



(22) Date de dépôt/Filing Date: 2007/05/24
(41) Mise à la disp. pub./Open to Public Insp.: 2008/05/08
(62) Demande originale/Original Application: 2 653 248
(30) Priorité/Priority: 2006/05/25 (US60/808/430)

(51) Cl.Int./Int.Cl. *C12Q 1/68* (2006.01),
A01H 1/02 (2006.01), *A01H 1/04* (2006.01),
A01H 5/00 (2006.01), *C12N 5/04* (2006.01)

(71) Demandeur/Applicant:
MONSANTO TECHNOLOGY LLC, US

(72) Inventeurs/Inventors:
BALEY, GEORGE JAMES, US;
BUTRUILLE, DAVID VINCENT, US;
EATHINGTON, SAMUEL R., US;
HAVERDINK, MICHAEL D., US;
KRUGER, WARREN M., US;
LEDEAUX, JOHN ROBERT, US;

...

(74) Agent: MCFADDEN, FINCHAM

(54) Titre : PROCEDE D'IDENTIFICATION DE LOCUS QUANTITATIFS RESISTANT AUX MALADIES DANS LE SOJA,
ET COMPOSITIONS ASSOCIEES
(54) Title: A METHOD TO IDENTIFY DISEASE RESISTANT QUANTITATIVE TRAIT LOCI IN SOYBEAN AND
COMPOSITIONS THEREOF

(57) **Abrégé/Abstract:**

A method for producing an Asian Soybean Rust (ASR) resistant soybean plant comprising the steps of: a) selecting a plant using marker assisted selection from a plurality of plants by detecting the presence of a marker in linkage group C2, wherein the plant has an ASR resistant locus obtainable from P1462312, and the marker is mapped within 10 centimorgans or less from a SNP marker selected from the group consisting of NS0099746, NS0123747, NS0126598, NS0128378, NS0096829, NS0125408, NS0098902, NS0099529, NS0097798, NS0137477, NS0095322, NS0136101, NS0098982, NS0103749, NS0118897, NS0119715 and NS0130920; b) generating an ASR resistant progeny plant from the selected plant in a breeding program.



(72) **Inventeurs(suite)/Inventors(continued):** CONCIBIDO, VERGEL C., US; NARVEL, JAMES, US; PITKIN, JOHN W., US; TAMULONIS, JOHN, US; XIE, CHONGQING, US

ABSTRACT

A method for producing an Asian Soybean Rust (ASR) resistant soybean plant comprising the steps of: a) selecting a plant using marker assisted selection from a plurality of plants by detecting the presence of a marker in linkage group C2, wherein the plant has an ASR resistant locus obtainable from PI462312, and the marker is mapped within 10 centimorgans or less from a SNP marker selected from the group consisting of NS0099746, NS0123747, NS0126598, NS0128378, NS0096829, NS0125408, NS0098902, NS0099529; NS0097798, NS0137477, NS0095322, NS0136101, NS0098982, NS0103749, NS0118897, NS0119715 and NS0130920; b) generating an ASR resistant progeny plant from the selected plant in a breeding program.

A Method to Identify Disease Resistant Quantitative Trait Loci in Soybean and
Compositions Thereof

5

This is a division of Canadian Patent Application 2,653,248 filed May 24, 2007.

10

Sequence Listing

A sequence listing is contained in the file named "pa_53517.txt" which is
65,000 bytes (measured in MS-Windows) and was created on May 22, 2007 and is
located in computer readable form on a diskette filed herewith.

15

Field of the Invention

The present invention is in the field of plant breeding and disease resistance.
More specifically, the invention relates to a method for screening plants from the
genus *Glycine* containing quantitative trait loci that are associated with disease
20 resistance and methods for breeding disease resistant *Glycine* plants. The disease can
be caused by a fungus, virus, bacterium, or invertebrate animal. The invention further
relates to the use of accession germplasm containing quantitative trait loci (QTL)
conferring disease resistance for introgression into elite germplasm in a breeding
program for resistance to the fungal pathogen, *Phakopsora pachyrhizi*.

25

Background of the Invention

The soybean, *Glycine max* (L.) Merrill, is one of the major economic crops
grown worldwide as a primary source of vegetable oil and protein (Sinclair and
Backman, *Compendium of Soybean Diseases*, 3rd Ed. APS Press, St. Paul, MN, p.
30 106. (1989)). The growing demand for low cholesterol and high fiber diets has also
increased soybean's importance as a health food.

Soybean yields in the United States are reduced each year by diseases. High
yields per hectare are critical to a farmer's profit margin, especially during periods of
low prices for soybean. The financial loss caused by soybean diseases is important to

rural economies and to the economies of allied industries in urban areas. The effects of these losses are eventually felt throughout the soybean market worldwide.

Estimates of loss due to disease in the United States and Ontario vary from year to year and by disease. From 1999 to 2002 soybean yield loss estimates were in the
 5 range of 8 million metric tons to 10 million metric tons in the United States and 90,000 to 166,000 metric tons in Ontario (Wrather *et al.*, Online. *Plant Health Progress* doi:10.1094/PHP-2003-0325-01-RV).

Asian Soybean Rust (herein referred to as ASR) has been reported in the Eastern and Western Hemispheres. In the Eastern Hemisphere, ASR has been
 10 reported in Australia, China, India, Japan, Taiwan and Thailand. In the Western Hemisphere, ASR has been observed in Brazil, Columbia, Costa Rica and Puerto Rico. ASR can be a devastating disease, causing yield losses of up to 70 to 80% as reported in some fields in Taiwan. Plants that are heavily infected have fewer pods and smaller seeds that are of poor quality (Frederick *et al.*, *Mycology* 92: 217-227
 15 (2002)). ASR was first observed in the United States in Hawaii in 1994. ASR was later introduced into the continental United States in the fall of 2004, presumably as a consequence of tropical storm activity. Model predictions indicated that ASR had been widely dispersed throughout the southeastern United States, and subsequent field and laboratory observations confirmed this distribution.

20 Two species of fungi, *Phakopsora pachyrhizi* Sydow and *Phakopsora meibomiae* (Arthur) Arthur, cause ASR. Unlike other rusts, *P. pachyrhizi* and *P. meibomiae* infect an unusually broad range of plant species. *P. pachyrhizi* is known to naturally infect 31 species in 17 genera of legumes and 60 species in 26 other genera have been infected under controlled conditions. *P. meibomiae* naturally
 25 infects 42 species in 19 genera of legumes, and 18 additional species in 12 other genera have been artificially infected. Twenty-four plant species in 19 genera are hosts for both species (Frederick *et al.*, *Mycology* 92: 217-227 (2002)).

Soybean plants resistant to ASR have been identified. Four dominant, independently inherited race-specific QTL for resistance to *P. pachyrhizi*, herein
 30 designated ASR resistance locus 1, ASR resistance locus 2, ASR resistance locus 3, and ASR resistance locus 4, have been identified in PI 200492, PI 230970, PI 462312 (Ankur), and PI 459025B, respectively. These lines, as well as seven others, are suspected of containing QTL for ASR resistance. PI 239871A and PI 239871B (*G. soja*), PI 230971 and PI 459024B, and the cultivars Taita Kaohsiung-5, Tainung-4,

and Wayne have been used as differentials to identify nine races at the Asian Vegetable Research and Development Center, in Taiwan. The predominant race was compatible with three or more of the differentials, indicating that some races already possess multiple virulence factors to known and suspected genes for resistance.

5 Resistance also occurs among the wild *Glycine* spp. from Australia. Rate-reducing resistance has also been demonstrated. However, it is difficult to evaluate this type of resistance because the rate of rust development is dependent on soybean development and maturity (Sinclair *et al.*, eds., Soybean rust workshop. College of Agricultural, Consumer, and Environmental Sciences. Natl. Soybean Res. Lab. Publ. 1 (1996)).

10 Evaluating plants that could potentially contain QTL conferring resistance to ASR can be time consuming and require large amounts of biologically contained space. Culturing *P. pachyrhizi* requires the use of an approved biological containment hood. In addition, greenhouses and growth chambers used to grow plants for ASR resistance testing will have to be constructed in a manner that prevents the accidental
15 release of the organism, especially in locations in which the organism has still not yet been observed. Different cultures of *P. pachyrhizi* may possess different virulence factors. Over time, new strains of *P. pachyrhizi* may be introduced into the United States. Therefore, any breeding program designed to breed resistance into soybean against ASR will need to be able to respond rapidly to changes in the *P. pachyrhizi*
20 population. Also, breeding for soybean crops used in other geographic locations will require selecting resistance to the specific strains that affect those regions, in addition to providing those agronomic characteristics that are preferred by these farmers in that region. Therefore, there is a great need for a rapid, time and cost efficient high throughput method for screening germplasm resistant to ASR. This method must not
25 only provide speed and efficiency, but must also be able to be performed with a minimal amount of space, allowing for the screening of many samples at one time.

The present invention provides a method for screening and selecting a soybean plant comprising QTL for disease resistance.

Summary of the Invention

The present invention provides a method for assaying soybean plants for disease resistance, immunity, or susceptibility comprising: (a) detaching a plant tissue from the soybean plant; (b) cultivating said tissue in a media; (c) exposing said tissue to a plant pathogen; and (d) assessing said tissue for resistance, immunity, or susceptibility to disease caused by the pathogen. Additionally, the plant response to the pathogen can be evaluated by the following steps (e) isolating nucleic acids (DNA and/or RNA) from said plant; (f) assaying said nucleic acids (DNA, RNA, and/or cDNA) for the presence of one or more molecular markers for a quantitative trait locus associated with said resistance, immunity, or susceptibility; and (g) selecting said plant for use in a breeding program. Determination of resistance, immunity, or susceptibility of a plant to a particular pathogen is obvious to anyone skilled in the art. The plant tissue can be leaf, vascular tissue, flower, pod, root, stem, seed, or a portion thereof, or a cell isolated from the tissue. Exposing said tissue to a plant pathogen is accomplished by a means selected from the group consisting of (a) direct application of the pathogen to the tissue; (b) inclusion of the pathogen in the culture media; and (c) inclusion of an agent that is effectively contaminated with the pathogen and serves to inoculate the tissue. The plant pathogen can be a fungus, virus, bacterium, or invertebrate animal. The plant pathogen exposure can be in the form of pathogen macromolecules, cells, tissues, whole organism or combinations thereof, wherein the pathogen, and parts thereof, is either living or dead so long that the material mediates an immune response in the host tissue. Pathogen macromolecules relevant for the present invention include, but are not limited to, toxins, cell walls or membranes, antigens, and polysaccharides.

The present invention further comprises a QTL that confers disease resistance to a fungal pathogen selected from the group consisting of *Phakopsora pachyrhizi*, *Phakopsora meibomia* (Asian Soybean Rust), *Colletotrichum truncatum*, *Colletotrichum dematium* var. *truncatum*, *Glomerella glycines* (Soybean Anthracnose), *Phytophthora sojae* (Phytophthora root and stem rot), *Sclerotinia sclerotiorum* (Sclerotinia stem rot), *Fusarium solani* f. sp. *glycines* (sudden death syndrome), *Fusarium* spp. (Fusarium root rot), *Macrophomina phaseolina* (charcoal rot), *Septoria glycines*, (Brown Spot), *Pythium aphanidermatum*, *Pythium debaryanum*, *Pythium irregulare*, *Pythium ultimum*, *Pythium myriotylum*, *Pythium*

torulosum (Pythium seed decay), *Diaporthe phaseolorum* var. *sojae* (Pod blight),
Phomopsis longicola (Stem blight), *Phomopsis* spp. (Phomopsis seed decay),
Peronospora manshurica (Downy Mildew), *Rhizoctonia solani* (Rhizoctonia root and
stem rot, Rhizoctonia aerial blight), *Phialophora gregata* (Brown Stem Rot),
5 *Diaporthe phaseolorum* var. *caulivora* (Stem Canker), *Cercospora kikuchii* (Purple
Seed Stain), *Alternaria* sp. (Target Spot), *Cercospora sojina* (Frogeye Leafspot),
Sclerotium rolfsii (Southern blight), *Arkoala nigra* (Black leaf blight), *Thielaviopsis*
basicola, (Black root rot), *Choanephora infundibulifera*, *Choanephora trispora*
(Choanephora leaf blight), *Leptosphaerulina trifolii* (Leptosphaerulina leaf spot),
10 *Mycoleptodiscus terrestris* (Mycoleptodiscus root rot), *Neocosmospora vasinfecta*
(Neocosmospora stem rot), *Phyllosticta sojicola* (Phyllosticta leaf spot),
Pyrenochaeta glycines (Pyrenochaeta leaf spot), *Cylindrocladium crotalariae* (Red
crown rot), *Dactuliochaeta glycines* (Red leaf blotch), *Spaceloma glycines* (Scab),
Stemphylium botryosum (Stemphylium leaf blight), *Corynespora cassiicola* (Target
15 spot), *Nematospora coryli* (Yeast spot), and *Phymatotrichum omnivorum* (Cotton
Root Rot).

The present invention further comprises a QTL that confers disease resistance
to a viral pathogen selected from the group consisting of *Alfavirus* (Alfafa mosaic
virus, AMV), *Comovirus* (bean pod mottle virus, BPMV), *Potyvirus* (bean yellow
20 mosaic virus, BYMV), *Bromovirus* (cowpea chlorotic mottle virus, CCMV),
Begomovirus (mung bean yellow mosaic virus, MYMV), *Potyvirus* (peanut mottle
virus, PeMoV), *Potyvirus* (peanut stripe virus, PStV), *Cucumovirus* (peanut stunt
virus, PSV), *Caulimovirus* (soybean chlorotic mottle virus, SbCMV), *Begomovirus*
(soybean crinkle leaf virus, SCLV), *Luteovirus* (soybean dwarf virus, SbDV),
25 *Potyvirus* (soybean mosaic virus, SMV), *Nepovirus* (soybean severe stunt virus,
SSSV), and *Nepovirus* (tobacco ringspot virus, TRSV).

The present invention further comprises a QTL that confers disease resistance
to a bacterial pathogen selected from the group consisting of *Bacillus subtilis*
(Bacillus seed decay), *Pseudomonas savastanoi* pv. *glycinea* (Bacterial blight),
30 *Pseudomonas syringae* subsp. *syringae* (Bacterial crinkle-leaf), *Xanthomonas*
axonopodis pv. *glycines*, (Bacterial pustule), *Curtobacterium flaccumfaciens* pv.
flaccumfaciens, (Bacterial tan spot), *Curtobacterium flaccumfaciens* pv.
flaccumfaciens, *Ralstonia solanacearum*, (Bacterial wilt), and *Pseudomonas syringae*
pv. *tabaci* (Wildfire).

The present invention further comprises a QTL that confers disease resistance to a invertebrate pathogen selected from the group consisting of *Aphis glycines* (Soybean aphid), *Heterodera glycines* (Soybean cyst nematode), *Meloidogyne arenaria*, *Meloidogyne hapla*, *Meloidogyne incognita*, *Meloidogyne javanica* (Root
 5 knot nematode), *Hoplolaimus Columbus*, *Hoplolaimus galeatus*, *Hoplolaimus magnistylus* (Lance nematode), *Pratylenchus* spp. (Lesion nematode), *Paratylenchus projectus*, *Paratylenchus tenuicaudatus* (Pin nematode), *Rotylenchulus reniformis* (Reniform nematode), *Criconemella ornata* (Ring nematode), *Hemicycliophora* spp. (Sheath nematode), *Heliocotylenchus* spp. (Spiral nematode), *Belonolaimus gracilis*,
 10 *Belonolaimus longicaudatus* (Sting nematode), *Quinisulcius acutus*, *Tylenchorhynchus* spp. (Stunt nematode), and *Paratrachodorus minor* (Stubby root nematode).

The present invention also provides selected soybean tissue and plants that are resistant to *Phakopsora pachyrhizi*, *Phakopsora meibomia* (Asian Soybean Rust), *Colletotrichum truncatum*, *Colletotrichum dematium* var. *truncatum*, *Glomerella*
 15 *glycines* (Soybean Anthracnose), *Phytophthora sojae* (Phytophthora root and stem rot), *Sclerotinia sclerotiorum* (Sclerotinia stem rot), *Fusarium solani* f. sp. *glycines* (sudden death syndrome), *Fusarium* spp. (Fusarium root rot), *Macrophomina phaseolina* (charcoal rot), *Septoria glycines*, (Brown Spot), *Pythium aphanidermatum*, *Pythium debaryanum*, *Pythium irregulare*, *Pythium ultimum*, *Pythium myriotylum*,
 20 *Pythium torulosum* (Pythium seed decay), *Diaporthe phaseolorum* var. *sojae* (Pod blight), *Phomopsis longicola* (Stem blight), *Phomopsis* spp. (Phomopsis seed decay), *Peronospora manshurica* (Downy Mildew), *Rhizoctonia solani* (Rhizoctonia root and stem rot, Rhizoctonia aerial blight), *Phialophora gregata* (Brown Stem Rot), *Diaporthe phaseolorum* var. *caulivora* (Stem Canker), *Cercospora kikuchii* (Purple
 25 Seed Stain), *Alternaria* sp. (Target Spot), *Cercospora sojina* (Frogeye Leafspot), *Sclerotium rolfsii* (Southern blight), *Arkoala nigra* (Black leaf blight), *Thielaviopsis basicola*, (Black root rot), *Choanephora infundibulifera*, *Choanephora trispora* (Choanephora leaf blight), *Leptosphaerulina trifolii* (Leptosphaerulina leaf spot), *Mycoleptodiscus terrestris* (Mycoleptodiscus root rot), *Neocosmospora vasinfecta*
 30 (Neocosmospora stem rot), *Phyllosticta sojicola* (Phyllosticta leaf spot), *Pyrenochaeta glycines* (Pyrenochaeta leaf spot), *Cylindrocladium crotalariae* (Red crown rot), *Dactuliochaeta glycines* (Red leaf blotch), *Spaceloma glycines* (Scab), *Stemphylium botryosum* (Stemphylium leaf blight), *Corynespora cassiicola* (Target spot), *Nematospora coryli* (Yeast spot), *Phymatotrichum omnivorum* (Cotton Root

Rot), *Alfavirus* (Alfafa mosaic virus, AMV), *Comovirus* (bean pod mottle virus, BPMV), *Potyvirus* (bean yellow mosaic virus, BYMV), *Bromovirus* (cowpea chlorotic mottle virus, CCMV), *Begomovirus* (mung bean yellow mosaic virus, MYMV), *Potyvirus* (peanut mottle virus, PeMoV), *Potyvirus* (peanut stripe virus, PStV), *Cucumovirus* (peanut stunt virus, PSV), *Caulimovirus* (soybean chlorotic mottle virus, SbCMV), *Begomovirus* (soybean crinkle leaf virus, SCLV), *Luteovirus* (soybean dwarf virus, SbDV), *Potyvirus* (soybean mosaic virus, SMV), *Nepovirus* (soybean severe stunt virus, SSSV), *Nepovirus* (tobacco ringspot virus, TRSV), *Bacillus subtilis* (Bacillus seed decay), *Pseudomonas savastanoi* pv. *glycinea* (Bacterial blight), *Pseudomonas syringae* subsp. *syringae* (Bacterial crinkle-leaf), *Xanthomonas axonopodis* pv. *glycines*, (Bacterial pustule), *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, (Bacterial tan spot), *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, *Ralstonia solanacearum*, (Bacterial wilt), *Pseudomonas syringae* pv. *tabaci* (Wildfire), *Aphis glycines* (Soybean aphid), *Heterodera glycines* (Soybean cyst nematode), *Meloidogyne arenaria*, *Meloidogyne hapla*, *Meloidogyne incognita*, *Meloidogyne javanica* (Root knot nematode), *Hoplolaimus Columbus*, *Hoplolaimus galeatus*, *Hoplolaimus magnistylus* (Lance nematode), *Pratylenchus* spp. (Lesion nematode), *Paratylenchus projectus*, *Paratylenchus tenuicaudatus* (Pin nematode), *Rotylenchulus reniformis* (Reniform nematode), *Criconebella ornata* (Ring nematode), *Hemicycliophora* spp. (Sheath nematode), *Heliocotylenchus* spp. (Spiral nematode), *Belonolaimus gracilis*, *Belonolaimus longicaudatus* (Sting nematode), *Quinisulcius acutus*, *Tylenchorhynchus* spp. (Stunt nematode), or *Paratrachodorus minor* (Stubby root nematode).

The present invention further provides that the selected plant is from the group consisting of members of the genus *Glycine*, more specifically from the group consisting of *Glycine arenaria*, *Glycine argyrea*, *Glycine canescens*, *Glycine clandestine*, *Glycine curvata*, *Glycine cyrtoloba*, *Glycine falcate*, *Glycine latifolia*, *Glycine latrobeana*, *Glycine max*, *Glycine microphylla*, *Glycine pescadrensis*, *Glycine pindanica*, *Glycine rubiginosa*, *Glycine soja*, *Glycine* sp., *Glycine stenophita*, *Glycine tabacina* and *Glycine tomentella*.

The present invention further provides that the media used in the method for selection is comprised of water that is untreated, distilled or deionized. The media can contain any ingredients necessary to sustain the pathogen or plant tissue, so long

as the ingredients do not interfere with the expression of resistance as conferred by the QTL.

The present invention further provides a soybean plant selected using said method.

5 The present invention also provides a QTL that is selected from the group consisting of *Phytophthora* (root rot) infection tolerance locus, *Fusarium solani* f. sp. *glycines* (sudden death syndrome) resistance locus, *Cercospora sojina* (Frogeye leaf spot) resistance locus, *Phialophora gregata* (brown stem rot) resistance locus, *Sclerotinia* (stem rot) resistance locus, ASR resistance locus 1, ASR resistance locus
10 2, ASR resistance locus 3, ASR resistance locus 4, ASR resistance locus 5, ASR resistance locus 6, ASR resistance locus 7, ASR resistance locus 8, ASR resistance locus 9, ASR resistance locus 10, ASR resistance locus 11, ASR resistance locus 12, and ASR resistance locus 13.

The present invention further provides that the selected plant contains one or
15 more fungal disease resistance QTL, including ASR resistance locus 1, ASR resistance locus 2, ASR resistance locus 3, ASR resistance locus 4, ASR resistance locus 5, ASR resistance locus 6, ASR resistance locus 7, ASR resistance locus 8, ASR resistance locus 9, ASR resistance locus 10, ASR resistance locus 11, ASR resistance locus 12, and ASR resistance locus 13.

20 The present invention further provides one or more single nucleotide polymorphism (SNP) marker loci for ASR resistance locus 1, wherein said SNP marker is selected from the group consisting of NS0093250, NS0119710, NS0103004, NS0099454, NS0102630, NS0102915, NS0102913, NS0123728, NS0129943, NS0102168, NS0092723, NS0098177, NS0127343, and NS0101121.
25 One or more SNP marker loci for ASR resistance locus 3 are also provided, wherein said SNP marker is selected from the group consisting of NS0099746, NS0123747, NS0126598, NS0128378, NS0096829, NS0125408, NS0098902, NS0099529, NS0097798, NS0137477, NS0095322, NS0136101, and NS0098982. An exemplary SNP marker locus, NS0103033, is provided for ASR resistance locus 5, ASR
30 resistance locus 6, ASR resistance locus 7, ASR resistance locus 8, and ASR resistance locus 9. Another exemplary SNP marker locus, NS0124935, is provided for ASR resistance locus 10, ASR resistance locus 11, ASR resistance locus 12, and ASR resistance locus 13. Further, one or more markers mapped within 10

centimorgans or less from said marker molecules can be used for the selection and introgression of ASR resistance loci.

The present invention further provides a method for selecting and introgressing ASR resistance in soybean comprising: (a) isolating nucleic acids from a plurality of soybean plants; (b) detecting in said isolated nucleic acids the presence of one or more marker molecules associated with ASR resistance loci 1-13, wherein said marker molecule is selected from the group consisting of SEQ ID NOs: 67 through 99, and any one marker molecule mapped within 10 centimorgans or less from said marker molecules; and (c) selecting a soybean plant comprising said one or more marker molecules, thereby selecting an ASR resistant soybean plant.

