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[Continued on next page]

(54) Title: SOYBEAN MARKERS LINKED TO SCN RESISTANCE

ID	Product code	Size category	Mass (milligrammes)	Material type	Color	Texture	Permeability (%)	LOC	Product	Author	Comments
A1	polyester	3	4407	NYLON	yellowish (C4-1040)	Translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4407-1040-1040
A2	nylon	2	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A3	nylon	not available	4000-4500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4000-4500-1040
A4	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A5	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A6	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A7	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A8	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A9	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A10	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A11	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A12	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A13	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A14	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A15	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A16	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A17	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A18	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A19	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A20	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A21	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A22	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A23	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A24	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A25	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A26	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A27	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A28	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A29	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040
A30	nylon	3	4500-5500	nylon 6,6	yellowish (C4-1040)	translucent	1.3	non-absorbent	0.002	Wang et al., 1995	Mass: 4500-5500-1040

FIG. 1a

(57) Abstract: This disclosure concerns compositions and methods for identifying the SCN resistant phenotype in soybean. In some embodiments, the disclosure concerns methods for performing marker assisted breeding and selection of plants carrying one or more determinants of SCN resistance in soybean.

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— *with sequence listing part of description (Rule 5.2(a))*

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SOYBEAN MARKERS LINKED TO SCN RESISTANCE**PRIORITY CLAIM**

This application claims the benefit of U.S. Provisional Patent Application
5 Serial No. 61/410,783, filed November 5, 2010.

TECHNICAL FIELD

The present disclosure relates to plant disease resistance. In some
embodiments, the disclosure relates to Soybean cyst nematode (SCN) resistance in
10 soybean. In particular embodiments, the disclosure relates to compositions and
methods for identifying an SCN resistance trait in an organism, for example, molecular
markers that are tightly linked to SCN resistance. Further embodiments relate to
compositions and methods for introducing an SCN resistance trait into a host organism,
for example, by using molecular markers tightly linked to SCN resistance.

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BACKGROUND

The soybean, *Glycine max*, is one of the major economic crops grown
worldwide as a primary source of vegetable oil and protein. Growing demand for low
cholesterol and high fiber diets has increased soybean's importance as a food. Over
20 10,000 soybean varieties have now been introduced into the United States, of which a
limited number form the genetic base of cultivars developed from hybridization and
selection programs. Johnson and Bernard, *The Soybean*, Norman Ed., Academic Press,
N.Y., pp. 1-73, 1963.

Soybean cyst nematode, (SCN, *Heterodera glycines* (HG) Ichinohe) is the
25 single most damaging pest affecting soybean in the U.S. as well as in most of the other
top soybean-producing countries of the world. The estimated yield reduction in the
United States was between approximately 2.9 and 3.4 million tons in 2003 and 2004,
which resulted in an estimated annual loss of approximately \$1.5 billion. Wrather *et al.*
(2001); Wrather and Koenning (2006). The SCN phenotype is a very complex trait,
30 which is controlled by multiple genes, both recessive and dominant. Concibido *et al.*
(2004). SCN phenotyping is time consuming, cost and labor intensive.

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SCN infection causes various symptoms that may include chlorosis of the leaves and stems, root necrosis, loss in seed yield, and suppression of root and shoot growth. The aboveground symptoms of SCN infection are not unique to SCN infection, and could be confused with nutrient deficiency, particularly iron deficiency, stress from drought, herbicide injury or another disease. The first signs of infection are groups of plants with yellowing leaves that have stunted growth. The pathogen may also be difficult to detect on the roots, since stunted roots are also a common symptom of stress or plant disease. Adult females and cysts of SCN are about 1/32 inch long and, thus, visible without magnification. Observation of adult females and cysts on the roots is the only accurate way to detect and diagnose SCN infection in the field.

The presence of SCN is usually not obvious at the time of initial soil infestation. The SCN population density must increase in the soil until it is sufficient to cause above-ground symptoms on plants or a decrease in yield. Population densities may take several years to reach significant numbers. Thus, current SCN damage is the result of infestations that have been growing for several years. Although soybean is the primary host of SCN, other legumes can serve as hosts, for example: green beans, snap beans, dry beans, red beans, lima beans, mung beans, bush beans, Adzuki beans, garden peas, and cowpeas. There are thirty days in the SCN life cycle. Thus, a single growing season encompasses multiple generations of the parasite. Moreover, SCN eggs may remain intact in soil for several years before hatching.

In the past, an SCN population was given a "race" designation by comparing its reproduction on a set of four soybean germplasm lines with that on a standard SCN-susceptible soybean cultivar. The most commonly used race scheme identified 16 races of SCN. The race designation allowed nematologists and soybean breeders to share information about the ability of certain SCN populations to reproduce on soybean varieties that contain certain genes for resistance to SCN.

In 2003, the HG Type Test was developed to replace the race test. This new test includes seven sources of resistance (germplasm lines) and the results are shown as a percentage, indicating how much the nematode population from a soil sample increased on each of the seven lines. This test indicates which sources of resistance would be good for a particular field being tested, and which would be poor. Since the genetic sources of resistance are currently limited in commercially available soybean

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varieties, it is important to rotate these “sources of resistance” to delay the build-up of a virulent SCN population.

Shortly after the discovery of SCN in the United States, sources of SCN resistance were identified. Ross and Brim (1957) *Plant Dis. Rep.* 41:923-4. Some lines, such as Peking and PI 88788, were quickly incorporated into breeding programs. Peking became widely used as a source of resistance due to its lack of agronomically undesirable traits, with Pickett as the first SCN resistant cultivar released. The recognition that certain SCN resistant populations could overcome resistant cultivars led to an extensive screen for additional sources of SCN resistance. PI 88788 emerged as a popular source of race 3 and 4 resistance, even though it had a cyst index greater than 10% (but less than 20%) against race 4, and Peking and its derivatives emerged as a popular source for races 1 and 3. PI 437654 was subsequently identified as having resistance to all known races and its SCN resistance was backcrossed into Forrest. Currently, there are more than 130 PIs known to have SCN resistance. PI 209332 and PI 90763 are other exemplary SCN resistant soybean breeding lines. Not all varieties with the same source of resistance have comparable yields, nor do they respond identically to SCN.

Resistant soybean varieties are the most effective tool available for management of SCN. SCN densities usually decrease when resistant soybeans are grown because most SCN juveniles are unable to feed and develop on the roots of the resistant varieties. However, in any naturally infested field, a few SCN juveniles (< 1%) will be able to reproduce on the resistant varieties currently available. The number of SCN juveniles that can reproduce on resistant soybean varieties can increase when resistant varieties are grown repeatedly. Eventually, the SCN population may be able to reproduce as well on a resistant variety as a susceptible variety if SCN-resistant soybeans are grown every time soybeans are produced in an infested field. Fortunately, the number of SCN juveniles that can reproduce on resistant varieties declines when susceptible soybean varieties are grown because these nematodes do not compete well for food with the other SCN juveniles in the soil that cannot feed on the resistant varieties.

SCN race 3 is considered to be the most prominent race in the Midwestern soybean producing states. Considerable effort has been devoted to the genetics and

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breeding for resistance to race 3. While both Peking and PI 88788 are resistant to SCN race 3, classical genetics studies suggest that they harbor different genes for race 3 resistance. Rao-Arelli and Anand (1988) *Crop Sci.* 28:650-2. Race 3 resistance is probably under the control of three or four different genes. *Id.*; see also Mansur *et al.* (1993) *Crop Sci.* 33:1249-53. One major SCN resistance QTL that maps to linkage group G is rhg1. Concibido *et al.* (1996) *Theor. Appl. Genet.* 93:234-41. Other SCN resistance QTLs map to linkage groups A2, C1, M, D, J, L25, L26, and K. *Id.*; U.S. Patent 5,491,081. SCN resistance QTLs behave in a race-specific manner, at least by accounting for different proportions of the total phenotypic variation with respect to different SCN races. Concibido *et al.* (1997) *Crop Sci.* 37:258-64. However, the rhg1 locus on linkage group G may be necessary for the development of resistance to any of the identified SCN races. *But see* Qui *et al.* (1999) *Theor. Appl. Genet.* 98:356-64.

Markers that are linked to SCN traits include RFLPs, SSRs and SNPs. The SNP markers identified in this disclosure can be used to do SCN genotyping to support a breeding program. Using the presently disclosed SNP markers to perform SCN genotyping in support of a breeding program provides: cost and time savings, early selection of desired progeny, and more accurate and rapid commercialization of SCN resistant soybean varieties.

20

DISCLOSURE

Molecular markers that are linked to an SCN phenotype may be used to facilitate marker-assisted selection for the SCN resistance trait in soybean. Marker-assisted selection provides significant advantages with respect to time, cost, and labor, when compared to SCN phenotyping. Surprisingly, it is disclosed herein that among 15 SNP markers identified to be within or near SCN disease resistance QTL regions in the soybean genome that were polymorphic in parent genotypes, only three were linked to the SCN resistance trait. These three SNP markers, then, offer superior utility in marker-assisted selection of SCN resistant soybean varieties.

Described herein are nucleic acid molecular markers that are linked to (*e.g.*, linked, tightly linked, or extremely tightly linked) an SCN resistance phenotype. In particular examples, the molecular markers may be SNP markers. Also described herein are methods of using nucleic acid molecular markers that are linked to an SCN

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resistance phenotype, for example and without limitation, to identify plants with an SCN resistance phenotype, to introduce an SCN resistance phenotype into new plant genotypes (*e.g.*, through marker-assisted breeding or genetic transformation), and to cultivate plants that are likely to have an SCN resistance phenotype.

5 Further described are means for introducing an SCN phenotype to soybean and means for identifying plants having an SCN phenotype. In some examples, a means for introducing an SCN phenotype to soybean may be a marker that is linked (*e.g.*, linked, tightly linked, or extremely tightly linked) to an SCN phenotype. In some examples, a means for identifying plants having an SCN phenotype may be a probe
10 that specifically hybridizes to a marker that is linked (*e.g.*, linked, tightly linked, or extremely tightly linked) to an SCN phenotype.

Also described herein are plants and plant materials that are derived from plants having an SCN phenotype as identified using molecular markers described herein. Thus, soybean plants that are produced by marker-assisted selection using one or more
15 molecular marker(s) that are linked to an SCN resistance phenotype are described.

BRIEF DESCRIPTION OF THE FIGURES

FIGS. 1a and 1b include a list of QTLs associated with SCN resistance that have been reported in the SCN literature.

20 FIG. 2 includes a representation of the soybean genome, including chromosomes and linkage groups (LGs).

FIG. 3 includes a representation of soybean chromosome 18 (linkage group G), and QTLs and QTL intervals associated with SCN resistance and SNPs located therein.

25 FIG. 4 includes a representation of soybean chromosome 8 (linkage group A₂), and QTLs and QTL intervals associated with SCN resistance and SNPs located therein.

FIG. 5 includes a representation of soybean chromosome 11 (linkage group B₁), and QTLs and QTL intervals associated with SCN resistance and SNPs located therein.

30 FIG. 6 includes a representation of soybean chromosome 20 (linkage group I), and QTLs and QTL intervals associated with SCN resistance and SNPs located therein.

FIG. 7 includes clusters of 24 soybean SCN related cultivars or parental lines on four SNP loci. Also included is a table showing the 24 soybean cultivars and SCN

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mapping parents used. In the table, the first row of samples and the last two samples in the second row were SCN susceptible (green), and the first ten samples in the second row were SCN resistant (yellow). The last three samples in the second row were parental lines of two SCN mapping populations.

5 FIG. 8 includes clusters of 96 lines on three SNPs loci that showed co-segregation with the SCN resistance trait.

FIG. 9 includes the distribution of the SCN indexes assigned to mapping populations. The histogram shows a range from 0.01 to 3.8, with a mean of 0.63, and a median of 0.465.

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SEQUENCE LISTING

The nucleic acid sequences listed in the accompanying sequence listing are shown using standard letter abbreviations for nucleotide bases, as defined in 37 C.F.R. § 1.822. Only one strand of each nucleic acid sequence is shown, but the
15 complementary strand is understood to be included by any reference to the displayed strand. In the accompanying sequence listing:

SEQ ID NO:1 shows a primer sequence used in a KBiosciences Competitive Allele-Specific PCR SNP genotyping system (KASPar™) assay that is specific for the rhg1-3995 allele: GAAGGTGACCAAGTTCATGCTGGAATTATGTTGGGTTTT
20 TTTTCTTTCTGT.

SEQ ID NO:2 shows a second primer sequence used in a KASPar™ assay that is specific for the rhg1-3995 allele: GAAGGTCGGAGTCAAC
GGATTGAATTATGTTGGGTTTTTTTCTTTCTGG.

SEQ ID NO:3 shows a common reverse primer sequence used in a KASPar™
25 assay for rhg1-3995: GCCCAGAAAAAAGGGATAAATAACGGATA.

SEQ ID NO:4 shows a primer sequence used in a KASPar™ assay that is specific for the NCSB_004074 allele: GAAGGTGACCAAGTT
CATGCTATTATGTTGTAACACAA ATTTGCACCTCAT.

SEQ ID NO:5 shows a second primer sequence used in a KASPar™ assay that
30 is specific for the NCSB_004074 allele: GAAGGTCGGAGTCA
ACGGATTATGTTGTAACACAAATTTGCACCTCAG.

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SEQ ID NO:6 shows a common reverse primer sequence used in a KASPar™ assay for NCSB_004074: CTATACTAAATCGTAATTCCATTGTAT.

SEQ ID NO:7 shows a primer sequence used in a KASPar™ assay that is specific for the BARC_010889-01691 allele: GAAGGTGACCAA
5 GTTCATGCTGAAAAAATAAAATTGATCATCACATATGGTTAG.

SEQ ID NO:8 shows a second primer sequence used in a KASPar™ assay that is specific for the BARC_010889-01691 allele: GAAGGTGCG
GAGTCAACGGATTGAAAAAATAAAATTGATCATCACATATGGTTAA.

SEQ ID NO:9 shows a common reverse primer sequence used in a KASPar™
10 assay for BARC_010889-01691: TAAGTGAGGGCAATGTATTAGTATYAAGTA.

SEQ ID NO:10 shows a marker NCSB_004074 sequence:
CACGATTTTGTGTGTACATAAATTACTATACTAAATCGTAATTCCA
TTGTATTAC[A/C]TGAGGTGCAAATTTGTGTTACAACATAATTGTAATTTTA
TTGTACGATAAAAACTATAAC.

15 SEQ ID NO:11 shows a marker BARC_010889-01691 sequence:
CTCTTCACACCTTTAAGGAAGTTAGTACCATTCCACTATTCAAGTATTTTTT
TTAATTCAAAATTATTAAGTGAGGGCAATGTATTAGTATNAAGTA[C/T]TA
ACCATATGTGATGATCAATTTTATTTTTTTCATGGCTTTGTCGAAAGTAACA
TTATATTGTGGTTTTAAATGAAAATCTGTGATTTGCAT.

20 SEQ ID NO:12 shows a marker rhg1-3995 sequence:
TCTGATAACTATGACAGCATCTTCCAAGATAATGACTTCCAAGTTCCAACA
CTGGCTCTGTACATTTGAACTAATTTTATATCATTTATCTATTGTGATTGAA
ATATAAAATTGAAGTGATGTGAACAATACAAATCACATCTTGAATTAAAA
TATCTAACAACCTGGAACAAATAAGAGGCCAGAAAAAAGGGATAAATAA
25 CGGATAACAAG[A/C]CAGAAAGAAAAAAAAACCCAACATAATTCCAACCTC
AAAATTCACCTCAATAAAAAAGTTTAACATGTAAATTTACTTGGAACAAAA
CTCATAACCAATAATAATAATAAAAGAAATCAGTTTTATAGCATTA
TTTGGGATGCTCTGCTTGTATGCAAATGGCACAACCTTACCCTCAAGATTG
CAAAACACAGATGAGTAACAGATGCAATGTGAATCAATAAAAAAGTATTGT
30 TCGGTTGTTGATGACACAACCTTACTCATAAAAAATGCAT.

