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ORIGINAL PAPER

Genetic mapping revealed two loci for soybean aphid resistance in PI 567301B

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Abstract The soybean aphid (*Aphis glycines* Matsumura) is the most damaging insect pest of soybean [*Glycine max* (L.) Merr.] in North America. New soybean aphid biotypes have been evolving quickly and at least three confirmed biotypes have been reported in USA. These biotypes are capable of defeating most known aphid resistant soybean genes indicating the need for identification of new genes. Plant Introduction (PI) 567301B was earlier identified to have antixenosis resistance against biotype 1 and 2 of the soybean aphid. Two hundred and three $F_{7:9}$ recombinant inbred lines (RILs) developed from a cross of soybean aphid susceptible cultivar Wyandot and resistant PI 567301B were used for mapping aphid resistance genes using the quantitative trait loci (QTL) mapping approach. A subset of 94 RILs and 516 polymorphic SNP makers were used to construct a genome-wide molecular linkage map. Two candidate QTL regions for aphid resistance were identified on this linkage map. Fine mapping of the QTL regions was conducted with SSR markers using all 203 RILs. A major gene on chromosome 13 was mapped near the previously identified *Rag*2 gene. However, an earlier study revealed that the detached leaves of PI 567301B had no resistance against the soybean aphids while the detached leaves of PI 243540 (source of *Rag*2) maintained aphid resistance. These results and the earlier finding that PI 243540 showed antibiosis resistance and PI 567301B showed antixenosis type resistance, indicating that the aphid resistances in the two PIs are not controlled by the same gene. Thus, we have mapped a new gene near the *Rag*2 locus for soybean aphid resistance that should be useful in breeding for new aphid-resistant soybean cultivars. Molecular markers closely linked to this gene are available for marker-assisted breeding. Also, the minor locus found on chromosome 8 represents the first reported soybean aphid-resistant locus on this chromosome.

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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Introduction

The soybean aphid (*Aphis glycines* Matsumura) is native to southeastern and eastern Asia including China, Japan, Korea, the Philippines, Indonesia, Malaysia, Thailand, Vietnam, and Russia (Wu et al. 2004). The soybean aphid has become the most damaging insect pest influencing soybean yield in the USA since it was first found in the Midwest in 2000 (Ragsdale et al. 2004; Kim et al. 2008; Mian et al. 2008a; Zhang et al. 2010). The current distribution also includes Australia and Canada (Venette and Ragsdale 2004; OMAFRA 2009). The feeding of soybean aphid by sucking plant sap can result in significant water and nutrients loss from leaves and stems (Krupke et al. 2005).

Severe aphid infestations of soybean may cause many observable symptoms or phenotypic manifestations such as curling, wilting, yellowing, plant stunting, reduced pod and seed numbers, and smaller seeds (Krupke et al. 2005; Mian et al. 2008a). Yield losses of more than 50% due to soybean aphids were reported in Minnesota in 2001(Ostlie 2002) and up to 58% in China (Wang et al. 1996). In addition, soybean aphids can transmit several plant viruses such as *Alfalfa mosaic virus*, *Soybean dwarf virus*, *Tobacco ringspot virus*, and *Soybean mosaic virus* to soybean (Iwaki et al. 1980; Hartman et al. 2001; Hill et al. 2001; Clark and Perry 2002; Mueller and Grau 2007). Hence, an infestation of soybean aphid may result in significant reduction of total yield as well as seed quality, which is an important issue for food grade soybeans.

Host-plant resistance is a promising method of pest control, which can reduce cost and environmental pollution from the use of chemicals for controlling soybean aphids. There are three types of host plant resistance to insects: antibiosis, antixenosis, and tolerance (Painter 1951). Antibiosis influences insect biology such as reproduction, is often caused by toxic chemicals or secondary metabolites produced by resistant plants, and consequently results in a reduction of number of insects. The insects can be repelled from the host plant with antixenosis resistance, which is also known as non-preference for specific plants. Last, tolerance is the ability to withstand infestation of insects without significant yield loss. Thus, yield and dry matter studies are necessary to confirm tolerance (Mensah et al. 2005). The first step for controlling insect by host-plant resistance is to find the genotypes with insect resistance.

A number of soybean germplasm lines with aphid resistance have been identified by different research groups. Hill et al. (2004) reported three ancestors of North American soybean cultivars with aphid resistance. They include two late-maturing cultivars Dowling (maturity group [MG] VIII) and Jackson (MG VII) with antibiosis resistance, and one plant introduction PI 71506 with antixenosis or non-preference resistance. Four early-maturing (MG III) soybean PIs originally from Shandong province of China were identified as aphid resistant (Mensah et al. 2005). PI 567541B and PI 567598B have antibiosis resistance while PI 567543C and PI 567597C possess antixenosis resistance. Hesler et al. (2007) also discovered two aphid-resistant genotypes: PI 595099 with antixenosis resistance and PI 230977 with antibiosis resistance.

