

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
12 February 2009 (12.02.2009)

PCT

(10) International Publication Number
WO 2009/021153 A2

(51) International Patent Classification:
A01H 1/00 (2006.01) *C12Q 1/68* (2006.01)
A01H 1/04 (2006.01)

(74) Agent: **LI, Chunping**; Monsanto Technology LLC, 800 North Lindbergh Boulevard, Attention: Nancy Huelskamp, Mail Zone E2na, St. Louis, MO 63167 (US).

(21) International Application Number:
PCT/US2008/072544

(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(22) International Filing Date: 8 August 2008 (08.08.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/963,936 8 August 2007 (08.08.2007) US

(71) Applicant (*for all designated States except US*): **MONSANTO TECHNOLOGY LLC**; 800 North Lindbergh Boulevard, Mail Zone E1na, St. Louis, MO 63167 (US).

(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **CONCIBIDO, Vergel** [US/US]; Monsanto Technology LLC, 800 North Lindbergh Boulevard, St. Louis, MO 63167 (US). **NARVEL, James** [US/US]; Monsanto Technology LLC, 800 North Lindbergh Boulevard, St. Louis, MO 63167 (US). **YATES, Jennifer** [US/US]; Monsanto Technology LLC, 800 North Lindergh Boulevard, St. Louis, MO 63167 (US). **BOERMA, Henry, Roger** [US/US]; Monsanto Technology LLC, 800 North Lindbergh Boulevard, St. Louis, MO 63167 (US).

Published:

- without international search report and to be republished upon receipt of that report
- with sequence listing part of description published separately in electronic form and available upon request from the International Bureau

(54) Title: METHODS TO IDENTIFY SOYBEAN APHID RESISTANT QUANTITATIVE TRAIT LOCI IN SOYBEAN AND COMPOSITIONS THEREOF

(57) Abstract: The present invention is in the field of plant breeding and aphid resistance. More specifically, the invention includes a method for breeding soybean plants containing quantitative trait loci that are associated with resistance to aphids, *Aphis glycines*. The invention further includes method for monitoring the introgression quantitative trait loci (QTL) conferring aphid resistance into elite germplasm in a breeding program.



WO 2009/021153 A2

IN THE UNITED STATES PATENT OFFICE
APPLICATION FOR PATENT FOR

METHODS TO IDENTIFY SOYBEAN APHID RESISTANT QUANTITATIVE TRAIT
LOCI IN SOYBEAN AND COMPOSITIONS THEREOF

INVENTORS:

VERGEL CONCIBIDO

JAMES NARVEL

JENNIFER YATES

HENRY ROGER BOERMA

METHODS TO IDENTIFY SOYBEAN APHID RESISTANT QUANTITATIVE TRAIT LOCI IN SOYBEAN AND COMPOSITIONS THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

5

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 60/963936 filed Aug 8, 2007. The entirety of the application is hereby incorporated by reference.

10

INCORPORATION OF THE SEQUENCE LISTING

A sequence listing is contained in the file named "53776seqlisting.txt" which is 80,088 bytes (measured in MS-Windows) and was created on August 7, 2008. This electronic sequence listing is electronically filed herewith and is incorporated herein by
15 reference.

FIELD OF THE INVENTION

20

The present invention is in the field of plant breeding. More specifically, the invention includes methods and compositions for screening plants from the genus *Glycine* with markers associated with quantitative trait loci that are related to the aphid resistance in *Glycine* plants. The invention further includes methods and compositions of genomic regions for screening plants from the genus *Glycine* associated with aphid resistance.

BACKGROUND OF THE INVENTION

25

Soybean, *Glycine max* (L.) Merril, is a major economic crop worldwide and is a primary source of vegetable oil and protein (Sinclair and Backman, *Compendium of Soybean Diseases*, 3rd Ed. APS Press, St. Paul, MN, p. 106. (1989). The growing demand for low cholesterol and high fiber diets has also increased soybean's importance as a health food.

Soybean varieties grown in the United States have a narrow genetic base. Six introductions, 'Mandarin,' 'Manchu,' 'Mandarin' (Ottawa), 'Richland,' 'AK' (Harrow), and 'Mukden,' contributed nearly 70% of the germplasm represented in 136 cultivar releases. To date, modern day cultivars can be traced back from these six soybean strains from China. In a study conducted by Cox *et al.*, *Crop Sci.* 25:529-532 (1988), the soybean germplasm is comprised of 90% adapted materials, 9% unadapted, and only 1% from exotic species. The genetic base of cultivated soybean could be widened through exotic species. In addition, exotic species may possess such key traits as disease, stress, and insect resistance.

Soybean aphid, *Aphis glycines* Matsumura, was identified as new insect pest of soybeans in 2001 and spread to over 21 states in the United States and 3 Canadian provinces by 2003 (Vennette *et al.* *Ann Entomol Soc Am* 97:217-226 (2004)). High yields are critical to a farmer's profit margin. Soybean aphid can cause over 50% yield losses (Wang *et al.*, *Plant Protect* 20:12-13 (1994)). In addition to the decrease in yield, an increase in insecticide use can also decrease a farmer's profit margin. Over 7 million acres of soybean in the North Central U.S. were sprayed with insecticide to control soybean aphids in 2003; the estimated cost of the insecticide treatments was \$84 - \$105 million in the North Central region alone in 2003 (Landis *et al.* NCR-125 Arthropod biological control: state reports for 2003; Li *et al.*, *Mol Breeding* 19:25-34 (2007)).

Soybean aphids can directly damage the plant by removing significant amounts of water and nutrients causing the leaves to yellow and wilt. Additionally, aphids excrete honeydew, a sugar-rich sticky substance, on to the leaves and plants. Honeydew often leads to the development of sooty mold, which affects photosynthesis resulting significant yield losses (Gomez *et al.*, *Environ Exp Bot* 55: 77-86 (2006)). Soybean aphids vector a number of viruses that can stunt plant growth, distorts leaves, cause mottling of leaves and stem, reduce pod number and cause discoloration in the seed. Viruses transmitted via soybean aphid include, Soybean mosaic virus, yellow mosaic virus, tobacco etch virus and tobacco vein mottling virus (Wang *et al.* *Plant Dis* 90: 920-926 (2006)).

Host plant resistance to insect are often quantitatively inherited traits and not major resistance gene. Stacking quantitative resistances is more durable than a major gene for

resistance, but is difficult to identify and incorporate multiple quantitative resistances into a single soybean variety. Molecular markers associated with insect resistance offers breeders a more efficient method to work with quantitative traits and insect resistance. Aphid resistance genes and QTLs in soybean are known. Examples of which including *Rag1* was identified in the soybean variety Dowling and mapped to linkage group M (US Patent Application No. 11/158,307). Additionally, quantitative trait loci associated with aphid resistance were identified in Plant Introduction (PI) 567598B and mapped linkage groups B2, D1b, J and K (PCT/US2006/019200).

There is a need in the art of plant breeding to identify additional quantitative trait loci associated with aphid resistance in soybean. Additionally, there is a need for rapid, cost-efficient method to assay the absence or presence of aphid resistance loci in soybean. The present invention provides a method for screening and selecting a soybean plant comprising a quantitative trait loci associated with aphid resistance using single nucleotide polymorphism (SNP) technology.

SUMMARY OF THE INVENTION

The present invention provides methods for producing aphid resistance in soybean plants. The present invention relates to methods to determine the presence or absence of quantitative trait loci conferring aphid resistance in soybean plants, including but not limited to exotic germplasm, populations, lines, elite lines, cultivars and varieties. The present invention is not limited to any one type of aphid resistant trait, such as antibiosis, antixenosis or repellency of aphids. More particularly, the invention relates to methods involving for identifying molecular markers associated with aphid resistance quantitative trait loci (QTL). The present invention relates to the use of molecular markers to screen and select for aphid resistance within soybean plants, including but not limited to exotic germplasm, populations, lines, elite lines, and varieties.

In a preferred embodiment, the present invention further provides quantitative trait loci associated with resistance to one or more of arthropods including but not limited to Coleoptera, examples of which including *Cerotoma* sp. such as bean leaf beetle (*Cerotoma trifurcata*), *Diabrotica* sp. such as spotted cucumber beetle (*Diabrotica undecimpunctata howardi*), *Epicauta* sp. such as blister beetle (*Epicauta pestifera*), *Popilli* sp. such as Japanese beetle (*Popillia japonica*), *Dectes* sp. such as soybean stem borer (*Dectes texanus texanus*), and *Colaspis* sp. such as grape colaspis (*Colaspis brunnea*), etc.; Orthoptera, examples of which including *Melanoplus* sp. such as red-legged grasshopper (*Melanoplus femurrubrum*), and *Shistocerca* sp. such as American locust (*Shistocerca Americana*), etc.; Lepidoptera, examples of which including *Plathypen* sp. such as green cloverworm (*Plathypena scabra*), *Pseudoplusia* sp. such as soybean looper (*Pseudoplusia includens*), *Anticarsia* sp. such as velvetbean caterpillar (*Anticarsia gemmatilis*), *Epargyreus* sp. such as Silverspotted skipper (*Epargyreus clarus*), *Estigmene* sp. such as saltmarsh caterpillar (*Estigmene acrea*), *Spodoptera* sp. such as beet armyworm (*Spodoptera exigua*), *Heliothis* sp. such as Corn earworm (*Heliothis zea*), and *Matsumuraeses* sp. such as bean podworm (*Matsumuraeses phaseoli*); Hemiptera, examples of which including *Acrosternum* sp. such as green stink bugs (*Acrosternum hilare*), *Euschistus* sp. such as brown stink bug (*Euschistus servus*), *Nezara* sp. such as southern stinkbug (*Nezara viridula*); Homoptera, examples of which including

Spissistilus sp. such as threecornered alfalfa hopper (*Spissistilus festinus*), and *Aphis* sp. such as soybean aphid (*Aphis glycines*); Thysanoptera, examples of which including *Sericothrips* sp. such as soybean thrips (*Sericothrips variabilis*).

In a preferred embodiment, the present invention further provides loci associated with resistance to nematodes, including, but not limited to *Heterodera* sp. such as soybean cyst nematode (*Heterodera glycines*), *Belonolaimus* sp. such as sting nematode (*Belonolaimus longicaudatus*), *Rotylenchulus* sp. such as reniform nematode (*Rotylenchulus reniformis*), *Meloidogyne* sp. such as southern root-knot nematode (*Meloidogyne incognita*), peanut root-knot nematode (*Meloidogyne arenaria*) and the Javanese root-knot nematode (*Meloidogyne javanica*).

The present invention relates to producing aphid resistant plants, populations, lines, elite lines, and varieties. More particularly, the present invention includes a method of introgressing an aphid resistant allele into a soybean plant comprising (A) crossing at least one first soybean plant comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 81 through SEQ ID NO: 120 with at least one second soybean plant in order to form a segregating population, (B) screening the segregating population with one or more nucleic acid markers to determine if one or more soybean plants from the segregating population contains the nucleic acid sequence, and (C) selecting from the segregation population one or more soybean plants comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 81 through SEQ ID NO: 120 .

The present invention includes a method of introgressing an allele into a soybean plant comprising: (A) crossing at least one aphid resistant soybean plant with at least one aphid susceptible soybean plant in order to form a segregating population; (B) screening said segregating population with one or more nucleic acid markers to determine if one or more soybean plants from said segregating population contains an aphid resistant allele, wherein said aphid resistance allele is an allele selected from the group consisting of aphid resistance allele 1, aphid resistance allele 2, aphid resistance allele 3, aphid resistance allele 4, aphid resistance allele 5, aphid resistance allele 6, aphid resistance allele 7, aphid resistance allele 8, aphid resistance allele 9, aphid resistance allele 10, aphid resistance allele 11, aphid

resistance allele 12, aphid resistance allele 13, aphid resistance allele 14, aphid resistance allele 15, aphid resistance allele 16, aphid resistance allele 17, aphid resistance allele 18, aphid resistance allele 19, aphid resistance allele 20, aphid resistance allele 21, aphid resistance allele 22, aphid resistance allele 23, aphid resistance allele 24, aphid resistance allele 25, aphid resistance allele 26, aphid resistance allele 27, aphid resistance allele 28, aphid resistance allele 29, aphid resistance allele 30, aphid resistance allele 31, aphid resistance allele 32, aphid resistance allele 33, aphid resistance allele 34, aphid resistance allele 35, aphid resistance allele 36, aphid resistance allele 37 and aphid resistance allele 38, aphid resistance allele 39, and aphid resistance allele 40.

The present invention includes an elite soybean plant comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 81 through SEQ ID NO: 120.

BRIEF DESCRIPTION OF NUCLEIC ACID SEQUENCES

SEQ ID NO: 1 is a forward PCR primer for the amplification of SEQ ID NO: 81.

SEQ ID NO: 2 is a reverse PCR primer for the amplification of SEQ ID NO: 81. SEQ

ID NO: 3 is a forward PCR primer for the amplification of SEQ ID NO: 82.

5 SEQ ID NO: 4 is a reverse PCR primer for the amplification of SEQ ID NO: 82.

SEQ ID NO: 5 is a forward PCR primer for the amplification of SEQ ID NO: 83.

SEQ ID NO: 6 is a reverse PCR primer for the amplification of SEQ ID NO: 83.

SEQ ID NO: 7 is a forward PCR primer for the amplification of SEQ ID NO: 84.

SEQ ID NO: 8 is a reverse PCR primer for the amplification of SEQ ID NO: 84.

10 SEQ ID NO: 9 is a forward PCR primer for the amplification of SEQ ID NO: 85.

SEQ ID NO: 10 is a reverse PCR primer for the amplification of SEQ ID NO: 85.

SEQ ID NO: 11 is a forward PCR primer for the amplification of SEQ ID NO: 86.

SEQ ID NO: 12 is a reverse PCR primer for the amplification of SEQ ID NO: 86.

SEQ ID NO: 13 is a forward PCR primer for the amplification of SEQ ID NO: 87.

15 SEQ ID NO: 14 is a reverse PCR primer for the amplification of SEQ ID NO: 87.

SEQ ID NO: 15 is a forward PCR primer for the amplification of SEQ ID NO: 88.

SEQ ID NO: 16 is a reverse PCR primer for the amplification of SEQ ID NO: 88.

SEQ ID NO: 17 is a forward PCR primer for the amplification of SEQ ID NO: 89.

SEQ ID NO: 18 is a reverse PCR primer for the amplification of SEQ ID NO: 89.

20 SEQ ID NO: 19 is a forward PCR primer for the amplification of SEQ ID NO: 90.

SEQ ID NO: 20 is a reverse PCR primer for the amplification of SEQ ID NO: 90.

SEQ ID NO: 21 is a forward PCR primer for the amplification of SEQ ID NO: 91.

SEQ ID NO: 22 is a reverse PCR primer for the amplification of SEQ ID NO: 91.

SEQ ID NO: 23 is a forward PCR primer for the amplification of SEQ ID NO: 92.

25 SEQ ID NO: 24 is a reverse PCR primer for the amplification of SEQ ID NO: 92.

SEQ ID NO: 25 is a forward PCR primer for the amplification of SEQ ID NO: 93.

SEQ ID NO: 26 is a reverse PCR primer for the amplification of SEQ ID NO: 93.

SEQ ID NO: 27 is a forward PCR primer for the amplification of SEQ ID NO: 94.

