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POURRIDIE PHYTOPHTHOREEN**

(54) **Title: METHODS AND COMPOSITIONS FOR SELECTING SOYBEAN PLANTS RESISTANT TO PHYTOPHTHORA ROOT ROT**

(57) **Abrégé/Abstract:**

The present invention relates to the field of plant breeding and disease resistance. More specifically, the invention includes a method for breeding soybean plants containing quantitative trait loci (QTL) for resistance to the Phytophthora root rot (PRR) caused by Phytophthora sojae. The invention further includes the use of molecular markers in the introgression of PRR resistance QTL into soybean plants.

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APPLICATION FOR LETTERS PATENT

FOR

5 **METHODS AND COMPOSITIONS FOR SELECTING SOYBEAN PLANTS
RESISTANT TO PHYTOPHTHORA ROOT ROT**

BY

10

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METHODS AND COMPOSITIONS FOR SELECTING SOYBEAN PLANTS RESISTANT TO PHYTOPHTHORA ROOT ROT

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FIELD OF THE INVENTION

The present invention is in the field of plant breeding and disease resistance. More specifically, the invention includes a method of breeding plants from the genus *Glycine* containing quantitative trait loci (QTL) that are associated with disease resistance to the pathogen *Phytophthora sojae* (Kauffman & Gerdemann). The invention relates to the use of genetic markers to identify QTL for disease resistance. The invention further relates to the use of genetic markers for the introgression of resistance to *Phytophthora sojae* into elite germplasm in a breeding program.

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BACKGROUND OF THE INVENTION

Phytophthora sojae (Kauffman & Gerdemann) is an oomycete pathogen which causes extensive damage to roots and stems of soybean plants (*Glycine max*) (Zhang *et al.*, *MPMI*, 19:1302-1310 (2006)). Symptoms of Phytophthora Root Rot (PRR) caused by *P. sojae* include yellowing and wilting of leaves and browning of lower stems and branches (Demirbas *et al.*, *Crop Sci.* 41:1220-1227 (2001)). PRR results in annual worldwide soybean crop losses of \$1 to \$2 billion (Zhang *et al.*, *MPMI*, 19: 1302-1310 (2006)). Soybean PRR resistance or susceptibility depends on a system of signaling between pathogen and host. Certain quantitative trait loci (QTL) can confer resistance to PRR. Pathogen avirulence (*Avr*) and host resistance (*Rps*) quantitative trait loci determine the interaction of different *P. sojae* races and soybean

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cultivars (Valer *et al.*, *FEMS Microbiol Lett.*, 265:60-68 (2006)). Eight loci have been identified which provide race-specific resistance to PRR, and two of these loci, *Rps1* and *Rps3*, have been identified as having multiple alleles which are designated by a letter following the locus number (Ferro *et al.*, *Crop Sci.*, 46:2427-2436 (2006)).

5 The *Rps1* locus includes, for example, *Rps1a*, *Rps1b*, *Rps1c*, *Rps1d*, and *Rps1k*, and the *Rps3* locus includes, for example, *Rps3a*, *Rps3b*, and *Rps3c*. Map-based cloning has attempted to characterize the *Rps1k* region (Bhattacharyya, M.K. *et al.*, *Theor Appl Genet*, 111:75-86 (2005), U.S. Patent 7,256,323). Planting soybean cultivars with race-specific resistance genes has been the primary means of controlling PRR
10 (Ferro *et al.*, *Crop Sci.*, 46:2427-2436 (2006)). Over fifty *P. sojae* races have been identified, and *Rps* loci can provide resistance to more than one *P. sojae* race. Examples include, but are not limited to, the following: *Rps1k* can provide resistance to *P. sojae* races 1 and 4, *Rps1c* can provide resistance to *P. sojae* races 1 and 3, and *Rps8* can provide resistance to *P. sojae* races 1, 4, 7, and 25. Plant breeders are able
15 to use molecular markers as an indirect means to select plants with alleles resistant to PRR races of concern (Demirbas *et al.*, *Crop Sci.*, 41:1220-1227 (2001)).

Breeding for PRR resistant soybeans can be greatly facilitated by the use of marker-assisted selection for PRR resistance alleles. Genetic markers used in soybean breeding programs to detect, select for, and introgress PRR resistant plants have
20 included simple sequence repeats (SSRs), restriction fragment length polymorphisms (RFLPs), and single nucleotide polymorphisms (SNPs). SSR and SNP markers have been provided for PRR resistance loci on Linkage Groups B1, G, K, and M (U.S. Patent Application Serial No. 11/199,819 (filed August 8, 2005)). RFLP markers, SSR markers, and isozyme markers have been provided for PRR resistance loci
25 located on Linkage Group A2 (U.S. Patent Application Serial No. 10/436,376 (filed May 12, 2003)). SSR markers have been provided for PRR resistance loci located on Linkage Group F (U.S. Patent Application Serial No. 10/778,018 (filed February 12, 2004)). Linkage groups are described by Cregan *et al.* (*Crop Sci.*, 39:1464-1490 (1999)). To date, a SNP-based marker set for *Rps1* on Linkage Group N, *Rps3* on
30 Linkage Group F, and *Rps8* on Linkage Group F is lacking.

Of the classes of markers, SNPs have characteristics which make them preferential to other genetic markers in detecting, selecting for, and introgressing PRR resistance in a soybean plant. SNPs are preferred because technologies are available for automated, high-throughput screening of SNP markers, which can decrease the

time to select for and introgress PRR resistance in soybean plants. Further, SNP markers are ideal because the likelihood that a particular SNP allele is derived from independent origins in the extant population of a particular species is very low. As such, SNP markers are useful for tracking and assisting introgression of PRR resistance alleles, particularly in the case of PRR resistance haplotypes. A need exists for a SNP based marker set to screen for resistance to PRR races with agronomic importance. *Rps1*, *Rps3*, and *Rps8* provide resistance to PRR races that are a significant source of damage to soybean crops. The present invention provides a SNP-based marker set for *Rps1* on Linkage Group N, *Rps3* on Linkage Group F, and *Rps8* on Linkage Group F.

The present invention provides and includes a method for screening and selecting a soybean plant comprising at least one PRR resistance QTL. The invention includes SNP markers for the detection of, selection for, and introgression of PRR resistance QTL from PRR resistant soybean plants.

SUMMARY OF THE INVENTION

The present invention includes a method of selecting for and introgressing an allele into a soybean plant comprising (A) crossing at least one PRR resistant soybean plant with at least one other soybean plant in order to form a population, (B) screening said population with at least one nucleic acid marker selected from the group comprising SEQ ID NO: 1 to SEQ ID NO: 16 and SEQ ID NO: 81 to SEQ ID NO: 84, (C) selecting from said population one or more soybean plants comprising at least one genotype corresponding to a PRR resistant soybean plant.

The present invention further comprises an elite soybean plant produced by such method.

The present invention includes a method of introgressing an allele into a soybean plant comprising: (A) crossing at least one PRR resistant soybean plant with at least one other soybean plant in order to form a population, (B) screening said population with at least one nucleic acid marker, (C) selecting from said population one or more soybean plants comprising a haplotype associated with PRR resistance, wherein said PRR resistance haplotype is selected from the group consisting of 1, 2, or 3 PRR resistant loci where one or more haplotypes at one or more loci are selected from the group *Rps1*, *Rps3*, and *Rps8*, and the one or more haplotypes are selected based on the haplotype of the PRR resistant soybean plants.

The present invention further comprises an elite soybean plant produced by said method.

One embodiment of the present invention provides a method for introgressing an allele into a soybean plant comprising: a. crossing at least one *Phytophthora* root rot (PRR) resistant soybean plant with at least one other soybean plant to form a population; b. screening said population for the presence of at least one nucleic acid marker that comprises SEQ ID NO:10; c. selecting from said population, on the basis of said presence of the at least one nucleic acid marker, one or more soybean plants for comprising a haplotype associated with PRR resistance, wherein said haplotype corresponds to a *Rpslc* locus and comprises SEQ ID NO: 10; wherein at least one of said at least one marker is located within 10 cM of said resistance haplotype.

The present invention includes a substantially purified nucleic acid molecule comprising a nucleic acid sequence selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 116.

Further, the present invention provides assays for detection of the PRR resistance QTLs, *Rps1*, *Rps3*, and *Rps8*.

In accordance with one embodiment of the present invention, there is provided a cell of an elite soybean plant produced by: a. crossing at least one *Phytophthora* root rot (PRR) resistant soybean plant with at least one other soybean plant to form a population; b. screening the population with at least one nucleic acid marker; c. selecting from the population, on the basis of the at least one nucleic acid marker, one or more soybean plants comprising a haplotype associated with PRR resistance, the haplotype corresponding to the *Rps1* locus comprising NS0138011, NS0202603 and NS0203225.

In another embodiment of the present invention, there is provided a set of oligonucleotides comprising: a. a pair of oligonucleotide primers wherein each of the primers comprises at least 12 contiguous nucleotides and wherein the pair of primers permit PCR amplification of a DNA segment comprising a soybean genomic DNA polymorphism associated with *Phytophthora* root rot (PRR) resistance locus *Rps1* comprising NS0138011, NS0202603 and NS0203225; and b. at least one detector oligonucleotide that permits detection of a polymorphism in the amplified segment, wherein the sequence of the detector oligonucleotide is at least 95 percent identical to a sequence of the same number of consecutive nucleotides in either strand of a segment of soybean DNA that include or are immediately adjacent to the polymorphism of step (A).

One embodiment of the present invention provides a set of oligonucleotides comprising: a. a pair of oligonucleotide primers wherein each of said primers comprises at least 12 contiguous nucleotides and wherein said pair of primers permit PCR amplification of a DNA segment comprising a soybean genomic DNA polymorphism associated with *Phytophthora* root rot (PRR) resistance locus *Rps1* comprising SEQ ID NO: 10; and b. at least one detector oligonucleotide that permits detection of a polymorphism in said amplified segment, wherein the sequence of said detector oligonucleotide is at least 95 percent identical to a sequence of the same number of consecutive nucleotides in either strand of a segment of soybean DNA that include or are immediately adjacent to said soybean genome DNA

polymorphism of step (a), wherein said detector oligonucleotide is at least 95% identical to SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:111 or SEQ ID NO:112.

Also provided in one embodiment is a method of introgressing an allele into a soybean plant comprising: a. providing a population of soybean plants; b. genotyping at least one soybean plant in the population for the presence of a soybean genomic nucleic acid marker that comprises SEQ ID NO:10; c. selecting from said population, on the basis of said presence of the at least one nucleic acid marker, one or more soybean plants comprising a haplotype associated with *Phytophthora* root rot (PRR) resistance, said haplotype corresponding to the *Rpslc* locus comprising SEQ ID NO:10; wherein providing a population comprises crossing a PRR resistant soybean plant with a second soybean plant to form a population.

BRIEF DESCRIPTION OF THE NUCLEIC ACID SEQUENCES

SEQ ID NO: 1 is a genomic sequence derived from *Glycine max* associated with the PRR resistance locus *Rps1*.

5 SEQ ID NO: 2 is a genomic sequence derived from *Glycine max* associated with the PRR resistance locus *Rps1*.

SEQ ID NO: 3 is a genomic sequence derived from *Glycine max* associated with the PRR resistance locus *Rps1*.

SEQ ID NO: 4 is a genomic sequence derived from *Glycine max* associated with the PRR resistance locus *Rps1*.

10 SEQ ID NO: 5 is a genomic sequence derived from *Glycine max* associated with the PRR resistance locus *Rps1*.

SEQ ID NO: 6 is a genomic sequence derived from *Glycine max* associated with the PRR resistance locus *Rps1*.

