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ORIGINAL PAPER

Genetic mapping of a novel gene for soybean aphid resistance in soybean (*Glycine max* [L.] Merr.) line P203 from China

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Abstract The soybean aphid (SA: *Aphis glycines* Matsumura) is a worldwide pest of soybean (*Glycine max* [L.] Merr.). The objectives of this study were to identify the type of aphid resistance and the resistance phenotype in soybean line 'P203', and to map the relative position of the gene involved. Compared with cultivars 'P746' and 'Dongnong 47', P203 was demonstrated to possess antixenosis resistance. P203 prevented aphids from reproducing in a choice test, but the resistance level decreased significantly in a no-choice test at 11 and 21 days after infestation. Analysis of 273 Dongnong 47/P203 F_2 plants and confirmed using 260 $F_{2:3}$ families revealed that a single dominant gene from P203 was positioned between marker loci Sat_377 and Satt409 on chromosome 8. The gene was further mapped to a 1.57 Mb interval flanked by marker loci BARCSOYSSR_08_1451 and BARCSOYSSR_08_1527. We developed five new SSR markers in the target interval and the resistance locus mapped between new markers SSR_08_75 and SSR_08_88 with the genetic distance of 1.1 and 1.0 cM corresponding to a physical distance of 192 kb on the Williams 82 8X draft genome assembly (Glyma1.01). A single serine/threonine protein kinase gene is present in this region, suggesting that the SA resistance mechanism in P203 may be different from those previously reported. Therefore, the resistance gene could very well be novel, and could be valuable in soybean aphid resistance breeding programs.

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Introduction

The soybean aphid (SA: *Aphis glycines* Matsumura), which is native to Asia, has become the most damaging insect pest affecting soybean yield in the USA since it was first discovered in the American midwest in 2000 (Ragsdale et al. 2004). In addition to causing direct damage by feeding, SA can transmit plant viruses, such as *Soybean mosaic virus* and *Alfalfa mosaic virus*, which distort growth and further reduce yield in soybean (Davis et al. 2005). Severe infestations of SA can reach densities of several thousand per plant during its reproductive stage, and have caused yield losses of >50 % in Minnesota (USA) (Ostlie 2002), and 52 % in China (Wang et al. 1994). Development of soybean varieties that are resistant to SA is the most effective and environmentally safe way to resolve this problem (Luginbill 1969). It is, therefore, imperative to identify genes for SA resistance from diverse germplasm sources and incorporate them into agriculturally important cultivars.

There are three major types of host plant resistance to insects: antibiosis, antixenosis and tolerance (Painter 1951). Antibiosis is defined by the way in which the host plant affects the biology, life cycle and abundance of the insect. Antixenosis refers to a host effect on pest behavior, which discourages feeding and/or oviposition. Antixenosis was suggested by Kogan and Ortman (1978) as a substitute for 'non-preference'. The distinction between the two is based on whether a choice (antixenosis) or no-choice (antibiosis) assay is used to quantify insect resistance (Hill et al. 2004; Mensah et al. 2005). Lastly, tolerance is the ability to withstand insect infestation without significant yield loss (Smith 2005).

In recent years, a number of genes for SA resistance have been identified in soybean. SA resistance in the two

soybean cultivars, 'Dowling' and 'Jackson', is controlled by a single dominant gene (Hill et al. 2006a, b). The resistance gene in Dowling, *Rag1*, and the resistance gene in Jackson (unnamed) were both mapped to the same region on chromosome 7 [linkage group (LG) M] (Li et al. 2007). Similarly, a single, dominant gene, *Rag2* in plant introduction (PI) 243540 was mapped to chromosome 13 (LG F) (Mian et al. 2008b), and *Rag2* in PI 200538 has been fine-mapped to a 54 kb region (Kim et al. 2010b). It has been determined that aphid resistance in PI 567541B and PI 567598B is controlled by two recessive genes (Mensah et al. 2008). A quantitative trait locus (QTL)—genetic mapping study has suggested that the two genes in PI 567541B are located on chromosomes 7 and 13. The gene on chromosome 7 was mapped to the same genomic region as *Rag1* and was later designated *rag1*, and the gene on chromosome 13 was named *rag4* (Zhang et al. 2009). Using the same approach, a major gene, *Rag3*, which confers antixenosis resistance, was isolated from PI 567543C, and mapped to chromosome 16 (LG J) (Mensah et al. 2005; Zhang et al. 2010). Two QTLs, qRa_1 on chromosome 8 (LG A2) and qRa_2 on chromosome 13, have been identified as being associated with the antibiosis type of resistance to SA in the cultivar 'Zhongdou 27' (Meng et al. 2011). In PI 567301B, a major QTL *Rag5* was mapped on chromosome 13 and a minor SA resistance locus on chromosome 8 (Jun et al. 2012).

