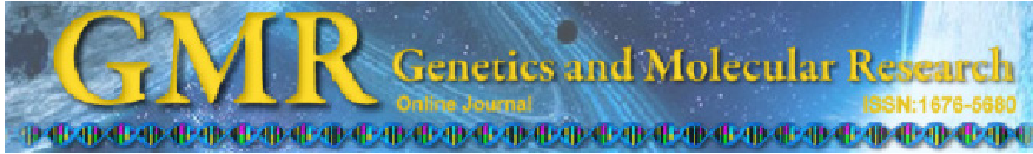

Mapping an aphid resistance gene in soybean [*Glycine max* (L.) Merr.] P746

L. Xiao, Y.P. Zhong, B. Wang and T.L. Wu

Legume Biotechnology Laboratory of School of Agriculture and Biology, Shanghai Jiaotong University, Shanghai, China

Corresponding author: T.L. Wu
E-mail: tianlongwu@263.net

Genet. Mol. Res. 13 (4): 9152-9160 (2014)
Received March 28, 2014
Accepted October 23, 2014
Published November 7, 2014
DOI http://dx.doi.org/10.4238/2014.November.7.2

ABSTRACT. Soybean aphid (SA: *Aphis glycines* Matsumura) is one of the most serious pests of soybean [*Glycine max* (L.) Merr.] worldwide. A single dominant gene was found to control SA resistance in soybean line P746, which exhibits antibiosis resistance. This study aimed to define the location of the SA resistance gene in P746. A $F_{2:3}$ mapping population, including 312 individuals, was created based on the cross of P746 and 'Dongnong 47'. Combined with bulked segregant analysis, all of the 1015 simple sequence repeats (SSR) from the soybean consensus map were used to locate the tentative genomic region of the SA resistance gene in P746. The effort resulted in the mapping of R_P746, the SA resistance gene in P746, and was flanked on either side by Satt334 and Satt335 on chromosome 13. By chromosome walking with SSRs from BARCSOYSSR_1.0, R_P746 was mapped between BARCSOYSSR_13_1278 and BARCSOYSSR_13_1363, with distances of 4.2 and 2.6 cM, respectively. The results indicate that R_P746 might be different to the SA resistance genes previously reported. The markers that are closely linked to R_P746 are expected to be useful for marker assisted selection in future soybean aphid resistance breeding programs.

Key words: Soybean; Aphid resistance; Bulked segregant analysis; P746; Simple sequence repeat marker; R_P746

 ©FUNPEC-RP www.funpecrp.com.br

INTRODUCTION

The soybean aphid (SA), *Aphis glycines* Matsumura, is the principal pest of soybean [*Glycine max* (L.) Merr.] worldwide. As an insect pest native to Asia, SA has been found to be a common soybean pest in China, and has also been reported by other Asian countries (Ragsdale et al., 2004; Tilmon et al., 2011). Recently, this species has rapidly spread to other soybean-producing areas as an exotic (invasive) insect, threatening global soybean production (Venette and Ragsdale, 2004; OMAFRA, 2009). Severe infestations of SA are characterized by thousands of individuals being present on individual soybean plants during the reproductive stage of the pest. Feeding by aphids directly results in devastating crop yield losses, by affecting various yield components, including plant height, number of nodes and pods per plant, seed size, and bean quality (Ostlie, 2001; Beckendorf et al., 2008). Furthermore, soybean aphids potentially boost plant viral transmission, also causing a reduction in soybean yield and seed quality (Hartman et al., 2001; Davis et al., 2005). In addition to the agronomic impacts, this soybean pest is a potential factor leading to a cascade of ecological problems through its invasion of new areas (Ragsdale et al., 2011). Chemical management is a general method of eradicating soybean aphids from fields. For instance, nearly 3 million hectares of soybeans in the USA were estimated to be sprayed with insecticides to control insect pests in 2003 (Landis et al., 2003). However, insecticide applications are always associated with cost considerations and ecological concerns, because of the simultaneous non-selective elimination of natural enemies of the soybean aphid, such as spider mites (Smith and Krischik, 1999). Therefore, the utilization of host plant resistance is considered to be potentially a sustainable and effective way of resolving this problem.

