphid, whereas PI 567324 is a maturity group IV accession from China that has antixenosis resistance against the biotypes 1 and 2 of the soybean aphid (Mian *et al.*, 2008b).

Evaluation of soybean aphid resistance was performed using choice tests in both greenhouse and field environments at the Ohio Agricultural Research and Development Center (OARDC), Wooster, OH, USA. The colony of biotype 2 soybean aphid used in this study was established in a growth chamber by collecting aphids from a naturally infested soybean field during the summer of 2005. The colony had been maintained on seedlings of soybean cultivar Williams 82 in growth chambers at temperatures between 22 and 24 °C, with 15 h of light daily, and 60–70% relative humidity (Mian *et al.*, 2008b).

In the summer of 2010, a field aphid test was performed with 184 F<sub>7</sub> RILs and the parents of the Wyandot × PI 567324 population in a large aphid-proof polypropylene cage with a 0.49-mm size mesh (Redwood Empire Awning Co., Santa Rosa, CA, USA). The cage helped to prevent aphids from escaping and inhibited the entry of predators into the cage. The soil type of the field was Riddles silt loam. The F<sub>7</sub> RIL seeds developed by single-seed decent were planted with 10-cm spacing between plants and 76 cm between rows. Each of the F<sub>7</sub> 184 RIL was represented by a single plant grown from a F<sub>7</sub> seed. Ten replications of each parent were randomly placed among the RILs. Each seedling was manually infested at the V1 stage (Fehr *et al.*, 1971) with five wingless adult aphids following installation of the cage (Jun *et al.*, 2012). In the spring of 2011, the greenhouse aphid screening was performed in a greenhouse with 184 F<sub>7,8</sub> families derived from the same 184 RILs tested in the field and their parents. Eight seeds of each RIL family were planted in a 10.16-cm diameter × 8.89 cm deep plastic pot and were thinned to four seedlings at the unifoliate stage of growth. The pots were organized on a rectangular bench top to touch the neighboring pots along the rows as well as columns. Ten pots of each of the parents were also included and randomly distributed among the RIL families as checks for reference points for evaluation of aphid resistance. Five wingless adult aphids were transferred to each plant at the V1 stage. The greenhouse was maintained at approximately 24/20 °C day/night temperatures, and the plants were kept under 15-h light daily (Mian *et al.*, 2008b). Aphid resistance for each plant was evaluated at 2 and 4 weeks after infestation in both the field and greenhouse tests, resulting in two sets of field scores (Field2W and Field4W) and two sets of greenhouse scores (GH2W and GH4W) based on the criteria for aphid resistance used by Jun *et al.* (2012). Although ratings at both time periods used a five-point scale, the calibration of the scale differed. At 2 weeks after aphid infestation, each line was rated by assigning aphid density scores ranging from 1 to 5, where 1 = <10 aphids per plant, 2 = 10–25 aphids per plant, 3 = 25–50 aphids per plant, 4 = 50–100 aphids per plant and 5 = >100 aphids per plant. Aphid scores at 4 weeks were recorded using a scale in which 1 = <25 aphids per plant, 2 = 25–100 aphids per plant, 3 = 100–200 aphids per plant, 4 = 200–500 aphids per plant and 5 = >500 aphids per plant.

### DNA extraction and molecular marker genotyping

Before aphid infestation, young leaves from each RIL and the parental seedlings in the field cage were collected in 2-ml tubes and were immediately frozen in liquid nitrogen. The frozen leaf tissues were lyophilized in a freeze dryer (SP Industries Inc., Stone Ridge, NY, USA) for 3 days and then were ground into fine powder using a Mixer Mill (Retsch, Model MM301, Hannover, Germany). The genomic DNA was extracted using a slightly modified CTAB protocol (Saghai-Marooif *et al.*, 1984), and was treated with RNase enzyme (QIAGEN Inc., Valencia, CA, USA) at 37 °C for 1 h.

The DNA quality was checked by a 1% agarose gel electrophoresis with ethidium bromide.