In North America, three known biotypes have been identified in Illinois (biotype 1), Ohio (biotype 2), and Indiana (biotype 3) (Kim et al. 2008; Hill et al. 2010). In Ohio, SA has overcome *Rag1* resistance in Dowling (Kim et al. 2008). *Rag2* has been found to provide resistance to SA in Illinois and Ohio, while *Rag3* showed resistance to an unknown SA in Michigan and all three aphid biotypes identified (Hill et al. 2010; Zhang et al. 2010). *rag1* and *rag4* conferring broader resistance than Dowling were resistant to Michigan SA (Zhang et al. 2009). *Rag5* had SA resistance to Illinois and Ohio biotype (Jun et al. 2012). So far, the SA biotypes occurring in China are unknown and little is known about genetic mapping of the resistance to SA in China except that Meng et al. (2011) reported that qRa_1 and qRa_2 conferred resistance to the Ohio biotype.

It is well known that China is famous for the rich soybean germplasm resources. It is reasonable to believe that sources of the aphid resistance are abundant in Chinese soybean germplasm. P203 was found to be resistant to a SA biotype collected from Shanghai (Wu et al. 2009). The objectives of this study were to characterize the nature of soybean resistance to SA from Shanghai, determine the mode of its inheritance in line P203, and fine map the gene from this accession on the soybean genome.

Materials and methods

Mapping population and insects

Soybean cultivar P203 (resistant to aphids) was crossed with Dongnong 47 (susceptible to aphids) in 2008. Seeds from individual F_1 hybrid plants were harvested separately. Two hundred and seventy-three F_2 and $F_{2:3}$ families together with the parental lines were used to characterize and map the gene for resistance to aphids from Shanghai. P203 is a high yielding summer soybean cultivar maturity group VIII from Anhui province, China. Dongnong 47 is a spring-cultivated soybean accession in the early maturity group that is validated by Heilongjiang Variety Approval Committee and the validation code is 'Heishendou 2004006'. The SA used to infestation in this study is a single clone obtained from the field at Shanghai Jiaotong University and maintained in a room at the Legume Biotechnology Laboratory of Shanghai Jiaotong University by feeding on plants of Dongnong 47 at 22 °C.

Identification of aphid resistance in P203

Aphid choice tests

Soybean cultivars P203, P746 and Dongnong 47 were sown in 15-cm-deep × 4-cm diameter plastic containers filled with a mixture of peat, vermiculite, and perlite at a ratio of 1:1:1 (by vol.) in a greenhouse under a 12-hour photoperiod at 27 °C and 70 % relative humidity at Shanghai Jiaotong University (121°E, 31°N) in June 2009. In addition to cultivars P203 and Dongnong 47, cultivar P746 was included as a resistant control in the SA choice- and no-choice tests.

P746 is a summer soybean cultivar from Anhui province, China. The cultivars were sown in plastic containers described as in Xiao et al. (2012). Each experimental unit consisted of four plants. The experiment was arranged in a randomized complete block design with three replicates. Fifteen days after sowing, each plant was infested with five adult wingless soybean aphids. The number of SA per plant was counted at 11 and 21 days after infestation. The damage index (DI) for each line was calculated with the following formula (Mensah et al. 2005): DI = (scale value × number of plants in the category)/(4 × total number of plants) × 100. The aphid resistance scale values are described below. The value for DI falls between 0 (for no infestation) and 100 (for the most severe damage). A DI of ≤30 % was classified as resistant and a DI of ≥30 % was classified as susceptible (Mensah et al. 2005).

No-choice tests

Lines P203, P746 and Dongnong 47 were evaluated in no-choice tests under the environmental conditions described

above in July 2009. Each pot was set up as described for the choice tests with three replicates in a randomized complete block design. Fifteen days after sowing, each plant was infested with five adult wingless SA. Each plant was enclosed in a 0.3 mm size nylon net mesh supported by a stick to restrict the movement of SA after infestation. The number of SA per plant was counted 11 and 21 days after infestation, respectively. The DI for each line was calculated by the formula as for the choice tests.

Evaluation of aphid resistance

The parental lines, 15 F_1 plants and 273 F_2 plants were evaluated for aphid resistance in a choice test in the summer of 2010. Seeds were sown in 15-cm × 4-cm diameter plastic containers in the greenhouse as described above. Fifteen plants each of the parental lines were also randomly planted as resistance and susceptible controls. Fourteen days after sowing, each plant was infested with five adult wingless SA. Aphid resistance for each plant was rated 21 days after infestation using the visual scale of 0–4 as developed by Mensah et al. (2005) with a slight modification. Plants were scored as: 0 = 0–10 aphids per plant; 1 = 11–100 aphids per plant; 2 = 101–300 aphids per plant; 3 = 301–800 aphids per plant; 4 = more than 800 aphids per plant. Plants that had a score of 0, 1 or 2 were considered resistant, and plants with a score of 3 or 4 were considered susceptible. After scoring, plants were sprayed with an insecticide to ensure that they were able to set seed.