In 2006, a new biotype of soybean aphid called "Ohio" biotype or "Biotype 2" capable of defeating the *Rag*1 gene was found in Ohio (Mian et al. 2008a; Kim et al. 2008). Following the discovery of a soybean aphid biotype in USA, Mian et al. (2008a) identified three aphid-resistant PIs with resistance to both biotypes (IL, biotype 1 and OH, biotype 2) of the soybean aphid. PI 243540 revealed strong antibiosis resistance in a no-choice test and the other two PIs (PI 567301B and PI 567324) showed antixenosis resistance. Kim et al. (2008) reported that PI 200538 and PI 567597C were also resistant to both biotypes of the aphid.

The aphid-resistant genes *Rag*1 and *Rag*?, which are present in Dowling and Jackson, respectively, were both controlled by a single dominant gene (Hill et al. 2006a, b) and they were mapped to the same genomic region between Satt463 and Satt435 on soybean chromosome 7 (Li et al. 2007). Kang et al. (2008) identified a single dominant gene controlling aphid resistance in PI 243540. Subsequently, this gene was mapped to chromosome 13 and named *Rag*2 (Mian et al. 2008b). Interestingly, the aphid resistance gene in PI 200538 was also mapped to the same genomic region with *Rag*2, although it is not clear whether the aphid resistance genes from the two different sources are identical (Hill et al. 2009; Kim et al. 2010). Kim et al. (2010) performed fine mapping of the gene in PI 200538 to a 54-kb interval while the fine mapping of the *Rag*2 gene in PI 243540 is currently in progress. Aphid resistance in soybean can also be controlled by more than one gene or quantitative trait loci (QTLs). Mensah et al. (2008) reported that two recessive genes were involved in aphid resistance in PI 567541B and PI 567598B each. Two QTLs, located on chromosome 7 and 13, controlling the aphid resistance in PI 567541B were detected in both field and greenhouse trials using a mapping population of 228 F3 derived lines (Zhang et al. 2009). Another aphid resistance gene, *Rag*3, controlled by a major gene in PI 567543C was mapped in an interval between Sat_339 and Satt414 on chromosome 16 (Zhang et al. 2010).

Aphid-resistant soybean cultivars with single gene (e.g., *Rag*1 and *Rag*2) resistance have been developed and cultivars with the *Rag*1 gene were commercially available in 2010. However, a soybean aphid biotype (biotype 2) capable of defeating the *Rag*1 gene has already been found in Ohio (Kim et al. 2008). More recently, a new soybean aphid biotype was discovered from the overwintering host glossy buckthorn (*Frangula alnus*) which can easily colonize plants with the *Rag2* resistance gene, and this has been named "Biotype 3" (Hill et al. 2010). The presence of such soybean aphid biotypes decreases the utility and effectiveness of most single gene (R-gene) for aphid resistance identified so far. Therefore, it is necessary to identify new sources of aphid resistance, particularly the ones with multigenic resistance. Multigenic or QTL type resistance to pests is known to be more durable than most single gene resistance (Palloix et al. 2009).

While both PI 243540 and PI 567301B had resistance to both biotype 1 and 2, PI 243540 showed strong antibiosis and PI 567301B showed antixenosis type of resistance (Mian et al. 2008a). The aphid resistance in PI 243540 was reported to be controlled by a single gene (Kang et al. 2008;

Mian et al. 2008b). The objectives of this study were to genetically map the aphid resistance gene(s) in PI 567301B and to compare their genomic locations with previously mapped aphid-resistant genes.

Materials and methods

Mapping population and DNA extraction

A population of 203 $F_{7:9}$ recombinant inbred lines (RILs) derived from a cross of 'Wyandot' × PI 567301B by single seed descent and the two parental lines were used for this study. PI 567301B is a MG IV accession from China with strong antixenosis resistance to the biotypes 1 and 2 of soybean aphid, while Wyandot is a soybean aphid susceptible cultivar developed in Ohio (Mian et al. 2008a). Before aphid infestation, a young leaf from each RIL and the parents 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) for 2 days and then were ground into fine powder using a Mixer Mill (Model MM301; Retsch, Hannover, Germany). The genomic DNA was extracted using a modified CTAB protocol (Mian et al. 2008b).