The present invention further provides for a soybean plant selected using said method.

Detailed Description Of The Invention

The present invention provides a method for screening soybean plants for resistance, immunity, or susceptibility to a fungal disease. In a preferred embodiment the plant is selected from the genus *Glycine*. The wild perennial soybeans belong to the subgenus *Glycine* and have a wide array of genetic diversity. The cultivated soybean (*Glycine max* (L.) Merr.) and its wild annual progenitor (*Glycine soja* (Sieb. and Zucc.)) belong to the subgenus *Soja*, contain $2n = 40$ chromosomes, are cross-compatible, usually produce vigorous fertile F1 hybrids, and carry similar genomes. Crosses between cultivated *Glycine* species and wild perennial *Glycine* species are possible, the success of which is variable amongst accessions. Investigations have shown that several wild perennial *Glycine* accessions carry resistance to brown spot, soybean rust, root rot, yellow mosaic virus, and powdery mildew. There are more than 100,000 *Glycine max* accessions, probably less than 10,000 *Glycine soja* accessions and approximately, 3500 accessions of perennial *Glycine* species in germplasm collections throughout the world. The exact numbers are unknown. Major *Glycine* collections exist in Australia, Brazil, China, Germany, India, Indonesia, Japan, Russia, South Korea, and the United States. Many other smaller but important collections exist throughout Asia and Europe. It is not known how many of the accessions are duplicated among collections. The USDA Soybean Germplasm Collection is one of the largest collections and the largest outside Asia (Verma *et al.*, eds., Soybean: Genetics, Molecular Biology and Biotechnology (1996)). It currently contains 20,765 accessions, comprised of 19 species collections, including 18,680 accessions of *Glycine max* and 1,166 accessions of *Glycine soja* as well as perennial *Glycine* species.

In a preferred embodiment, a soybean plant is assayed for disease resistance, immunity, or susceptibility comprising: (a) detaching a plant tissue from the soybean plant; (b) cultivating said tissue in a media; (c) exposing said tissue to a plant pathogen; and (d) assessing said tissue for resistance, immunity, or susceptibility to disease caused by the pathogen. Additionally, the plant response to the pathogen can be evaluated by the following steps (e) isolating nucleic acids from said plant; (f) assaying said nucleic acids for the presence of one or more molecular markers for a quantitative trait locus associated with said resistance, immunity, or susceptibility; and (g) selecting said plant for use in a breeding program. Determination of

resistance, immunity, or susceptibility of a plant to a particular pathogen is obvious to anyone skilled in the art. The plant tissue can be leaf, vascular tissue, flower, pod, root, stem, seed, or a portion thereof, or a cell isolated from the tissue. Exposing said tissue to a plant pathogen is accomplished by a means selected from the group

5 consisting of (a) direct application of the pathogen to the tissue; (b) inclusion of the pathogen in the culture media; and (c) inclusion of an agent that is effectively contaminated with the pathogen and serves to inoculate the tissue. The plant pathogen can be a fungus, virus, bacterium, or invertebrate animal. The plant pathogen exposure can be in the form of pathogen macromolecules, cells, tissues,

10 whole organism or combinations thereof, wherein the pathogen, and parts thereof, is either living or dead so long that the material mediates an immune response in the host tissue. Pathogen macromolecules relevant for the present invention include, but are not limited to, toxins, cell walls or membranes, antigens, and polysaccharides.

In a preferred embodiment, the leaf tissue may comprise a cotyledon leaf,

15 unifoliate leaf, a trifoliate leaf, and prophylls. There are four types of soybean leaves: 1) the first pair of simple cotyledons or seed leaves, 2) second pair of simple primary leaves, also known as unifoliate leaves, 3) trifoliate foliage leaves, and 4) prophylls, which are plant parts resembling leaves. The unifoliate leaves occur at the first node above the cotyledons. All other leaves would be trifoliate, wherein the first pair to

20 emerge following the unifoliate leaves are the first trifoliate leaves, which are followed by the emergence of the second trifoliate leaves and then the third trifoliate leaves (H.R. Boerma and J.E. Specht (ed.) Soybean Monograph, 3rd Edition, Am. Soc. Agron., Madison, WI (2004)).

In a preferred embodiment, the present invention enables a soybean plant to be

25 assayed for resistance, immunity, or susceptibility to a fungal disease. Soybean diseases caused by fungi include, but are not limited to, *Phakopsora pachyrhizi*, *Phakopsora meibomiae* (Asian Soybean Rust), *Colletotrichum truncatum*, *Colletotrichum dematium* var. *truncatum*, *Glomerella glycines* (Soybean Anthracnose), *Phytophthora sojae* (Phytophthora root and stem rot), *Sclerotinia sclerotiorum* (Sclerotinia stem rot), *Fusarium solani* f. sp. *glycines* (sudden death syndrome), *Fusarium* spp. (Fusarium root rot), *Macrophomina phaseolina* (charcoal rot), *Septoria glycines*, (Brown Spot), *Pythium aphanidermatum*, *Pythium debaryanum*, *Pythium irregulare*, *Pythium ultimum*, *Pythium myriotylum*, *Pythium torulosum* (Pythium seed decay), *Diaporthe phaseolorum* var. *sojae* (Pod blight),

30

Phomopsis longicola (Stem blight), *Phomopsis* spp. (Phomopsis seed decay),
Peronospora manshurica (Downy Mildew), *Rhizoctonia solani* (Rhizoctonia root and
stem rot, Rhizoctonia aerial blight), *Phialophora gregata* (Brown Stem Rot),
Diaporthe phaseolorum var. *caulivora* (Stem Canker), *Cercospora kikuchii* (Purple
5 Seed Stain), *Alternaria* sp. (Target Spot), *Cercospora sojina* (Frogeye Leafspot),
Sclerotium rolfsii (Southern blight), *Arkoala nigra* (Black leaf blight), *Thielaviopsis*
basicola, (Black root rot), *Choanephora infundibulifera*, *Choanephora trispora*
(Choanephora leaf blight), *Leptosphaerulina trifolii* (Leptosphaerulina leaf spot),
Mycoleptodiscus terrestris (Mycoleptodiscus root rot), *Neocosmospora vasinfecta*
10 (Neocosmospora stem rot), *Phyllosticta sojicola* (Phyllosticta leaf spot),
Pyrenochaeta glycines (Pyrenochaeta leaf spot), *Cylindrocladium crotalariae* (Red
crown rot), *Dactuliochaeta glycines* (Red leaf blotch), *Spaceloma glycines* (Scab),
Stemphylium botryosum (Stemphylium leaf blight), *Corynespora cassiicola* (Target
spot), *Nematospora coryli* (Yeast spot), and *Phymatotrichum omnivorum* (Cotton
15 Root Rot).

In a preferred embodiment, the present invention enables a soybean plant to be
assayed for resistance, immunity, or susceptibility to a viral disease. Soybean
diseases caused by viruses include, but are not limited to, *Alfamovirus* (Alfafa mosaic
virus, AMV), *Comovirus* (bean pod mottle virus, BPMV), *Potyvirus* (bean yellow
20 mosaic virus, BYMV), *Bromovirus* (cowpea chlorotic mottle virus, CCMV),
Begomovirus (mung bean yellow mosaic virus, MYMV), *Potyvirus* (peanut mottle
virus, PeMoV), *Potyvirus* (peanut stripe virus, PStV), *Cucumovirus* (peanut stunt
virus, PSV), *Caulimovirus* (soybean chlorotic mottle virus, SbCMV), *Begomovirus*
(soybean crinkle leaf virus, SCLV), *Luteovirus* (soybean dwarf virus, SbDV),
25 *Potyvirus* (soybean mosaic virus, SMV), *Nepovirus* (soybean severe stunt virus,
SSSV), and *Nepovirus* (tobacco ringspot virus, TRSV).

In a preferred embodiment, the present invention enables a soybean plant to
be assayed for resistance, immunity, or susceptibility to a bacterial disease. Soybean
diseases caused by bacteria include, but are not limited to, *Bacillus subtilis* (Bacillus
30 seed decay), *Pseudomonas savastanoi* pv. *glycinea* (Bacterial blight), *Pseudomonas*
syringae subsp. *syringae* (Bacterial crinkle-leaf), *Xanthomonas axonopodis* pv.
glycines, (Bacterial pustule), *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*,
(Bacterial tan spot), *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, *Ralstonia*
solanacearum, (Bacterial wilt), and *Pseudomonas syringae* pv. *tabaci* (Wildfire).

In a preferred embodiment, the present invention enables a soybean plant to be assayed for resistance, immunity, or susceptibility to an animal pest disease. Soybean diseases caused by animal pests include, but are not limited to *Aphis glycines* (Soybean aphid), *Heterodera glycines* (Soybean cyst nematode), *Meloidogyne arenaria*, *Meloidogyne hapla*, *Meloidogyne incognita*, *Meloidogyne javanica* (Root knot nematode), *Hoplolaimus Columbus*, *Hoplolaimus galeatus*, *Hoplolaimus magnistylus* (Lance nematode), *Pratylenchus* spp. (Lesion nematode), *Paratylenchus projectus*, *Paratylenchus tenuicaudatus* (Pin nematode), *Rotylenchulus reniformis* (Reniform nematode), *Criconemella ornata* (Ring nematode), *Hemicycliophora* spp. (Sheath nematode), *Heliocotylenchus* spp. (Spiral nematode), *Belonolaimus gracilis*, *Belonolaimus longicaudatus* (Sting nematode), *Quinisulcius acutus*, *Tylenchorhynchus* spp. (Stunt nematode), and *Paratrichodorus minor* (Stubby root nematode).

The invention further provides a method for selection and introgression of QTL for disease resistance in soybean comprising: (a) isolating nucleic acids from a plurality of soybean plants; (b) detecting in said isolated nucleic acids the presence of one or more marker molecules associated with disease resistance QTL; and (c) selecting a soybean plant comprising said one or more marker molecules, thereby selecting a disease resistant soybean plant.

The disease resistance QTL of the present invention may be introduced into an elite *Glycine max* line. An "elite line" is any line that has resulted from breeding and selection for superior agronomic performance. Examples of elite lines are lines that are commercially available to farmers or soybean breeders such as HARTZ™ variety H4994, HARTZ™ variety H5218, HARTZ™ variety H5350, HARTZ™ variety H5545, HARTZ™ variety H5050, HARTZ™ variety H5454, HARTZ™ variety H5233, HARTZ™ variety H5488, HARTZ™ variety HLA572, HARTZ™ variety H6200, HARTZ™ variety H6104, HARTZ™ variety H6255, HARTZ™ variety H6586, HARTZ™ variety H6191, HARTZ™ variety H7440, HARTZ™ variety H4452 Roundup Ready™, HARTZ™ variety H4994 Roundup Ready™, HARTZ™ variety H4988 Roundup Ready™, HARTZ™ variety H5000 Roundup Ready™, HARTZ™ variety H5147 Roundup Ready™, HARTZ™ variety H5247 Roundup Ready™, HARTZ™ variety H5350 Roundup Ready™, HARTZ™ variety H5545 Roundup Ready™, HARTZ™ variety H5855 Roundup Ready™, HARTZ™ variety H5088 Roundup Ready™, HARTZ™ variety H5164 Roundup Ready™,

HARTZ™ variety H5361 Roundup Ready™, HARTZ™ variety H5566 Roundup Ready™, HARTZ™ variety H5181 Roundup Ready™, HARTZ™ variety H5889 Roundup Ready™, HARTZ™ variety H5999 Roundup Ready™, HARTZ™ variety H6013 Roundup Ready™, HARTZ™ variety H6255 Roundup Ready™, HARTZ™ variety H6454 Roundup Ready™, HARTZ™ variety H6686 Roundup Ready™, HARTZ™ variety H7152 Roundup Ready™, HARTZ™ variety H7550 Roundup Ready™, HARTZ™ variety H8001 Roundup Ready™ (HARTZ SEED, Stuttgart, Arkansas, USA); A0868, AG0202, AG0401, AG0803, AG0901, A1553, A1900, AG1502, AG1702, AG1901, A1923, A2069, AG2101, AG2201, AG2205, A2247, AG2301, A2304, A2396, AG2401, AG2501, A2506, A2553, AG2701, AG2702, AG2703, A2704, A2833, A2869, AG2901, AG2902, AG2905, AG3001, AG3002, AG3101, A3204, A3237, A3244, AG3301, AG3302, AG3006, AG3203, A3404, A3469, AG3502, AG3503, AG3505, AG3305, AG3602, AG3802, AG3905, AG3906, AG4102, AG4201, AG4403, AG4502, AG4603, AG4801, AG4902, AG4903, AG5301, AG5501, AG5605, AG5903, AG5905
 A3559, AG3601, AG3701, AG3704, AG3750, A3834, AG3901, A3904, A4045 AG4301, A4341, AG4401, AG4404, AG4501, AG4503, AG4601, AG4602, A4604, AG4702, AG4703, AG4901, A4922, AG5401, A5547, AG5602, AG5702, A5704, AG5801, AG5901, A5944, A5959, AG6101, AJW2600C0R, FPG26932, QR4459 and QP4544 (Asgrow Seeds, Des Moines, Iowa, USA); DKB26-52, DKB28-51, DKB32-52, DKB08-51, DKB09-53, DKB10-52, DKB18-51, DKB26-53, DKB29-51, DKB42-51, DKB35-51 DKB34-51, DKB36-52, DKB37-51, DKB38-52, DKB46-51, DKB54-52 and DeKalb variety CX445 (DeKalb, Illinois, USA); 91B91, 92B24, 92B37, 92B63, 92B71, 92B74, 92B75, 92B91, 93B01, 93B11, 93B26, 93B34, 93B35, 93B41, 93B45, 93B51, 93B53, 93B66, 93B81, 93B82, 93B84, 94B01, 94B32, 94B53, 94M80 RR, 94M50 RR, 95B71, 95B95, 95M81 RR, 95M50 RR, 95M30 RR, 9306, 9294, 93M50, 93M93, 94B73, 94B74, 94M41, 94M70, 94M90, 95B32, 95B42, 95B43 and 9344 (Pioneer Hi-bred International, Johnston, Iowa, USA); SSC-251RR, SSC-273CNRR, AGRA 5429RR, SSC-314RR, SSC-315RR, SSC-311STS, SSC-320RR, AGRA5432RR, SSC-345RR, SSC-356RR, SSC-366, SSC-373RR and AGRA5537CNRR (Schlessman Seed Company, Milan, Ohio, USA); 39-E9, 44-R4, 44-R5, 47-G7, 49-P9, 52-Q2, 53-K3, 56-J6, 58-V8, ARX A48104, ARX B48104,

ARX B55104 and GP530 (Armor Beans, Fisher, Arkansas, USA); HT322STS, HT3596STS, L0332, L0717, L1309CN, L1817, L1913CN, L1984, L2303CN, L2495, L2509CN, L2719CN, L3997CN, L4317CN, RC1303, RC1620, RC1799, RC1802, RC1900, RC1919, RC2020, RC2300, RC2389, RC2424, RC2462, RC2500, RC2504, RC2525, RC2702, RC2964, RC3212, RC3335, RC3354, RC3422, RC3624, RC3636, RC3732, RC3838, RC3864, RC3939, RC3942, RC3964, RC4013, RC4104, RC4233, RC4432, RC4444, RC4464, RC4842, RC4848, RC4992, RC5003, RC5222, RC5332, RC5454, RC5555, RC5892, RC5972, RC6767, RC7402, RT0032, RT0041, RT0065, RT0073, RT0079, RT0255, RT0269, RT0273, RT0312, RT0374, RT0396, RT0476, RT0574, RT0583, RT0662, RT0669, RT0676, RT0684, RT0755, RT0874, RT0907, RT0929, RT0994, RT0995, RT1004, RT1183, RT1199, RT1234, RT1399, RT1413, RT1535, RT1606, RT1741, RT1789, RT1992, RT2000, RT2041, RT2089, RT2092, RT2112, RT2127, RT2200, RT2292, RT2341, RT2430, RT2440, RT2512, RT2544, RT2629, RT2678, RT2732, RT2800, RT2802, RT2822, RT2898, RT2963, RT3176, RT3200, RT3253, RT3432, RT3595, RT3836, RT4098, RX2540, RX2944, RX3444 and TS466RR (Croplan Genetics, Clinton, Kentucky, USA); 4340RR, 4630RR, 4840RR, 4860RR, 4960RR, 4970RR, 5260RR, 5460RR, 5555RR, 5630RR and 5702RR (Delta Grow, England, Arkansas, USA); DK3964RR, DK3968RR, DK4461RR, DK4763RR, DK4868RR, DK4967RR, DK5161RR, DK5366RR, DK5465RR, DK55T6, DK5668RR, DK5767RR, DK5967RR, DKXTJ446, DKXTJ448, DKXTJ541, DKXTJ542, DKXTJ543, DKXTJ546, DKXTJ548, DKXTJ549, DKXTJ54J9, DKXTJ54X9, DKXTJ554, DKXTJ555, DKXTJ55J5 and DKXTJ5K57 (Delta King Seed Company, McCrory, Arkansas, USA); DP 3861RR, DP 4331 RR, DP 4546RR, DP 4724 RR, DP 4933 RR, DP 5414RR, DP 5634 RR, DP 5915 RR, DPX 3950RR, DPX 4891RR, DPX 5808RR (Delta & Pine Land Company, Lubbock, Texas, USA); DG31T31, DG32C38, DG3362NRR, DG3390NRR, DG33A37, DG33B52, DG3443NRR, DG3463NRR, DG3481NRR, DG3484NRR, DG3535NRR, DG3562NRR, DG3583NRR, DG35B40, DG35D33, DG36M49, DG37N43, DG38K57, DG38T47, SX04334, SX04453 (Dyna-gro line, UAP-MidSouth, Cordova, Tennessee, USA); 8374RR CYSTX, 8390 NNRR, 8416RR, 8492NRR and 8499NRR (Excel Brand, Camp Point, Illinois, USA); 4922RR, 5033RR, 5225RR and 5663RR (FFR Seed, Southhaven, Mississippi, USA); 3624RR/N, 3824RR/N, 4212RR/N, 4612RR/N, 5012RR/N, 5212RR/N and 5412RR/STS/N (Garst Seed Company, Slater, Iowa, USA); 471, 4R451, 4R485,

4R495, 4RS421 and 5R531 (Gateway Seed Company, Nashville, Illinois, USA); H-3606RR, H-3945RR, H-4368RR, H-4749RR, H-5053RR and H-5492RR (Golden Harvest Seeds, Inc., Pekin, Illinois, USA); HBK 5324, HBK 5524, HBK R4023, HBK R4623, HBK R4724, HBK R4820, HBK R4924, HBK R4945CX, HBK R5620 and
5 HBK R5624 (Hornbeck Seed Co. Inc., DeWitt, Arkansas, USA); 341 RR/SCN, 343 RR/SCN, 346 RR/SCN, 349 RR, 355 RR/SCN, 363 RR/SCN, 373 RR, 375 RR, 379 RR/SCN, 379+ RR/SCN, 380 RR/SCN, 380+ RR/SCN, 381 RR/SCN, 389 RR/SCN, 389+ RR/SCN, 393 RR/SCN, 393+ RR/SCN, 398 RR, 402 RR/SCN, 404 RR, 424 RR, 434 RR/SCN and 442 RR/SCN (Kruger Seed Company, Dike, Iowa, USA);
10 3566, 3715, 3875, 3944, 4010 and 4106 (Lewis Hybrids, Inc., Ursa, Illinois, USA); C3999NRR (LG Seeds, Elmwood, Illinois, USA); Atlanta 543, Austin RR, Cleveland VIIRR, Dallas RR, Denver RRSTS, Everest RR, Grant 3RR, Olympus RR, Phoenix IIIIRR, Rocky RR, Rushmore 553RR and Washington IXRR (Merschman Seed Inc., West Point, Iowa, USA); RT 3304N, RT 3603N, RT 3644N, RT 3712N, RT 3804N,
15 RT 3883N, RT 3991N, RT 4044N, RT 4114N, RT 4124N, RT 4201N, RT 4334N, RT 4402N, RT 4480N, RT 4503N, RT 4683N, RT 4993N, RT 5043N, RT 5204, RT 5553N, RT 5773, RT4731N and RTS 4824N (MFA Inc., Columbia, Missouri, USA); 9A373NRR, 9A375XRR, 9A385NRS, 9A402NRR, 9A455NRR, 9A485XRR and 9B445NRS (Midland Genetics Group L.L.C., Ottawa, Kansas, USA); 3605nRR,
20 3805nRR, 3903nRR, 3905nRR, 4305nRR, 4404nRR, 4705nRR, 4805nRR, 4904nRR, 4905nRR, 5504nRR and 5505nRR (Midwest Premium Genetics, Concordia, Missouri, USA); S37-N4, S39-K6, S40-R9, S42-P7, S43-B1, S49-Q9, S50-N3, S52-U3 and S56-D7 (Syngenta Seeds, Henderson, Kentucky, USA); NT-3707 RR, NT-3737 RR/SCN, NT-3737+RR/SCN, NT-3737sc RR/SCN, NT-3777+ RR, NT-3787
25 RR/SCN, NT-3828 RR, NT-3839 RR, NT-3909 RR/SCN/STS, NT-3909+ RR/SCN/ST, NT-3909sc RR/SCN/S, NT-3919 RR, NT-3922 RR/SCN, NT-3929 RR/SCN, NT-3999 RR/SCN, NT-3999+ RR/SCN, NT-3999sc RR/SCN, NT-4040 RR/SCN, NT-4040+ RR/SCN, NT-4044 RR/SCN, NT-4122 RR/SCN, NT-4414 RR/SCN/STS, NT-4646 RR/SCN and NT-4747 RR/SCN (NuTech Seed Co., Ames,
30 Iowa, USA); PB-3494NRR, PB-3732RR, PB-3894NRR, PB-3921NRR, PB-4023NRR, PB-4394NRR, PB-4483NRR and PB-5083NRR (Prairie Brand Seed Co., Story City, Iowa, USA); 3900RR, 4401RR, 4703RR, 4860RR, 4910, 4949RR, 5250RR, 5404RR, 5503RR, 5660RR, 5703RR, 5770, 5822RR, PGY 4304RR, PGY 4604RR, PGY 4804RR, PGY 5622RR and PGY 5714RR (Progeny Ag Products,

Wynne, Arkansas, USA); R3595RCX, R3684Rcn, R3814RR, R4095Rcn, R4385Rcn and R4695Rcn (Renze Hybrids Inc., Carroll, Iowa, USA); S3532-4, S3600-4, S3832-4, S3932-4, S3942-4, S4102-4, S4542-4 and S4842-4 (Stine Seed Co., Adel, Iowa, USA); 374RR, 398RRS (Taylor Seed Farms Inc., White Cloud, Kansas, USA); USG 5002T, USG 510nRR, USG 5601T, USG 7440nRR, USG 7443nRR, USG 7473nRR, USG 7482nRR, USG 7484nRR, USG 7499nRR, USG 7504nRR, USG 7514nRR, USG 7523nRR, USG 7553nRS and USG 7563nRR (UniSouth Genetics Inc., Nashville, Tennessee, USA); V38N5RS, V39N4RR, V42N3RR, V48N5RR, V284RR, V28N5RR, V315RR, V35N4RR, V36N5RR, V37N3RR, V40N3RR, V47N3RR, and V562NRR (Royster-Clark Inc., Washington C.H., Ohio, USA); RR2383N, 2525NA, RR2335N, RR2354N, RR2355N, RR2362, RR2385N, RR2392N, RR2392NA, RR2393N, RR2432N, RR2432NA, RR2445N, RR2474N, RR2484N, RR2495N and RR2525N (Willcross Seed, King City Seed, King City, Missouri, USA); 1493RR, 1991NRR, 2217RR, 2301NRR, 2319RR, 2321NRR, 2341NRR, 2531NRR, 2541NRR, 2574RR, 2659RR, 2663RR, 2665NRR, 2671NRR, 2678RR, 2685RR, 2765NRR, 2782NRR, 2788NRR, 2791NRR, 3410RR, 3411NRR, 3419NRR, 3421NRR, 3425NRR, 3453NRR, 3461NRR, 3470CRR, 3471NRR, 3473NRR, 3475RR, 3479NRR, 3491NRR, 3499NRR, WX134, WX137, WX177 and WX300 (Wilken Seeds, Pontiac, Illinois, USA). An elite plant is a representative plant from an elite line.