For the genetic map construction, a total of 184 RILs and the two parental lines were genotyped using a custom Oligo Pool Assay containing 384 single-nucleotide polymorphisms (SNPs) selected from the Universal Soy Linkage Panel (USLP) 1.0 developed by Hyten *et al.* (2010) at the Molecular and Cellular Imaging Center (MCIC) at OARDC, Wooster, OH, USA. The genotype data for each SNP marker were analyzed using the GenomeStudio Data analysis software (Illumina Inc., San Diego, CA, USA), which generates homozygous and heterozygous genotype clusters. The SSR marker analysis was performed to saturate three candidate QTL regions on chromosomes 6 and 13. The primer information such as sequences and genomic locations of the SSR markers was obtained from the BARCSOYSSR\_1.0 soybean SSR database (Song *et al.*, 2010). PCR reactions were performed in 20 µl reactions containing 25–50 ng of template DNA, 1 × PCR buffer, 2.5 mM Mg<sup>2+</sup>, 200 µM deoxyribonucleotide triphosphates, 100 nM of forward and reverse primers, and 1.0 units of *Taq* DNA polymerase (GenScript USA Inc., Piscataway, NJ, USA). The PCR cycles consisted of initial denaturation at 95 °C for 5 min, followed by 32 cycles of 20 s denaturation at 94 °C, 20 s annealing temperature between 50 and 60 °C depending on the optimum annealing temperature for each primer pair, and 20 s extension at 72 °C. The PCR reaction finished with a final 10 min extension at 72 °C on a thermal cycler (Techne Inc., model TC-512, Burlington, NJ, USA). The PCR fragments were resolved on a 4% 3:1 agarose gel (RPI Corp., Mount Prospect, IL, USA).

### Statistical analysis

The aphid score data from the field and greenhouse tests were analyzed using the PROC GLM procedure of SAS 9.2 (SAS Institute Inc., Cary, NC, USA). Pearson correlation coefficients among the four aphid scores (Field2W, Field4W, GH2W and GH4W) were calculated using the PROC CORR function of SAS 9.2. Association between each marker genotype and phenotypic scores was tested by single-factor analysis of variance (single-factor ANOVA) using the PROC GLM procedure of SAS 9.2 at the 0.05 significance level. The single-factor ANOVA was separately conducted on the four sets of aphid scores with the SNP genotype data from 184 RILs of the mapping population.

The linkage map was constructed with the maximum likelihood mapping function using JoinMap 4.0 (Van Ooijen, 2006). A logarithm (base 10) of the odds (LOD) score of 3.0 as a significance threshold and a maximum genetic distance of 50 cM were used to group all markers to chromosomes (or linkage groups). Identification of QTL associated with soybean aphid resistance was initially conducted using interval mapping methods, followed by composite interval mapping (CIM) with MapQTL 5.0 software (Van Ooijen, 2004). A permutation test with 1000 replicates was performed to empirically determine the genome-wide LOD threshold at the 5% probability level for each phenotypic trait individually. The phenotypic variation and additive effect explained by each locus were estimated from the value expressed by the QTL peaks obtained from CIM. The LOD graphs for significant QTL were generated using MapChart 2.2 software with a 2-LOD support interval (Voorrips, 2002).

Allelic effects between two QTL identified on chromosome 13 were estimated using the PROC GLM procedure of SAS 9.2, and means were separated by least significant difference at a 5% significance level. Additive × additive (AA) interaction effects were calculated using QTLNetwork v2.1 (Yang *et al.*, 2008). An experiment-wise Type I error rate of  $\alpha = 0.05$  was used for putative QTL detection, and QTL effects were estimated using the Markov chain Monte Carlo method at the 0.05 significance level. Critical F-values were obtained based on permutation testing using 1000 permutations.

### No-choice assays with detached leaf and whole plant

To compare the aphid resistance in PI 567324 with the *Rag2* aphid resistance, no-choice (antibiosis) aphid resistance tests based on two types of assays (detached leaves and whole plants) were performed with four genotypes (Wyandot, PI 567324, PI 243540 and PI 200538) in a USDA greenhouse at the OARDC in Wooster, OH, USA. The greenhouse was maintained at temperatures of 24/20 °C day/night, under a 15-h light photoperiod. For the detached leaf test, a single leaf was placed on moist filter paper in a Petri dish. For the whole plants, a single leaf on an intact plant was locked in a 'clip cage' to

prevent aphids from escaping. Cages were 42 mm in width, 55 mm in height and 18 mm thick, and had one hole covered with fine mesh to allow air circulation. Three wingless adult aphids were manually transferred to each single leaf before locking the clip cage. The experiment was performed for 6 days in three replications. The number of aphids from detached leaf and whole plant tests was visually counted. The aphid data were analyzed using the PROC GLM procedure of SAS 9.2, and means were separated by least significant difference at a 5% significance level.

