Title: Methods and compositions to select cotton plants resistant to cotton root knot nematode

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Related Art:
METHODS AND COMPOSITIONS TO SELECT COTTON PLANTS RESISTANT TO COTTON ROOT KNOT NEMATODE

The present invention is in the field of plant breeding and disease resistance. More specifically, the invention provides a method for breeding cotton plants containing one or more quantitative trait loci (QTL) that are associated with resistance to Root Knot Nematode (RKN), a disease associated with *Meloidogyne incognita*. The invention further provides germplasm and the use of germplasm containing quantitative trait loci (QTL) conferring disease resistance for introgression into elite germplasm in a breeding program, thus producing novel elite germplasm comprising one or more RKN resistance QTL.
METHODS AND COMPOSITIONS TO SELECT COTTON PLANTS RESISTANT TO COTTON ROOT KNOT NEMATODE

CROSS-REFERENCES TO RELATED APPLICATIONS
[0001] This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 61/092649 filed on 28-Aug-2008. The entirety of the application is hereby incorporated by reference.

INCORPORATION OF SEQUENCE LISTING
[0002] A sequence listing containing the file named "pa_53664.txt" which is 70.5 kilobytes (measured in MS-Windows®) and created on August 25th, 2009, comprises 103 nucleotide sequences, and is herein incorporated by reference in its entirety.

FIELD OF INVENTION
[0003] The present invention is in the field of plant breeding and disease resistance. More specifically, the invention includes a method for breeding cotton plants containing quantitative trait loci that are associated with resistance to cotton root knot nematode (RKN), a disease associated with Meloidogyne incognita (Kofoid and White) Chitwood. The invention further includes germplasm and the use of germplasm containing quantitative trait loci (QTL) conferring disease resistance for introgression into elite germplasm in a breeding program thus producing novel elite germplasm comprising one or more cotton RKN resistance QTL.
BACKGROUND OF THE INVENTION

[0004] Cotton root knot nematode (RKN) is a destructive nematode which forms galls on the roots of cotton plants. The causative agent is *Meloidogyne incognita* (Kofoid and White) Chitwood, a nematode which can infect a variety of plant species. Nutrient and water uptake are decreased in infected plants, and plants may become susceptible to other pathogens, especially *Fusarium* wilt (Minton, N.A. and Minton E.B., *Phytopathology* 56:319-322 (1966)). Consequently, yield is decreased in plants infected with RKN. In the USA alone, an estimated 10.93% of cotton yield loss in 2004 was attributed to RKN (Blasingame and Patel, 2005). RKN is wide-spread throughout the US Cotton Belt. Methods to mitigate RKN damage include rotating cotton crops with non-susceptible crops and application of costly nematicides. However, the most effective way for cotton growers to reduce yield loss and crop damage due to RKN is to grow RKN resistant cotton cultivars. Therefore, a need exists for development of such RKN resistant cotton varieties and for methods to accelerate development of such varieties. Genetic markers can be used by plant breeders as an indirect means to select plants with favorable alleles. A major RKN resistance locus has been reported on Chromosome A11 (Kai, W. *et al.* *Theor. Appl. Genet.* 113:73-80 (2006)). Breeding for RKN resistant cotton varieties can be greatly facilitated by the use of marker-assisted selection for RKN resistance alleles. RKN resistance in cotton has been reported in different germplasm lines such as Auburn 623 RNR and Acala NemX. However, commercial cultivars with RKN resistance are limited. Identification of genetic markers associated with RKN resistance is of great value in a cotton breeding program. RAPD, AFLP, and RGA markers for identifying RKN resistant plants have been identified in a study using near-isogenic lines (NILs) (Niu, C. *et al.*, *Crop Science* 47:951-960 (2007)). Genetic markers associated with RKN resistance in plants have also included SSR markers (Wang, C. *et al.* *Theor. Appl. Genet.* 112:770-777 (2006)).

[0005] Of the classes of markers, SNPs have characteristics which make them preferential to other genetic markers in detecting, selecting for, and introgressing RKN resistance in a cotton plant. SNPs are preferred because technologies are available for automated, high-throughput screening of SNP markers, which can decrease the time to select for and introgress RKN resistance in soybean plants. Further, SNP markers are ideal because the likelihood that a particular SNP allele is derived from independent origins in the extant population of a particular species is very low. As such, genetically linked SNP markers are useful for tracking and assisting introgression of RKN resistance alleles, particularly in the case of RKN resistance haplotypes validated to exist in the resistant donor parent. A need exists for a SNP based marker set for identifying cotton plants resistant to RKN. The present invention provides a SNP based marker for identifying plants resistant to RKN.
BRIEF SUMMARY OF THE INVENTION

[0006] The present invention provides a method of introgressing an allele associated with Root Knot Nematode (RKN) resistance into a cotton plant comprising the steps of: (A) providing a population of cotton plants; B) genotyping at least one cotton plant in the population with respect to a cotton genomic nucleic acid marker selected from the group comprising SEQ ID NOs: 1-38 and C) selecting from the population at least one cotton plant comprising at least one allele associated with RKN resistance. The population provided may be derived by crossing at least one RKN resistant cotton plant with at least one RKN sensitive plant to form a population.