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

It is further understood that a soybean plant of the present invention may exhibit the characteristics of any maturity group. The pollen from the selected soybean plant can be cryopreserved and used in crosses with elite lines from other

maturity groups to introgress a the fungal disease resistance locus into a line that would not normally be available for crossing in nature. Pollen cryopreservation techniques are well known in the art (Tyagi *et al.*, *Cryo Letters*, 24: 119-124 (2003), Liang *et al.*, *Acta Botanica Sinica*, 35: 733-738 (1993), and Honda *et al.*, *Euphytica* 5 126: 315-320 (2002)).

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 10 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. Herein, a plant disease can be caused by a fungi, virus, bacterium or invertebrate animal.

A number of molecular genetic maps of *Glycine* have been reported (Mansur *et al.*, *Crop Sci.* 36: 1327-1336 (1996), Shoemaker *et al.*, *Genetics* 144: 329-338 15 (1996), Shoemaker *et al.*, *Crop Science* 32: 1091-1098 (1992), Shoemaker *et al.*, *Crop Science* 35: 436-446 (1995), Tinley *et al.*, *J. Cell Biochem. Suppl.* 14E: 291 (1990), Cregan *et al.*, *Crop Science* 39:1464-1490 (1999)). *Glycine max*, *Glycine soja* and *Glycine max* x. *Glycine soja* share linkage groups (Shoemaker *et al.*, *Genetics* 144: 329-338 (1996)). As used herein, reference to the linkage groups, G; 20 C2; J; and N of *Glycine max* refers to the linkage group that corresponds to linkage groups, G; C2; J; and N from the genetic map of *Glycine max* (Mansur *et al.*, *Crop Science*. 36: 1327-1336 (1996), Cregan *et al.*, *Crop Science* 39:1464-1490 (1999), and Soybase, Agricultural Research Service, United States Department of Agriculture).

An allele of a QTL can, of course, comprise multiple genes or other genetic 25 factors even within a contiguous genomic region or linkage group, such as a haplotype. As used herein, an allele of a QTL can therefore encompasses 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 30 embodiment of the present invention the allele of a QTL comprises one or more genes or other genetic factors that are also capable of exhibiting allelic variation. The use of the term "an allele of a QTL" is thus not intended to exclude a QTL that comprises more than one gene or other genetic factor. Specifically, an "allele of a QTL" in the present in the invention can denote a haplotype within a haplotype window wherein a

phenotype 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 said polymorphisms indicate identity by descent. A haplotype within that window can be defined by the unique fingerprint of alleles at each marker.

5 As used herein, an allele is one of several alternative forms of a gene occupying a given locus on a chromosome. When all the alleles present at a given locus on a chromosome are the same, that plant is homozygous at that locus. If the alleles present at a given locus on a chromosome differ, that plant is heterozygous at that locus.

10 Plants of the present invention can be part of or generated from a breeding program. The choice of breeding method depends on the mode of plant reproduction, the heritability of the trait(s) being improved, and the type of cultivar used commercially (*e.g.*, F_1 hybrid cultivar, pureline cultivar, etc.). A cultivar is a race or variety of a plant that has been created or selected intentionally and maintained
15 through cultivation.

Selected, non-limiting approaches for breeding the plants of the present invention are set forth below. A breeding program can be enhanced using marker assisted selection (MAS) of the progeny of any cross. It is further understood that any commercial and non-commercial cultivars can be utilized in a breeding program.

20 Factors such as, for example, emergence vigor, vegetative vigor, stress tolerance, disease resistance, branching, flowering, seed set, seed size, seed density, standability, and threshability etc. will generally dictate the choice.

For highly heritable traits, a choice of superior individual plants evaluated at a single location will be effective, whereas for traits with low heritability, selection
25 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 embodiment a backcross or recurrent breeding program is undertaken.

The complexity of inheritance influences choice of the breeding method.

30 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. The use of recurrent selection in self-pollinating crops depends on the ease of pollination, the

frequency of successful hybrids from each pollination event, and the number of hybrid offspring from each successful cross.

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
5 deficient in traits may be used as parents to produce new populations for further selection.

One method of identifying a superior plant is to observe its performance relative to other experimental plants and to a widely grown standard cultivar. If a
10 single observation is inconclusive, replicated observations can provide a better estimate of its genetic worth. A breeder can select and cross two or more parental lines, followed by repeated selfing and selection, producing many new genetic combinations.

The development of new soybean cultivars requires the development and
15 selection of soybean varieties, the crossing of these varieties and selection of superior hybrid crosses. The hybrid seed can be produced by manual crosses between selected male-fertile parents or by using male sterility systems. Hybrids are selected for certain single gene traits such as pod color, flower color, seed yield, pubescence color or herbicide resistance which indicate that the seed is truly a hybrid. Additional data
20 on parental lines, as well as the phenotype of the hybrid, influence the breeder's decision whether to continue with the specific hybrid cross.

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

Pedigree breeding is used commonly for the improvement of self-pollinating crops. Two parents who possess favorable, complementary traits are crossed to produce an F_1 . An F_2 population is produced by selfing one or several F_1 's. Selection
30 of the best individuals in the best families is selected. Replicated testing of families can begin in the F_4 generation to improve the effectiveness of selection for traits with low heritability. At an advanced stage of inbreeding (*i.e.*, F_6 and F_7), the best lines or mixtures of phenotypically similar lines are tested for potential release as new cultivars.

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. The resulting plant is expected to have the attributes of the recurrent parent (*e.g.*,
 5 cultivar) and the desirable trait transferred from 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 parent is expected to have the attributes of the recurrent parent (*e.g.*, cultivar) and the desirable trait transferred from the donor parent.

10 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
 15 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.

In a multiple-seed procedure, soybean breeders commonly harvest one or more pods from each plant in a population and thresh them together to form a bulk.
 20 Part of the bulk is used to plant the next generation and part is put in reserve. The procedure has been referred to as modified single-seed descent or the pod-bulk technique.

The multiple-seed procedure has been used to save labor at harvest. It is considerably faster to thresh pods with a machine than to remove one seed from each
 25 by hand for the single-seed procedure. The multiple-seed procedure also makes it possible to plant the same number of seed of a population each generation of inbreeding.

Descriptions of other breeding methods that are commonly used for different traits and crops can be found in one of several reference books (*e.g.*, Fehr, *Principles*
 30 *of Cultivar Development* Vol. 1, pp. 2-3 (1987)).

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 embodiment of the present invention, the plant part is a seed.

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

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

The present invention also provides for QTL conferring resistance to Asian Soybean Rust, including ASR resistance locus 1, ASR resistance locus 2, ASR resistance locus 3, ASR resistance locus 4, ASR resistance locus 5, ASR resistance locus 6, ASR resistance locus 7, ASR resistance locus 8, ASR resistance locus 9, ASR resistance locus 10, ASR resistance locus 11, ASR resistance locus 12, and ASR resistance locus 13. Four dominant and independently inherited loci for resistance to *P. pachyrhizi*, herein designated ASR resistance locus 1 through 4, have been identified in PI 200492, PI 230970, PI 462312 (Ankur), and PI 459025B, respectively. In the present invention, ASR resistance locus 1 has been localized to linkage group G

of soybean. SNP markers used to monitor the introgression of ASR resistance locus 1 are selected from the group consisting of NS0093250, NS0119710, NS0103004, NS0099454, NS0102630, NS0102915, NS0102913, NS0123728, NS0129943, NS0102168, NS0092723, NS0098177, NS0127343 and NS0101121. The ASR resistance locus 1 SNP marker DNA sequences (presented as SEQ ID NOs: 67 through 80) can be amplified using the primers indicated as SEQ ID NOs: 1 through 28 and detected with probes indicated as SEQ ID NOs: 100 through 127. In the present invention, ASR resistance locus 2 is most likely located on linkage group J, near or within the disease resistance cluster containing Brown Stem Rot, Soybean Cyst Nematode resistance and Frog Eye Leaf Spot resistance; or linkage group N. In the present invention, ASR resistance locus 3 is localized to linkage group C2. SNP markers used to monitor the introgression of ASR resistance locus 3 are selected from the group consisting of NS0099746, NS0123747, NS0126598, NS0128378, NS0096829, NS0125408, NS0098902, NS0099529, NS0097798, NS0137477, NS0095322, NS0136101, NS0098982, NS0103749, NS0118897, NS0119715, and NS0130920. These marker DNA sequences (presented as SEQ ID NOs: 81 through 97) can be amplified using the primers indicated as SEQ ID NOs: 29 through 62 and detected with probes indicated as SEQ ID NOs: 128 through 161. In the present invention, ASR resistance locus 4 is likely located on linkage group N.

The present invention also provides for haplotypes that confer resistance to ASR that were identified in association studies. These genome-wide surveys revealed two SNP markers associated with ASR resistance located in two different windows on chromosome 13. In the first haplotype window, the SNP marker used to monitor the introgression of ASR resistance locus 5, ASR resistance locus 6, ASR resistance locus 7, ASR resistance locus 8, and ASR resistance locus 9 is NS0103033. This SNP marker DNA sequences (presented as SEQ ID NO: 98) can be amplified using the primers indicated as SEQ ID NOs: 63 and 64 and detected with probes indicated as SEQ ID NOs: 162 and 163. In the second haplotype window, the SNP marker used to monitor the introgression of ASR resistance locus 10, ASR resistance locus 11, ASR resistance locus 12, and ASR resistance locus 13 is NS0124935. This SNP marker DNA sequences (presented as SEQ ID NO: 99) can be amplified using the primers indicated as SEQ ID NOs: 65 and 66 and detected with probes indicated as SEQ ID NOs: 164 and 165.

It is further understood, that one or more markers mapped within 10 centimorgans or less from said marker molecules can be used for the selection and introgression of ASR resistance loci.

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

Nucleic acid molecules or fragments thereof are capable of specifically hybridizing to other nucleic acid molecules under certain circumstances. As used herein, two nucleic acid molecules are capable of specifically hybridizing to one
10 another if the two molecules are capable of forming an anti-parallel, double-stranded nucleic acid structure. A nucleic acid molecule is the "complement" of another nucleic acid molecule if they exhibit complete complementarity. As used herein, molecules are exhibit "complete complementarity" when every nucleotide of one of the molecules is complementary to a nucleotide of the other. Two molecules are
15 "minimally complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low-stringency" conditions. Similarly, the molecules are "complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under conventional "high-stringency" conditions.
20 Conventional stringency conditions are described by Sambrook *et al.*, In: *Molecular Cloning, A Laboratory Manual, 2nd Edition, Cold Spring Harbor Press, Cold Spring Harbor, New York (1989)*, and by Haymes *et al.*, In: *Nucleic Acid Hybridization, A Practical Approach*, IRL Press, Washington, DC (1985). Departures from complete complementarity are therefore permissible, as long as such departures do not
25 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
30 sequence that will specifically hybridize to the complement of the nucleic acid sequence to which it is being compared under high stringency conditions. The nucleic-acid probes and primers of the present invention can hybridize under stringent conditions to a target DNA sequence. The term "stringent hybridization conditions" is defined as conditions under which a probe or primer hybridizes specifically with a

target sequence(s) and not with non-target sequences, as can be determined empirically. The term "stringent conditions" is functionally defined with regard to the hybridization of a nucleic-acid probe to a target nucleic acid (i.e., to a particular nucleic-acid sequence of interest) by the specific hybridization procedure discussed in

5 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 *Nucl. Acids Res.* 12:203-213, (1984), and Wetmur *et al.*, *J. Mol. Biol.* 31:349-370 (1968). 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

10 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 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

15 stringency conditions at about 65°C. Both temperature and salt may be varied, or either the temperature or the salt concentration may be held constant while the other variable is changed.

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

20 DNA (e.g., sonicated salmon sperm DNA) with washing at 0.5x SSC, 0.5% SDS at 65°C, for high stringency.

It is contemplated that lower stringency hybridization conditions such as lower hybridization and/or washing temperatures can be used to identify related sequences having a lower degree of sequence similarity if specificity of binding of the probe or

25 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. Detection of DNA segments via hybridization is well-known to those of skill in the art, and thus depending on the application envisioned, one will desire to employ

30 varying hybridization conditions to achieve varying degrees of selectivity of probe towards target sequence and the method of choice will depend on the desired results.

As used herein, an agent, be it a naturally occurring molecule or otherwise may be "substantially purified", if desired, referring to a molecule separated from

substantially all other molecules normally associated with it in its native state. More preferably a substantially purified molecule is the predominant species present in a preparation. A substantially purified molecule may be greater than 60% free, preferably 75% free, more preferably 90% free, and most preferably 95% free from
 5 the other molecules (exclusive of solvent) present in the natural mixture. The term "substantially purified" is not intended to encompass molecules present in their native state.

The agents of the present invention will preferably be "biologically active" with respect to either a structural attribute, such as the capacity of a nucleic acid to
 10 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,
 15 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), European Patent 144914), chemical labels (U.S. Patent 4,582,789, U.S. Patent
 20 4,563,417), modified bases (European Patent 119448).

In a preferred embodiment, 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: 67 through SEQ ID NO: 99 or complements thereof or fragments of either
 25 under moderately stringent conditions, for example at about 2.0 x SSC and about 65°C. In a particularly preferred embodiment, 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: 67 through SEQ ID NO: 99 or complements or fragments of either under high stringency conditions. In one aspect of the present invention, a preferred marker
 30 nucleic acid molecule of the present invention has the nucleic acid sequence set forth in SEQ ID NO: 67 through SEQ ID NO: 99 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% or 90% and 100% sequence identity with the nucleic acid sequence set forth in SEQ ID NO: 67 through

SEQ ID NO: 99 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: 67 through SEQ ID NO: 99 or complement thereof 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: 67 through SEQ ID NO: 99 or complement thereof or fragments of either.

Additional genetic markers can be used to select plants with an allele of a QTL associated with fungal 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.

Markers, such as simple sequence repeat markers (SSR), AFLP markers, RFLP markers, RAPD markers, phenotypic markers, SNPs, isozyme markers, microarray transcription profiles that are genetically linked to or correlated with alleles of a QTL of the present invention can be utilized (Walton, *Seed World* 22-29 (July, 1993), Burow *et al.*, *Molecular Dissection of Complex Traits*, 13-29, ed. Paterson, CRC Press, New York (1988)). Methods to isolate such markers are known in the art. For example, locus-specific SSR markers can be obtained by screening a genomic library for microsatellite repeats, sequencing of "positive" clones, designing primers which flank the repeats, and amplifying genomic DNA with these primers. The size of the resulting amplification products can vary by integral numbers of the basic repeat unit. To detect a polymorphism, PCR products can be radiolabeled,

separated on denaturing polyacrylamide gels, and detected by autoradiography. Fragments with size differences >4 bp can also be resolved on agarose gels, thus avoiding radioactivity.

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 or other means.

The most preferred 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 Appln. 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, 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.

In lieu of PCR, alternative methods, such as the "Ligase Chain Reaction" (LCR) may be used (Barany, *Proc. Natl. Acad. Sci. (U.S.A.)* 88:189-193 (1991). LCR uses two pairs of oligonucleotide probes to exponentially amplify a specific target. The sequence of each pair of oligonucleotides is selected to permit the pair to hybridize to abutting sequences of the same strand of the target. Such hybridization forms a substrate for a template-dependent ligase. As with PCR, the resulting products thus serve as a template in subsequent cycles and an exponential amplification of the desired sequence is obtained.

The "Oligonucleotide Ligation Assay" (OLA) may alternatively be employed (Landegren *et al.*, *Science* 241:1077-1080 (1988)). The OLA protocol uses two oligonucleotides that are designed to be capable of hybridizing to abutting sequences of a single strand of a target. OLA, like LCR, is particularly suited for the detection of point mutations. Unlike LCR, however, OLA results in "linear" rather than exponential amplification of the target sequence.

Schemes based on ligation of two (or more) oligonucleotides in the presence of a nucleic acid having the sequence of the resulting "di-oligonucleotide", thereby amplifying the di-oligonucleotide, are also known (Wu *et al.*, *Genomics* 4:560-569

(1989), and may be readily adapted to the purposes of the present invention.

Other known nucleic acid amplification procedures, such as allele-specific oligomers, branched DNA technology, transcription-based amplification systems, or isothermal amplification methods may also be used to amplify and analyze such polymorphisms (U.S. Patent 5,130,238, European Patent 329,822, U.S. Patent 5,169,766, European Patent 359,789, Kwoh, *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:1173-1177 (1989) European Patent 368,906, Walker *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 89:392-396 (1992).

10

Polymorphisms can also be identified by Single Strand Conformation Polymorphism (SSCP) analysis. SSCP is a method capable of identifying most sequence variations in a single strand of DNA, typically between 150 and 250 nucleotides in length (Elles, *Methods in Molecular Medicine: Molecular Diagnosis of Genetic Diseases*, Humana Press (1996); Orita *et al.*, *Genomics* 5: 874-879 (1989)). Under denaturing conditions a single strand of DNA will adopt a conformation that is uniquely dependent on its sequence conformation. This conformation usually will be different, even if only a single base is changed. Most conformations have been reported to alter the physical configuration or size sufficiently to be detectable by electrophoresis.

20

A central attribute of SNPs is that the site of the polymorphism is at a single nucleotide. SNPs are more stable than other classes of polymorphisms. Their spontaneous mutation rate is approximately 10^{-9} (Kornberg, *DNA Replication*, W. H. Freeman & Co., San Francisco (1980)). As SNPs result from sequence variation, new polymorphisms can be identified by sequencing random genomic or cDNA molecules. SNPs can also result from deletions, point mutations and insertions. That said, SNPs are also advantageous as markers since they are often diagnostic of "identity by descent" because they rarely arise from independent origins. Any single base alteration, whatever the cause, can be a SNP. SNPs occur at a greater frequency than other classes of polymorphisms and can be more readily identified. In the present invention, a SNP can represent a single indel event, which may consist of one or more base pairs, or a single nucleotide polymorphism.

30

SNPs can be characterized using any of a variety of methods. Such methods include the direct or indirect sequencing of the site, the use of restriction enzymes

where the respective alleles of the site create or destroy a restriction site, the use of allele-specific hybridization probes, the use of antibodies that are specific for the proteins encoded by the different alleles of the polymorphism, or by other biochemical interpretation. SNPs can be sequenced using a variation of the chain termination method (Sanger *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 74: 5463-5467 (1977)) in which the use of radioisotopes are replaced with fluorescently-labeled dideoxy nucleotides and subjected to capillary based automated sequencing (U.S. Patent 5,332,666; U.S. Patent 5,821,058). Automated sequencers are available from, for example, Applied Biosystems, Foster City, CA (3730x1 DNA Analyzer), Beckman Coulter, Fullerton, CA (CEQ™ 8000 Genetic Analysis System) and LI-COR, Inc., Lincoln, NE (4300 DNA Analysis System).

Approaches for analyzing SNPs can be categorized into two groups. The first group is based on primer-extension assays, such as solid-phase minisequencing or pyrosequencing. In the solid-phase minisequencing method, a DNA polymerase is used specifically to extend a primer that anneals immediately adjacent to the variant nucleotide. A single labeled nucleoside triphosphate complementary to the nucleotide at the variant site is used in the extension reaction. Only those sequences that contain the nucleotide at the variant site will be extended by the polymerase. A primer array can be fixed to a solid support wherein each primer is contained in four small wells, each well being used for one of the four nucleoside triphosphates present in DNA. Template DNA or RNA from each test organism is put into each well and allowed to anneal to the primer. The primer is then extended one nucleotide using a polymerase and a labeled di-deoxy nucleotide triphosphate. The completed reaction can be imaged using devices that are capable of detecting the label which can be radioactive or fluorescent. Using this method several different SNPs can be visualized and detected (Syvänen *et al.*, *Hum. Mutat.* 13: 1-10 (1999)). The pyrosequencing technique is based on an indirect bioluminometric assay of the pyrophosphate (PPi) that is released from each dNTP upon DNA chain elongation. Following Klenow polymerase mediated base incorporation, PPi is released and used as a substrate, together with adenosine 5-phosphosulfate (APS), for ATP sulfurylase, which results in the formation of ATP. Subsequently, the ATP accomplishes the conversion of luciferin to its oxi-derivative by the action of luciferase. The ensuing light output

becomes proportional to the number of added bases, up to about four bases. To allow processivity of the method dNTP excess is degraded by apyrase, which is also present in the starting reaction mixture, so that only dNTPs are added to the template during the sequencing procedure (Alderborn *et al.*, *Genome Res.* 10: 1249-1258 (2000)). An
 5 example of an instrument designed to detect and interpret the pyrosequencing reaction is available from Biotage, Charlottesville, VA (PyroMark MD).

A more recent SNP detection method, based on primer-extension assays is the GOOD assay. The GOOD assay (Sauer *et al.*, *Nucleic Acids Res.* 28: e100 (2000)) is an allele-specific primer extension protocol that employs MALDI-TOF (matrix-
 10 assisted laser desorption/ionization time-of-flight) mass spectrometry. The region of DNA containing a SNP is amplified first by PCR amplification. Residual dNTPs are destroyed using an alkaline phosphatase. Allele-specific products are then generated using a specific primer, a conditioned set of a-S-dNTPs and a-S-ddNTPs and a fresh DNA polymerase in a primer extension reaction. Unmodified DNA is removed by
 15 5'phosphodiesterase digestion and the modified products are alkylated to increase the detection sensitivity in the mass spectrometric analysis. All steps are carried out in a single vial at the lowest practical sample volume and require no purification. The extended reaction can be given a positive or negative charge and is detected using mass spectrometry (Sauer *et al.*, *Nucleic Acids Res.* 28: e13 (2000)). An instrument in
 20 which the GOOD assay is analyzed is for example, the autoflex® MALDI-TOF system from Bruker Daltonics (Billerica, MA).

The second group, which is based on recognition of heteroduplex DNA molecules, includes oligonucleotide hybridization, Taq-Man assays, molecular beacons, electronic dot blot assays and denaturing high-performance liquid
 25 chromatography. Oligonucleotide hybridizations can be performed in mass using micro-arrays (Southern, *Trends Genet.* 12: 110-115 (1996)). Taq-Man ® assays, or Real Time PCR, detects the accumulation of a specific PCR product by hybridization and cleavage of a double-labeled fluorogenic probe during the amplification reaction. A Taq-Man assay includes four oligonucleotides, two of which serve as PCR primers
 30 and generate a PCR product encompassing the polymorphism to be detected. The other two are allele-specific fluorescence-resonance-energy-transfer (FRET) probes. FRET probes incorporate a fluorophore and a quencher molecule in close proximity so that the fluorescence of the fluorophore is quenched. The signal from a FRET probes is generated by degradation of the FRET oligonucleotide, so that the

fluorophore is released from proximity to the quencher, and is thus able to emit light when excited at an appropriate wavelength. In the assay, two FRET probes bearing different fluorescent reporter dyes are used, where a unique dye is incorporated into an oligonucleotide that can anneal with high specificity to only one of the two alleles.

5 Useful reporter dyes include 6-carboxy-4,7,2',7'-tetrachlorofluorecein (TET), 2'-chloro-7'-phenyl-1,4-dichloro-6-carboxyfluorescein (VIC) and 6-carboxyfluorescein phosphoramidite (FAM). A useful quencher is 6-carboxy-N,N,N',N'-tetramethylrhodamine (TAMRA). Annealed (but not non-annealed) FRET probes are degraded by TAQ DNA polymerase as the enzyme encounters the 5' end of the

10 annealed probe, thus releasing the fluorophore from proximity to its quencher. Following the PCR reaction, the fluorescence of each of the two fluorescers, as well as that of the passive reference, is determined fluorometrically. The normalized intensity of fluorescence for each of the two dyes will be proportional to the amounts of each allele initially present in the sample, and thus the genotype of the sample can

15 be inferred. An example of an instrument used to detect the fluorescence signal in Taq-Man ® assays, or Real Time PCR are the 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA).