DETAILED DESCRIPTION

I. Overview of several embodiments

Particular embodiments include three exemplary SNP markers (rhg1-3995, BARC_010889_01691, and NCSB_004074) that show co-segregation with the soybean cyst nematode (SCN) resistance trait in 96 tested soybean lines. Markers that co-segregate with SCN resistance are linked to this trait and, therefore, may be useful in marker-assisted selection and breeding. Also disclosed herein is a strategy used to identify these three exemplary SNP markers linked to SCN resistance. The physical map positions of these three exemplary SNP markers in the *Glycine max* genome are provided. Using the three exemplary SNP markers described herein, a specific assay using KBiosciences Competitive Allele-Specific PCR SNP genotyping system (KASPar™) was developed to rapidly and accurately identify plants carrying the SCN resistance trait. While embodiments of the invention are described with reference to three exemplary SNP markers linked to SCN resistance, those of skill in the art will appreciate that additional, equivalent markers may be identified using the techniques described herein. SNP markers linked to SCN resistance may be used, for example, in SCN genotyping to select SCN resistant individuals from soybean breeding populations.

Soybean cyst nematode (SCN) resistance is a very complex trait. SCN infestation may be caused by one or more different *Heterodera glycines* races, the resistance for each of which may require different resistant genes located on different linkage groups. See Table 1. The three markers disclosed in Table 1 are all located in linkage group G. The SCN resistance gene(s) in linkage group G is thought to be responsible for resistance to races 3 and 14.

The strategy described herein is used to identify markers in other linkage groups (for example, A₂, B₁, and I) that are linked to SCN resistance. Thus, methods for identifying such markers are also provided. The general strategy is also used to map other traits of interest. The strategy is more efficient than traditional mapping strategies and may be particularly useful in molecular breeding programs.

Table 1: Sources of SCN resistance

Resistant Germplasm	<i>H. glycines</i> Races	Linkage groups (LG)
PI 88788	3, 14	G
Peking	1, 3, and 5	G, A2, and B
PI 437654	All	G (Rhg1), A2 (Rhg4), B, C1, L25, L26, M, and D1a
PI 90763	3	
PI 438489B	1, 2, 3, 5, and 14	G, E, A1, B1, and C1
PI 89772	1, 2, 3, and 5	G, E, A1, C1, C2, and D1a
PI209332	All	G (Rhg1), and A2 (Rhg4)
PUSCN14	3	A, G, B, I, and F
Hartwig	3	
Forrest	3	G and A2
Pyramid	3, 14 (from PI 88788)	A2, D, and G

5 II. Terms

Mapping population: As used herein, the term “mapping population” may refer to a plant population used for gene mapping. Mapping populations are typically obtained from controlled crosses of parent genotypes. Decisions on the selection of parents and mating design for the development of a mapping population, and the type of markers used, depend upon the gene to be mapped, the availability of markers, and the molecular map. The parents of plants within a mapping population must have sufficient variation for the trait(s) of interest at both the nucleic acid sequence and phenotype level. Variation of the parents’ nucleic acid sequence is used to trace recombination events in the plants of the mapping population. The availability of informative polymorphic markers is dependent upon the amount of nucleic acid sequence variation.

Backcrossing: Backcrossing methods may be used to introduce a nucleic acid sequence into plants. The backcrossing technique has been widely used for decades to introduce new traits into plants. N. Jensen, Ed., *Plant Breeding Methodology*, John Wiley & Sons, Inc., 1988. In a typical backcross protocol, the original variety of

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interest (recurrent parent) is crossed to a second variety (non-recurrent parent) that carries a gene of interest to be transferred. The resulting progeny from this cross are then crossed again to the recurrent parent, and the process is repeated until a plant is obtained wherein essentially all of the desired morphological and physiological characteristics of the recurrent plant are recovered in the converted plant, in addition to the transferred gene from the non-recurrent parent.

KBiosciences Competitive Allele-Specific PCR SNP genotyping system (KASPar™): KASPar™ is a commercially available homogeneous fluorescent system for determining SNP genotypes (KBiosciences Ltd., Hoddesdon, UK). A KASPar™ assay comprises an SNP-specific “assay mix,” which contains three unlabelled primers, and a “reaction mix,” which contains all the other required components, for example, a universal fluorescent reporting system. In addition to these mixes, the user provides, *inter alia*, a FRET-capable plate reader, microtiter plate(s), and DNA samples that contain about 5 ng/L DNA.

A typical KASPar™ assay comprises the steps of: allele-specific primer design (*e.g.*, using PrimerPicker™, which is a free service available through the internet at the KBiosciences website), preparation of reaction mix including the allele-specific primers, admixing the reaction mix to DNA samples in a microtiter plate, thermocycling, reading the plate in a fluorescent plate reader, and plotting and scoring the fluorescent data. Data from each sample are plotted together on a two-dimensional graph, where the x- and y-axes correspond to FAM and VIC fluorescence values. Samples having the same SNP genotype cluster together on the plot (*i.e.*, A/A, A/a, and a/a). More technical information about the KASPar system, including a guide of solutions to common problems, is obtainable from KBiosciences Ltd. (*e.g.*, the *KASPar SNP Genotyping System Reagent Manual*).

Linked, tightly linked, and extremely tightly linked: As used herein, linkage between genes or markers may refer to the phenomenon in which genes or markers on a chromosome show a measurable probability of being passed on together to individuals in the next generation. The closer two genes or markers are to each other, the closer to (1) this probability becomes. Thus, the term “linked” may refer to one or more genes or markers that are passed together with a gene with a probability greater than 0.5 (which is expected from independent assortment where markers/genes are

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located on different chromosomes). When the presence of a gene contributes to a phenotype in an individual, markers that are linked to the gene may be said to be linked to the phenotype. Thus, the term “linked” may refer to a relationship between a marker and a gene, or between a marker and a phenotype.

5 Because the proximity of two genes or markers on a chromosome is directly related to the probability that the genes or markers will be passed together to individuals in the next generation, the term “linked” may also refer herein to one or more genes or markers that are located within about 2.0 Mb of one another on the same chromosome. Thus, two “linked” genes or markers may be separated by about 2.1 Mb,
10 2.00 Mb, about 1.95 Mb, about 1.90 Mb, about 1.85 Mb, about 1.80 Mb, about 1.75 Mb, about 1.70 Mb, about 1.65 Mb, about 1.60 Mb, about 1.55 Mb, about 1.50 Mb, about 1.45 Mb, about 1.40 Mb, about 1.35 Mb, about 1.30 Mb, about 1.25 Mb, about 1.20 Mb, about 1.15 Mb, about 1.10 Mb, about 1.05 Mb, about 1.00 Mb, about 0.95 Mb, about 0.90 Mb, about 0.85 Mb, about 0.80 Mb, about 0.75 Mb, about 0.70 Mb,
15 about 0.65 Mb, about 0.60 Mb, about 0.55 Mb, about 0.50 Mb, about 0.45 Mb, about 0.40 Mb, about 0.35 Mb, about 0.30 Mb, about 0.25 Mb, about 0.20 Mb, about 0.15 Mb, about 0.10 Mb, about 0.05 Mb, about 0.025 Mb, and about 0.01 Mb. Particular examples of markers that are “linked” to the SCN phenotype in soybean include nucleotide sequences on chromosome 18 of the soybean genome.

20 As used herein, the term “tightly linked” may refer to one or more genes or markers that are located within about 0.5 Mb of one another on the same chromosome. Thus, two “tightly linked” genes or markers may be separated by about 0.6 Mb, about 0.55 Mb, 0.5 Mb, about 0.45 Mb, about 0.4 Mb, about 0.35 Mb, about 0.3 Mb, about 0.25 Mb, about 0.2 Mb, about 0.15 Mb, about 0.1 Mb, and about 0.05 Mb.

25 As used herein, the term “extremely tightly linked” may refer to one or more genes or markers that are located within about 100 kb of one another on the same chromosome. Thus, two “extremely tightly linked” genes or markers may be separated by about 125 kb, about 120 kb, about 115 kb, about 110 kb, about 105 kb, 100 kb, about 95 kb, about 90 kb, about 85 kb, about 80 kb, about 75 kb, about 70 kb, about 65
30 kb, about 60 kb, about 55 kb, about 50 kb, about 45 kb, about 40 kb, about 35 kb, about 30 kb, about 25 kb, about 20 kb, about 15 kb, about 10 kb, about 5 kb, and about 1 kb.

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Particular examples of markers that are “extremely tightly linked” to the SCN phenotype in soybean include rhg1-3995, BARC_010889_01691, and NCSB_004074.

In view of the foregoing, it will be appreciated that markers linked to a particular gene or phenotype include those markers that are tightly linked, and those
5 markers that are extremely tightly linked, to the gene or phenotype. Linked, tightly linked, and extremely tightly genetic markers of the SCN phenotype may be useful in marker-assisted breeding programs to identify SCN resistant soybean varieties, and to breed this trait into other soybean varieties to confer SCN resistance.

Locus: As used herein, the term “locus” refers to a position on the genome that
10 corresponds to a measurable characteristic (*e.g.*, a trait). An SNP locus is defined by a probe that hybridizes to DNA contained within the locus.

Marker: As used herein, a marker refers to a gene or nucleotide sequence that can be used to identify plants having a particular allele. A marker may be described as a variation at a given genomic locus. A genetic marker may be a short DNA sequence,
15 such as a sequence surrounding a single base-pair change (single nucleotide polymorphism, or “SNP”), or a long one, for example, a microsatellite/simple sequence repeat (“SSR”). A “marker allele” refers to the version of the marker that is present in a particular individual.

The term marker as used herein may refer to a cloned segment of soybean
20 chromosomal DNA (for example, a segment including rhg1-3995, BARC_010889_01691, or NCSB_004074), and may also or alternatively refer to a DNA molecule that is complementary to a cloned segment of soybean chromosomal DNA (for example, DNA complementary to a segment including rhg1-3995, BARC_010889_01691, or NCSB_004074).

25 In some embodiments, the presence of a marker in a plant may be detected through the use of a nucleic acid probe. A probe may be a DNA molecule or an RNA molecule. RNA probes can be synthesized by means known in the art, for example, using a DNA molecule template. A probe may contain all or a portion of the nucleotide sequence of the marker and additional, contiguous nucleotide sequence from
30 the plant genome. This is referred to herein as a “contiguous probe.” The additional, contiguous nucleotide sequence is referred to as “upstream” or “downstream” of the original marker, depending on whether the contiguous nucleotide sequence from the

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plant chromosome is on the 5' or the 3' side of the original marker, as conventionally understood. As is recognized by those of ordinary skill in the art, the process of obtaining additional, contiguous nucleotide sequence for inclusion in a marker may be repeated nearly indefinitely (limited only by the length of the chromosome), thereby
5 identifying additional markers along the chromosome. All above-described markers may be used in some embodiments of the present invention.

An oligonucleotide probe sequence may be prepared synthetically or by cloning. Suitable cloning vectors are well-known to those of skill in the art. An oligonucleotide probe may be labeled or unlabeled. A wide variety of techniques exist
10 for labeling nucleic acid molecules, including, for example and without limitation: radiolabeling by nick translation, random priming, tailing with terminal deoxytransferase, or the like, where the nucleotides employed are labeled, for example, with radioactive ³²P. Other labels which may be used include, for example and without limitation: Fluorophores (*e.g.*, FAM and VIC), enzymes, enzyme substrates, enzyme
15 cofactors, enzyme inhibitors, and the like. Alternatively, the use of a label that provides a detectable signal, by itself or in conjunction with other reactive agents, may be replaced by ligands to which receptors bind, where the receptors are labeled (for example, by the above-indicated labels) to provide detectable signals, either by themselves, or in conjunction with other reagents. *See, e.g.*, Leary *et al.* (1983) *Proc.*
20 *Natl. Acad. Sci. USA* 80:4045-9.

A probe may contain a nucleotide sequence that is not contiguous to that of the original marker; this probe is referred to herein as a "noncontiguous probe." The sequence of the noncontiguous probe is located sufficiently close to the sequence of the original marker on the genome so that the noncontiguous probe is genetically linked to
25 the same gene or trait (*e.g.*, SCN resistance). For example, in some embodiments, a noncontiguous probe is located within 500 kb, 450 kb, 400 kb, 350 kb, 300 kb, 250 kb, 200 kb, 150 kb, 125 kb, 100 kb, 0.9 kb, 0.8 kb, 0.7 kb, 0.6 kb, 0.5 kb, 0.4 kb, 0.3 kb, 0.2 kb, or 0.1 kb of the original marker on the soybean genome.

A probe may be an exact copy of a marker to be detected. A probe may also be
30 a nucleic acid molecule comprising, or consisting of, a nucleotide sequence which is substantially identical to a cloned segment of the subject organism's (for example, soybean) chromosomal DNA. As used herein, the term "substantially identical" may

refer to nucleotide sequences that are more than 85% identical. For example, a substantially identical nucleotide sequence may be 85.5%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 99.5% identical to the reference sequence.

5 A probe may also be a nucleic acid molecule that is “specifically hybridizable” or “specifically complementary” to an exact copy of the marker to be detected (“DNA target”). “Specifically hybridizable” and “specifically complementary” are terms that indicate a sufficient degree of complementarity such that stable and specific binding occurs between the nucleic acid molecule and the DNA target. A nucleic acid
10 molecule need not be 100% complementary to its target sequence to be specifically hybridizable. A nucleic acid molecule is specifically hybridizable when there is a sufficient degree of complementarity to avoid non-specific binding of the nucleic acid to non-target sequences under conditions where specific binding is desired, for example, under stringent hybridization conditions.

15 Hybridization conditions resulting in particular degrees of stringency will vary depending upon the nature of the hybridization method of choice and the composition and length of the hybridizing nucleic acid sequences. Generally, the temperature of hybridization and the ionic strength (especially the Na^+ and/or Mg^{++} concentration) of the hybridization buffer will determine the stringency of hybridization, though wash
20 times also influence stringency. Calculations regarding hybridization conditions required for attaining particular degrees of stringency are known to those of ordinary skill in the art, and are discussed, for example, in Sambrook *et al.* (ed.) *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, chapters 9 and 11; and Hames and Higgins
25 (eds.) *Nucleic Acid Hybridization*, IRL Press, Oxford, 1985. Further detailed instruction and guidance with regard to the hybridization of nucleic acids may be found, for example, in Tijssen, “Overview of principles of hybridization and the strategy of nucleic acid probe assays,” in *Laboratory Techniques in Biochemistry and Molecular Biology - Hybridization with Nucleic Acid Probes*, Part I, Chapter 2,
30 Elsevier, NY, 1993; and Ausubel *et al.*, Eds., *Current Protocols in Molecular Biology*, Chapter 2, Greene Publishing and Wiley-Interscience, NY, 1995.

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As used herein, “stringent conditions” encompass conditions under which hybridization will only occur if there is less than 50% mismatch between the hybridization molecule and the DNA target. “Stringent conditions” include further particular levels of stringency. Thus, as used herein, “moderate stringency” conditions
5 are those under which molecules with more than 50% sequence mismatch will not hybridize; conditions of “high stringency” are those under which sequences with more than 20% mismatch will not hybridize; and conditions of “very high stringency” are those under which sequences with more than 10% mismatch will not hybridize.

The following are representative, non-limiting hybridization conditions.

10 Very High Stringency (detects sequences that share at least 90% sequence identity): Hybridization in 5x SSC buffer at 65°C for 16 hours; wash twice in 2x SSC buffer at room temperature for 15 minutes each; and wash twice in 0.5x SSC buffer at 65°C for 20 minutes each.

High Stringency (detects sequences that share at least 80% sequence identity):
15 Hybridization in 5x-6x SSC buffer at 65-70°C for 16-20 hours; wash twice in 2x SSC buffer at room temperature for 5-20 minutes each; and wash twice in 1x SSC buffer at 55-70°C for 30 minutes each.

Moderate Stringency (detects sequences that share at least 50% sequence identity): Hybridization in 6x SSC buffer at room temperature to 55°C for 16-20
20 hours; wash at least twice in 2x-3x SSC buffer at room temperature to 55°C for 20-30 minutes each.

With respect to all probes discussed, *supra*, the probe may comprise additional nucleic acid sequences, for example, promoters, transcription signals, and/or vector sequences. Any of the probes discussed, *supra*, may be used to define additional
25 markers that are tightly linked to a gene involved in SCN resistance, and markers thus identified may be equivalent to exemplary markers named in the present disclosure, and thus are within the scope of the invention.

Marker-assisted breeding: As used herein, the term “marker-assisted breeding” may refer to an approach to breeding directly for one or more complex traits (*e.g.*, SCN
30 resistance). In current practice, plant breeders attempt to identify easily detectable traits, such as flower color, seed coat appearance, or isozyme variants that are linked to an agronomically desired trait. The plant breeders then follow the agronomic trait in

the segregating, breeding populations by following the segregation of the easily detectable trait. However, there are very few of these linkage relationships available for use in plant breeding.