In progeny tests, 10–12 F_3 seeds from each F_2 plant were sown and the F_3 plants were evaluated individually for SA resistance as described above. Thirteen families of $F_{2:3}$ seeds could not be harvested because of drought or aphid damage. The remaining 260 $F_{2:3}$ lines and the parental lines were planted in 48-pot plastic inserts in flats without drainage holes in the same greenhouse during the early spring of 2011. An F_2 plant producing only susceptible F_3 plants (scores of 3 or 4) was confirmed as homozygous susceptible. An F_2 plant producing only resistant F_3 plants (scores of 0, 1 or 2) was confirmed as homozygous resistant. An F_2 plant resulting in a combination of resistant and susceptible progeny was confirmed as heterozygous. Thus, 260 $F_{2:3}$ families were used to inform and confirm the F_2 phenotype for mapping purposes.

Bulked segregant analysis

Young leaves were sampled from each F_2 plant and the two parental lines for DNA extraction using the CTAB method (Keim and Shoemaker 1988). The DNA was adjusted to a final concentration of 25 ng/μL. Equal amounts of DNA from ten resistant plants with a score of 0 or 1 and ten susceptible plants with a score of 3 or 4 from the F_2 population were pooled to form two DNA pools for bulked segregant analysis (BSA). The DNA pools were used to identify simple sequence repeat (SSR) markers linked to the gene for SA resistance in line P203 (Michelmore et al. 1991).

Molecular marker genotyping

A total of 1,015 primers selected from Song et al. (2004) were obtained from soybase (http://www.soybase.org) and were used to screen for polymorphisms in the two parental lines and the two DNA bulks. Approximately, 400 SSR markers between Sat_377 and Satt409 were obtained from the BARCSOYSSR_1.0, hereafter referred to as BSSR (Song et al. 2010) to screen the linked markers for further fine mapping. An additional 144 SSR loci between BSSR_08_1451 and BSSR_08_1527 based on Williams 82 sequence (Song et al. 2010) were developed with SSR hunter analysis software (http://www.bio-soft.net) for the flanking regions of repeat motifs that consisted of either eight or more dinucleotide repeats, or eight or more trinucleotide repeats. The forward and reverse primer sequences of the five most useful of the 144 new SSRs (SSR_08_64, SSR_08_75, SSR_08_88, SSR_08_133 and SSR_08_143) are listed in Table 1.

Amplification reactions contained 1× PCR buffer, 30 mM $MgCl_2$, 3 mM dNTP, 50 to 250 ng of template DNA, 2 μM of each primer, and 2.5 U of *Taq* polymerase (Shanghai Lifefeng Biotechnology Co., Ltd.) in a total volume of 10 μL. The reaction mixture was denatured at 94 °C for 4 min, followed by 30 cycles of denaturation at 94 °C for 25 s, annealing at 47 °C for 25 s, and extension at 68 °C for 25 s, with a final extension at 72 °C for 10 min. The PCR products were separated on a 6 % denaturing polyacrylamide gel in tris–borate-ethylenediaminetetraacetic acid (TBE) buffer and fragments visualized by silver-staining (Bassam et al. 1991).

Statistics and linkage analysis

The number of aphids per plant and the DI from the choice and no-choice tests were analyzed using the PROCEDURE GLM procedure in SAS statistical software V9.1 (SAS Institute 2002). Means for SA number and DI at days 11 and 21, respectively, were separated by least significant difference (LSD) tests at the 5 % probability level.

The Chi-square test was performed to test the goodness of fit for the observed segregation among F_2 plants and $F_{2:3}$ families with different genetic ratios. The segregation ratio of alleles at each locus was determined by Chi square to identify whether the loci met the expected ratio of 1:2:1 or 3:1 with a significance threshold of $P \leq 0.05$. Analysis of linkage between the aphid resistance locus and associated

Table 1 PCR primer sequences used in this study to develop SSR markers to map the soybean aphid resistance gene in soybean line P203

Marker	Forward primer (5′–3′)	Reverse primer (5′–3′)
SSR_08_64	AATACCACATTGAAGTTACACA	GCAAAGATGTGCCCTTGATATA
SSR_08_75	GGTTTGGTATTAAATTGTGGTA	TTTAGTACGAAGTTATGTGGGC
SSR_08_88	AGGTGGAACATACAGTAAGAAA	GATTAGTTGATTTTGATCTGGT
SSR_08_133	AAAGAGTCATAAGGGAGGGG	CTTCAAATGACGAAATTACTCT
SSR_08_143	TTTCTTCTCTAGGGGAACTTTA	CGATTCACTTTTCTTTTTTTCA

SSR marker loci in the mapping population was performed with the program MAPMAKER/EXP3.0 (Lander et al. 1987) with a logarithm of the odds difference (LOD) score ≥3.0. A linkage map was drawn using the software MapChart 2.0 (Voorrips 2002).