Soybean aphid resistance evaluation

The choice tests in both greenhouse and field trials were performed to evaluate aphid resistance of the soybean lines to biotype 2 of the soybean aphid. The soybean aphid used in this study was established in a growth chamber at Ohio Agricultural Research and Development Center (OARDC), Wooster, OH, during the summer of 2005 by collecting aphids from a nearby soybean field. The colony was maintained on seedlings of soybean cultivar Williams 82 in growth chambers maintained at temperatures between 22 and 24°C with 15 h of light daily and 60–70% relative humidity (Mian et al. 2008a). In the fall of 2009, the greenhouse trial was performed with 203 $F_{7:9}$ RILs and the parents of the Wyandot × PI 567301B population in a USDA greenhouse at OARDC, Wooster, OH. In this test, four seedlings per line were grown in a 10.16 cm in diameter and 8.89 cm deep plastic pot. Each plant was infested with five wingless adult aphids at the V1 stage. Two parents (each with ten pots) were also included as checks. 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. 2008a). In the summer of 2010, a field aphid test was performed in a field at OARDC in a large polypropylene cage with 0.49-mm size mesh aphid proof cage (Redwood Empire Awning Co., Santa Rosa, CA, USA) to prevent aphids from escaping and to restrict entry of predators in the cage from outside. The soil type of the field was Riddles silt loam. Six seedlings of each soybean line were grown in a hill plot with 10 cm spacing between hills and 30 cm between rows. Ten replications of each parent were placed among the RILs at random. Five wingless adult aphids were transferred to each seedling at the V1-stage with the cage in place. The soybean aphid resistance of each line was evaluated by scoring at 2 and 4 weeks after infestation, resulting in two sets of greenhouse (GH2W and GH4W) and two sets of field (Field2W and Field4W) scores. Aphid resistance was evaluated by assigning scores between 1 and 5 based on the rating criteria used by Mian et al. (2008a) with a slight modification according to scoring time. Two weeks after aphid infestation, each line was evaluated by assigning aphid scores ranging from 1 to 5, where 1 $\leq$ 10 aphids per plant, 2 = 10 to 25 aphids per plant, 3 = 25 to 50 aphids per plant, 4 = 50 to 100 aphids per plant, and 5 $\geq$ 100 aphids per plant. In contrast with 2-week scoring, the scores of 4 weeks were recorded using a scale of 1–5, where 1 $\leq$ 25 aphids per plant, 2 = 25 to 100 aphids per plant, 3 = 100 to 200 aphids per plant, 4 = 200 to 500 aphids per plant, and 5 $\geq$ 500 aphids per plant.

Molecular marker genotyping and linkage analysis

For construction of the genetic map, 94 RILs were randomly selected out of the 203 RILs of the mapping population. The selected RILs and the two parental lines were genotyped with SNP markers using the GoldenGate assay for a Universal Soy Linkage Panel (USLP 1.0) containing 1536 SNP loci well distributed throughout the 20 soybean chromosomes (Hyten et al. 2010). The SSR marker genotyping was conducted to saturate the candidate QTL regions. The primer sequences and location of the SSR markers were obtained from BARCSOYSSR_1.0 soybean SSR database (Song et al. 2010). PCR amplifications were performed in 20-µl reactions containing 50 ng of template DNA, 1× PCR buffer, 2.5 mM Mg^{2+}, 200 µM dNTP, 100 nM of forward and reverse primers, and 1.0 unit of *Taq* DNA polymerase (GenScript USA Inc., Piscataway, NJ). The PCR cycles consisted of initial denaturation at 95°C for 5 min, followed by 32 cycles of 30 s denaturation at 94°C, 30 s annealing temperature between 50–60°C depending on the optimum annealing temperature for each primer pair, and 30 s extension at 72°C. The PCR reaction was finished with a final 10-min extension at 72°C on a thermalcycler (Techne Inc., model TC-512). The PCR products were analyzed in 4% 3:1 agarose gel (RPI corp., Mount Prospect, IL). The linkage map was constructed with the Kosambi mapping function using JoinMap 4.0 (Van Ooijen 2006). A logarithm of odds (LOD) score of 3.0 as a threshold and a maximum genetic distance of 50 cM were used to assign all markers to chromosomes (or linkage groups).

Table 1 Summary of aphid scores in the greenhouse and field using 203 $F_{7:9}$ recombinant inbred lines (RILs) and the two parents

Phenotype	Parents		$F_{7:9}$ RIL population			
	PI567301B	Wyandot	N[a]	Mean	SD	Range (Min–Max)
GH2W	1.0a	3.9b	203	2.38	1.42	1–5
GH4W	1.0a	4.8b	203	3.09	1.78	1–5
Field2W	1.2a	4.5b	203	3.41	1.62	1–5
Field4W	1.2a	4.9b	203	3.43	1.62	1–5

Means followed by different letters for each phenotype are significantly different at $P < 0.0001$