Marker-assisted breeding provides a time- and cost-efficient process for improvement of plant varieties. Several examples of the application of marker-assisted breeding involve the use of isozyme markers. *See, e.g.,* Tanksley and Orton, eds. (1983) *Isozymes in Plant Breeding and Genetics*, Amsterdam: Elsevier. One example is an isozyme marker associated with a gene for resistance to a nematode pest in tomato. The resistance, controlled by a gene designated *Mi*, is located on chromosome 6 of tomato and is very tightly linked to *Aps1*, an acid phosphatase isozyme. Use of the *Aps1* isozyme marker to indirectly select for the *Mi* gene provided the advantages that segregation in a population can be determined unequivocally with standard electrophoretic techniques; the isozyme marker can be scored in seedling tissue, obviating the need to maintain plants to maturity; and co-dominance of the isozyme marker alleles allows discrimination between homozygotes and heterozygotes. *See* Rick (1983) in Tanksley and Orton, *supra*.

Quantitative trait locus: As used herein, the term “Quantitative trait locus” (QTL) may refer to stretches of DNA that have been identified as likely DNA sequences (*e.g.,* genes, non-coding sequences, and/or intergenic sequences) that underlie a quantitative trait, or phenotype, that varies in degree, and can be attributed to the interactions between two or more DNA sequences (*e.g.,* genes, non-coding sequences, and/or intergenic sequences) or their expression products and their environment. Quantitative trait loci (QTLs) can be molecularly identified to help map regions of the genome that contain sequences involved in specifying a quantitative trait.

As used herein, the term “QTL interval” may refer to stretches of DNA that are linked to the genes that underlie the QTL trait. A QTL interval is typically, but not necessarily, larger than the QTL itself. A QTL interval may contain stretches of DNA that are 5' and/or 3' with respect to the QTL.

Sequence identity: The term “sequence identity” or “identity,” as used herein in the context of two nucleic acid or polypeptide sequences, may refer to the residues in the two sequences that are the same when aligned for maximum correspondence over a specified comparison window.

As used herein, the term “percentage of sequence identity” may refer to the value determined by comparing two optimally aligned sequences (*e.g.*, nucleic acid sequences) over a comparison window, wherein the portion of the sequence in the comparison window may comprise additions or deletions (*i.e.*, gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleotide or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the comparison window, and multiplying the result by 100 to yield the percentage of sequence identity.

Methods for aligning sequences for comparison are well-known in the art. Various programs and alignment algorithms are described in, for example: Smith and Waterman (1981) *Adv. Appl. Math.* 2:482; Needleman and Wunsch (1970) *J. Mol. Biol.* 48:443; Pearson and Lipman (1988) *Proc. Natl. Acad. Sci. U.S.A.* 85:2444; Higgins and Sharp (1988) *Gene* 73:237-44; Higgins and Sharp (1989) *CABIOS* 5:151-3; Corpet *et al.* (1988) *Nucleic Acids Res.* 16:10881-90; Huang *et al.* (1992) *Comp. Appl. Biosci.* 8:155-65; Pearson *et al.* (1994) *Methods Mol. Biol.* 24:307-31; Tatiana *et al.* (1999) *FEMS Microbiol. Lett.* 174:247-50. A detailed consideration of sequence alignment methods and homology calculations can be found in, *e.g.*, Altschul *et al.* (1990) *J. Mol. Biol.* 215:403-10.

The National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST™; Altschul *et al.* (1990)) is available from several sources, including the National Center for Biotechnology Information (Bethesda, MD), and on the internet, for use in connection with several sequence analysis programs. A description of how to determine sequence identity using this program is available on the internet under the “help” section for BLAST™. For comparisons of nucleic acid sequences, the “Blast 2 sequences” function of the BLAST™ (Blastn) program may be employed using the default BLOSUM62 matrix set to default parameters. Nucleic acid sequences with even greater similarity to the reference sequences will show increasing percentage identity when assessed by this method.

Single-nucleotide polymorphism: As used herein, the term “single-nucleotide polymorphism” (SNP) may refer to a DNA sequence variation occurring when a single

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nucleotide in the genome (or other shared sequence) differs between members of a species or paired chromosomes in an individual. Within a population, SNPs can be assigned a minor allele frequency that is the lowest allele frequency at a locus that is observed in a particular population. This is simply the lesser of the two allele
5 frequencies for single-nucleotide polymorphisms. Different populations are expected to exhibit at least slightly different allele frequencies. Particular populations may exhibit significantly different allele frequencies. In some examples, markers linked to SCN resistance are SNP markers.

SNPs may fall within coding sequences of genes, non-coding regions of genes,
10 or in the intergenic regions between genes. SNPs within a coding sequence will not necessarily change the amino acid sequence of the protein that is produced, due to degeneracy of the genetic code. An SNP in which both forms lead to the same polypeptide sequence is termed “synonymous” (sometimes called a silent mutation). If a different polypeptide sequence is produced, they are termed “non-synonymous.” A
15 non-synonymous change may either be missense or nonsense, where a missense change results in a different amino acid, and a nonsense change results in a premature stop codon. SNPs that are not in protein-coding regions may still have consequences for gene splicing, transcription factor binding, or the sequence of non-coding RNA. SNPs are usually biallelic and thus easily assayed in plants and animals.
20 Sachidanandam (2001) *Nature* 409:928-33.

Trait or phenotype: The terms “trait” and “phenotype” are used interchangeably herein. For the purposes of the present disclosure, a trait of particular interest is SCN resistance.

25 **III. *QTL-based identification of markers linked to a trait of interest***

A. Overview

In some embodiments, a trait (*e.g.*, SCN resistance) is mapped using a strategy that is different from traditional mapping approaches. For example, a trait may be mapped according to a strategy that, for the sake of convenience, may be described as
30 comprising four steps. In a first step, QTL interval target regions that correspond to a trait to be mapped may be determined. In a second step, markers (*e.g.*, SNP markers) may be selected which are located within or near determined QTL intervals of the

target genome (e.g., soybean genome). In a third step, specific primers may be designed that facilitate the genotyping of individual subjects with respect to selected markers. In particular examples, specific primers are designed for use in a KASPar™ genotyping assay. In a fourth step, populations that show segregation for the trait may be screened using the specific primers to identify those markers that are linked to the trait.

B. Markers linked to a trait of interest and the identification thereof

Determination of QTL interval target regions and identification of markers.

QTLs may be determined by any technique available to those of skill in the art. For example, the physical positions of a QTL that corresponds to a particular trait of interest may be initially determined by reference to the location of genes that are known to contribute to the particular trait. In some embodiments, SCN resistance genes may be identified on at least four regions on chromosome 8, 11, 18, and 20, respectively. *See, e.g.,* Concibido *et al.* (1996) *Theor. Appl. Genet.* 93:234-41, Concibido *et al.* (1997) *Crop Sci.* 37:258-64, Meksem *et al.* (1999) *Theor. Appl. Genet.* 99:1131-42, Qui *et al.* (1999) *Theor. Appl. Genet.* 98:356-64, Meksem *et al.* (2001) *Mol. Breeding* 7:63-71, Li *et al.* (2009) *Mol. Breeding* 24:63-76, Wu *et al.* (2009) *Theor. Appl. Genet.* 118:1093-105; U.S. Patents 5,491,081, 6,096,944, 6,162,967, 6,271,437, 6,284,948, 6,300,541, 6,538,175, 7,154,021, 7,485,770; U.S.S.N.s 20020129402, 20020144310, 20030005491, 20030135881, 20060225150, 20060253919, 20080072352, and 20090100537; and International PCT Publication Nos. WO1995020669A2, WO2001051627A2, and WO2008153804A2. In some embodiments, the initially identified QTLs are grouped or divided into a less complicated or extensive list of QTLs that may have boundaries in the genome that are the same or different than the boundaries of the initially identified QTLs.

In some embodiments, a region of DNA may be selected that is likely to contain markers that are linked to the QTL trait. This region may be referred to as a QTL interval. For example, a QTL interval may be a region of DNA that includes the QTL and additional genomic DNA that is near the QTL in either, or both, the 5' and 3' directions. In some embodiments, a QTL interval may be about 4 Mb, about 3.5 Mb, about 3 Mb, about 2.5 Mb, about 2 Mb, about 1.5 Mb, or about 1 Mb.

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In particular embodiments, the target genome may be searched to identify markers that are physically located in, near, or between the QTLs and QTL intervals. If a reference map containing the location of known markers is available for the target genome, the reference map may be used to identify markers. Nucleic acid sequences of the target genome may also be searched, for example, by software such as BLAST™. In some embodiments, SNP markers may be identified. In some embodiments, markers may be identified that are physically located in, near, or between QTLs and QTL intervals of the soybean genome that correspond to the SCN resistance trait. In particular examples, identified SNP markers that are physically located in, near, or between QTLs and QTL intervals of the soybean genome that correspond to the SCN resistance trait may be selected from the group consisting of the markers listed in Table 2.

In other embodiments, particular markers may be selected from the identified markers that are physically located in, near, or between QTLs and QTL intervals that correspond to a trait of interest, which markers are polymorphic among the parental lines from which a mapping population will be generated. Polymorphism of a given marker among the parental lines is directly related to the ability to trace recombination events in a mapping population produced from the parental lines.

In particular examples, polymorphic markers among parental soybean lines are selected to screen SCN resistance mapping populations to determine which, if any, of the polymorphic markers are linked to the SCN resistance trait. Such markers may segregate so that one allele of the SNP marker appears exclusively in SCN resistant individuals, and the other allele of the SNP marker appears exclusively in SCN susceptible individuals. Mapping populations may be generated by crossing one variety that is SCN resistant with another variety that is SCN susceptible. In embodiments, a mapping population may comprise about 10, about 20, about 30, about 40, about 50, about 60, about 70, about 80, about 90, about 95, about 100, about 150, about 200, about 250, about 300, about 350, about 400, about 450, about 500, or more individuals. In some embodiments, SCN resistant soybean germplasm 98860-71 may be crossed with one or more SCN susceptible germplasm(s) (e.g., 75213 and 6CH026-035) to create mapping populations.

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In some embodiments, the polymorphic markers may be single nucleotide polymorphisms (SNPs) linked to or within the gene or QTL corresponding to the SCN resistance trait of interest. These SNP markers may be detected by sequencing through the region containing the gene or QTL using any DNA sequencing methods known in the art, including but not limited to Sanger sequencing or high throughput sequencing (“Next Generation”) methodologies that enable short or long sequence reads through the region of interest. In such embodiments, where genotyping by sequencing is used for the detection of SNP markers, primers corresponding to the flanking sequences of the region containing the SNPs in gene or QTL of interest may be used for the sequencing chemistries in order to sequence through the region of interest. In such embodiments, when different genotypes are used for sequencing through the region of interest for the detection of SNPs exemplified herein, other SNPs may be identified in addition to the SNPs exemplified herein. In such embodiments, the SNPs exemplified herein by themselves (individual SNPs) or in combination with other SNPs linked to exemplified sequences (haplotypes) may be utilized for differentiating genotypes towards marker assisted selection of plants for the SCN resistance trait of interest.

Primer design and linkage screening.

Oligonucleotide probes (*e.g.*, primers) may be designed to specifically detect markers that are physically located in, near, or between QTLs and QTL intervals that correspond to a trait of interest. In general, an oligonucleotide probe may be designed that specifically hybridizes to only one allele of a marker. In some embodiments, two oligonucleotide probes are designed to detect an SNP marker, such that each specifically hybridizes to the SNP allele to which the other probe does not specifically hybridize. As is understood by those of skill in the art, the length or composition of oligonucleotide probes for a particular marker may be varied according to established principles without rendering the probe non-specific for one allele of the marker.

In some embodiments, the oligonucleotide probes may be primers. In specific embodiments, primers may be designed to detect markers in a KASPar™ genotyping assay. In particular embodiments, primers may be designed to detect markers linked to the SCN resistance phenotype in soybean using a KASPar™ genotyping assay. In these and further embodiments, the detection system may provide a high-throughput and convenient format for genotyping individuals in a mapping population, which may

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greatly facilitate the identification of individuals carrying a particular gene or trait, and may also greatly facilitate the implementation or execution of a marker-assisted selection program.

In specific embodiments, the oligonucleotide probes may be primers designed to detect markers in a TAQMAN[®] genotyping assay. This method utilizes primers specific to the marker closely linked to the SCN resistance gene and fluorescent labeled probes containing a single nucleotide polymorphism (SNP). The SNP probe associated with resistance is labeled with a fluorescent dye such as FAM while the probe associated with susceptibility is labeled with a different fluorescent dye such as VIC.

The data is analyzed as the presence or absence of a fluorescent dye signal. The detection system may provide a high-throughput and convenient format, such as multiplexing for genotyping individuals in a mapping population, which may greatly facilitate the identification of individuals carrying a particular gene or trait, and may also greatly facilitate the implementation or execution of a marker-assisted selection program.

Additional markers may be identified as equivalent to any of the exemplary markers named herein (e.g., markers listed in Table 3, such as, for example, rhg1-3995, BARC_010889_01691, and NCSB_004074), for example, by determining the frequency of recombination between the exemplary marker and an additional marker.

Such determinations may utilize a method of orthogonal contrasts based on the method of Mather (1931), *The Measurement of Linkage in Heredity*, Methuen & Co., London, followed by a test of maximum likelihood to determine a recombination frequency. Allard (1956) *Hilgardia* 24:235-78. If the value of the recombination frequency is less than or equal to 0.10 (i.e., 10%), then the additional marker is considered equivalent to the particular exemplary marker for the purposes of use in the presently disclosed methods.

Markers that are linked to any and all SCN resistance genes may be identified in embodiments of the invention. Further, markers that control any and all of resistance contributing loci for all SCN *HG* races may be identified in embodiments of the invention.

A means for providing SCN resistance in soybean may be an SNP marker allele, the detection of which SNP marker allele in soybean plants belonging to, or

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derived from, germplasm 98860-71 provides at least a strong indication that the plant comprising the nucleic acid sequence has the SCN resistance phenotype. In some examples, a means for providing SCN resistance in soybean is a marker selected from the group consisting of the markers listed in Table 3. In particular examples, a means
5 for providing SCN resistance in soybean is a marker selected from the group consisting of rhg1-3995, BARC_010889_01691, and NCSB_004074.

A means for identifying soybean plants having the SCN resistance phenotype may be a molecule that presents a detectable signal when added to a sample obtained from a soybean plant belonging to, or derived from, germplasm 98860-71 having the
10 SCN resistance genotype, but which means does not present a detectable signal when added to a sample obtained from a soybean plant of belonging to, or derived from, germplasm 98860-71 that does not have the SCN resistance phenotype. Specific hybridization of nucleic acids is a detectable signal, and a nucleic acid probe that specifically hybridizes to an SNP marker allele that is linked to the SCN resistance
15 phenotype may therefore be a means for identifying soybean plants having the SCN resistance phenotype. In some examples, a means for identifying soybean plants having the SCN resistance phenotype is a probe that specifically hybridizes to a marker that is linked to the SCN resistance phenotype.

20 C. Methods of using markers linked to a trait of interest

Methods of using nucleic acid molecular markers that are linked to a trait of interest (*e.g.*, SCN resistance in soybean) to identify plants having the trait of interest may result in a cost savings for plant developers, because such methods may eliminate the need to phenotype individual plants generated during development (for example, by
25 crossing soybean plant varieties having SCN resistance with vulnerable plant varieties).

In particular embodiments, markers linked to SCN resistance in soybean may be used to transfer segment(s) of DNA that contain one or more determinants of SCN resistance. In particular embodiments, the markers may be selected from a group of markers comprising the markers listed in Table 3 and markers that are their
30 equivalents. In some embodiments, a marker may be selected from the group consisting of rhg1-3995, BARC_010889_01691, and NCSB_004074. In some embodiments, a method for using markers linked to SCN resistance in soybean to

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transfer segment(s) of DNA that contain one or more determinants of SCN resistance may comprise analyzing the genomic DNA of two parent plants with probes that are specifically hybridizable to markers linked to the SCN resistance phenotype; sexually crossing the two parental plant genotypes to obtain a progeny population, and
5 analyzing those progeny for the presence of the markers linked to the SCN resistance phenotype; backcrossing the progeny that contain the markers linked to the SCN resistance phenotype to the recipient genotype to produce a first backcross population, and then continuing with a backcrossing program until a final progeny is obtained that comprises any desired trait(s) exhibited by the parent genotype and the SCN resistance
10 phenotype. In particular embodiments, individual progeny obtained in each crossing and backcrossing step are selected by SCN marker analysis at each generation. In some embodiments, analysis of the genomic DNA of the two parent plants with probes that are specifically hybridizable to markers linked to SCN resistance phenotype reveals that one of the parent plants comprises fewer of the linked markers to which the
15 probes specifically hybridize, or none of the linked markers to which the probes specifically hybridize. In some embodiments, individual progeny obtained in each cross and/or backcross are selected by the sequence variation of individual plants.