Results

Antixenosis aphid resistance in line P203

Soybean line P746 was previously shown to possess antibiosis resistance to SA (Xiao et al. 2012). SA resistance in soybean line P203 was compared with P746 and Dongnong 47 in aphid choice tests and no-choice tests. In choice tests, the average number of SA per plant and the DI was significantly lower for P203 than Dongnong 47 but higher than for P746 at both 11 and 21 days following infestation (Fig. 1). In no-choice tests, however, the resistance level was significantly reduced for both SA and DI when compared with P746 at day 21 (Fig. 1). The average number of SA increased to 210 per P203 plant and DI was 28.5 %. Therefore, the data strongly indicated antixenosis resistance in P203, prevented aphids from reproduction in choice test, but tended to be susceptible in no-choice test.

Genetic analysis

The SA score for P203 was 1 or 2 (Fig. 2a), while the score for Dongnon 47 was 3 (Fig. 2b). All F_1 plants were resistant to SA (Fig. 2c). The frequency distributions of the F_2 SA scores are shown in Fig. 2d. Most F_2 plants had definite aphid scores of 0, 1, 3, or 4, and 12 plants had inconclusive phenotypes. Of these 12 plants, nine plants were confirmed as susceptible with all susceptible progeny in the corresponding $F_{2:3}$ families and three plants were confirmed to be heterozygous. The segregation ratio of resistant to susceptible plants in the F_2 population was 208:65 (3.2:1). This segregation pattern fit a ratio of resistant:susceptible of 3:1, indicating that SA resistance in P203 is controlled by a single dominant gene ($\chi^2 = 0.21$; $P = 0.65$). The 260 $F_{2:3}$ families also segregated in a 60:138:62 (resistant:segregating:susceptible) ratio, failed to reject the 1:2:1 genetic ratio ($\chi^2 = 1.02$; $P = 0.60$), supporting the mode of monogenic inheritance found in the F_2 phenotype analyses.

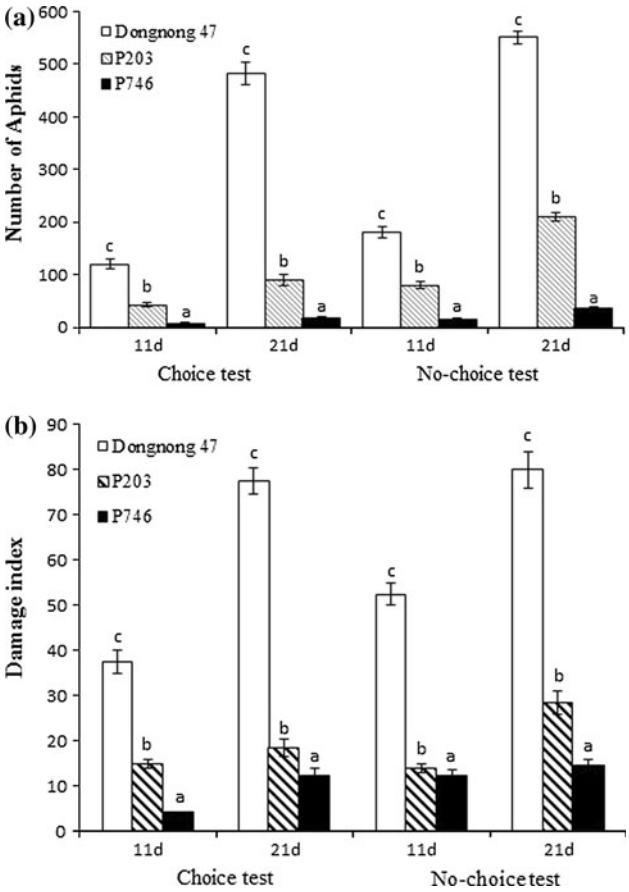

Fig. 1 Mean number of soybean aphid per plant **(a)** and damage index **(b)** 11 and 21 days after infestation with SA in choice and no-choice tests with soybean lines P203, P746 and Dongnong 47. Values represent the mean and standard error of four replicates. Means labeled with the same letter are not significantly different ($P = 0.05$)

Mapping the aphid resistance locus using SSRs from Song et al. (2004)

A total of 1015 SSR markers available from Song et al. (2004) were screened for polymorphism in the parental