To date, several SA resistance genes have been identified in soybean. The resistance genes *Rag1* in Dowling and *Rag* in Jackson were mapped to the same genomic region on soybean chromosome 7 [linkage group M (LG M); Li et al., 2007]. The dominant resistant gene *Rag2* in PI 243540 was mapped to soybean chromosome 13 (LG F) (Rouf Mian et al., 2008). Interestingly, PI 200538 was considered to be an additional source of *Rag2* because the resistance locus in PI 200538 was located in the same region as *Rag2*, with both genes exhibiting very similar resistant abilities (Hill et al., 2009). In 2010, *Rag2* was mapped into a 54-kb region in PI 200538 (Kim et al., 2010b). Another important antixenosis resistance gene, *Rag3* in PI 567543C, was found to be chromosome 16 (LG J) (Mensah et al., 2005; Zhang et al., 2010). Using the quantitative trait loci (QTL) mapping approach, 2 recessive genes (*rag1* and *rag4*) in PI 567541B were anchored on chromosomes 7 and 13, respectively (Zhang et al., 2009). In PI 567301B, 2 genes for SA resistance were identified using the QTL mapping approach. A major QTL for aphid antixenosis, *Rag5*, was mapped near the *Rag2* locus on chromosome 13, while a minor locus was found on chromosome 8 (LG A2) (Jun et al., 2012). In addition, 2 QTLs, qRa_1 on chromosome 8 and qRa_2 on chromosome 13, were found to be associated with SA resistance in the soybean cultivar ‘Zhongdou 27’ (Meng et al., 2011).

In 2012, we identified a single dominant gene responsible for excellent aphid resistance in a Chinese soybean cultivar P746 (Xiao et al., 2012). As a subsequent study, our primary goal here was to molecularly map the gene in P746 using simple sequence repeats (SSR) markers to provide valuable information for utilizing and cloning this gene in the future.

MATERIAL AND METHODS

Mapping population

Three hundred and twelve $F_{2:3}$ plants derived from 'Dongnong 47' (susceptible to aphids) and P746 (resistant to aphids) were used to map the gene for resistance to Shanghai SA. Two hundred and thirteen out of the 312 $F_{2:3}$ individuals have been previously described (Xiao et al., 2012).

SA resistance evaluation

In July 2010, SA resistance of 99 F_2 plants with the parents were evaluated in non-choice tests in the greenhouse, as previously described (Xiao et al., 2012). Two weeks after sowing, each plant was inoculated with 5 wingless SA, and separated from other plants with a mesh bag to prevent aphids from escaping. The SA clone used for infestation was obtained from nearby naturally infested soybean fields at Shanghai Jiaotong University, China. SA resistance for each plant was visually rated at 3 weeks after inoculation, using a scale of 0-4 developed by Mensah et al. (2005). In progeny tests conducted in April 2012, 12 $F_{2:3}$ seeds from the corresponding F_2 plant were sown, and the $F_{2:3}$ plants were separately evaluated for SA resistance, as described by Xiao et al. (2012). 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 producing only susceptible F_3 plants (scores of 3 or 4) was confirmed as homozygous susceptible. An F_2 plant resulting in a combination of resistant and susceptible progeny was confirmed as heterozygous.

Bulked segregant analysis and SSR marker genotyping

Young leaves from each F_2 plant and 2 parental lines were sampled for DNA extraction with the CTAB method (Keim et al., 1988) and diluted to 25 ng/µL for SSR genotyping. Equal amounts of DNA from 10 resistant plants (with a score of 0 or 1) and 10 susceptible plants (with a score of 3 or 4) from the F_2 population were pooled to form 2 DNA bulks for bulked segregant analysis (BSA) (Michelmore et al., 1991).

A total of 1015 primers selected from the integrated soybean map of Song et al. (2004) were used to screen polymorphisms in the 2 parents and 2 bulks. Corresponding the physical position of Satt334 on chromosome 13 in BARCSOYSSR_1.0, about 200 SSR markers were selected every 100 kb on chromosome 13, based on the Williams 82 assembly (Glyma1), to screen the linked marker flanked on the other side and to conduct further mapping (Schmutz et al., 2010).

Polymerase chain reactions (PCR) were performed in 10 µL volumes with 50 to 250 ng template DNA, 2 µM of each primer, 30 mM $MgCl_2$, 3 mM of each deoxyribonucleotide triphosphate, 2.5 U *Taq* polymerase, and 1X PCR buffer. Conditions for PCR amplification consisted of an initial denaturing step of 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 step at 72°C for 10 min. The amplification products were separated on 6% denatured polyacrylamide gel with 1X Tris-borate-ethylenediaminetetraacetic acid buffer at a constant power of 45 W for 2 h. After electrophoresis, the gel was stained with silver (Bassam et al., 1991), and photographed with a digital camera.