Molecular beacons are oligonucleotide probes that form a stem-and-loop structure and possess an internally quenched fluorophore. When they bind to

20 complementary targets, they undergo a conformational transition that turns on their fluorescence. These probes recognize their targets with higher specificity than linear probes and can easily discriminate targets that differ from one another by a single nucleotide. The loop portion of the molecule serves as a probe sequence that is complementary to a target nucleic acid. The stem is formed by the annealing of the

25 two complementary arm sequences that are on either side of the probe sequence. A fluorescent moiety is attached to the end of one arm and a nonfluorescent quenching moiety is attached to the end of the other arm. The stem hybrid keeps the fluorophore and the quencher so close to each other that the fluorescence does not occur. When the molecular beacon encounters a target sequence, it forms a probe-target hybrid that

30 is stronger and more stable than the stem hybrid. The probe undergoes spontaneous conformational reorganization that forces the arm sequences apart, separating the fluorophore from the quencher, and permitting the fluorophore to fluoresce (Bonnet et al., 1999). The power of molecular beacons lies in their ability to hybridize only to target sequences that are perfectly complementary to the probe sequence, hence

permitting detection of single base differences (Kota *et al.*, *Plant Mol. Biol. Rep.* 17: 363-370 (1999)). Molecular beacon detection can be performed for example, on the Mx4000® Multiplex Quantitative PCR System from Stratagene (La Jolla, CA).

The electronic dot blot assay uses a semiconductor microchip comprised of an
 5 array of microelectrodes covered by an agarose permeation layer containing streptavidin. Biotinylated amplicons are applied to the chip and electrophoresed to selected pads by positive bias direct current, where they remain embedded through interaction with streptavidin in the permeation layer. The DNA at each pad is then hybridized to mixtures of fluorescently labeled allele-specific oligonucleotides.
 10 Single base pair mismatched probes can then be preferentially denatured by reversing the charge polarity at individual pads with increasing amperage. The array is imaged using a digital camera and the fluorescence quantified as the amperage is ramped to completion. The fluorescence intensity is then determined by averaging the pixel count values over a region of interest (Gilles *et al.*, *Nature Biotech.* 17: 365-370
 15 (1999)).

A more recent application based on recognition of heteroduplex DNA molecules uses denaturing high-performance liquid chromatography (DHPLC). This technique represents a highly sensitive and fully automated assay that incorporates a Peltier-cooled 96-well autosampler for high-throughput SNP analysis. It is based on
 20 an ion-pair reversed-phase high performance liquid chromatography method. The heart of the assay is a polystyrene-divinylbenzene copolymer, which functions as a stationary phase. The mobile phase is composed of an ion-pairing agent, triethylammonium acetate (TEAA) buffer, which mediates the binding of DNA to the stationary phase, and an organic agent, acetonitrile (ACN), to achieve subsequent
 25 separation of the DNA from the column. A linear gradient of CAN allows the separation of fragments based on the presence of heteroduplexes. DHPLC thus identifies mutations and polymorphisms that cause heteroduplex formation between mismatched nucleotides in double-stranded PCR-amplified DNA. In a typical assay, sequence variation creates a mixed population of heteroduplexes and homoduplexes
 30 during reannealing of wild-type and mutant DNA. When this mixed population is analyzed by DHPLC under partially denaturing temperatures, the heteroduplex molecules elute from the column prior to the homoduplex molecules, because of their reduced melting temperatures (Kota *et al.*, *Genome* 44: 523-528 (2001)). An example

of an instrument used to analyze SNPs by DHPLC is the WAVE® HS System from Transgenomic, Inc. (Omaha, NE).

A microarray-based method for high-throughput monitoring of plant gene expression can be utilized as a genetic marker system. This 'chip'-based approach involves using microarrays of nucleic acid molecules as gene-specific hybridization targets to quantitatively or qualitatively measure expression of plant genes (Schena *et al.*, *Science* 270:467-470 (1995); Shalon, Ph.D. Thesis, Stanford University (1996)). Every nucleotide in a large sequence can be queried at the same time. Hybridization can be used to efficiently analyze nucleotide sequences. Such microarrays can be probed with any combination of nucleic acid molecules. Particularly preferred combinations of nucleic acid molecules to be used as probes include a population of mRNA molecules from a known tissue type or a known developmental stage or a plant subject to a known stress (environmental or man-made) or any combination thereof (*e.g.* mRNA made from water stressed leaves at the 2 leaf stage). Expression profiles generated by this method can be utilized as markers.

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

The genetic linkage of additional marker molecules can be established by a gene mapping model such as, without limitation, the flanking marker model reported by Lander and Botstein, *Genetics*, 121:185-199 (1989), and the interval mapping, based on maximum likelihood methods described by Lander and Botstein, *Genetics*, 121:185-199 (1989), 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 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
 5 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 and Botstein, *Genetics*, 121:185-199 (1989), and further described by Arús and Moreno-González,
 10 *Plant Breeding*, Hayward, Rosemark, 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 and Lander, *Genetics*, 139:1421-1428 (1995)).
 15 Multiple regression methods or models can be also be used, in which the trait is regressed on a large number of markers (Jansen, *Biometrics in Plant Breed*, van Oijen, Jansen (eds.) Proceedings of the Ninth Meeting of the Eucarpia Section Biometrics in Plant Breeding, The Netherlands, pp. 116-124 (1994); Weber and Wricke, *Advances in Plant Breeding*,
 20 Blackwell, Berlin, 16 (1994)). Procedures combining interval mapping with regression analysis, whereby the phenotype is regressed onto a single putative QTL at a given marker interval, and at the same time onto a number of markers that serve as 'cofactors,' have been reported by Jansen and Stam, *Genetics*, 136:1447-1455 (1994) and Zeng, *Genetics*, 136:1457-1468 (1994). Generally, the use of cofactors reduces
 25 the bias and sampling error of the estimated QTL positions (Utz and Melchinger, *Biometrics in Plant Breeding*, van Oijen, Jansen (eds.) Proceedings of the Ninth Meeting of the Eucarpia Section Biometrics in Plant Breeding, The Netherlands, pp.195-204 (1994), thereby improving the precision and efficiency of QTL mapping (Zeng, *Genetics*, 136:1457-1468 (1994)). These models can be extended to multi-
 30 environment experiments to analyze genotype-environment interactions (Jansen *et al.*, *Theo. Appl. Genet.* 91:33-37 (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 of plant*

chromosomes. chromosome structure and function: Impact of new concepts J.P. Gustafson and R. Appels (eds.). Plenum Press, New York, pp. 157-173 (1988)).

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

10 An F_2 population is the first generation of selfing after the hybrid seed is produced. Usually a single F_1 plant is selfed to generate a population segregating for all the genes in Mendelian (1:2:1) fashion. Maximum genetic information is obtained from a completely classified F_2 population using a codominant marker system (Mather, Measurement of Linkage in Heredity: Methuen and Co.,
 15 (1938)). In the case of dominant markers, progeny tests (e.g F_3 , BCF_2) are required to identify the heterozygotes, thus making it equivalent to a completely classified F_2 population. However, this procedure is often prohibitive because of the cost and time involved in progeny testing. Progeny testing of F_2 individuals is often used in map construction where phenotypes do not
 20 consistently reflect genotype (e.g. disease resistance) or where trait expression is controlled by a QTL. Segregation data from progeny test populations (e.g. F_3 or BCF_2) can be used in map construction. Marker-assisted selection can then be applied to cross progeny based on marker-trait map associations (F_2 , F_3), where linkage groups have not been completely disassociated by recombination events (i.e.,
 25 maximum disequilibrium).

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 by using RIL because all loci are homozygous or nearly so. Under conditions of tight
 30 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. (U.S.A.)* 89:1477-1481 (1992)). However, as the distance between markers becomes larger

(*i.e.*, loci become more independent), the information in RIL populations decreases dramatically when compared to codominant markers.

Backcross populations (*e.g.*, generated from a cross between a successful variety (recurrent parent) and another variety (donor parent) carrying a trait not present in the former) can be utilized as a mapping population. A series of backcrosses to the recurrent parent can be made to recover most of its desirable traits. Thus a population is created 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 have contrasting polymorphic marker alleles (Reiter *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 89:1477-1481 (1992)). Information obtained from backcross populations using either codominant or dominant markers is less than that obtained from F₂ populations because one, rather than two, recombinant gametes are sampled per plant. Backcross populations, however, are more informative (at low marker saturation) when compared to RILs as the distance between linked loci increases in RIL populations (*i.e.* about .15% recombination). Increased recombination can be beneficial for resolution of tight linkages, but may be undesirable in the construction of maps with low marker saturation.

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

Bulk segregant analysis (BSA) is a method developed for the rapid identification of linkage between markers and traits of interest (Michelmore, *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 88:9828-9832 (1991)). 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 (*i.e.* heterozygous). Regions unlinked to the target region will not differ between the bulked samples of many individuals in BSA.

An alternative to traditional QTL mapping involves achieving higher resolution by mapping haplotypes, versus individual markers (Fan *et al.* 2006

Genetics). This approach tracks blocks of DNA known as haplotypes, as defined by polymorphic markers, which are assumed to be identical by descent in the mapping population. This assumption results in a larger effective sample size, offering greater resolution of QTL. Methods for determining the statistical significance of a correlation between a phenotype and a genotype, in this case a haplotype, may be determined by any statistical test known in the art and with any accepted threshold of statistical significance being required. The application of particular methods and thresholds of significance are well within the skill of the ordinary practitioner of the art.

The SNP markers of the present invention can be used to isolate or substantially purify an allele of a QTL that is also located on linkage group associated with ASR resistance locus 1, ASR resistance locus 2, ASR resistance locus 3, ASR resistance locus 4, ASR resistance locus 5, ASR resistance locus 6, ASR resistance locus 7, ASR resistance locus 8, ASR resistance locus 9, ASR resistance locus 10, ASR resistance locus 11, ASR resistance locus 12, and ASR resistance locus 13. Construction of an overlapping series of clones (a clone contig) across the region can provide the basis for a physical map encompassing an allele of a fungal disease resistance QTL that are located on a linkage group associated with ASR resistance locus 1, ASR resistance locus 2, ASR resistance locus 3, ASR resistance locus 4, ASR resistance locus 5, ASR resistance locus 6, ASR resistance locus 7, ASR resistance locus 8, ASR resistance locus 9, ASR resistance locus 10, ASR resistance locus 11, ASR resistance locus 12, and ASR resistance locus 13. The yeast artificial chromosome (YAC) cloning system has facilitated chromosome walking and large-size cloning strategies. A sequence tag site (STS) content approach utilizing the markers of the present invention can be used for the construction of YAC clones across chromosome regions. Such an STS content approach to the construction of YAC maps can provide a detailed and ordered STS-based map of any chromosome region, including the region encompassing the allele of a QTL is also located on a linkage group associated with ASR resistance locus 1, ASR resistance locus 2, ASR resistance locus 3, ASR resistance locus 4, ASR resistance locus 5, ASR resistance locus 6, ASR resistance locus 7, ASR resistance locus 8, ASR resistance locus 9, ASR resistance locus 10, ASR resistance locus 11, ASR resistance locus 12, and ASR resistance locus 13. YAC maps can be supplemented by detailed physical maps are constructed across the region by using BAC, PAC, or bacteriophage P1 clones that

contain inserts ranging in size from 70 kb to several hundred kilobases (Cregan,
Theor. Appl. Gen. 78:919-928 (1999), Sternberg, *Proc. Natl. Acad. Sci.* 87:103-107
 (1990), Sternberg, *Trends Genet.* 8:11-16 (1992); Sternberg *et al.*, *New Biol.* 2:151-
 162 (1990); Ioannou *et al.*, *Nat. Genet.* 6:84-89 (1994); Shizuya *et al.*, *Proc. Natl.*
 5 *Acad. Sci.* 89:8794-8797 (1992)).

Overlapping sets of clones are derived by using the available markers of the
 present invention to screen BAC, PAC, bacteriophage P1, or cosmid libraries. In
 addition, hybridization approaches can be used to convert the YAC maps into BAC,
 10 PAC, bacteriophage P1, or cosmid contig maps. Entire YACs and products of inter-
Alu-PCR as well as primer sequences from appropriate STSs can be used to screen
 BAC, PAC, bacteriophage P1, or cosmid libraries. The clones isolated for any region
 can be assembled into contigs using STS content information and fingerprinting
 approaches (Sulston *et al.*, *Comput. Appl. Biosci.* 4:125-132 (1988)).

15 The degeneracy of the genetic code, which allows different nucleic acid
 sequences to code for the same protein or peptide, is known in the literature. As used
 herein a nucleic acid molecule is degenerate of another nucleic acid molecule when
 the nucleic acid molecules encode for the same amino acid sequences but comprise
 different nucleotide sequences. An aspect of the present invention is that the nucleic
 20 acid molecules of the present invention include nucleic acid molecules that are
 degenerate of the nucleic acid molecule that encodes the protein(s) of the quantitative
 trait alleles.

Another aspect of the present invention is that the nucleic acid molecules of
 the present invention include nucleic acid molecules that are homologues of the
 25 nucleic acid molecule that encodes the one or more of the proteins associated with the
 QTL.

Exogenous genetic material may be transferred into a plant by the use of a
 DNA plant transformation vector or construct designed for such a purpose. A
 particularly preferred subgroup of exogenous material comprises a nucleic acid
 30 molecule of the present invention. Design of such a vector is generally within the
 skill of the art (See, *Plant Molecular Biology: A Laboratory Manual*, eds. Clark,
 Springer, New York (1997), Examples of such plants, include, without limitation,
 alfalfa, *Arabidopsis*, barley, *Brassica*, broccoli, cabbage, citrus, cotton, garlic, oat,
 oilseed rape, onion, canola, flax, maize, an ornamental plant, pea, peanut, pepper,

potato, rice, rye, sorghum, soybean, strawberry, sugarcane, sugarbeet, tomato, wheat, poplar, pine, fir, eucalyptus, apple, lettuce, lentils, grape, banana, tea, turf grasses, sunflower, oil palm, *Phaseolus* etc.

A construct or vector may include the endogenous promoter of the fungal
 5 disease resistance QTL of the present invention. The characteristic of fungal disease resistance might best be achieved by expressing the identified QTL protein with the endogenous promoter. Alternatively, a heterologous promoter may be selected to express the protein or protein fragment of choice. These promoters may be operably linked to a polynucleotide sequence encoding the protein corresponding to the fungal
 10 resistance QTL. The heterologous promoter may be one that is selected based upon maturation or flowering time, in that timing of expression of the desired protein may be critical to the parameters affecting the fungal disease resistance trait. Effective expression of the fungal disease resistance QTL may require promoters that express in specific tissue types as well.

15 Alternatively, the promoters may be operably linked to other nucleic acid sequences, such as those encoding transit peptides, selectable marker proteins, or antisense sequences. The promoters may be selected on the basis of the cell type into which the vector will be inserted or on the basis of its regulatory features. Examples of such features include enhancement of transcriptional activity, inducibility, tissue-
 20 specificity, and developmental stage-specificity. In plants, promoters that are inducible, of viral or synthetic origin, constitutively active, temporally regulated, and spatially regulated have been described (Poszkowski, *et al.*, *EMBO J.*, 3: 2719, 1989; Odell, *et al.*, *Nature*, 313:810, 1985; Chau *et al.*, *Science*, 244:174-181, 1989). Often-used constitutive promoters include the CaMV 35S promoter (Odell, *et al.*, *Nature*,
 25 313: 810, 1985), the enhanced CaMV 35S promoter, the Figwort Mosaic Virus (FMV) promoter (Richins, *et al.*, *Nucleic Acids Res.* 20: 8451, 1987), the nopaline synthase (nos) promoter (Shaw *et al.*, *Nucleic Acids Res.* 12: 7831-7846 (1984)) and the octopine synthase (ocs) promoter.

Useful inducible promoters include promoters induced by salicylic acid or
 30 polyacrylic acids (PR-1; Williams, *et al.*, *Biotechnology* 10:540-543, 1992), induced by application of safeners (substituted benzenesulfonamide herbicides; Hershey and Stoner, *Plant Mol. Biol.* 17: 679-690, 1991), heat-shock promoters (Ou-Lee *et al.*, *Proc. Natl. Acad. Sci U.S.A.* 83: 6815, 1986; Ainley *et al.*, *Plant Mol. Biol.* 14: 949, 1990), a nitrate-inducible promoter derived from the spinach nitrite reductase

transcribable polynucleotide sequence (Back *et al.*, *Plant Mol. Biol.* 17: 9, 1991), hormone-inducible promoters (Yamaguchi-Shinozaki *et al.*, *Plant Mol. Biol.* 15: 905, 1990), and light-inducible promoters associated with the small subunit of RuBP carboxylase and LHCP families (Kuhlemeier *et al.*, *Plant Cell* 1: 471, 1989; 5 Feinbaum *et al.*, *Mol. Gen. Genet.* 226: 449-456, 1991; Weisshaar, *et al.*, *EMBO J.* 10: 1777-1786, 1991; Lam and Chua, *J. Biol. Chem.* 266: 17131-17135, 1990; Castresana *et al.*, *EMBO J.* 7: 1929-1936, 1988; Schulze-Lefert, *et al.*, *EMBO J.* 8: 651, 1989).

Particularly preferred promoters in the recombinant vector include the 10 nopaline synthase (NOS) promoter (Ebert *et al.*, 1987), the octopine synthase (OCS) promoter (which is carried on tumor-inducing plasmids of *Agrobacterium tumefaciens*), the caulimovirus promoters such as the cauliflower mosaic virus (CaMV) 19S promoter (Lawton *et al.*, 1987), the CaMV 35S promoter (Odell *et al.*, 1985), the figwort mosaic virus 35S-promoter (Walker *et al.*, 1987); the light- 15 inducible promoter from the small subunit of ribulose-1,5-bisphosphate carboxylase (ssRUBISCO); the EIF-4A promoter from tobacco (Mandel, *et al.*, *Plant Mol. Biol.* 29: 995-1004, 1995); the chitinase promoter from *Arabidopsis* (Samac, *et al.*, *Plant Cell*, 3:1063-1072, 1991); the LTP (Lipid Transfer Protein) promoters from broccoli (Pyee, *et al.*, *Plant J.*, 7: 49-59, 1995); petunia chalcone isomerase (Van Tunen, *et al.*, 20 *EMBO J.* 7: 1257, 1988); bean glycine rich protein 1 (Keller, *et al.*, *EMBO L.*, 8: 1309-1314, 1989); the Potato patatin (Wenzler, *et al.*, *Plant Mol. Biol.*, 12: 41-50, 1989); the *Arabidopsis* Actin 7 promoter (Genbank accession U27811.1 GI:1002528, 17-APR-1997 and PCT application: WO0144457A2); the *Arabidopsis* Actin 8 promoter (An *et al.*, *Plant J.* 10:107- 121 (1996) and PCT application: 25 WO0144457A2); the *Arabidopsis* Rubisco small subunit 4 promoter (Krebbers *et al.*, *Plant Mol. Biol.* 11:745-759 (1988)); the *Brassica napin* gene promoter (U.S. Patent 5,420,034); the *Arabidopsis* Suc2 promoter (Truernit *et al.*, *Planta* 196: 564-570 (1995)); *Arabidopsis* elongation factor EF-1 30 alpha promoter (Axelos *et al.*, *Mol. Gen. Genet.* 219: 106-112 (1989)); and the *Glycine max* 7S alpha beta conglycin promoter, Sphas (Doyle *et al.*, *J. Biol. Chem.* 261: 9228-9238 (1986)).

Constructs of the present invention may also include additional 5' untranslated regions (5' UTR) or leaders of an mRNA polynucleotide molecule or gene which can play an important role in translation initiation. Some 5' UTRs may act as translational enhancers and may also be incorporated as part of the recombinant vector. For example, non-translated 5' leader polynucleotide molecules derived from heat shock protein genes have been demonstrated to enhance gene expression in plants (see for example, U.S. Patent 5,659,122, and U.S. Patent 5,362,865). Thus the recombinant vector may preferably contain one or more 5' non-translated leader sequences which serve to enhance expression of the nucleic acid sequence. Such enhancer sequences may be desirable to increase or alter the translational efficiency of the resultant mRNA. Preferred 5' nucleic acid sequences include the *Arabidopsis* Actin 7 leader (Genbank accession U27811.1 GI:1002528, 17-APR-1997 and PCT application: WO0144457A2); the *Arabidopsis* Actin 8 leader (An *et al.*, *Plant J.* 10: 107-121 (1996) and PCT application: WO0144457A2); the *Arabidopsis* Rubisco small subunit 4 leader (Krebbers *et al.*, *Plant Mol. Biol.* 11: 745-759 (1988)); the *Brassica napin* gene leader (U.S. Patent 5,420,034); the *Arabidopsis* Suc2 leader (Truernit *et al.*, *Planta* 196: 564-570 (1995)); the *Petunia hybrida* Hsp70 gene leader (Winter *et al.*, *Mol. Gen. Genet.* 211: 315-319 (1988)); the *Arabidopsis* EPSPS gene leader (Klee *et al.*, *Mol. Gen. Genet.* 210: 437-442 (1987)); the *Arabidopsis* elongation factor EF-1 alpha leader (Axelos *et al.*, *Mol. Gen. Genet.* 219: 106-112 (1989)); and the *Glycine max* 7s alpha beta conglycin leader (Doyle *et al.*, *J. Biol. Chem.* 261: 9228-9238 (1986)). These additional upstream regulatory polynucleotide molecules may be derived from a source that is native or heterologous with respect to the other elements present on the construct.

In addition, constructs may include additional regulatory polynucleotide molecules from the 3'-untranslated region (3' UTR) of plant genes. A 3' UTR or terminator typically provides a transcriptional termination signal, and a polyadenylation signal which functions in plants to cause the addition of adenylate nucleotides to the 3' end of the mRNA. Usually, nucleic acid sequences located a few hundred base pairs downstream of the polyadenylation site serve to terminate

transcription. In addition, some 3' UTRs provide additional properties such as enhancing the stability of the mRNA as in the potato proteinase inhibitor II gene 3' UTR (An *et al.*, *The Plant Cell* 1: 115-122 (1989)). Other 3' UTRs may provide sequences that enhance degradation of the mRNA such as the 5'-UUAUUUAUU-3' motif shown to contribute to lower stability of RNA messages in animal cells (Zubiaga *et al.*, *Mol. Cell Biol.* 15: 2219-2230 (1995)). These additional downstream regulatory polynucleotide molecules may be derived from a source that is native or heterologous with respect to the other elements present on the construct.

Preferred 3' UTRs or terminators are the potato proteinase inhibitor II gene 3' UTR (An *et al.*, *The Plant Cell* 1: 115-122 (1989)); the pea Rubisco small subunit E9 terminator (Coruzzi *et al.*, *EMBO J.* 3: 1671-1679 (1984)); the cauliflower mosaic virus 35S terminator; the *Brassica napin* gene terminator (U.S. Patent 5,420,034); the *Glycine max* 7s α beta conglycin gene terminator (Doyle *et al.*, *J. Biol. Chem.* 261: 9228-9238 (1986)); the *Phaseolus vulgaris* Arc5 terminator (Goossens *et al.*, *Eur. J. Biochem.* 225: 787-795 (1994)); the *Agrobacterium tumefaciens* nopaline synthase terminator (Rojiyaa *et al.*, 1987, Genbank Accession E01312 and U.S. Patent Application US20020192813A1); and the *Glycine max* ADR12 gene terminator (Datta *et al.*, *Plant Mol. Biol.* 21:859-869 (1993)).