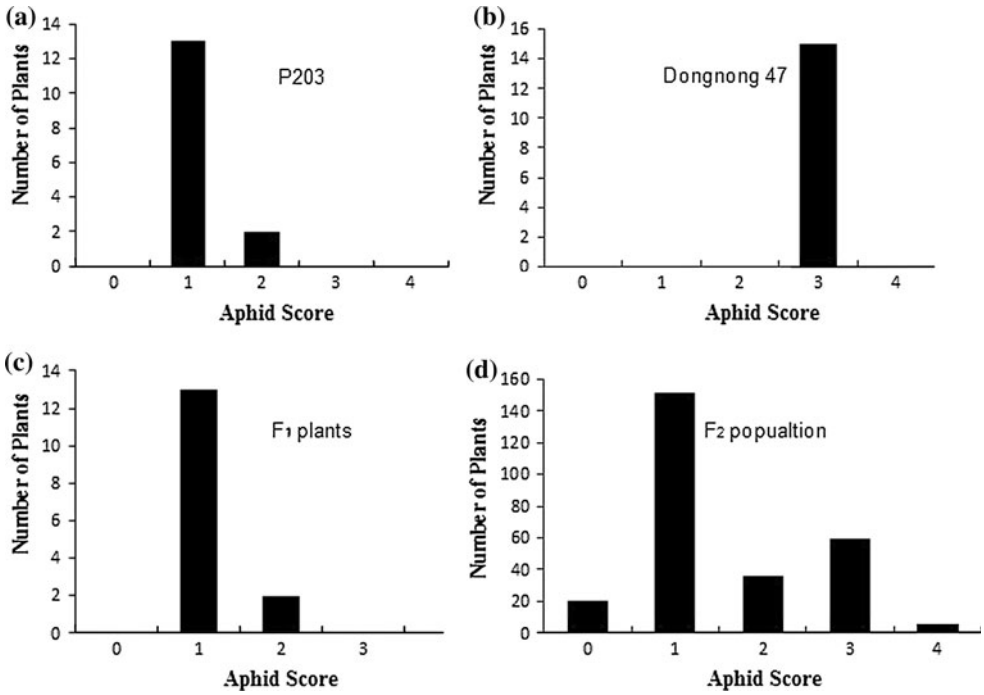

Fig. 2 Distribution of SA scores in mapping populations 21 days after infestation with SA: **a** resistant parent P203; **b** susceptible parent Dongnong 47; **c** F_1 plants; **d** F_2 population

lines, and 494 primer pairs (48.7 %) were found to be polymorphic. BSA identified four SSR markers, Satt437, Sct_194, Sat_377, and Satt409, which were found to be linked to the locus for SA resistance in P203. Data from all markers fit the expected co-dominant 1:2:1 segregation ratio (Table 2). The four markers were genotyped in the 260 F_2 plants. Linkage analysis showed that the SA resistance gene in P203 mapped to a region between Satt409 and Sat_377 on chromosome 8 (Data not shown).

Fine mapping using SSRs from the BSSR_1.0 soybean database

To further define the region between Satt409 and Sat_377, additional 400 SSR markers between the genomic interval were selected randomly from BSSR_1.0 soybean database to screen against the two parental lines. Seven markers, BSSR_08_1415, BSSR_08_1451, BSSR_08_1527, BSSR_08_1543, BSSR_08_1577, BSSR_08_1596 and BSSR_08_1653 were found to detect polymorphisms between Dongnong 47 and P203. These seven markers were then used to screen the 260 F_2 plants. Data from all SSR markers fit the 1:2:1 ratio expected at the 0.05 significance level, and showed no segregation distortion near the SA resistance gene in this population (Table 2). Thirteen plants had recombination events between BSSR_08_1451 and the resistance gene, and five plants had recombinant events between BSSR_08_1527 and the resistance gene. Linkage analysis showed that the resistance locus is in the interval between BSSR_08_1451 and BSSR_08_1527, with interlocus distances of 3.5 and 2.4 cM, respectively (Fig. 3a). The physical distance between BSSR_08_1451 and BSSR_08_1527 is approximately 1.57 Mb based on

Table 2 Chi-square analysis of the segregation ratios for linked SSR markers in the 260 $F_{2:3}$ lines

Marker locus	Number of F_2 lines for each genotype[a]				χ^2 1:2:1[b]	$P_{0.05}$
	R	H	S	–		
Satt437	78	112	68	2	5.26	0.07
Sct_194	64	122	74	0	1.75	0.42
Sat_377	62	127	69	2	0.44	0.80
BARCSOYSSR_08_1415	60	132	68	0	0.55	0.76
BARCSOYSSR_08_1451	63	130	67	0	0.12	0.94
SSR_08_64	58	135	67	0	1.01	0.60
SSR_08_75	61	135	64	0	0.45	0.80
SSR_08_88	56	142	62	0	2.49	0.29
SSR_08_133	57	141	62	0	2.05	0.36
SSR_08_143	58	140	62	0	1.66	0.44
BARCSOYSSR_08_1527	59	141	60	0	1.87	0.39
BARCSOYSSR_13_1543	59	144	57	0	3.04	0.22
BARCSOYSSR_13_1577	72	133	55	0	2.36	0.31
BARCSOYSSR_13_1596	72	132	56	0	2.03	0.36
BARCSOYSSR_13_1653	70	132	58	0	1.17	0.58
Satt409	71	126	63	0	0.74	0.69