SEQ ID NO: 28 is a reverse PCR primer for the amplification of SEQ ID NO: 94.
SEQ ID NO: 29 is a forward PCR primer for the amplification of SEQ ID NO: 95.
SEQ ID NO: 30 is a reverse PCR primer for the amplification of SEQ ID NO: 95.
SEQ ID NO: 31 is a forward PCR primer for the amplification of SEQ ID NO: 96.
5 SEQ ID NO: 32 is a reverse PCR primer for the amplification of SEQ ID NO: 96.
SEQ ID NO: 33 is a forward PCR primer for the amplification of SEQ ID NO: 97.
SEQ ID NO: 34 is a reverse PCR primer for the amplification of SEQ ID NO: 97.
SEQ ID NO: 35 is a forward PCR primer for the amplification of SEQ ID NO: 98.
SEQ ID NO: 36 is a reverse PCR primer for the amplification of SEQ ID NO: 98.
10 SEQ ID NO: 37 is a forward PCR primer for the amplification of SEQ ID NO: 99.
SEQ ID NO: 38 is a reverse PCR primer for the amplification of SEQ ID NO: 99.
SEQ ID NO: 39 is a forward PCR primer for the amplification of SEQ ID NO: 100.
SEQ ID NO: 40 is a reverse PCR primer for the amplification of SEQ ID NO: 100.
SEQ ID NO: 41 is a forward PCR primer for the amplification of SEQ ID NO: 101.
15 SEQ ID NO: 42 is a reverse PCR primer for the amplification of SEQ ID NO: 101.
SEQ ID NO: 43 is a forward PCR primer for the amplification of SEQ ID NO: 102.
SEQ ID NO: 44 is a reverse PCR primer for the amplification of SEQ ID NO: 102.
SEQ ID NO: 45 is a forward PCR primer for the amplification of SEQ ID NO: 103.
SEQ ID NO: 46 is a reverse PCR primer for the amplification of SEQ ID NO: 103.
20 SEQ ID NO: 47 is a forward PCR primer for the amplification of SEQ ID NO: 104.
SEQ ID NO: 48 is a reverse PCR primer for the amplification of SEQ ID NO: 104.
SEQ ID NO: 49 is a forward PCR primer for the amplification of SEQ ID NO: 105.
SEQ ID NO: 50 is a reverse PCR primer for the amplification of SEQ ID NO: 105.
SEQ ID NO: 51 is a forward PCR primer for the amplification of SEQ ID NO: 106.
25 SEQ ID NO: 52 is a reverse PCR primer for the amplification of SEQ ID NO: 106.
SEQ ID NO: 53 is a forward PCR primer for the amplification of SEQ ID NO: 107.
SEQ ID NO: 54 is a reverse PCR primer for the amplification of SEQ ID NO: 107.
SEQ ID NO: 55 is a forward PCR primer for the amplification of SEQ ID NO: 108.
SEQ ID NO: 56 is a reverse PCR primer for the amplification of SEQ ID NO: 108.

SEQ ID NO: 57 is a forward PCR primer for the amplification of SEQ ID NO: 109.

SEQ ID NO: 58 is a reverse PCR primer for the amplification of SEQ ID NO: 109.

SEQ ID NO: 59 is a forward PCR primer for the amplification of SEQ ID NO: 110.

SEQ ID NO: 60 is a reverse PCR primer for the amplification of SEQ ID NO: 110.

5 SEQ ID NO: 61 is a forward PCR primer for the amplification of SEQ ID NO: 111.

SEQ ID NO: 62 is a reverse PCR primer for the amplification of SEQ ID NO: 111

SEQ ID NO: 63 is a forward PCR primer for the amplification of SEQ ID NO: 112.

SEQ ID NO: 64 is a reverse PCR primer for the amplification of SEQ ID NO: 112.

SEQ ID NO: 65 is a forward PCR primer for the amplification of SEQ ID NO: 113.

10 SEQ ID NO: 66 is a reverse PCR primer for the amplification of SEQ ID NO: 113

SEQ ID NO: 67 is a forward PCR primer for the amplification of SEQ ID NO: 114.

SEQ ID NO: 68 is a reverse PCR primer for the amplification of SEQ ID NO: 114.

SEQ ID NO: 69 is a forward PCR primer for the amplification of SEQ ID NO: 115.

SEQ ID NO: 70 is a reverse PCR primer for the amplification of SEQ ID NO: 115.

15 SEQ ID NO: 71 is a forward PCR primer for the amplification of SEQ ID NO: 116.

SEQ ID NO: 72 is a reverse PCR primer for the amplification of SEQ ID NO: 116.

SEQ ID NO: 73 is a forward PCR primer for the amplification of SEQ ID NO: 117.

SEQ ID NO: 74 is a reverse PCR primer for the amplification of SEQ ID NO: 117.

SEQ ID NO: 75 is a forward PCR primer for the amplification of SEQ ID NO: 118.

20 SEQ ID NO: 76 is a reverse PCR primer for the amplification of SEQ ID NO: 118.

SEQ ID NO: 77 is a forward PCR primer for the amplification of SEQ ID NO: 119.

SEQ ID NO: 78 is a reverse PCR primer for the amplification of SEQ ID NO: 119.

SEQ ID NO: 79 is a forward PCR primer for the amplification of SEQ ID NO: 120.

SEQ ID NO: 80 is a reverse PCR primer for the amplification of SEQ ID NO: 120.

25 SEQ ID NO: 81 is a genomic sequence derived from *Glycine Max* corresponding to
aphid resistance locus 1.

SEQ ID NO: 82 is a genomic sequence derived from *Glycine Max* corresponding to
aphid resistance locus 1.

SEQ ID NO: 83 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 1.

SEQ ID NO: 84 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 1.

5 SEQ ID NO: 85 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 2.

SEQ ID NO: 86 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 3.

10 SEQ ID NO: 87 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 4.

SEQ ID NO: 88 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 5.

SEQ ID NO: 89 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 6.

15 SEQ ID NO: 90 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 7.

SEQ ID NO: 91 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 8.

20 SEQ ID NO: 92 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 8.

SEQ ID NO: 93 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 9.

SEQ ID NO: 94 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 10.

25 SEQ ID NO: 95 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 11.

SEQ ID NO: 96 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 12.

SEQ ID NO: 97 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 13.

SEQ ID NO: 98 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 14.

5 SEQ ID NO: 99 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 15.

SEQ ID NO: 100 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 16.

10 SEQ ID NO: 101 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 16.

SEQ ID NO: 102 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 16.

SEQ ID NO: 103 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 17.

15 SEQ ID NO: 104 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 18.

SEQ ID NO: 105 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 19.

20 SEQ ID NO: 106 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 20.

SEQ ID NO: 107 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 21.

SEQ ID NO: 108 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 21.

25 SEQ ID NO: 109 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 22.

SEQ ID NO: 110 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 23.

SEQ ID NO: 111 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 23.

SEQ ID NO: 112 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 24.

5 SEQ ID NO: 113 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 25.

SEQ ID NO: 114 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 26.

10 SEQ ID NO: 115 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 26.

SEQ ID NO: 116 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 27.

SEQ ID NO: 117 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 28.

15 SEQ ID NO: 118 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 28.

SEQ ID NO: 119 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 28.

20 SEQ ID NO: 120 is a genomic sequence derived from *Glycine Max* corresponding to aphid resistance locus 28.

SEQ ID NO: 121 is a probe for the detection of the SNP of SEQ ID NO: 81.

SEQ ID NO: 122 is a probe for the detection of the SNP of SEQ ID NO: 81.

SEQ ID NO: 123 is a probe for the detection of the SNP of SEQ ID NO: 82.

SEQ ID NO: 124 is a probe for the detection of the SNP of SEQ ID NO: 82.

25 SEQ ID NO: 125 is a probe for the detection of the SNP of SEQ ID NO: 83.

SEQ ID NO: 126 is a probe for the detection of the SNP of SEQ ID NO: 83.

SEQ ID NO: 127 is a probe for the detection of the SNP of SEQ ID NO: 84.

SEQ ID NO: 128 is a probe for the detection of the SNP of SEQ ID NO: 84.

SEQ ID NO: 129 is a probe for the detection of the SNP of SEQ ID NO: 85.

SEQ ID NO: 130 is a probe for the detection of the SNP of SEQ ID NO: 85.
SEQ ID NO: 131 is a probe for the detection of the SNP of SEQ ID NO: 86.
SEQ ID NO: 132 is a probe for the detection of the SNP of SEQ ID NO: 86.
SEQ ID NO: 133 is a probe for the detection of the SNP of SEQ ID NO: 87.
5 SEQ ID NO: 134 is a probe for the detection of the SNP of SEQ ID NO: 87.
SEQ ID NO: 135 is a probe for the detection of the SNP of SEQ ID NO: 88.
SEQ ID NO: 136 is a probe for the detection of the SNP of SEQ ID NO: 88.
SEQ ID NO: 137 is a probe for the detection of the SNP of SEQ ID NO: 89.
SEQ ID NO: 138 is a probe for the detection of the SNP of SEQ ID NO: 89.
10 SEQ ID NO: 139 is a probe for the detection of the SNP of SEQ ID NO: 90.
SEQ ID NO: 140 is a probe for the detection of the SNP of SEQ ID NO: 90.
SEQ ID NO: 141 is a probe for the detection of the SNP of SEQ ID NO: 91.
SEQ ID NO: 142 is a probe for the detection of the SNP of SEQ ID NO: 91.
SEQ ID NO: 143 is a probe for the detection of the SNP of SEQ ID NO: 92.
15 SEQ ID NO: 144 is a probe for the detection of the SNP of SEQ ID NO: 92.
SEQ ID NO: 145 is a probe for the detection of the SNP of SEQ ID NO: 93.
SEQ ID NO: 146 is a probe for the detection of the SNP of SEQ ID NO: 93.
SEQ ID NO: 147 is a probe for the detection of the SNP of SEQ ID NO: 94.
SEQ ID NO: 148 is a probe for the detection of the SNP of SEQ ID NO: 94.
20 SEQ ID NO: 149 is a probe for the detection of the SNP of SEQ ID NO: 95.
SEQ ID NO: 150 is a probe for the detection of the SNP of SEQ ID NO: 95.
SEQ ID NO: 151 is a probe for the detection of the SNP of SEQ ID NO: 96.
SEQ ID NO: 152 is a probe for the detection of the SNP of SEQ ID NO: 96.
SEQ ID NO: 153 is a probe for the detection of the SNP of SEQ ID NO: 97.
25 SEQ ID NO: 154 is a probe for the detection of the SNP of SEQ ID NO: 97.
SEQ ID NO: 155 is a probe for the detection of the SNP of SEQ ID NO: 98.
SEQ ID NO: 156 is a probe for the detection of the SNP of SEQ ID NO: 98.
SEQ ID NO: 157 is a probe for the detection of the SNP of SEQ ID NO: 99.
SEQ ID NO: 158 is a probe for the detection of the SNP of SEQ ID NO: 99.

SEQ ID NO: 159 is a probe for the detection of the SNP of SEQ ID NO: 100.
SEQ ID NO: 160 is a probe for the detection of the SNP of SEQ ID NO: 100.
SEQ ID NO: 161 is a probe for the detection of the SNP of SEQ ID NO: 101.
SEQ ID NO: 162 is a probe for the detection of the SNP of SEQ ID NO: 101.
5 SEQ ID NO: 163 is a probe for the detection of the SNP of SEQ ID NO: 102.
SEQ ID NO: 164 is a probe for the detection of the SNP of SEQ ID NO: 102.
SEQ ID NO: 165 is a probe for the detection of the SNP of SEQ ID NO: 103.
SEQ ID NO: 166 is a probe for the detection of the SNP of SEQ ID NO: 103.
SEQ ID NO: 167 is a probe for the detection of the SNP of SEQ ID NO: 104.
10 SEQ ID NO: 168 is a probe for the detection of the SNP of SEQ ID NO: 104.
SEQ ID NO: 169 is a probe for the detection of the SNP of SEQ ID NO: 105.
SEQ ID NO: 170 is a probe for the detection of the SNP of SEQ ID NO: 105.
SEQ ID NO: 171 is a probe for the detection of the SNP of SEQ ID NO: 106.
SEQ ID NO: 172 is a probe for the detection of the SNP of SEQ ID NO: 106.
15 SEQ ID NO: 173 is a probe for the detection of the SNP of SEQ ID NO: 107.
SEQ ID NO: 174 is a probe for the detection of the SNP of SEQ ID NO: 107.
SEQ ID NO: 175 is a probe for the detection of the SNP of SEQ ID NO: 108.
SEQ ID NO: 176 is a probe for the detection of the SNP of SEQ ID NO: 108.
SEQ ID NO: 177 is a probe for the detection of the SNP of SEQ ID NO: 109.
20 SEQ ID NO: 178 is a probe for the detection of the SNP of SEQ ID NO: 109.
SEQ ID NO: 179 is a probe for the detection of the SNP of SEQ ID NO: 110.
SEQ ID NO: 180 is a probe for the detection of the SNP of SEQ ID NO: 110.
SEQ ID NO: 181 is a probe for the detection of the SNP of SEQ ID NO: 111.
SEQ ID NO: 182 is a probe for the detection of the SNP of SEQ ID NO: 111.
25 SEQ ID NO: 183 is a probe for the detection of the SNP of SEQ ID NO: 112.
SEQ ID NO: 184 is a probe for the detection of the SNP of SEQ ID NO: 112.
SEQ ID NO: 185 is a probe for the detection of the SNP of SEQ ID NO: 113.
SEQ ID NO: 186 is a probe for the detection of the SNP of SEQ ID NO: 113.
SEQ ID NO: 187 is a probe for the detection of the SNP of SEQ ID NO: 114.

SEQ ID NO: 188 is a probe for the detection of the SNP of SEQ ID NO: 114.
SEQ ID NO: 189 is a probe for the detection of the SNP of SEQ ID NO: 115.
SEQ ID NO: 190 is a probe for the detection of the SNP of SEQ ID NO: 115.
SEQ ID NO: 191 is a probe for the detection of the SNP of SEQ ID NO: 116.
5 SEQ ID NO: 192 is a probe for the detection of the SNP of SEQ ID NO: 116.
SEQ ID NO: 193 is a probe for the detection of the SNP of SEQ ID NO: 117.
SEQ ID NO: 194 is a probe for the detection of the SNP of SEQ ID NO: 117.
SEQ ID NO: 195 is a probe for the detection of the SNP of SEQ ID NO: 118.
SEQ ID NO: 196 is a probe for the detection of the SNP of SEQ ID NO: 118.
10 SEQ ID NO: 197 is a probe for the detection of the SNP of SEQ ID NO: 119.
SEQ ID NO: 198 is a probe for the detection of the SNP of SEQ ID NO: 119.
SEQ ID NO: 199 is a probe for the detection of the SNP of SEQ ID NO: 120.
SEQ ID NO: 200 is a probe for the detection of the SNP of SEQ ID NO: 120.
SEQ ID NO: 201 is a probe for the detection of the SNP of SEQ ID NO: 82.
15 SEQ ID NO: 202 is a probe for the detection of the SNP of SEQ ID NO: 82.
SEQ ID NO: 203 is a probe for the detection of the SNP of SEQ ID NO: 83.
SEQ ID NO: 204 is a probe for the detection of the SNP of SEQ ID NO: 83.
SEQ ID NO: 205 is a probe for the detection of the SNP of SEQ ID NO: 84.
SEQ ID NO: 206 is a probe for the detection of the SNP of SEQ ID NO: 84.
20 SEQ ID NO: 207 is a probe for the detection of the SNP of SEQ ID NO: 100.
SEQ ID NO: 208 is a probe for the detection of the SNP of SEQ ID NO: 100.
SEQ ID NO: 209 is a probe for the detection of the SNP of SEQ ID NO: 111.
SEQ ID NO: 210 is a probe for the detection of the SNP of SEQ ID NO: 111.
SEQ ID NO: 211 is a probe for the detection of the SNP of SEQ ID NO: 117.
25 SEQ ID NO: 212 is a probe for the detection of the SNP of SEQ ID NO: 117.
SEQ ID NO: 213 is a probe for the detection of the SNP of SEQ ID NO: 119.
SEQ ID NO: 214 is a probe for the detection of the SNP of SEQ ID NO: 119.
SEQ ID NO: 215 is a probe for the detection of the SNP of SEQ ID NO: 120.
SEQ ID NO: 216 is a probe for the detection of the SNP of SEQ ID NO: 120.