A vector or construct may also include regulatory elements derived from the introns of certain genes. Examples of such include the Adh intron 1 (Callis *et al.*, *Genes and Develop.* 1:1183-1200 (1987); the sucrose synthase intron (Vasil *et al.*, *Plant Physiol.* 91:1575-1579 (1989); and the TMV omega element (Gallie *et al.*, *The Plant Cell* 1:301-311 (1989)). Preferred introns are the *Arabidopsis* Actin 7 intron (Genbank accession U27811.1 GI:1002528, 17-APR-1997 and PCT application: WO200144457A2); the *Arabidopsis* Actin 8 intron (An *et al.*, *Plant J.* 10:107-121 (1996) and PCT application: WO200144457A2); and the *Arabidopsis* elongation factor EF-1 alpha intron (Axelos *et al.*, *Mol. Gen. Genet.* 219: 106-112 (1989)). These and other regulatory elements may be included when appropriate.

A vector or construct may also include a selectable marker. Selectable markers may also be used to select for plants or plant cells that contain the exogenous genetic material. Examples of such include, but are not limited to, a neo gene

(Potrykus *et al.*, *Mol. Gen. Genet.* 199:183-188 (1985)), which codes for kanamycin resistance and can be selected for using kanamycin, G418, etc.; a bar gene which codes for bialaphos resistance; a mutant EPSP synthase gene (Hinchee *et al.*, *Bio/Technology* 6:915-922 (1988)), which encodes glyphosate resistance; a nitrilase gene which confers resistance to bromoxynil (Stalker *et al.*, *J. Biol. Chem.* 263:6310-6314 (1988)); a mutant acetolactate synthase gene (ALS) which confers imidazolinone or sulphonylurea resistance (for example, U.S. Patent 6,222,100); a methotrexate resistant DHFR gene (Thillet *et al.*, *J. Biol. Chem.* 263:12500-12508 (1988)); Dicamba tolerance conferred, for example, by a gene for dicamba monooxygenase (DMO) from *Pseudomonas maltophilia* (US Patent Application 20030135879).

A vector or construct may also include a screenable marker. Screenable markers may be used to monitor expression. Exemplary screenable markers include a β -glucuronidase or uidA gene (GUS) which encodes an enzyme for which various chromogenic substrates are known (Jefferson, *Plant Mol. Biol. Rep.* 5:387-405 (1987); Jefferson *et al.*, *EMBO J.* 6:3901-3907 (1987)); an R-locus gene, which encodes a product that regulates the production of anthocyanin pigments (red color) in plant tissues (Dellaporta *et al.*, *Stadler Symposium* 11:263-282 (1988)); a β -lactamase gene (Sutcliffe *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 75:3737-3741 (1978)), a gene which encodes an enzyme for which various chromogenic substrates are known (*e.g.*, PADAC, a chromogenic cephalosporin); a luciferase gene (Ow *et al.*, *Science* 234:856-859 (1986)); a xylE gene (Zukowsky *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 80:1101-1105 (1983), which encodes a catechol dioxygenase that can convert chromogenic catechols; an α -amylase gene (Ikata *et al.*, *Bio/Technol.* 8:241-242 (1990)); a tyrosinase gene (Katz *et al.*, *J. Gen. Microbiol.* 129:2703-2714 (1983)), which encodes an enzyme

capable of oxidizing tyrosine to DOPA and dopaquinone which in turn condenses to melanin; and an α -galactosidase.

Any of the techniques known in the art for introduction of transgenes into plants may be used to prepare a plant resistant to fungal disease in accordance with the invention. Suitable methods for transformation of plants are believed to include
 5 virtually any method by which DNA can be introduced into a cell, such as by electroporation as illustrated in U.S. Patent No. 5,384,253; microprojectile bombardment as illustrated in U.S. Patent Nos. 5,015,580; 5,550,318; 5,538,880; 6,160,208; 6,399,861; and 6,403,865; Agrobacterium-mediated transformation as
 10 illustrated in U.S. Patent Nos. 5,635,055; 5,824,877; 5,591,616; 5,981,840; and 6,384,301; and protoplast transformation as illustrated in U.S. Patent No. 5,508,184. Through the application of techniques such as these, the cells of virtually any plant species may be stably transformed, and these cells developed into transgenic plants. Techniques useful in the context of cotton transformation are disclosed in U.S. Patent
 15 Nos. 5,846,797, 5,159,135, 5,004,863, and 6,624,344; and techniques for transforming Brassica plants in particular are disclosed, for example, in U.S. Patent 5,750,871; and techniques for transforming soybean are disclosed in for example in Zhang et al. (*Plant Cell Tissue Organ Cult* 56:37–46 (1999) and US Patent 6,384,301.

Having now generally described the invention, the same will be more readily
 20 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

25

Example 1: Breeding of Near-Isogenic Lines containing ASR resistance loci

One thousand, four hundred single nucleotide polymorphism (SNP) markers, randomly distributed across the 20 linkage groups of the soybean genetic linkage map, were used to identify SNP markers tightly linked to the ASR resistance locus 1
 30 locus. A panel of soybean lines consisting of near-isogenic lines (NILs) developed from a cross between Williams 82 and ASR resistance locus 1 donor, PI 200492. Derivative lines of PI 200492 were used to identify SNP markers that were polymorphic between Williams 82 and PI 200492. These polymorphic SNP markers

were then used to identify the map location of ASR resistance locus 1 using a segregating backcross population, L85-2378. L85-2378 was developed by crossing Williams 82 with PI 200492 and five backcross cycles, or essentially 6 doses of Williams 82, were made to recover most of Williams 82's desirable traits. Thus L85-2378 is created consisting of individuals nearly like the recurrent parent, Williams 82, but each individual NIL carries varying amounts or mosaic of genomic regions from the donor parent, PI 200492.

The entire population was genotyped with the polymorphic SNP markers identified above and was subsequently evaluated for soybean rust resistance using a greenhouse assay. Associations between SNP marker genotype and soybean rust resistance phenotype were evaluated. SNP markers found to be in high linkage disequilibrium with ASR resistance locus 1 disease phenotypic response were NS0093250, NS0119710, NS0103004, NS0099454, NS0102630, NS0102915, NS0102913, NS0123728, NS0129943, NS0102168, NS0092723, NS0098177, NS0127343, and NS0101121, and are presented in Table 1 and indicated as SEQ ID NOs: 67 through 80. All of these SNP markers map to a region on linkage group G of the public soybean genetic linkage map. Table 1 lists sequences for PCR amplification primers, indicated as SEQ ID NOs: 1 through 28, and probes, indicated as SEQ ID NOs: 100 through 127, corresponding to these SNP markers. Two SNP markers were identified as being useful in monitoring the positive introgression of ASR resistance locus 1 and correspond to SNP markers NS0102913 and NS0129943 and correspond to SEQ ID NO: 73 and SEQ ID NO: 75, respectively.

The efficacy of ASR resistance locus 1 against soybean rust isolates from Alabama was also evaluated in the following F2:3 populations: AG4403 x PI 200492, AG3302 x PI 200492, AG3201 x PI 200492, AG26932 x PI 200492, AG2402 x PI 200492. In each of the populations, a 3:1 segregation ratio was observed indicating a single dominant gene inheritance pattern.

Following the procedure described for ASR resistance locus 1, the ASR resistance locus 3 locus was mapped using NILs developed from the cross between Williams 82 and the donor parent, PI 462312, followed by five backcross cycles, or essentially 6 doses of Williams 82, were made to recover most of Williams 82's desirable traits. Thus L85-2378 is created consisting of individuals nearly like the recurrent parent, Williams 82 but each individual near isogenic line carries varying amounts or mosaic of genomic regions from the donor parent, PI 200492. The entire

population was genotyped with the set of polymorphic SNP markers identified above and was subsequently evaluated for soybean rust resistance using a greenhouse assay. Associations between SNP marker genotype and soybean rust resistance phenotype were evaluated. SNP markers found to be in high linkage disequilibria with ASR resistance locus 3 were NS0099746, NS0123747, NS0126598, NS0128378, NS0096829, NS0125408, NS0098902, NS0099529, NS0097798, NS0137477, NS0095322, NS0136101, and NS0098992, and are presented in Table 1 and indicated as SEQ ID NOs: 81 through 93. These markers were all mapping to LG C2 of the public soybean genetic map. Table 1 lists sequences for PCR amplification primers, indicated as SEQ ID NOs: 29 through 54, and probes, indicated as SEQ ID NOs: 128 through 153, corresponding to these SNP markers. The marker used to monitor the introgression of ASR resistance locus 3 corresponds to SNP marker NS0137477 and is indicated as SEQ ID NO: 90. To confirm the putative location of ASR resistance locus 3, a segregating F3:4 population was developed between AVRDC-8 and AG4403. AVRDC-8 is line developed by Asian Vegetable Research and Development Center in Taiwan by crossing Ankur (ASR resistance locus 3 containing line) and PI 230970 (ASR resistance locus 2 donor). This population is currently being genotyped for SNP markers and evaluated for resistance reaction against a soybean rust isolate from Loxley, AL to validate the location of ASR resistance locus 3.

The approximate locations of ASR resistance locus 2 and ASR resistance locus 4 were later determined based upon a survey of polymorphisms between a panel of PI lines that are known to contain either ASR resistance locus 2 or ASR resistance locus 4, PI 230970, PI 459025B, the donor of ASR resistance locus 2 and ASR resistance locus 4, respectively, and other lines that were reported in literature to contain either QTL. Based on the polymorphism survey, any polymorphic SNP marker is a candidate region near the ASR resistance loci. For ASR resistance locus 2, two candidate regions were identified and the locus is most likely located on linkage group J, near or within the disease resistance cluster Brown Stem Rot, Soybean Cyst Nematode resistance and Frog Eye Leaf Spot, or within linkage group N. The ASR resistance locus 4 is likely located on linkage group N.

Table 1. SNP markers for identification and selection of ASR resistance locus 1 and ASR resistance locus 3.

MARKER	SEQ ID	SEQ ID FORWARD PRIMER	SEQ ID REVERSE PRIMER	SEQ ID PROBE 1	SEQ ID PROBE 2
NS0093250	67	1	2	100	101
NS0119710	68	3	4	102	103
NS0103004	69	5	6	104	105
NS0099454	70	7	8	106	107
NS0102630	71	9	10	108	109
NS0102915	72	11	12	110	111
NS0102913	73	13	14	112	113
NS0123728	74	15	16	114	115
NS0129943	75	17	18	116	117
NS0102168	76	19	20	118	119
NS0092723	77	21	22	120	121
NS0098177	78	23	24	122	123
NS0127343	79	25	26	124	125
NS0101121	80	27	28	126	127
NS0099746	81	29	30	128	129
NS0123747	82	31	32	130	131
NS0126598	83	33	34	132	133
NS0128378	84	35	36	134	135
NS0096829	85	37	38	136	137
NS0125408	86	39	40	138	139
NS0098902	87	41	42	140	141
NS0099529	88	43	44	142	143
NS0097798	89	45	46	144	145
NS0137477	90	47	48	146	147
NS0095322	91	49	50	148	149
NS0136101	92	51	52	150	151
NS0098982	93	53	54	152	153

5 Example 2: Collection and propagation of spores.

Asian Soybean Rust urediniospores from *Phakopsora pachyrhizi* were collected from infected plants at the Monsanto Loxley Agronomy station (Loxley, AL), herein referred to as the Loxley strain.

Soybean plants were inoculated by spraying the underside of the leaves with spores suspended in water containing 0.01% Tween-20. Lesion development was visible without magnification at around 7 to 10 days with sporulation occurring at 12 to 14 days after infection. Spores from the infected plants were collected and

resuspended in sterile deionized water containing 0.01% Tween 20. The spore concentration was determined using a hemacytometer.

Example 3: Detached leaf assay for Asian Soybean Rust Resistance

5 Two types of leaf tissue were assessed for ASR disease phenotyping. Unifoliate leaves, seven to ten days after emergence, or V3 trifoliolate leaves, twenty-one to twenty-eight after emergence, were assessed. At about two days after emergence from the soil, the soybean plant bears a pair of unifoliolate leaves which are fully unfurled about five days later and constitute the first 'true leaves'. At about
10 seven days after emergence, the trifoliolate leaves appear (comprising three leaves at the end of one petiole). Three sets emerge in sequence and the first trifoliolate leaves are denoted as the V1 stage, and are fully expanded at ten days after emergence. The next two V stages occur a week apart. Notably, the leaves are inoculated for disease after they have both unfurled and hardened, i.e. not new and green. The unifoliate
15 tend to harden very quickly, around 8-10d after emergence, while V2 and V3 trifoliate leaves may not even unfurl completely until up to 24-28 days after emergence.

Three 3.2 cm diameter Watmann #1 filter papers are placed in each of 6 wells of a 6-well tissue culture plate (well volume is 15.5 milliliters). The leaves are cut into 3 centimeter by 3 centimeter pieces and placed on top of the Watmann filter
20 papers with the bottom (stomatal side) of the leaf facing upwards. Approximately 2.0 milliliters of sterile deionized water is put into each well of the 6-well tissue culture plate. Asian Soybean Rust urediniospores from *Phakopsora pachyrhizi* are suspended in sterile deionized water containing 0.01% tween 20 at a concentration of 1×10^5 urediniospores per milliliter. Approximately 50 microliters of spore suspension is
25 applied to each leaf piece using an airbrush (Model Badger 155 Anthem, Badger Air-Brush Co., Franklin Park, IL) with a compressor (Model TC-20, Airbrush Depot, San Diego, CA) setting of 1 kilogram per square centimeter to wetness. The 6-well plate is then sealed with parafilm and placed in a growth chamber set to 22 degrees Celsius, with a photoperiod of 12 hours daylength. The plates are checked every 2 or 3 days to
30 monitor the progression of disease and to assure the wells have not dried out. Deionized water is added to make up the original volume in the well when needed or incubator relative humidity is adjusted to approximately 80%. Early symptoms of developing lesions should be evident under a dissecting microscope about 3 to 5 days after inoculation. Sporulating lesions should be evident 9 to 14 days after inoculation.

Average soybean rust severity scores are calculated from multiple trials. The rust severity score uses a rating scale from 1 to 5; 1 - being immune, 2 - demonstrating red/brown lesions over less than 50% of the leaf area, 3 - demonstrating red/brown lesions over greater than 50% of the leaf area, 4 - demonstrating tan lesions over less than 50% of the leaf area and 5 – demonstrating tan lesions over greater than 50% of the leaf area. Leaf sections can remain viable in this assay for up to 2 months.

Experiments using Asian Soybean Rust susceptible soybean, Lee 74 demonstrate consistently high levels of infection for each assay performed. Further experiments evaluating putative resistant germplasm were able to differentiate tolerant from susceptible accessions as demonstrated in Table 2. Accession PI 200487 demonstrated a slow rust resistance phenotype. Efforts are underway to identify markers that will be used in the introgression of the resistance locus identified in PI 200487 into elite germplasm.

In addition, comparison of ASR evaluation of unifoliate and trifoliate leaf tissue showed it takes approximately 45 days from seed to data point for trifoliate and approximately 23 days for unifoliate. By cutting the assay time in half, this significantly economizes the detached leaf assay and time required to determine disease resistance rating. By saving 3 weeks, plants can be propagated on a faster time scale and susceptible plants can be culled sooner, saving field and greenhouse space.

Table 2. Average rust score for resistant and susceptible accessions as determined using unifoliate and trifoliate leaf tissue; “-” indicates the assay was not performed.

Accession	Average Rust Severity Score Detached Unifoliate	Average Rust Severity Score Detached Leaf
Lee 74	5.0	5
PI 200487	1.89	2.25
PI 200492 (ASR resistance locus 1)	1.00	2
PI 200499	-	5
PI 230970	2.5	3
PI 368038	-	3
PI 368039	-	2
PI 462312	-	2
PI 547875	-	2
PI 547878	-	4.25
PI 547879	-	5
Tiana	-	5
Williams	-	5
AVRDC-8	1.8	2.25
Dowling	-	5

- 5 Example 4: Testing of Elite crosses for resistance to *P. pachyrizi* with introgressed ASR resistance locus 1, ASR resistance locus 2 and ASR resistance locus 3.

Crosses with donor resistant parent line, PI 200492, containing ASR resistance locus 1 were performed with various elite lines of soybean to monitor the positive introgression of ASR resistance locus 1. Leaf assays for resistance to the Loxley strain were performed using lines derived from crosses with the resistant parent line accession, PI 200492 (ASR resistance locus 1) as well as known resistant accessions (PI 230970 (ASR resistance locus 2) and PI 462312 (ASR resistance locus 3)) and susceptible elite lines. The resistance scores for all lines tested are presented in Table 3. Average rust severity scores were derived from 4 plants, each with 4 replications and rated on 4 different days (10DAI, 17DAI, 24DAI, 32DAI).

Table 3. Average Rust Severity Score of ASR backcross events and elite lines.

Cross	Progeny From Cross	ASR resistance locus Loci	Average Rust Severity Score
Multiple crosses to introgress both ASR resistance locus 2 and ASR resistance locus 3	AVRDC-8	ASR resistance locus 2/ASR resistance locus 3	1.7
Known Susceptible Line	Dowling	Susceptible	5
GL_AG4801//L85-2378/L86-1752	JN1137.1	ASR resistance locus 1 (MAS)	1
GL_AG4801//L85-2378/L86-1753	JN1137.2	ASR resistance locus 1 (MAS)	5
GL_AG4801//L85-2378/L86-1754	JN1137.3	ASR resistance locus 1 (MAS)	1
GL_AG4801//L85-2378/L86-1755	JN1137.4	ASR resistance locus 1 (MAS)	1
GL_AG4801//L85-2378/L86-1752	JN1153.1	ASR resistance locus 1 (MAS)	1
GL_AG4801//L85-2378/L86-1753	JN1153.2	ASR resistance locus 1 (MAS)	1
GL_AG4801//L85-2378/L86-1754	JN1153.3	ASR resistance locus 1 (MAS)	1
GL_AG4801//L85-2378/L86-1755	JN1153.4	ASR resistance locus 1 (MAS)	1
GL_AG4801//L85-2378/L86-1752	JN1160.1	ASR resistance locus 1 (MAS)	4.8
GL_AG4801//L85-2378/L86-1752	JN1160.2	ASR resistance locus 1 (MAS)	1
GL_AG4801//L85-2378/L86-1752	JN1160.3	ASR resistance locus 1 (MAS)	1
GL_AG4801//L85-2378/L86-1752	JN1160.4	ASR resistance locus 1 (MAS)	1
GL_AG4801//L85-2378/L86-1752	JN1163.1	ASR resistance locus 1 (MAS)	1
GL_AG4801//L85-2378/L86-1752	JN1163.2	ASR resistance locus 1 (MAS)	4.8
GL_AG4801//L85-2378/L86-1752	JN1163.3	ASR resistance locus 1 (MAS)	1
GL_AG4801//L85-2378/L86-1752	JN1163.4	ASR resistance locus 1 (MAS)	5
GL_AG5501//L85-2378/L86-1752	JN1691.1	ASR resistance locus 1 (MAS)	4.5
GL_AG5501//L85-2378/L86-1752	JN1691.2	ASR resistance locus 1 (MAS)	4.6
GL_AG5501//L85-2378/L86-1752	JN1691.3	ASR resistance locus 1 (MAS)	4.6
GL_AG5501//L85-2378/L86-1752	JN1691.4	ASR resistance locus 1 (MAS)	3
GL_AG5501//L85-2378/L86-1752	JN1692.1	ASR resistance locus 1 (MAS)	1.1
GL_AG5501//L85-2378/L86-1752	JN1692.2	ASR resistance locus 1 (MAS)	1
GL_AG5501//L85-2378/L86-1752	JN1692.3	ASR resistance locus 1 (MAS)	1
GL_AG5501//L85-2378/L86-1752	JN1692.4	ASR resistance locus 1 (MAS)	1
GL_AG5501//L85-2378/L86-1752	JN1742.1	ASR resistance locus 1 (MAS)	1
GL_AG5501//L85-2378/L86-1752	JN1742.2	ASR resistance locus 1 (MAS)	1
GL_AG5501//L85-2378/L86-1752	JN1742.3	ASR resistance locus 1 (MAS)	1
GL_AG5501//L85-2378/L86-1752	JN1742.4	ASR resistance locus 1 (MAS)	1
GL_AG5501//L85-2378/L86-1752	JN1765.1	ASR resistance locus 1 (MAS)	1.2
GL_AG5501//L85-2378/L86-1752	JN1765.2	ASR resistance locus 1 (MAS)	1
GL_AG5501//L85-2378/L86-1752	JN1765.3	ASR resistance locus 1 (MAS)	1
GL_AG5501//L85-2378/L86-1752	JN1765.4	ASR resistance locus 1 (MAS)	1
GL_AG5501//L85-2378/L86-1752	JN1774.1	ASR resistance locus 1 (MAS)	1
GL_AG5501//L85-2378/L86-1752	JN1774.2	ASR resistance locus 1 (MAS)	1
GL_AG5501//L85-2378/L86-1752	JN1774.3	ASR resistance locus 1 (MAS)	1
GL_AG5501//L85-2378/L86-1752	JN1774.4	ASR resistance locus 1 (MAS)	1
GL_CGL4504D0C//L85-2378/L86-1752	JN1866.1	ASR resistance locus 1 (MAS)	1
GL_CGL4504D0C//L85-2378/L86-1752	JN1866.2	ASR resistance locus 1 (MAS)	1
GL_CGL4504D0C//L85-2378/L86-1752	JN1866.3	ASR resistance locus 1 (MAS)	1
GL_CGL4504D0C//L85-2378/L86-1752	JN1866.4	ASR resistance locus 1 (MAS)	1
GL_CGL5400E1X//L85-2378/L86-1752	JN2242.1	ASR resistance locus 1 (MAS)	1
GL_CGL5400E1X//L85-2378/L86-1752	JN2242.2	ASR resistance locus 1 (MAS)	1
GL_CGL5400E1X//L85-2378/L86-1752	JN2242.3	ASR resistance locus 1 (MAS)	1

GL_CGL5400E1X//L85-2378/L86-1752	JN2242.4	ASR resistance locus 1 (MAS)	1
GL_CGL5400E1X//L85-2378/L86-1752	JN2243.1	ASR resistance locus 1 (MAS)	1
GL_CGL5400E1X//L85-2378/L86-1752	JN2243.2	ASR resistance locus 1 (MAS)	2.4
GL_CGL5400E1X//L85-2378/L86-1752	JN2243.3	ASR resistance locus 1 (MAS)	1
GL_CGL5400E1X//L85-2378/L86-1752	JN2243.4	ASR resistance locus 1 (MAS)	1.3
GL_CGL5400E1X//L85-2378/L86-1752	JN2250.1	ASR resistance locus 1 (MAS)	1
GL_CGL5400E1X//L85-2378/L86-1752	JN2250.2	ASR resistance locus 1 (MAS)	1
GL_CGL5400E1X//L85-2378/L86-1752	JN2250.3	ASR resistance locus 1 (MAS)	1.2
GL_CGL5400E1X//L85-2378/L86-1752	JN2250.4	ASR resistance locus 1 (MAS)	1
GL_AG4403//L85-2378/L86-1752	JN774.1	ASR resistance locus 1 (MAS)	1
GL_AG4403//L85-2378/L86-1752	JN774.2	ASR resistance locus 1 (MAS)	1.1
GL_AG4403//L85-2378/L86-1752	JN774.3	ASR resistance locus 1 (MAS)	1
GL_AG4403//L85-2378/L86-1752	JN774.4	ASR resistance locus 1 (MAS)	1

Lines containing the ASR resistance locus 1 locus showed greatest resistance to the Loxley strain. Introgression of the ASR resistance locus 1 was confirmed by MAS.

5

Example 5: Testing of Soybean Accessions for ASR Resistance using the Detached Leaf Assay

Seven hundred putative ASR resistant accessions were identified based upon greenhouse assays, using a mixed population of ASR isolates of foreign origin. Leaf assays for resistance to ASR were performed as described in Example 3 using a subset of two hundred and fifty of the seven hundred USDA putative resistant accessions. A complementary set of two hundred and fifty ASR susceptible accessions from the USDA were selected for comparison in the leaf assay based upon matching maturities and geographic origins to the two hundred and fifty resistant accessions. The average rust severity scores of the most resistant accessions (those exhibiting an average rust severity score from 1 to 2) is presented in Table 4 below. One thousand, four hundred SNP markers, distributed every 5 centimorgans across the 20 linkage groups of the soybean genetic linkage map, will be used to identify markers useful in following the introgression of the ASR resistance loci possessed by the resistant accessions into elite germplasm.