[a] *R* SSR allele from the resistant parent, *S* SSR allele from the susceptible parent, *H* SSR alleles from both resistant and susceptible parents, – missing data

[b] Expected segregation = 1:2:1 = R:H:S

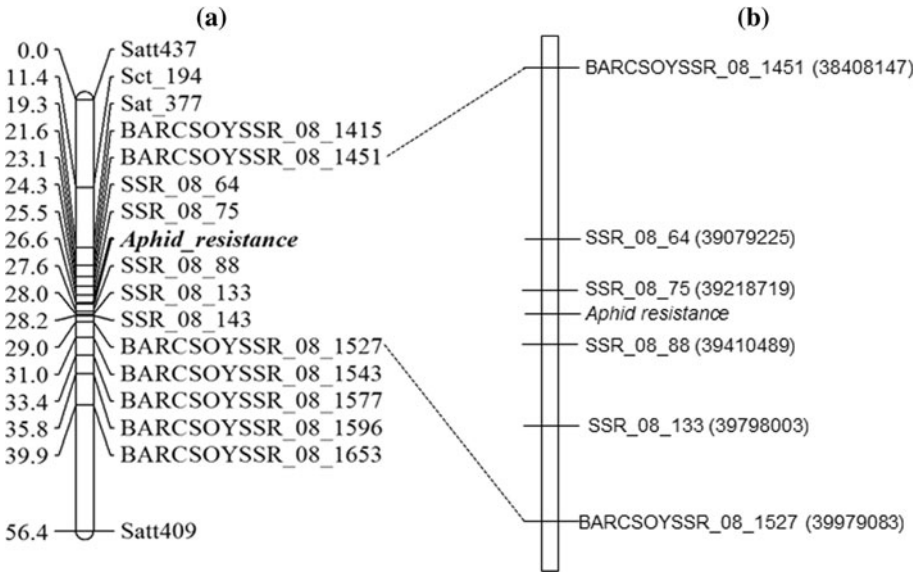

Fig. 3 **a** Linkage map of chromosome 8 indicating position of the SA resistance locus based on 260 $F_{2:3}$ plants from a cross between Dongnong 47 and P203; **b** expanded map showing the SA resistance gene in P203 located between BSSR_08_1451 and BSSR_08_1527. Numbers in brackets indicate the physical position of the markers in base pairs (bp)

the Williams 82 genomic sequence (from 38,408,147 to 39,979,083 bp) (Fig. 3b).

Fine mapping with newly-developed SSR markers

To better define the position of SA resistance gene within the 1.57 Mb, 140 SSRs (SSR_08_1–SSR_08_140) were developed between BSSR_08_1451 and BSSR_08_1527 by SSR hunter analysis software (http://www.bio-soft.net). Five of these SSR markers, SSR_08_64, SSR_08_75, SSR_08_88, SSR_08_133 and SSR_08_143 showed polymorphism between the two parents. No segregation distortion was found when these markers were scored in the 260 F_2 lines (Table 2).

The right border of the position of the target gene was defined by analysis of lines 26 and 83 (Table 3). L26 (heterozygous) was segregating for marker alleles from BSSR_08_1451 to SSR_08_88, and had homozygous resistant alleles for markers from BSSR_08_1527 to SSR_08_133. L83 (homozygous resistant) was segregating from BSSR_08_1527 to SSR_08_88, and was homozygous resistant from BSSR_08_1451 to SSR_08_75, demonstrating that the target gene was to the left of SSR_08_88. Lines 129, 173, 206 and 212 (all heterozygotes) were segregating for marker alleles from BSSR_08_1527 to SSR_08_88, while L129 was homozygous resistant from BSSR_08_1451 to SSR_08_75; L173 and 206 were homozygous from alleles from the susceptible parent from BSSR_08_1451 to SSR_08_75. These results indicated that the target gene must be to the right of SSR_08_75 (Table 3). Therefore, we were able to map the SA resistance gene in P203 to a region 1.1 cM from SSR_08_75 and 1.0 cM from SSR_08_88, a ~192 kb interval from 39,218,719 to 39,410,489 bp based on the Williams 82 8X assembly (Glyma1.0) (Fig. 3).

Discussion

Resistance to aphids is one result of co-evolution of plant and aphid. The discovery of aphid-resistant sources will be useful in the isolation and characterization of resistance genes. Antibiosis and antixenosis are two major mechanisms of plant resistance to insects (Painter 1951). Currently, SA-resistant soybean cultivars have been screened in choice and no-choice tests in the US. The cultivars Dowling, Jackson, PI 200538, PI 243540, PI 567541B, PI 567598B, PI 230977, and P746 possess antibiosis resistance; cultivars PI 71506, PI 567301B, PI 567324, PI 567543C, PI 567597C and PI 595099 possess antixenosis resistance (Hill et al. 2004; Mensah et al. 2005; Mian et al. 2008a; Hesler et al. 2007; Xiao et al. 2012). Valuable SA-resistant germplasm has been discovered by screening hundreds of genetic sources from China. Soybean cultivar P203 was identified as being resistant to SA from Shanghai (Wu et al. 2009). In the present study, we found that SA population development on plants of Dongnong 47 and P203 was significantly different. P203 should be useful to soybean breeders especially in the development of SA-resistant soybean cultivars.