In some embodiments, markers linked to the SCN resistance phenotype may be used to introduce one or more determinants of SCN resistance into a plant (*e.g.*,
20 soybean) by genetic transformation. In particular embodiments, the markers may be selected from a group of markers comprising the markers listed in Table 3 and markers that are their equivalents. In some embodiments, a method for introducing one or more determinants of SCN resistance into a plant by genetic recombination may comprise
25 analyzing the genomic DNA of a plant (*e.g.*, soybean) with probes that are specifically hybridizable to markers linked to the SCN resistance phenotype to identify one or more determinants of SCN resistance in the plant; isolating a segment of the genomic DNA of the plant comprising the markers linked to the SCN resistance phenotype, for example, by extracting the genomic DNA and digesting the genomic DNA with one or more restriction endonuclease enzymes; optionally amplifying the isolated segment of
30 DNA; introducing the isolated segment of DNA into a cell or tissue of a host plant; and analyzing the DNA of the host plant with probes that are specifically hybridizable to markers linked to the SCN resistance phenotype to identify the one or more

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determinants of SCN resistance in the host plant. In particular embodiments, the isolated segment of DNA may be introduced into the host plant such that it is stably integrated into the genome of the host plant.

In some embodiments, markers that are linked to the SCN resistance phenotype may be used to introduce one or more determinants of SCN resistance into other organisms, for example, plants. In particular embodiments, the markers can be selected from a group of markers listed in Table 3 and markers that are their equivalents. In some embodiments, a method for introducing one or more determinants of SCN resistance into an organism other than soybean may comprise analyzing the genomic DNA of a plant (*e.g.*, a soybean plant) with probes that are specifically hybridizable to markers linked to the SCN resistance phenotype to identify one or more determinants of SCN resistance in the plant; isolating a segment of the genomic DNA of the plant comprising the one or more determinants of SCN resistance, for example, by extracting the genomic DNA and digesting the genomic DNA with one or more restriction endonuclease enzymes; optionally amplifying the isolated segment of DNA; introducing the isolated segment of DNA into an organism other than soybean; and analyzing the DNA of the organism other than soybean with probes that are specifically hybridizable to markers linked to the SCN resistance phenotype to identify the one or more determinants of SCN resistance in the organism. In other embodiments, the isolated segment of DNA may be introduced into the organism such that it is stably integrated into the genome of the organism.

In some embodiments, markers that are linked to the SCN resistance phenotype may be used to identify a plant with one or more determinants of SCN resistance. In some embodiments, the plant may be a soybean plant. For example, the plant may be a soybean plant of germplasm 98860-71. In particular embodiments, nucleic acid molecules (*e.g.*, genomic DNA or mRNA) may be extracted from a plant. The extracted nucleic acid molecules may then be contacted with one or more probes that are specifically hybridizable to markers linked to the SCN resistance phenotype. Specific hybridization of the one or more probes to the extracted nucleic acid molecules is indicative of the presence of one or more determinants of SCN resistance in the plant.

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In some embodiments, markers that are linked to multiple determinants of SCN resistance may be used simultaneously. In other embodiments, markers that are linked to only one determinant of SCN resistance may be used. In specific examples, markers that are linked to SCN resistance with respect to one or more particular SCN
5 *HG* races (e.g., race 1, race 2, race 3, race 5, and race 14) may be used simultaneously. For example, a plurality of markers that are linked to SCN resistance with respect to different SCN *HG* races may be used simultaneously.

The following examples are provided to illustrate certain particular features and/or embodiments. These examples should not be construed to limit the disclosure
10 to the particular features or embodiments described.

EXAMPLES

Example 1: Materials and Methods

24 soybean cultivars and SCN mapping parents were used to identify markers
15 linked to the SCN resistance phenotype. 14 of the cultivars were SCN susceptible: 75110, 75155, 75163, 99630, 99726, 95895-755PRU, 99345-31, 75192, 75209, 75159, Essex, Williams82, 75213, and 6CH026-035. 10 of the cultivars were SCN resistant: Maverick, Peking, PI209332, PI437654, 99811, 99294, Forrest, PI88788, PI437654, and 98860-71.

20 SCN bioassay: SCN bioassays were performed to generate phenotype information of mapping populations produced by crossing SCN resistant soybean variety 98860-71 with SCN susceptible soybean varieties 75213 and 6CH026-035. The phenotype information of the mapping population used is listed in Table 3. The industry does not have a uniform approach to categorizing resistance levels in soybean
25 varieties. Therefore, resistance levels were categorized in terms of "SCN score:" SCN score 0-10 = R (resistance); SCN score 10.1-29.9 = MR (medium resistance); SCN score 30.0-59.9 = MS (medium susceptible); and 60+ = S (susceptible). SCN index values were determined by comparing testing lines to known susceptible and resistant lines. The index score was directly based on the percentage of SCN susceptibility
30 observed for the sample. For example, if a testing line had 10 cysts on each of 9 plants, and Williams (susceptible) had 100 cysts on each of 9, then the testing line was

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categorized with an index of 10%. The final index was the average of the scores of the 9 plants.

KASPar™ reactions: KASPar™ primers were designed using PrimerPicker™ tool in KLIMST™ (KBioscience Laboratory Management System) by providing DNA sequences with SNPs. Three primers, A1 (Allele specific primer 1), A2 (Allele specific primer 2), and C (common reverse primer) were designed for each SNP sequence based on KASPar™ chemistry. An assay mix of each KASPar™ reaction was prepared as in the KASPar™ SNP Genotyping System v2.0. The final reaction volume was 5 µL per reaction, including 1 µL DNA template (5 ng/µL), 2.5 µL 2X Reaction Mix, 0.06875 µL Assay mix, 0.04 µL 50 mM MgCl₂, and 1.39125 µL ddH₂O. The assay was carried out in 384-well format. The thermocycle conditions used during the assay were according to the manufacturer's instructions: 94°C for 15 minutes; 20 cycles of 94°C for 10 seconds, 57°C for 5 seconds, and 72°C for 10 seconds; and 22 cycles of 94°C for 10 seconds, 57°C for 20 seconds, and 72°C for 40 seconds. PCR plates were centrifuged, and allele-specific FAM and VIC intensities were read on a spectrofluorometer (Tecan GENios™, Männedorf, Switzerland) at room temperature. Data were directly loaded and analyzed on KLIMST™ using Kluster Caller™.

Example 2: Identification of physical positions of QTLs and QTL intervals that are linked to SCN resistance genes

QTLs that are involved in SCN resistance were initially identified by studying the SCN literature. The initially identified SCN-associated QTLs found in the SCN literature are listed in FIGS. 1a and 1b.

From the list of QTLs that were initially identified in the SCN literature, several distinct QTL intervals that are involved in resistance to different SCN races were determined by reference to the soybean genome map. See, *e.g.*, FIG. 2. For example, QTL intervals on linkage group (LG) G were determined as shown in FIG. 3; QTL intervals on LG A₂ were determined as shown in FIG. 4; QTL intervals on LG B₁ were determined as shown in FIG. 5; and QTL intervals on LG I were determined as shown in FIG. 6. Table 2 lists exemplary QTLs and their corresponding determined QTL intervals that are associated with resistance to different SCN races.

**Example 3: Identification of SNP markers that are physically located
in/near/between the QTLs and QTL intervals that are
linked to SCN resistance genes**

5 The soybean genome was searched using BLAST™ for SNP markers that are physically located in, near, or between the QTL intervals that were determined. It was hypothesized that some of these SNP markers may be linked to the SCN resistance phenotype. A total of 79 SNP markers were selected for an initial screen using 24 soybean lines (14 SCN susceptible and 10 SCN resistant) to determine which, if any, of
10 these SNP markers are linked to the SCN resistance phenotype. 25 of the 79 markers were located on LG G, 12 of the markers were located on LG A₂, 22 of the markers were located on LG B₁, and 20 of the markers were located on LG I. All of the 79 selected markers are listed in Table 2.

15 Table 2: List of the 79 SNPs for screening with 24 soybean lines

Marker	SNP allele	Linkage group	Chromosome
BARC_018419_02911	[C/T]	A2	8
BARC_025811_05088	[C/T]	A2	8
BARC_040339_07714	[A/G]	A2	8
NCSB_001710	[A/T]	A2	8
NCSB_001716	[T/C]	A2	8
NCSB_001717	[A/C]	A2	8
NCSB_001718	[A/G]	A2	8
NCSB_001719	[A/C]	A2	8
BARC_007704_00081	[T/A]	B1	11
BARC_010169_00537	[C/T]	B1	11
BARC_013547_01157	[A/T]	B1	11
BARC_018557_03202	[A/G]	B1	11
BARC_018649_03221	[C/T]	B1	11
BARC_025703_04999	[C/G]	B1	11
BARC_035379_07178	[G/T]	B1	11
BARC_904050_01007	[A/T]	B1	11
NCSB_002644	[A/G]	B1	11
NCSB_002645	[A/G]	B1	11
NCSB_002646	[A/G]	B1	11

Marker	SNP allele	Linkage group	Chromosome
NCSB_002647	[A/T]	B1	11
NCSB_002648	[T/G]	B1	11
NCSB_002649	[T/C]	B1	11
NCSB_002650	[C/G]	B1	11
NCSB_002651	[T/C]	B1	11
NCSB_002652	[T/C]	B1	11
NCSB_002653	[A/C]	B1	11
NCSB_002654	[A/C]	B1	11
NCSB_002655	[A/C]	B1	11
NCSB_002656	[A/G}	B1	11
NCSB_002657	[A/C]	B1	11
BARC_003180_00257	[C/T]	G	18
BARC_010889_01691	[C/T]	G	18
BARC_012237_01755	[A/C]	G	18
BARC_015377_01829	[A/C]	G	18
BARC_027452_06569	[A/T]	G	18
BARC_028299_05817	[C/G]	G	18
BARC_035305_07162	[A/T]	G	18
BARC_G01475_00237	[A/C]	G	18
NCSB_004072	[A/G]	G	18
NCSB_004073	[A/G]	G	18
NCSB_004074	[A/C]	G	18
NCSB_004078	[A/G]	G	18
NCSB_004079	[C/G]	G	18
NCSB_004080	[C/G]	G	18
NCSB_004081	[A/G]	G	18
NCSB_004082	[T/C]	G	18
NCSB_004083	[A/G]	G	18
NCSB_004084	[A/T]	G	18
NCSB_004085	[A/T]	G	18
NCSB_004086	[A/T]	G	18
NCSB_004097	[T/C]	G	18
NCSB_004098	[T/C]	G	18
NCSB_004107	[T/G]	G	18
NCSB_004108	[A/C]	G	18

Marker	SNP allele	Linkage group	Chromosome
NCSB_004109	[C/G]	G	18
rhg1_2564	[G/-]	G	18
rhg1_3995	[A/C]	G	18
rhg1_689	[A/C]	G	18
rhg1_757	[T/C]	G	18
NCSB_004874	[A/G]	I	20
NCSB_004875	[T/G]	I	20
NCSB_004877	[A/T]	I	20
NCSB_004879	[A/G]	I	20
NCSB_004882	[A/T]	I	20
NCSB_004883	[A/G]	I	20
NCSB_004884	[T/G]	I	20
NCSB_004886	[T/C]	I	20
NCSB_004887	[T/G]	I	20
NCSB_004889	[A/G]	I	20
NCSB_004890	[T/C]	I	20
NCSB_004891	[T/C]	I	20
NCSB_004893	[T/C]	I	20
NCSB_004894	[T/C]	I	20
NCSB_004895	[A/G]	I	20
NCSB_004897	[T/C]	I	20
NCSB_004898	[A/T]	I	20
NCSB_004899	[A/C]	I	20
NCSB_004900	[A/T]	I	20
NCSB_004903	[T/G]	I	20

Example 4: KASPar™ assay development

Initial screening of the 79 SNP markers in the parental 24 lines was performed using KASPar™ genotyping assays. 75 of the SNP markers were validated.

21 SNP markers on LG G (Gm 18) were validated: NCSB_004072, BARC_015277_01929, NCSB_004073, NCSB_004074, NCSB_004075, NCSB_004076, NCSB_004077, NCSB_004078, NCSB_004079, NCSB_004080, NCSB_004081, NCSB_004082, NCSB_004083, NCSB_004084, NCSB_004085,

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NCSB_004086, NCSB_004097, NCSB_004098, NCSB_004107, NCSB_004108, and NCSB_004109.

12 SNP markers on LG A2 (Gm 08) were validated: BARC_025911_05089, BARC_019419_02921, NCSB_001710, NCSB_001711, NCSB_001712, NCSB_001713, NCSB_001714, NCSB_001715, NCSB_001716, NCSB_001717, NCSB_001718, and NCSB_001719.

22 SNP markers on LG B1 (Gm 11) were validated: NCSB_002644, NCSB_002645, NCSB_002646, NCSB_002647, NCSB_002648, NCSB_002649, NCSB_002650, NCSB_002651, NCSB_002652, NCSB_002653, NCSB_002654, NCSB_002655, NCSB_002656, NCSB_002657, BARC_007704_00091, BARC_010269_00537, BARC_904050_01007, BARC_019557_03202, BARC_018649_03221, BARC_025703_04999, BARC_013547_01157, and BARC_035379_07178.

20 SNP markers on LG I (Gm 20) were validated: NCSB_004974, NCSB_004975, NCSB_004977, NCSB_004979, NCSB_004882, NCSB_004883, NCSB_004884, NCSB_004886, NCSB_004887, NCSB_004889, NCSB_004880, NCSB_004882, NCSB_004883, NCSB_004884, NCSB_004885, NCSB_004887, NCSB_004888, NCSB_004899, NCSB_004900, and NCSB_004903.

The initial screening identified 44 of the SNP markers as polymorphic among the 24 parental lines. 24 polymorphic markers were selected (NCSB_001716 (LG A₂), NCSB_002645 (B₁), NCSB_002646 (B₁), NCSB_002648 (B₁), NCSB_002651 (B₁), NCSB_002652 (B₁), NCSB_002654 (B₁), NCSB_002656 (B₁), BARC_013547_01157 (B₁), NCSB_004073 (G), NCSB_004074 (G), NCSB_004078 (G), NCSB_004080 (G), NCSB_004084 (G), NCSB_004085 (G), NCSB_004097 (G), NCSB_004109 (G), BARC_012237_01755 (G), rgh1-689 (G), rgh1-757 (G), rgh1-2564 (G), rgh1-3995 (G), and NCSB_004900 (I)) for further linkage testing with mapping populations produced by crossing SCN resistant soybean variety 98860-71 with SCN susceptible soybean varieties 75213 and 6CH026-035. FIG. 7 shows representative genotyping data from the KASPar™ assay for four of the polymorphic markers.

15 of the SNP markers were polymorphic between SCN resistant and susceptible parents. These 15 polymorphic SNP markers were subsequently screened against 93 individuals in the mapping populations.

Of the fifteen SNP markers that were polymorphic among the parental lines that were tested against individuals of the mapping population, only three SNPs showed co-segregation with the SCN resistance trait: NCSB_004074, BARC_010889_01691, and rhg1-3995. FIG. 8. The KASPar™ primer sequences that were used to genotype individuals for these three markers are listed in Table 3.