15 SEQ ID NO: 7 is a genomic sequence derived from *Glycine max* associated with the PRR resistance locus *Rps1*.

SEQ ID NO: 8 is a genomic sequence derived from *Glycine max* associated with the PRR resistance locus *Rps1*.

SEQ ID NO: 9 is a genomic sequence derived from *Glycine max* associated with the PRR resistance locus *Rps1*.

20 SEQ ID NO: 10 is a genomic sequence derived from *Glycine max* associated with the PRR resistance locus *Rps1*.

SEQ ID NO: 11 is a genomic sequence derived from *Glycine max* associated with the PRR resistance loci *Rps3* and *Rps8*.

25 SEQ ID NO: 12 is a genomic sequence derived from *Glycine max* associated with the PRR resistance loci *Rps3* and *Rps8*.

SEQ ID NO: 13 is a genomic sequence derived from *Glycine max* associated with the PRR resistance loci *Rps3* and *Rps8*.

SEQ ID NO: 14 is a genomic sequence derived from *Glycine max* associated with the PRR resistance loci *Rps3* and *Rps8*.

30 SEQ ID NO: 15 is a genomic sequence derived from *Glycine max* associated with the PRR resistance loci *Rps3* and *Rps8*.

SEQ ID NO: 16 is a genomic sequence derived from *Glycine max* associated with the PRR resistance loci *Rps3* and *Rps8*.

SEQ ID NO: 17 is a forward PCR primer for amplifying SEQ ID NO: 1.

SEQ ID NO: 18 is a reverse PCR primer for amplifying SEQ ID NO: 1.
 SEQ ID NO: 19 is a forward PCR primer for amplifying SEQ ID NO: 2.
 SEQ ID NO: 20 is a reverse PCR primer for amplifying SEQ ID NO: 2.
 SEQ ID NO: 21 is a forward PCR primer for amplifying SEQ ID NO: 3.
 5 SEQ ID NO: 22 is a reverse PCR primer for amplifying SEQ ID NO: 3.
 SEQ ID NO: 23 is a forward PCR primer for amplifying SEQ ID NO: 4.
 SEQ ID NO: 24 is a reverse PCR primer for amplifying SEQ ID NO: 4.
 SEQ ID NO: 25 is a forward PCR primer for amplifying SEQ ID NO: 5.
 SEQ ID NO: 26 is a reverse PCR primer for amplifying SEQ ID NO: 5.
 10 SEQ ID NO: 27 is a forward PCR primer for amplifying SEQ ID NO: 6.
 SEQ ID NO: 28 is a reverse PCR primer for amplifying SEQ ID NO: 6.
 SEQ ID NO: 29 is a forward PCR primer for amplifying SEQ ID NO: 7.
 SEQ ID NO: 30 is a reverse PCR primer for amplifying SEQ ID NO: 7.
 SEQ ID NO: 31 is a forward PCR primer for amplifying SEQ ID NO: 8.
 15 SEQ ID NO: 32 is a reverse PCR primer for amplifying SEQ ID NO: 8.
 SEQ ID NO: 33 is a forward PCR primer for amplifying SEQ ID NO: 9.
 SEQ ID NO: 34 is a reverse PCR primer for amplifying SEQ ID NO: 9.
 SEQ ID NO: 35 is a forward PCR primer for amplifying SEQ ID NO: 10.
 SEQ ID NO: 36 is a reverse PCR primer for amplifying SEQ ID NO: 10.
 20 SEQ ID NO: 37 is a forward PCR primer for amplifying SEQ ID NO: 11.
 SEQ ID NO: 38 is a reverse PCR primer for amplifying SEQ ID NO: 11.
 SEQ ID NO: 39 is a forward PCR primer for amplifying SEQ ID NO: 12.
 SEQ ID NO: 40 is a reverse PCR primer for amplifying SEQ ID NO: 12.
 SEQ ID NO: 41 is a forward PCR primer for amplifying SEQ ID NO: 13.
 25 SEQ ID NO: 42 is a reverse PCR primer for amplifying SEQ ID NO: 13.
 SEQ ID NO: 43 is a forward PCR primer for amplifying SEQ ID NO: 14.
 SEQ ID NO: 44 is a reverse PCR primer for amplifying SEQ ID NO: 14.
 SEQ ID NO: 45 is a forward PCR primer for amplifying SEQ ID NO: 15.
 SEQ ID NO: 46 is a reverse PCR primer for amplifying SEQ ID NO: 15.
 30 SEQ ID NO: 47 is a forward PCR primer for amplifying SEQ ID NO: 16.
 SEQ ID NO: 48 is a reverse PCR primer for amplifying SEQ ID NO: 16.
 SEQ ID NO: 49 is a probe for detecting the PRR resistance locus of SEQ ID
 NO: 1.

SEQ ID NO: 50 is a second probe for detecting the PRR resistance locus of SEQ ID NO: 1.

SEQ ID NO: 51 is a probe for detecting the PRR resistance locus of SEQ ID NO: 2.

5 SEQ ID NO: 52 is a second probe for detecting the PRR resistance locus of SEQ ID NO: 2.

SEQ ID NO: 53 is a probe for detecting the PRR resistance locus of SEQ ID NO: 3.

10 SEQ ID NO: 54 is a second probe for detecting the PRR resistance locus of SEQ ID NO: 3.

SEQ ID NO: 55 is a probe for detecting the PRR resistance locus of SEQ ID NO: 4.

SEQ ID NO: 56 is a second probe for detecting the PRR resistance locus of SEQ ID NO: 4.

15 SEQ ID NO: 57 is a probe for detecting the PRR resistance locus of SEQ ID NO: 5.

SEQ ID NO: 58 is a second probe for detecting the PRR resistance locus of SEQ ID NO: 5.

20 SEQ ID NO: 59 is a probe for detecting the PRR resistance locus of SEQ ID NO: 6.

SEQ ID NO: 60 is a second probe for detecting the PRR resistance locus of SEQ ID NO: 6.

SEQ ID NO: 61 is a probe for detecting the PRR resistance locus of SEQ ID NO: 7.

25 SEQ ID NO: 62 is a second probe for detecting the PRR resistance locus of SEQ ID NO: 7.

SEQ ID NO: 63 is a probe for detecting the PRR resistance locus of SEQ ID NO: 8.

30 SEQ ID NO: 64 is a second probe for detecting the PRR resistance locus of SEQ ID NO: 8.

SEQ ID NO: 65 is a probe for detecting the PRR resistance locus of SEQ ID NO: 9.

SEQ ID NO: 66 is a second probe for detecting the PRR resistance locus of SEQ ID NO: 9.

SEQ ID NO: 67 is a probe for detecting the PRR resistance locus of SEQ ID NO: 10.

SEQ ID NO: 68 is a second probe for detecting the PRR resistance locus of SEQ ID NO: 10.

5 SEQ ID NO: 69 is a probe for detecting the PRR resistance loci of SEQ ID NO: 11.

SEQ ID NO: 70 is a second probe for detecting the PRR resistance loci of SEQ ID NO: 11.

10 SEQ ID NO: 71 is a probe for detecting the PRR resistance loci of SEQ ID NO: 12.

SEQ ID NO: 72 is a second probe for detecting the PRR resistance loci of SEQ ID NO: 12.

SEQ ID NO: 73 is a probe for detecting the PRR resistance loci of SEQ ID NO: 13.

15 SEQ ID NO: 74 is a second probe for detecting the PRR resistance loci of SEQ ID NO: 13.

SEQ ID NO: 75 is a probe for detecting the PRR resistance loci of SEQ ID NO: 14.

20 SEQ ID NO: 76 is a second probe for detecting the PRR resistance loci of SEQ ID NO: 14.

SEQ ID NO: 77 is a probe for detecting the PRR resistance loci of SEQ ID NO: 15.

SEQ ID NO: 78 is a second probe for detecting the PRR resistance loci of SEQ ID NO: 15.

25 SEQ ID NO: 79 is a probe for detecting the PRR resistance loci of SEQ ID NO: 16.

SEQ ID NO: 80 is a second probe for detecting the PRR resistance loci of SEQ ID NO: 16.

30 SEQ ID NO: 81 is a genomic sequence derived from *Glycine max* associated with the PRR resistance locus *Rps1*.

SEQ ID NO: 82 is a genomic sequence derived from *Glycine max* associated with the PRR resistance locus *Rps1*.

SEQ ID NO: 83 is a genomic sequence derived from *Glycine max* associated with the PRR resistance locus *Rps1*.

SEQ ID NO: 84 is a genomic sequence derived from *Glycine max* associated with the PRR resistance locus *Rps1*.

SEQ ID NO: 85 is a forward PCR primer for amplifying SEQ ID NO: 81.

SEQ ID NO: 86 is a reverse PCR primer for amplifying SEQ ID NO: 81.

5 SEQ ID NO: 87 is a forward PCR primer for amplifying SEQ ID NO: 82.

SEQ ID NO: 88 is a reverse PCR primer for amplifying SEQ ID NO: 82.

SEQ ID NO: 89 is a forward PCR primer for amplifying SEQ ID NO: 83.

SEQ ID NO: 90 is a reverse PCR primer for amplifying SEQ ID NO: 83.

SEQ ID NO: 91 is a forward PCR primer for amplifying SEQ ID NO: 84.

10 SEQ ID NO: 92 is a reverse PCR primer for amplifying SEQ ID NO: 84.

SEQ ID NO: 93 is a probe for detecting the PRR resistance locus of SEQ ID NO: 81.

SEQ ID NO: 94 is a second probe for detecting the PRR resistance locus of SEQ ID NO: 81.

15 SEQ ID NO: 95 is a probe for detecting the PRR resistance locus of SEQ ID NO: 82.

SEQ ID NO: 96 is a second probe for detecting the PRR resistance locus of SEQ ID NO: 82.

20 SEQ ID NO: 97 is a probe for detecting the PRR resistance locus of SEQ ID NO: 83.

SEQ ID NO: 98 is a second probe for detecting the PRR resistance locus of SEQ ID NO: 83.

SEQ ID NO: 99 is a probe for detecting the PRR resistance locus of SEQ ID NO: 84.

25 SEQ ID NO: 100 is a second probe for detecting the PRR resistance locus of SEQ ID NO: 84.

SEQ ID NO: 101 is a third probe for detecting the PRR resistance locus of SEQ ID NO: 8.

30 SEQ ID NO: 102 is a fourth probe for detecting the PRR resistance locus of SEQ ID NO: 8.

SEQ ID NO: 103 is a third probe for detecting the PRR resistance locus of SEQ ID NO: 10.

SEQ ID NO: 104 is a fourth probe for detecting the PRR resistance locus of SEQ ID NO: 10.

SEQ ID NO: 105 is a third probe for detecting the PRR resistance loci of SEQ ID NO: 14.

SEQ ID NO: 106 is a fourth probe for detecting the PRR resistance loci of SEQ ID NO: 14.

5 SEQ ID NO: 107 is a third probe for detecting the PRR resistance loci of SEQ ID NO: 15.

SEQ ID NO: 108 is a fourth probe for detecting the PRR resistance loci of SEQ ID NO: 15.

10 SEQ ID NO: 109 is a fifth probe for detecting the PRR resistance locus of SEQ ID NO: 8.

SEQ ID NO: 110 is a sixth probe for detecting the PRR resistance locus of SEQ ID NO: 8.

SEQ ID NO: 111 is a fifth probe for detecting the PRR resistance locus of SEQ ID NO: 10.

15 SEQ ID NO: 112 is a sixth probe for detecting the PRR resistance locus of SEQ ID NO: 10.

SEQ ID NO: 113 is a fifth probe for detecting the PRR resistance loci of SEQ ID NO: 14.