In this study, a linkage map of the region on chromosome 8 surrounding the resistance locus was constructed with 16 SSR markers. The total genetic distance encompassed by these marker loci was 56.4 cM, with an average distance of 3.53 cM between adjacent SSR loci. The marker order of the loci in the new map was also consistent with those of the Soybean Consensus Map (Song et al. 2004). The effort to fine map the target locus was greatly enhanced by the availability of the public soybean genome sequence (Schmutz et al. 2010), as well as the high level of

Table 3 Phenotypic and genotypic analyses of the representative recombinant lines crossed at BSSR_08_1451–BSSR_08_1527 interval

Line	Phenotype[a]	SSR marker						
		BSSR_08_1451	SSR_08_64	SSR_08_75	SSR_08_88	SSR_08_133	SSR_08_143	BSSR_08_1527
L26	H	H	H	H	H	R	R	R
L83	R	R	R	R	H	H	H	H
L89	H	H	H	H	H	R	R	R
L108	H	R	H	H	H	H	H	H
L114	H	R	R	H	H	H	H	H
L129	H	R	R	R	H	H	H	H
L140	H	S	S	H	H	H	H	H
L173	H	S	S	S	H	H	H	H
L206	H	S	S	S	H	H	H	H
L212	H	R	R	R	H	H	H	H

[a] *R* resistant plant or the SSR allele from P203, *H* heterozygous plant or the SSR alleles from heterozygous plants, *S* susceptible plant or the SSR allele from 'Dongnong 47'

polymorphism (48.7 %) between the parental lines P203 and Dongnong 47.

Recently, two SA resistance genes, qRa_1 (Meng et al. 2011) and a minor QTL (Jun et al. 2012), have been mapped on chromosome 8. Although qRa_1, which was mapped close to Satt470 on chromosome 8, was found to give SA resistance in Zhongdou 27, we concluded that the gene in P203 was different from qRa_1 for the following reasons. Firstly, qRa_1 was mapped to a region around Satt470 (Fig. 4); however in our study, Satt470 was not shown to be linked with the SA resistance in P203. Secondly, Zhongdou 27 conferred antibiosis resistance to SA while P203 showed antixenosis type resistance, indicating that SA resistance in the two soybean lines is not controlled by the same gene (Meng et al. 2011). Jun et al. (2012) reported that a minor QTL controlling SA resistance on chromosome 8 was mapped close to the interval between BSSR_08_1095 and BSSR_08_1110 (from 19,718,493 to 20,047,898 bp), which is located north of the SA resistance in P203 in this study (Fig. 4). Thus, we conclude that the resistance in P203, which we mapped to chromosome 8, is likely different than the other two resistance loci previously reported on this chromosome. However, due to differences in experimental conditions between studies, more direct comparisons of the sources of the resistances may be required for conclusive evidence.

Almost all reported aphid resistance genes in soybean, as well as aphid resistance genes in other crops, are predicted to encode nucleotide-binding site leucine-rich repeat (NBS-LRR) proteins (Jun et al. 2012; Kim et al. 2010a, b; Zhang et al. 2010). In *Medicago truncatula*, *AKR* to the blue green aphid (BGA, *Acyrthosiphon kondoi* Shinji), *TTR* to the spotted alfalfa aphid (SAA, *Therioaphis trifolii* f. *maculate*), *RAP*1 to the pea aphid (PA, *Acyrthosiphon pisum* Harris) and *AIN* to BGA and PA are predicted to reside in a cluster of NBS-LRR genes on chromosome 3 (Klingler