Table 3: The KASPar™ primer sequences of SNP markers

rhg1-3995A1	GAAGGTGACCAAGTTCATGCTGGAATTATGTTGGGTTTTTTTTTCTTTCTGT (SEQ ID NO:1)
rhg1-3995A2	GAAGGTCGGAGTCAACGGATTGAATTATGTTGGGTTTTTTTTTCTTTCTGG (SEQ ID NO:2)
rhg1-3995C1	GCCCAGAAAAAAGGGATAAATAACGGATA (SEQ ID NO:3)
NCSB_004074A1	GAAGGTGACCAAGTTCATGCTATTATGTTGTAACACAAATTTGCACCTCAT (SEQ ID NO:4)
NCSB_004074A2	GAAGGTCGGAGTCAACGGATTATGTTGTAACACAAATTTGCACCTCAG (SEQ ID NO:5)
NCSB_004074C1	CTATACAATAAATCGTAATTCATTGTAT (SEQ ID NO:6)
BARC_010889-01691A1	GAAGGTGACCAAGTTCATGCTGAAAAAATAAAATTGATCATCATATGGTTAG (SEQ ID NO:7)
BARC_010889-01691A2	GAAGGTCGGAGTCAACGGATTGAAAAAATAAAATTGATCATCATATGGTTAA (SEQ ID NO:8)
BARC_010889-01691C1	TAAGTGAGGGCAATGTATTAGTATYAAGTA (SEQ ID NO:9)

Using the genome nucleic acid sequence of soybean cultivar Williams 82 as a reference, BARC_010889_01691 is located on chromosome 18 at 1,674,511 bp; NCSB_004074 is located on chromosome 18 at 1,663,671 bp; and rhg1-3995 is located on chromosome 18 at 1,714,741 bp. All three linked markers (NCSB_004074, BARC_010889_01691, and rhg1-3995) are located either within the *rhg1* locus (rhg1-3995), or close to it on linkage group G (BARC_010889_01691 and NCSB_004074).

For the resistant and medium resistant phenotypes, all three linked marker genotypes were congruent with the phenotype. With respect to the susceptible lines,

BARC_010889_01691 had 5 mismatches with phenotypes, NSCB_004074 had 9 mismatches, and rhg1-3995 had 6 mismatches. See Table 4.

Table 4: Comparison of the phenotype score and genotype scores of 93 lines derived from two mapping populations plus 3 parents. R = resistance; MR = medium resistance; S = susceptible; MS = medium susceptible; and X = inconsistency.

Sample	SCN Resistance	SCN Score	rhg1_3995	NCSB_004074	BARC_010889_01691
40779	R	1.00	C:C	A:A	T:T
40785	R	1.60	C:C	A:A	T:T
29110	R	2.30	C:C	A:A	T:T
40780	R	2.30	C:C	A:A	T:T
29148	R	2.50	C:C	A:A	T:T
40781	R	2.50	C:C	A:A	T:T
40799	R	2.50	C:C	A:A	T:T
29226	R	3.10	C:C	A:A	T:T
40910	R	4.00	C:C	A:A	T:T
29040	R	4.10	C:A	A:A	T:T
40908	R	4.50	C:C	A:A	T:T
19152	R	4.70	C:C	A:A	T:T
29149	R	4.80	C:C	A:A	T:T
29023	R	5.60	C:C	A:A	T:T
40907	R	5.80	C:C	A:A	T:T
40959	R	6.10	C:C	A:A	T:T
29181	R	6.50	C:C	A:A	T:T
29151	R	6.60	C:C	A:A	T:T
19209	R	6.90	C:C	A:A	T:T
40989	R	7.10	C:C	A:A	T:T
29189	R	7.60	C:C	A:A	T:T
21692	R	8.30	C:C	A:A	T:T
29089	R	8.80	C:C	A:A	T:T
21553	R	9.00	C:C	A:A	T:T
40833	R	9.20	C:C	A:A	T:T
21642	R	9.60	C:C	A:A	T:T
29228	R	9.70	C:C	A:A	T:T
40957	R	9.80	C:C	A:A	T:T

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Sample	SCN Resistance	SCN Score	rhg1_3995	NCSB_004074	BARC_010889_01691
29191	MR	10.2	C:C	A:A	T:T
40935	MR	10.7	C:C	A:A	T:T
98860-71(P1-R)	R/MR	11.3	C:C	A:A	T:T
21648	R/MR	11.8	C:C	A:A	T:T
19155	MR	12.4	C:C	A:A	T:T
40808	MR	12.6	C:C	A:A	T:T
40940	MR	13.0	C:C	A:A	T:T
40831	MR	13.7	C:C	A:A	T:T
40932	MR	14.5	C:C	A:A	T:T
29180	MR	16.9	C:C	A:A	T:T
40937	MR	17.9	C:C	A:A	T:T
40958	MR	19.6	C:C	A:A	T:T
40936	MR	21.5	C:C	A:A	T:T
41022	X	22.7	C:C	A:A	T:T
19182	MR	23.3	C:C	A:A	T:T
40810	X	27.5	C:C	A:A	T:T
41015	X	27.6	C:C	A:A	T:T
29039	MS	31.70	A:A	C:C	C:C
29212	MS	37.50	C:C	A:A	T:T
40905	X	39.70	A:A	A:A	C:C
40834	X	46.50	A:A	C:C	C:C
40906	MS	46.60	A:A	A:A	C:C
41016	MS	46.80	A:A	C:C	C:C
29179	MS	48.10	A:A	C:C	C:C
29119	MS	59.50	A:A	C:C	C:C
29021	MS	59.80	A:A	C:C	C:C
29142	S	61.90	A:A	C:C	C:C
40939	S	66.40	A:A	C:C	C:C
41026	S	67.90	C:C	C:C	C:C
40909	S	68.90	A:A	C:C	C:C
29150	S	70.60	A:A	C:C	C:C
40942	S	71.20	A:A	A:A	C:C
40711	S	71.50	C:C	A:A	T:T
40931	S	73.80	A:A	C:C	C:C

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Sample	SCN Resistance	SCN Score	rhg1_3995	NCSB_004074	BARC_010 889_01691
21633	S	75.00	C:C	A:A	T:T
29190	S	79.70	A:A	C:C	C:C
29229	S	79.90	A:A	C:C	C:C
40938	S	80.10	A:A	C:C	C:C
40873	X	81.50	C:C	A:A	T:T
29222	S	81.90	A:A	C:C	C:C
40941	S	84.00	A:A	C:C	C:C
40934	S	85.70	A:A	C:C	C:C
29376	S	88.90	A:A	C:C	C:C
75213(P2-S)	S	88.90	A:A	C:C	C:C
40990	S	90.10	A:A	C:C	C:C
29224	S	90.60	A:A	C:C	C:C
29399	S	93.50	A:A	C:C	C:C
40798	X	96.40	A:A	A:A	C:C
21693	X	98.00	A:A	C:C	C:C
21684	X	98.10	A:A	C:C	C:C
6CH026-035 (P3-S)	S	101.20	A:A	C:C	C:C
41014	S	109.40	A:A	C:C	C:C
41027	S	125.60	A:A	C:C	C:C
29514	S	130.00	A:A	C:C	C:C
21688	S	133.90	A:A	C:C	C:C
40992	S	134.40	A:A	C:C	C:C
21700	S	134.60	A:A	C:C	C:C
40991	S	138.80	A:A	C:C	C:C
40782	S	147.00	A:A	C:C	C:C
29639	S	163.20	A:A	C:C	C:C
40783	S	178.20	C:C	A:A	T:T
40778	S	187.40	A:A	C:C	C:C
21683	S	204.40	A:A	C:C	C:C
40809	S	207.70	A:A	C:C	C:C
40835	S	212.90	A:A	C:C	C:C
40832	S	215.20	A:A	C:C	C:C
21694	S	254.90	A:A	C:C	C:C
21698	S	380.00	A:A	C:C	C:C

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Once we have identified any susceptible genotypes with these 3 SNP markers, there is a 0% false negative rate. In other words, we can identify with perfect accuracy the SCN susceptible phenotype using the 3 markers. We also can predict the SCN resistant genotype with a “false positive” rate of about 10-18% (5 or 9 divided by 51, the total number of susceptible samples). Therefore, of the SCN resistant genotypes identified, only 5-9% of them would be expected to exhibit a SCN susceptible phenotype.

10

Example 5: SNP markers in LG A₂, LG B₁, and LG I that are linked to the SCN resistance phenotype

The soybean genome is searched using BLAST™ for SNP markers that are physically located in, near, or between QTL intervals associated with SCN resistance on linkage groups A₂, B₁, and I. A list of SNP markers is produced by the BLAST™ search. A plurality of SNP markers are selected for an initial screen using SCN susceptible and SCN resistant soybean lines to determine which, if any, of these SNP markers in linkage groups A₂, B₁, and I are linked to the SCN resistance phenotype.

Initial screening of the selected SNP markers in the parental lines is performed using KASPar™ genotyping assays. A set of the selected SNP markers are validated, a subset of which are identified as polymorphic among the parental lines. At least one of the polymorphic SNP markers is/are used for linkage testing with mapping populations produced by crossing an SCN resistant soybean variety with one or more SCN susceptible soybean varieties. One or more of these polymorphic SNP markers are screened against individuals in the mapping populations.

SNPs that co-segregate with the SCN resistance trait in individuals of the mapping populations are identified as markers on linkage groups A₂, B₁, and I that are linked to SCN resistance in the SCN resistant parent variety. The linked marker genotypes match the phenotypes observed in the individuals of the mapping population.

30

Example 6: SNP markers linked to the SCN resistance phenotype in germplasm JTN-5109

A plurality of SNP markers that are physically located in, near, or between QTL intervals associated with SCN resistance (for example, SNP markers selected from the group of markers listed in Table 3) are selected for an initial screen using SCN resistant soybean variety JTN-5109 and SCN susceptible soybean lines to determine which, if any, of these SNP markers are linked to the SCN resistance phenotype in soybean variety JTN-5109.

Initial screening of the selected SNP markers in the parental lines is performed using KASPar™ genotyping assays. A set of the selected SNP markers are validated, a subset of which are identified as polymorphic among soybean variety JTN-5109 and the SCN susceptible parental lines. At least one of the polymorphic SNP markers is/are used for linkage testing with mapping populations produced by crossing soybean variety JTN-5109 with one or more SCN susceptible soybean varieties. These one or more polymorphic SNP markers are screened against individuals in the mapping populations.

SNPs that co-segregate with the SCN resistance trait in individuals of the mapping populations are identified as markers that are linked to SCN resistance in soybean variety JTN-5109. The linked marker genotypes match the phenotypes observed in the individuals of the mapping population.

Example 7: SNP markers that are linked to the SCN resistance phenotype in *HG* races other than race 3

Mapping populations are developed specifically for an *HG* race other than race 3 by crossing an SCN resistant soybean variety selected from the group consisting of PI 88788, Peking, PI 437654, PI 90763, PI 438489B, PI 89772, PI209332, PUSCN14, Hartwig, Forrest, and Pyramid with one or more SCN susceptible soybean varieties.

The soybean genome is searched using BLAST™ for SNP markers that are physically located in, near, or between QTL intervals associated with SCN resistance with respect to the specific *HG* race. A list of SNP markers is produced by the BLAST™ search. A plurality of SNP markers are selected for an initial screen using the selected SCN resistant soybean variety and SCN susceptible varieties to determine

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which, if any, of the SNP markers are linked to the SCN resistance phenotype with respect to the specific *HG* race.

Initial screening of the selected SNP markers in the parental lines is performed using KASPar™ genotyping assays. A set of the selected SNP markers are validated, a
5 subset of which are identified as polymorphic among the parental lines. At least one of the polymorphic SNP markers is/are used for linkage testing with mapping populations produced by crossing the selected SCN resistant soybean variety with one or more SCN susceptible soybean varieties. These one or more polymorphic SNP markers are screened against individuals in the mapping populations.

10 SNPs that co-segregate with the SCN resistance trait in individuals of the mapping populations are identified as markers that are linked to SCN resistance in the SCN resistant parent variety with respect to the specific *HG* race. The linked marker genotypes match the phenotypes observed in the individuals of the mapping population.

15

CLAIMS

What may be claimed is:

- 5 1. A method for identifying a plant comprising at least one determinant of
SCN resistance in a soybean variety, the method comprising:
isolating nucleic acid molecules from a plant; and
screening the isolated nucleic acid molecules for a marker linked to the SCN resistance
phenotype in the soybean variety or an equivalent thereof, wherein the marker
10 is genetically linked to a marker selected from the group consisting of
NCSB_004074, BARC_010889_01691, and rhg1-3995, and the presence of
the marker is indicative of at least one determinant of SCN resistance in the
soybean variety.
- 15 2. The method according to claim 1, wherein the marker linked to the
SCN resistance phenotype in the soybean variety or equivalent thereof is in a soybean
linkage group (LG) selected from the group consisting of LG G, LG A₂, LG B₁, and
LG I.
- 20 3. The method according to claim 2, wherein the marker linked to the
SCN resistance phenotype in the soybean variety or equivalent thereof is in soybean
linkage group G.
4. The method according to claim 1, wherein the isolated nucleic acid
25 molecules are genomic DNA.
5. The method according to claim 1, wherein the SCN resistance
phenotype is SCN resistance to SCN race 3.
- 30 6. The method according to claim 1, wherein the soybean variety is
soybean variety 98860-71.

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7. The method according to claim 6, wherein the marker linked to the SCN resistance phenotype in the soybean variety or equivalent thereof is selected from the group consisting of NCSB_004074, BARC_010889_01691, and rhg1-3995.

5 8. The method according to claim 1, wherein screening the isolated nucleic acid molecules for a marker linked to the SCN resistance phenotype in the soybean variety or equivalent thereof is performed using competitive allele-specific polymerase chain reaction.

10 9. The method according to claim 6, wherein the at least one determinant of SCN resistance in the soybean variety is *rhg1*.

10. The method according to claim 1, further comprising determining the genotype of the plant for the marker linked to the SCN resistance phenotype in the
15 soybean variety or equivalent thereof.

11. A plant identified by the method according to claim 1.

12. A plant comprising the at least one determinant of SCN resistance in a
20 soybean variety of claim 1, wherein the plant is not *Glycine max*.

13. A method for producing an SCN resistant soybean plant, the method comprising:
crossing a soybean plant having the trait of SCN resistance with a soybean plant from a
25 soybean variety of interest;
using marker-assisted selection to identify an F₁ soybean plant comprising a marker
linked to the SCN resistance phenotype in the soybean plant having the trait of
SCN resistance or equivalent thereof, wherein the marker is genetically linked
to a marker selected from the group consisting of NCSB_004074,
30 BARC_010889_01691, and the F₁ soybean plant has any desirable traits of the
soybean variety of interest; and

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propagating the identified F₁ soybean plant, thereby producing an SCN resistant soybean plant.

14. The method according to claim 13, wherein the marker linked to the
5 SCN resistance phenotype in the soybean plant having the trait of SCN resistance or equivalent thereof is in a soybean linkage group (LG) selected from the group consisting of LG G, LG A₂, LG B₁, and LG I.

15. The method according to claim 14, wherein the marker linked to the
10 SCN resistance phenotype in the soybean plant having the trait of SCN resistance or equivalent thereof is in soybean linkage group G.

16. The method according to claim 13, wherein the soybean variety of
interest is an SCN susceptible soybean variety.

15

17. The method according to claim 13, wherein the SCN resistance is
resistance to SCN race 3.

18. The method according to claim 13, wherein the soybean plant having
20 the trait of SCN resistance is a soybean plant of variety 98860-71.

19. The method according to claim 18, wherein the marker linked to the
SCN resistance phenotype in the soybean plant having the trait of SCN resistance or
equivalent thereof is selected from the group consisting of NCSB_004074,
25 BARC_010889_01691, and rhg1-3995.

20. The method according to claim 13, wherein marker-assisted selection is
performed using competitive allele-specific polymerase chain reaction.

30 21. An SCN resistant soybean plant produced by the method according to
claim 13.

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22. A method for introducing SCN resistance to a soybean plant, the method comprising introducing a marker linked to the SCN resistance phenotype in soybean variety 98860-71 or an equivalent thereof into an SCN susceptible soybean plant.

5

23. The method according to claim 22, wherein the marker linked to the SCN resistance phenotype in soybean variety 98860-71 or an equivalent thereof is selected from the group consisting of NCSB_004074, BARC_010889_01691, and rhg1-3995.

10

24. A nucleic acid probe substantially identical to a probe selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, and SEQ ID NO:9.

15

25. A nucleic acid probe that is specifically hybridizable to a stretch of contiguous nucleotides in the Williams 82 soybean genome comprising base pair 1,674,511 on chromosome 18.

20

26. A nucleic acid probe that is specifically hybridizable to a stretch of contiguous nucleotides in the Williams 82 soybean genome comprising base pair 1,663,671 on chromosome 18.

25

27. A nucleic acid probe that is specifically hybridizable to a stretch of contiguous nucleotides in the Williams 82 soybean genome comprising base pair 1,714,741 on chromosome 18.

30

28. A method for producing an SCN resistant plant, the method comprising:
introducing into a plant of interest at least one means for providing SCN resistance in soybean, thereby producing an SCN resistant plant.

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29. The method of claim 28, further comprising propagating the plant of interest.