[a] Number of plants used for aphid resistance test

Statistical analysis and QTL mapping

Association between molecular marker genotype and soybean aphid resistance scores was tested by single-factor analysis of variance (single factor ANOVA) at the 0.05 significance level using the PROC GLM procedure of SAS (SAS Institute 2002). The single-factor ANOVA was separately performed for the four sets of phenotypic scores (GH2W, GH4W, Field2W, and Field4W) with the SNP genotype data obtained from the GoldenGate assay for 94 RILs of the mapping population. Correlation coefficients among the four sets of aphid scores were calculated using the PROC CORR procedure of SAS. Potential two-way epistasis between two marker loci was tested by two-way ANOVA at the 0.05 significance level using the PROC GLM procedure of SAS. Identification of aphid resistance loci was initially performed using interval mapping (IM) methods. Subsequently, composite interval mapping (CIM) was conducted using multiple QTL mapping (MQM) method with MapQTL 5.0 software (Van Ooijen 2004). The genome-wide LOD threshold at 5% significance level was determined by a 1000 permutation test for each trait individually. The phenotypic variation explained by each locus was calculated from the value at the QTL peaks indicated by CIM.

Results

Evaluation of phenotypic trait

The soybean aphid scores for the 203 RILs derived from the cross between Wyandot and PI 567301B ranged from 1 to 5 in two greenhouse (GH2W and GH4W) as well as in two field (Field2W and Field4W) screenings, but the mean values of aphid scores in the field were relatively higher than those in the greenhouse (Table 1). The frequency distributions of aphid scores for the 203 RILs for each scoring time are shown in Fig. 1. The means of the scores for parents were significantly different in all four sets ($P < 0.0001$) (Table 1; Fig. 1). The correlations between greenhouse and field phenotypic scores were highly significant at the 0.0001 probability level (Table 2). The frequency distributions of the aphid scores were close to bimodal distribution, indicating that one or two major genes may be involved in aphid resistance of the mapping population. The frequency distribution for the 2-week and 4-week greenhouse scores was somewhat different. In contrast, the frequency distributions of aphid scores for the Field2W and Field4W screenings were very similar (Fig. 1).

Linkage analysis and QTL mapping

The genotyping with a USLP 1.0 containing 1536 SNP loci revealed 706 polymorphic SNPs (45.9%) between Wyandot and PI 567301B that also segregated among the subset of 94 RILs of the population. Among the 706 polymorphic

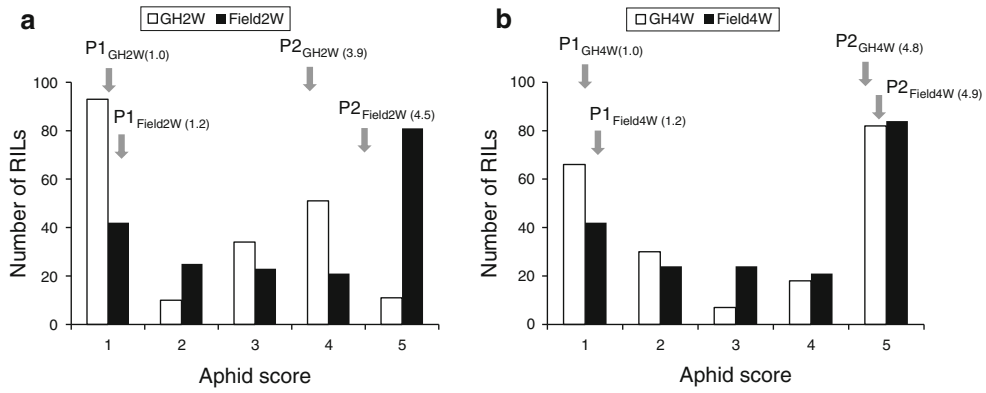

Fig. 1 Frequency distribution of 203 recombinant inbred lines (RILs) derived from a cross of Wyandot × PI 567301B for soybean aphid resistance: **a** 2-week scoring in the field and greenhouse, **b** 4-week scoring in the field and greenhouse. *Arrows* indicate the means of the scores for parents: P1 (PI 567301B) and P2 (Wyandot)

Table 2 Pearson correlation coefficients among four phenotypic scores of 203 recombinant inbred lines (RILs) of the Wyandot × PI567301B population

	GH2W	GH4W	Field2W	Field4W
GH2W		0.889	0.765	0.791
GH4W			0.823	0.863
Field2W				0.967
Field4W				

GH2W 2-week scoring in the greenhouse, *GH4W* 4-week scoring in the greenhouse, *Field2W* 2-week scoring in the Field cage, *Field4W* 4-week scoring in the Field cage

Correlation coefficients among phenotypic data are significant at $P < 0.0001$

SNPs, a total of 516 SNP loci that showed a good fit to 1:1 segregation ratio as expected for the RIL population and had less than 10% missing data were used to construct the genetic linkage map for the population spanning 2005.3 cM (Table 3; Supplementary Fig. 1). The linkage map of 516 SNP markers was grouped into the 20 chromosomes (or linkage groups) with the mean length of 100.3 cM, and the average distance between mapped SNP loci was 5.0 cM with a range from 0.0 to 30.8 cM (Table 3; Supplementary Fig. 1). The total length of genetic map and the average length of chromosome nearly corresponded to the Soybean Consensus Map 4.0 (Hyten et al. 2010) that spanned 2296.4 cM with the mean chromosome length of 114.8 cM. Most SNPs were very evenly distributed across the 20 chromosomes and the marker order of the loci was also in accordance with those of the Soybean Consensus Map 4.0. However, several large genetic gaps (≥30 cM) and rearrangements of marker orders between the SNPs were observed in some chromosomal regions.