20 SEQ ID NO: 114 is a sixth probe for detecting the PRR resistance loci of SEQ ID NO: 14.

SEQ ID NO: 115 is a fifth probe for detecting the PRR resistance locus of SEQ ID NO: 15.

SEQ ID NO: 116 is a sixth probe for detecting the PRR resistance locus of SEQ ID NO: 15.

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DETAILED DESCRIPTION OF THE INVENTION

The definitions and methods provided define the present invention and guide those of ordinary skill in the art in the practice of the present invention. Unless otherwise noted, terms are to be understood according to conventional usage by those of ordinary skill in the relevant art. Definitions of common terms in molecular biology may also be found in Alberts *et al.*, Molecular Biology of The Cell, 3rd Edition, Garland Publishing, Inc.: New York, 1994; Rieger et al., Glossary of Genetics: Classical and Molecular, 5th edition, Springer-Verlag: New York, 1991; and Lewin, Genes V, Oxford University Press: New York, 1994. The nomenclature for DNA bases as set forth at 37 CFR § 1.822 is used.

An “allele” refers to an alternative sequence at a particular locus; the length of an allele can be as small as 1 nucleotide base, but is typically larger.

A “locus” is a position on a genomic sequence that is usually found by a point of reference; e.g., a short DNA sequence that is a gene, or part of a gene or intergenic region. The loci of this invention comprise one or more polymorphisms in a population; i.e., alternative alleles present in some individuals.

As used herein, “polymorphism” means the presence of one or more variations of a nucleic acid sequence at one or more loci in a population of one or more individuals. The variation may comprise but is not limited to one or more base changes, the insertion of one or more nucleotides or the deletion of one or more nucleotides. A polymorphism may arise from random processes in nucleic acid replication, through mutagenesis, as a result of mobile genomic elements, from copy number variation and during the process of meiosis, such as unequal crossing over, genome duplication and chromosome breaks and fusions. The variation can be commonly found or may exist at low frequency within a population, the former having greater utility in general plant breeding and the latter may be associated with rare but important phenotypic variation. Useful polymorphisms may include single nucleotide polymorphisms (SNPs), insertions or deletions in DNA sequence (Indels), simple sequence repeats of DNA sequence (SSRs) a restriction fragment length polymorphism, and a tag SNP. A genetic marker, a gene, a DNA-derived sequence, a haplotype, a RNA-derived sequence, a promoter, a 5' untranslated region of a gene, a 3' untranslated region of a gene, microRNA, siRNA, a QTL, a satellite marker, a transgene, mRNA, ds mRNA, a transcriptional profile, and a methylation pattern may comprise polymorphisms.

As used herein, “marker” means a polymorphic nucleic acid sequence or nucleic acid feature. A marker may be represented by one or more particular variant sequences, or by a consensus sequence. In another sense, a “marker” is an isolated variant or consensus of such a sequence. In a broader aspect, a “marker” can be a detectable characteristic that can be used to discriminate between heritable differences between organisms. Examples of such characteristics may include genetic markers, protein composition, protein levels, oil composition, oil levels, carbohydrate composition, carbohydrate levels, fatty acid composition, fatty acid levels, amino acid composition, amino acid levels, biopolymers, pharmaceuticals, starch composition, starch levels, fermentable starch, fermentation yield, fermentation efficiency, energy yield, secondary compounds, metabolites, morphological characteristics, and agronomic characteristics.

As used herein, “marker assay” means a method for detecting a polymorphism at a particular locus using a particular method, e.g. measurement of at least one phenotype (such as seed color, flower color, or other visually detectable trait), restriction fragment length polymorphism (RFLP), single base extension, electrophoresis, sequence alignment, allelic specific oligonucleotide hybridization (ASO), random amplified polymorphic DNA (RAPD), microarray-based technologies, and nucleic acid sequencing technologies, etc.

As used herein, “typing” refers to any method whereby the specific allelic form of a given soybean genomic polymorphism is determined. For example, a single nucleotide polymorphism (SNP) is typed by determining which nucleotide is present (i.e. an A, G, T, or C). Insertion/deletions (Indels) are determined by determining if the Indel is present. Indels can be typed by a variety of assays including, but not limited to, marker assays.

As used herein, the term “adjacent”, when used to describe a nucleic acid molecule that hybridizes to DNA containing a polymorphism, refers to a nucleic acid that hybridizes to DNA sequences that directly abut the polymorphic nucleotide base position. For example, a nucleic acid molecule that can be used in a single base extension assay is “adjacent” to the polymorphism.

As used herein, “interrogation position” refers to a physical position on a solid support that can be queried to obtain genotyping data for one or more predetermined genomic polymorphisms.

As used herein, “consensus sequence” refers to a constructed DNA sequence

which identifies SNP and Indel polymorphisms in alleles at a locus. Consensus sequence can be based on either strand of DNA at the locus and states the nucleotide base of either one of each SNP in the locus and the nucleotide bases of all Indels in the locus. Thus, although a consensus sequence may not be a copy of an actual DNA
5 sequence, a consensus sequence is useful for precisely designing primers and probes for actual polymorphisms in the locus.

As used herein, the term “single nucleotide polymorphism,” also referred to by the abbreviation “SNP,” means a polymorphism at a single site wherein said polymorphism constitutes a single base pair change, an insertion of one or more base
10 pairs, or a deletion of one or more base pairs.

As used herein, the term “haplotype” means a chromosomal region within a haplotype window defined by at least one polymorphic molecular marker. The unique marker fingerprint combinations in each haplotype window define individual haplotypes for that window. Further, changes in a haplotype, brought about by
15 recombination for example, may result in the modification of a haplotype so that it comprises only a portion of the original (parental) haplotype operably linked to the trait, for example, via physical linkage to a gene, QTL, or transgene. Any such change in a haplotype would be included in our definition of what constitutes a haplotype so long as the functional integrity of that genomic region is unchanged or
20 improved.

As used herein, the term “haplotype window” means a chromosomal region that is established by statistical analyses known to those of skill in the art and is in linkage disequilibrium. Thus, identity by state between two inbred individuals (or two gametes) at one or more molecular marker loci located within this region is taken as
25 evidence of identity-by-descent of the entire region. Each haplotype window includes at least one polymorphic molecular marker. Haplotype windows can be mapped along each chromosome in the genome. Haplotype windows are not fixed per se and, given the ever-increasing density of molecular markers, this invention anticipates the number and size of haplotype windows to evolve, with the number of windows
30 increasing and their respective sizes decreasing, thus resulting in an ever-increasing degree confidence in ascertaining identity by descent based on the identity by state at the marker loci.

As used herein, “genotype” means the genetic component of the phenotype and it can be indirectly characterized using markers or directly characterized by

nucleic acid sequencing. Suitable markers include a phenotypic character, a metabolic profile, a genetic marker, or some other type of marker. A genotype may constitute an allele for at least one genetic marker locus or a haplotype for at least one haplotype window. In some embodiments, a genotype may represent a single locus
5 and in others it may represent a genome-wide set of loci. In another embodiment, the genotype can reflect the sequence of a portion of a chromosome, an entire chromosome, a portion of the genome, and the entire genome.

As used herein, “phenotype” means the detectable characteristics of a cell or organism which can be influenced by gene expression.

10 As used herein, “linkage” refers to relative frequency at which types of gametes are produced in a cross. For example, if locus A has genes “A” or “a” and locus B has genes “B” or “b” and a cross between parent I with AABB and parent B with aabb will produce four possible gametes where the genes are segregated into AB, Ab, aB and ab. The null expectation is that there will be independent equal
15 segregation into each of the four possible genotypes, i.e. with no linkage $\frac{1}{4}$ of the gametes will of each genotype. Segregation of gametes into a genotypes differing from $\frac{1}{4}$ are attributed to linkage.

As used herein, “linkage disequilibrium” is defined in the context of the relative frequency of gamete types in a population of many individuals in a single
20 generation. If the frequency of allele A is p, a is p', B is q and b is q', then the expected frequency (with no linkage disequilibrium) of genotype AB is pq, Ab is pq', aB is p'q and ab is p'q'. Any deviation from the expected frequency is called linkage disequilibrium. Two loci are said to be “genetically linked” when they are in linkage disequilibrium.

25 As used herein, “quantitative trait locus (QTL)” means a locus that controls to some degree numerically representable traits that are usually continuously distributed.

As used herein, “resistance allele” means the isolated nucleic acid sequence that includes the polymorphic allele associated with resistance to PRR.

30 As used herein, the term “soybean” means *Glycine max* and includes all plant varieties that can be bred with soybean, including wild soybean species.

As used herein, the term “comprising” means “including but not limited to”.

As used herein, the term “elite line” means any line that has resulted from breeding and selection for superior agronomic performance. An elite plant is any plant from an elite line. Non-limiting examples of elite soybean varieties that are

commercially available to farmers or soybean breeders include AG00802, A0868, AG0902, A1923, AG2403, A2824, A3704, A4324, A5404, AG5903 and AG6202 (Asgrow Seeds, Des Moines, Iowa, USA); BPR0144RR, BPR 4077NRR and BPR 4390NRR (Bio Plant Research, Camp Point, Illinois, USA); DKB17-51 and DKB37-51 (DeKalb Genetics, DeKalb, Illinois, USA); and DP 4546 RR, and DP 7870 RR (Delta & Pine Land Company, Lubbock, Texas, USA); JG 03R501, JG 32R606C ADD and JG 55R503C (JGL Inc., Greencastle, Indiana, USA); NKS13-K2 (NK Division of Syngenta Seeds, Golden Valley, Minnesota, USA); 90M01, 91M30, 92M33, 93M11, 94M30, 95M30 and 97B52 (Pioneer Hi-Bred International, Johnston, Iowa, USA); SG4771NRR and SG5161NRR/STS (Soygenetics, LLC, Lafayette, Indiana, USA); S00-K5, S11-L2, S28-Y2, S43-B1, S53-A1, S76-L9 and S78-G6 (Syngenta Seeds, Henderson, Kentucky, USA). An elite plant is a representative plant from an elite variety.

The present invention provides SNP genetic markers useful for screening and selecting for PRR resistance at the *RpsI* locus located on Linkage Group N (LG N) (Cregan, *et al. Crop Sci.* 39:1464-1490 (1999)). The present invention also provides SNP DNA markers useful for screening and selecting for PRR resistance at the *Rps3* locus located on Linkage Group F (LG F) and the *Rps8* locus located on Linkage Group F (LG F) (Cregan, *et al. Crop Sci.* 39:1464-1490 (1999)). The SNP markers are useful for monitoring the selection for and introgression of the PRR resistance loci from PRR resistant sources. As used herein, the *RpsI* locus includes, for example, *Rps1a*, *Rps1b*, *Rps1c*, *Rps1d*, and *Rps1k*. Further, as used herein, the *Rps3* locus includes, for example, *Rps3a*, *Rps3b*, and *Rps3c*.

The present invention also includes a method of selecting or introgressing a PRR resistant allele into a soybean plant comprising: (A) crossing at least one PRR resistant soybean plant with at least one other soybean plant in order to form a population; (B) screening the population with one or more nucleic acid markers to determine if one or more soybean plants from the population contains the allele of the PRR resistance source.

SNP markers used to monitor the selection or introgression of the PRR resistance locus 1 (*RpsI*) include those selected from the group consisting of NS0099413, NS0102174, NS0118166, NS0102920, NS0114258, NS0118976, NS0119981, NS0119335, NS0201536, NS0138011, NS0202603, NS0203225, NS0129030, and NS0127084. Sources of *RpsI* include both accession germplasm,

such as plant introductions, and elite germplasm. Sources include, but are not limited to, Williams 82, L75-3735, and elite varieties with demonstrated PRR resistance.