30. The method of claim 28, wherein the plant of interest is a legume.

5

31. The method of claim 30, wherein the plant of interest is selected from the group consisting of soybean, green beans, snap beans, dry beans, red beans, lima beans, mung beans, bush beans, Adzuki beans, garden peas, and cowpeas.

10 32. A method for identifying a plant comprising at least one determinant of SCN resistance in a soybean variety, the method comprising:
isolating nucleic acid molecules from a plant; and
contacting the isolated nucleic acid molecules with means for identifying soybean
plants having the SCN resistance phenotype to produce a detectable signal that
15 is indicative of the presence of at least one determinant of SCN resistance in a soybean variety in the plant.

33. A method for transferring at least one determinant of SCN resistance in a soybean variety, the method comprising:

- 20 (a) analyzing with probes that are specifically hybridizable to at least one marker that is linked to the SCN resistance phenotype in the soybean variety or an equivalent thereof the genomic DNA of a first plant with a donor genotype and the DNA of a second plant with a recipient genotype;
- (b) sexually crossing the two parental plant genotypes to obtain a progeny
25 population;
- (c) analyzing the progeny population for the presence of the at least one marker that is linked to the SCN resistance phenotype in the soybean variety or an equivalent thereof;
- (d) backcrossing an individual from the progeny population that comprises the at
30 least one marker that is linked to the SCN resistance phenotype in the soybean variety or an equivalent thereof to the recipient genotype to produce a next generation population;

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- (e) determining if a member of the next generation population comprises a desired trait from the recipient genotype and the marker that is linked to the SCN resistance phenotype in the soybean variety or an equivalent thereof; and
- (f) if no member of the next generation population comprises the desired trait from the recipient genotype and the marker that is linked to the SCN resistance phenotype in the soybean variety or an equivalent thereof, repeating steps (d) and (e) until an individual is identified that comprises the desired trait from the recipient genotype and the marker that is linked to the SCN resistance phenotype in the soybean variety or an equivalent thereof.

10

34. The method of 33, wherein individual progeny obtained in each crossing and backcrossing step are selected by SCN marker analysis at each generation.

35. The method according to claim 33, wherein the soybean variety is soybean variety 98860-71.

36. A method for introducing at least one determinant of SCN resistance in a soybean variety into a host organism by genetic transformation, the method comprising:

20 analyzing the genomic DNA of a plant with a probe that is specifically hybridizable to a marker linked to the SCN resistance phenotype in the soybean variety or an equivalent thereof to identify the at least one determinant of SCN resistance in the soybean variety in the plant;

isolating a segment of the genomic DNA of the plant that specifically hybridizes to the probe that is specifically hybridizable to a marker linked to the SCN resistance phenotype in the soybean variety or an equivalent thereof;

25 introducing the isolated segment of genomic DNA into the host organism; and analyzing the DNA of the host organism with a probe that is specifically hybridizable to a marker linked to the SCN resistance phenotype in the soybean variety or an equivalent thereof to identify the at least one determinant of SCN resistance in the soybean variety in the host organism.

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37. The method according to claim 36, wherein the isolated segment of DNA is stably integrated into the genome of the host organism.

38. The method according to claim 37, wherein the host organism is a
5 legume.

39. The method according to claim 38, wherein the host organism is selected from the group consisting of soybean, green beans, snap beans, dry beans, red beans, lima beans, mung beans, bush beans, Adzuki beans, garden peas, and cowpeas..

10

LG	Predicted gene	Race significant	Peak marker interval	Marker type	Cross	Source	Percent effect	LOD	P-value	Authors	Reference
A1	unknown	3	A487	RELP	Williams S2 x (Harwig)	Peeling, PI437554	1.3	not available	0.0006	Welling et al., 1996	Theor. Appl. Genet. 92:83-86
A1	unknown	2	A262-5a550	RELP/SSR	Hamilton x PI436465	PI436465	7.4	2.9	not available	Yue et al., 2003a	Theor. Appl. Genet. 102:921-928
A2	Rhg4	not available	PBL724_05-TEE	RELP	P 35C115 x BARC21R(4)	not available	not available	not available	not available	Melism et al., 1992	Theor. Appl. Genet. 95:136-138
A2	Rhg4	3	PBL755a	RELP	Essex x Forrest	Peeling	24	not available	0.0001	Cheng et al., 1997	Crop Sci. 37:955-971
A2	Rhg4	3	h305	Morphological	(Peeling) x Essex	Peeling	25	not available	0.0001	Mahalingam and Okunribido, 1995	Genes. Mol. 45:425-442
A2	Rhg4	2	EscoV266405	AFLP	(Forrest) x Essex	Peeling	15	not available	0.0001	Melism et al., 2001	Theor. Appl. Genet. 102:713-717
A2	Rhg4	1.3	SCAR545553	SCAR	US71331 x P437554	Peeling, PI437554, P437553	115-118.9	not available	0.0001	Heer et al., 1998	Mol. Breed. 4:352-357
A2	Rhg4	3	A395	RELP	W35-15 x (H86-1430)	PI206332	15	2.9	0.002	Cordoba et al., 1994	Crop Sci. 34:340-346
A2	Rhg4	2	18205	Morphological	ESR1(1) x (PI437554)	PI437554	9.0	not available	0.005	Wells et al., 1992	Theor. Appl. Genet. 91:571-581
A2	Rhg4	1.3	PBL755a	RELP	ESR1(1) x (PI437554)	PI437554	15.0-18.0	not available	0.0001	Webb, 2003	U.S. Patent 6,536,175 B1
A2	unknown	1	8aM167-AM153402		Essex x P437554	Essex, PI437554	5.1		0	Wu et al., 2005	Theor. Appl. Genet. 118:1051-1055
A2	unknown	2	EsacV266405_3aM167		Essex x P437554	Essex, PI437554			0	Wu et al., 2005	Theor. Appl. Genet. 118:1051-1055
A2	Rhg4	1.3	8aM134-2aM532-8aL405	SSR	PI404155A x Magellan	PI404155A	9	5.5		Guo et al., 2005	Crop Sci. 45:224-233
A2		3	8aL400-2aM424	SSR	Hamilton x PI30753	PI30753	17.7	14.5		Guo et al., 2005	Theor. Appl. Genet. 111:565-571
B1	unknown	3	A305	RELP	Williams S2 x (Harwig)	Peeling, PI437554	91	not available	0.0001	Welling et al., 1995	Theor. Appl. Genet. 92:82-85
B1	unknown	1.2, 5	A305-2aM533	RELP/SSR	Hamilton x (PI30753)	PI30753	5.8-15.6	2.9-5.3	not available	Yue et al., 2003b	Crop Sci. 41:1535-1555
B1		1.5	3aM555-2aM1C1037		Essex x P437554	Essex, PI437554	2.7-11.0		0	Wu et al., 2005	Theor. Appl. Genet. 118:1051-1055
B1		2.1	EscoV266405_3aM154		Essex x P437554	Essex, PI437554	1.1		0	Wu et al., 2005	Theor. Appl. Genet. 118:1051-1055
B1		2.5	3aM453	SSR	PI404155A x Magellan	PI404155A	11-13	3.5-6.7		Guo et al., 2005	Crop Sci. 45:224-233
B1		2.2	3aM452-2aM553	SSR	Hamilton x PI30753	PI30753	5.7-11.2	5.5-6.0		Guo et al., 2005	Theor. Appl. Genet. 111:565-571
B1	unknown	1.2, 5	3aM522-2aL123	SSR	Hamilton x (PI436465)	PI436465	7.4-12.7	2.7-4.2	not available	Yue et al., 2003a	Theor. Appl. Genet. 102:921-928
B1 (L25)	unknown	1.2, 5, 14	A567a	RELP	ESR1(1) x (PI437554)	PI437554	2.0-7.0	not available	0.0001	Webb, 2003	U.S. Patent 6,536,175 B1

FIG. 1a

B1 (B)	unknown	3	ASST		RFLP	Williams S2 x (Hartwig)	Peking, PI437654	1.0	not available	0.0001	Vierling et al., 1995	Theor. Appl. Genet. 92:93-95
B2	unknown	1.3	ASB3		RFLP	(Peking) x Essex	Peking	9.2-21.0	not available	0.005-0.0001	Qui et al., 1999	Theor. Appl. Genet. 98:355-354
B2	unknown	1.3	Salt168-A329		RFLP/PCR	Hamilton x (PI4354855)	PI4354855	8.1-11.7	2.5-2.8	not available	Yue et al., 2003a	Theor. Appl. Genet. 102:921-928
C1	unknown	2.14	A453-Q46356		RFLP/PCR	Hamilton x (PI4354856)	PI4354856	10.2-11.1	2.5-3.5	not available	Yue et al., 2003a	Theor. Appl. Genet. 102:921-928
C1	unknown	1.2	PHC2550		RFLP	ESR101 x (PI437654)	PI437654	1.3-2.0	not available	0.02	Wang et al., 2003	U.S. Patent 6,338,175 B1
C1	unknown	3	Salt140-E46404175		RFLP	Essex x PI437654	Essex, PI437654			0	Wu et al., 2005	Theor. Appl. Genet. 115:1052-1105
C2	unknown	3	A125_1		RFLP	PI455515 x (A81255022)	A81255022	5.0	4.4	not available	Wang et al., 2001	Theor. Appl. Genet. 103:551-556
C2	unknown	3	ASB5		RFLP	(Peking) x Essex	Peking	8.0	not available	0.005	Mahalingam and Skomposka, 1995	Breeding Sci. 45:435-443
C2	unknown	1.4	A426		RFLP	(87-233) x Hutcheson	Peking, PI35755, PI9763	6.5	not available	0.006	Heer et al., 1998	Mol. Breed. 4:359-357
C2	unknown	1.5	Salt127-Q46202		PCR	Hamilton x (PI4354856)	P4354856	7.1-8.3	3.2-6.3	not available	Yue et al., 2003a	Theor. Appl. Genet. 102:921-928
D1a	unknown	2.5-1.4	A336-K457		RFLP	Hamilton x (PI4354856)	P4354856	7.4-10.7	4.1-6.5	not available	Yue et al., 2003a	Theor. Appl. Genet. 102:921-928
D1a	unknown	5	Salt142-Q46355		PCR	Hamilton x (PI437654)	P437654	7.8	3.3	not available	Yue et al., 2003b	Crop Sci. 41:1555-1555
D1a	unknown	2	ANT61265-Q46356		RFLP/PCR	Essex x PI437654	Essex, PI437654		not available	0.000038	Wu et al., 2003	Theor. Appl. Genet. 115:1052-1105
D1a	unknown	3	Salt172-Q46119		RFLP	Essex x PI437654	Essex, PI437654		not available	0	Wu et al., 2003	Theor. Appl. Genet. 115:1052-1105
D2	unknown	1.4	Salt162		PCR	(Hartwig) x ER 92-21583	Peking, PI437654	41.1	not available	40.0001	Schuster et al., 2001	Theor. Appl. Genet. 102:921-928
D2	unknown	6	A354-2		RFLP	Evans x (P437654)	P437654	11	not available	0.001	Cordisco et al., 1995b	Crop Sci. 35:1543-1550
D2	unknown	1	B132-Q46371		RFLP/PCR	Hamilton x (PI437654)	P437654	9.7	4.5	not available	Yue et al., 2003b	Crop Sci. 41:1555-1555
E	unknown	2.14	A555-Q46451		RFLP/PCR	Hamilton x (PI4354855)	P4354855	8.0-18.7	2.5-5.0	not available	Yue et al., 2003a	Theor. Appl. Genet. 102:921-928
E	unknown	3	Eng107-Q4655_1		RFLP	(PI455515) x A81255022	P455515	23	3.1	not available	Wang et al., 2003	Theor. Appl. Genet. 103:551-556
E	unknown	3	A135-Q46231		RFLP/PCR	Hamilton x (PI437654)	P437654	15.7	3.5	not available	Yue et al., 2003b	Crop Sci. 41:1555-1555
E	unknown	5	Salt575-Q46234		PCR	Hamilton x PI30753	P30753	12.5	7.2		Gao et al., 2005	Theor. Appl. Genet. 111:565-571
E	unknown		Essex/MS125-B46111230		RFLP/PCR	G503 PI455515 x A81255022	P455515	0.84		<0.0001	Katella et al., 2005	Crop Sci. 45:2473-2491
F	unknown	3	G15		RFLP	(Peking) x Essex	Peking	6.0	not available	0.02	Mahalingam and Skomposka, 1995	Breeding Sci. 45:435-443
F	unknown	3	A112		RFLP	Williams S2 x (Hartwig)	Peking, PI437654	1.0	not available	0.002	Vierling et al., 1995	Theor. Appl. Genet. 92:93-95
F	unknown	1.5	KD02		RFLP	(87-233) x Hutcheson	Peking, PI35755, PI9763	5.5-7.2	not available	0.02	Heer et al., 1998	Mol. Breed. 4:359-357

FIG. 1a (cont.)

G	Fig. 1	3	K069	RELIP	M35-15 x M25-1433	P. 205332	15	not available	0.003	Corcibko et al., 1994	Comp. Sci. 34:240-246
G	Fig. 1	3	O103450	RAPD	Essex x (Farreri)	Peking	14	not available	0.0001	Chang et al., 1997	Comp. Sci. 37:565-571
G	Fig. 1	1,2,6	C006	RELIP	Essex x (Peking)	Peking	17.2-16.2	not available	<0.0001	Corcibko et al., 1997	Comp. Sci. 37:459-464
G	unknown	1,2,6	A376	RELIP	Essex x (Peking)	Peking	10.2-17.5	not available	0.001-0.0001	Corcibko et al., 1997	Comp. Sci. 37:459-464
G	Fig. 1	3	Essex/K069	ASLP	(Farreri) x Essex	Peking	25.2	not available	0.0001	Mattson et al., 2001	Theor. Appl. Genet. 103:715-717
G	Fig. 1	3	S00356	2BR	Flier x (Hartwig)	Peking, P. 437654	29	not available	0.0001	Frasnu et al., 1999	Comp. Sci. 36:482-487
G	Fig. 1	1,2	K069	RELIP	(J37-233) x Huxfordson	Peking, P. 437654, P. 437655	5.3-11.3	not available	0.07-0.002	Heer et al., 1998	Mol. Breed. 4:349-357
G	Fig. 1	1,2,6	C006	RELIP	Essex x (P. 205332)	P. 205332	35.2-54.0	not available	0.0001	Corcibko et al., 1995b	Comp. Sci. 36:1643-1650
G	Fig. 1	3	PhC031543	RELIP	BOR101 x (P. 437654)	P. 437654	22	not available	<0.05	Wade et al., 1995	Theor. Appl. Genet. 91:574-581
G	Fig. 1	1,2,3,14	PhC031543	RELIP	BOR101 x (P. 437654)	P. 437654	10.3-43.0	not available	0.0001	Wade, 2003	U.S. Patent 6,533,175 B1
G	unknown	1,2,3,5	S011-30-507312	3BR	Hamilton x (P. 438485)	P. 438485	5.8-15.8	2.3-9.1	not available	Xue et al., 2003a	Theor. Appl. Genet. 102:521-529
G	unknown	3	S011356-507472	RELIP	(P. 438485) x A3-155022	P. 438485	27	3.5	not available	Wang et al., 2001	Theor. Appl. Genet. 103:551-556
G	Fig. 1	2,6	C006	RELIP	Essex x (P. 437654)	P. 437654	25.2-26.3	not available	<0.0001	Corcibko et al., 1997	Comp. Sci. 37:253-264
G	Fig. 1	1,2,3,5	B051-S00309	RELIP/3BR	Hamilton x (P. 437654)	P. 437654	4.5-25.6	2.5-13.7	not available	Yue et al., 2003b	Comp. Sci. 41:1595-1596
G	Fig. 1	1,2,6	C006	RELIP	Essex x (P. 437654)	P. 437654	15.3-44.9	15.9	<0.0001	Corcibko et al., 1997	Comp. Sci. 37:253-264
G	Fig. 1	3	S011356-507472	RELIP	Essex x (P. 437654)	Essex x (P. 437654)			0	Wu et al., 2005	Theor. Appl. Genet. 118:1031-1105
G	Fig. 1	2,3,5	P011-H05-S01235	RELIP	Essex x (P. 437654)	Essex x (P. 437654)	15.5-17.0-26.5		0	Wu et al., 2005	Theor. Appl. Genet. 118:1031-1105
G	Chr. 10/11/12	Chr. 10/11/12	S011356-507472	2BR	S011356-507472 x P. 437654 (S. 5081)	S. 5081	10.1	2.45		Wang et al., 2007	Theor. Appl. Genet. 114:451-473
G	Fig. 1	3	S011356-507472	3BR	PhC031543 x Huxfordson	P. 437654	20.2	10		Guo et al., 2006	Comp. Sci. 46:224-233
G	Fig. 1	2,5	S011356-507472	3BR	PhC031543 x Huxfordson	P. 437654	12.5-13	7.1-13.3		Guo et al., 2006	Comp. Sci. 46:224-233
G	Fig. 1	2,3,5	S011356-507472	3BR	Hamilton x (P. 437654)	P. 437654	14.7-22.1-13.0	7.9-22.1-7.1		Guo et al., 2005	Theor. Appl. Genet. 111:565-571
G			Essex/S011356-507472	AFLP/3BR	G. 4323 x (P. 438485) x A3-155022	P. 438485	92	<0.0001		Kaballa et al., 2005	Comp. Sci. 45:2473-2481
G			Essex/S011356-507472	AFLP/3BR	G. 4323 x (P. 438485) x A3-155022	P. 438485	0.81	<0.0001		Kaballa et al., 2005	Comp. Sci. 45:2473-2481
H	unknown	1,3	B072	RELIP	(Peking) x Essex	Peking	5.0-13.0	not available	0.001-0.002	Guo et al., 1999	Theor. Appl. Genet. 98:355-364
H		4	S011356-507472		Essex x (P. 437654)	Essex, P. 437654	1		0.00052	Wu et al., 2005	Theor. Appl. Genet. 118:1031-1105