[0007] In one aspect, the cotton plants selected by the methods of the present invention exhibit a resistant reaction rating to RKN of no worse than about 2.0 using indexing scale of 0-5, where 0 is nematode free plant and 5 = 100% roots with galls.

[0008] In one aspect, the method of the present invention further comprises the step (d) of assaying the selected cotton plant for resistance to a RKN disease inducing pathogen. In a further aspect, the genotype is determined by an assay which is selected from the group consisting of single base extension (SBE), allele-specific primer extension sequencing (ASPE), DNA sequencing, RNA sequencing, microarray-based analyses, universal PCR, allele specific extension, hybridization, mass spectrometry, ligation, extension-ligation, and Flap Endonuclease-mediated assays. In a further aspect, the cotton genomic nucleic acid marker is SEQ ID NO: 33.

[0009] The present invention also provides for an elite cotton plant produced by: a) providing a population of cotton plants; b) genotyping at least one cotton plant in the population with respect to a cotton genomic nucleic acid marker selected from the group comprising SEQ ID NOs: 1-38; and c) selecting from the population at least one cotton plant comprising at least one allele associated with RKN resistance. The elite cotton plant of the present invention can exhibit a transgenic trait. The transgenic trait is selected from the group consisting of 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/or seedling growth control, enhanced animal and human nutrition, low raffinose, environmental stress resistance, increased digestibility, improved processing traits, improved flavor, nitrogen fixation, hybrid seed production, and reduced allergenicity. The herbicide tolerance can be selected from the group consisting of glyphosate, dicamba, glufosinate, sulfonylurea, bromoxynil, 2, 4, Dichlorophenoxyacetic acid, and norflurazon herbicides.

[0010] The present invention further provides a method of introgressing at least one RKN resistance allele into a cotton plant comprising a) providing a population of cotton plants, b)
screening the population with at least one nucleic acid marker, c) selecting from the population one or more cotton plants comprising one or more alleles associated with RKN resistance. In one aspect, the selected cotton plants exhibit a resistant reaction rating to RKN of no worse than about 2.0. The present invention further provides for a cotton plant produced by a) providing a population of cotton plants, b) screening the population with at least one nucleic acid marker, c) selecting from the population one or more cotton plants comprising one or more alleles associated with RKN resistance.

[0011] The invention further provides a substantially purified nucleic acid molecule for the detection of loci related to RKN resistance comprising a nucleic acid molecule selected from the group consisting of SEQ ID NOs: 1-62 and complements thereof. The invention further provides an isolated nucleic acid molecule for detecting a molecular marker representing a polymorphism in cotton DNA, wherein the nucleic acid molecule comprises at least 15 nucleotides that include or are adjacent to the polymorphism, wherein the nucleic acid molecule is at least 90 percent identical to a sequence of the same number of consecutive nucleotides in either strand of DNA that include or are adjacent to the polymorphism, and wherein the molecular marker is selected from the group consisting of SEQ ID NOs: 1-38. In one aspect, the isolated nucleic acid further comprises a detectable label or provides for incorporation of a detectable label. In a further aspect, the detectable label is selected from the group consisting of an isotope, a fluorophore, an oxidant, a reductant, a nucleotide and a hapten.

[0012] The present invention further provides a set of oligonucleotides comprising a) a pair of oligonucleotide primers wherein each of the primers comprises at least 12 contiguous nucleotides and wherein the pair of primers permit PCR amplification of a DNA segment comprising a molecular marker selected from the group consisting of SEQ ID NOs: 1-38 and b) at least one detector oligonucleotide that permits detection of a polymorphism in the amplified segment, wherein the sequence of the detector oligonucleotide is at least 95 percent identical to a sequence of the same number of consecutive nucleotides in either strand of a segment of cotton DNA that include or are adjacent to the polymorphism of step (a).
BRIEF DESCRIPTION OF THE NUCLEIC ACIDS

[0013] SEQ ID NO: 1 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromsome 11.

[0014] SEQ ID NO: 2 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromsome 11.

[0015] SEQ ID NO: 3 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromsome 11.

[0016] SEQ ID NO: 4 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromsome 11.

[0017] SEQ ID NO: 5 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromsome 11.

[0018] SEQ ID NO: 6 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromsome 11.

[0019] SEQ ID NO: 7 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromsome 11.

[0020] SEQ ID NO: 8 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromsome 11.

[0021] SEQ ID NO: 9 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromsome 11.

[0022] SEQ ID NO: 10 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromsome 11.