## RESULTS

### Evaluation of phenotypic trait

The frequency distributions of aphid scores for the 184 RILs from the cross between Wyandot and PI 567324 at each screening time are shown in Figure 1. Scores ranged from 1 to 5 in both field (Field2W and Field4W) and greenhouse (GH2W and GH4W) scorings. The average aphid scores for the two parents were significantly different in all trials at the 0.0001 probability level (Table 1). There were highly significant ( $P < 0.001$ ) correlations between corresponding field and greenhouse aphid scores with Pearson correlation coefficients ( $r$ ) values ranging between 0.73 and 0.89. The frequency distributions of aphid scores for the Field2W and GH2W data sets were somewhat different, revealing that the Field2W trial had relatively higher mean aphid scores and a greater ratio of susceptible plants compared with the GH2W scoring, whereas the frequency distributions for the Field4W and GH4W aphid scores were similar (Table 1 and Figure 1). These results indicate that the field environment might be more favorable for aphid growth than greenhouse conditions at the early stage.

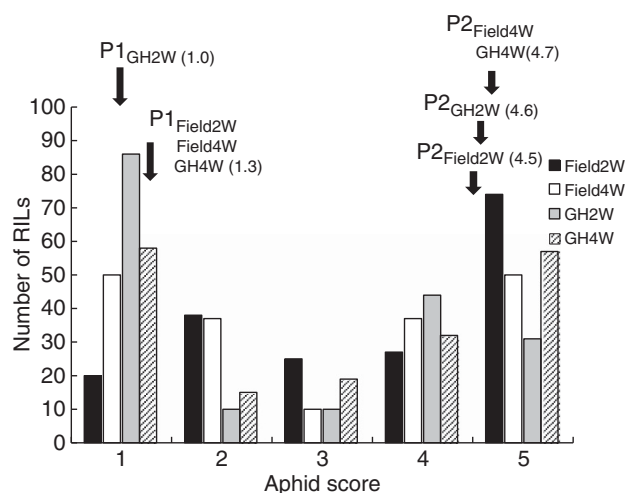
### Genetic linkage analysis

A total of 175 (45.6%) of the 384 SNPs tested were polymorphic between Wyandot and PI 567324, and these polymorphic SNPs segregated among the 184 RILs of the population. Among the 175 polymorphic SNPs, 170 SNP loci that had less than 10% missing data and that showed a good fit to the 1:1 segregation ratio expected for an advanced RIL population were used for construction of the genetic map for the population. A total of 34 linkage groups encompassing all 20

chromosomes of soybean were created, spanning approximately 1505 cM (Supplementary Figure 1). The number of SNPs mapped per chromosome ranged from 4 to 12, with an average of eight SNPs. The mean chromosome length was 75.3 cM, with a range of 48.2–142.3 cM, and the average genetic distance between SNP markers was 9.4 cM, with a range from 0.0–44.1 cM. The marker orders for the SNPs mapped in this population were in agreement with those of the Soybean Consensus Map 4.0 (Hyten *et al.*, 2010), except for two regions which showed inverted marker order: the BARC-020735-04704—BARC-029491-06207 interval on chromosome 10 and the BARC-042299-08241—BARC-035379-07181 interval on chromosome 11.