FIG. 1b

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I	unknown	♀	KD11	REL	(Peking) x Essex	Peking	11	not available	0.001	Qui et al., 1999	Ther. Appl. Genet. 99:255-264
I		Ruby-en(7)	Gall102-Gall148	30R	30C-30(G.max) x P454-9355 (G. 503)	G 503	6.7	2.57		Winter et al., 2007	Ther. Appl. Genet. 114:461-472
I		2.5.14	Gall_255-Gall_195		Essex x P437554	Essex, P437554	2.5-2.3		0	Va et al., 2005	Ther. Appl. Genet. 118:193-195
I		Chatham	Gall102-Gall133	30R	30C-30(G.max) x P454-9355 (G. 503)	G 503	7.6	2.75		Winter et al., 2007	Ther. Appl. Genet. 114:461-472
J	unknown	3	B032	REL	M81-15 x (M85-1433)	P 209332	38	5.1	<0.0001	Corcibao et al., 1994	Crop Sci. 34:240-246
J	unknown	3	B032	REL	Evans x (P 209332)	P 209332	36	not available	<0.0001	Corcibao et al., 1996	Crop Sci. 36:1643-1650
J	unknown	3	B032	REL	Evans x (P 30753)	P 30753	18.8	not available	<0.0001	Corcibao et al., 1997	Crop Sci. 37:255-264
J (L35)	unknown	2.1.4	K079	REL	B08101 x (P 437554)	P 437554	3.0-5.0	not available	0.0001	Webb, 2003	U.S. Patent 5,533, 175 B1
J		2.3	Gall547-Gall_224	30R	Hamilton x P130757	P 30757	7.8-11.2	4.5-1.5		Gui et al., 2005	Ther. Appl. Genet. 111:555-571
L	unknown	4	A013	REL	Evans x (P 209332)	P 209332	24	not available	0.002	Corcibao et al., 1996	Crop Sci. 36:1643-1650
L		3	Gall_286-Gall229	30R	Hamilton x P130757	P 30757	4	3		Gui et al., 2005	Ther. Appl. Genet. 111:555-571
M	unknown	1.2.5	A131	REL	(J87-233) x Hutchinson	Peking, P138758, P30753	5.4-6.9	not available	0.02	Heer et al., 1998	Mol. Breed. 4:355-357
M	unknown	3	ph0203018	REL	B08101 x (P 437554)	P 437554	7.0	not available	<0.05	Webb et al., 1999	Ther. Appl. Genet. 91:474-481
M	unknown	1.2.5.14	ph0203018	REL	B08101 x (P 437554)	P 437554	0.0-14.0	not available	0.0001	Webb, 2003	U.S. Patent 5,533, 175 B1
N	unknown	6 or 21	A230	REL	Evans x (Peking)	Peking	14.3	not available	<0.0001	Corcibao et al., 1997	Crop Sci. 37:255-264
N		5	Gall_286-Gall249	30R	P404198A x flagellon	P 404198A	9.5	3		Gui et al., 2005	Crop Sci. 45:224-233
K		1	Gall55-Gall39		Essex x P437554	Essex, P437554	1		0.000001	Va et al., 2005	Ther. Appl. Genet. 118:193-195
K		Chatham	Gall56-Gall195	30R	30C-30(G.max) x P454-9355 (G. 503)	G 503	7.1	2.05		Winter et al., 2007	Ther. Appl. Genet. 114:461-472
O		Ruby-en	Gall17-Gall455	30R	30C-30(G.max) x P454-9355 (G. 503)	G 503	5.1	2.02		Winter et al., 2007	Ther. Appl. Genet. 114:461-472

FIG. 1b (cont.)

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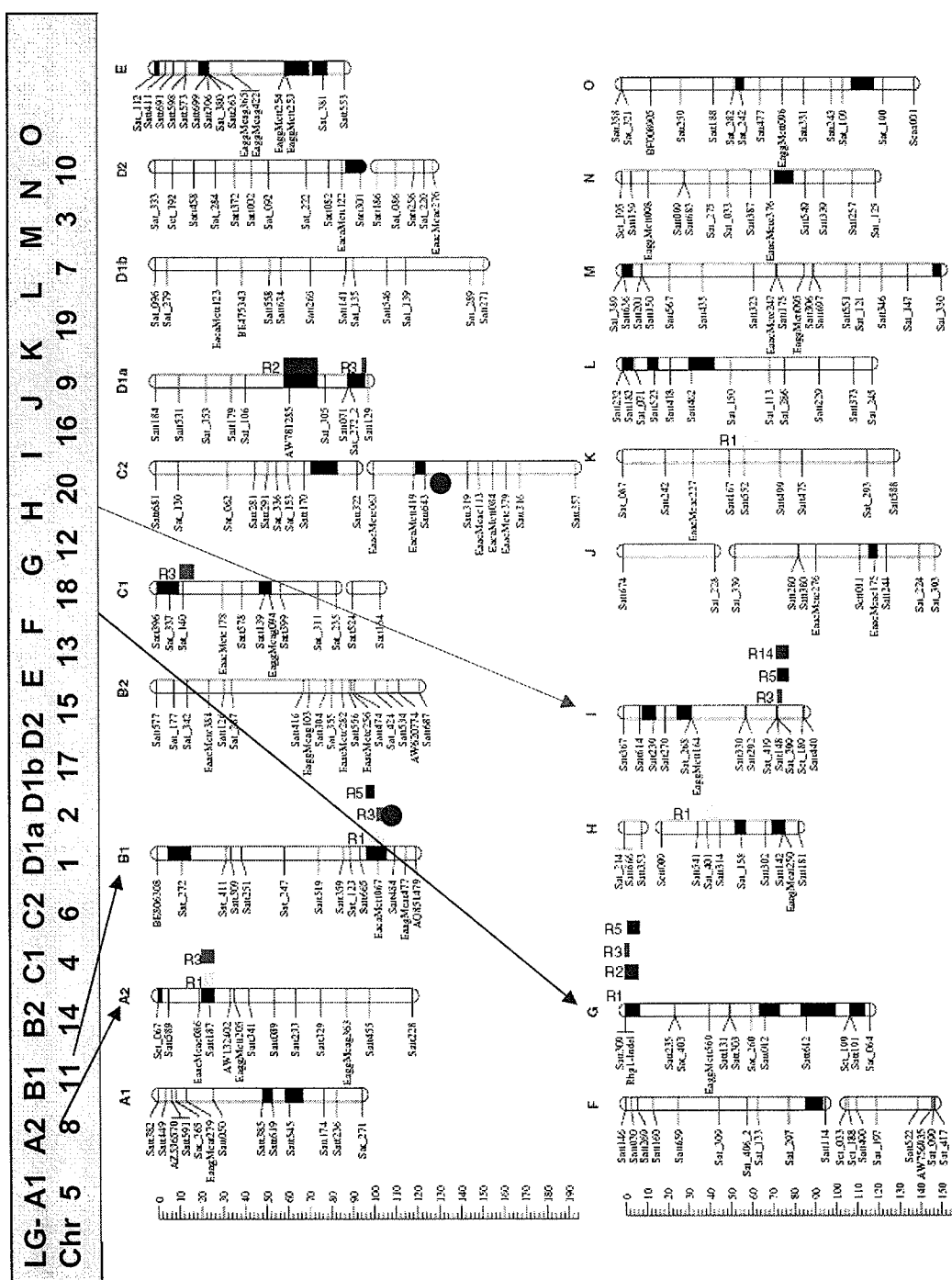