SEQ ID NO: 217 is a probe for the detection of the SNP of SEQ ID NO: 82.

SEQ ID NO: 218 is a probe for the detection of the SNP of SEQ ID NO: 83.

SEQ ID NO: 219 is a probe for the detection of the SNP of SEQ ID NO: 84.

SEQ ID NO: 220 is a probe for the detection of the SNP of SEQ ID NO: 100.

5

SEQ ID NO: 221 is a probe for the detection of the SNP of SEQ ID NO: 111.

SEQ ID NO: 222 is a probe for the detection of the SNP of SEQ ID NO: 117.

SEQ ID NO: 223 is a probe for the detection of the SNP of SEQ ID NO: 119.

SEQ ID NO: 224 is a probe for the detection of the SNP of SEQ ID NO: 120.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides 28 aphid resistance loci that are located on linkage group A1, B1, B2, C1, D1a, D1b, E, F, G, H, I, and O in the soybean genome that are not previously associated with aphid resistance (Table 1). The present invention also provides for quantitative trait loci (QTL) alleles capable of conferring resistance to soybean aphid. Alleles that are located at aphid resistance locus 1, aphid resistance locus 2, aphid resistance locus 3, aphid resistance locus 4, aphid resistance locus 5, aphid resistance locus 6, aphid resistance locus 7, aphid resistance locus 8, aphid resistance locus 9, aphid resistance locus 10, aphid resistance locus 11, aphid resistance locus 12, aphid resistance locus 13, aphid resistance locus 14, aphid resistance locus 15, aphid resistance locus 16, aphid resistance locus 17, aphid resistance locus 18, aphid resistance locus 19, aphid resistance locus 20, aphid resistance locus 21, aphid resistance locus 22, aphid resistance locus 23, aphid resistance locus 24, aphid resistance locus 25, aphid resistance locus 26, aphid resistance locus 27, and aphid resistance locus 28 are provided.

In the present invention, aphid resistance locus 1 is located on linkage group J. SNP markers used to monitor the introgression of aphid resistance locus 1 are SEQ ID NO: 81 through SEQ ID NO: 84. SNP marker DNA sequences associated with aphid resistance locus 1 (SEQ ID NO: 81 through SEQ ID NO: 84) can be amplified using the primers indicated as SEQ ID NO: 1 through SEQ ID NO: 8 and detected with probes indicated as SEQ ID NO: 121 through SEQ ID NO: 128, SEQ ID NO: 201 through SEQ ID NO: 206, and SEQ ID NO: 217 through SEQ ID NO: 219.

In the present invention, aphid resistance locus 2 is located on linkage group E. SNP marker used to monitor the introgression of aphid resistance locus 2 is SEQ ID NO: 85. SNP marker DNA sequences associated with aphid resistance locus 2 (SEQ ID NO: 85) can be amplified using the primers indicated as SEQ ID NO: 9 through SEQ ID NO: 10 and detected with probes indicated as SEQ ID NO: 129 through SEQ ID NO: 130.

In the present invention, aphid resistance locus 3 is located on linkage group E. SNP marker used to monitor the introgression of aphid resistance locus 3 is SEQ ID NO: 86. SNP marker DNA sequences associated with aphid resistance locus 3 (SEQ ID NO: 86) can be

amplified using the primers indicated as SEQ ID NO: 11 through SEQ ID NO: 12 and detected with probes indicated as SEQ ID NO: 131 through SEQ ID NO: 132.

In the present invention, aphid resistance locus 4 is located on linkage group E. SNP marker used to monitor the introgression of aphid resistance locus 4 is SEQ ID NO: 87. SNP marker DNA sequences associated with aphid resistance locus 4 (SEQ ID NO: 87) can be amplified using the primers indicated as SEQ ID NO: 13 through SEQ ID NO: 14 and detected with probes indicated as SEQ ID NO: 133 through SEQ ID NO: 134.

In the present invention, aphid resistance locus 5 is located on linkage group B1. SNP marker used to monitor the introgression of aphid resistance locus 5 is SEQ ID NO: 88. SNP marker DNA sequences associated with aphid resistance locus 5 (SEQ ID NO: 88) can be amplified using the primers indicated as SEQ ID NO: 15 through SEQ ID NO: 16 and detected with probes indicated as SEQ ID NO: 135 through SEQ ID NO: 136.

In the present invention, aphid resistance locus 6 is located on linkage group N. SNP marker used to monitor the introgression of aphid resistance locus 6 is SEQ ID NO: 89. SNP marker DNA sequences associated with aphid resistance locus 6 (SEQ ID NO: 89) can be amplified using the primers indicated as SEQ ID NO: 17 through SEQ ID NO: 18 and detected with probes indicated as SEQ ID NO: 137 through SEQ ID NO: 138.

In the present invention, aphid resistance locus 7 is located on linkage group G. SNP marker used to monitor the introgression of aphid resistance locus 7 is SEQ ID NO: 90. SNP marker DNA sequences associated with aphid resistance locus 7 (SEQ ID NO: 90) can be amplified using the primers indicated as SEQ ID NO: 19 through SEQ ID NO: 20 and detected with probes indicated as SEQ ID NO: 139 through SEQ ID NO: 140.

In the present invention, aphid resistance locus 8 is located on linkage group N. SNP markers used to monitor the introgression of aphid resistance locus 8 are SEQ ID NO: 91 through SEQ ID NO 92. SNP marker DNA sequences associated with aphid resistance locus 8 (91 through SEQ ID NO 92) can be amplified using the primers indicated as SEQ ID NO: 21 through SEQ ID NO: 24 and detected with probes indicated as SEQ ID NO: 141 through SEQ ID NO: 144.

In the present invention, aphid resistance locus 9 is located on linkage group N. SNP marker used to monitor the introgression of aphid resistance locus 9 is SEQ ID NO: 93. SNP marker DNA sequences associated with aphid resistance locus 9 (SEQ ID NO: 93) can be amplified using the primers indicated as SEQ ID NO: 25 through SEQ ID NO: 26 and
5 detected with probes indicated as SEQ ID NO: 145 through SEQ ID NO: 146.

In the present invention, aphid resistance locus 10 is located on linkage group A1. SNP marker used to monitor the introgression of aphid resistance locus 10 is SEQ ID NO: 94. SNP marker DNA sequences associated with aphid resistance locus 10 (SEQ ID NO: 94) can be amplified using the primers indicated as SEQ ID NO: 27 through SEQ ID NO: 28 and
10 detected with probes indicated as SEQ ID NO: 147 through SEQ ID NO: 148.

In the present invention, aphid resistance locus 11 is located on linkage group A1. SNP marker used to monitor the introgression of aphid resistance locus 11 is SEQ ID NO: 95. SNP marker DNA sequences associated with aphid resistance locus 11 (SEQ ID NO: 95) can be amplified using the primers indicated as SEQ ID NO: 29 through SEQ ID NO: 30 and
15 detected with probes indicated as SEQ ID NO: 149 through SEQ ID NO: 150.

In the present invention, aphid resistance locus 12 is located on linkage group D1a. SNP marker used to monitor the introgression of aphid resistance locus 12 is SEQ ID NO: 96. SNP marker DNA sequences associated with aphid resistance locus 12 (SEQ ID NO: 96) can be amplified using the primers indicated as SEQ ID NO: 31 through SEQ ID NO: 32 and
20 detected with probes indicated as SEQ ID NO: 151 through SEQ ID NO: 152.

In the present invention, aphid resistance locus 13 is located on linkage group C2. SNP marker used to monitor the introgression of aphid resistance locus 13 is SEQ ID NO: 97. SNP marker DNA sequences associated with aphid resistance locus 13 (SEQ ID NO: 97) can be amplified using the primers indicated as SEQ ID NO: 33 through SEQ ID NO: 34 and
25 detected with probes indicated as SEQ ID NO: 153 through SEQ ID NO: 154.

In the present invention, aphid resistance locus 14 is located on linkage group H. SNP marker used to monitor the introgression of aphid resistance locus 14 is SEQ ID NO: 98. SNP marker DNA sequences associated with aphid resistance locus 14 (SEQ ID NO: 98) can

be amplified using the primers indicated as SEQ ID NO: 35 through SEQ ID NO: 36 and detected with probes indicated as SEQ ID NO: 155 through SEQ ID NO: 156.

In the present invention, aphid resistance locus 15 is located on linkage group H. SNP marker used to monitor the introgression of aphid resistance locus 15 is SEQ ID NO: 99. 5 SNP marker DNA sequences associated with aphid resistance locus 15 (SEQ ID NO: 99) can be amplified using the primers indicated as SEQ ID NO: 37 through SEQ ID NO: 38 and detected with probes indicated as SEQ ID NO: 157 through SEQ ID NO: 158.

In the present invention, aphid resistance locus 16 is located on linkage group D2. SNP marker used to monitor the introgression of aphid resistance locus 16 is SEQ ID NO: 100 through SEQ ID NO: 102. 10 SNP marker DNA sequences associated with aphid resistance locus 16 (SEQ ID NO: 100 through SEQ ID NO: 102) can be amplified using the primers indicated as SEQ ID NO: 39 through SEQ ID NO: 44 and detected with probes indicated as SEQ ID NO: 159 through SEQ ID NO: 162, SEQ ID NO: 207 through SEQ ID NO: 208, and SEQ ID NO: 220.

15 In the present invention, aphid resistance locus 17 is located on linkage group F. SNP marker used to monitor the introgression of aphid resistance locus 17 is SEQ ID NO: 103. SNP marker DNA sequences associated with aphid resistance locus 17 (SEQ ID NO: 103) can be amplified using the primers indicated as SEQ ID NO: 45 through SEQ ID NO: 46 and detected with probes indicated as SEQ ID NO: 165 through SEQ ID NO: 166.

20 In the present invention, aphid resistance locus 18 is located on linkage group F. SNP marker used to monitor the introgression of aphid resistance locus 18 is SEQ ID NO: 104. SNP marker DNA sequences associated with aphid resistance locus 18 (SEQ ID NO: 104) can be amplified using the primers indicated as SEQ ID NO: 47 through SEQ ID NO: 48 and detected with probes indicated as SEQ ID NO: 167 through SEQ ID NO: 168.

25 In the present invention, aphid resistance locus 19 is located on linkage group I. SNP marker used to monitor the introgression of aphid resistance locus 19 is SEQ ID NO: 105. SNP marker DNA sequences associated with aphid resistance locus 19 (SEQ ID NO: 105) can be amplified using the primers indicated as SEQ ID NO: 49 through SEQ ID NO: 50 and detected with probes indicated as SEQ ID NO: 169 through SEQ ID NO: 170.

In the present invention, aphid resistance locus 20 is located on linkage group D1b. SNP marker used to monitor the introgression of aphid resistance locus 20 is SEQ ID NO: 106. SNP marker DNA sequences associated with aphid resistance locus 20 (SEQ ID NO: 106) can be amplified using the primers indicated as SEQ ID NO: 51 through SEQ ID NO: 52 and detected with probes indicated as SEQ ID NO: 171 through SEQ ID NO: 172.

In the present invention, aphid resistance locus 21 is located on linkage group D1b. SNP markers used to monitor the introgression of aphid resistance locus 21 are SEQ ID NO: 107 through SEQ ID NO. 108. SNP markers DNA sequences associated with aphid resistance locus 21 (SEQ ID NO: 107 through SEQ ID NO. 108). can be amplified using the primers indicated as SEQ ID NO: 53 through SEQ ID NO: 56 and detected with probes indicated as SEQ ID NO: 173 through SEQ ID NO: 176.

In the present invention, aphid resistance locus 22 is located on linkage group O. SNP marker used to monitor the introgression of aphid resistance locus 22 is SEQ ID NO: 109. SNP marker DNA sequences associated with aphid resistance locus 22 (SEQ ID NO: 109) can be amplified using the primers indicated as SEQ ID NO: 57 through SEQ ID NO: 58 and detected with probes indicated as SEQ ID NO: 177 through SEQ ID NO: 178.

In the present invention, aphid resistance locus 23 is located on linkage group O. SNP markers used to monitor the introgression of aphid resistance locus 23 are SEQ ID NO: 110 through SEQ ID NO: 111. SNP marker DNA sequences associated with aphid resistance locus 23 (SEQ ID NO: 110 through SEQ ID NO: 111) can be amplified using the primers indicated as SEQ ID NO: 59 through SEQ ID NO: 62 and detected with probes indicated as SEQ ID NO: 179 through SEQ ID NO: 182, SEQ ID NO: 209 through SEQ ID NO: 210, and SEQ ID NO: 221..

In the present invention, aphid resistance locus 24 is located on linkage group C1. SNP marker used to monitor the introgression of aphid resistance locus 24 is SEQ ID NO: 112. SNP marker DNA sequences associated with aphid resistance locus 24 (SEQ ID NO: 112) can be amplified using the primers indicated as SEQ ID NO: 63 through SEQ ID NO: 64 and detected with probes indicated as SEQ ID NO: 183 through SEQ ID NO: 184.

In the present invention, aphid resistance locus 25 is located on linkage group C1. SNP marker used to monitor the introgression of aphid resistance locus 25 is SEQ ID NO: 113. SNP marker DNA sequences associated with aphid resistance locus 25 (SEQ ID NO: 113) can be amplified using the primers indicated as SEQ ID NO: 65 through SEQ ID NO: 66 and detected with probes indicated as SEQ ID NO: 185 through SEQ ID NO: 186.

In the present invention, aphid resistance locus 26 is located on linkage group C1. SNP markers used to monitor the introgression of aphid resistance locus 26 are SEQ ID NO: 114 through SEQ ID NO: 115. SNP marker DNA sequences associated with aphid resistance locus 26 (SEQ ID NO: 114 through SEQ ID NO: 115) can be amplified using the primers indicated as SEQ ID NO: 67 through SEQ ID NO: 70 and detected with probes indicated as SEQ ID NO: 187 through SEQ ID NO: 190.

In the present invention, aphid resistance locus 27 is located on linkage group K. SNP marker used to monitor the introgression of aphid resistance locus 27 is SEQ ID NO: 116. SNP marker DNA sequences associated with aphid resistance locus 27 (SEQ ID NO: 116) can be amplified using the primers indicated as SEQ ID NO: 71 through SEQ ID NO: 72 and detected with probes indicated as SEQ ID NO: 191 through SEQ ID NO: 192.

In the present invention, aphid resistance locus 28 is located on linkage group B2. SNP markers used to monitor the introgression of aphid resistance locus 28 are SEQ ID NO: 117 through SEQ ID NO: 120. SNP marker DNA sequences associated with aphid resistance locus 28 (SEQ ID NO: 117 through SEQ ID NO: 120) can be amplified using the primers indicated as SEQ ID NO: 73 through SEQ ID NO: 80 and detected with probes indicated as SEQ ID NO: 193 through SEQ ID NO: 200, SEQ ID NO: 211 – 216, and SEQ ID NO: 222-223.