### QTL analysis

Three chromosomal regions were found to be significantly associated with soybean aphid resistance using single-factor ANOVA; one was found on chromosome 6 and the other two were on chromosome 13. The two SNPs (BARC-025707-05008 and BARC-051071-10973), which were located about 5.4 cM apart on chromosome 6, based on the Soybean Consensus Map 4.0 (Hyten *et al.*, 2010), showed a significant association ( $P < 0.05$ ) with aphid resistance scores for the two field tests. The genomic region flanked by the two significant SNPs was saturated by genotyping 184 RILs using eight SSR markers from the BARCSOYSSR\_1.0 soybean SSR database (Figure 2). Reanalysis revealed that SSR marker BARCSOYSSR\_06\_0998 (QTL\_6\_1) was significantly associated with aphid resistance in all of the assays ( $P < 0.01$ ), explaining 4.2–11.3% of the variation in the aphid resistance scores (Table 2). This marker accounted for 11.3 and 7.7% of the phenotypic variation in the Field2W and Field4W data sets, respectively ( $P < 0.001$ ), compared with 5.1% and 4.2% for GH2W and GH4W, respectively ( $P < 0.01$ ; Table 2). In contrast, two SNPs (BARC-058031-15072 and BARC-024045-04714) on chromosome 13, which are approximately 37.3 cM apart according to the Soybean Consensus Map 4.0, showed highly significant association ( $P < 0.0001$ ) with aphid resistance for all four aphid scoring times on the basis of single-factor ANOVA. The allele for soybean aphid resistance at the QTL on chromosome 6 (QTL\_6\_1) was contributed by the susceptible parent (Wyandot), whereas the alleles for aphid resistance at the two QTL on chromosome 13 (QTL\_13\_1 and QTL\_13\_2) were contributed by the resistant parent (PI 567324; Table 2). Eleven SSR markers on chromosome 13 were used for construction of a dense genetic map; three and eight markers were mapped to two separate genomic regions near BARC-058031-15072



**Figure 1** Frequency distribution of 184 recombinant inbred lines (RILs) derived from the Wyandot × PI 567324 cross for soybean aphid resistance in the field and greenhouse tests: Field2W, aphid scores after 2 weeks in the field cage; Field4W, aphid scores after 4 weeks in the field cage; GH2W, aphid scores after 2 weeks in the greenhouse; and GH4W, aphid scores after 4 weeks in the greenhouse. The means of the aphid scores for PI 567324 (P1) and Wyandot (P2) were indicated by arrows.

**Table 1** Summary of aphid scores in the field and greenhouse tests using 184 RILs and the two parents

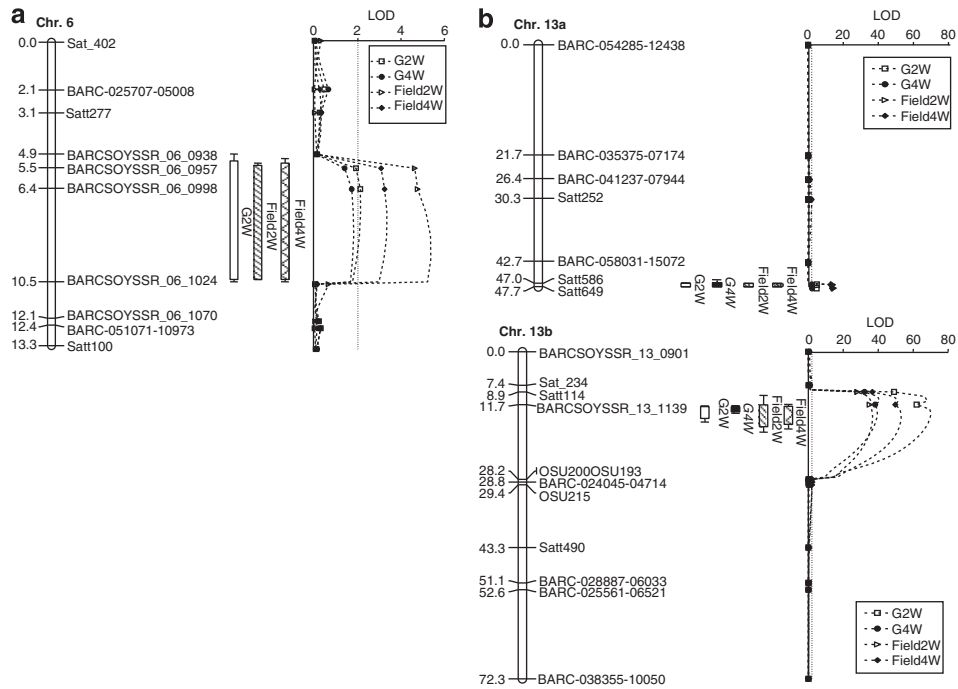
Phenotype <sup>a</sup>	Parents <sup>b</sup>		RIL population	
	PI 567324	Wyandot	Mean	s.d.
Field2W	1.3a	4.5b	3.53	1.46
Field4W	1.3a	4.7b	3.00	1.61
GH2W	1.0a	4.6b	2.58	1.65
GH4W	1.3a	4.7b	3.08	1.68

Abbreviation: RIL, recombinant inbred line.