20

Table 4. Average Rust Severity Score ASR Resistant Accessions.

Accession	Average Rust Severity Score
PI200488	1.0
PI200492	1.0
PI203398	1.0
PI307884B	1.0
PI416764	1.0
PI416826A	1.0
PI417117	1.0
PI417132	1.0
PI423967	1.0
PI506947	1.0
PI507009	1.0
PI507259	1.0
PI561305	1.0
PI567031B	1.0
PI567034	1.0
PI567056A	1.0
PI567058D	1.0
PI567190	1.0
PI605773	1.0
PI605829	1.0
PI605865B	1.0
PI379620	1.3
PI416873B	1.3
PI417128	1.3
PI417463	1.3
PI567123A	1.3
PI578457A	1.3
PI615437	1.3
PI379621	1.3
PI567102B	1.3
PI594172A	1.3
PI628932	1.3
PI079648	1.5
PI291309C	1.5
PI416886	1.5
PI417503	1.5
PI506491	1.5
PI506677	1.5
PI506695	1.5
PI507193	1.5
PI567046A	1.5
PI567053	1.5
PI567189A	1.5

PI605891B	1.5
PI200455	1.8
PI232989	1.8
PI594494A	1.8
PI597405D	1.8
PI069533	2.0
PI084674	2.0
PI230970	2.0
PI291278	2.0
PI341252	2.0
PI417126	2.0
PI417134	2.0
PI417208	2.0
PI423923	2.0
PI437609A	2.0
PI471900	2.0
PI497969	2.0
PI506628	2.0
PI547875	2.0
PI567024	2.0
PI567025A	2.0
PI578471A	2.0
PI594512C	2.0
PI594561	2.0
PI605781A	2.0
PI605838	2.0
PI606405	2.0
PI606440A	2.0
PI615445	2.0

In addition, SNP markers distributed proximal and distal to ASR resistance locus 3 were genotyped for a set of eighty-nine resistant accessions. Four additional SNP markers (NS0103749, NS0118897, NS0119715, and NS0130920) were found to be associated with ASR resistance locus 3 and are listed in Table and indicated as SEQ ID NOs: 94 through 97. Table 5 lists sequences for PCR amplification primers, indicated as SEQ ID NOs: 55 through 62, and probes, indicated as SEQ ID NOs: 154 through 161, corresponding to these SNP markers.

This information will be used to identify novel resistance sources useful in prioritizing the introgression of the ASR and other pathogen resistance loci.

Table 5. SNP markers for identification and selection of ASR resistance locus 3.

MARKER	SEQ ID	SEQ ID FORWARD PRIMER	SEQ ID REVERSE PRIMER	SEQ ID PROBE 1	SEQ ID PROBE 2
NS0103749	94	55	56	154	155
NS0118897	95	57	58	156	157
NS0119715	96	59	60	158	159
NS0130920	97	61	62	160	161

Example 6. Using association studies to identify QTL that confer fungal disease resistance

- 5 To identify regions or genes associated with the disease is the first step toward developing resistant varieties. Four loci for rust resistance (ASR resistance locus 1, ASR resistance locus 2, ASR resistance locus 3, ASR resistance locus 4) were previously identified. In this example, linkage disequilibrium and haplotype association mapping were applied to a case-control data sample from soybean
- 10 germplasm.
- Four hundred ninety-two soybean lines (246 resistant-susceptible pairs) were scored for rust resistance as well as fingerprinted using 797 SNPs. Disease resistance was scored in 1 to 5 scales to a mixture of *Phakopsora pachyrhizi* isolates, with less than 3 as resistant and greater than 4 as susceptible. Specifically, case-control testing,
- 15 Fishers' exact test, single marker F-test, and haplotype trend regression were explored on window sizes of 3, 5 and 9 consecutive SNPs. Multiple testing results significantly associate two SNP markers from two separate haplotype windows, referred to herein as in fungal disease resistance haplotype windows 1 and 2, on chromosome 13 24-45 cM) with resistance to fungal disease. The SNP markers NS0103033 and NS0124935
- 20 are located in fungal disease resistance haplotype windows 1 and 2 respectively. The primers for NS0103033 (SEQ ID NO: 98) are indicated in SEQ ID NOs: 63 and 64 and the probes are indicated in SEQ ID NOs: 162 and 163. The primers for NS0124935 (SEQ ID NO: 99) are indicated in SEQ ID NOs: 65 and 66 and the probes are indicated in SEQ ID NOs: 164 and 165. Resistance scores for each of the
- 25 haplotypes and the marker allele for each haplotype are indicated in Table 5. Each window is designated by five SNP markers and the alleles for each is indicated as haplotype sequence. The allele for NS0103033 in haplotype window 1 and NS0124935 in haplotype window 2 are indicated in bold. For NS0103033, the SNP is

actually a 9-bp indel where “Z” represents the deletion (*****) and “W” represents the insertion (GAAGTGGAT).

Varieties containing resistant haplotypes from haplotype window 1 and/or 2 are indicated in table 6. This mapping effort has identified additional ASR disease resistance QTLs in addition to the previously defined ASR resistance loci.

Table 5. Summary scoring for lines containing resistant haplotypes in ASR resistance haplotype windows 1 and 2. A resistance score of 0 indicates the line was resistant and a score of 1 indicates the line was designated susceptible.

ASR resistance locus	Haplotype Window 1	Haplotype sequence	Resistance Score	
			0	1
5	Haplotype 1	AAZA?	5	0
6	Haplotype 2	AGWGA	26	10
7	Haplotype 3	AGWGG	34	15
8	Haplotype 4	TAZAG	5	0
9	Haplotype 5	TAZGA	13	5

ASR resistance locus	Haplotype Window 1	Haplotype sequence	Resistance Score	
			0	1
10	Haplotype 6	CGTTG	8	1
11	Haplotype 7	GGTTC	26	11
12	Haplotype 8	GGCCC	12	6
13	Haplotype 9	GGT-C	4	0

10

Table 6. Disease ratings for resistant germplasm containing haplotypes in ASR resistance windows 1 and/or 2 on chromosome 13.

Line	Rating	Resistance haplotype from haplotype window 1	Resistance haplotype from haplotype window 2
PI164885	2.5	X	X
PI165524	2	X	X
PI166028	2		X
PI189968	2	X	X
PI200446	2	X	
PI200488	2.5		X
PI205901B	2.5	X	
PI222549	2.5	X	
PI224270	2.5	X	
PI227331	2.5	X	X
PI229333	2.5		X
PI238109	2.3	X	
PI240667A	1	X	
PI258383	2	X	
PI291309C	2	X	
PI341252	2.5	X	X
PI374189	2.3	X	
PI398335	2	X	
PI399070	2.5	X	
PI407831	2.5	X	
PI407833C	2		X
PI407845A	2.5	X	
PI407858	2.3	X	X
PI407881	2.3	X	
PI408088	2.3	X	
PI408134B	2	X	
PI408272B	2	X	
PI417122	2.5	X	
PI417126	2.5	X	
PI417235	2	X	
PI417335	2.3	X	
PI423717	2	X	
PI423722	2.3	X	
PI423730B	2.3	X	
PI423852	2.3	X	X
PI424190	2.5	X	
PI434973A	2.5	X	
PI437110A	2.3	X	
PI437437A	1.5		X
PI437740B	2.3	X	X
PI437921	2	X	
PI437982	2.3	X	X
PI438073	2.3	X	

PI438371	2.5	X	
PI438480	2.5	X	
PI479735	2.3	X	
PI497965	2.5	X	
PI506737	2	X	
PI506863	2	X	
PI507142	2.5		X
PI508269	2	X	
PI548325	2	X	
PI561289	2	X	X
PI561329	2.5	X	
PI561330A	2		X
PI561337	2		X
PI561377	2.3		X
PI566978	2.5	X	
PI567010B	2.3	X	
PI567093B	2	X	X
PI567104B	2.5	X	X
PI567108B	2.5	X	X
PI567129	2.3	X	X
PI567140B	2.5	X	
PI567174C	2.3		X
PI567175C	2	X	X
PI567300A	2	X	
PI567409A	2.3	X	
PI567470	2	X	
PI567473C	2.5	X	
PI567474	2.3	X	
PI567489A	2	X	
PI567507B	2	X	
PI567554A	2	X	
PI567560	2.5	X	X
PI567561	2.5	X	
PI567675	2.3		X
PI567692	2	X	X
PI567718	2	X	X
PI567780A	2.3	X	
PI578305B	2.5	X	
PI587598A	2.5	X	
PI587914B	2		X
PI587922A	2		X
PI587935A	2.3		X
PI588000	2.5		X
PI588034	2.5		X
PI592962B	2.3	X	
PI594525	2.5	X	X
PI594538A	2	X	X
PI594767B	1	X	
PI597480A	2.3	X	
PI603293B	2.3	X	

PI603296	2.5		X
PI603429D	2.5	X	
PI603564A	2.3	X	
PI603612	2.3	X	X
PI603704A	2.5	X	X
PI605891B	2.5	X	
PI628870	1.5		X
PI628932	2.4	X	

Having illustrated and described the principles of the present invention, it should be apparent to persons skilled in the art that the invention can be modified in arrangement and detail without departing from such principles. We claim all

5 modifications that are within the spirit and scope of the appended claims.

<110> MONSANTO TECHNOLOGY LLC

<120> A Method to Identify Disease Resistant Quantitative Trait
Loci in Soybean and Compositions Thereof

<130> 06624-0044.2DIV

<140> UNKNOWN

<141> 2007-05-24

<150> 2,643,248

<151> 2007-05-24

<150> US 60/808,430

<151> 2006-05-25

<160> 165

<210> 1

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 1

cacctgttgc ttctccacca t

21

<210> 2

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 2

gggtggtgct cttggaggta

20

<210> 3

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 3

taaagcaaaa ggacgttacg acaa

24

<210> 4

<211> 23

<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 4

gcattgattt gtccacgaag aac

23

<210> 5
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 5

ttgcaatttt ttatatcttg atttcacat

29

<210> 6
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 6

gcgaagaatc aaaactgggc aaa

23

<210> 7
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 7

aagtggagga ggaaatgatg ga

22

<210> 8
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 8

caagggccct gattatgtga a

21

<210> 9
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 9

acaaggacaa ggctatgaga agtaaga

27

<210> 10
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 10

ggccatgaat caagccactt

20

<210> 11
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 11

gagtttagatt tatccggcaa cga

23

<210> 12
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 12

cccgaagaga tgtcatgtta acaa

24

<210> 13
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 13
gggttacctt catagctgct attttc 26

<210> 14
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 14
gggcacaaca cctgaatgg 19

<210> 15
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 15
ccgcagtgaa tcaagtaatt agttaataa 29

<210> 16
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 16
aaggtttagt tcgatcagtg attttga 27

<210> 17
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 17
ggtttcagat attttgataa gctaattaga tg 32

<210> 18
<211> 26
<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 18

caagaactca cattcctcag atgaag

26

<210> 19

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 19

agggcctatt gtgatgatta agga

24

<210> 20

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 20

aaatctgaaa aagcatccca aaag

24

<210> 21

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 21

gtaataatcc caaacatddd tcttcga

27

<210> 22

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 22

gggatgagtt gaattaatddd tcaaagta

28

<210> 23
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 23

gcatgctgct tttaactatg aaacat

26

<210> 24
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 24

gttgatgaag tatacaattt catttgca

28

<210> 25
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 25

aaaccaagtg aggagacatt aattca

26

<210> 26
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 26

ggctgagttg ggттаататс acatt

25

<210> 27
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 27

aaaaagttga atgacacct gcatt

25

<210> 28

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 28

catttgctt ttgcaggcta atctaa

26

<210> 29

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 29

atgggcaaca gttgtcatat gg

22

<210> 30

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 30

tgatgatggc atggaattat tacc

24

<210> 31

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 31

atttttggtta cctctctttc cttcaa

26

<210> 32

<211> 26

<212> DNA

<213> Artificial Sequence

<220>
<223> artificial

<400> 32
ttattaccaa catccaaaca cacaca 26

<210> 33
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 33
gtggcaagga aataatcagt agcttt 26

<210> 34
<211> 31
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 34
atccttaaca tgatttatgt tgtaatttgt g 31

<210> 35
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 35
ggaggaaggg tatgcaactt ttac 24

<210> 36
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 36
catttcttca acatccgaac caa 23

<210> 37
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 37

cataagacgc gttaaacgtc agtactt

27

<210> 38
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 38

ccaacgatct tgctaattag cacata

26

<210> 39
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 39

cgaggttggt agccgttgga

20

<210> 40
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 40

accaatcaac ctttctttat cgtttt

26

<210> 41
<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 41

tgtggtaatg cattttcttg gtctt

25

<210> 42
 <211> 26
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> artificial

<400> 42

gaacaggttc caacactaat gtgagt

26

<210> 43
 <211> 23
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> artificial

<400> 43

tggaagcaat gtcaatcaat tca

23

<210> 44
 <211> 22
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> artificial

<400> 44

tccatggcat ccttaagggt aa

22

<210> 45
 <211> 25
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> artificial

<400> 45

caattttatt cttggcacct tcatt

25

<210> 46
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>

<223> artificial

<400> 46

gtgaagtgta ttccagtgggt gtga

24

<210> 47

<211> 24

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 47

cgcatatcaa caggacagac aaaa

24

<210> 48

<211> 28

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 48

cgttgagagt actattaata gccctcaa

28

<210> 49

<211> 26

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 49

caggcgatcc ctaattataa ttatcc

26

<210> 50

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 50

cgcaggaggg acatggtaa

19

<210> 51

<211> 25
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 51

ggagacagtc atgacatgca tattg

25

<210> 52
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 52

tcaactgcat tgttgcttta atttct

26

<210> 53
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 53

accgtgtcct taaagctttc ca

22

<210> 54
<211> 27
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 54

aaggttatat aaatcaaggg gaatgct

27

<210> 55
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 55

cgccctgggag caacaagat

19

<210> 56
 <211> 21
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> artificial

<400> 56

ttcgaagaat gggagcagaa a

21

<210> 57
 <211> 26
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> artificial

<400> 57

tggagatcat ctataccgaa tggatt

26

<210> 58
 <211> 30
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> artificial

<400> 58

tgtcttgata attacacagc tctgatacaa

30

<210> 59
 <211> 20
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> artificial

<400> 59

gaaatgcggg catatatgca

20

<210> 60
 <211> 29
 <212> DNA
 <213> Artificial Sequence

<220>

<223> artificial

<400> 60

gcttctggtt ctagttctaa cttctacca

29

<210> 61

<211> 17

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 61

gggtcgggtc gaaccaa

17

<210> 62

<211> 25

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 62

cgaatattca gtgaaacggg ttaaa

25

<210> 63

<211> 21

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 63

aggcggcttg aagaatttga c

21

<210> 64

<211> 23

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 64

ttagc caaaa tccatagcag gaa

23

<210> 65

<211> 22

<212> DNA
 <213> Artificial Sequence

<220>
 <223> artificial

<400> 65

aacatcaggg tcagcattcc at 22

<210> 66
 <211> 24
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> artificial

<400> 66

cacaatatgg tcagacagct ttcc 24

<210> 67
 <211> 492
 <212> DNA
 <213> Glycine max

<400> 67

aacaaactcc aacaccccaa cgacgtcatt catggaagga cgctgggtcc catcctcatg 60
 caaacagctt agcgcaacct cgccaaatct gttcaaacac tgagttgcta tctggccctt 120
 tagttttgca tccacaatgg caccagaga acccttctga tatagatgct tcgccaatc 180
 aaccagtgac acctgttgct tctccaccat gcggagcaaa ggctgtctcc cagacaatac 240
 ctccaagagc accaccccaa aggagtacac gtcagacttc tcagtcaaac gctgtcgttt 300
 gtaatactcc gggctctaat atccaacgct acctttcacc tgagtgtctc catgggtcat 360
 tgaagaacca atgggcccac ttcgggataa cccgaagtct gaaaccttgg ccaccattt 420
 ctcaccaat aagatgttgg tgctcttcac gtcacggtga atgatcatgt gcttcgcacc 480
 cgtatgcaga ta 492

<210> 68
 <211> 655
 <212> DNA
 <213> Glycine max

<400> 68

attgataata taaagcaaaa ggacgttacg acaaaaagct cgttcccat gaatagttct 60
 tcgtggacaa atcaatgctc tgtagtactg ttggcttaga ttaattttac tacggattta 120

cctacggtca caaaattgac tgctgaaaat ctttacagta cttcatcttt ttcatacatt 180
 aaaaattttac tttatttctt atatatatat tattagtata atcaattatg catatataacc 240
 aaccgatcga gcccttgctt catttccaaa caatatggca tacgaaaatc tatttattat 300
 atagagatta aatgtgaaca cttcttagtt cttaactcgt gtggttgtat tattatgaaa 360
 caccagcctc ttcggctata ctcaattcta ttctattaag atcttgggta aagttttaca 420
 ttcactctta ttttaacagt atcctatata agaaaatagg caaaaagatg ccacagctgc 480
 attaatttgc tagaaaaata taggtgacta gcctgtaaaa ttatgtcaat ctcttgctct 540
 gctaccttat caagccgcac tgttgcactg attgcatgta gaggcacgc aacagcactt 600
 aatttgcgag atactggtag tatatgattt taattggacc ccaacaagcc atttt 655

<210> 69
 <211> 668
 <212> DNA
 <213> Glycine max

<400> 69

acttttgtac aagctcatca atgtatatca tagtttttga aatcggtaat gaaccaatgc 60
 aaaaaatggg ttaaaccggt atttcttttag gagatattag gtgaattgga tgattaagga 120
 aaaatggaag gaaaaaatat cacaagttta attcctgcta ataaaattaa tattttaaca 180
 actaacatth gctcatataa aaaaccccaa tattttttta aatttaactt aaagcatttt 240
 taagttaact aaaaacatat ttaataagaa ttaaaatagt tgtaaattat tttattaatt 300
 attattaatt actcttataa aacatataat ttaatcatta tatcaatttg caatttttta 360
 tatcttgatt tcacataatc ttatattaac ttcttggttt cttttttatt ctagatgtaa 420
 acttggtatg aagtgattht actgggtttt gaccagtttt gattcttcgc caattccttg 480
 aacgatthtg tagtttatth atatcaaaca ctaaataaat tcatcagttt tctgggtcaa 540
 accaacaat ttgatctggc atcttataac acaattgttt atggaaaaca catctaattg 600
 gattaaacaa ggacatcacg caacttggca gttaccactt ctttgcttt gctccaatat 660
 ttttatth 668

<210> 70
 <211> 612
 <212> DNA
 <213> Glycine max

<400> 70

ctagtgggtga caatattcct gaaaggggtg gcaacaatgg tgggaggteg ggtaatccag 60
 gaatgggagg aagaagacca ccagggtgtt ttggattttg aagtggagga ggaaatgatg 120

gatctatttt tggaggaaat atgtgtttca cataatcagg gcccttgatg ttttgaatgc 180
ttgggtttttc gttgcaaaga actggctgtc taagaggctt gaatgagaaa aagccggctg 240
agaatatgtg tagtccttcc tttctggact tgaggcacag tgatgaagaa gttgctgctg 300
aggcaacagc acaataaggc tcactgctat ttattagttt cacagtgcac ccttttattc 360
ttttcacatg tttgctcact gagaaaggca actgcacctt gaactcccca tgctcatctg 420
tcttcacttc tttcctaaac cttggctttg acctcccata cccatctttg cattctacag 480
caactgatgc acctgccatc atgaatatag aaaaacataa gacaacatga cttcataatc 540
aaaattttaga gcatgtgtgt aagagttaag agatatgaaa ctgaaccttt ttaagaaaaa 600
taagtcaagc ag 612

<210> 71
<211> 941
<212> DNA
<213> Glycine max
<400> 71

caagcttggt gcctgcagaa aaagcacatg ggatagttgt taatagtttt gaagagttgg 60
aagcagaata tgttgaagag tgtcaaagat ttacggacca tagggatatg tgtgttgggc 120
ctgtgtcgct gtcaaataag gatgacaagg acaaggctat gagaagtaag agaaactcaa 180
gtgatattga gagtgagtat gtgaagtggc ttgattcatg gcctccgagg tcagtgattt 240
atgtttgcct tggtagccta aaccgtgcaa cgccagagca gttgatagag ctcggttag 300
gattggaagc gacaaaaagg ccattcattt ggggtgcttag aggtgcatat ggaagagagg 360
agatggagaa gtggctggtg gaagatgggt ttgaagagag ggtgaaaggg agagggttt 420
tgatcaaggg ttgggtgccca caagtgttga tcttatcaca tagagcaata ggagcgttca 480
tgacacattg cggatggaat tccacactcg aagggtttg tgctggcgtg ccgttggtta 540
cttttcctct gtttgctgag cagttcatca atgagaaact tgtacaagtg gtgaagattg 600
gcgtgagtgt gggagctgaa tctgttggtc acttgggtga agaagataag tctcgggttc 660
aggtgaccag agaaaatggt ctggattcta ttgaaaggta atgggagaaat ggccaaaaaa 720
aaaaaaaaata taggaaaggg ctttaaagta ttccgccatt ggcagggaaa gcaaaaaaaaa 780
aagtgggttt tttttctcac atggctctac tcattgggcc atataccttt ggagggttaa 840
ccaagtttaa ccagggttct attttttggt ttcaacacca attgcttttc tcaagggtca 900
accttaaacc caatttgtct tccgaaagaa tttttttttt a 941

<210> 72

<211> 652
 <212> DNA
 <213> Glycine max

<400> 72

```
taattatatt atttgat ttttattcat gacatttatt ttatataatt ttttcttagt 60
ttgggtcaaatt attatcatcc ttttcattat ctactaata aggtggattt tttttgtttg 120
acaaaatttc ttttttcaga ttgggtcaaag ctaaagaaga tagaggagtt agatttatcc 180
ggcaacgaat ttaagggacc acttccctcg tcttttggtta acatgacatc tcttcgggag 240
ttggaaattt ctcataatca cttcattgga aatttcgatt ctaacattgc aagccttaca 300
tcacttgaat attttggttt tacagaaaac caatttgaag ttctgtttc tttctcaaca 360
tttgccaatc attcaaagat caagttgatc gacgggtggag gaaacagatt catattggac 420
tcacaacata gtttaccaac ttggattcca aaatttcagt tacaagagct tagtgtgtct 480
tcaacaactg aaactaagtc tcttccactc cccaattttc ttctatacca aaacagttta 540
atcagcctag acttcagtag ttggaagttg gaaggagact ttctttattg gttgttgga 600
aacaacacaa aaatgactga agctctgttt agaaattgct ctttactgg tg 652
```

<210> 73
 <211> 671
 <212> DNA
 <213> Glycine max

<400> 73

```
aaggattgat gtataaagct attgctgttg ccaacaatgc tgctgagaat gacactgcaa 60
agctcacata gaaaacatac atgtccacca aactaccatc gtctacatga gtgtgtgaat 120
catttggtat aatactaggt ggaggattgc aactttttga cagtggaggt ccacaaagga 180
aggggttacc ttcatagctg ctattttcaa aggtcgagaa ttgtcctttc cattcaggtg 240
ttgtgcccga taagttgttg tgtgccacac tgaatacttc aaggagaggt agcttactca 300
gttgaggagg aatttgacca ctcaacttgt taaatgaaag gtctaaactc tctgtttgta 360
ccaaattgga gaatgtagct ggaatttgcc caatcaaatc attatgagac aagttcaatg 420
ctcgaattct tgtcaaattt ccaagatcaa atgggatatt ccattcagt ttattgtggg 480
acaagtcaat tccagacata taagcaagga tgctccttgt gtaagtgtcg gttctcttct 540
ttgaagtga atttactttc tcttccacat ttggtaattg tgatgggaaa attttatttt 600
ggcctgtaga accccaaccg gacaatcttt ccaaaaaccg ttcaggatcc ttattctcaa 660
aagacatttt t 671
```