The present invention also provides a soybean plant comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 81 through SEQ ID NO: 120 and complements thereof. In one aspect, the soybean plant comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 nucleic acid sequences selected from the group consisting of SEQ ID NO: 81 through SEQ ID NO: 120, fragment thereof, and complements thereof.

The present invention also provides a soybean plant comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, or 28 aphid resistance loci where one or more alleles at one or more of their loci are selected from the group consisting of aphid resistance allele 1, aphid resistance allele 2, aphid resistance allele 3, aphid resistance allele 4, aphid resistance allele 5, aphid resistance allele 6, aphid resistance allele 7, aphid resistance allele 8, aphid resistance allele 9, aphid resistance allele 10, aphid resistance allele 11, aphid resistance allele 12, aphid resistance allele 13, aphid resistance allele 14, aphid resistance allele 15, aphid resistance allele 16, aphid resistance allele 17, aphid resistance allele 18, aphid resistance allele 19, aphid resistance allele 20, aphid resistance allele 21, aphid resistance allele 22, aphid resistance allele 23, aphid resistance allele 24, aphid resistance allele 25, aphid resistance allele 26, aphid resistance allele 27, aphid resistance allele 28, aphid resistance allele 29, aphid resistance allele 30, aphid resistance allele 31, aphid resistance allele 32, aphid resistance allele 33, aphid resistance allele 34, aphid resistance allele 35, aphid resistance allele 36, aphid resistance allele 37, aphid resistance allele 38, aphid resistance allele 39, and aphid resistance allele 40. Such alleles may be homozygous or heterozygous.

As used herein, aphid refers to any of various small, soft-bodied, plant-sucking insects of the Order *Homoptera*, further of the family *Aphididae*, wherein examples of *Aphididae* include but are not limited to the genus of *Acyrtosiphon*, *Allocotaphis*, *Amphorophora*, *Anoecia*, *Anuraphis*, *Aphidounguis*, *Aphidura*, *Aphis*, *Asiphonaphis*, *Astegopteryx*, *Aulacorthum*, *Betacallis*, *Betulaphis*, *Boernerina*, *Brachycaudus*, *Brachycorynella*, *Brevicoryne*, *Calaphis*, *Callipterinella*, *Callipterus*, *Cavariella*, *Cerataphis*, *Ceratovacuna*, *Chaetomyzus*, *Chaetosiphon*, *Chaitophorus*, *Chaitoregma*, *Chromaphis*, *Cinara*, *Clethrobis*, *Clydesmithia*, *Coloradoa*, *Cornaphis*, *Cryptomyzus*, *Crypturaphis*, *Doralis*, *Doraphis*, *Drepanaphis*, *Drepanosiphoniella*, *Drepanosiphum*, *Dysaphis*, *Eomacrosiphum*, *Epipemphigus*, *Ericolophium*, *Eriosoma*, *Essigella*, *Euceraphis*, *Eulachnus*, *Eumyzus*, *Eutrichosiphum*, *Fimbriaphis*, *Fullawaya*, *Geopemphigus*, *Glyphina*, *Gootiella*, *Greenidea*, *Grylloprociphilus*, *Hamamelistes*, *Hannabura*, *Hormaphis*, *Hyadaphis*, *Hyalomyzus*,

Hyalopterus, *Hyperomyza*, *Hyperomyzus*, *Hysteroneura*, *Illinoia*, *Indiaphis*, *Indomasonaphis*, *Kakimia*, *Lachnus*, *Laingia*, *Lambersaphis*, *Latgerina*, *Longicaudus*, *Longistigma*, *Macromyzus*, *Macrosiphoniella*, etc. while even further any one or more of the following genus species of *Aphididae*, examples of which including soybean aphid *Aphis glycines*,
 5 Bean aphid *Aphis fabae*, Cotton aphid *Aphis gossypii*, Rose aphid *Macrosiphum rosae*, green peach aphid *Myzus persicae*, corn leaf aphid *Rhopalosiphum maidis*, spotted alfalfa aphid *Therioaphis maculata*, wooly apple aphid *Eriosoma lanigerum* and the like.

As used herein, soybean aphid, *Aphis glycines*, and *Aphis glycines* Matasamura refers to an aphid that feeds on soybean. However, any aphid that is found on and feeds on a soybean
 10 plant, such as the bean aphid *Aphis fabae* is a target for aphid resistance in soybean and is within the scope of the invention. A soybean plant of the present invention can be resistant to one or more aphids infesting a soybean plant. In one aspect, the present invention provides plants resistant to aphids as well as methods and compositions for screening soybean plants for resistance or susceptibility to aphids, caused by the genus *Aphis*. In a preferred aspect,
 15 the present invention provides methods and compositions for screening soybean plants for resistance or susceptibility to *Aphis glycines*.

In an aspect, the plant is selected from the genus *Glycine*. *Glycine* plants, including but not limited to exotic germplasm, populations, lines, elite lines, cultivars and varieties.

As uses herein, soybean plant refers to a plant of the family Fabaceae, herein uses in
 20 the broadest sense and includes but is not limited to any species of soybean, examples of which including a *Glycine* species. A soybean plant may be a *Glycine arenaria*, *Glycine argyrea*, *Glycine canescens*, *Glycine clandestine*, *Glycine curvata*, *Glycine cyrtoloba*, *Glycine falcate*, *Glycine latifolia*, *Glycine latrobeana*, *Glycine max*, *Glycine microphylla*, *Glycine pescadrensis*, *Glycine pindanica*, *Glycine rubiginosa*, *Glycine soja*, *Glycine sp.*,
 25 *Glycine stenophita*, *Glycine tabacina*, and *Glycine tomentella*.

Plants of the present invention can be a soybean plant that is very resistant, resistant, substantially resistant, mid-resistant, comparatively resistant, partially resistant, mid-susceptible, or susceptible.

As used herein, the term resistant, resistance and host plant resistance refers the ability of a host plant to prevent or reduce infestation and damage of a pest from the group comprising insects, nematodes, pathogens, fungi, viruses, and diseases.

As used herein, the term antixenosis or non-preference resistance refers to the ability of a plant to ability to repel insects, causing a reduction in egg laying and feeding.

As used herein, the term antibiosis refers the ability of a plant to reduce survival, growth, or reproduction of insects that feed on it.

As used herein, the term tolerance refers to the ability of host plant to produce a larger yield of good quality than other plants when being fed upon by similar numbers of insects.

In a preferred aspect, the present invention provides a soybean plant to be assayed for resistance or susceptibility to aphids by any method to determine whether a soybean plant is very resistant, resistant, moderately resistant, moderately susceptible, or susceptible.

In this aspect, a plant is assayed for aphid resistance or susceptibility by visually estimating the number of aphids on a plant (Mensah *et al. Crop Sci* 25:2228-2233 (2005)).

As used herein, aphid resistance refers to preventing or inhibiting the ability of aphids to cause damage, such as reducing feeding, delaying growth and developing, reducing fecundity and the like, to a host plant.

In another aspect, the soybean plant can show a comparative resistance compared to a non-resistant control soybean plant. In this aspect, a control soybean plant will preferably be genetically similar except for the aphid resistant allele or alleles in question. Such plants can be grown under similar conditions with equivalent or near equivalent exposure to the pest. In this aspect, the resistant plant or plants has significantly fewer aphids per plant or damage per plant on resistant plants compared to known susceptible plants, and equivalent number of aphids or damage per plant compared to known resistant plants

As used herein, the terms quantitative trait loci and QTL refer to a genomic region affecting the phenotypic variation in continuously varying traits like yield or resistance. A QTL can comprise multiple genes or other genetic factors even within a contiguous genomic region or linkage group.

As used herein, the terms single nucleotide polymorphism and SNP refer to a single base difference between two DNA sequences.

As used herein, the term oligonucleotide refers to a molecule comprised of two or more deoxyribonucleotides or ribonucleotides.

5 As used herein, the term primer refers to an oligonucleotide complementary to a given nucleotide sequence and that is needed to initiate replication by a polymerase.

As used herein, the term probe refers to an oligonucleotide that is capable of hybridizing to another oligonucleotide of interest. A probe may be a single-stranded or double stranded oligonucleotide. Probes are useful for detection, identification or isolation of
10 particular nucleotide sequence.

As used herein, the term gene refers to a nucleic acid sequence that comprises introns, untranslated regions and control regions, and coding sequences necessary for the production of RNA, a polypeptide or a pre-cursor of a polypeptide.

As used herein, the term marker, DNA marker, and genetic marker refers to a trait,
15 including genetic traits such as DNA sequences loci alleles chromosome features isozyme, and morphological traits that can be used to detect the presence or location of a gene or trait in an individual or in a population.

As used herein, a diagnostic marker refers to a genetic marker that can detect or identify a trait, examples of which including aphid resistance, rust resistance and yield.

20 A resistance QTL of the present invention may be introduced into an elite soybean line. Herein, "line" refers to a group of individual plants from the similar parentage with similar traits. An "elite line" is any line that has resulted from breeding and selection for superior agronomic performance. Additionally, an elite line is sufficiently homogenous and homozygous to be used for commercial production. Elite lines may be used in the further
25 breeding efforts to develop new elite lines.

An aphid resistance QTL of the present invention may also be introduced into an soybean line comprising one or more transgenes conferring transgenic plant that contains one or more genes for herbicide tolerance, increased yield, insect control, fungal disease resistance, virus resistance, nematode resistance, bacterial disease resistance, mycoplasma

disease resistance, modified oils production, high oil production, high protein production, germination and seedling growth control, enhanced animal and human nutrition, low raffinose, environmental stress resistant, increased digestibility, industrial enzymes, pharmaceutical proteins, peptides and small molecules, improved processing traits, improved flavor, nitrogen fixation, hybrid seed production, reduced allergenicity, biopolymers, and biofuels among others. These agronomic traits can be provided by the methods of plant biotechnology as transgenes in soybean.

An aphid resistant QTL allele or alleles can be introduced from any plant that contains that allele (donor) to any recipient soybean plant. In one aspect, the recipient soybean plant can contain additional aphid resistant loci. In another aspect, the recipient soybean plant can contain a transgene. In another aspect, while maintaining the introduced QTL, the genetic contribution of the plant providing the aphid resistant QTL can be reduced by back-crossing or other suitable approaches. In one aspect, the nuclear genetic material derived from the donor material in the soybean plant can be less than or about 50%, less than or about 25%, less than or about 13%, less than or about 5%, 3%, 2% or 1%, but that genetic material contains the aphid resistant locus or loci of interest.

Plants containing one or more aphid resistant loci described can be donor plants. Aphid plants containing resistant loci can be, examples of which including screened for by using a nucleic acid molecule capable of detecting a marker polymorphism associated with resistance. In one aspect, a donor plant is PI 594427C. In a preferred aspect, a donor plant is the source for aphid resistance loci 1, 2, 4, 6, 7, 8, 9, 11, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 27, and 28. In another aspect, a donor plant is soybean variety MV0031. In another preferred aspect, a donor plant is the source for aphid resistance loci 2, 5, 8, 10, 25, and 26. In another aspect, a donor plant is soybean variety CNS (PI 548445). In another preferred aspect, a donor plant is the source for aphid resistance loci 3, 12, 14, and 24. A donor plant can be a susceptible line. In one aspect, a donor plant can also be a recipient soybean plant.

As used herein, a maturity group refers to an industry division of groups of varieties based range in latitude which the plant is best adapted and most productive. Soybean varieties are classified into 13 recognized maturity groups with the designations ranging from

maturity groups 000, 00, 0, and I through X, wherein 000 represents the earliest maturing variety and X represents the latest maturing variety. Soybean plants in maturity groups 000 to IV have indeterminate plant habit, while soybean plants in maturity groups V through X have determinate plant habit. Herein, determinate growth habit refers to a cease vegetative growth after the main stem terminates in a cluster of mature pods. Herein, indeterminate growth habit refers to the development of leaves and flowers simultaneously throughout a portion of their reproductive period, with one to three pods at the terminal apex. Early maturity varieties (000 to IV) are adapted to northern latitudes with longer day lengths with the maturity designation increasing in southern latitudes with shorter day lengths

Herein, relative maturity refers to a soybean plant maturity group subdividing a maturity group into tenths, for example III.5. Relative maturity provided a more exact maturity. The number following the decimal point refers to the relative earliness or lateness with a maturity group, examples of which including IV.2 is an early group IV variety and IV.9 is a late group IV.

It is further understood that a soybean plant of the present invention may exhibit the characteristics of any relative maturity group. In an aspect, the relative maturity group is selected from the group consisting of 000.1-000.9, 00.1-00.9, 0.1-0.9, I.1-I.9, II.1-II.9, III.1-III.9, IV.1-IV.9, V.1-V.9, VI.1-VI.9, VII.1-VII.9, VIII.1-VIII.9, IX.1-IX.9, and X.1-X.9. The pollen for selected soybean plant can be cryopreserved and used in crosses with soybean lines from other maturity groups to introgress an aphid resistance locus in a line that would not normally be available for crossing in nature. Pollen cryopreservation techniques are well known in the art (Tyagi and Hymowitz, *Cryo letters* 24: 119-124 (2003), Liang *et al. Acta Botanica Sinica* 35: 733-738 (1993)).

The aphid resistance effect of the QTL can vary based on parental genotype and on the environmental factors in which the aphid resistance is measured. It is within the skill of those in the art of plant breeding and without undue experimentation to use methods described herein to select from populations of plants or from a collection of parental genotypes those that when containing an aphid resistance locus result in enhanced aphid

resistance relative to the parental genotype. Herein, an infestation can be caused by insects, fungi, virus, bacterium or invertebrate animal.

A number of molecular genetic maps of *Glycine* have been reported (Mansur *et al.*, *Crop Sci.* 36: 1327-1336 (1996), Shoemaker *et al.*, *Genetics* 144: 329-338 (1996),;

5 Shoemaker *et al.*, *Crop Science* 32: 1091-1098 (1992), Shoemaker *et al.*, *Crop Science* 35: 436-446 (1995), ; Tinley and Rafalski, *J. Cell Biochem. Suppl.* 14E: 291 (1990),); Cregan *et al.*, *Crop Science* 39:1464-1490 (1999). *Glycine max*, *Glycine soja* and *Glycine max* x. *Glycine soja* share linkage groups (Shoemaker *et al.*, *Genetics* 144: 329-338 (1996). A linkage group (LG) is a set of genes that tend to be inherited together from generation to
10 generation. As used herein, reference to the linkage groups (LG), J, E, B1, N, A1, D1a_Q, H, D1, F, I D1b+W, O C1 and B2 of *Glycine max* refers to the linkage group that corresponds to linkage groups, J, E, B1, N, A1, D1a_Q, H, D1, F, I D1b+W, O C1 and B2 from the genetic map of *Glycine max* (Mansur *et al.*, *Crop Science* 36: 1327-1336 (1996); Cregan *et al.*, *Crop Science* 39:1464-1490 (1999), and Soybase, Agricultural Research Service, United States
15 Department of Agriculture).

An allele of a QTL can, of course, comprise multiple genes or other genetic factors even within a contiguous genomic region or linkage group, such as a haplotype. A linkage group is a group loci carried on the same chromosome. A haplotype is set of genetic markers associated with closely linked segments of DNA on one chromosome and tend to be inherited
20 as a unit. As used herein, an allele of a resistance locus can therefore encompass more than one gene or other genetic factor wherein each individual gene or genetic component is also capable of exhibiting allelic variation and wherein each gene or genetic factor is also capable of eliciting a phenotypic effect on the quantitative trait in question. In an aspect of the present invention the allele of a QTL comprises one or more genes or other genetic factors
25 that are also capable of exhibiting allelic variation. The use of the term "an allele of a QTL" is thus not intended to exclude a QTL that comprises more than one gene or other genetic factor. Specifically, an "allele of a QTL" in the present invention can denote a haplotype within a haplotype window wherein a phenotype can be pest resistance. A haplotype window is a contiguous genomic region that can be defined, and tracked, with a set of one or more

polymorphic markers wherein the polymorphisms indicate identity by descent. A haplotype within that window can be defined by the unique fingerprint of alleles at each marker. As used herein, an allele is one of several alternative forms of a gene occupying a given locus on a chromosome. When all the alleles present at a given locus on a chromosome are the same, that plant is homozygous at that locus. If the alleles present at a given locus on a chromosome differ, that plant is heterozygous at that locus. Plants of the present invention may be homozygous or heterozygous at any particular aphid resistance locus or for a particular polymorphic marker.