Two genomic regions on the genetic map were found to be significantly associated with soybean aphid resistance from single-factor ANOVA and were located on chromosomes 8 and 13. The seven SNP markers in the interval between BARC-050235-09520 and BARC-024045-04714 on chromosome 13 revealed highly significant association ($P < 0.0001$) with aphid resistance scores for all four sets of aphid scores (Fig. 2d). Among these SNPs, BARC-060107-16382 showed the most significant association, explaining 83–90% of phenotypic variation in the aphid resistance scores (Table 4). On the other hand, only one SNP (BARC-063283-18296) marker on chromosome 8

Table 3 Summary of soybean genetic map of the recombinant inbred line (RIL) mapping population derived from a cross between Wyandot and PI567301B, including chromosome length, total number of SNP marker mapped, total number of SNP locus mapped separately and average distance between SNP loci

Chromosome	Linkage group	Length (cM)	Total number of SNP marker mapped	Total number of SNP locus located separately	Average distance between SNP loci (cM)
1	D1a	105.8	24	20	5.3
2	D1b	80.3	41	30	2.7
3	N	82.0	23	16	5.1
4	C1	126.3	29	22	5.7
5	A1	122.1	19	15	8.1
6	C2	92.4	28	22	4.2
7	M	105.5	25	22	4.8
8	A2	117.7	33	29	4.1
9	K	100.9	33	26	3.9
10	O	78.5	23	17	4.6
11	B1	109.1	17	17	6.4
12	H	56.5	24	16	3.5
13	F	127.9	34	22	5.8
14	B2	88.5	19	16	5.5
15	E	116.2	21	16	7.3
16	J	89.2	22	18	5.0
17	D2	70.0	16	16	4.4
18	G	97.6	40	21	4.6
19	L	114.3	21	17	6.7
20	I	124.5	24	21	5.9
Average		100.3	26.0	20.0	5.0
Total		2005.3	516	399	