<210> 74
 <211> 619
 <212> DNA
 <213> Glycine max

<400> 74

```

aaatctctca ggtcattgct aatctgctaa taattaatat ttacttatgt aataatttct 60
ctgactatat ttgtgttaac tttgcgttga aattcaccat atgcaggaca cttcaactag 120
aatacataga tctctttctg atccactggc ccattgctac taagcctgga aaagttgtgt 180
accccatgga agtatcagag atagtggaa ttgacatgaa ggggtgtgtgg ggatccatgg 240
aggagtgcc a gagacttggc ctcaccaaag ccattggagt cagcaacttc tccatcaaga 300
agcttgaaaa attgctctct tttgctacca tccctccgc agtgaatcaa gtaattagtt 360
aataattaag ttgatcacac attagtttaa ttacctgact gatccactga tcaaaatcac 420
tgatcgaact aaaccttaat caatccttaa caggtagaag ttaaccttgg gtggcaacaa 480
cagaagctta gagatttctg caaggaaaag ggtattactg taactgcctt ctcaccctg 540
aggaaggggtg ctagtagggg tgctaatttt gtgctggaca atgatgtaat caaagaattg 600
gcagatgctc atggcaaga 619

```

<210> 75
 <211> 978
 <212> DNA
 <213> Glycine max

<400> 75

```

ttgcatgctt ttagtagatg aattacacat aagtaaaaca agtaaaattt atacaagtga 60
ccagggtataa aatgatggcc acttgcaagt tagatatgca tatacataaa gtaaaagtaa 120
actgtttcca gtaaaaactt gtgctcaaaa gaaccaacag tggttcacta aaatcttatt 180
agtccagcat agggcataaa gatgtgtatt aaaccatcac aatatgcaca acattcgccg 240
agaggtttca gatattttga taagctaatt agatgataat aataataatc agatgacact 300
gctcttctgc aatgaaagac ttcactctgag gaatgtgagt tcttgatatt ttaaataata 360
ggagcatata ggatatagca taggcagaga cagcaaacg gctacagttg ttgatcaatc 420
agattactgg atgtggttct ttaccttcta tcccaggaa tgaatcatta tagccttatt 480
ataggatatct ctccaatct gacatatatc tcctttccta gggatataaa cagcaaatca 540
aaacctttaa caaaaggaaa gtattcactt acaatgatag caccaacagg gtccatccaa 600
tcatcaacat aatttgccaa aagtgcagca ataaggccaa tgatattagt gatcacatca 660
aaaaagtgat cctgggcata ggctttaatt atctcattgg taaaagaacg acagtaaatc 720

```

atcagcagga atttcaccaa agtcactgaa agcataatgc ccacaacca gcgctcttgt 780
 tccttgggtca agttgaatgc attttcctgg aaatgtcatt acgagacaca ttagaaatac 840
 ctagaaaaaa aagagatcct aaatgcaact aaatgaaaat ttaatgtttt agcagtttgg 900
 gtgtcagatt tgcctaaaaa aatagagcaa gaatcaactt acagaagata ttaatgtgcg 960
 ggtagactcc aagattat 978

<210> 76
 <211> 845
 <212> DNA
 <213> Glycine max
 <400> 76

ttgcctgcag gcatagtcca tctgaggata ttactagtat tttgtatgat gtgtttgact 60
 attttgagga tgtcagggag caaggtggga ggggtgttgt gcactgttgt caaggggtgt 120
 ctaggtctac ctcttgggtc attgcttacc ttatgtggag ggaagggcag agttttgatg 180
 atgcttttca gtttgtgaag gcggcgagag ggattgctga tccaaatatg gggtttgctt 240
 gccagctgtt gcagtgccag aagaggggtc atgctgtccc tcttagccct agctctttgc 300
 tgaggatgta tagaattgct ccccatcgc cgtatgatcc tttgcatctt gttccaaaaa 360
 tgttgggtga tccttcatta gctgcattgg actccagagg tgcgtttatc gtgcacattc 420
 cttcagcaat atatgttttg gtgggtaaga actgtgaggg caccatggag agggacgcca 480
 gaggggctgt tggccagatt gtccgggtatg agaaggtaca agggcctatt gtgatgatta 540
 aggaaggtga ggagcctgtt tacttttggg atgctttttc agattttctg cccttaatgg 600
 acaaactctgg caaatctggc agtaggatag aagatggaaa actatttggt ttgcctggta 660
 agaggagagt agatgcatac aatgttgatt acgaagtttt cacaaaagca atcacgggag 720
 ggcttcgtgc cttcttttgg ttcattctgg tgaacatgaa acccatttgc ctgcaaggga 780
 aaatatattg agtgtaaggc gtaaagtttc ccattggtac atggaaagag ttgttacggt 840
 ccctc 845

<210> 77
 <211> 533
 <212> DNA
 <213> Glycine max
 <400> 77

tcaaaccatg ttaaactgtc tatttttctt ctccctctgt tcgcacagct tctaagcatg 60
 gcattcctca aattggtgcc aaattgcaag aatttctggc tgatttttgg aatataattt 120
 tacttcggaa gagggaggag caacttcaat aaaaaatact ttctaatttg aaattttaac 180

tccactatTTT tataagtaat aatcccaaac atTTTTcttc gatttttcaa tagcaatccc 240
 tcctctttcc tctactttga aaattaattc aactcatccc agtttagaat ttgtgattta 300
 aaattttgat catagaaatt aggaatatac agtaaaactt tatttggggc taagaaaatg 360
 ataaccagaa aaggaataaa acataaaaga caacaattaa ttgaaacata catgtcaata 420
 attagtttaa taaggacgct cacagccaca tccgttgatg gttttccact gttattactt 480
 agtctagttg atgctgtagt agcattggta ttcggggaat aagcctctga gca 533

<210> 78
 <211> 645
 <212> DNA
 <213> Glycine max

<400> 78
 aggtaatcct aatgcatgct gcttttaact atgaaacatt aaattcctat aagttatatg 60
 cattgcgaag agttctgtgc aaatatgcaa atgaaattgt atacttcata aacaagtata 120
 cagaatacta cacttgaaga ttgtataagt cttagcctat ggtgttttat tttgtgtcat 180
 caaatactat accaaattgg atcacattat cagcagaaaa tcataatata taagttttaa 240
 tccccaaaat gcaaggtttc aaagaatgac tgaacaaaat caatgagaaa caacactcca 300
 gcacaatttg tagacagttg agcataaact aataaaaata tatttcaatt agtaaagaag 360
 ttgtcaaaac ctaacctttt gcttgatcca gctatgtcgt ttgccactgc gaggacaaag 420
 aagcacttgc actgaagagt gcttaaaaaa tctacgagtc tcctcatcat gagttgccat 480
 gacaccatcc tgaacgtaaa gtccaagagt atctcaaaac taaagctaaa agggaaaatc 540
 ataaatatta tatttggatt aatgaaaaaa aataacttaa taatctaagt ctttcttgaa 600
 ataaagtaaa aatcagtagt catacatgag aaagtgttaa tgaag 645

<210> 79
 <211> 1178
 <212> DNA
 <213> Glycine max

<400> 79
 tctcgggtag ttgagtctta gtgagttagt taatgacaac taattacagc tgtcatagct 60
 aacagctttg tctatataaa cagatctgta ctctggagtt agttgggtta atgaaactaa 120
 agtttttttc ctctttcatt ttctgttatt aaaaatcttc tctcaagatt ctattaacgg 180
 acatacaagc actcgtaata ataaactaag gaataatatg gtagaatact tacatgtaaa 240
 gattaatatg ttctagcata tttatttcct atgacatggc aatgcctctc attatttgtc 300

```

aaatttgtgc ttctggggtt tgattatttt acactcaact agcttttcag tcagtttctt 360
tcttgaacaa ttgtattata agcttttgtc atactcatga aagaaaaata agctaatacca 420
aagatgtact aatgtgtaac atgatttctt tatttgtctt cttactacag atacaaacca 480
agtgaggaga cattaattca gatagatctc ttttgctcaa ccatgattgc tgaatgtgat 540
attaacccaa ctcagccgtg gtcactagct ttgaatcgac aatctgggtg atcaaataca 600
tctcctttac ctgtatctac ctttgccctc gagtcccttg taaagtcatt aagttatgtg 660
cgttccctag ttgctcaaca cattcctaaa cggctttttc aaccagcttc atttgctgga 720
ccaccttcat cgggacagtc actaccgaca ttgtcatctt tgctgagtaa atccttcaat 780
tcccaactaa ctctgcaag tattccagaa acaccaagtt ctgcaagtgt tccaaaaaca 840
ctagagaagg attcaagtgc tctatctgtt tcaaggttat caaaaatcga aaaagctaata 900
gaaacagatg aactaggggt cattgctcat gatgttctaa aatggcgctg gcttgaggaa 960
ccacagtcac catctatagg gactgaaaag taataatcta tgtttcatgt tggtttaggt 1020
gtcttggtag tggccactgt agtttatatc tctactttac ctgtagttt tttcccactg 1080
tagtttcaga aattgacttt gttatttaac atttttaatg cagtgatcga gctgtgaatt 1140
ctcaagacat gacagcacat agtttcttag aaataggt 1178

```

```

<210>      80
<211>      807
<212>      DNA
<213>      Glycine max

<400>      80

```

```

tctacttgta ggagatatag aatctaagat gaaggggcaa ccatggaaat tttttggaac 60
cgatgatatg ccttacctgg atcagctatt gcagtcttct cctgtaacac caataactaa 120
ttctgactct gctcgccccc atctgagagc aataacagca tctaaacgca caaaaccggg 180
ctctcgtcag atttggcatg ttcttatatc aacttgcatt tttgatattt ttctttttct 240
aattctttta tatttaaggt catgccacaa taaatcttgc ataatttatt tttagccaaa 300
tgttatataa aaaagttgaa tgatcacctg cattatagtt aataaggtta ttgtgaaatt 360
aaagtactta caatctaaaa ccaatgggtt atagaatatt agattagcct gcaaaagaca 420
aatgtattac attgtcatgt cctgtaggct tctatgaaaa taaaaataaa ttttagtgta 480
agaattataa ttatccattg cttttaatga acagaaaatt tcttattgat taattgactg 540
ggggggaggt gctgatcttg ttcttcccac ttcataatat ctttttattc ttcaaagaaa 600
tagagcaagt aatctttatg cagtagtttg gggttctgtt tggttcatta accactcatt 660
cccataaaga ctgtagtggt ccatgaatta tcgtttgttt atatgggtcaa aattatgtga 720

```

taagtatttta ttgctactc tacagtttga tactctctct ttggggataa atgcttgagt 780
actcttttttc gcccaagatg cataaaa 807

<210> 81
<211> 1395
<212> DNA
<213> Glycine max

<400> 81

acttgccctga gagtgttggt gcttctgaac aggctgcatg ttcatcacat ttgaaagaaa 60
ctggttggaac acctactctt gatgcatctc aaccagccc aactgctact cccagagata 120
ttgaggcttt tggccgatct ctaagaccaa acattgtttt gaatcataat ttctccttgt 180
tggatcaagt tcaatctgca agaaacatgg agactgatcc tagtaatcgg gatgtcaaga 240
gattgaaagt ttctgataat atggtggtgg acaaacagct ggtagattcc aaccatgggc 300
aacagttgtc atatgggtat gataatgtgg tcaaagatgg gtggtcaggt aataattcca 360
tgccatcatc agatcctaata atgctaagct ttccaacaaa gccacttgat ggacagtaca 420
caaatgcatc ttctcaagag gaggttggtt atggtaaaaa aattgctctt aatgttgctg 480
acagtaacaa agcagcctct gttaaaagtg attattctct ggtaaactct caaatggcac 540
catcatgggt tgagcgatat ggaactttta aaaatggtaa gatgttgcca atgtacaatg 600
cacagaaaat gactgctgct aagataatgg accagccttt cattgtagca aaccaattca 660
gatagtttgc gctttcataa ttcagtagag caaattcaga gtgtcagtga tgctcagcta 720
agtaatgcta gtgaaagtcc aatgcctgct ttagctgcaa ataagcatgc agactctcag 780
ttatcgacac ctgctgttga acctgactta cttattatga gaccgaagaa gcgaaaaagt 840
gccacatctg aactcatacc atggcataaa gaactgttac agggttctga aaggcttcga 900
gatatcaggt gggtgccaaa actaagtgat ttaatgtgct tatttttcgg tgttgctatt 960
gttgggtgtag taaaagatcc catgtctcca gttgatattg tgttgtttca attgttttga 1020
aagaaaacgg tgtgtttcca tagtgtcagt atgactatct taatattggt ttatgtttat 1080
caatatatca agtatttggt ttctataac ttaaaatttc ttactatgtg gcagtgtggc 1140
agaattagac tgggctcaaa gtgcaagcag attgattgaa aaggtttggt tataataaaa 1200
tcagtctacg catgaatcta taattctata atttatgagt tcactttact ctgtataatt 1260
ataattatag gttgaagaca gtgtggaggt agttgaagat ttgccagcag tgggtgaagtc 1320
aaaaagaaga cttgtcttgt actactcagc ttatgcagca acaacttagt cctcctccag 1380
ctgcaggcag gcgag 1395

<210> 82
 <211> 618
 <212> DNA
 <213> Glycine max

<400> 82

```

atttcttata ctcaaatttt tggtagctct ctttccttca ataaaatttc ttcttttata   60
catgtgtgtg tgtgtgtttg gatgttggtg ataaatttct gccagaggat ttgaagatga  120
agagtccata agtttggtga ttacttgata caatctaata gagtatttta accggcccat  180
tttttttctt gggctaaagt gatgtaacat ctaacaagtg ttgaggagat aaaacatttt  240
caaggagttt gattggttga tatctagagc aattgtaggg ttttattgta ttcattgatgc  300
ttcttaataca ttcaaattgt ttgtgccttt tcatgttata gctttgtgaa gaggagttac  360
tcaaggaaga agcgctttta gtaaaaaaac aacttatattc ctttagtttt attaatgact  420
tgtatgcaga ttggacaaca ctttagggat ggctacttgc ataaagaaga atttaagata  480
gtttatgttg ctccaatgaa ggtatgttga tgcttttgtt tttctttaca tttctctatt  540
cagatttgct ttttgttccc tgcatttggt tgccattact catttctaag tatagattct  600
tgctctttcc aggctttg                                     618

```

<210> 83
 <211> 1085
 <212> DNA
 <213> Glycine max

<400> 83

```

tctttcgcca gcttgctgcc tgcagtaaga tccaaccttt atggtgggat tctatgtcct   60
ggataaatct caaagggtgct atgcctttga gtacaaaaca gctgttcatg cagtactcct  120
ttttgcaaga agatggtaga aggaatagga ggtggcaata ctggtgggtg gctatcactt  180
ggtctacttg gcagcttcga aaccgaattc tgttctctgg agcgactttt gatggaaaca  240
aattgggtga agacgccact ttcttaattgt ggacttggct tcataatttg gagaaggatt  300
ttactattca ttccaatcac tgggtccagta acttcaaaca tcattttttg cagtagtagg  360
ggtgtttttt gggttcatat cacatgtatt ttcttcccat attttggttc ctaatcagac  420
ttatgttgcc tgattgagga accatattta tgggtgtctaa ctctgtattt agtacctctg  480
gtacttttct aatatatata tagttttatc ttgctgac aaataaaaaa aagtactcat  540
ttgtctattc ctgtaaagtg gcaaggaaat aatcagtagc tttaaaatca tctgatgtgt  600
ggatgtgaca caaattacaa cataaatcat gttaaggata tgaaaagtat gtactcatta  660
gtctcttcca tcaactaagc aaagcaacat ggaaatcatc tgtagctgag agtgactatt  720

```

```

aaattgtaag atttggaggt catggacctc atgtttatag ggacagtaaa attatccaat 780
taccctaaacc tttagacaat ctgaaatgca cacataatat ggtacaacag ttctaaatgg 840
ggcaggtaag tagcattcat tgctcaatat gtctataatt caagaatgaa gctttacatt 900
tagtgcatat ttggacctca gattggctta tttttgcttt aagaaaagct tatgatcaaa 960
atgttcaata aaaaacttaa agcttctttt tttttttcag tgtaaaatag tcagaaattc 1020
agaaccagat tgcacttagc cagttgtata taaaactatc caatggccat aaagaagaca 1080
gatca 1085

```

```

<210>      84
<211>      1052
<212>      DNA
<213>      Glycine max
<400>      84

```

```

aagttcactc ttaactaatg ttttttcact gtattcccta gctatatttc agactgggtgt 60
gtgacagtct ttttttggtc atagatattg cggaagcttg aagaacgtgg ggctgacctc 120
gaccgcttgt atgagatgga ggaaaaagac attggggcat taattcggtta tgcgcctgga 180
ggaaggggat gcaactttta ctagaatgat tttcgaagat ttccatcaga ggttggttcg 240
gatggtgaag aaatgctgat taatgttttc ttatcccttc cccttttttag ttgggtcaagc 300
aacacctagg gtattttcca tcacttcagt tatcagcaac tgtgagtcca attaccagaa 360
ctgtgttgaa ggtatttcat gatgaagatt tttttttcca gactgctcag ttgacatttt 420
ttcattgatt tcatcacatc aaaaagcctt gataccta atctgcatcac cactcattat 480
tttcagggtg atctgggtcat tacgcctggt ttcatgttga aagatcggtt tcatgggtact 540
gctcaacggt ggtggatttt ggtagagggt aataaatttt catgtgatga ttgggtcacat 600
tgtaaatcc ttgggtttttg ttaaaaactc tgatctcttg ttataaaagg agaaatttat 660
caagatgaag agaaagactt tcaaagagaa aggaggatga ggaatcctcc taaacaaagg 720
aacaaaacag aaaacaacta ggaagaaaga gataatcaga gaaacaaatc ttcccagttg 780
ctcgatataa ctttcagtga aaatgctaaa gaaacccctt ttaaagcaaa tagatactga 840
gcacctgatc ttataccaaa tcatgtgacg tgctaaagaa acctccttta aaaatactag 900
aacagcttgt agcatatgta gcagatttat acaaaaaatt agcttcttta cttctgtcaa 960
aaccttgaaa accaatcatc gataattggt tttgagactt aggacacacc caacattaac 1020
tgaaaatgct gaataagtaa tgccaggagg gg 1052

```

```

<210>      85

```

<211> 855
 <212> DNA
 <213> Glycine max

<400> 85

```

tagaattaca ggtctggaga agtatctgaa gactgtagat tcggtgcggg attggattct   60
gtttcatata tacttttttta acaacataag ttaatttttc atatagtttt ttattttaatt  120
ttataaatat tttgaataaa accaaaaata tatgtaagtc gttcgtacat aagacgcgtt  180
aaacgtcagt acttaataat aataatatag tgtaagaaac tcaactgggg aagtgcataa  240
aaaaataaaa gtataaatac aagaaaaatg aactaagaaa gtgtgtactt atgtgctaata  300
tagcaagatc gttggaacaa aaagccaaat tgactggtag tttctcgtaa atttcttcaa  360
ttttcattgt ttcgttaaata actagtggca tgtccgtcaa aagtcaaaag ccacatattg  420
atgaaattgt gttgtagaaa taattaatta attacttgca gagcaaattc cctccacaat  480
ttttcttttt ttctctaccc aagagacttc ctttcaactc agatactctt tgattctctt  540
caggaaaaca tcaactaatt aaaatctaata tttgtctttg atactctttg tccgcggaat  600
tcaccacccc caccttctca atttgtttgc tttctgcttt cttacctctt ttttctcaga  660
tttcatttgg ttgatccttt cttcaattct tcttctgggt ttgtagttgt ttttttatct  720
gacttgtgtt tctaaaatcc atgaaccgta tgtgatttcc agtgtctttt tctttttcca  780
gattcccaga gagaaaaaag aaaaaatcct tttgtttgtg tgagactgta aggatcaatt  840
ggttgagtto tccta                                     855

```

<210> 86
 <211> 1066
 <212> DNA
 <213> Glycine max

<400> 86

```

gtatggggcg attcaggagg tggaatctgc aatacaagag cttgaaggga acaatgaggg   60
gaatgtaatg ttgacagaaa ctgttggacc tgaacacata gccgaggttg ttagccgttg  120
gactgggtata cctgtgacaa ggcttggcca aaacgataaa gaaagggttg ttggtcttgc  180
tgacagattg caccagagag ttgtggggca agaccaagca gttaatgctg ttgctgaagc  240
tgtgctgaga tcaagagctg ggcttgggaag acctcagcaa ccaactgggt ccttcttgtt  300
cttgggtcca actggtgttg gcaagactga gctttcaaag gcacttgctg agcaactctt  360
cgatgacgaa aatcaattgg tgagaattga catgtctgaa tacatggaac aacactctgt  420
ttcgcggttg attggtgcac caccagggtg tgtggattga cattttcaca tttcagttta  480
ttgttagttt tctgtatgaa ctacagataa ctgactcatt gtttcgactt tcaggatatgt  540

```

```

tggacatgaa gaaggaggtc aactaactga agctataagg cggaggcctt atagtgtggt 600
actctttgat gaagtggaaa aggcacacac atctgtgttt aacactctcc ttcaagtctt 660
ggatgatggg aggttaactg atggccaagg ccgtactgtg gacttccgaa acactgtcat 720
tatcatgacc tccaaccttg gtgcagagca tctcctcact ggactttcag gaaaatcttc 780
aatgcaagta gcccgtgata gagtgatgca agaggatatgt ctcttgacac catttgttta 840
atatgtatga caaagggtctt tgtgctgtgt ttgacttgt gaccttgtct gttgaatttg 900
ttgtaacagg tgaggaggca ttttaggcca gagttgttga accggctcga tgaaattggt 960
gtatttgatc ctctttcaca cgagcaacta aggaagggtca caagggtaca aatgaaggac 1020
gttgctagtc gtcttgctga gagaggaata gccattggca gtgacc 1066

```

```

<210>      87
<211>      890
<212>      DNA
<213>      Glycine max

<220>
<221>      unsure
<222>      (719)..(719)
<223>      n = a, t, g, or g

<400>      87

```

```

attatgaagt atgtgacaga attgtgtttt caatatattt ctgacaatta gggattgcac 60
gggaaaatga acagcgacca gagaagttat ccttaaagaa gcaattgaag cggaaatgtc 120
tagaacaaaa tccaatctt gtccaaaacc cagttatgtg tggatatgga gtgggtgctg 180
ccagtaacca gcccatggaa atcttgaact gtagccaacc aagtgagaac ttgcttatat 240
attaactttc tgaggaatac aataaaaaaa aattattttt ccttgaagtg atatgttttt 300
tcctgtcata cttggtatat tggatttagg aagtccttca aatgtatatc acagcttata 360
ttacatgctc tcttgtggta atgcattttc ttgggtcttaa agattttggc cattttagta 420
gataatgtca aggtagtgag atttgagaat tagtgctctt agctgtactc acattagtgt 480
tggaacctgt tcttcctact tgtttatgtt tattgagaca ggtaccatgg cttgtggcaa 540
ggtgatattt tctaattgga tataaatata acctataaaa atgtagaccc tttatgagcc 600
tggaggatca aagaatggaa aatggaattt ggtttattac attcataggg gcgaaatgaa 660
atatgctgca tcatgattac cggcagacta aatcccaata aatcatcctt ttttctgana 720
ggaatggtec cgcccagtta ggaaaaaact acaggatatct tttagaccgtt tgtggaagct 780
ctatgagtcg gttaaaccgc taactctatt cttttatatg caagggtgtct tctttttcga 840
gtaaacaaat caaatctctt aaaaaaaagc tccggataac ttatgtttca 890