Statistical analysis and mapping

The chi-square test was performed to test the goodness of fit for the observed segregation among F_2 and $F_{2:3}$ plants with different genetic ratios. The SSR marker data and the aphid phenotypes of the 312 $F_{2:3}$ plants were combined for linkage analysis by the MAPMAKER/EXP3.0 program (Lander et al., 1987) with a logarithm of the odds score ≥3.0. The recombination percentage was converted to genetic distance by the Kosambi mapping function (Kosambi, 1944). A linkage map was drawn using the MapChart 2.0 software (Voorrips, 2002).

RESULTS

Segregation of the population mapped for aphid resistance

Most F_2 plants had definite aphid scores of 0, 1, 3, or 4; however, four plants had obscure phenotypes. The 4 plants were confirmed as susceptible, and grouped with all-susceptible progeny in the corresponding $F_{2:3}$ families. Of the 99 F_2 individuals, 73 F_2 plants were resistant, while 26 plants were susceptible. Chi-square analysis produced a ratio of 3R:1S, indicating the action of a single dominant gene controlling SA resistance in P746 (Table 1; $\chi^2 = 0.08$; P = 0.78). Chi-square analysis of the segregation of 99 $F_{2:3}$ families in progeny tests failed to reject the 1:2:1 ratio of resistant to segregated to susceptible $F_{2:3}$ families (Table 2; $\chi^2 = 0.11$; P = 0.95), confirming the monogenic dominant inheritance found in the F_2 phenotype analysis. The 99 $F_{2:3}$ plants and 213 $F_{2:3}$ plants identified in a previous study (Xiao et al., 2012) were used to map the SA resistance gene in P746.

Table 1. Segregation of soybean aphid (SA) resistance in soybean 'Dongnong 47' x P746 F_2 plants 21 days after infestation by Shanghai SA in summer 2010.

F_2 population	No. of F_2 plants	Observed[a]	Expected	χ^2	$P_{0.05}$
		R:S	R:S		
801	52	36:16	39:13	0.92	0.34
802	47	37:10	35.25:11.75	0.35	0.55
Total	99	73:26	74.25:24.75	0.08	0.78

[a]R = resistant; S = susceptible.

Table 2. Segregation of soybean aphid (SA) resistance in 99 'Dongnong 47' x P746 $F_{2:3}$ families 21 days after infestation by Shanghai SA in spring 2011.

F_2 family	No. of $F_{2:3}$ families	Observed[a]	Expected	χ^2	$P_{0.05}$
		R:H:S	R:H:S		
801	52	12:24:16	13:26:13	0.92	0.63
802	47	13:24:10	11.75:23.5:11.75	0.40	0.82
Total	99	25:48:26	24.75:49.5:24.75	0.11	0.95

[a]R = resistant; H = heterozygous; S = susceptible.

Mapping of the SA resistance locus using 1015 SSRs

A total of 1015 SSR markers (Song et al., 2004) were screened for parental polymorphism. As a result, 465 polymorphic markers were obtained and used in preliminary link-

 ©FUNPEC-RP www.funpecrp.com.br

age analysis with the BSA method. The BSA identified 5 SSR markers (Satt648, Satt684, SOYNOD26A, Satt334, and Satt335; Figure 1) that showed polymorphism between the 2 bulks. Satt648, Satt684, and SOYNOD26A proved to be false positive, because no association was found for the resistant and susceptible bulks with the parental lines (data not shown). Therefore, the SA resistance gene in P746 was found to be linked with Satt334 and Satt335 on chromosome 13. The genetic patterns of the 2 markers were then examined in the 312 $F_{2:3}$ plants. Linkage analysis indicated that Satt335 and Satt334 flanked either side of the aphid resistance gene.