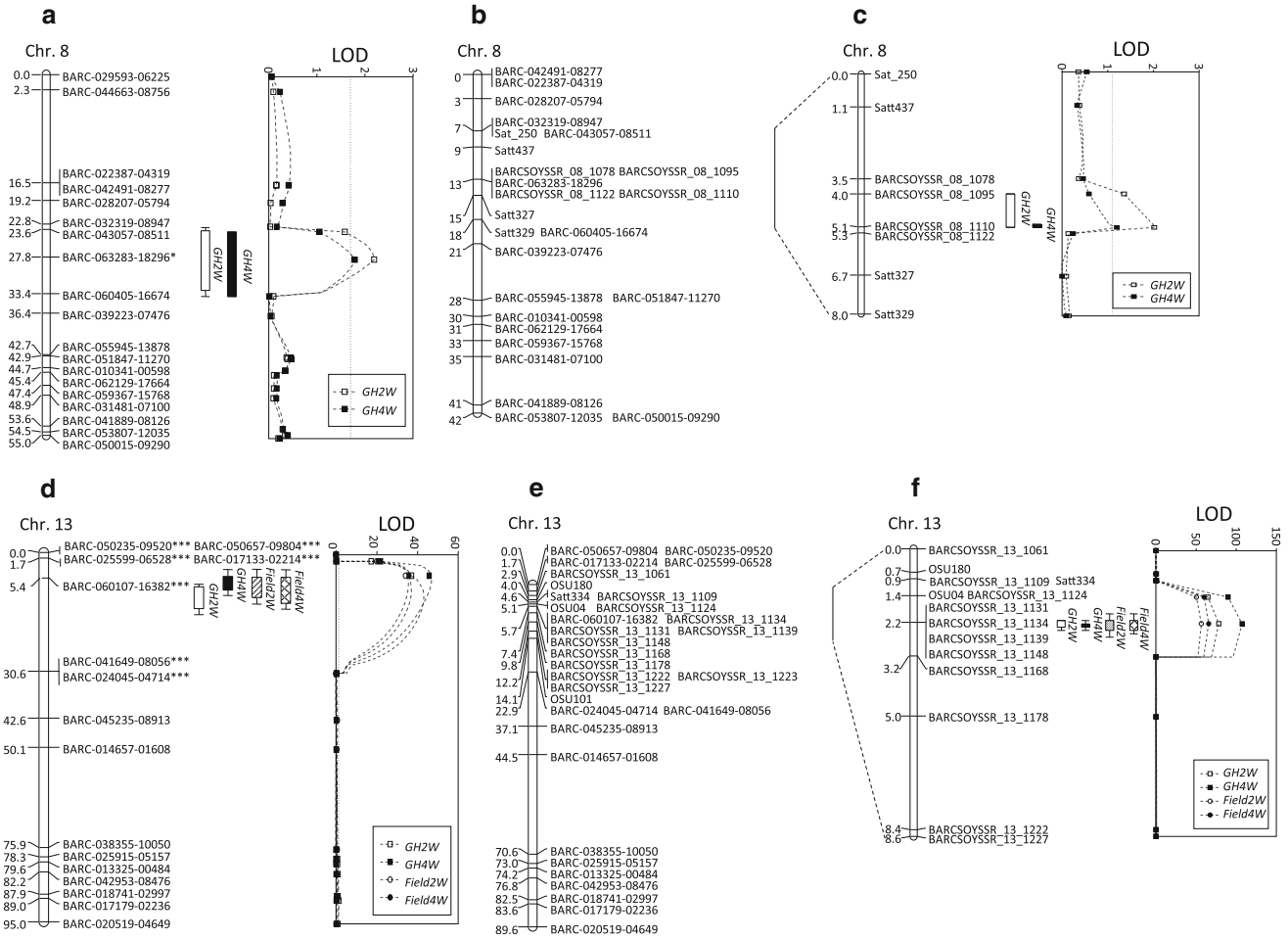

Fig. 2 Location of quantitative trait loci (QTL) associated with soybean aphid resistance on chromosome 8 and 13 using composite interval mapping method. **a** and **d** show maps generated using 94 RILs of Wyandot × PI 567301B mapping population and SNP markers from the Goldengate assay. **b** and **e** show saturated maps using additional SSR markers on the **a** and **d** maps, and **c** and **f** show maps spanning the QTL regions on chromosome 8 and 13 using 203 RILs. The *asterisk* signs in the two maps **a** and **d** indicate that the markers revealed significant association with aphid resistance by single-factor ANOVA: * and ***, significance at $P < 0.05$ and $P < 0.0001$, respectively. In QTL LOD graphs, LOD thresholds for 94 and 203 RILs are 1.8 and 1.1, respectively

showed significant linkage with aphid resistance, accounting for 6.3–9.8% of phenotypic variation in the aphid resistance scores (Table 4; Fig. 2a). This significant marker explained 9.8 and 8.0% of the phenotypic variation for GH2W and GH4W, respectively ($P < 0.01$), compared with 6.7 and 6.3% for Field2W and Field4W, respectively ($P < 0.05$) (Table 4). However, no significant epistatic interaction was detected between BARC-060107-16382 and BARC-063283-18296.

The QTL mapping with the CIM method was initially conducted for the two chromosomes, 8 and 13, that revealed significant association with aphid resistance by single-factor ANOVA using 94 RILs of the population. Two different loci were detected—one locus was closely positioned near the BARC-060107-16382 on chromosome 13, which was consistently significant for all aphid scores (Fig. 2d). The locus explained a large amount of phenotypic variation ranging from 83 to 90%. The second locus was identified on chromosome 8, but it was significant only in the greenhouse aphid scores. The QTL peak was located at BARC-063283-18296 which is same locus detected by single-factor ANOVA, accounting for a small proportions (10.5% for GH2W and 8.6% for GH4W) of the total phenotypic variation (Fig. 2a). The two QTL regions identified by initial QTL mapping using the subset of 94 RILs were densely populated with SSR markers by genotyping 203 RILs with 8 and 14 SSR markers located near the SNP BARC-063283-18296 on chromosome 8 and BARC-060107-16382 on chromosome 13, respectively, and new genetic maps of the two regions were constructed (Fig. 2c, f). The QTL analysis was performed using the saturated linkage map and the aphid scores of the 203 RILs. The locus on chromosome 13 was closely linked to four SSR markers (BARCSOYSSR_13_1131, BARCSOYSSR_13_1134, BARCSOYSSR_13_1139, and

Table 4 SNP markers associated with soybean aphid resistance in the 94 $F_{7:9}$ recombinant inbred lines (RILs) of the Wyandot × PI 567301B population using single-factor ANOVA