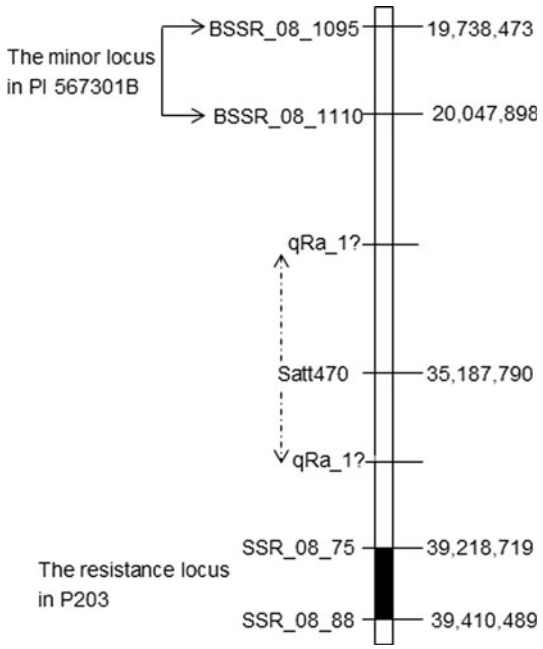

Fig. 4 The physical position of flanking SSR markers and the aphid resistance genes mapped on chromosome 8 in three soybeans cultivars (Zhongdou 27, PI 567301B and P203) based on Williams 82 sequence. Numbers indicate the physical location of the SSR markers based on soybean chromosome 8 sequence of Glyma1.01. The dotted arrows indicate that qRa_1 may be located north or south of Satt470 being associated with qRa_1 in Zhongdou 27, and the genetic distance between qRa_1 and Satt470 is unknown (Meng et al. 2011). *Solid arrows* show the putative minor QTL in PI 567301B (Jun et al. 2012). The *solid black* region represents the SA resistance in P203 in this study

Table 4 The function annotation of the five predicted genes in the 192 kb region between SSR_08_75 and SSR_08_88 on soybean chromosome 8 based on Williams 82 sequence in this study

Gene ID	Function annotation
Glyma08g39700	BURP domain, polygalacturonase
Glyma08g39710	Radical SAM superfamily, catalytic activity
Glyma08g39731	Sentrin/SUMO-specific protease, cysteine protease
Glyma08g39751	Serine-threonine protein kinase, LRR protein kinase family
Glyma08g39770	Aldehyde dehydrogenase-related, aldehyde dehydrogenase family

et al. 2005, 2007, 2009; Stewart et al. 2009). The *Mi* gene from tomato (*Solanum lycopersicum* L.) and *Vat* from melon (*Cucumis melo* L.) encode NBS-LRR proteins conferring resistance against *Macrosiphum euphorbiae* and *Aphis gossypii*, respectively (Rossi et al. 1998; Dogimont et al. 2009). In addition, *Bph14* in rice (*Oryza sativa* L.) also encodes an R protein that confers resistance to the brown planthopper (*Nilaparvata lugens Stål*) (Du et al. 2009). None of the genes described above encode a serine/threonine protein kinase which is the most likely candidate gene in the region of chromosome 8 where the resistance found in P203 is located. Therefore, the resistance in P203 may be especially important in developing SA resistance cultivars.

In this study, the current gene annotation of the 192 kb region between SSR_08_75 and SSR_08_88 based on Williams 82 (Glyma1) predicts the presence of five candidate genes. The function annotation of the five genes is listed in Table 4. Of the five candidate genes, Glyma08g39750 is the strongest candidate for a resistance gene as it is predicted to encode a Ser/Thr protein kinase belonging to the family of transmembrane pattern recognition receptors (PRRs). These recognize conserved pathogen-associated molecular patterns (PAMPs), providing broad spectrum disease resistance, as the first layer in the plant immune system (Jones and Dangl 2006). Our research indicates that soybean P203 can provide resistance to different SA isolates from five locations in China (Shandong, Heilongjiang, Jilin, Yunnan, Guizhou), while the other cultivars including Dowling, PI 243540, PI 567543C and PI 567541B could not (unpublished data). This result supports the concept that the SA resistance gene in P203 encodes a Ser/Thr protein kinase that provides a broad spectrum resistance. Our results indicated that the SA resistance gene in P203 is different from the others mapped previously (Jun et al. 2012; Kim et al. 2010a, b; Meng et al. 2011; Zhang et al. 2009, 2010). After consultation with the Soybean Genetics Committee about appropriate nomenclature, the gene we describe has been provisionally named [Rag6]_P203. We are conducting ongoing research to clone this gene and validate the function.

The SA biotypes occurring in China are unknown; therefore, it is important to identify the SA biotypes in China and to compare them with the SA biotypes found in the USA to understand and improve crop resistance. The struggle between aphids and plants is never-ending due to the hosts and the aphids engage each other in a constant evolutionary arms race. The breakdown of resistance genes by insects has frequently occurred, especially when resistance is conditioned by a single gene (Burd and Porter 2006; Haley et al. 2004). However, the probability of breakdown of resistance genes encoding LRR protein kinase would be much lower than that of the NBS-LRR genes (i.e. *Rag1–Rag5*) (Lacombe et al. 2010). Identification and mapping of SA resistance genes will help in the understanding of the coevolution soybean resistance and SA biotypes and in developing resistant soybean varieties efficiently.

In this study, we mapped the SA resistance found in P203 to chromosome 8. Additionally, we developed new SSR markers to fine map the gene to the interval SSR_08_75-SSR_08_88. Fine mapping of the SA resistance gene in P203 will greatly facilitate the molecular identification of the gene which will contribute to a better understanding of the mechanism of SA defense. The SSR markers we developed will also be useful in marker-assisted breeding programs to facilitate incorporating this gene into new, better adapted cultivars.

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