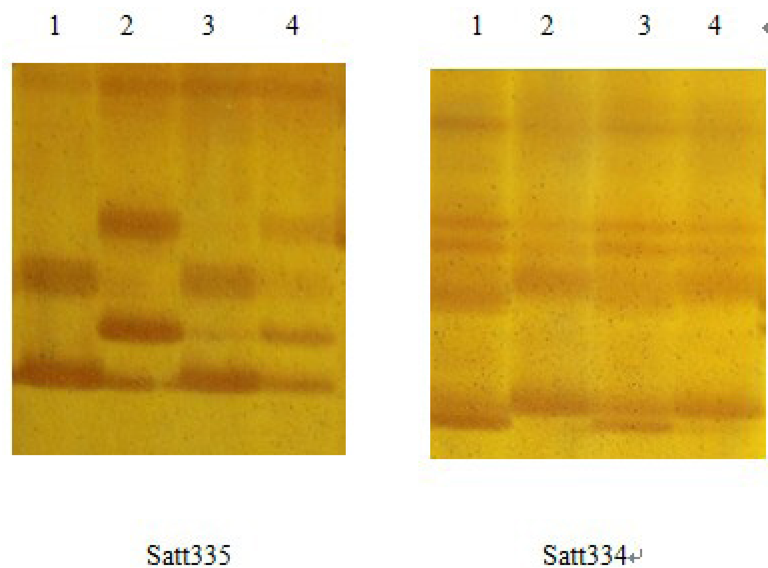

Figure 1. Photographic images of polyacrylamide gels following electrophoresis of Satt335 and Satt334 produced from 'Dongnong 47', P746, susceptible F_2 plant bulks and resistant F_2 plant bulks; *lane 1* = 'Dongnong 47'; *lane* 2 = 746; *lane 3* = susceptible bulk; *lane 4* = resistant bulk.

Mapping using SSRs from the BARCSOYSSR_1.0 soybean database

Of the 200 SSRs on chromosome 13, BARCSOYSSR_13_1129, BARCSOYSSR_13_1136, and BARCSOYSSR_13_1508 were found to generate polymorphic products between the 2 bulks and the parental lines. The 312 $F_{2:3}$ individuals were assessed for the genotypes of the 3 markers. The linkage analysis indicated that R_P746 was mapped between Satt335 and BARCSOYSSR_13_1508 (Figure 2). The SSR markers in this interval were selected randomly to screen the polymorphic markers between P746 and 'Dongnong 47'. Four polymorphic markers (BARCSOYSSR_13_1278, BARCSOYSSR_13_1363, BARCSOYSSR_13_175, and BARCSOYSSR_13_1389) were further used to genotype the 312 $F_{2:3}$ individuals. Data from all of the markers fitted the expected 1:2:1 ratio at the 0.05 significance level (Table 3). In addition, the linkage map containing R_P746 was constructed based on the 9 SSR markers. The genetic distance covered by the markers was 33.7 cM. The order of SSR markers in the map was in agreement with BARCSOYSSR_1.0 (Song et al., 2010). The 2 flanking markers with the closest linkage to the resistance locus are BARCSOYSSR_13_1278

and BARCSOYSSR_13_1363, which were located at a genetic distance of 4.2 and 2.6 cM, respectively (Figure 2). The physical distance approximately corresponded to 1.65 Mb, based on the Williams 82 sequence (from 31,803,199 to 33,448,837 bp).

Table 3: Chi-square analysis of the segregation ratios for the linked markers in 312 $F_{2:3}$ lines.

Locus	Number of F_2 lines for each genotype[a]				χ^2 1:2:1[b]	$P_{0.05}$	χ^2 3:1[c]	$P_{0.05}$
	A	H	B	-				
Satt334	72	162	76	2	0.83	0.66	0.22	0.64
BARCSOYSSR_13_1129	73	162	77	0	0.56	0.75	0.02	0.90
BARCSOYSSR_13_1136	68	167	75	2	2.17	0.34	0.11	0.74
Satt335	72	155	85	0	2.11	0.35	0.84	0.36
BARCSOYSSR_13_1278	72	157	83	0	0.79	0.67	0.43	0.51
BARCSOYSSR_13_1363	70	155	87	0	2.29	0.32	1.39	0.24
BARCSOYSSR_13_1375	77	151	84	0	0.64	0.73	0.62	0.43
BARCSOYSSR_13_1389	76	153	83	0	0.42	0.81	0.43	0.51
BARCSOYSSR_13_1508	78	153	81	0	0.17	0.92	0.15	0.70

[a]A = the SSR allele of the resistant parent; B = the SSR allele of the susceptible parent; H = the SSR alleles from both resistant and susceptible parents; and (-) = missing data. [b]Expected segregation =1:2:1 = A:H:B. [c]Expected segregation = 3:1 = [A+H]:B.