Trials[a]	Chromosome	Position (cM)[b]	Marker[c]	R^{2d}	*P* value
GH2W	8	94.1	BARC-063283-18296	9.8	0.0027
	13	55.8	BARC-060107-16382	86.2	<0.0001
GH4W	8	94.1	BARC-063283-18296	8.0	0.0068
	13	55.8	BARC-060107-16382	91.0	<0.0001
Field2W	8	94.1	BARC-063283-18296	6.7	0.014
	13	55.8	BARC-060107-16382	83.6	<0.0001
Field4W	8	94.1	BARC-063283-18296	6.3	0.017
	13	55.8	BARC-060107-16382	85.5	<0.0001

[a] GH2W: 2-week scoring in the greenhouse, GH4W: 4-week scoring in the greenhouse, Field2W: 2-week scoring in the Field cage, and Field4W: 4-week scoring in the Field cage

[b] Position of significant SNPs is based on the Soybean Consensus Map 4.0

[c] Marker that revealed the highest R^2 value among significant markers

[d] Percentage of phenotypic variation explained by the QTL detected

BARCSOYSSR_13_1148) mapped at the same location indicated by the initial QTL mapping and explained 75-91% of phenotypic variations (Fig. 2f). Although the 4 SSR markers were apparently mapped together in the linkage of this RIL population, they cover the genomic region of about 512 kb based on the BARCSOYSSR_1.0 soybean SSR database (Song et al. 2010). In the test using additional markers and more RILs, the locus on chromosome 8 was closely mapped to the BARCSOYSSR_08_1095-BARCSOYSSR_08_1110 interval (Fig. 2c). However, it revealed somewhat smaller portion of total phenotypic variations of 4.6 and 2.4% for GH2W and GH4W, respectively (data not shown).

Discussion

The soybean aphid resistance in PI567301B was reported as antixenosis-type resistance (Mian et al. 2008a) and a choice test was performed both in the greenhouse and field using the same infestation method. In comparison with the aphid resistance test in the greenhouse, relatively high mean of aphid scores and rapid growth of susceptible plants at the early stage (2 weeks) were observed in the field test. Zhang et al. (2009) reported that the field environment might be more favorable for aphid growth than greenhouse conditions. In a previous study, temperature has been reported to be significantly related to soybean aphid population growth, indicating the greatest growth rates at 25°C (Mccornack et al. 2004). Thus, our results indicate that the temperature condition in our field test was probably more favorable for the aphid growth than that in the greenhouse. However, it is difficult to fully explain the favorable environment for aphid growth in the field with only temperature as a factor since the greenhouse used for the aphid resistance test was also maintained at 24°C for optimum aphid development. Therefore, it is likely that other factors might have positively influenced aphid growth in the field. In spite of the difference, there was a very high correlation between greenhouse and field tests for both 2- and 4-week scorings.

To identify QTLs associated with aphid resistance, a linkage map was constructed using 516 SNP loci distributed throughout the 20 chromosomes. The average distance between mapped SNP loci was 5.0 cM. The molecular linkage map in this study nearly corresponded to the Soybean Consensus Map 4.0 recently published for the total length of genetic map and the average length of chromosomes. Assuming approximately more than 2500 cM genome size of soybean, at least 250 markers distributed every 10-cM interval on each chromosome are necessary for a genome-wide QTL detection study (Hyten et al. 2010). Thus, the genetic map in this study had enough power for the identification of the aphid resistance genes in our mapping population. However, the linkage analysis in our study also left a few things to be improved, for instance, unattached genomic regions to their corresponding chromosomes, and segregation distortion of some molecular markers. A total of 706 SNP loci showing polymorphism between two parents were obtained from the high-throughput genotyping using 1536 SNPs. Out of these polymorphic SNPs, 190 loci were not used for the linkage analysis because the markers revealed segregation distortions and (or) had more than 10% missing data. Although these excluded markers have resulted in several genetic gaps between two loci or uncombined chromosomes in our linkage map, inaccurate mapping caused by the overestimated recombination frequency among markers was minimized (Zhang et al. 2007). Few inconsistent marker orders, compared with the reference soybean map, were also observed in most chromosomes.

Discordant marker orders have been reported in previous mapping studies (Mian et al. 2008b; Vuong et al. 2010; Zhang et al. 2010). Such differences might have been due to chromosomal mutations such as insertions, deletions, inversions, and translocations in either parents of the mapping population (Stam 1993; Williams et al. 1995). The population structure and size and different mapping algorithm used in mapping may also account for some of the observed differences (Marinov et al. 1999; Vuong et al. 2010).