```

<210> 88
 <211> 640
 <212> DNA
 <213> Glycine max

<400> 88

```

aataaatgtc atggactatg atatacctga gcttggtgtt tttattcaag aggaacatca   60
gcaatttgtc aaggatactt gcattggtaa gggagtgtca ccagaaggca agtgtatgtc  120
agaagattgt gcagtaaadc atgatccaat gtcattgtcac tttgagaatg acttgaaccc  180
ccgaagagat tcaaatactaa gaactatgga agcaatgtca atcaattcaa atgggccaga  240
gtttgaatct aaacctctta ccttaagga tgccatggaa ttttatgatt caagagggtt  300
agtgatggat ggtgaagagg attcaggata caatatttca attgaccacc tcacaaagaa  360
gacaatacca gagaccatta gagagggtgag acactttaat ctcatccttg tgcattttac  420
gtctttggac gaggagttaa ttgtttttta gaatattgcc actaagacat taatacttat  480
attctaagca atataaaata tactgtggac tcgtcttctc ttttagcatg gttggtgaaa  540
ccccctacat caatgtacat cttctctttt gtttcatatg cttgattgta tgattgataa  600
aagattgaaa caagacttaa taatcatata gggagttacc                               640

```

<210> 89
 <211> 993
 <212> DNA
 <213> Glycine max

<400> 89

```

atcattttca aagagtgtat attttttttt tttaaategc tgagttccta aatataatcc   60
aaacactgaa ttgaggagtc aagtgtgtgt tgtgtaagac attgcaaaat aagttaccac  120
aaattcaccg aagtttcata gatattgtct tattgttatt tgatcctgaa acatgctagc  180
aggattaata aaaagaataa aaatgttacc agctgcacta gtatagtttt gatcctgtca  240
tcctttctag caatggttcc attccttgaa tacacttcat ctgaatgacc aattttattc  300
ttggcacctt cattcttttc aatggaatca atgttggtgg agctcacacc actggaatac  360
acttcacccg aatgaccaat tttattcttg gaaccttcat tcttttcaat ggaatcaatg  420
ttggtggagc tcacaacact tgaatacact tcacccaaat gaccaatttt attcttggca  480
ccttcatttt tttcaatgca atcaatgttg atagagctca caacacttga agtcagctcc  540
atgatctgct cagactttgt tcctttgtca tcaattgcat cctcagtagt tgtctctggc  600
atatcttcat aagtagagag ttgacagaa tcgctgaaag aaactctttt aatttttggc  660

```

gttattgggc tttctaactt agaaacatct gattcaacca ttgacataga aaatctttgt 720
 atcggaccag gttggataaa aaaatttcta ccttttgacc aaattttgtt agagtagtct 780
 ttggttgtec tccatctctt cagtttcgtg ctgccactgc tactttggct actggaagag 840
 cctttaaagg tattaagttt caattcatcc gtttcgctcg atgtggaatt tggagagacc 900
 ctctcaagct ccagaacaga atttgagacc tgccttttcc cccaagatc cttgggtgga 960
 tgttgcccc aaagctatct cttactgaag gaa 993

<210> 90
 <211> 804
 <212> DNA
 <213> Glycine max
 <400> 90

caagcttgca tgcctgcagt aaatatgcca tcaatatgag caagtgagca acagcctaaa 60
 aattggtgga atgtttatgg ttagagattt aattggtttg catgattttg gtatttctat 120
 ttattcataa tttggagaca cggatcatat aacttttagga ttagatcaa gtagagtggc 180
 tttaaaataa atcaggaatt gaatttaatt agttgtagat caaatttagt tggggttctt 240
 gcactagtaa agttatgatt cttatgggtt tcagtaggag ctaatcctgt attttgaatt 300
 tacttatata gaatttgatc atttcagaat gatctgttag gtttcattta ccaccctta 360
 cataccattt tttatcttca catctaaatt cttcttcctc tttggcgcat atcaacagga 420
 cagacaaaat aaaatttaac ctgccttttt tagaagataa gttttgaggg ctattaatag 480
 tactctcaac gggtatgctt ttgacttagt tgaacatttg ttcattgctt ttgattggct 540
 cccccagtct tctcaatagc agtatcatca tcaacacaat cattctcctc tgccactacc 600
 aactagtgcg gagactactc ctaactctcc ttgcgcaact ccatttcctc cgactttgtt 660
 tccacttgat gatctcccca ttgaacttca taaaaatata tgttccactc aatacatctc 720
 attatgttac cttaacctta acctatcatt gtttgtctct ttctattat gctagtcttt 780
 tgtgtttgat cctgtcatcc tgag 804

<210> 91
 <211> 749
 <212> DNA
 <213> Glycine max
 <400> 91

tggcgacca gaatggggac tgccgtccac agttccaatg atgacacttt acagtccata 60
 tcattcaaat tttgttgga cttcatatca tcatccagga gctgaatcta ttaatcagat 120
 gtggtcgacg aatgctcatc acttgcaggc gatccctaata tataattatc ctcatcctta 180

```

tagttatacc tatgattata attatacagc tcccgttggt caccattacc atgtccctcc 240
tgcgccattg tttcataact ctttggttca aaattctgtg ggagatgaaa ataatggagc 300
aactaccaat agtactgttg ctacaatttc agatgaagct agagaggaaa ttctggtgaa 360
atttcgaaac tttaaacaat ttgacgttgt tgaagacgtt tcagaccatc actttgttaa 420
tgccgactct tccatgcaac aggtagcctt ttaatgcaat ttttttcttc cagttttaat 480
cttaattatt taggtttaca tacagtcaat ttatttcatg aacagcattc aaagaattgg 540
gctaaaagaa tccagggaga gtggaagtca ttggagaagg atttaccagg tgagagttag 600
ttcgattagt attcatgtca taaattggct aaaatatttt ccttatctat ccaacttttt 660
gtatttaatt aaattgattt cttttacaag ccaatggatt tggactggat gaattggatt 720
cccaatttgg cattaaatat gctaaaggg 749

```

```

<210>      92
<211>      685
<212>      DNA
<213>      Glycine max

```

```

<400>      92

```

```

acccttcaga actgaatatt gctaacacac aaggcaacaa ccaaacttac acgacaaaca 60
tccacactaa aactgaaaaa cacaccttct tgcttggttca tcccgaagtt tggcatcaaa 120
ttctttgcta ggaggatact ttggcaaact tgaaggatca caaggtagag gctttgttgt 180
aaagaactaa atagcaagca aaccaaaagg agacagtcac gacatgcata ttgaagtatc 240
agcaacaata atttacctcc aaaataaaaat agaaattaaa gcaacaatgc agttgagtaa 300
gagataatga ataaatacac acgaatatgc gataacatat caggatatat aagtgaggac 360
ttgcaataat agtaacaata agaattttct actatattac attcttggag gcaatacagg 420
ttaggacttg caacaatagc aacaataaag agacaacaaa ggagcatcaa tttgattaaa 480
gcaaaaggaa aacatgcaca aaaatgacaa aaaattccaa ggttgtcata ttttgagaaa 540
tacacaacct aagtttgaaa tataatttaa tccaactctt ttaccaaca tgaacatcca 600
ctcttcgtta tgtgtactga ctgatattat aaaatgtact caagtagtct gaaaaattca 660
tcatgaattt catacctcac tcttc 685

```

```

<210>      93
<211>      652
<212>      DNA
<213>      Glycine max

```

```

<400>      93

```

```

attgtgattt tcactggttt gtgagagtgc aaaagaattg ttcagttgaa tgtgcaaaat   60
tgcttggatc agttgaaatg cacctatgaa tttgtatttt tcttttttat gacaaagggc  120
atgtagaata tgattatatt ttgtttgaat agtgtggggg agcattactg tttttttttt  180
tttggaaaaa aaaatctgat gtggtagtgg tgtctgattc acatgtggaa aattcttatg  240
gattgggaaa gaatattgat tgtttccttt tctcacagtg ctggtgggtga aagcagtgga  300
ttccttgcac tcagagttca gggctgtgga taatttggtt gtgtgcaata ccaaccgtgt  360
ccttaaagct ttccagaatg ctcgagttgg atcccatgta agcattcccc ttgatttata  420
taacctttat gcaaatgtac atttaatatg atgctcaatg ctcaagggtt caaggctaata  480
aaacttgtaa actgttttga ttgtaattgg tagagatgtc ctttaagcca ttgggctgat  540
cttgatgcct ttatgtattt tgacattttt accaaaaaca taactaatat aggaacccaa  600
aaacttagga ttcgattagg gagaacctaa ggctgccccat taaaacttga gc          652

```

```

<210>      94
<211>      821
<212>      DNA
<213>      Glycine max

<220>
<221>      unsure
<222>      (798)..(798)
<223>      n = a, t, c, or g

<400>      94

```

```

attccataac ggtttgcaac tcttgaagat cgtgactctg gtcgtgtcac tcctgcgtat   60
cgcgccctggg agcaacaaga ttagttgttc ctctcatggc ttcaatccac cgtttctgct  120
cccatcttc gaaatttcat cggctgcact agtttgtggc ttctctagga caaaatccac  180
aactattttc atgctcatac aaatgcaaag gcacggccac ttcgtacaga gctgcatcaa  240
ctcactcttg aaggtcgtac tatttctgat tatttgactg agattcagaa tcttgttgat  300
tcttttactg ctattgggtga tccaatttct atttgcaaac atgttgacat tattattgaa  360
gaatgtgtac cagaaaacta tgagtcctct gtttcgcaca tcaataatag atctgaacct  420
ctcactattg atgaaatcaa aactgttctt ctcggtcatg aggctcagat tgacaaattc  480
aggaagaagg cagtggtttc ggttaatggt gcttccacat cactgtgtc ttctgtgact  540
aatccatctc atgctaattt tggaggtttc agaatcagaa tcagagtcag tataaaaaaca  600
gaggacgtag cagtattcag tgttacatct gtcagaagtt tggtcatgat gttgccaaact  660
gctggcacag gccctcaact tcctatgctc tgctccttat cctatgttgg cacaatttcc  720
caccatgcct cagctttatt ccaatttctt tggagctgct ctgcatttcc ctcttatctg  780

```

tttatgcagg ctctgtntc tcaacaatgc cagcagccac t 821

<210> 95
 <211> 738
 <212> DNA
 <213> Glycine max

<400> 95

ctctagagtc gaccctgcag ttattatagt gacagaagag gaaaagtcac cagactcat 60
 atgtaaaaga aaagatagaa aatgatgaaa ggacaaggga aaaacactac actaattaag 120
 tcaatttcat tacatcatta gaaaaggaca tgagggggga aaagaaaatg ttgtgaggca 180
 atttacatgg gtatttgagg tgggaacata atcttttagta cataccaaat ttctgtgtat 240
 acattatagc atgtcccttt tttttttttt ctattctcaa gttgaattgg acagtaatag 300
 attaatgttg taggatgcca agcacacaaa cattgagaat atattgatct tggctggaga 360
 tcatctatac cgaatggatt acatggacct tgtgcagtta aacatgttaa ttttttgtat 420
 cagagctgtg taattatcaa gacaattaag gcaattatat taggctttaa gttcttctca 480
 ctacttgtta ttgaaattta ttactaattg tattctatga atatttttca tcattcacia 540
 aatcaaggca attaattaac acaaaaaaaaa ctagttctat gtttcttttc tcttaccctt 600
 cttatgttca tacaccaagt cttttgaata atttttgcca aaggttccaa agtttttaga 660
 cacaccgagt tcatacatgg agtgtttctt aattttattt tcacaatttt tgctgattat 720
 ctatctttta taattaat 738

<210> 96
 <211> 710
 <212> DNA
 <213> Glycine max

<400> 96

tgtgatgctg tcaagagtgt tgtctacaaa ttttgcaaac cgagatacta gatgcctcaa 60
 aattaagata ttaatttcag caataggtaa tgaataattt cttatggatg acagtctaga 120
 ttctctctc aaaaggagaa tcttctaaac aaactcattt taactcttga aattgtaagt 180
 attaattacg ttaatctttt tattaattac tttaatattg tccctttttt gtgaatactt 240
 atttataaaa cagacaacaa aaataagaat tttgtgaagt caaagattaa aatgattggc 300
 gcatagactc taaagcaaat gggagtttac tcaaaatgtt tttctttttc aattgggtcta 360
 tatagtgaca tactatttta gtggccacta gattgtcatg agagtggaca taaaatggac 420
 caaatcagcc atatcaggta tgtgggaagc gcactcatat agctaagtgc attgtttcca 480
 catgaaattt ctactcttt tgagactcaa aagtaacctat ctttagctaa tgctgcatg 540

aaatgcgggc atatatgcac atgggtcctt tcagattata atgtggtaga agttagaact 600
 agaaccagaa gctataactaa ccttggtaat tcgacaaaaa actaccaggt cgcgagagag 660
 gttctggctg gggctttctt ctccttctat tgtgtccatc taaacgtttt 710

<210> 97
 <211> 954
 <212> DNA
 <213> Glycine max

<400> 97

agtcgacctg caggaaaagt tgtaaataag gtaattaaat gctacaatat aatgattgac 60
 aaaaacatta tctgtttagg aacaaatgga ttatatTTTA tgagacttaa acattgatga 120
 aaaaaatggt caagcatcaa aagttgttat cagtcaatat ctctgtcaat aaatatccag 180
 gacaagagtt gtgagccaac aatgaccatt cgTTTTgtaa tctgttatta tagcatatta 240
 ttttgccctc tctataagag ggattaactc tttgactgaa aatacagaga aatattttac 300
 aagccataaa tgcatttatt tggcctaaaa aacttaacac tttctttttg gccatacttg 360
 tccgtgaaaa acgaaatttg ttggaatttt tcagggccaa aactgaaaat taaaaattaa 420
 attgaaaaaa tgaaaatagc agataaaacg atttgaatga aagtaaaaat ttgtgagggg 480
 attagagaat gttgagtaga gtgaaaatgg atcacgtacc gaataggaat gaggaacaag 540
 agagagctcc gagaataacg aggatccctt cgccaagaaa cccttcaggc ttcagccttc 600
 accgagaagc cgatcgagga acaggaaccc tagctgtcgt gaacgagaga gagtcgcgag 660
 tgcagttcac tgaatattca gctcacaact tttttttccc gaaacgggtc gggtcgaacc 720
 aaaccggtta caaaacggag cggtttttaa cccgtttcac tgaatattcg gttcactgaa 780
 tttgattagt ttgatcccta aaattaaaga ttaaattaat cactagtaat aattgtacca 840
 aacttgacag acacacaccc tatcagtaag agctagtcta cctcggccta gaatacagac 900
 taattaccca aaggttccag agaacaaatg aagtactgca ggcattgcaag ctgg 954

<210> 98
 <211> 730
 <212> DNA
 <213> Glycine max

<400> 98

ttttattttc aaattcttct tgttacaaaa actttttaac tttttgatgg gtaaaatgta 60
 caaataaacc attaatTTTg agcacttatt gatccttcac gaggttttct tctaaataaa 120
 gttatacaca ggtatggatc gttgtatgca agaccacttc ttgagaagct tagggaagat 180

```

ggtgtataca tgaggcctct gggtaatgtc atttatctgt tgtgtggacc ctgcacatct 240
ccagaagttt gcaatcaact actcgtcaaa cttcttaggc ggcttgaaga atttgacgta 300
tgtaagaatt gaagtggatg aagtggataa gtctaataatt cctgctatgg attttggcta 360
agtttattgc tactattatt ttttgtgggg gaggaggaag gggcatgaat tgtaaatacca 420
agggttgcaa ttagcttggg tttttttcaa ttcagttgat gtattttatt tataaataaa 480
aatgttatct tatttcaatt cccaccctcc ccccatTTTT aagatgttat taataaaaaat 540
gttaaagtat ttaatcattt tctttcaata tttttggaaa aaaaatcaag ttatgtaatt 600
aaaaatatat attaactgga aacaaaacca gaataaatta aatagtcaat aggtctagta 660
acaattgaat agagtgtgag taattgtaaa tttttggaaa taatttttta attttatggg 720
ataaaaaaat 730

```

```

<210>      99
<211>      1061
<212>      DNA
<213>      Glycine max

<400>      99

```

```

agcagagtgc aacaaataga aaagggaaaa catccatttc aatagaaaga aaggttgagc 60
tgcaaattgt tgattaccaa tgcaatgggc ttcttccatc attatcagga acatcagggt 120
cagcattcca ttttgaaaca acgtggtaaa ggaaagctgt ctgaccatat tgtgcagcaa 180
catgcgttgt ctgtgtccaa gcaaatcata attagtaaaa ttaacattta cagccacacg 240
cttgtgcttc aagtaaaaca agaatcccaa actaccaaatt tagcagatgt aaccagaaat 300
agacagccag gtataagaga tacataacat gtcctaaatg ccttacccca gcaatctctt 360
gaataaaggg aaaatgaatc acagcaatct aacctgatat ccattcatat cggcagcact 420
cactcgagca cctcctgga gtaaaagtgc cgcaacctga atagcacccc gaaccgcact 480
ccaatgtaag gctgtctggc cagtatgatc cgttgcatTT acatctcccc catgctgcca 540
accacaaatc aaccagttaa ttaggataa gactaattcc atacttaata tcaagccaaa 600
tttggtaca tacattcaat tgcaaaacaa acaaacacca cattcgattt ccatttccat 660
aacacaaaca tgacctacaa gccaacctga gctgagctga gctgagctga gctgagctaa 720
gctactttta caaattcaaa ccaccaaaaa catcataatc aaatgttctt ctcttctaca 780
tctatatcat tccttcctta aacagcaata agtaactaat tcttacattt tcttttcttg 840
gagtaaaaaa atagtaattc agcgagtgcc aaaataacat acgcattcaa acaagcacia 900
taaaagcaaa gtaagcaaag caaacacaat tcaacaaacc gatcaaagaa cctcaatgat 960
gtactgagca gcggcagtgc gattgttgag agcagcccac tgaagcgcgt agtatcccaa 1020

```

cccgctcgggc tccggtgacgg ggcaaccctc ttgctccacc a 1061

<210> 100
<211> 13
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 100

tctgggagggc agc 13

<210> 101
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 101

tgggagacag cctt 14

<210> 102
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 102

ctcggtcccc atgaa 15

<210> 103
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 103

aagctcggtc tccatg 16

<210> 104
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 104

agatgtaaac ttgttataaa g 21

<210> 105
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 105

ttgttatgaa gtgatttta 19

<210> 106
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 106

tttggaggaa atatg 15

<210> 107
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 107

tttttggagg aagtatg 17

<210> 108
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 108

ctcactctca atatc 15

<210> 109

<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 109

cactctcaag atcac

15

<210> 110
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 110

agtggtcct taaat

15

<210> 111
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 111

agtggtcct caaat

15

<210> 112
<211> 13
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 112

aaggtcgaaa att

13

<210> 113
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 113

aggtcgagaa ttgt 14

<210> 114
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 114

cagtcaggta attaaa 16

<210> 115
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 115

cagtcacgta attaa 15

<210> 116
<211> 13
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 116

actgcccttc tgc 13

<210> 117
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 117

acactgctct tctg 14

<210> 118
<211> 16
<212> DNA
<213> Artificial Sequence

<220>

<223> artificial

<400> 118

aggtgaagag cctgtt

16

<210> 119

<211> 15

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 119

aggtgaggag cctgt

15

<210> 120

<211> 16

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 120

tttcaataac aatccc

16

<210> 121

<211> 13

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 121

caatagcaat ccc

13

<210> 122

<211> 15

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 122

tgacacataac tcttc

15

<210> 123

<211> 14

<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 123

tgcacagaac tctt

14

<210> 124
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 124

tggttgagca aaag

14

<210> 125
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 125

tggttgaaca aaag

14

<210> 126
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 126

ttgtaagtac tttaatttc

19

<210> 127
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 127

agattgtaag cactttaa

18

<210> 128
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 128

aatgtgatca aagatg 16

<210> 129
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 129

aatgtggtca aagat 15

<210> 130
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 130

cacacatgta tataagaag 19

<210> 131
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 131

cacacatgta taaaaga 17

<210> 132
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 132
cacatcctca catcag 16

<210> 133
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 133
acatccacac atcag 15

<210> 134
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 134
ctctgatgga atcat 15

<210> 135
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 135
tgatggaaat cttc 14

<210> 136
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 136
cttccccatt tgagttt 17

<210> 137
<211> 16
<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 137

ttccccagtt gagttt

16

<210> 138

<211> 15

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 138

ttgtcacggg tatac

15

<210> 139

<211> 16

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 139

tgtcacaggt atacca

16

<210> 140

<211> 15

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 140

tctactcaaa tggcc

15

<210> 141

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> artificial

<400> 141

attatctact aaaatggcc

19

<210> 142
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 142

ccagagtatg aatcta 16

<210> 143
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 143

ccagagtttg aatcta 16

<210> 144
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 144

tcaatgaaat caatgttg 18

<210> 145
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 145

ttcaatggaa tcaatg 16

<210> 146
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 146
aaggcaggtt aaattt 16

<210> 147
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 147
aaaaggcagg ctaaatt 16

<210> 148
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 148
cagctcccgt tggt 14

<210> 149
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 149
agctcctggt gttca 15

<210> 150
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 150
ttggatgtaa attattgt 18

<210> 151
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 151

atgtaaacta ttgttgct 18

<210> 152
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 152

aatgctgagt tggatc 16

<210> 153
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 153

atgctcgagt tggat 15

<210> 154
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 154

ctctcgtggc ttca 14

<210> 155
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 155

ctctcatggc ttcaa 15

<210> 156
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 156

ttgtgcagtt aaacat 16

<210> 157
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 157

tgtgcaggta aacat 15

<210> 158
<211> 14
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 158

catgggtcct ttca 14

<210> 159
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 159

atgggtccat tcaga 15

<210> 160
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 160

ccgttctgta accgg 15

<210> 161
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 161

tccgttttgt aaccg 15

<210> 162
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 162

attgaagtgg ataagtc 17

<210> 163
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 163

ttgaagtgga tgaagtg 17

<210> 164
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 164

taccacgttg tttcaaa 17

<210> 165
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> artificial

<400> 165

tttaccacat tgtttc

CLAIMS:

1. A method for producing an Asian Soybean Rust (ASR) resistant soybean plant comprising the steps of:
 - a) selecting a plant using marker assisted selection from a plurality of plants by detecting the presence of a marker in linkage group C2, wherein said plant has an ASR resistant locus obtainable from PI462312, and said marker is mapped within 10 centimorgans or less from a SNP marker selected from the group consisting of NS0099746, NS0123747, NS0126598, NS0128378, NS0096829, NS0125408, NS0098902, NS0099529; NS0097798, NS0137477, NS0095322, NS0136101, NS0098982, NS0103749, NS0118897, NS0119715 and NS0130920;
 - b) generating an ASR resistant progeny plant from said selected plant in a breeding program.
2. The method of claim 1, wherein said ASR resistant locus is locus 3.
3. The method of claim 1, wherein said SNP marker is selected from the group consisting of NS0137477, NS0103749, NS0118897, NS0119715 and NS0130920.
4. The method of claim 3, wherein said SNP marker is NS0137477.
5. The method of claim 1, wherein said ASR resistant progeny plant further comprises one or more ASR resistance loci selected from the group consisting of ASR resistance loci 1, 2 and 4-13.
6. The method of claim 1, wherein said ASR resistant progeny plant is resistant to the pathogen *Phakopsora meibomiaae*.
7. The method of claim 1, wherein said ASR resistant progeny plant is immune to ASR.

8. A plant cell from a plant produced by the method of claim 1.
9. The method of claim 1, wherein said ASR resistant progeny plant is resistant to the pathogen *Phakopsora pachyrhizi*.
10. The method of claim 1, wherein said marker is mapped within 5 centimorgans or less from said SNP markers.
11. The method of claim 5, wherein said ASR resistance locus 1 is localized to linkage group G and is mapped within 10 centimorgans or less from one or more SNP markers selected from the group consisting of NS0093250, NS0119710, NS0103004, NS0099454, NS0102630, NS0102915, NS0102913, NS0123728, NS0129943, NS0102168, NS0092723, NS0098177, NS0127343 and NS0101121, said ASR resistance loci 5-9 are localized to chromosome 13 and are mapped within 10 centimorgans or less from SNP marker NS0103033, and said ASR resistance loci 10-13 is localized to chromosome 13 and are mapped within 10 centimorgans or less from SNP marker NS0124935.
12. The method of claim 10, wherein said 10 centimorgans or less is 5 centimorgans or less.