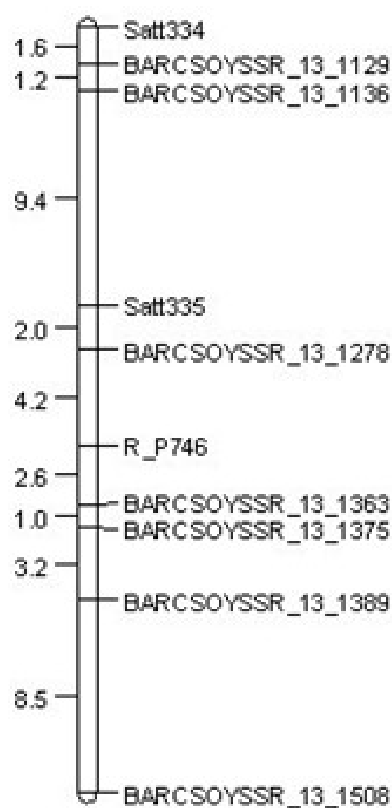

Figure 2. Genetic linkage map for R_P746 based on 312 $F_{2:3}$ lines derived from 'Dongnong 47' and P746. Distance between neighboring markers on the left is in centiMorgans (cM).

DISCUSSION

In this study, the linkage map was constructed with 9 SSR markers on chromosome 13. No segregation distortion was found in these markers. The total genetic distance covered by the markers was 33.7 cM, with an average distance of 3.75 cM between adjacent SSR loci. The marker order of the loci in the new map was also consistent with those of the Soybean Map BARCSOYSSR_1.0. No chromosomal alternations (such as inversions and translocations) were observed in the population that was mapped in this study.

Kanazin et al. (1996) reported that resistance gene analogs are conserved and clustered in soybean. At present, 9 SA-resistance genes have been identified, with 4 SA resistance genes being mapped to 4 different genomic regions on chromosome 13 (Zhang et al., 2009; Kim et al., 2010b; Meng et al., 2011; Jun et al., 2012). *rag4* in PI 567541B, which provides antibiosis SA resistance, was mapped, and found to be linked with Satt649 (Zhang et al., 2009). qRa_2, which is located close to Satt114 of LG F, was found to confer antibiosis aphid resistance in "Zhongdou 27" (Meng et al., 2011). Glyma13g26000 is the only nucleotide-binding site-leucine-rich repeat (NBS-LRR) candidate *Rag2* that also confers antibiosis resistance (Kim et al., 2010b). *Rag5* in PI 567301B confers antixenosis type resistance, and is physically located between 29,036,526 and 29,548,838 bp on chromosome 13, based on Williams 82 8X draft assembly (Glyma 1) (Jun et al., 2012). In this study, R_P746 was mapped to an interval corresponding to the reported 1.65 Mb sequence between 31,803,199 bp and 33,448,837 bp on chromosome 13 in Williams 82. The results indicated that R_P746 was completely different to all previously genes. To correctly map R_P746, additional polymorphic markers, especially single nucleotide polymorphism markers, must be explored or developed for this region.

To date, almost all aphid resistance genes that have been mapped in soybean and *Medicago truncatula* have been predicted to encode NBS-LRR proteins (Klingler et al., 2005; Kim et al., 2010a,b; Zhang et al., 2010; Jun et al., 2012). Furthermore, 2 aphid resistance genes that have been cloned in plants are members of the NBS-LRR family, including the *Mi-1.2* gene resistant to the potato aphid (*Macrosiphum euphorbiae*) in tomato (*Lycopersicum esculentum* Mill.) and the *Vat* gene, which confers resistance to *Ashbya gossypii* in melon (*Cucumis melo* L.) (Rossi et al., 1998; Dogimont et al., 2009). In addition, *Bph14* in rice (*Oryza sativa* L.) also encodes a NBS-LRR disease resistance protein that confers resistance to the brown planthopper (*Nilaparvata lugens* Stål) (Du et al., 2009). Unexpectedly, in this study, NBS-LRR type genes were not recorded in this 1.65-Mb region spanning the SA resistance locus. Instead, more than 10 Serine/Threonine protein kinase genes were recorded, which confer broad-spectrum disease resistance by perceiving pathogen associated molecular patterns (PAMPs), which lead to PAMP-triggered immunity (Dodds and Rathjen, 2010).