The present invention also provides for parts of the plants of the present invention.

Plant parts, without limitation, include seed, endosperm, ovule and pollen. In a particularly preferred aspect of the present invention, the plant part is a seed.

Plants or parts thereof of the present invention may be grown in culture and regenerated. Methods for the regeneration of *Glycine max* plants from various tissue types and methods for the tissue culture of *Glycine max* are known in the art (See, examples of which including Widholm *et al.*, *In Vitro Selection and Culture-induced Variation in Soybean*, In *Soybean: Genetics, Molecular Biology and Biotechnology*, Eds. Verma and Shoemaker, CAB International, Wallingford, Oxon, England (1996). Regeneration techniques for plants such as *Glycine max* can use as the starting material a variety of tissue or cell types. With *Glycine max* in particular, regeneration processes have been developed that begin with certain differentiated tissue types such as meristems, Cartha *et al.*, *Can. J. Bot.* 59:1671-1679 (1981), hypocotyl sections, Cameya *et al.*, *Plant Science Letters* 21: 289-294 (1981), and stem node segments, Saka *et al.*, *Plant Science Letters*, 19: 193-201 (1980); Cheng *et al.*, *Plant Science Letters*, 19: 91-99 (1980). Regeneration of whole sexually mature *Glycine max* plants from somatic embryos generated from explants of immature *Glycine max* embryos has been reported (Ranch *et al.*, *In Vitro Cellular & Developmental Biology* 21: 653-658 (1985). Regeneration of mature *Glycine max* plants from tissue culture by organogenesis and embryogenesis has also been reported (Barwale *et al.*, *Planta* 167: 473-481 (1986); Wright *et al.*, *Plant Cell Reports* 5: 150-154 (1986).

The present invention also provides an aphid resistant soybean plant selected for by screening for aphid resistance or susceptibility in the soybean plant, the selection comprising interrogating genomic nucleic acids for the presence of a marker molecule that is genetically linked to an allele of a QTL associated with aphid resistance in the soybean plant, where the allele of a QTL is also located on a linkage group associated with aphid resistant soybean.

The present invention includes a method of introgressing an aphid resistant allele into a soybean plant comprising (A) crossing at least one first soybean plant comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 81 through SEQ ID NO: 120 with at least one second soybean plant in order to form a segregating population, (B) screening the segregating population with one or more nucleic acid markers to determine if one or more soybean plants from the segregating population contains the nucleic acid sequence, and (C) selecting from the segregation population one or more soybean plants comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 81 through SEQ ID NO: 120 .

The present invention includes a method of introgressing an allele into a soybean plant comprising: (A) crossing at least one aphid resistant soybean plant with at least one aphid sensitive soybean plant in order to form a segregating population; (B) screening said segregating population with one or more nucleic acid markers to determine if one or more soybean plants from said segregating population contains an aphid resistant allele, wherein said aphid resistance allele is an allele selected from the group consisting of aphid resistance allele 1, aphid resistance allele 2, aphid resistance allele 3, aphid resistance allele 4, aphid resistance allele 5, aphid resistance allele 6, aphid resistance allele 7, aphid resistance allele 8, aphid resistance allele 9, aphid resistance allele 10, aphid resistance allele 11, aphid resistance allele 12, aphid resistance allele 13, aphid resistance allele 14, aphid resistance allele 15, aphid resistance allele 16, aphid resistance allele 17, aphid resistance allele 18, aphid resistance allele 19, aphid resistance allele 20, aphid resistance allele 21, aphid resistance allele 22, aphid resistance allele 23, aphid resistance allele 24, aphid resistance allele 25, aphid resistance allele 26, aphid resistance allele 27, aphid resistance allele 28, aphid resistance allele 29, aphid resistance allele 30, aphid resistance allele 31, aphid

resistance allele 32, aphid resistance allele 33, aphid resistance allele 34, aphid resistance allele 35, aphid resistance allele 36, aphid resistance allele 37, aphid resistance allele 38, aphid resistance allele 39, and aphid resistance allele 40.

The present invention includes nucleic acid molecules. Such molecules include those
5 nucleic acid molecules capable of detecting a polymorphism genetically or physically linked to aphid resistance loci. Such molecules can be referred to as markers. Additional markers can be obtained that are linked to aphid resistance locus 1, aphid resistance locus 2, aphid resistance locus 3, aphid resistance locus 4, aphid resistance locus 5, aphid resistance locus 6, aphid resistance locus 7, aphid resistance locus 8, aphid resistance locus 9, aphid resistance
10 locus 10, aphid resistance locus 11, aphid resistance locus 12, aphid resistance locus 13, aphid resistance locus 14, aphid resistance locus 15, aphid resistance locus 16, aphid resistance locus 17, aphid resistance locus 18, aphid resistance locus 19, aphid resistance locus 20, aphid resistance locus 21, aphid resistance locus 22, aphid resistance locus 23, aphid resistance locus 24, aphid resistance locus 25, aphid resistance locus 26, aphid
15 resistance locus 27, and aphid resistance locus 28 by available techniques. In one aspect, the nucleic acid molecule is capable of detecting the presence or absence of a marker located less than 50, 40, 30, 20, 10, 5, 2, or 1 centimorgans from an aphid resistance loci. In another aspect, a marker exhibits a LOD score of 2 or greater, 3 or greater, or 4 or greater with aphid resistance, measuring using MapManager or QGene Version 3 and default parameters. In
20 another aspect, the nucleic acid molecule is capable of detecting a marker in a locus selected from the group aphid resistance locus 1, aphid resistance locus 2, aphid resistance locus 3, aphid resistance locus 4, aphid resistance locus 5, aphid resistance locus 6, aphid resistance locus 7, aphid resistance locus 8, aphid resistance locus 9, aphid resistance locus 10, aphid resistance locus 11, aphid resistance locus 12, aphid resistance locus 13, aphid resistance
25 locus 14, aphid resistance locus 15, aphid resistance locus 16, aphid resistance locus 17, aphid resistance locus 18, aphid resistance locus 19, aphid resistance locus 20, aphid resistance locus 21, aphid resistance locus 22, aphid resistance locus 23, aphid resistance locus 24, aphid resistance locus 25, aphid resistance locus 26, aphid resistance locus 27, and aphid resistance locus 28. In a further aspect, a nucleic acid molecule is selected from the

group consisting of SEQ ID NO: 81 through SEQ ID NO: 120, fragments thereof, complements thereof, and nucleic acid molecules capable of specifically hybridizing to one or more of these nucleic acid molecules.

In a preferred aspect, a nucleic acid molecule of the present invention includes those
5 that will specifically hybridize to one or more of the nucleic acid molecules set forth in SEQ ID NO: 81 through SEQ ID NO: 120 or complements thereof or fragments of either under moderately stringent conditions, for example at about 2.0 x SSC and about 65°C. In a particularly preferred aspect, a nucleic acid of the present invention will specifically hybridize to one or more of the nucleic acid molecules set forth in SEQ ID NO: 81 through
10 SEQ ID NO: 120 or complements or fragments of either under high stringency conditions. In one aspect of the present invention, a preferred marker nucleic acid molecule of the present invention has the nucleic acid sequence set forth in SEQ ID NO: 81 through SEQ ID NO: 120 or complements thereof or fragments of either. In another aspect of the present invention, a preferred marker nucleic acid molecule of the present invention shares between 80% and
15 100% or 90% and 100% sequence identity with the nucleic acid sequence set forth in SEQ ID NO: 81 through SEQ ID NO: 120 or complement thereof or fragments of either. In a further aspect of the present invention, a preferred marker nucleic acid molecule of the present invention shares between 95% and 100% sequence identity with the sequence set forth in SEQ ID NO: 81 through SEQ ID NO: 120 or complement thereof or fragments of either. In a
20 more preferred aspect of the present invention, a preferred marker nucleic acid molecule of the present invention shares between 98% and 100% sequence identity with the nucleic acid sequence set forth in SEQ ID NO: 81 through SEQ ID NO: 120 or complement thereof or fragments of either.

Nucleic acid molecules or fragments thereof are capable of specifically hybridizing to
25 other nucleic acid molecules under certain circumstances. As used herein, two nucleic acid molecules are capable of specifically hybridizing to one another if the two molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure. A nucleic acid molecule is the “complement” of another nucleic acid molecule if they exhibit complete complementarity. As used herein, molecules are exhibit “complete complementarity” when

every nucleotide of one of the molecules is complementary to a nucleotide of the other. Two molecules are "minimally complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low-stringency" conditions. Similarly, the molecules are "complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under conventional "high-stringency" conditions. Conventional stringency conditions are described by Sambrook *et al.*, In: *Molecular Cloning, A Laboratory Manual, 2nd Edition, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989)*, and by Haymes *et al.*, In: *Nucleic Acid Hybridization, A Practical Approach*, IRL Press, Washington, DC (1985). Departures from complete complementarity are therefore permissible, as long as such departures do not completely preclude the capacity of the molecules to form a double-stranded structure. In order for a nucleic acid molecule to serve as a primer or probe it need only be sufficiently complementary in sequence to be able to form a stable double-stranded structure under the particular solvent and salt concentrations employed.

As used herein, a substantially homologous sequence is a nucleic acid sequence that will specifically hybridize to the complement of the nucleic acid sequence to which it is being compared under high stringency conditions. The nucleic-acid probes and primers of the present invention can hybridize under stringent conditions to a target DNA sequence. The term "stringent hybridization conditions" is defined as conditions under which a probe or primer hybridizes specifically with a target sequence(s) and not with non-target sequences, as can be determined empirically. The term "stringent conditions" is functionally defined with regard to the hybridization of a nucleic-acid probe to a target nucleic acid (i.e., to a particular nucleic-acid sequence of interest) by the specific hybridization procedure discussed in Sambrook *et al.*, 1989, at 9.52-9.55. See also, Sambrook *et al.*, 1989 at 9.47-9.52, 9.56-9.58; Kanehisa 1984 *Nucl. Acids Res.* 12: 203-213; and Wetmur *et al.* 1968 *J. Mol. Biol.* 31:349-370. Appropriate stringency conditions that promote DNA hybridization are, examples of which including 6.0 x sodium chloride/sodium citrate (SSC) at about 45° C, followed by a wash of 2.0 x SSC at 50°C, are known to those skilled in the art or can be found in Current

Protocols in Molecular Biology, John Wiley & Sons, N.Y., 1989, 6.3.1-6.3.6. Examples of which including the salt concentration in the wash step can be selected from a low stringency of about 2.0 x SSC at 50°C to a high stringency of about 0.2 x SSC at 50°C. In addition, the temperature in the wash step can be increased from low stringency conditions at room
5 temperature, about 22°C, to high stringency conditions at about 65°C. Both temperature and salt may be varied, or either the temperature or the salt concentration may be held constant while the other variable is changed.

Examples of which including hybridization using DNA or RNA probes or primers can be performed at 65°C in 6x SSC, 0.5% SDS, 5x Denhardt's, 100 µg/mL nonspecific DNA
10 (e.g., sonicated salmon sperm DNA) with washing at 0.5x SSC, 0.5% SDS at 65°C, for high stringency.

It is contemplated that lower stringency hybridization conditions such as lower hybridization and/or washing temperatures can be used to identify related sequences having a lower degree of sequence similarity if specificity of binding of the probe or primer to target
15 sequence(s) is preserved. Accordingly, the nucleotide sequences of the present invention can be used for their ability to selectively form duplex molecules with complementary stretches of DNA, RNA, or cDNA fragments.

A fragment of a nucleic acid molecule can be any sized fragment and illustrative fragments include fragments of nucleic acid sequences set forth in SEQ ID NO: 81 through
20 SEQ ID NO: 120 and complements thereof. In one aspect, a fragment can be between 15 and 25, 15 and 30, 15 and 40, 15 and 50, 15 and 100, 20 and 25, 20 and 30, 20 and 40, 20 and 50, 20 and 100, 25 and 30, 25 and 40, 25 and 50, 25 and 100, 30 and 40, 30 and 50, and 30 and 100. In another aspect, the fragment can be greater than 10, 15, 20, 25, 30, 35, 40, 50, 100, or 250 nucleotides.

25 Additional genetic markers can be used to select plants with an allele of a QTL associated with aphid resistance of soybean of the present invention. Examples of public marker databases include, for example: Soybase, Agricultural Research Service, United States Department of Agriculture.

A genetic marker is a DNA sequence that has a known location on a chromosome and associated with a particular trait or gene. Genetic markers associated with aphid resistance can be used to determine whether an individual plant is resistant to aphids.

Genetic markers of the present invention include "dominant" or "codominant" markers. "Codominant markers" reveal the presence of two or more alleles (two per diploid individual). "Dominant markers" reveal the presence of only a single allele. The presence of the dominant marker phenotype (*e.g.*, a band of DNA) is an indication that one allele is present in either the homozygous or heterozygous condition. The absence of the dominant marker phenotype (*e.g.*, absence of a DNA band) is merely evidence that "some other" undefined allele is present. In the case of populations wherein individuals are predominantly homozygous and loci are predominantly dimorphic, dominant and codominant markers can be equally valuable. As populations become more heterozygous and multiallelic, codominant markers often become more informative of the genotype than dominant markers.

Markers, such as single sequence repeat markers (SSR), AFLP markers, RFLP markers, RAPD markers, phenotypic markers, SNPs, isozyme markers, microarray transcription profiles that are genetically linked to or correlated with alleles of a QTL of the present invention can be utilized (Walton, 1993; Burow *et al.* 1988). Methods to isolate such markers are known in the art.

The detection of polymorphic sites in a sample of DNA, RNA, or cDNA may be facilitated through the use of nucleic acid amplification methods. Such methods specifically increase the concentration of polynucleotides that span the polymorphic site, or include that site and sequences located either distal or proximal to it. Such amplified molecules can be readily detected by gel electrophoresis, fluorescence detection methods, or other means.

A method of achieving such amplification employs the polymerase chain reaction (PCR) (Mullis *et al.* 1986 Cold Spring Harbor *Symp. Quant. Biol.* 51:263-273; European Patent No. 50,424; European Patent No. 84,796; European Patent No. 258,017; European Patent No. 237,362; European Patent No. 201,184; U.S. Patent No. 4,683,202; U.S. Patent 4,582,788; and U.S. Patent No. 4,683,194), using primer pairs that are capable of hybridizing to the proximal sequences that define a polymorphism in its double-stranded form.

For the purpose of QTL mapping, the markers included should be diagnostic of origin in order for inferences to be made about subsequent populations. SNP markers are ideal for mapping because the likelihood that a particular SNP allele is derived from independent origins in the extant populations of a particular species is very low. As such, SNP markers are useful for tracking and assisting introgression of QTLs, particularly in the case of haplotypes.