Aphid scores ranged from 1 to 5 for all of phenotypes: 1 = <10 aphids, 3 = 25–50 aphids and 5 = >100 aphids per plant for aphid score at 2 weeks, and 1 = <25 aphids, 3 = 100–200 aphids and 5 = >500 aphids per plant for aphid score at 4 weeks.

<sup>a</sup>Field2W: aphid scores after 2 weeks in the field cage, Field4W: aphid scores after 4 weeks in the field cage, GH2W: aphid scores after 2 weeks in the greenhouse, and GH4W: aphid scores after 4 weeks in the greenhouse.

<sup>b</sup>Means followed by different letters for each phenotype are significantly different at  $P < 0.0001$ .



**Figure 2** Localization of quantitative trait loci (QTL) for soybean aphid resistance on chromosomes 6 and 13 by composite interval mapping QTL analysis using 184 recombinant inbred lines (RILs). (a and b) show maps spanning the QTL regions on chromosomes 6 and 13 using a logarithm (base 10) of the odds (LOD) score of 3.0 as a threshold. In QTL LOD graphs, 95% genome-wide LOD significant threshold value is 2.0 (shown by the dotted line).

**Table 2** Summary of QTL associated with soybean aphid resistance identified in the 184 RILs of the Wyandot × PI 567324 population using single-factor ANOVA and composite interval mapping methods

Phenotype <sup>a</sup>	Marker <sup>b</sup>	Chr.	QTL name	Position (bp) <sup>c</sup>	Single-factor ANOVA		Composite interval mapping		
					R <sup>2d</sup>	P	LOD <sup>e</sup>	R <sup>2d</sup>	a <sup>f</sup>
Field2W	BARCSOYSSR_06_0998	6	QTL_6_1	18713522	11.3	<0.0001	4.8	11.6	-0.50
	BARCSOYSSR_13_1139	13	QTL_13_1	29274967	58.1	<0.0001	35.9	42.7	0.99
Field4W	Satt649	13	QTL_13_2	12953321	29.7	<0.0001	14.7	13.1	0.55
	BARCSOYSSR_06_0998	6	QTL_6_1	18713522	7.7	0.0002	3.2	8.0	-0.46
GH2W	BARCSOYSSR_13_1139	13	QTL_13_1	29274967	70.3	<0.0001	50.3	54.4	1.23
	Satt649	13	QTL_13_2	12953321	25.3	<0.0001	14.2	9.3	0.51
GH4W	BARCSOYSSR_06_0998	6	QTL_6_1	18713522	5.1	0.0027	2.1	5.4	-0.39
	BARCSOYSSR_13_1139	13	QTL_13_1	29274967	80.6	<0.0001	63.5	70.6	1.43
GH4W	Satt649	13	QTL_13_2	12953321	13.4	<0.0001	4.5	2.1	0.25
	BARCSOYSSR_06_0998	6	QTL_6_1	18713522	4.2	0.0068	1.7	4.4	-0.35
GH4W	BARCSOYSSR_13_1139	13	QTL_13_1	29274967	65.1	<0.0001	38.9	56.9	1.30
	Satt649	13	QTL_13_2	12953321	11.4	<0.0001	2.4	2.1	0.25

Abbreviations: ANOVA, analysis of variance; QTL, quantitative trait loci; RIL, recombinant inbred line.  
<sup>a</sup>Field2W: aphid scores after 2 weeks in the field cage, Field4W: aphid scores after 4 weeks in the field cage, GH2W: aphid scores after 2 weeks in the greenhouse, and GH4W: aphid scores after 4 weeks in the greenhouse.  
<sup>b</sup>Marker that revealed the highest R<sup>2</sup> value among significant markers.  
<sup>c</sup>Start position of significant markers is based on the Soybean Consensus Map 4.0 and BARCSOYSSR\_1.0 soybean SSR database.  
<sup>d</sup>Percentage of phenotypic variation explained by the QTL detected in our mapping population.  
<sup>e</sup>Genome-wide LOD thresholds for 184 RILs is 2.0.  
<sup>f</sup>Additive effect. A positive value indicates that resistance alleles were derived from the resistant parent PI 567324, whereas a negative symbol implies that resistance alleles were contributed by the susceptible parent Wyandot.