Although two biotypes of SA have been discovered in North America, the biotype of SA in China remains undetermined. It is important to characterize the frequencies and distribution of the biotypes in China. It is also important to identify whether P746 could provide resistance to SA from other locations.

The identified resistance gene, together with the SSR-based molecular map, is valuable for soybean breeders in the development of SA resistant soybean cultivars. In particular, the multiple molecular markers linked to this resistance gene are anticipated to greatly facilitate work integrating a selection of resistance genes with the aim of providing a broader spectrum of resistance to multiple SA biotypes, in addition to making adaptation to resistance genes more complicated for aphid populations.

 ©FUNPEC-RP www.funpecrp.com.br

ACKNOWLEDGMENTS

Research supported by grants from the National Natural Science Foundation of China (#31071435), Ministry of Education Fund on New Staff (#20100073120059), the Key Projects of Breeding of New Cultivars (#2011ZX08004-004), and the Shanghai Horticulture Key Discipline Construction Fund (#B209).

REFERENCES

Bassam BJ, Caetano-Anolles G and Gresshoff PM (1991). Fast and sensitive silver staining of DNA in polyacrylamide gels. *Anal. Biochem.* 196: 80-83.

Beckendorf EA, Catangui MA and Riedell WE (2008). Soybean aphid feeding injury and soybean yield, yield components, and seed composition. *Agron. J.* 100: 237-246.

Davis JA, Radcliffe EB and Ragsdale DW (2005). Soybean aphid, *Aphis glycines* Matsumura, a new vector of potato virus Y in potato. *Am. J. Potato Res.* 82: 197-201.

Dodds PN and Rathjen JP (2010). Plant immunity: towards an integrated view of plant-pathogen interactions. *Nat. Rev. Genet.* 11: 539-548.

Dogimont C, Bendahmane A, Pitrat M, Burget-Bigeard E, et al. (2009). Gene resistant to *Aphis gossypii*. *U. S. Patent* 7: 576-264.

Du B, Zhang WL, Liu BF, Hu J, et al. (2009). Identification and characterization of Bph14, a gene conferring resistance to brown planthopper in rice. *Proc. Natl. Acad. Sci. U. S. A.* 106: 22163-22168.

Hartman GL, Domier LL, Wax LM and Helm CG (2001). Occurrence and Distribution of *Aphis glycines* on Soybeans in Illinois in 2000 and its Potential Control. Available at [http://www.plantmanagementnetwork.org/pub/php/brief/aphisglycines]. Accessed January 2, 2009.

Hill CB, Kim KS, Crull L, Diers BW, et al. (2009). Inheritance of resistance to the soybean aphid in soybean PI 200538. *Crop Sci.* 49: 1193-1200.

Jun TH, Rouf Mian MA and Michel AP (2012). Genetic mapping revealed two loci for soybean aphid resistance in PI 567301B. *Theor. Appl. Genet.* 124: 13-22.

Kanazin V, Marek LF and Shoemaker RC (1996). Resistance gene analogs are conserved and clustered in soybean. *Proc. Natl. Acad. Sci. U. S. A.* 93: 11746-11750.

Keim P, Olson TC and Shoemaker RC (1988). A rapid protocol for isolating soybean DNA. *Soybean Genet. Newsl.* 15: 150-152.

Kim KS, Bellendir S, Hudson KA, Hill CB, et al. (2010a). Fine mapping the soybean aphid resistance gene Rag1 in soybean. *Theor. Appl. Genet*. 120: 1063-1071.

Kim KS, Hill CB, Hartman GL, Hyten DL, et al. (2010b). Fine mapping of the soybean aphid-resistance gene Rag2 in soybean PI 200538. *Theor. Appl. Genet.* 121: 599-610.

Klingler J, Creasy R, Gao L, Nair RM, et al. (2005). Aphid resistance in *Medicago truncatula* involves antixenosis and phloem-specific, inducible antibiosis, and maps to a single locus flanked by NBS-LRR resistance gene analogs. *Plant Physiol.* 137: 1445-1455.

Kosambi DD (1944). The estimation of map distances from recombination values. *Ann. Eugen.* 12: 172-175.