The genetic linkage of additional marker molecules can be established by a gene mapping model such as, without limitation, the flanking marker model reported by Lander *et al.* (Lander *et al.* 1989 *Genetics*, 121:185-199), and the interval mapping, based on maximum likelihood methods described therein, and implemented in the software package MAPMAKER/QTL (Lincoln and Lander, *Mapping Genes Controlling Quantitative Traits Using MAPMAKER/QTL*, Whitehead Institute for Biomedical Research, Massachusetts, (1990). Additional software includes Qgene, Version 3, Department of Plant Breeding and Biometry, 266 Emerson Hall, Cornell University, Ithaca, NY). Use of Qgene software is a particularly preferred approach.

A maximum likelihood estimate (MLE) for the presence of a marker is calculated, together with an MLE assuming no QTL effect, to avoid false positives. A $\log_{10}$ of an odds ratio (LOD) is then calculated as: $\text{LOD} = \log_{10} (\text{MLE for the presence of a QTL} / \text{MLE given no linked QTL})$. The LOD score essentially indicates how much more likely the data are to have arisen assuming the presence of a QTL versus in its absence. The LOD threshold value for avoiding a false positive with a given confidence, say 95%, depends on the number of markers and the length of the genome. Graphs indicating LOD thresholds are set forth in Lander *et al.* (1989), and further described by Arús and Moreno-González, *Plant Breeding*, Hayward, Bosemark, Romagosa (eds.) Chapman & Hall, London, pp. 314-331 (1993).

Additional models can be used. Many modifications and alternative approaches to interval mapping have been reported, including the use of non-parametric methods (Kruglyak *et al.* 1995 *Genetics*, 139:1421-1428). Multiple regression methods or models can be also be used, in which the trait is regressed on a large number of markers (Jansen, *Biometrics in Plant Breed*, van Oijen, Jansen (eds.) Proceedings of the Ninth Meeting of the Eucarpia

Section Biometrics in Plant Breeding, The Netherlands, pp. 116-124 (1994); Weber and Wricke, *Advances in Plant Breeding*, Blackwell, Berlin, 16 (1994)). Procedures combining interval mapping with regression analysis, whereby the phenotype is regressed onto a single putative QTL at a given marker interval, and at the same time onto a number of markers that serve as 'cofactors,' have been reported by Jansen *et al.* (Jansen *et al.* 1994 *Genetics*, 136:1447-1455) and Zeng (Zeng 1994 *Genetics* 136:1457-1468). Generally, the use of cofactors reduces the bias and sampling error of the estimated QTL positions (Utz and Melchinger, *Biometrics in Plant Breeding*, van Oijen, Jansen (eds.) Proceedings of the Ninth Meeting of the Eucarpia Section Biometrics in Plant Breeding, The Netherlands, pp.195-204 (1994), thereby improving the precision and efficiency of QTL mapping (Zeng 1994). These models can be extended to multi-environment experiments to analyze genotype-environment interactions (Jansen *et al.* 1995 *Theor. Appl. Genet.* 91:33-3).

Selection of appropriate mapping populations is important to map construction. The choice of an appropriate mapping population depends on the type of marker systems employed (Tanksley *et al.*, *Molecular mapping in plant chromosomes. chromosome structure and function: Impact of new concepts* J.P. Gustafson and R. Appels (eds.). Plenum Press, New York, pp. 157-173 (1988)). Consideration must be given to the source of parents (adapted vs. exotic) used in the mapping population. Chromosome pairing and recombination rates can be severely disturbed (suppressed) in wide crosses (adapted x exotic) and generally yield greatly reduced linkage distances. Wide crosses will usually provide segregating populations with a relatively large array of polymorphisms when compared to progeny in a narrow cross (adapted x adapted).

An F₂ population is the first generation of selfing after the hybrid seed is produced. Usually a single F₁ plant is selfed to generate a population segregating for all the genes in Mendelian (1:2:1) fashion. Maximum genetic information is obtained from a completely classified F₂ population using a codominant marker system (Mather, Measurement of Linkage in Heredity: Methuen and Co., (1938)). In the case of dominant markers, progeny tests (e.g. F₃, BCF₂) are required to identify the heterozygotes, thus making it equivalent to a completely classified F₂ population. However, this procedure is often prohibitive because of

the cost and time involved in progeny testing. Progeny testing of F₂ individuals is often used in map construction where phenotypes do not consistently reflect genotype (*e.g.* pest resistance) or where trait expression is controlled by a QTL. Segregation data from progeny test populations (*e.g.* F₃ or BCF₂) can be used in map construction. Marker-assisted selection can then be applied to cross progeny based on marker-trait map associations (F₂, F₃), where linkage groups have not been completely disassociated by recombination events (*i.e.*, maximum disequilibrium).

Recombinant inbred lines (RIL) (genetically related lines; usually >F₅, developed from continuously selfing F₂ lines towards homozygosity) can be used as a mapping population. Information obtained from dominant markers can be maximized by using RIL because all loci are homozygous or nearly so. Under conditions of tight linkage (*i.e.*, about <10% recombination), dominant and co-dominant markers evaluated in RIL populations provide more information per individual than either marker type in backcross populations (Reiter *et al.* 1992 *Proc. Natl. Acad. Sci.(USA)* 89:1477-1481). However, as the distance between markers becomes larger (*i.e.*, loci become more independent), the information in RIL populations decreases dramatically.

Backcross populations (*e.g.*, generated from a cross between a successful variety (recurrent parent) and another variety (donor parent) carrying a trait not present in the former) can be utilized as a mapping population. A series of backcrosses to the recurrent parent can be made to recover most of its desirable traits. Thus a population is created comprising of individuals nearly like the recurrent parent but each individual carries varying amounts or mosaic of genomic regions from the donor parent. Backcross populations can be useful for mapping dominant markers if all loci in the recurrent parent are homozygous and the donor and recurrent parent have contrasting polymorphic marker alleles (Reiter *et al.* 1992).

Information obtained from backcross populations using either codominant or dominant markers is less than that obtained from F₂ populations because one, rather than two, recombinant gametes are sampled per plant. Backcross populations, however, are more informative (at low marker saturation) when compared to RILs as the distance between linked loci increases in RIL populations (*i.e.* about .15% recombination). Increased

recombination can be beneficial for resolution of tight linkages, but may be undesirable in the construction of maps with low marker saturation.

Near-isogenic lines (NIL) created by many backcrosses to produce an array of individuals that are nearly identical in genetic composition except for the trait or genomic region under interrogation can be used as a mapping population. In mapping with NILs, only a portion of the polymorphic loci are expected to map to a selected region.

Bulk segregant analysis (BSA) is a method developed for the rapid identification of linkage between markers and traits of interest (Michelmore *et al.* 1991 *Proc. Natl. Acad. Sci. (U.S.A.)* 88:9828-9832). In BSA, two bulked DNA samples are drawn from a segregating population originating from a single cross. These bulks contain individuals that are identical for a particular trait (resistant or susceptible to particular pests) or genomic region but arbitrary at unlinked regions (*i.e.* heterozygous). Regions unlinked to the target region will not differ between the bulked samples of many individuals in BSA.

An alternative to traditional QTL mapping involves achieving higher resolution by mapping haplotypes, versus individual markers (Fan *et al.* 2006 *Genetics* 172:663-686). This approach tracks blocks of DNA known as haplotypes, as defined by polymorphic markers, which are assumed to be identical by descent in the mapping population. This assumption results in a larger effective sample size, offering greater resolution of QTL. Methods for determining the statistical significance of a correlation between a phenotype and a genotype, in this case a haplotype, may be determined by any statistical test known in the art and with any accepted threshold of statistical significance being required. The application of particular methods and thresholds of significance are well within the skill of the ordinary practitioner of the art.

Plants of the present invention can be part of or generated from a breeding program. The choice of breeding method depends on the mode of plant reproduction, the heritability of the trait(s) being improved, and the type of cultivar used commercially (*e.g.*, F₁ hybrid cultivar, pureline cultivar, etc). A cultivar is a race or variety of a plant species that has been created or selected intentionally and maintained through cultivation.

Selected, non-limiting approaches for breeding the plants of the present invention are set forth below. A breeding program can be enhanced using marker assisted selection (MAS) on the progeny of any cross. It is understood that nucleic acid markers of the present invention can be used in a MAS (breeding) program. It is further understood that any commercial and non-commercial cultivars can be utilized in a breeding program. Factors such as, examples of which including emergence vigor, vegetative vigor, stress tolerance, disease resistance, branching, flowering, seed set, seed size, seed density, standability, and threshability etc. will generally dictate the choice.

For highly heritable traits, a choice of superior individual plants evaluated at a single location will be effective, whereas for traits with low heritability, selection should be based on mean values obtained from replicated evaluations of families of related plants. Popular selection methods commonly include pedigree selection, modified pedigree selection, mass selection, and recurrent selection. In a preferred aspect, a backcross or recurrent breeding program is undertaken.

The complexity of inheritance influences choice of the breeding method. Backcross breeding can be used to transfer one or a few favorable genes for a highly heritable trait into a desirable cultivar. This approach has been used extensively for breeding disease and insect-resistant cultivars. Various recurrent selection techniques are used to improve quantitatively inherited traits controlled by numerous genes.

Breeding lines can be tested and compared to appropriate standards in environments representative of the commercial target area(s) for two or more generations. The best lines are candidates for new commercial cultivars; those still deficient in traits may be used as parents to produce new populations for further selection.

The development of new elite soybean hybrids requires the development and selection of elite inbred lines, the crossing of these lines and selection of superior hybrid crosses. The hybrid seed can be produced by manual crosses between selected male-fertile parents or by using male sterility systems. Additional data on parental lines, as well as the phenotype of the hybrid, influence the breeder's decision whether to continue with the specific hybrid cross.

Pedigree breeding and recurrent selection breeding methods can be used to develop cultivars from breeding populations. Breeding programs combine desirable traits from two or more cultivars or various broad-based sources into breeding pools from which cultivars are developed by selfing and selection of desired phenotypes. New cultivars can be evaluated to determine which have commercial potential.

Backcross breeding has been used to transfer genes for a simply inherited, highly heritable trait into a desirable homozygous cultivar or inbred line, which is the recurrent parent. The source of the trait to be transferred is called the donor parent. After the initial cross, individuals possessing the phenotype of the donor parent are selected and repeatedly crossed (backcrossed) to the recurrent parent. The resulting plant is expected to have most attributes of the recurrent parent (*e.g.*, cultivar) and, in addition, the desirable trait transferred from the donor parent.

The single-seed descent procedure in the strict sense refers to planting a segregating population, harvesting a sample of one seed per plant, and using the one-seed sample to plant the next generation. When the population has been advanced from the F₂ to the desired level of inbreeding, the plants from which lines are derived will each trace to different F₂ individuals. The number of plants in a population declines each generation due to failure of some seeds to germinate or some plants to produce at least one seed. As a result, not all of the F₂ plants originally sampled in the population will be represented by a progeny when generation advance is completed.

Descriptions of other breeding methods that are commonly used for different traits and crops can be found in one of several reference books (Allard, "Principles of Plant Breeding," John Wiley & Sons, NY, U. of CA, Davis, CA, 50-98, 1960; Simmonds, "Principles of crop improvement," Longman, Inc., NY, 369-399, 1979; Snee and Hendriksen, "Plant breeding perspectives," Wageningen (ed), Center for Agricultural Publishing and Documentation, 1979; Fehr, *In: Soybeans: Improvement, Production and Uses*, 2nd Edition, *Manograph.*, 16:249, 1987; Fehr, "Principles of variety development," *Theory and Technique*, (Vol. 1) and *Crop Species Soybean* (Vol. 2), Iowa State Univ., Macmillan Pub. Co., NY, 360-376, 1987).

It is further understood, that the present invention provides bacterial, viral, microbial, insect, mammalian and plant cells comprising the nucleic acid molecules of the present invention.

As used herein, a "nucleic acid molecule," be it a naturally occurring molecule or otherwise may be "substantially purified", if desired, referring to a molecule separated from substantially all other molecules normally associated with it in its native state. More preferably a substantially purified molecule is the predominant species present in a preparation. A substantially purified molecule may be greater than 60% free, preferably 75% free, more preferably 90% free, and most preferably 95% free from the other molecules (exclusive of solvent) present in the natural mixture. The term "substantially purified" is not intended to encompass molecules present in their native state.

The agents of the present invention will preferably be "biologically active" with respect to either a structural attribute, such as the capacity of a nucleic acid to hybridize to another nucleic acid molecule, or the ability of a protein to be bound by an antibody (or to compete with another molecule for such binding). Alternatively, such an attribute may be catalytic, and thus involve the capacity of the agent to mediate a chemical reaction or response.

The agents of the present invention may also be recombinant. As used herein, the term recombinant means any agent (*e.g.* DNA, peptide etc.), that is, or results, however indirect, from human manipulation of a nucleic acid molecule.

The agents of the present invention may be labeled with reagents that facilitate detection of the agent (*e.g.* fluorescent labels (Prober *et al.* 1987 Science 238:336-340; Albarella *et al.*, European Patent No. 144914), chemical labels (Sheldon *et al.*, U.S. Patent No. 4,582,789; Albarella *et al.*, U.S. Patent No. 4,563,417), modified bases (Miyoshi *et al.*, European Patent No. 119448).

Having now generally described the invention, the same will be more readily understood through reference to the following examples which are provided by way of illustration, and are not intended to be limiting of the present invention, unless specified.

EXAMPLES

Example 1: Identifying antixenosis type aphid resistance in PI594427C

Soybean breeders evaluate soybean germplasm for genetic traits that confer resistance to attack and injury by aphids. Host plant resistance is classified as antixenosis, antibiosis or tolerance. Antixenosis, also referred to as non-preference, is the ability of a plant to repel insects, causing a reduction in oviposition or feeding. Dowling, CNS (PI 548445). Jackson, PI 597727C, PI 594403 and Williams were evaluated for antixenosis type resistance.

Antixenosis resistance is typical evaluated choice experiments where insects can select between at least two host plants.

PI 594427C was identified to have resistance to aphids in choice field tests conducted at Michigan State University over three field season. In the choice field tests, Dowling, CNS, Jackson, PI 597727C, PI 594403 and Williams were grown and enclosed in a single cage. Two field collected aphids were placed on each plant. Aphids were able to move freely from plant to plant, therefore the study evaluated the aphid plant preference. Plants were individually scored at 2, 3, 4, or 5 wks after infestation, depending on when symptoms were first observed. Plants were given a visual rating ranging from 0 to 4 (Table 1).

Table 1: Description of rating scale used for aphid resistance phenotyping

Rating		Symptoms
0	Very Resistant	No aphids
1	Resistant	Fewer than 100 aphids
2	Moderately Resistant	101-300 aphids
3	Moderately Susceptible	300-800 aphids
4	Susceptible	>800 aphids

Each week, the plants were also assigned a damage index (DI), which is calculated using the following formula:

$$DI = \frac{\sum(\text{each scale} \times \text{no. of plants in the scale})}{4 \times \text{total no. of plants evaluated}} \times 100$$

A higher damage index corresponds to a more susceptible plant.

Over three years of field tests, PI 594427C consistently showed equivalent or lower aphid ratings and damage indices than known aphid resistant varieties, Jackson and Dowling (Table 2). CNS and Williams were sensitive to aphid infestation.

Table 2. Average soybean aphid rating and damage index for aphid-resistant and – susceptible soybean genotypes tested in triplicate in field cages over 3 years at Michigan State University.