and BARC-024045-04714, respectively (Figure 2). SSR markers BARCSOYSSR\_13\_1139 (QTL\_13\_1) and Satt649 (QTL\_13\_2) had the highest levels of association ( $P < 0.0001$ ), accounting for 58.1–80.6% and 11.4–29.7% of phenotypic variation in the aphid resistance scores, respectively (Table 2). The peaks of the two QTL were estimated to be located 16 321 646 bp (~20 cM) apart on the same

chromosome, based on the BARCSOYSSR\_1.0 soybean SSR database and the Soybean Consensus Map 4.0.

The QTL mapping with the interval mapping and CIM methods was performed using the saturated linkage maps for chromosomes 6 and 13, where significant association with aphid resistance had been detected by single-factor ANOVA. Two different QTL were mapped to

chromosome 13 in the same chromosomal regions identified by the single-marker analysis (Figure 2). QTL\_13\_1 explained a large amount of phenotypic variation, ranging from 42.7 to 70.6% (Table 2). The LOD scores from the QTL peaks for all aphid scoring times were significantly higher than the genome-wide LOD threshold value of 2.0 obtained by permutation testing (Figure 2). QTL\_13\_2 accounted for 2.1–13.1% of phenotypic variation. On chromosome 6, one QTL was mapped in the interval between the markers BARCSOYSSR\_06\_0938 and BARCSOYSSR\_06\_1024, except with the GH4W aphid score, for which the LOD threshold was below 2.0 (Table 2 and Figure 2).

The mean aphid scores for the four possible allelic combinations of the two QTL identified on chromosome 13 are shown in Table 3. The genotype  $R_1R_1R_2R_2$ , with homozygous resistant alleles at both QTL, provided greater aphid resistance than any other combination of alleles ( $P < 0.05$ ), indicating a dominant additive (A) effect of the two loci. On the contrary, only one significant AA interaction was detected involving QTL\_13\_1 and QTL\_13\_2 (Table 4). However, the epistasis effect for soybean aphid resistance was significant only under the field conditions, explaining 6.7% and 2.0% of the phenotype variation for Field2W and Field4W, respectively. In both field trials, the negative epistatic effects of AA interaction revealed that two loci with homozygous alleles from the resistant parent PI 567324 could decrease aphid densities, which was consistent in direction with the additive (A) effects of both QTL.

## DISCUSSION

We performed molecular mapping to find soybean aphid-resistant loci in a RIL population derived from a cross of Wyandot  $\times$  PI

**Table 3 Summary of allelic additive (A) effects according to all four possible genotypes**

Genotype <sup>a</sup>		Aphid resistance scores <sup>b</sup>			
QTL_13_1	QTL_13_2	Field2W	Field4W	GH2W	GH4W
$r_1r_1$	$r_2r_2$	4.87 (46)a	4.72 (46)a	4.37 (46)a	4.74 (46)a
$r_1r_1$	$R_2R_2$	4.58 (33)a	4.12 (33)b	3.97 (33)b	4.33 (33)a
$R_1R_1$	$r_2r_2$	3.75 (32)b	2.69 (32)c	1.65 (31)c	2.29 (31)b
$R_1R_1$	$R_2R_2$	1.89 (64)c	1.31 (64)d	1.03 (63)d	1.63 (63)c

<sup>a</sup>QTL\_13\_1: BARCSOYSSR\_13\_1139 on chromosome 13,  $r_1$ : susceptible allele, and  $R_1$ : resistant allele; QTL\_13\_2: Satt649 on chromosome 13,  $r_2$ : susceptible allele, and  $R_2$ : resistant allele.

<sup>b</sup>Field2W: aphid scores after 2 weeks in the field cage, Field4W: aphid scores after 4 weeks in the field cage, GH2W: aphid scores after 2 weeks in the greenhouse, and GH4W: aphid scores after 4 weeks in the greenhouse. Means followed by the same letters in the phenotype columns are not significantly different by the least significant difference at  $P = 0.05$ . The numbers in parentheses refer to number of plants for each genotype generated by combination of alleles from two quantitative trait loci.