Lander ES, Green P, Abrahamson J, Barlow A, et al. (1987). MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. *Genomics* 1: 174-181.

Landis D, Brewer M and Heimpel G (2003). NCR-125 Arthropod Biological Control: State Reports for 2003. Available at [http://www.cips.msu.edu/ncr125/StateRpts2003MIhtm]. Accessed March 23, 2006.

Li Y, Hill CB, Carlson SR, Diers BW, et al. (2007). Soybean aphid resistance genes in the soybean cultivars Dowling and Jackson map to linkage group M. *Mol. Breed.* 19: 25-34.

Meng F, Han Y, Teng W, Li Y, et al. (2011). QTL underlying the resistance to soybean aphid (*Aphis glycines* Matsumura) through isoflavone-mediated antibiosis in soybean cultivar ʻZhongdou 27ʼ. *Theor. Appl. Genet*. 123: 1459-1465.

Mensah C, DiFonzo C, Nelson RL and Wang D (2005). Resistance to soybean aphid in early maturing soybean germplasm. *Crop Sci.* 45: 2228-2233.

Michelmore RW, Paran I and Kesseli RV (1991). Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci. U. S. A.* 88: 9828-9832.

 ©FUNPEC-RP www.funpecrp.com.br

OMAFRA (2009). Soybean Insects and Pests: Soybean Aphid. Agronomy Guide for Field Crops-Publications 811. Available at [http://www.oma-fra.gov.on.ca/english/crops/pub811/13soybean.htm]. Accessed February 14, 2011.

Ostlie K (2001). Soybean Aphid Reduces Yields: Harvest Results from Insecticide Strip Trials. University of Minnesota, St. Paul. Available at [www.soybeans.umn.edu/crop/insects/aphid/studyresults.htm]. Accessed July 12, 2008.

Ragsdale DW, Voegtlin DJ and O'neil RJ (2004). Soybean aphid biology in North America. *Annu. Entomol. Soc. Am.* 97: 204-208.

Ragsdale DW, Landis DA, Brodeur J, Heimpel GE, et al. (2011). Ecology and management of the soybean aphid in North America. *Annu. Rev. Entomol.* 56: 375-399.

Rossi M, Goggin FL, Milligan SB, Kaloshian I, et al. (1998). The nematode resistance gene Mi of tomato confers resistance against the potato aphid. *Proc. Natl. Acad. Sci. U. S. A.* 95: 9750-9754.

Rouf Mian MA, Kang ST, Beil SE and Hammond RB (2008). Genetic linkage mapping of the soybean aphid resistance gene in PI 243540. *Theor. Appl. Genet.* 117: 955-962.

Schmutz J, Cannon SB, Schlueter J, Ma J, et al. (2010). Genome sequence of the palaeopolyploid soybean. *Nature* 463: 178-183.

Smith SF and Krischik VA (1999). Effects of systemic imidacloprid on *Coleomegilla maculata* (Coleoptera: Coccinellidae). *Environ. Entomol.* 28: 1189-1195.

Song QJ, Marek LF, Shoemaker RC, Lark KG, et al. (2004). A new integrated genetic linkage map of the soybean. *Theor. Appl. Genet.* 109: 122-128.

Tilmon K, Hodgson E, O'Neal M and Ragsdale D (2011). Biology of the soybean aphid, *Aphis glycines* (Hemiptera: Aphididae) in the United States. *J. Integrated Pest Manag.* 2: 1-7.

Venette RC and Ragsdale DW (2004). Assessing the invasion by soybean aphid (Homoptera: Aphididae): where will it end? *Ann. Entomol. Soc. Am.* 97: 219-226.

Voorrips RE (2002). MapChart: software for the graphical presentation of linkage maps and QTLs. *J. Hered.* 93: 77-78.

Xiao L, Zhong Y, Zhang J, Wang B, et al. (2012). Inheritance of resistance to *Aphis glycines* in soybean P746 from China. *J. Econ. Entomol.* 105: 2167-2171.

Zhang G, Gu C and Wang D (2009). Molecular mapping of soybean aphid resistance genes in PI 567541B. *Theor. Appl. Genet.* 118: 473-482.

Zhang G, Gu C and Wang D (2010). A novel locus for soybean aphid resistance. *Theor. Appl. Genet.* 120: 1183-1191.

 ©FUNPEC-RP www.funpecrp.com.br