PRR resistance SNP marker DNA sequences (SEQ ID NO: 1 through 10 and SEQ ID NO: 81 through 84) can be amplified using the primers indicated as SEQ ID NO: 17

5 through 36 and SEQ ID NO: 85 through 92 and detected with probes indicated as SEQ ID NO: 49 through 68 and SEQ ID NO: 93 through 100, wherein the corresponding primer and probe sets provide assays for the detection of PRR resistance or susceptibility in *Glycine max*. Determination of resistance or susceptibility of a plant to a particular pathogen is obvious to anyone skilled in the art.

10 In the present invention, a PRR resistance locus 3 (*Rps3*) is located on Linkage Group F (Cregan, *et al. Crop Sci.* 39:1464-1490 (1999)). SNP markers used to monitor the introgression of *Rps3* can be selected from the group consisting of NS0114683, NS0101324, NS0102483, NS0119333, NS0102262, and NS0116265. Sources of *Rps3* include both accession and elite germplasm. Sources of *Rps3*
15 include, but are not limited to, L83-570, L89-1541, L92-7857, Ivory, and elite varieties with demonstrated PRR resistance. In the present invention, a PRR resistance locus 8 (*Rps8*) is located on Linkage Group F (Cregan, *et al. Crop Sci.* 39:1464-1490 (1999)). SNP markers used to monitor the introgression of PRR resistance locus 8 can be selected from the group consisting of NS0114683,
20 NS0101324, NS0102483, NS0119333, NS0102262, and NS0116265. Sources of *Rps8* include accession germplasm. Sources of *Rps8* include, but are not limited to, PI399703 and other varieties with known PRR resistance.

In one aspect, the present invention provides methods and compositions for screening soybean plants for resistance or susceptibility to PRR, caused by the species
25 *Phytophthora sojae*. In another aspect, the present invention provides methods and compositions for selecting PRR resistant plants. In a preferred aspect, the present invention provides methods and compositions for selecting for and introgressing PRR resistance into soybean plants. The PRR resistance alleles of the present invention may be introduced into an elite *Glycine max* line.

30 As used herein, PRR refers to any PRR race, variant or isolate. A soybean plant of the present invention can be resistant to one or more oomycete capable of causing or inducing PRR. In one aspect, the present invention provides plants resistant to and methods and compositions for screening soybean plants for resistance or susceptibility to *Phytophthora sojae* races 1 through 55. In another aspect, the

present invention provides plants resistant to and methods and compositions for screening soybean plants for resistance or susceptibility to *Phytophthora sojae* race 1. In additional aspect, the present invention provides plants resistant to and methods and compositions for screening soybean plants for resistance or susceptibility to

5 *Phytophthora sojae* race 3. In a further aspect, the present invention provides plants resistant to and methods and compositions for screening soybean plants for resistance or susceptibility to *Phytophthora sojae* race 4. The invention further provides plants resistant to and methods and compositions for screening soybean plants for resistance or susceptibility to *Phytophthora sojae* race 7. The invention further provides plants

10 resistant to and methods and compositions for screening soybean plants for resistance or susceptibility to *Phytophthora sojae* race 17. The invention further provides plants resistant to and methods and compositions for screening soybean plants for resistance or susceptibility to *Phytophthora sojae* race 25.

The PRR resistance alleles of the present invention may also be introduced

15 into an elite *Glycine max* transgenic plant that contains one or more genes for herbicide tolerance, increased yield, insect control, fungal disease resistance, virus resistance, nematode resistance, bacterial disease resistance, mycoplasma disease resistance, modified oils production, high oil production, high protein production, germination and seedling growth control, enhanced animal and human nutrition, low

20 raffinose, environmental stress resistance, increased digestibility, industrial enzymes, pharmaceutical proteins, peptides and small molecules, improved processing traits, improved flavor, nitrogen fixation, hybrid seed production, reduced allergenicity, biopolymers, and biofuels among others. These agronomic traits can be provided by the methods of plant biotechnology as transgenes in *Glycine max*.

25 A disease resistance allele or alleles can be introduced from any plant that contains that allele (donor) to any recipient soybean plant. In one aspect, the recipient soybean plant can contain additional PRR resistance loci. In another aspect, the recipient soybean plant can contain a transgene. In another aspect, while maintaining the introduced PRR resistance allele, the genetic contribution of the plant providing

30 the disease resistance allele can be reduced by back-crossing or other suitable approaches. In one aspect, the nuclear genetic material derived from the donor material in the soybean plant can be less than or about 50%, less than or about 25%, less than or about 13%, less than or about 5%, 3%, 2% or 1%, but that genetic material contains the PRR resistance locus or loci of interest.

It is further understood that a soybean plant of the present invention may exhibit the characteristics of any relative maturity group. In an aspect, the maturity group is selected from the group consisting of 000, 00, 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10.

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

Plants or parts thereof of the present invention may be grown in culture and regenerated. Methods for the regeneration of *Glycine max* plants from various tissue
10 types and methods for the tissue culture of *Glycine max* are known in the art (See, for example, Widholm *et al.*, *In Vitro Selection and Culture-induced Variation in Soybean*, In Soybean: Genetics, Molecular Biology and Biotechnology, Eds. Verma and Shoemaker, CAB International, Wallingford, Oxon, England (1996). Regeneration techniques for plants such as *Glycine max* can use as the starting
15 material a variety of tissue or cell types. With *Glycine max* in particular, regeneration processes have been developed that begin with certain differentiated tissue types such as meristems, Cartha *et al.*, *Can. J. Bot.* 59:1671-1679 (1981), hypocotyl sections, Cameya *et al.*, *Plant Science Letters* 21: 289-294 (1981), and stem node segments, Saka *et al.*, *Plant Science Letters*, 19: 193-201 (1980); Cheng *et al.*, *Plant Science
20 Letters*, 19: 91-99 (1980). Regeneration of whole sexually mature *Glycine max* plants from somatic embryos generated from explants of immature *Glycine max* embryos has been reported (Ranch *et al.*, *In Vitro Cellular & Developmental Biology* 21: 653-658 (1985). Regeneration of mature *Glycine max* plants from tissue culture by organogenesis and embryogenesis has also been reported (Barwale *et al.*, *Planta* 167:
25 473-481 (1986); Wright *et al.*, *Plant Cell Reports* 5: 150-154 (1986).

Plants containing one or more PRR resistance loci described can be donor plants. Soybean plants containing resistance loci can be, for example, screened for by using a nucleic acid molecule capable of detecting a marker polymorphism associated or genetically linked with each of the resistance alleles.

30 As used herein, an allele of a disease resistance locus can encompass more than one gene or other genetic factor where each individual gene or genetic component is also capable of exhibiting allelic variation and where each gene or genetic factor is also capable of eliciting a phenotypic effect on the quantitative trait in question. In an aspect of the present invention the resistance allele comprises one

or more genes or other genetic factors that are also capable of exhibiting allelic variation. The use of the term "a resistance allele" does not exclude a genomic region that comprises more than one gene or other genetic factor. Specifically, a "disease resistance allele" in the present in the invention can denote a haplotype allele within a
5 haplotype window or genomic region wherein a phenotype associated with said haplotype allele can be disease resistance. A haplotype window is a contiguous genomic region that can be defined, and tracked, with a set of one or more polymorphic markers wherein the polymorphisms indicate identity by descent. A haplotype within that window can be defined by the unique fingerprint of alleles at
10 each marker. When all the alleles present at a given locus on a chromosome are the same, that plant is homozygous at that locus. If the alleles present at a given locus on a chromosome differ, that plant is heterozygous at that locus. Plants of the present invention may be homozygous or heterozygous at any particular PRR resistance locus or for a particular polymorphic marker.

15 The present invention includes isolated nucleic acid molecules. Such molecules include those nucleic acid molecules capable of detecting a polymorphism genetically or physically linked to a PRR resistance locus. Additional markers can be obtained that are genetically linked to *Rps1*, *Rps3*, and *Rps8*, by available techniques. In one aspect, the nucleic acid molecule is capable of detecting the presence or
20 absence of a marker located less than 30, 25, 20, 10, 5, 2, or 1 centimorgans from a PRR resistance locus. In another aspect, the nucleic acid molecule is capable of detecting a marker in a locus selected from the group *Rps1*, *Rps3*, and *Rps8*. In a further aspect, a nucleic acid molecule is selected from the group consisting of SEQ ID NO: 1 to SEQ ID NO: 116, fragments thereof, complements thereof, and nucleic
25 acid molecules capable of specifically hybridizing to one or more of these nucleic acid molecules.

In a preferred aspect, a nucleic acid molecule of the present invention includes those that will specifically hybridize to one or more of the nucleic acid molecules set forth in SEQ ID NO: 1 through SEQ ID NO: 116 or complements thereof or
30 fragments of either under moderately stringent conditions, for example at about 2.0 x SSC and about 65°C. In a particularly preferred aspect, a nucleic acid of the present invention will specifically hybridize to one or more of the nucleic acid molecules set forth in SEQ ID NO: 1 through SEQ ID NO: 116 or complements or fragments of either under high stringency conditions. In one aspect of the present invention, a

preferred marker nucleic acid molecule of the present invention has the nucleic acid sequence set forth in SEQ ID NO: 1 through SEQ ID NO: 116 or complements thereof or fragments of either. In another aspect of the present invention, a preferred marker nucleic acid molecule of the present invention shares between 80% and 100%
5 or 90% and 100% sequence identity with the nucleic acid sequence set forth in SEQ ID NO: 1 through SEQ ID NO: 116 or complement thereof or fragments of either. In a further aspect of the present invention, a preferred marker nucleic acid molecule of the present invention shares between 95% and 100% sequence identity with the sequence set forth in SEQ ID NO: 1 through SEQ ID NO: 116 or complement thereof
10 or fragments of either. In a more preferred aspect of the present invention, a preferred marker nucleic acid molecule of the present invention shares between 98% and 100% sequence identity with the nucleic acid sequence set forth in SEQ ID NO: 1 through SEQ ID NO: 116 or complement thereof or fragments of either.

Nucleic acid molecules or fragments thereof are capable of specifically
15 hybridizing to other nucleic acid molecules under certain circumstances. As used herein, two nucleic acid molecules are capable of specifically hybridizing to one another if the two molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure. A nucleic acid molecule is the "complement" of another nucleic acid molecule if they exhibit complete complementarity. As used herein,
20 molecules exhibit "complete complementarity" when every nucleotide of one of the molecules is complementary to a nucleotide of the other. Two molecules are "minimally complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low-stringency" conditions. Similarly, the molecules are "complementary" if they
25 can hybridize to one another with sufficient stability to permit them to remain annealed to one another under conventional "high-stringency" conditions.

Conventional stringency conditions are described by Sambrook *et al.*, In: *Molecular Cloning, A Laboratory Manual, 2nd Edition*, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989), and by Haymes *et al.*, In: *Nucleic Acid Hybridization, A Practical Approach*, IRL Press, Washington, DC (1985). Departures from complete
30 complementarity are therefore permissible, as long as such departures do not completely preclude the capacity of the molecules to form a double-stranded structure. In order for a nucleic acid molecule to serve as a primer or probe it need

only be sufficiently complementary in sequence to be able to form a stable double-stranded structure under the particular solvent and salt concentrations employed.