Three soybean aphid resistance genes were identified and mapped on three different genomic regions recently (Li et al. 2007; Mian et al. 2008b; Zhang et al. 2010). In our study, two loci associated with the soybean aphid resistance in PI 567301B were identified using 203 RIL mapping population. One locus considered as a major gene in this population was detected on chromosome 13 that was closely linked to four SSR markers mapped at the same location and physically located between 29,036,526–29,548,838 bp on chromosome 13 based on the BARCSOYSSR_1.0 soybean SSR database and Williams 82 8X draft assembly (Glyma 1). This locus was very close to the aphid resistance gene, *Rag*2. In previous studies, the *Rag*2 gene from PI 200538 (Kim et al. 2010) and PI 243540 (Mian et al. 2008b) were mapped to almost same region on chromosome 13, but the relationship between gene(s) from the two sources is unknown. The location of the *Rag*2 gene from PI 200538 was between positions 29,212,318–29,266,469 bp on chromosome 13 based on the locations of flanking markers after fine mapping of the gene region. Fine mapping for the *Rag*2 gene from PI 243540 has not been reported, but the location was originally reported as the interval between Satt334 and Sct_033 (28,415,888–30,739,587 bp) on chromosome 13 by Mian et al. (2008b). Additional fine mapping or allelic test using progenies derived from each cross might be needed to identify allelic relationship among these resistance genes in close proximity (Zhang et al. 2009). Seven candidate resistance genes (Glyma13g25920, Glyma 13g25950, Glyma13g26000, Glyma13g26140, Glyma13g 26230, Glyma13g26250, and Glyma13g26310) were annotated in the 512-kb region spanning the major locus on chromosome 13 from the Williams 82 sequence, encoding F-Box/Leucine rich repeat (LRR) protein or LRR-containing protein. These genes can produce disease resistance proteins such as the coiled-coil (CC)-nucleotide-binding site (NBS)-LRR or toll-interleukin receptor (TIR)-NBS-LRR proteins might be associated with soybean aphid resistance at this locus.

Besides the major locus, one additional putative locus was detected on chromosome 8, explaining a small proportion of total phenotypic variations. The locus was significant in both greenhouse and field conditions by single-factor ANOVA. This locus, however, showed some inconsistency across environmental conditions. The significant aphid resistance was limited only to the greenhouse conditions based on the CIM method, but slightly lower LOD scores than the LOD threshold were also observed in the field environments. This result implies that this minor QTL might be sensitive to environmental effects (Pilet-Nayel et al. 2005; Suenaga et al. 2005). Zhang et al. (2009) reported some inconsistent effects of a minor QTL on chromosome 13 among greenhouse and field environments also. In this study, a negative additive effect indicates that resistance alleles were derived from susceptible parent Wyandot, and no interaction was detected between QTLs detected in chromosomes 8 and 13 using two-way ANOVA. Previous studies have reported a number of QTLs associated with insect resistance in soybean where resistance alleles at the QTLs were contributed by the susceptible parent (Rector et al. 2000; Boerma and Walker 2005).

Interestingly, a QTL for isoflavone content in soybean was mapped by Zeng et al. (2009) on chromosome 8 within 10 cM of the minor QTL for aphid resistance identified in this study. Isoflavone has been previously reported to protect soybean from pests and pathogenic microbes (Morris et al. 1991; Benhamou and Nicole 1999; Carrao-Panizzi and Kitamura 1995). Zeng et al. (2009) have also reported a QTL for isoflavone content on chromosome 13 within 10 cM of the major aphid resistance QTL found in this study. Therefore, a potential underlying relationship between isoflavone content and the aphid resistance QTLs warrants further investigation.

The major gene on chromosome 13 identified in this population was mapped near the *Rag*2 gene. However, a recent study revealed that the detached leaves of PI 567301B had no resistance against the soybean aphid, while the detached leaves of PI 243540 (source of *Rag*2) maintained their aphid resistance in the same study (Michel et al. 2010). Thus, the soybean aphid resistance in PI 243540 was active in both detached leaves and whole plants, whereas the aphid resistance of PI 567301B was only effective in whole plants. In addition, the other source of *Rag*2 (PI 200538) also revealed aphid resistance in detached leaves (data not shown). These results coupled with the earlier finding that PI 243540 and PI 200538 showed antibiosis resistance and that PI 567301B showed antixenosis type resistance, indicate that the aphid resistance in PI 567301B is not same as the two PIs having antibiosis resistance. Thus we have mapped a new soybean aphid resistance gene near the *Rag*2 locus that should be useful in breeding for aphid-resistant soybean cultivars. Using the convention specified by the Soybean Genetics Committee, the gene symbol for the major gene on chromosome 13 identified in PI 567301B has been tentatively proposed as *Rag*5, where 'ag' standing for *Aphis glycines*, and the number 5 indicating that it is the fifth major soybean gene identified for resistance to the soybean aphid.

A request has been submitted to the Soybean Genetics Committee for approval of this gene symbol. Molecular markers closely linked to this gene are available that should facilitate marker-assisted breeding for this resistance gene. Also, the minor QTL found on chromosome 8 for aphid resistance, if validated, will represent the first soybean aphid resistance gene on this chromosome.

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