FIG. 2

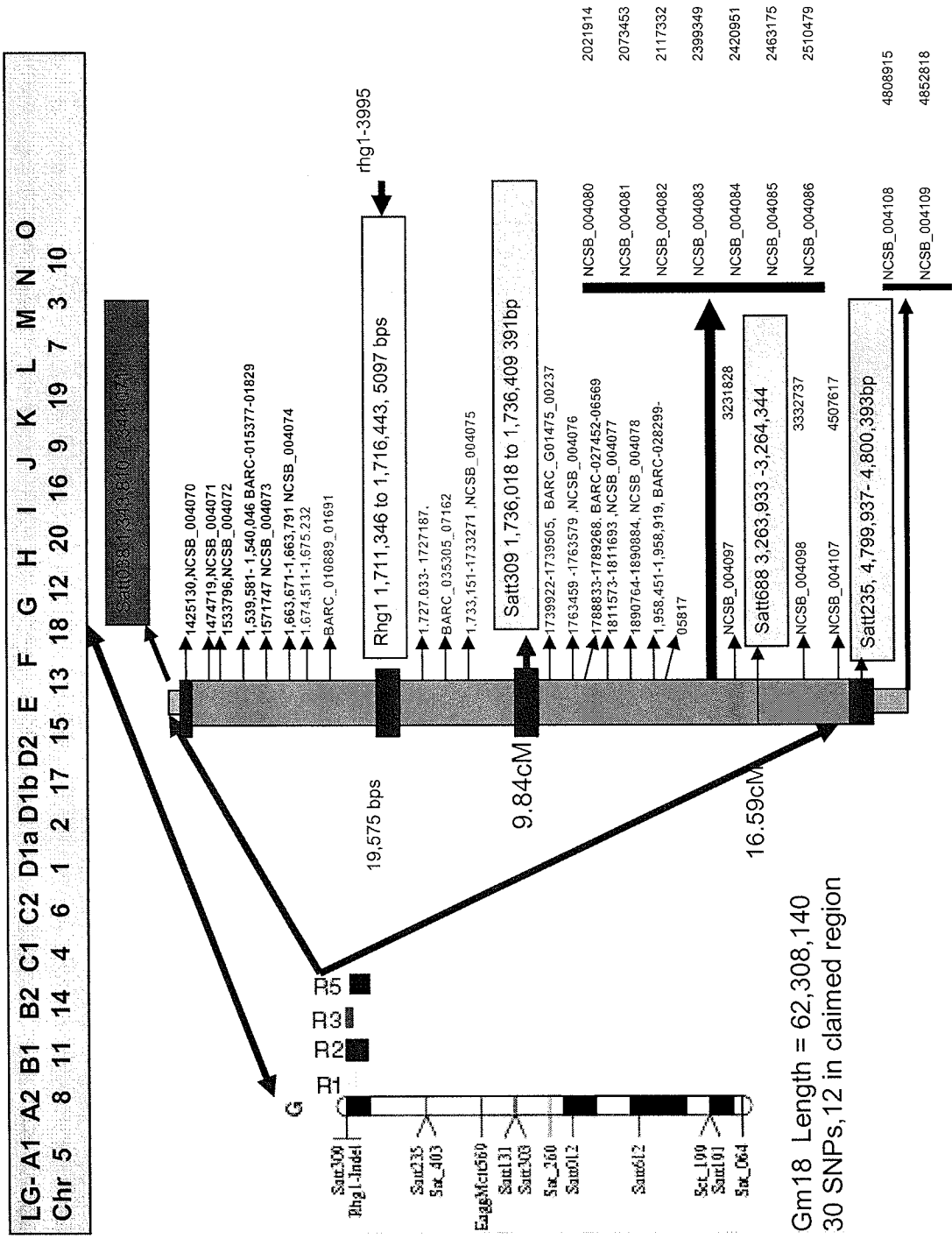


FIG. 3

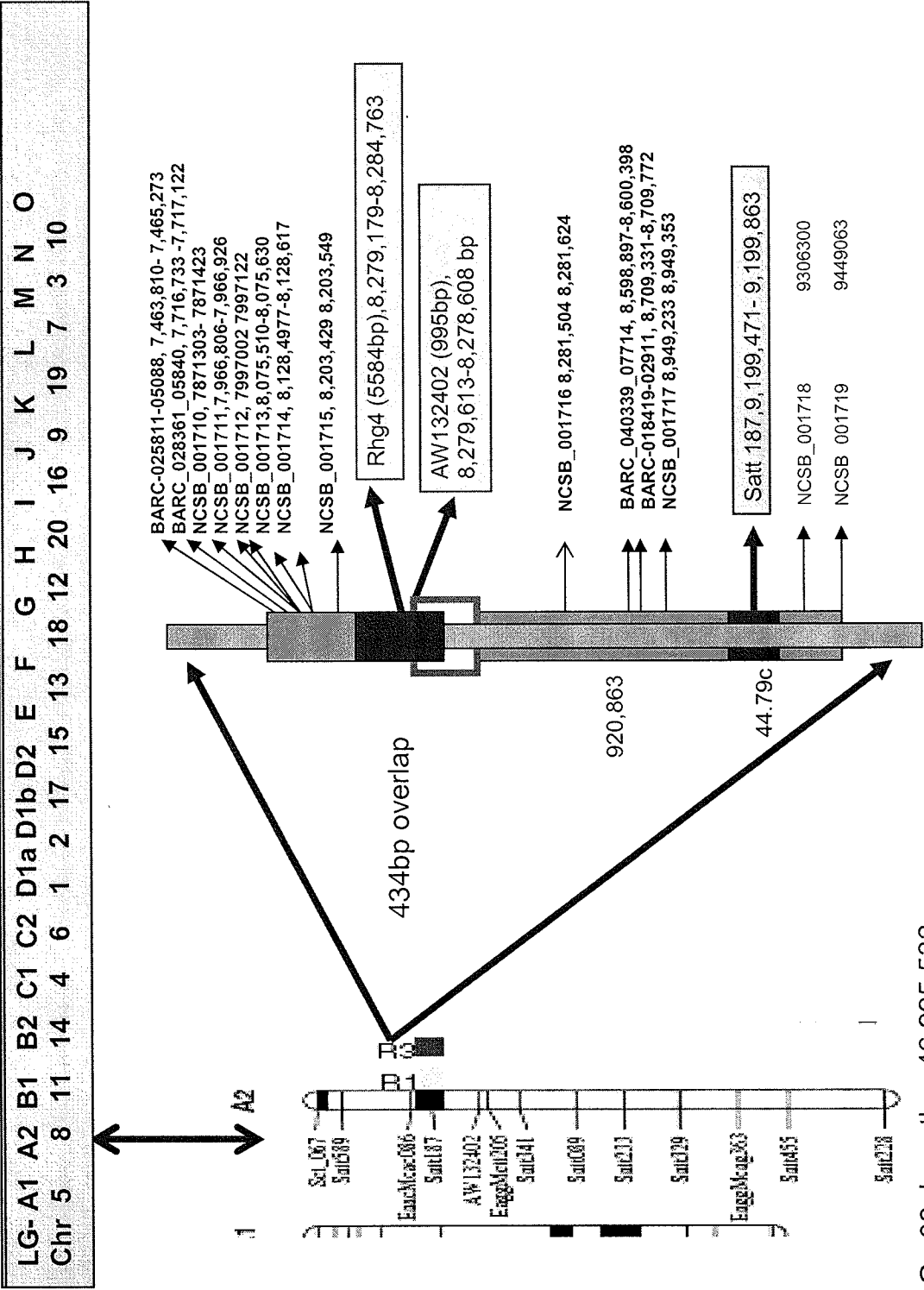


FIG. 4

Gm08 Length = 46,995,532

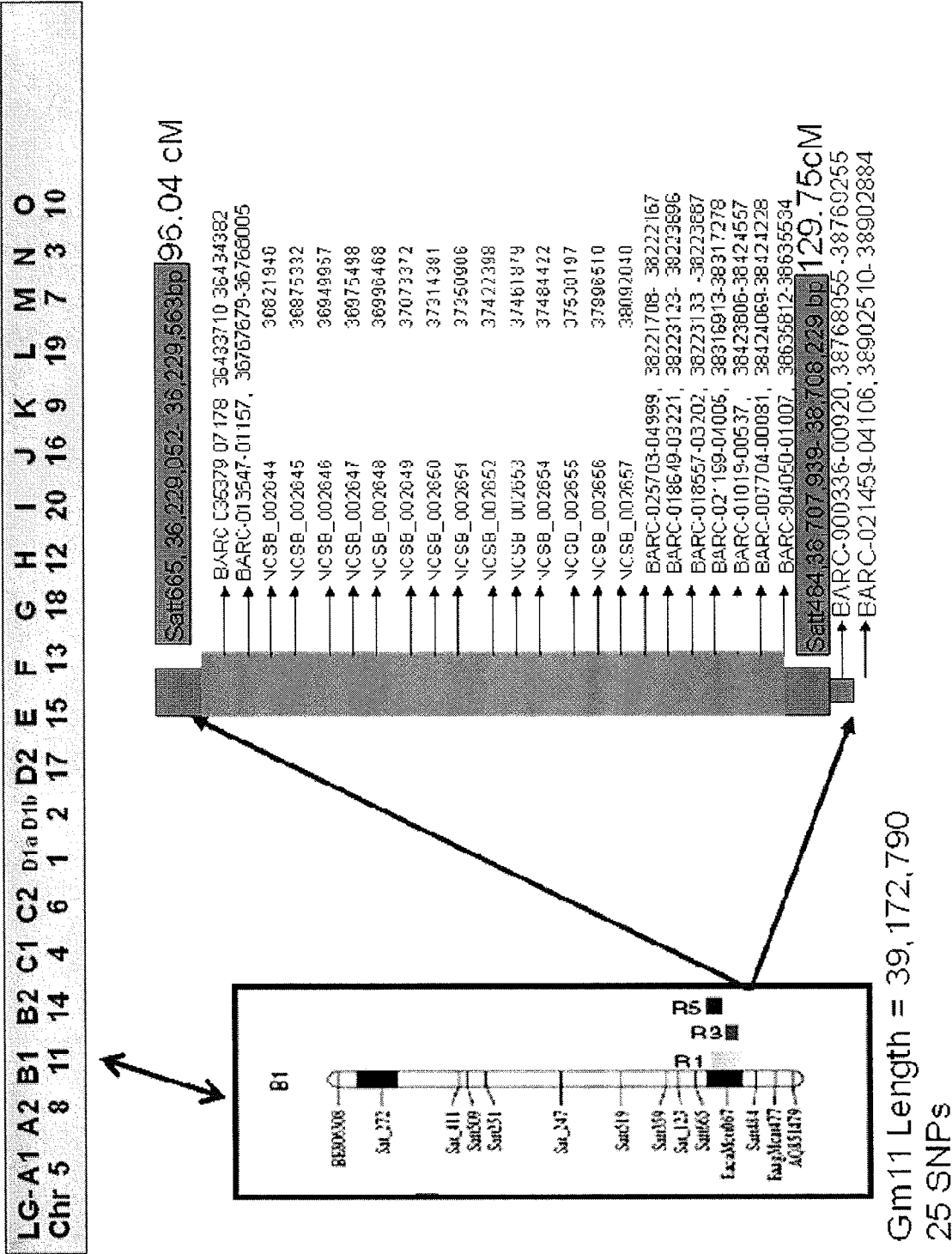


FIG. 5

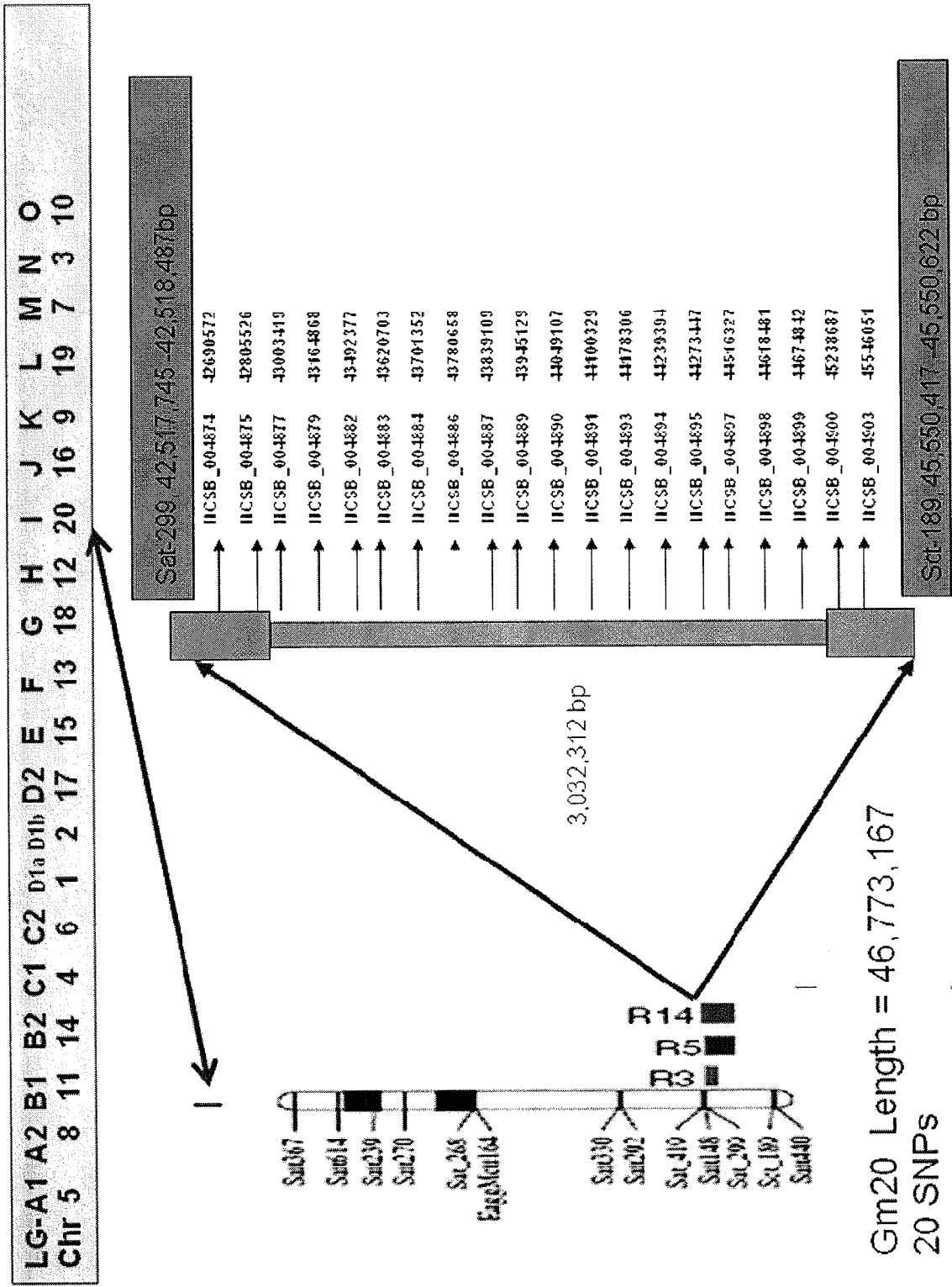
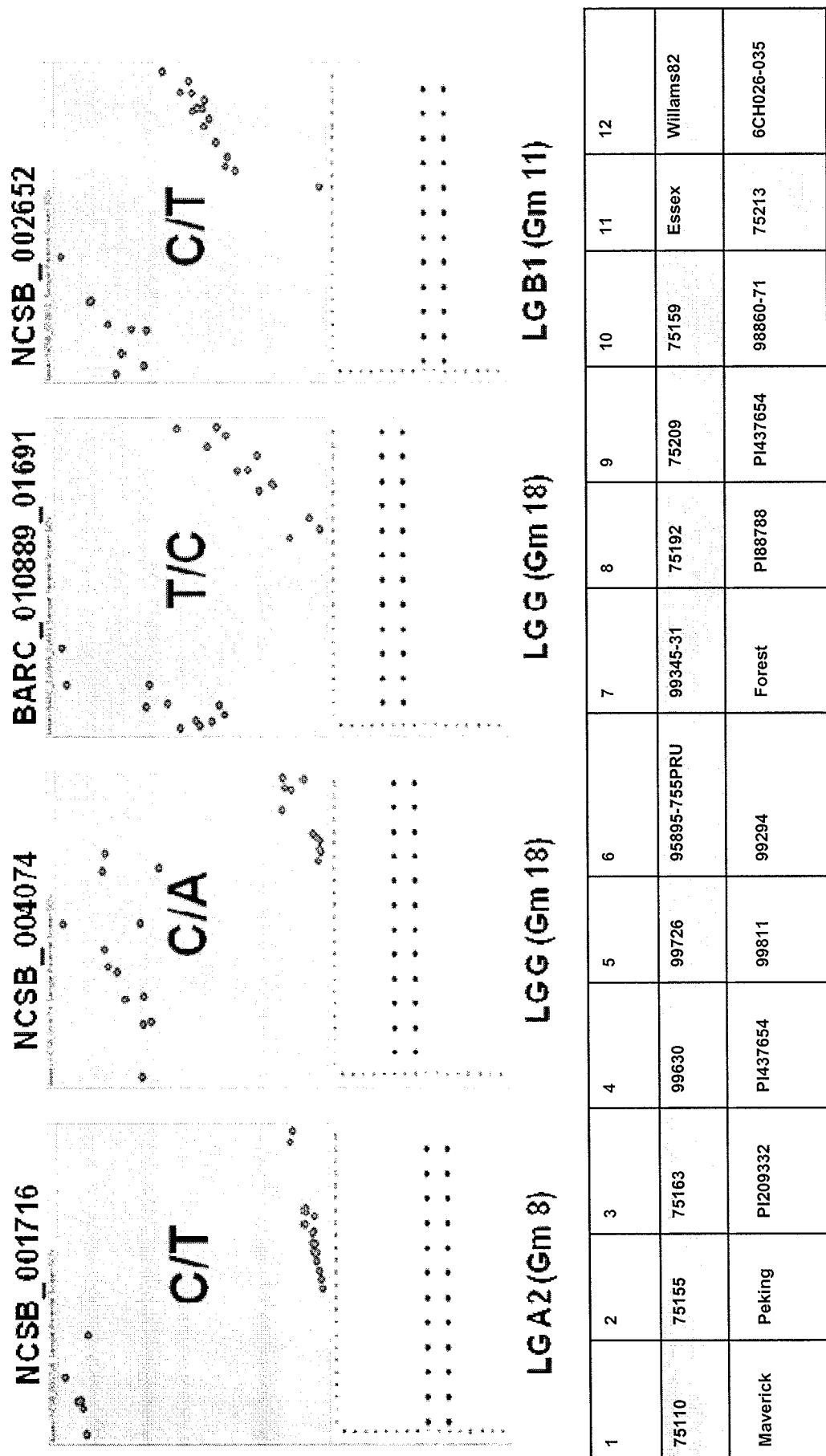
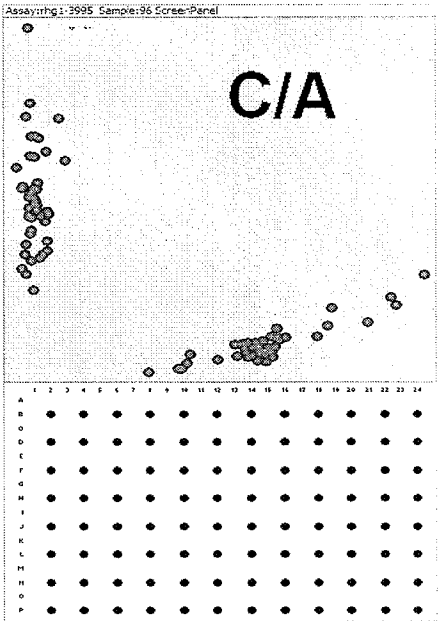


FIG. 6

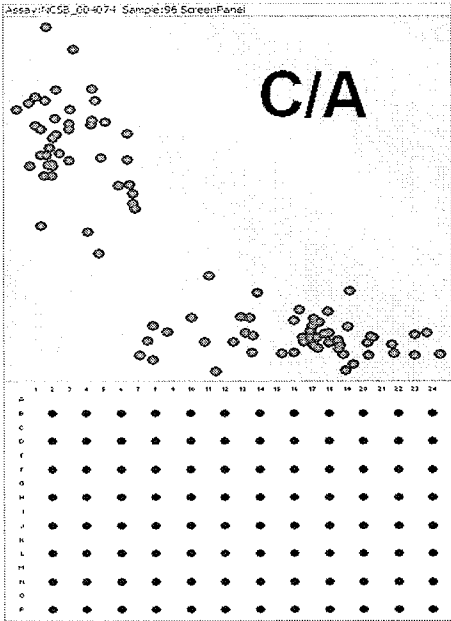


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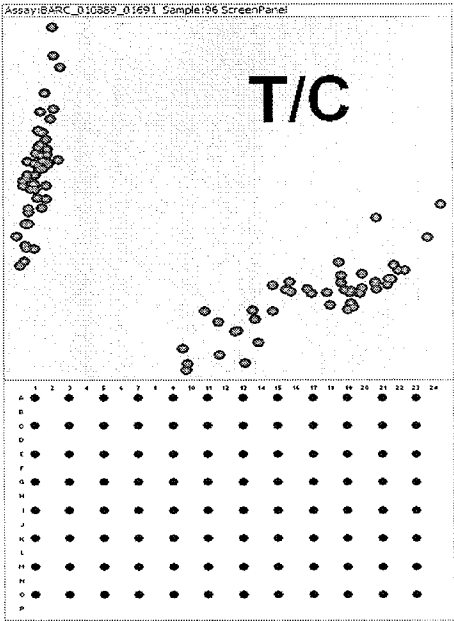
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NCSB_004074



BARC_010889_01691



LG G (Gm 18)

FIG. 8

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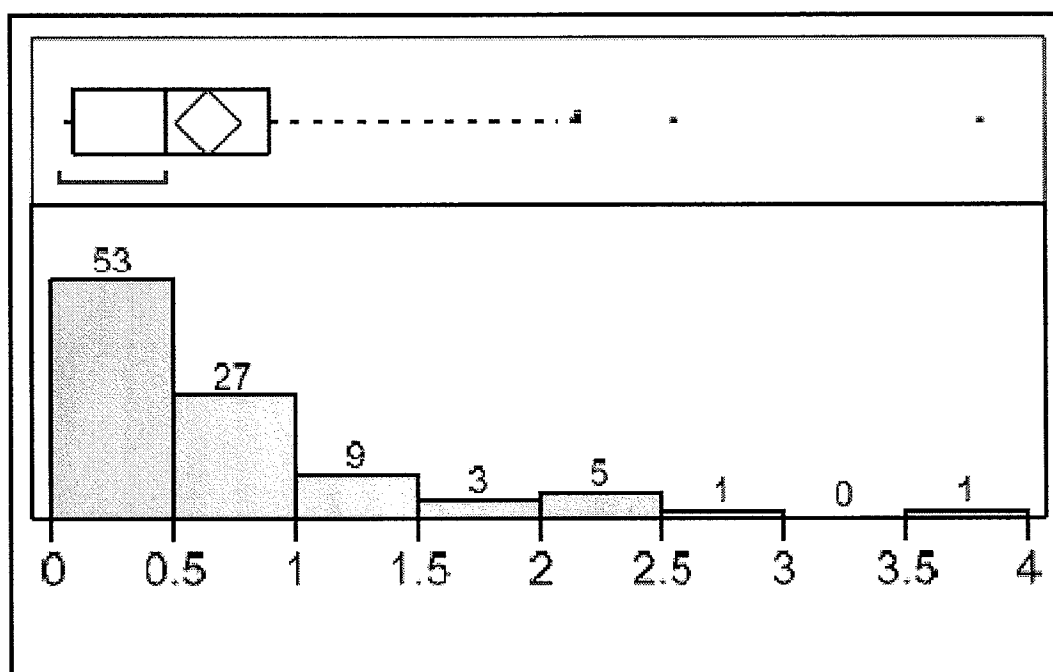


FIG. 9