As used herein, a substantially homologous sequence is a nucleic acid sequence that will specifically hybridize to the complement of the nucleic acid sequence to which it is being compared under high stringency conditions. The
5 nucleic-acid probes and primers of the present invention can hybridize under stringent conditions to a target DNA sequence. The term "stringent hybridization conditions" is defined as conditions under which a probe or primer hybridizes specifically with a target sequence(s) and not with non-target sequences, as can be determined
10 empirically. The term "stringent conditions" is functionally defined with regard to the hybridization of a nucleic-acid probe to a target nucleic acid (i.e., to a particular nucleic-acid sequence of interest) by the specific hybridization procedure discussed in Sambrook *et al.*, 1989, at 9.52-9.55. See also, Sambrook *et al.*, 1989 at 9.47-9.52, 9.56-9.58; Kanehisa 1984 *Nucl. Acids Res.* 12:203-213; and Wetmur *et al.* 1968 *J.*
15 *Mol. Biol.* 31:349-370. Appropriate stringency conditions that promote DNA hybridization are, for example, 6.0 x sodium chloride/sodium citrate (SSC) at about 45° C, followed by a wash of 2.0 x SSC at 50°C, are known to those skilled in the art or can be found in *Current Protocols in Molecular Biology*, John Wiley & Sons, N.Y., 1989, 6.3.1-6.3.6. For example, the salt concentration in the wash step can be
20 selected from a low stringency of about 2.0 x SSC at 50°C to a high stringency of about 0.2 x SSC at 50°C. In addition, the temperature in the wash step can be increased from low stringency conditions at room temperature, about 22°C, to high stringency conditions at about 65°C. Both temperature and salt may be varied, or either the temperature or the salt concentration may be held constant while the other
25 variable is changed.

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

30 It is contemplated that lower stringency hybridization conditions such as lower hybridization and/or washing temperatures can be used to identify related sequences having a lower degree of sequence similarity if specificity of binding of the probe or primer to target sequence(s) is preserved. Accordingly, the nucleotide sequences of

the present invention can be used for their ability to selectively form duplex molecules with complementary stretches of DNA, RNA, or cDNA fragments.

A fragment of a nucleic acid molecule can be any sized fragment and illustrative fragments include fragments of nucleic acid sequences set forth in SEQ ID NO: 1 to SEQ ID NO: 116 and complements thereof. In one aspect, a fragment can be between 15 and 25, 15 and 30, 15 and 40, 15 and 50, 15 and 100, 20 and 25, 20 and 30, 20 and 40, 20 and 50, 20 and 100, 25 and 30, 25 and 40, 25 and 50, 25 and 100, 30 and 40, 30 and 50, and 30 and 100. In another aspect, the fragment can be greater than 10, 15, 20, 25, 30, 35, 40, 50, 100, or 250 nucleotides.

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

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

In another embodiment, markers, such as single sequence repeat markers (SSR), AFLP markers, RFLP markers, RAPD markers, phenotypic markers, isozyme markers, single nucleotide polymorphisms (SNPs), insertions or deletions (Indels), single feature polymorphisms (SFPs, for example, as described in Borevitz et al. 2003 Gen. Res. 13:513-523), microarray transcription profiles, DNA-derived sequences, and RNA-derived sequences that are genetically linked to or correlated with alleles of a QTL of the present invention can be utilized.

In one embodiment, nucleic acid-based analyses for the presence or absence of the genetic polymorphism can be used for the selection of seeds in a breeding population. A wide variety of genetic markers for the analysis of genetic

polymorphisms are available and known to those of skill in the art. The analysis may be used to select for genes, QTL, alleles, or genomic regions (haplotypes) that comprise or are linked to a genetic marker.

Herein, nucleic acid analysis methods are known in the art and include, but are not limited to, PCR-based detection methods (for example, TaqMan assays), microarray methods, and nucleic acid sequencing methods. In one embodiment, the detection of polymorphic sites in a sample of DNA, RNA, or cDNA may be facilitated through the use of nucleic acid amplification methods. Such methods specifically increase the concentration of polynucleotides that span the polymorphic site, or include that site and sequences located either distal or proximal to it. Such amplified molecules can be readily detected by gel electrophoresis, fluorescence detection methods, or other means.

A method of achieving such amplification employs the polymerase chain reaction (PCR) (Mullis *et al.* Cold Spring Harbor Symp. Quant. Biol. 51:263-273 (1986); European Patent 50,424; European Patent 84,796; European Patent 258,017; European Patent 237,362; European Patent 201,184; U.S. Patent 4,683,202; U.S. Patent 4,582,788; and U.S. Patent 4,683,194), using primer pairs that are capable of hybridizing to the proximal sequences that define a polymorphism in its double-stranded form.

Polymorphisms in DNA sequences can be detected or typed by a variety of effective methods well known in the art including, but not limited to, those disclosed in U.S. Patents 5,468,613 and 5,217,863; 5,210,015; 5,876,930; 6,030,787; 6,004,744; 6,013,431; 5,595,890; 5,762,876; 5,945,283; 5,468,613; 6,090,558; 5,800,944; and 5,616,464.

However, the compositions and methods of this invention can be used in conjunction with any polymorphism typing method to type polymorphisms in soybean genomic DNA samples. These soybean genomic DNA samples used include but are not limited to soybean genomic DNA isolated directly from a soybean plant, cloned soybean genomic DNA, or amplified soybean genomic DNA.

For instance, polymorphisms in DNA sequences can be detected by hybridization to allele-specific oligonucleotide (ASO) probes as disclosed in U.S. Patents 5,468,613 and 5,217,863. US Patent 5,468,613 discloses allele specific oligonucleotide hybridizations where single or multiple nucleotide variations in nucleic acid sequence can be detected in nucleic acids by a process in which the

sequence containing the nucleotide variation is amplified, spotted on a membrane and treated with a labeled sequence-specific oligonucleotide probe.

Target nucleic acid sequence can also be detected by probe ligation methods as disclosed in U.S. Patent 5,800,944 where sequence of interest is amplified and
5 hybridized to probes followed by ligation to detect a labeled part of the probe.

Microarrays can also be used for polymorphism detection, wherein oligonucleotide probe sets are assembled in an overlapping fashion to represent a single sequence such that a difference in the target sequence at one point would result in partial probe hybridization (Borevitz et al., Genome Res. 13:513-523 (2003); Cui et al., Bioinformatics 21:3852-3858 (2005). On any one microarray, it is expected there
10 will be a plurality of target sequences, which may represent genes and/or noncoding regions wherein each target sequence is represented by a series of overlapping oligonucleotides, rather than by a single probe. This platform provides for high throughput screening a plurality of polymorphisms. A single-feature polymorphism
15 (SFP) is a polymorphism detected by a single probe in an oligonucleotide array, wherein a feature is a probe in the array. Typing of target sequences by microarray-based methods is disclosed in US Patents 6,799,122; 6,913,879; and 6,996,476.

Target nucleic acid sequence can also be detected by probe linking methods as disclosed in U.S. Patent 5,616,464 employing at least one pair of probes having
20 sequences homologous to adjacent portions of the target nucleic acid sequence and having side chains which non-covalently bind to form a stem upon base pairing of said probes to said target nucleic acid sequence. At least one of the side chains has a photoactivatable group which can form a covalent cross-link with the other side chain member of the stem.

Other methods for detecting SNPs and Indels include single base extension
25 (SBE) methods. Examples of SBE methods include, but are not limited, to those disclosed in U.S. Patents 6,004,744; 6,013,431; 5,595,890; 5,762,876; and 5,945,283. SBE methods are based on extension of a nucleotide primer that is adjacent to a polymorphism to incorporate a detectable nucleotide residue upon extension of the
30 primer. In certain embodiments, the SBE method uses three synthetic oligonucleotides. Two of the oligonucleotides serve as PCR primers and are complementary to sequence of the locus of soybean genomic DNA which flanks a region containing the polymorphism to be assayed. Following amplification of the region of the soybean genome containing the polymorphism, the PCR product is

mixed with the third oligonucleotide (called an extension primer) which is designed to hybridize to the amplified DNA adjacent to the polymorphism in the presence of DNA polymerase and two differentially labeled dideoxynucleosidetriphosphates. If the polymorphism is present on the template, one of the labeled
5 dideoxynucleosidetriphosphates can be added to the primer in a single base chain extension. The allele present is then inferred by determining which of the two differential labels was added to the extension primer. Homozygous samples will result in only one of the two labeled bases being incorporated and thus only one of the two labels will be detected. Heterozygous samples have both alleles present, and will
10 thus direct incorporation of both labels (into different molecules of the extension primer) and thus both labels will be detected.

In a preferred method for detecting polymorphisms, SNPs and Indels can be detected by methods disclosed in U.S. Patents 5,210,015; 5,876,930; and 6,030,787 in which an oligonucleotide probe having a 5' fluorescent reporter dye and a 3' quencher
15 dye covalently linked to the 5' and 3' ends of the probe. When the probe is intact, the proximity of the reporter dye to the quencher dye results in the suppression of the reporter dye fluorescence, e.g. by Forster-type energy transfer. During PCR forward and reverse primers hybridize to a specific sequence of the target DNA flanking a polymorphism while the hybridization probe hybridizes to polymorphism-containing
20 sequence within the amplified PCR product. In the subsequent PCR cycle DNA polymerase with 5' → 3' exonuclease activity cleaves the probe and separates the reporter dye from the quencher dye resulting in increased fluorescence of the reporter.

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

30 The genetic linkage of additional marker molecules can be established by a gene mapping model such as, without limitation, the flanking marker model reported by Lander *et al.* (Lander *et al.*, *Genetics*, 121:185-199 (1989)), and the interval mapping, based on maximum likelihood methods described therein, and implemented in the software package MAPMAKER/QTL (Lincoln and Lander, *Mapping Genes*

Controlling Quantitative Traits Using MAPMAKER/QTL, Whitehead Institute for Biomedical Research, Massachusetts, (1990)). Additional software includes Qgene, Version 2.23 (1996), Department of Plant Breeding and Biometry, 266 Emerson Hall, Cornell University, Ithaca, NY). Use of Qgene software is a particularly preferred

5 approach.

A maximum likelihood estimate (MLE) for the presence of a marker is calculated, together with an MLE assuming no QTL effect, to avoid false positives. A $\log_{10}$ of an odds ratio (LOD) is then calculated as: $\text{LOD} = \log_{10} (\text{MLE for the presence of a QTL} / \text{MLE given no linked QTL})$. The LOD score essentially indicates how

10 much more likely the data are to have arisen assuming the presence of a QTL versus in its absence. The LOD threshold value for avoiding a false positive with a given confidence, say 95%, depends on the number of markers and the length of the genome. Graphs indicating LOD thresholds are set forth in Lander *et al.* (1989), and further described by Arús and Moreno-González, *Plant Breeding*, Hayward,

15 Bosemark, Romagosa (eds.) Chapman & Hall, London, pp. 314-331 (1993).

Additional models can be used. Many modifications and alternative approaches to interval mapping have been reported, including the use of non-parametric methods (Kruglyak *et al.*, *Genetics*, 139:1421-1428 (1995)). Multiple regression methods or models can be also be used, in which the trait is regressed on a

20 large number of markers (Jansen, *Biometrics in Plant Breed*, van Oijen, Jansen (eds.) Proceedings of the Ninth Meeting of the Eucarpia Section Biometrics in Plant Breeding, The Netherlands, pp. 116-124 (1994); Weber and Wricke, *Advances in Plant Breeding*, Blackwell, Berlin, 16 (1994)). Procedures combining interval mapping with regression analysis, whereby the phenotype is regressed onto a single

25 putative QTL at a given marker interval, and at the same time onto a number of markers that serve as 'cofactors,' have been reported by Jansen *et al.* (Jansen *et al.*, *Genetics*, 136:1447-1455 (1994)) and Zeng (Zeng, *Genetics* 136:1457-1468 (1994)). Generally, the use of cofactors reduces the bias and sampling error of the estimated QTL positions (Utz and Melchinger, *Biometrics in Plant Breeding*, van Oijen, Jansen

30 (eds.) Proceedings of the Ninth Meeting of the Eucarpia Section Biometrics in Plant Breeding, The Netherlands, pp.195-204 (1994), thereby improving the precision and efficiency of QTL mapping (Zeng 1994). These models can be extended to multi-environment experiments to analyze genotype-environment interactions (Jansen *et al.*, *Theor. Appl. Genet.* 91:33-3 (1995)).