**Table 4 Significant additive-by-additive (AA) interaction effects for soybean aphid resistance between two major QTL detected on chromosome 13 in Wyandot  $\times$  PI567324 RILs population**

Traits <sup>a</sup>	QTL <sub>i</sub>	Interval	QTL <sub>j</sub>	Interval	AA <sub>ij</sub>		$h^2$ aa (%) <sup>f</sup>
					Effect <sup>b</sup>	P-value	
Field2W	QTL_13_1	BARCSOYSSR_13_1139~BARCSOYSSR_13_1343	QTL_13_2	Satt586- Satt649	-0.44	<0.001	6.7
Field4W	QTL_13_1	BARCSOYSSR_13_1139~BARCSOYSSR_13_1343	QTL_13_2	Satt586- Satt649	-0.26	<0.001	2.0

Abbreviations: QTL, quantitative trait loci; RIL, recombinant inbred line.

<sup>a</sup>Field2W: 2 weeks scoring in the field cage, Field4W: 4 weeks scoring in the field cage.

<sup>b</sup>AA<sub>ij</sub> is the effect of additive-by-additive interaction between QTL<sub>i</sub> and QTL<sub>j</sub>. Negative value indicates that PI 567324 two-locus genotypes have a positive effect to decrease the phenotypic value of aphid score.

<sup>c</sup>Phenotype variation that is explained by AA.

567324. In this study, three QTL associated with soybean aphid resistance in PI 567324 were identified using both single-marker analysis and the CIM method. Two QTL were localized on chromosome 13, but in different regions of that chromosome. The centromeric region (15 850 000–16 000 000 bp on chromosome 13) is located between the two QTL, approximately 3 cM from Satt649 based on the Soybean Consensus Map 4.0 and the Williams 82 soybean genomic sequences (<http://www.phytozome.net>). In our genetic map, the location of the centromere resulted in two unlinked chromosomal sections due to lack of polymorphic molecular markers. Ott *et al.* (2011) reported that centromeric regions of soybean chromosomes are characterized by low densities of markers and genes, which can make it difficult to map these regions.

The soybean aphid screening of the RIL population in the field was conducted on the F<sub>7</sub> plants where each F<sub>7</sub> plant represented an RIL, thus there was no statistical replication of the genotypes in this experiment. However, the aphid scores were collected twice—at 2 weeks then at 4 weeks after aphid infestation. The follow-up confirmation tests were conducted in a greenhouse using the average aphid scores from four plants for each F<sub>7,8</sub> family in a plastic pot. Again, the aphid scores were collected twice as mentioned above. The data from each of the four times were analyzed separately for QTL detection and the results consistently indicated the existence of the three QTL reported here (Table 2 and Figure 2). The aphid screening protocols used in this study are consistent with other recent publications on mapping aphid resistance in soybean (Li *et al.*, 2007; Mian *et al.*, 2008a; Zhang *et al.*, 2009, 2010; Jun *et al.*, 2012). The four different sets of aphid scores from two different environments served as repeats of the experiment. The positions of the two QTL on chromosome 13 were coincident to those of resistance genes previously detected in other aphid-resistant PIs. QTL\_13\_1 was very close to the *Rag2* gene (Mian *et al.*, 2008a; Kim *et al.*, 2010), and QTL\_13\_2 was close to the *rag4* gene for aphid resistance in PI 567541B (Zhang *et al.*, 2009). However, Mian *et al.* (2008b) reported that PI 567324 was moderately resistant to aphid biotypes 1 and 2, and Zhang *et al.* (2009, 2010) showed that the gene near Satt649 might provide resistance to an unknown aphid biotype that is unlike biotypes 1 and 2. Thus, the combination of the two chromosome 13 genes found in PI 567324 may provide resistance against a broader range of aphid biotypes.

PI 567324 has antixenosis (that is, non-preference) resistance, whereas PI 243540 possesses strong antibiosis resistance (Mian *et al.*, 2008b). In a recent study, Jun *et al.* (2012) reported that the mode of aphid resistance in PI 567301B is not the same as that in PI 243540, based on the results of detached leaf and whole plant no-choice assays, although two resistance genes were mapped to the same genomic region on chromosome 13. Interestingly, the same no-choice

assays performed with PI 567324 similarly revealed that the aphid resistance in PI 567324 was only active in whole plants, whereas two sources of *Rag2* maintained their resistance in both detached leaf and whole plant assays (Table 5). These results indicate that the QTL\_13\_1 locus identified in PI 567324 might be different from the *Rag2* locus, but it is not yet known whether QTL\_13\_1 in PI 567324 is different from the aphid resistance locus in PI 567301B on chromosome 13.