Line	Aphid rating (0-4)	Damage index [¶] (%)
Dowling	1.8	44
CNS	3.8	95
Jackson	1.9	48
PI 594427C	1.5	37
PI 594403	2.5	63
Williams	3.8	94

Example 2: Identifying antibiosis type aphid resistance in PI594427C

Host plant resistance is classified as antixenosis, antibiosis or tolerance. Antibiosis is the ability of a plant to reduce the survival, growth, or reproduction of insects that feed on it. Antibiosis is often caused by the production of toxic chemicals by the plant. Antibiosis type plant resistance is often evaluated in no-choice studies where insect are supplied a single food source. PI 594403, PI 71506, Williams, PI594403, PI 594427C, CNS, Jackson, and Dowling were evaluated for antibiosis resistance in no choice experiments.

Antibiosis resistance was identified in PI 594427C in no choice conducted at University of Illinois. Plants were grown in isolated cages in a choice situation. Three aphid

nymphs were placed on each plant. The number of aphids was counted at 14, 17, and 21 days after inoculation (Table 2). Four replications were performed for each entry. Aphids were able to readily reproduce on CNS under antixenosis conditions (Table 2), but not under antibiosis conditions. The level of aphid infestation was higher and the aphid age range was broader under the antixenosis evaluation compared to aphid infestation and age range under the antibiosis evaluation. The difference in aphid infestation level and aphid maturity may account for the differences observed on CNS in the antibiosis and antixenosis evaluations. Furthermore, geographic aphid biotypes from Illinois and Michigan may account for differences in reaction on CNS.

Table 3: Average number of soybean aphid for aphid-resistant and aphid-susceptible soybean genotypes tested at University of Illinois.

	No. of Aphids per Plant at:		
Line	14 d	17 d	21 d[*]
Pana	190	405	1076a
PI 594403	142	497	603a
PI 71506	200	316	294ab
Williams	126	251	236ab
PI 594403	36	83	59bc
PI 594427C	20	17	12cd
CNS	21	22	11cd
CNS	17	24	10cd
Jackson	14	5	6d
PI 594427C	17	12	5d
Dowling	7	3	3d
Dowling	6	3	3d

*Means followed by different letters are significantly different at 0.05 level.

5

Example 3: Aphid mapping studies

In order to map putative QTL to aphid resistance, a resistant line (PI 594427C) was crossed with a CNS or MV0031. Two mapping populations were developed to map putative QTLs linked with aphid resistance: PI594427C (aphid resistant) x MV0031 and PI594427C (aphid resistant) x CNS. F₃: PI594427CxMV0031 and F₄: PI594427CxNS populations were evaluated for aphid resistance phenotype in enclosed cages in East Lansing, MI. Three aphid nymphs were placed per plant and aphid density was rated at 3, 4, 5 weeks after inoculation. The rating scale was 0 -5 (Table 1). Twenty-eight aphid resistant loci were identified (Table 6 and 7).

10

The phenotype data from week 4 evaluation was used from QTL mapping studies (Tables 4 and 5). At week 4, the aphid resistant parent, PI594427C, was rated 1.5 and the

15

aphid sensitive parents, MV0031 and CNS, were rated 3.5 In addition to the above-described phenotyping, each population was genotyped with SNP markers: 181 polymorphic SNPs with the F₃: PI594427C×MV0031 population and 164 polymorphic SNPs with the F₄:

PI594427C×CNS population. Single marker and marker regression analyses were performed to determine QTL regions conditioning aphid resistance. Tables 6 and 7 list significant associations between genomic regions and aphid resistance along with diagnostic markers.

Table 4: Phenotype of F₄: PI594427C×CNS at week 4 after inoculation.

Aphid Rating	Number of Plants
0.5	0
1	4
1.5	8
2	32
2.5	64
3	70
3.5	6
4	0
Total (n)	184

Table 5: Phenotype of F₃: PI594427CxMV0031 at week 4 after inoculation.

Aphid Rating	Number of Plants
0.5	0
1	2
1.5	5
2	29
2.5	29
3	88
3.5	30
4	0
Total (n)	183

Table 6: Results of markers associated with aphid resistance determined (ns = not significant)

Population	Aphid Resistance Loci	LG	Marker	Allele	t-value*	p-value**	R-sq. value***	Marker Interval
PI 594427C x CNS	1	J	NS0115450	T	3.36(P<0.05)	0.00118	8	38-58 cM
PI 594427C x MV0031	1	J	NS0122151	A	5.51(P<0.05)	<0.0001	14	28-48 cM
PI 594427C x MV0031	2	E	NS0126797	G	2.49(P<0.05)	0.04961	3	30-50 cM
PI 594427C x CNS	2	E	NS0126797	A	2.01(P<0.05)	ns	3	30-50 cM
PI 594427C x CNS	3	E	NS0098210	C	2.78(P<0.05)	0.00741	5	76-96 cM
PI 594427C x CNS	4	E	NS0099483	C	2.64(P<0.05)	0.01348	5	111-131 cM
PI 594427C x MV0031	5	B1	NS0100200	A	2.04(P<0.05)	ns	2	44-64 cM
PI 594427C x CNS	6	N	NS0137720	C	ns	0.02978	4	0-10 cM
PI 594427C x CNS	7	G	NS0118422	T	ns	0.02406	4	77-97 cM
PI 594427C x MV0031	8	N	NS0125467	T	2.03(P<0.05)	ns	3	26-46 cM
PI 594427C x CNS	8	N	NS0129030	C	ns	0.04662	4	15-35 cM
PI 594427C x CNS	9	N	NS0098575	T	2.03(P<0.05)	0.04894	3	97-117 cM
PI 594427C x MV0031	10	A1	NS0129617	C	2.06(P<0.05)	0.04753	4	0-15 cM
PI 594427C x MV0031	11	A1	NS0130304	A	2.2(P<0.05)	ns	3	33-53 cM
PI 594427C x CNS	12	D1a	NS0095317	I	2.51(P<0.05)	0.00628	6	12-32 cM
PI 594427C x CNS	13	C2	NS0115731	A	ns	0.02083	4	54-74 cM
PI 594427C x CNS	14	H	NS0120346	T	2.28(P<0.05)	ns	3	1-13 cM
PI 594427C x CNS	15	H	NS0097165	A	2.03(P<0.05)	ns	3	62-82 cM
PI 594427C x MV0031	16	D2	NS0092748	T	4.44(P<0.05)	0.00008	10	0-18 cM
PI 594427C x MV0031	16	D2	NS0096662		4.44	0.00008		0-18 cM
PI 594427C x CNS	16	D2	NS0118525	T	2.37(P<0.05)	ns	3	0-10 cM
PI 594427C x MV0031	17	F	NS0099503	T	3.21(P<0.05)	0.0049	6	0-18 cM
PI 594427C x CNS	18	F	NS0123719	A	2.97(P<0.05)	0.0062	6	62-82 cM
PI 594427C x MV0031	19	I	NS0130766	A	2.47(P<0.05)	0.02812	4	0-14 cM
PI 594427C x CNS	20	D1b	NS0121903	C	3.23(P<0.05)	0.00633	6	30-50 cM
PI 594427C x CNS	21	D1b	NS0098438	A	2.01(P<0.05)	ns	2	124-144 cM
PI 594427C x CNS	21	D1b	NS0114263	C	ns	0.04948	3	91-111 cM

Table 6: Results of markers associated with aphid resistance determined (ns = not significant) (continued)

Population	Aphid Resistance Loci	LG	Marker	Allele	t-value*	p-value**	R-sq. value***	Marker Interval
PI 594427C x MV0031	22	O	NS0124919	C	2.06(P<0.05)	ns	4	50-70 cM
PI 594427C x CNS	23	O	NS0124051	C	2.72(P<0.05)	0.00485	6	120-140 cM
PI 594427C x CNS	24	C1	NS0124300	G	2.64(P<0.05)	0.02273	4	0-15 cM
PI 594427C x MV0031	25	C1	NS0093331	C	2.04(P<0.05)	ns	3	28-48 cM
PI 594427C x MV0031	26	C1	NS0097882	T	2.02(P<0.05)	ns	3	113-133 cM
PI 594427C x MV0031	26	C1	NS0136956	G	ns	0.00561	6	72-92 cM
PI 594427C x CNS	27	K	NS0098803	T	ns	0.02604	4	0-20 cM
PI 594427C x MV0031	28	B2	NS0092589	G	2.21(P<0.05)	0.03209	5	7-27 cM
PI 594427C x CNS	28	B2	NS0103077	D	2.18(P<0.05)	0.0415	4	0-10 cM

* Marker analysis was performed using a t-test. P-value ≤ 0.05 is significant.

**Marker analysis was performed using marker regression in MapManager QTX;

***R-squared value from marker regression in MapManager QTX

Table 7: Listing of SNP markers for aphid resistance loci 1-28

Aphid Resistance Loci	Marker	Resistance Allele	SEQ ID	SEQ ID Forward Primer	SEQ ID Reverse Primer	SEQ ID VIC probe	SEQ ID FAM probe
1	NS0115450	T	81	1	2	121	122
1	NS0122151	A	82	3	4	123	124
1	NS0125096		83	5	6	125	126
1	NS0120948		84	7	8	127	128
2	NS0126797	G	85	9	10	129	130
3	NS0098210	C	86	11	12	131	132
4	NS0099483	C	87	13	14	133	134
5	NS0100200	A	88	15	16	135	136
6	NS0137720	C	89	17	18	137	138
7	NS0118422	T	90	19	20	139	140
8	NS0125467	T	91	21	22	141	142
8	NS0129030	C	92	23	24	143	144
9	NS0098575	T	93	25	26	145	146
10	NS0129617	C	94	27	28	147	148
11	NS0130304	A	95	29	30	149	150
12	NS0095317	I	96	31	32	151	152
13	NS0115731	A	97	33	34	153	154
14	NS0120346	T	98	35	36	155	156
15	NS0097165	A	99	37	38	157	158
16	NS0092748	T	100	39	40	159	160
16	NS0096662		101	41	42	161	162
16	NS0118525	T	102	43	44	163	164
17	NS0099503	T	103	45	46	165	166
18	NS0123719	A	104	47	48	167	168
19	NS0130766	A	105	49	50	169	170
20	NS0121903	C	106	51	52	171	172
21	NS0098438	A	107	53	54	173	174
21	NS0114263	C	108	55	56	175	176
22	NS0124919	C	109	57	58	177	178
23	NS0124051	C	110	59	60	179	180
23	NS0118907		111	61	62	181	182
24	NS0124300	G	112	63	64	183	184

Table 7: Listing of SNP markers for aphid resistance loci 1-28 (continued)

Aphid Resistance Loci	Marker	Resistance Allele	SEQ ID	SEQ ID Forward Primer	SEQ ID Reverse Primer	SEQ ID VIC probe	SEQ ID FAM probe
25	NS0093331	C	113	65	66	185	186
26	NS0097882	T	114	67	68	187	188
27	NS0098803	T	116	71	72	191	192
28	NS0092589	G	117	73	74	193	194
28	NS0103077	D	118	75	76	195	196
28	NS0100457		119	77	78	197	198
28	NS0116259		120	79	80	199	200

SNP markers determined to be associated with region 1 are SEQ ID NO: 81 through SEQ ID NO: 84. SNP markers for region 1 are mapped to a region on linkage group J. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 1 through SEQ ID NO: 8 and probes indicated as SEQ ID NO: 121 through SEQ ID NO: 128.

5 A SNP marker determined to be associated with region 2 is SEQ ID NO: 85. A SNP marker for region 2 is mapped to a region on linkage group E. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 9 through SEQ ID NO: 10 and probes indicated as SEQ ID NO: 129 through SEQ ID NO: 130.

10 A SNP marker determined to be associated with region 3 is SEQ ID NO: 86. A SNP marker for region 3 is mapped to a region on linkage group E. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 11 through SEQ ID NO: 12 and probes indicated as SEQ ID NO: 131 through SEQ ID NO: 132.

15 A SNP marker determined to be associated with region 4 is SEQ ID NO: 87. A SNP marker for region 4 is mapped to a region on linkage group E. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 13 through SEQ ID NO: 14 and probes indicated as SEQ ID NO: 133 through SEQ ID NO: 134.

20 A SNP marker determined to be associated with region 5 is SEQ ID NO: 88. A SNP marker for region 5 is mapped to a region on linkage group B1. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 15 through SEQ ID NO: 16 and probes indicated as SEQ ID NO: 135 through SEQ ID NO: 136.

 A SNP marker determined to be associated with region 6 is SEQ ID NO: 89. A SNP marker for region 6 is mapped to a region on linkage group N. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 17 through SEQ ID NO: 18 and probes indicated as SEQ ID NO: 137 through SEQ ID NO: 138.

25 A SNP marker determined to be associated with region 7 is SEQ ID NO: 90. A SNP marker for region 7 is mapped to a region on linkage group G. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 19 through SEQ ID NO: 20 and probes indicated as SEQ ID NO: 139 through SEQ ID NO: 140.

SNP markers determined to be associated with region 8 are SEQ ID NO: 91 through SEQ ID NO: 92. SNP markers for region 8 are mapped to a region on linkage group N. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 21 through SEQ ID NO: 24 and probes indicated as SEQ ID NO: 141 through SEQ ID NO: 144.

5 A SNP marker determined to be associated with region 9 is SEQ ID NO: 93. A SNP marker for region 9 is mapped to a region on linkage group N. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 25 through SEQ ID NO: 26 and probes indicated as SEQ ID NO: 145 through SEQ ID NO: 146.

10 A SNP marker determined to be associated with region 10 is SEQ ID NO: 94. A SNP marker for region 10 is mapped to a region on linkage group A1. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 27 through SEQ ID NO: 28 and probes indicated as SEQ ID NO: 147 through SEQ ID NO: 148.

15 A SNP marker determined to be associated with region 11 is SEQ ID NO: 95. A SNP marker for region 11 is mapped to a region on linkage group A1. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 29 through SEQ ID NO: 30 and probes indicated as SEQ ID NO: 149 through SEQ ID NO: 150.

20 A SNP marker determined to be associated with region 12 is SEQ ID NO: 96. A SNP marker for region 12 is mapped to a region on linkage group D1a. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 31 through SEQ ID NO: 32 and probes indicated as SEQ ID NO: 151 through SEQ ID NO: 152.

 A SNP marker determined to be associated with region 13 is SEQ ID NO: 97. A SNP marker for region 13 is mapped to a region on linkage group C2. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 33 through SEQ ID NO: 34 and probes indicated as SEQ ID NO: 153 through SEQ ID NO: 154.

25 A SNP marker determined to be associated with region 14 is SEQ ID NO: 98. A SNP marker for region 14 is mapped to a region on linkage group H. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 35 through SEQ ID NO: 36 and probes indicated as SEQ ID NO: 155 through SEQ ID NO: 156.

A SNP marker determined to be associated with region 15 is SEQ ID NO: 99. A SNP marker for region 15 is mapped to a region on linkage group H. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 37 through SEQ ID NO: 38 and probes indicated as SEQ ID NO: 157 through SEQ ID NO: 158.

5 SNP markers determined to be associated with region 16 are SEQ ID NO: 100 through SEQ ID NO: 102. SNP markers for region 16 are mapped to a region on linkage group D2. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 39 through SEQ ID NO: 42 and probes indicated as SEQ ID NO: 159 through SEQ ID NO: 164.

10 A SNP marker determined to be associated with region 17 is SEQ ID NO: 103. A SNP marker for region 17 is mapped to a region on linkage group F. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 45 through SEQ ID NO: 46 and probes indicated as SEQ ID NO: 165 through SEQ ID NO: 166.

15 A SNP marker determined to be associated with region 18 is SEQ ID NO: 104. A SNP marker for region 18 is mapped to a region on linkage group F. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 47 through SEQ ID NO: 48 and probes indicated as SEQ ID NO: 167 through SEQ ID NO: 168.