Selection of appropriate mapping populations is important to map construction. The choice of an appropriate mapping population depends on the type of marker systems employed (Tanksley *et al.*, *Molecular mapping in plant chromosomes. chromosome structure and function: Impact of new concepts* J.P.

5 Gustafson and R. Appels (eds.). Plenum Press, New York, pp. 157-173 (1988)).

Consideration must be given to the source of parents (adapted vs. exotic) used in the mapping population. Chromosome pairing and recombination rates can be severely disturbed (suppressed) in wide crosses (adapted x exotic) and generally yield greatly reduced linkage distances. Wide crosses will usually provide segregating populations
10 with a relatively large array of polymorphisms when compared to progeny in a narrow cross (adapted x adapted).

Recombinant inbred lines (RIL) (genetically related lines; usually $>F_5$, developed from continuously selfing F_2 lines towards homozygosity) can be used as a mapping population. Information obtained from dominant markers can be maximized
15 by using RIL because all loci are homozygous or nearly so. Under conditions of tight linkage (*i.e.*, about $<10\%$ recombination), dominant and co-dominant markers evaluated in RIL populations provide more information per individual than either marker type in backcross populations (Reiter *et al.*, *Proc. Natl. Acad. Sci.(USA)*
89:1477-1481 (1992)). However, as the distance between markers becomes larger
20 (*i.e.*, loci become more independent), the information in RIL populations decreases dramatically.

Backcross populations (*e.g.*, generated from a cross between a successful variety (recurrent parent) and another variety (donor parent) carrying a trait not present in the former) can be utilized as a mapping population. A series of
25 backcrosses to the recurrent parent can be made to recover most of its desirable traits. Thus a population is created consisting of individuals nearly like the recurrent parent but each individual carries varying amounts or mosaic of genomic regions from the donor parent. Backcross populations can be useful for mapping dominant markers if all loci in the recurrent parent are homozygous and the donor and recurrent parent
30 have contrasting polymorphic marker alleles (Reiter *et al.* (1992)). Information obtained from backcross populations analyzed using either codominant or dominant markers is less than that obtained from F_2 populations because one, rather than two, recombinant gametes are sampled per plant. Backcross populations, however, are more informative (at low marker saturation) when compared to RILs as the distance

between linked loci increases in RIL populations (*i.e.* about .15% recombination). Increased recombination can be beneficial for resolution of tight linkages, but may be undesirable in the construction of maps with low marker saturation.

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

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

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

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

For highly heritable traits, a choice of superior individual plants evaluated at a single location will be effective, whereas for traits with low heritability, selection should be based on mean values obtained from replicated evaluations of families of related plants. Popular selection methods commonly include pedigree selection,

modified pedigree selection, mass selection, and recurrent selection. In a preferred aspect, a backcross or recurrent breeding program is undertaken.

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

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

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

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

The single-seed descent procedure in the strict sense refers to planting a segregating population, harvesting a sample of one seed per plant, and using the one-seed sample to plant the next generation. When the population has been advanced from the F_2 to the desired level of inbreeding, the plants from which lines are derived will each trace to different F_2 individuals. The number of plants in a population declines each generation due to failure of some seeds to germinate or some plants to produce at least one seed. As a result, not all of the F_2 plants originally sampled in the population will be represented by a progeny when generation advance is completed.

Descriptions of other breeding methods that are commonly used for different traits and crops can be found in one of several reference books (Allard, *Principles of*

Plant Breeding, John Wiley & Sons, NY, U. of CA, Davis, CA, 50-98 (1960);
Simmonds, *Principles of crop improvement*, Longman, Inc., NY, 369-399 (1979);
Sneep and Hendriksen, *Plant breeding perspectives*, Wageningen (ed), Center for
Agricultural Publishing and Documentation (1979); Fehr, *In: Soybeans: Improvement*,
5 *Production and Uses*, 2nd Edition, *Monograph.*, 16:249 (1987); Fehr, "Principles of
variety development," *Theory and Technique*, (Vol. 1) and *Crop Species Soybean*
(Vol. 2), Iowa State Univ., Macmillan Pub. Co., NY, 360-376 (1987).

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

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

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

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

such an attribute may be catalytic, and thus involve the capacity of the agent to mediate a chemical reaction or response.

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

The agents of the present invention may be labeled with reagents that facilitate detection of the agent (*e.g.* fluorescent labels (Prober *et al.*, *Science* 238:336-340 (1987); Albarella *et al.*, European Patent 144914), chemical labels (Sheldon *et al.*, U.S. Patent 4,582,789; Albarella *et al.*, U.S. Patent 4,563,417), modified bases (Miyoshi *et al.*, European Patent 119448).

Apparatus and methods for the high-throughput, non-destructive sampling of seeds have been described which would overcome the obstacles of statistical samples by allowing for individual seed analysis. For example, U.S. Patent Application Serial No. 11/213,435 (US2006/0046264) filed August 26, 2005 and U.S. Patent Application Serial No. 11/680,611 (US2007/0204366) filed March 2, 2007, disclose apparatus and systems for the automated sampling of seeds as well as methods of sampling, testing and bulking seeds.

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

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

EXAMPLES

Example 1. Validation of SNP markers for introgression of PRR resistance loci when used in conjunction with haplotype of known resistance source

Phytophthora sojae is the causal agent of Phytophthora root rot (PRR) and accounts for significant soybean yield loss in the United States. Planting resistant varieties is an effective method of controlling PRR. Breeding for PRR resistant

soybeans can be greatly facilitated by the use of marker-assisted selection for PRR resistance alleles. Segregating populations were generated to validate SNP markers that could be used to detect, select for, and introgress PRR resistance loci into plants

The phenotypic rating scale and definitions used herein for the following

- 5 Examples are included in Table 1. The percentage of plants surviving after inoculation with *P. sojae* is used for classification. This rating scale provides the basis for all disease ratings and determinations of resistance or susceptibility in the following Examples.

- 10 **Table 1:** Description of rating criteria used for PRR phenotyping.

Phenotypic Results	Rating
0-25% Dead	Resistant (R)
26-49% Dead	Heterozygous (H)
≥ 50% Dead	Susceptible (S)

- Segregating populations, involving crosses between a susceptible cultivar and a series of isolines containing *Rps* resistance alleles, were developed for genetic mapping in the summer of 2004 (Table 2). Individual F2 plants were tissue sampled and screened with markers polymorphic for the *Rps* resistant locus associated with the resistance source. F2:3 seed of the harvested F2 plants was analyzed for the PRR reaction. Results from validation experiments demonstrating of the utility of the markers are provided in Tables 3 through 10.

- 20 **Table 2:** Screening Populations for the PRR reaction

Resistant Parent	Resistant locus	Susceptible Parent
L75-3735	<i>Rps1c</i>	MV0030
Williams 82	<i>Rps1k</i>	MV0030
L83-570	<i>Rps3a</i>	MV0030
L89-1541	<i>Rps3b</i>	MV0030
L92-7857	<i>Rps3c</i>	MV0030
PI399703	<i>Rps8</i>	Williams

L75-3735 is a known source of *Rps1c*. From the L75-3735/MV0030 population, a total of 322 F2 plants were tissue sampled for marker screening. The seed harvested from each plant was used to plant and phenotype individual F3 plants from each F2-

derived family for reaction to PRR. Plants exhibiting a resistant reaction were found to have a different haplotype from that of susceptible plants when screened with genetic markers NS0102174, NS0118976, NS0118166, NS0099413, and NS0119335. The total number of families from the L75-3735/MV0030 population with the same

5 haplotype as the resistant parent was 41, and of these families, all 41 had a resistant reaction when phenotyped. From each family, an average of 10 F2 derived F3 plants (F2 families) were phenotyped. Table 3 provides an example of the PRR reaction and haplotype of two families from the L75-3735/MV0030 population.

Table 3. Validation of SNP markers used in conjunction with the haplotype of a known *RpsIc* source.

Family	Total Plants	Susc. Plants	Reaction	Pedigree	NS- 0102174	NS- 0118976	NS- 0118166	NS- 0099413	NS- 0119335
1	12	0	R	L75- 3735/MV0030	GG	TT	TT	CC	TT
2	11	10	S	L75- 3735/MV0030	CC	CC	CC	TT	AA

Williams 82 is a known source of *Rps1k*. From the Williams82/ MV0030 population, a total of 205 F2 plants were tissue sampled for marker screening. The seed harvested from each plant was used to plant and phenotype individual F3 plants from each F2-
5 derived family for reaction to PRR. Plants exhibiting a resistant reaction were found to have a different haplotype from that of susceptible plants when screened with genetic markers NS0118166, NS0102920, NS0114258, NS0102174, NS0119981, and NS0201536. The total number of families from the Williams 82/MV0030 population with the same haplotype as the resistant parent was 24, and of these families, all 24
10 had a resistant reaction when phenotyped. Table 4 provides an example of the PRR reaction and haplotype of two families from the Williams82/ MV0030 population.

Table 4. Validation of SNP markers used in conjunction with the haplotype of a known *Rps1k* source. (“*”) indicates a single nucleotide deletion)

Family	Total Plants	Susc. Plants	Reaction	Pedigree	NS- 0118166	NS- 0102920	NS- 0114258	NS- 0118976	NS- 0102174	NS- 0119981	NS- 0201536
1	12	0	R	Williams82/ MV0030	TT	CC	TT	TT	GG	CC	CGAG
2	10	10	S	Williams82/ MV0030	CC	TT	CC	CC	CC	AA	****

L83-570 is a known source of *Rps3a*. From the L83-570/MV0030 population, a total of 297 F2 plants were tissue sampled for marker screening. The seed harvested from each plant was used to plant and phenotype individual F3 plants from each F2-derived family for reaction to PRR. Plants exhibiting a resistant reaction were found to have a different haplotype than susceptible plants when screened with the genetic markers NS0101324 and NS0116265. The total number of families from the L83-570/MV0030 population with the same haplotype as the resistant parent was 64, and of these families, 58 were found to have a resistant reaction when phenotyped. Table 5 provides an example of the PRR reaction and haplotype for two families from the L83-570/MV0030 population.

Table 5. Validation of SNP markers used in conjunction with haplotype of a known *Rps3a* source.

Family	Total Plants	Susc. Plants	Reaction	Pedigree	NS-0101324	NS-0116265
1	11	0	R	L83-570/MV0030	AA	GG
2	10	9	S	L83-570/MV0030	GG	TT

L89-1541 is a known source of *Rps3b*. From the L89-1541/MV0030 population, a total of 345 F2 plants were tissue sampled for marker screening. The seed harvested from each plant was used to plant and phenotype individual F3 plants from each F2-derived family for reaction to PRR. Plants exhibiting a resistant reaction were found to have a different haplotype than susceptible plants when screened with the genetic markers NS0114683, NS0102483, NS0119333, and NS0102262. The total number of families from the L89-1541/MV0030 population with the same haplotype of the resistant parent was 88, and of these families, 47 were found to have a resistant reaction when phenotyped. Table 6 provides an example of the PRR reaction and haplotype of two families from the L89-1541/MV0030 population.