The putative QTL on chromosome 6 (QTL\_6\_1) was also significant in both field and greenhouse conditions based on the results of single-factor ANOVA. This locus has not been detected in the previous genetic studies for soybean aphid resistance. As mentioned in the results section, the allele for aphid resistance was contributed by the susceptible parent at this QTL. However, it is not unusual for susceptible soybean line to carry one or more QTL for insect resistance. Boerma and Walker (2005) in a review of insect resistance QTL in soybean, listed a number of QTL at which the alleles for resistance to the corn earworm (*Helicoverpa zea* (Boddie)) were contributed by the susceptible cultivar 'Cobb'. Interestingly, however, the corn earworm resistance QTL CEW8-3 (Terry *et al.*, 2000; Soybase; <http://www.soybase.org/>) was mapped on chromosome 6 within 1cM of this minor aphid resistance QTL, based on information from the Soybean Consensus Map 4.0 and BARC-SOYSSR\_1.0 soybean SSR database, suggesting the possibility of a common candidate resistance gene controlling resistance to insect pests from different orders. In addition, the Asian soybean rust (*Phakopsora pachyrhizi*) resistance genes *Rpp3* and *Rpp?* (Hyuuga; Hyten *et al.*, 2009; Monteros *et al.*, 2010) were mapped approximately 6cM away from the estimated location of aphid resistance QTL\_6\_1. However, this novel candidate QTL revealed some inconsistency across environmental conditions.

In the present study, a significant AA interaction was detected in only one combination of loci. This involved the main effects of QTL\_13\_1 and QTL\_13\_2, and no significant epistasis was detected in the absence of significant main effects. The epistatic effect for aphid resistance was only significant under the field conditions, indicating that the AA effect might be influenced by environment. Malmberg *et al.* (2005) reported that epistatic interaction QTL for fitness-related traits can vary depending on different growth conditions from epistatic QTL analysis using RILs of *Arabidopsis thaliana* grown in the field and greenhouse. Besides, Zhuang *et al.* (2002) suggested that the epistatic effects might be underestimated or go undetected due to increases in main effects in different environmental conditions. In this study, a slightly lower level of aphid pressure in the greenhouse relative to the field might have resulted in an increased effect from QTL\_13\_1, but a decreased effect from the smaller QTL\_13\_2, resulting in the loss of a significant epistatic effect between the two QTL.

**Table 5 Summary of DLT and WPT no-choice tests for soybean aphid resistance with four genotypes and biotype 2 of soybean aphid**

Genotype <sup>a</sup>	Number of aphids (DLT) <sup>b</sup>	Number of aphids (WPT) <sup>b</sup>
Wyandot	46.3a	55.3a
PI 567324	37.7b	5.0b
PI 243540	3.7c	0.0b
PI 200538	4.0c	0.0b

Abbreviations: DLT, detached leaf test; WPT, whole plant test.

<sup>a</sup>PI 243540 and PI 200538 are aphid-resistant soybean varieties with *Rag2* gene, revealing strong antibiosis resistance to biotype 2.

<sup>b</sup>Means followed by the same letters in the DLT or WPT columns are not significantly different at  $P=0.05$ .

In conclusion, we identified two major QTL for resistance to the soybean aphid on chromosome 13 using the 184 RILs from a cross between Wyandot and PI 567324. The effects of the two QTL were primarily additive when there were homozygous resistant alleles at both loci. In addition, a minor QTL (QTL\_6\_1) was detected on chromosome 6, where no other loci associated with soybean aphid resistance have been reported yet. These results indicate that PI 567324 possesses oligogenic resistance to the soybean aphid. The molecular markers closely linked to the QTL reported here will be useful for development of cultivars with oligogenic resistance to biotype 1 and 2 of soybean aphids. Cultivars with oligogenic resistance are expected to provide broader and more durable resistance against soybean aphids compared with cultivars with a single gene for aphid resistance.

#### DATA ARCHIVING

Data deposited in the Dryad repository: doi:10.5061/dryad.df47h.

#### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Notes: Trade and manufacturers' names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.

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