20 A SNP marker determined to be associated with region 19 is SEQ ID NO: 105. A SNP marker for region 19 is mapped to a region on linkage group I. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 49 through SEQ ID NO: 50 and probes indicated as SEQ ID NO: 169 through SEQ ID NO: 170.

A SNP marker determined to be associated with region 20 is SEQ ID NO: 106. A SNP marker for region 20 is mapped to a region on linkage group D1b. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 51 through SEQ ID NO: 52 and probes indicated as SEQ ID NO: 171 through SEQ ID NO: 172.

25 SNP markers determined to be associated with region 21 are SEQ ID NO: 107 through SEQ ID NO: 108. SNP markers for region 21 are mapped to a region on linkage group D1b. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO:

53 through SEQ ID NO: 56 and probes indicated as SEQ ID NO: 173 through SEQ ID NO: 176.

A SNP marker determined to be associated with region 22 is SEQ ID NO: 109. A SNP marker for region 22 is mapped to a region on linkage group O. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 57 through SEQ ID NO: 58 and probes indicated as SEQ ID NO: 177 through SEQ ID NO: 178.

SNP markers determined to be associated with region 23 are SEQ ID NO: 110 SEQ ID NO: 111. A SNP marker for region 23 is mapped to a region on linkage group O. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 59 through SEQ ID NO: 62 and probes indicated as SEQ ID NO: 179 through SEQ ID NO: 180.

A SNP marker determined to be associated with region 23 is SEQ ID NO: 111. A SNP marker for region 23 is mapped to a region on linkage group C1. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 61 through SEQ ID NO: 62 and probes indicated as SEQ ID NO: 181 through SEQ ID NO: 182.

A SNP marker determined to be associated with region 24 is SEQ ID NO: 112. A SNP marker for region 24 is mapped to a region on linkage group C1. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 63 through SEQ ID NO: 64 and probes indicated as SEQ ID NO: 183 through SEQ ID NO: 184.

A SNP marker determined to be associated with region 25 is SEQ ID NO: 113. A SNP marker for region 25 is mapped to a region on linkage group C1. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 65 through SEQ ID NO: 66 and probes indicated as SEQ ID NO: 185 through SEQ ID NO: 186.

SNP markers determined to be associated with region 26 are SEQ ID NO: 114 through SEQ ID NO: 116. SNP markers for region 26 are mapped to a region on linkage group C1. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 67 through SEQ ID NO: 70 and probes indicated as SEQ ID NO: 187 through SEQ ID NO: 190.

A SNP marker determined to be associated with region 27 is SEQ ID NO: 116. A SNP marker for region 27 is mapped to a region on linkage group K. Table 7 lists sequences

for PCR amplification primers, indicated as SEQ ID NO: 71 through SEQ ID NO: 72 and probes indicated as SEQ ID NO: 191 through SEQ ID NO: 192.

A SNP marker determined to be associated with region 28 is SEQ ID NO: 117. SNP markers for region 28 are mapped to a region on linkage group B2. Table 7 lists sequences for PCR amplification primers, indicated as SEQ ID NO: 73 through SEQ ID NO: 80 and probes indicated as SEQ ID NO: 193 through SEQ ID NO: 200.

Example 4. Oligonucleotide hybridization probes useful for detecting soybean plants with aphid resistance loci

Oligonucleotides can also be used to detect or type the polymorphisms associated with aphid resistance disclosed herein by hybridization-based SNP detection methods. Oligonucleotides capable of hybridizing to isolated nucleic acid sequences which include the polymorphism are provided. It is within the skill of the art to design assays with experimentally determined stringency to discriminate between the allelic states of the polymorphisms presented herein. Exemplary assays include Southern blots, Northern blots, microarrays, *in situ* hybridization, and other methods of polymorphism detection based on hybridization. Exemplary oligonucleotides for use in hybridization-based SNP detection are provided in Table 8. These oligonucleotides can be detectably labeled with radioactive labels, fluorophores, or other chemiluminescent means to facilitate detection of hybridization to samples of genomic or amplified nucleic acids derived from one or more soybean plants using methods known in the art.

Table 8. Oligonucleotide Hybridization Probes

Marker	Marker SEQ ID	SNP Position	Hybridization Probe	SEQ ID
NS0122151	82	62	CCTTGCAAGTCATGCT	201
NS0122151	82	62	CCTTGCAATGTCATGCT	202
NS0125096	83	139	AAGTTTATGATTTGAA	203
NS0125096	83	139	AAGTTTAAGATTTGAA	204
NS0120948	84	109	ATTCTTCAGCATGATC	205
NS0120948	84	109	ATTCTTCTGCATGATC	206
NS0092748	100	289	TACCTCTAAACTTGT	207
NS0092748	100	289	TACCTCTTAAACTTGT	208
NS0118907	111	449	CTCCAACCTATGATTG	209
NS0118907	111	449	CTCCAACATATGATTG	210
NS0092589	117	126	AGCCATCACAAGGAAA	211
NS0092589	117	126	AGCCATCATAAGGAAA	212
NS0100457	119	34	TTGGTCCTGCCGGTAA	213
NS0100457	119	34	TTGGTCCC GCCGGTAA	214
NS0116259	120	216	TGATAATGACTCCTGA	215
NS0116259	120	216	TGATAATAACTCCTGA	216

Example 5. Oligonucleotide probes useful for detecting soybean plants with aphid resistance loci by single base extension methods

Oligonucleotides can also be used to detect or type the polymorphisms associated with Soybean aphid resistance disclosed herein by single base extension (SBE)-based SNP detection methods. Exemplary oligonucleotides for use in SBE-based SNP detection are provided in Table 9. SBE methods are based on extension of a nucleotide primer that is hybridized to sequences adjacent to a polymorphism to incorporate a detectable nucleotide residue upon extension of the primer. It is also anticipated that the SBE method can use three synthetic oligonucleotides. Two of the oligonucleotides serve as PCR primers and are complementary to the sequence of the locus which flanks a region containing the polymorphism to be assayed. Exemplary PCR primers that can be used to type polymorphisms disclosed in this invention are provided in Table 7 in the columns labeled “Forward Primer SEQ ID” and “Reverse Primer SEQ ID”. Following amplification of the region containing the polymorphism, the PCR product is hybridized with an extension primer which anneals to the amplified DNA adjacent to the polymorphism. DNA polymerase and

two differentially labeled dideoxynucleoside triphosphates are then provided. If the polymorphism is present on the template, one of the labeled dideoxynucleoside triphosphates can be added to the primer in a single base chain extension. The allele present is then inferred by determining which of the two differential labels was added to the extension primer. Homozygous samples will result in only one of the two labeled bases being incorporated and thus only one of the two labels will be detected. Heterozygous samples have both alleles present, and will thus direct incorporation of both labels (into different molecules of the extension primer) and thus both labels will be detected.

Table 9. Probes (extension primers) for Single Base Extension (SBE) assays.

Marker	Marker SEQ ID	SNP Position	Probe (SBE)	SEQ ID
NS0122151	82	62	AGTAGTTACTCCCTTGC	217
NS0125096	83	139	TTCCAAAACCTCAAGTTT	218
NS0120948	84	109	GTCTGATTAATATTCTT	219
NS0092748	100	289	GCATTCCTCAATACCTC	220
NS0118907	111	449	AAAGAGAAAAGCTCCAA	221
NS0092589	117	126	GGCCAATAAAAAGCCAT	222
NS0100457	119	34	GGGAGCTAGATTTGGTC	223
NS0116259	120	216	TTTAATCAACATGATAA	224

5

Example 6: Confirmation of Selected Aphid Resistance Alleles

Forty soybean breeding lines were screened for antibiosis in a no-choices field study. Ten plants of each breeding line were planted in a plot. Individual plots were covered by a small insect cage. The cages were inoculated soybean aphids when the soybean plants reached the V1 stage. The plants were rated weekly as described in Table 1. The plants were assigned a damage index (DI) each week. Table 8 listed the average DI rating for soybean lines with various aphid resistance alleles.

In addition, the forty lines were screened for antibiosis in a no-choice greenhouse study. Several plants of each of the forty soybean breeding lines were cultivated in a greenhouse. Leaves were excised from each plant and inoculated with 2 soybean aphids in

15

closed container. The number of aphids on each leaf was counted after seven days. Table 10 listed the average number of aphids on soybean lines with various aphid resistance alleles.

Table 10: Confirmation of aphid resistance alleles 1, 16, 23, and 28. Soybean lines were screened for antibiosis to soybean aphid in no-choice greenhouse and field experiments.

Aphid Resistance Locus	Homozygous for Resistance Allele			Homozygous for Susceptible Allele		
	No. Plants	Greenhouse No. aphids	Field Damage Index	No. Plants	Greenhouse No. aphids	Field Damage Index
1	21	35	36	18	49	88
16	14	38	90	26	45	95
1+16	9	33	32	13	49	88
23	12	45	94	28	48	93
1+23	9	31	34	15	50	92
1+28	6	28.1	35.9	11	45	95
1+16+23	6	30	41	10	48	93
1+23+28	4	29	43	10	41	92
1+16+28	4	29	43	6	47	93
1+16+23+28	3	26	47	3	46	94
Check Varieties	No. Plants	Greenhouse No. aphids	Field Damage Index			
CNS	25	28.2	100.0			
PI594427C	19	20.8	25.0			
Jackson	25	32.4	21.7			
Dowling	9	20.8	-			
Williams	16	59.4375	-			

Aphid resistance allele 1 conferred aphids resistance in both the no-choice field and greenhouse tests. In addition, aphid resistance locus 16 conferred resistance in no-choice field tests. Moreover, aphid resistance allele 23 enhanced the aphid resistance conferred by aphid resistance allele 1. Similarly, aphid resistance allele 28 enhanced the aphid resistance conferred by aphid resistance allele 1. Soybean plant with three aphid resistance alleles had a higher level of aphid resistance compared soybean plants with one or two resistance alleles. Furthermore, soybean plants with four aphid resistance alleles possessed the highest level of aphids resistance.

Host plant resistance management methods may fall into three categories : (1) deploying single resistance alleles sequentially, (2) deploying multiple varieties with difference single resistance alleles through a seed mixtures or crop rotation, (3) stacking or combining different resistance allele into a single soybean line. Soybean plants may be bred for aphid resistance alleles 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20,

21, 22, 23, 24, 25, 26, 27, 28 singly or in combination depending on the aphid pressure of the geographic region and host plant resistance management plan.

Having illustrated and described the principles of the present invention, it should be apparent to persons skilled in the art that the invention can be modified in arrangement and
5 detail without departing from such principles. We claim all modifications that are within the spirit, scope and concept of the appended claims.

WE CLAIM:

1. A method of introgressing an allele into a soybean plant comprising:
 - A) Providing a population of soybean plants,
 - B) Genotyping at least one soybean plant in the population with respect to a soybean genomic nucleic acid marker selected from the group comprising SEQ ID NO: 81 through SEQ ID NO: 120,
 - C) Selecting from the population at least one soybean plant comprising at least one allele associated with aphid resistance.
2. The method according to Claim 1, wherein said selected soybean plants exhibit at least partial resistance to aphids.
3. The method according to Claim 1, wherein said selected soybean plants exhibit at least substantial resistance to aphids.
4. The method of claim 1, wherein the genotype is determined by an assay which is selected from the group consisting of single base extension (SBE), allele-specific primer extension sequencing (ASPE), DNA sequencing, RNA sequencing, micro-array based analyses, universal PCR, allele specific extension, hybridization, mass spectrometry, ligation, extension-ligation, and Flap-Endonuclease-mediated assays.
5. The method of claim 1 wherein the soybean genomic nucleic acid marker selected from the group comprising SEQ ID NO: 81-82.
6. An elite soybean plant obtained by the process of claim 1.
7. A method of introgressing an allele into a soybean plant comprising:

A) crossing at least one aphid resistant soybean plant with at least one aphid sensitive soybean plant in order to form a segregating population,

B) screening said segregating population with one or more nucleic acid markers to

determine if one or more soybean plants from said segregating population contains a aphid resistant allele, wherein said aphid resistant allele is an allele selected from the group consisting of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20,

21, 22, 23, 24, 25, 26, 27, and 28 aphid resistant loci where one or more alleles at one or more of their loci are selected from the group consisting of aphid resistance allele

1, aphid resistance allele 2, aphid resistance allele 3, aphid resistance allele 4, aphid resistance allele 5, aphid resistance allele 6, aphid resistance allele 7, aphid resistance allele 8, aphid resistance allele 9, aphid resistance allele 10, aphid resistance allele 11, aphid resistance allele 12, aphid resistance allele 13, aphid resistance allele 14, aphid resistance allele 15, aphid resistance allele 16, aphid resistance allele 17, aphid

resistance allele 18, aphid resistance allele 19, aphid resistance allele 20, aphid resistance allele 21, aphid resistance allele 22, aphid resistance allele 23, aphid resistance allele 24, aphid resistance allele 25, aphid resistance allele 26, aphid resistance allele 27, aphid resistance allele 28, aphid resistance allele 29, aphid resistance allele 30, aphid resistance allele 31, aphid resistance allele 32, aphid

resistance allele 33, aphid resistance allele 34, aphid resistance allele 35, and aphid resistance allele 36.

8. A method according to Claim 7, wherein at least one of said one or more markers is located within 30 cM of said resistant allele.

9. A method according to Claim 7, wherein at least one of said one or more markers is located within 20 cM of said resistant allele.

10. A method according to Claim 7, wherein at least one of said one or more markers is located within 2 cM of said resistant allele.

11. A method according to Claim 7, wherein at least one of said one or more markers is located within 1 cM of said resistant allele.
12. An elite soybean plant obtained by the method of Claim 9.
13. The elite soybean plant according to Claim 12, wherein said elite soybean plant exhibits a transgenic trait.
14. The elite soybean plant according to Claim 12, wherein said transgenic trait is selected from the group consisting of herbicide tolerance, pest resistance, oil composition, and others.
15. The elite soybean plant according to Claim 12, wherein said herbicide tolerance is selected from the group consisting of glyphosate, dicamba, glufosinate, sulfonyleurea, bromoxynil and norflurazon herbicides.
16. The elite soybean plant according to Claim 12, wherein said nucleic acid sequence is present as a single copy in said elite soybean plant.
17. The elite soybean plant according to Claim 12, wherein said nucleic acid sequence is present in two copies in said elite soybean plant.
18. The elite soybean plant of Claim 12, wherein said elite soybean plant exhibits at least partial resistance to aphid.
19. The elite soybean plant of Claim 16, wherein said elite soybean plant exhibits at least substantial resistance to aphids.

20. A substantially purified nucleic acid molecule for the detection of loci related to aphid resistance comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 175 and complements thereof.
21. A vector comprising the nucleic acid fragment of claim 20.
22. An expression product of the vector of claim 21 expressed in a host compatible with said vector.
23. An antibody to the expression product of claim 22.
24. A method of identifying an aphid resistance allele in a soybean plant comprising detecting a locus associated with a SNP marker selected from the group consisting of SEQ ID NO: 81 through SEQ ID NO: 120.
25. A set of oligonucleotides comprising:
- A) a pair of oligonucleotide primers wherein each of said primers comprises at least 12 contiguous nucleotides and wherein said pair of primers permit PCR amplification of a DNA segment comprising a soybean genomic DNA polymorphism associated with aphid resistance loci identified in Table 6 and 7; and
 - B) at least one detector oligonucleotide that permits detection of a polymorphism in said amplified segment, wherein the sequence of said detector oligonucleotide is at least 95 percent identical to a sequence of the same number of consecutive nucleotides in either strand of a segment of maize DNA that include or are immediately adjacent to said polymorphism of step (a).