Table 6. Validation of SNP markers used in conjunction with haplotype of known source of *Rps3b*.

Family	Total Plants	Susc. Plants	Reaction	Pedigree	NS-0114683	NS-0102483	NS-0119333	NS-0102262
1	12	0	R	L89-1541/MV0030	AA	GG	AA	AA
2	12	12	S	L89-1541/MV0030	CC	AA	CC	GG

L92-7857 is a known source of *Rps3c*. From the L92-7857/MV0030 population, a total of 340 F2 plants were tissue sampled for marker screening. The seed harvested from each plant was used to plant and phenotype individual F3 plants from each F2-derived family for reaction to PRR. Plants exhibiting a resistant reaction were found to have a different haplotype than susceptible plants when screened with genetic markers NS0114683, NS0102483, NS0119333, and NS0102262. The total number of families from the L92-7857/MV0030 population with the same haplotype of the resistant parent was 112, and of these families, 87 were found to have a resistant reaction when phenotyped. Table 7 provides an example of the PRR reaction and haplotype of two of the families from the L92-7857/MV0030 population.

Table 7. Validation of SNP markers used in conjunction with the haplotype of a known source of *Rps3c*.

Family	Total Plants	Susc. Plants	Reaction	Pedigree	NS-0114683	NS-0102483	NS-0119333	NS-0102262
1	11	0	R	L92-7857/MV0030	AA	GG	AA	AA
2	12	11	S	L92-7857/MV0030	AC	AG	AC	AG

PI399703 is a known source of *Rps8*. From the PI399703/Williams population, a total of 223 F2 plants were tissue samples for marker screening. The seed harvested from each plant was used to plant and phenotype individual F3 plants from each F2-derived family for reaction to PRR. Plants exhibiting a resistant reaction were found to have a different haplotype than susceptible plants when screened with genetic markers NS0114683, NS0102483, NS0119333, and NS0102262. The total number of families from the PI399703/Williams population with the same haplotype of the

resistant parent was 39, and of these families, all 39 had a resistant reaction when phenotyped. Table 8 provides an example of the PRR reaction and haplotype of two families from the PI399703/Williams population.

Table 8. Validation of SNP markers used in conjunction with the haplotype of a known source of *Rps8*.

Family	Total Plants	Susc. Plants	Reaction	Pedigree	NS- 0101324	NS- 0102262	NS- 0102483	NS- 0114683	NS- 0116265	NS- 0119333
1	6	0	R	PI399703/Williams	AA	GG	AA	CC	GG	CC
2	4	4	S	PI399703/Williams	GG	AA	GG	AA	TT	AA

An additional SNP marker, NS0138011, was identified for the detection of the *Rps1c* locus. Genotyping results of 100 soybean lines which were known to have either *Rps1a*, *Rps1k*, *Rps1c*, or to be susceptible to PRR were examined. Lines with *Rps1c* were found to have the AA allele when screened with the marker NS0138011. The other 85 lines, which had either PRR resistance loci *Rps1a*, *Rps1k*, or were susceptible to PRR, had the CC allele as provided in Table 9. Therefore, screening with the genetic marker NS0138011 can be used for detecting the *Rps1c* locus in a soybean breeding program.

Table 9. Validation of SNP marker NS0138011 in detecting *Rps1c*.

Allele	Number of lines	Locus
CC	85	<i>Rps1a</i> , <i>Rps1k</i> or susceptible
AA	15	<i>Rps1c</i>

Table 10 summarizes validation experiments and includes the SNP markers found to be useful in monitoring the selection or introgression of the PRR resistance locus *Rps1*, including *Rps1c* and *Rps1k* alleles into a soybean plant in a soybean breeding program. The haplotype of the known resistance source is used to determine which *Rps1* allele is selected for in a breeding program. Table 10 also includes SNP markers useful for monitoring the introgression of the PRR resistance loci *Rps3* and *Rps8* into a soybean plant in a soybean breeding program. PRR resistance locus *Rps3* includes, *Rps3a*, *Rps3b*, and *Rps3c*. The haplotype of the known resistance source is used to determine which *Rps3* allele is selected for in a breeding program. SNP markers found to be useful for screening for *Rps1* include NS0099413, NS0102174, NS0118166, NS0102920, NS0114258, NS0118976, NS0119981, NS0119335, NS0201536, NS0138011, NS0127084, NS0129030, NS0202603, and NS0203225. SNP markers found to be useful for screening for *Rps3* and *Rps8* include NS0114683, NS010324, NS0102483, NS0119333, NS0102262, and NS0116265. In a soybean breeding program, plants genotyped as homozygous or heterozygous for the resistant parent alleles, may be selected for advancement.

Table 10. SNP markers useful for introgression of PRR resistance loci *Rps1*, *Rps3*, and *Rps8* into soybean plants when used in conjunction with the known haplotype of the resistance source.

Marker	LG*	Chr. Pos.	<i>Rps</i> Locus	SEQ ID	SNP Position	Allele 1	Allele 2	SEQ ID Forward Primer	SEQ ID Reverse Primer	SEQ ID Probe 1	SEQ ID Probe 2
NS0099413	N	25.0	1	1	242	CC	TT	17	18	49	50
NS0102174	N	25.0	1	2	563	CC	GG	19	20	51	52
NS0118166	N	25.0	1	3	259	CC	TT	21	22	53	54
NS0102920	N	25.0	1	4	163	CC	TT	23	24	55	56
NS0114258	N	25.0	1	5	324	CC	TT	25	26	57	58
NS0118976	N	25.0	1	6	460	CC	TT	27	28	59	60
NS0119981	N	28.7	1	7	516	AA	CC	29	30	61	62
NS0119335	N	29.5	1	8	310	AA	TT	31	32	63	64
NS0202603	N	31.1	1	81	51	AA	GG	85	86	93	94
NS0138011	N	32.2	1	10	385	AA	CC	35	36	67	68
NS0201536	N	33.0	1	9	35-38	CGAG	****	33	34	65	66
NS0203225	N	33.2	1	82	192	AA	TT	87	88	95	96

NS0129030	N	34.7	1	83	324	CC	AA	89	90	97	98
NS0127084	N	41.4	1	84	858	CC	TT	91	92	99	100
NS0114683	F	100.6	3,8	11	368	AA	CC	37	38	69	70
NS0101324	F	100.8	3,8	12	84	AA	GG	39	40	71	72
NS0102483	F	101.7	3,8	13	172	AA	GG	41	42	73	74
NS0119333	F	101.7	3,8	14	607	AA	CC	43	44	75	76
NS0102262	F	103.0	3,8	15	131	AA	GG	45	46	77	78
NS0116265	F	113.8	3,8	16	719	GG	TT	47	48	79	80

*LG= Linkage Group

Example 2: Use of SNP markers to select for the *Rps1k* locus which confers resistance to PRR race 4.

SNP markers were used with knowledge of PRR resistant germplasm to breed for PRR resistance (Table 11). The F3 population derived from the cross of two PPR resistance sources AG3602, a source of *Rps1c*, and AG3505, a source of *Rps1k*, was screened with two SNP markers. A total of 466 individuals were screened. The SNP markers NS0119335 and NS0118166 were used to select for *Rps1k* based on the haplotype of AG3505, a source of resistance for PRR race 4. Sources of *Rps1* resistance include, but are not limited to AG3602, AG3505, and DKB28-53.

Table 11. Haplotypes of two sources of PRR resistance.

	Marker 1	Marker 2
Source	NS0119335	NS0118166
AG3602 (<i>Rps1c</i>)	TT	CC
AG3505 (<i>Rps1k</i>)	AA	TT

Example 3. Use of SNP markers to monitor the introgression of PRR resistance locus *Rps1k*.

F3 individuals derived from a cross between two soybean lines AG3602 (*Rps1c*) and AG3505 (*Rps1k*) were genotyped. The *Rps1k* locus provides resistance to PRR race 4. A total of 466 F3 plants are screened with two SNP markers NS0119335 and NS0118160. Table 12 reports the results of the phenotypic validation of the genotypic screening for PRR race 4. Screening with NS0119335 was 85.7% predictive of PRR reaction. Screening with NS0118160 was 83.8% predictive of PRR reaction.

Table 12. Results from phenotypic screening following selection based on resistance source haplotype.

Marker	Favorable Haplotype	Individuals selected based on haplotype	Individuals with Res. Reaction	% Resistant	Origin
NS0119335	AA	182	156	85.7	AG3602/AG3505
NS0118160	TT	185	155	83.8	AG3602/AG3505

Example 4. Use of SNP markers to select for *Rps3* or *Rps8* which confer resistance to PRR race 25.

F3 plants derived from the following breeding populations were genotyped with the SNP marker NS0114683. *Rps3* and *Rps8* provide resistance to PRR race 25. The breeding populations included a source of either *Rps3* or *Rps8*. Table 13 reports the results of the phenotypic validation of the genotypic screening for PRR race 25.

Screening with NS0114683 for *Rps3* was 96.4 to 100% predictive of the PRR reaction. Screening with NS0114683 for *Rps8* was 72.4 to 80.8% predictive of the PRR reaction.

Table 13. SNP marker NS-0114683 was used to select plants with haplotype matching resistance source. Phenotypic screening with PRR race 25 was then conducted.

Favorable Haplotype	Individuals selected based on haplotype	Individuals with Res. Reaction	% Resistant	Rps	Origin
CC	17	17	100.0	<i>Rps3</i>	Ivory/ DKB28-53
CC	48	47	97.9	<i>Rps3</i>	MV0033/CFN3303E3R
CC	28	27	96.4	<i>Rps3</i>	Ivory/ MV0036
CC	26	21	80.8	<i>Rps8</i>	DKB28-53/((Darby/OX-98317)/AG2703))
CC	29	21	72.4	<i>Rps8</i>	MV0039/((Darby/OX-98317)/MV0028))

Example 5. Introgression of race specific PRR resistance using SNP markers.

In this example, L75-3735 was the source of PRR resistance locus *Rps1c*.

Populations from a cross of L75-3735 with MV0030, a line susceptible to PRR race

3, were analyzed. Two markers, NS0099413 and NS0119335, were used to select

- 5 individuals with haplotypes matching that of the resistant parent. Individuals homozygous for the alleles present in the PRR resistant source (L75-3735) were chosen for advancement (Table 14).

Table 14. Use of two SNP markers to screen for resistance or susceptibility to PRR
10 race 3.

Pedigree	# Dead	Total	% Susc.	Reaction	NS0099413	NS0119335
L75-3735 (<i>Rps1c</i>)	0	11	0	R	CC	TT
MV0030	9	10	90	S	TT	AA
MV0030	5	10	50	S	TT	AA
L75- 3735/MV0030	0	10	0	R	CC	TT
L75- 3735/MV0030	10	10	100	S	TT	AA
L75- 3735/MV0030	5	10	50	S	CT	AT
L75- 3735/MV0030	4	10	40	H	CT	AT

Example 6. Use of SNP markers to select for PRR resistance

Further SNP markers were identified which flanked marker NS0138011. The alleles

of NS0138011 have been associated with *Rps1c*. The “A” allele has been shown to

- 15 indicate the presence of *Rps1c* and the “C” allele indicates the absence of *Rps1c*. The combination of markers NS0202603, NS0138011, and NS0203225 demonstrate utility for distinguishing multiple alleles at the *Rps1* locus including *rps* (susceptible), *Rps1a*, *Rps1c*, and *Rps1k*. In a study of 239 soybean lines, the haplotypes of three markers were effective in predicting the PRR reaction from 88 to 100% of the time

(Table 15). In a soybean breeding program, the three markers can be used to identify plants with resistance to *Rps1a*, *Rps1c*, and *Rps1k*, thus allowing for marker assisted selection.

- 5 **Table 15.** Ability of marker haplotypes of NS0202603, NS0138011, and NS0203225 to predict allele configuration of *Rps1* for PRR resistance.

Predicted <i>Rps1</i> allele	Haplotype NS0202603- NS0138011- NS0203225	# lines with haplotype	# lines that show phenotype of predicted allele*	% congruence
<i>rps</i>	GG CC AA	9	9	100
<i>Rps1a</i>	AA CC TT	9	8	89
<i>Rps1c</i>	AA AA AA	189	186	98
<i>Rps1k</i>	GG CC TT	32	28*	88

* 3 are heterozygous based on phenotype

- 10 Example 7. Use of SNP markers and knowledge of parental genotype to identify *Rps1c* Resistant Plants
- NS0129030 is useful for selecting for the *Rps1c* allele in populations of late Maturity Group 3 parents that have *Rps1c* and are crossed to susceptible Maturity Group 4 parents. For example, when AG3802, AG3905, AOX3903B0C, CFN3802A1X, or
- 15 AG3602 are used as *Rps1c* donors in crosses to the PRR-susceptible lines MV0097, MV0098, MV0099, or MV0022, all of the resistant parents have the “C” allele at NS0129030, while all of the susceptible parents have the “A” allele. Knowledge of the resistant source genotype is important since the association of the “C” allele with *Rps1c* is not present in all other lines, as the susceptible lines MV0101, MV0102, and
- 20 MV0103 also have the “C” allele at this locus. Therefore in a soybean breeding program, the marker NS0129030 can be used with knowledge of the resistant source genotype to select resistant plants.

- NS0127084 is also useful in many of the same populations. For example, the *Rps1c* donors AG3802, AG3905, AOX3903B0C, CFN3802C1X, or AG3602 all have
- 25 the “C” allele at the NS0127084 locus, while the susceptible lines MV0097, MV0099, and MV0100 all have the “T” allele. However, MV0098 also has the “C” allele, but lacks *Rps1c*. Therefore in a soybean breeding program, the marker NS0127084 can be used with knowledge of the resistant source genotype to select resistant plants.

Example 8. Use of SNP Markers to select for *Rps3c*

A population was provided from the cross of MV0031/BFN3205A0R, with BFN3205A0R as the heterozygous source of *Rps3c* which confers resistance to *Phytophthora sojae* race 25. The haplotypes of the parents and that of CFN3303E3R, a homozygous sister line of BFN3205A0R, are provided in Table 16. A total of 4,224 F2 seeds were non-destructively sampled and genotyped. Individual seeds were selected which were homozygous favorable for *Rps3c*. A total of 1018 seeds had the haplotype CCGGGG at the marker loci NS0119333, NS0102262, NS0116265 and were selected for planting. F2 seeds genotyped as homozygous favorable for *Rps3c* were planted in the Spring of 2006. F2:3 seed from selected plants were planted as progeny rows in 2006-2007. F2:4 seed bulked from selected progeny rows was grown in yield trials in 2007. Four lines selected for superior yield were evaluated for resistance to PRR race 25 to confirm presence of *Rps3c*. Pathology data are provided in Table 18. Pathology testing confirmed the marker assisted selection for resistance to *Phytophthora sojae* race 25. From this example, the markers provided have shown use for marker assisted selection for PRR resistance to *Phytophthora sojae* race 25.

Table 16. Haplotypes of MV0031, BFN3205A0R, and CFN3303E3R.

Line	NS0119333	NS0102262	NS0116265
MV0031	AA	AA	GG
BFN3205A0R	AC	AG	TT
CFN3303E3R	CC	GG	GG

20

Table 17. Pathology screening for *Rps3* with *Phytophthora sojae* race 25.

Line	Replications	Total Plants	Susceptible Plants
1	3	24	0
2	3	24	0
3	3	24	0
4	3	24	0

Example 9. Oligonucleotide hybridization probes useful for detecting soybean plants with PRR resistance loci

- 5 Oligonucleotides can also be used to detect or type the polymorphisms associated with PRR resistance disclosed herein by hybridization-based SNP detection methods. Oligonucleotides capable of hybridizing to isolated nucleic acid sequences which include the polymorphism are provided. It is within the skill of the art to design assays with experimentally determined stringency to discriminate between the allelic
- 10 states of the polymorphisms presented herein. Exemplary assays include Southern blots, Northern blots, microarrays, *in situ* hybridization, and other methods of polymorphism detection based on hybridization. Exemplary oligonucleotides for use in hybridization-based SNP detection are provided in Table 18. These oligonucleotides can be detectably labeled with radioactive labels, fluorophores, or
- 15 other chemiluminescent means to facilitate detection of hybridization to samples of genomic or amplified nucleic acids derived from one or more soybean plants using methods known in the art.

Table 18. Oligonucleotide Hybridization Probes*

Marker	Marker SEQ ID	SNP Position	Hybridization Probe	SEQ ID Probe
NS0119335	8	310	TCTCAGAGTGGGTAGA	101
NS0119335	8	310	TCTCAGTGTGGGTAGA	102
NS0138011	10	385	GAATGAAAAATCTACT	103
NS0138011	10	385	GAATGACAAATCTACT	104
NS0119333	14	607	TAAGAACCCTCTCCAA	105
NS0119333	14	607	TAAGAAACCTCTCCAA	106
NS0102262	15	131	AAGCCTGACAATTGAT	107
NS0102262	15	131	AAGCCTAACAATTGAT	108

* 16mer spanning SNP

Example 10. Oligonucleotide probes useful for detecting soybean plants with PRR resistance loci by single base extension methods

- 5 Oligonucleotides can also be used to detect or type the polymorphisms associated with PRR resistance disclosed herein by single base extension (SBE)-based SNP detection methods. Exemplary oligonucleotides for use in SBE-based SNP detection are provided in Table 19. SBE methods are based on extension of a nucleotide primer that is hybridized to sequences adjacent to a polymorphism to incorporate a detectable
- 10 nucleotide residue upon extension of the primer. It is also anticipated that the SBE method can use three synthetic oligonucleotides. Two of the oligonucleotides serve as PCR primers and are complementary to the sequence of the locus which flanks a region containing the polymorphism to be assayed. Exemplary PCR primers that can be used to type polymorphisms disclosed in this invention are provided in Table 10 in
- 15 the columns labeled "Forward Primer SEQ ID" and "Reverse Primer SEQ ID". Following amplification of the region containing the polymorphism, the PCR product is hybridized with an extension primer which anneals to the amplified DNA adjacent to the polymorphism. DNA polymerase and two differentially labeled dideoxynucleoside triphosphates are then provided. If the polymorphism is present on
- 20 the template, one of the labeled dideoxynucleoside triphosphates can be added to the primer in a single base chain extension. The allele present is then inferred by determining which of the two differential labels was added to the extension primer. Homozygous samples will result in only one of the two labeled bases being

incorporated and thus only one of the two labels will be detected. Heterozygous samples have both alleles present, and will thus direct incorporation of both labels (into different molecules of the extension primer) and thus both labels will be detected.

5

Table 19. Probes (extension primers) for Single Base Extension (SBE) assays.

Marker	Marker SEQ ID	SNP Position	SBE Probe	SEQ ID Probe
NS0119335	8	310	AGACTCTCTCTCTCAGA	109
NS0119335	8	310	ATTGGATTTCTACCCAC	110
NS0138011	10	385	AAATTCCTGTGAATGAA	111
NS0138011	10	385	TTATTCAAAGTAGATTT	112
NS0119333	14	607	TTTTTTAAATTAAGAAC	113
NS0119333	14	607	TGAAAGTGTTGGAGAGG	114
NS0102262	15	131	AAGTACTCCCAAGCCTG	115
NS0102262	15	131	ACAAGACAATCAATTGT	116

10 The scope of the claims should not be limited by the preferred embodiments set forth herein, but should be given the broadest interpretation consistent with the description as a whole.

15

The embodiments of the present invention for which an exclusive property or privilege is claimed are defined as follows:

1. A method for introgressing an allele into a soybean plant comprising:
 - a. crossing at least one *Phytophthora* root rot (PRR) resistant soybean plant with at least one other soybean plant to form a population;
 - b. screening said population for the presence of at least one nucleic acid marker that comprises SEQ ID NO:10;
 - c. selecting from said population, on the basis of said presence of the at least one nucleic acid marker, one or more soybean plants for comprising a haplotype associated with PRR resistance, wherein said haplotype corresponds to a *Rpslc* locus and comprises SEQ ID NO: 10;wherein at least one of said at least one marker is located within 10 cM of said resistance haplotype.
2. The method according to Claim 1, where at least one of said at least one marker is located within 5 cM of said resistance haplotype.
3. The method according to Claim 1, where at least one of said at least one marker is located within 1 cM of said resistance haplotype.
4. The method according to Claim 1, where at least one of said at least one marker is located within 1 Mb of said resistance haplotype.
5. The method according to Claim 1, where at least one of said at least one marker is located within 100 Kb of said resistance haplotype.
6. The method according to Claim 1, where at least one of said at least one marker is located within 1 Kb of said resistance haplotype.
7. The method of Claim 1, wherein the source of *Rpslc* is elite germplasm.
8. The method of Claim 1, wherein the source of *Rpslc* is accession germplasm.
9. A set of oligonucleotides comprising:
 - a. a pair of oligonucleotide primers wherein each of said primers comprises at least 12 contiguous nucleotides and wherein said pair of primers permit PCR

amplification of a DNA segment comprising a soybean genomic DNA polymorphism associated with *Phytophthora* root rot (PRR) resistance locus *Rpsl* comprising SEQ ID NO: 10; and

- b. at least one detector oligonucleotide that permits detection of a polymorphism in said amplified segment, wherein the sequence of said detector oligonucleotide is at least 95 percent identical to a sequence of the same number of consecutive nucleotides in either strand of a segment of soybean DNA that include or are immediately adjacent to said soybean genome DNA polymorphism of step (a), wherein said detector oligonucleotide is at least 95% identical to SEQ ID NO:67, SEQ ID NO:68, SEQ ID NO:103, SEQ ID NO:104, SEQ ID NO:111 or SEQ ID NO:112.

- 10. A method of introgressing an allele into a soybean plant comprising:
 - a. providing a population of soybean plants;
 - b. genotyping at least one soybean plant in the population for the presence of a soybean genomic nucleic acid marker that comprises SEQ ID NO:10;
 - c. selecting from said population, on the basis of said presence of the at least one nucleic acid marker, one or more soybean plants comprising a haplotype associated with *Phytophthora* root rot (PRR) resistance, said haplotype corresponding to the *Rpslc* locus comprising SEQ ID NO:10;

wherein providing a population comprises crossing a PRR resistant soybean plant with a second soybean plant to form a population.

11. The method according to Claim 10, wherein said selected one or more soybean plants exhibit increased grain yield in the presence of PRR as compared to soybean plants lacking PRR resistance loci.

12. The method according to Claim 11, wherein said selected one or more soybean plants exhibit an increased grain yield of at least 0.5 Bu/A in the presence of PRR as compared to soybean plants lacking PRR resistance loci.

13. The method according to Claim 11, wherein said selected one or more soybean plants exhibit an increased grain yield of at least 1.0 Bu/A in the presence of PRR as compared to soybean plants lacking PRR resistance loci.

14. The method according to Claim 11, wherein said selected one or more soybean plants exhibit an increased grain yield of at least 1.5 Bu/A in the presence of PRR as compared to soybean plants lacking PRR resistance loci.