[0023] SEQ ID NO: 11 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromsome 11.

[0024] SEQ ID NO: 12 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromsome 11.

[0025] SEQ ID NO: 13 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromsome 11.

[0026] SEQ ID NO: 14 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromsome 11.

[0027] SEQ ID NO: 15 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromsome 11.

[0028] SEQ ID NO: 16 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromsome 11.

[0029] SEQ ID NO: 17 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromsome 11.
SEQ ID NO: 18 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 11.

SEQ ID NO: 19 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 11.

SEQ ID NO: 20 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 11.

SEQ ID NO: 21 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 11.

SEQ ID NO: 22 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 11.

SEQ ID NO: 23 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 11.

SEQ ID NO: 24 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 11.

SEQ ID NO: 25 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 11.

SEQ ID NO: 26 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 11.

SEQ ID NO: 27 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 11.

SEQ ID NO: 28 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 11.

SEQ ID NO: 29 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 11.

SEQ ID NO: 30 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 11.

SEQ ID NO: 31 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 11.

SEQ ID NO: 32 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 11.

SEQ ID NO: 33 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 11.

SEQ ID NO: 34 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 11.

SEQ ID NO: 35 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 11.
SEQ ID NO: 36 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 11.

SEQ ID NO: 37 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 11.

SEQ ID NO: 38 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 11.

SEQ ID NO: 39 is a forward PCR primer for the amplification of SEQ ID NO: 33.

SEQ ID NO: 40 is a reverse PCR primer for the amplification of SEQ ID NO: 33.

SEQ ID NO: 41 is a forward PCR primer for the amplification of SEQ ID NO: 36.

SEQ ID NO: 42 is a reverse PCR primer for the amplification of SEQ ID NO: 36.

SEQ ID NO: 43 is a forward PCR primer for the amplification of SEQ ID NO: 9.

SEQ ID NO: 44 is a reverse PCR primer for the amplification of SEQ ID NO: 9.

SEQ ID NO: 45 is a probe for detecting the RKN resistance locus of SEQ ID NO: 33.

SEQ ID NO: 46 is a second probe for detecting the RKN resistance locus of SEQ ID NO: 34.

SEQ ID NO: 47 is a probe for detecting the RKN resistance locus of SEQ ID NO: 36.

SEQ ID NO: 48 is a second probe for detecting the RKN resistance locus of SEQ ID NO: 36.

SEQ ID NO: 49 is a probe for detecting the RKN resistance locus of SEQ ID NO: 9.

SEQ ID NO: 50 is a second probe for detecting the RKN resistance locus of SEQ ID NO: 9.

SEQ ID NO: 51 is a forward single base extension probe for detecting the RKN resistance locus of SEQ ID NO: 33.

SEQ ID NO: 52 is a reverse single base extension probe for detecting the RKN resistance locus of SEQ ID NO: 33.

SEQ ID NO: 53 is a forward single base extension probe for detecting the RKN resistance locus of SEQ ID NO: 36.

SEQ ID NO: 54 is a reverse single base extension probe for detecting the RKN resistance locus of SEQ ID NO: 36.

SEQ ID NO: 55 is a forward single base extension probe for detecting the RKN resistance locus of SEQ ID NO: 9.

SEQ ID NO: 56 is a reverse single base extension probe for detecting the RKN resistance locus of SEQ ID NO: 9.

SEQ ID NO: 57 is a hybridization probe for detecting the RKN resistance locus of SEQ ID NO: 33.
SEQ ID NO: 58 is a second hybridization probe for detecting the RKN resistance locus of SEQ ID NO: 33.

SEQ ID NO: 59 is a hybridization probe for detecting the RKN resistance locus of SEQ ID NO: 36.

SEQ ID NO: 60 is a second hybridization probe for detecting the RKN resistance locus of SEQ ID NO: 36.

SEQ ID NO: 61 is a hybridization probe for detecting the RKN resistance locus of SEQ ID NO: 9.

SEQ ID NO: 62 is a second hybridization probe for detecting the RKN resistance locus of SEQ ID NO: 9.

SEQ ID NO: 63 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 64 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 65 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 66 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 67 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 68 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 69 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 70 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 71 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 72 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 73 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 74 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 75 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.
SEQ ID NO: 76 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 77 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 78 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 79 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 80 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 81 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 82 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 83 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 84 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 85 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 86 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 87 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 88 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 89 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 90 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 91 is a genomic sequence derived from *Gossypium hirsutum* associated with RKN resistance locus on Chromosome 7.

SEQ ID NO: 92 is a forward PCR primer for the amplification of SEQ ID NO: 73.

SEQ ID NO: 93 is a reverse PCR primer for the amplification of SEQ ID NO: 73.

SEQ ID NO: 94 is a forward PCR primer for the amplification of SEQ ID NO: 74.

SEQ ID NO: 95 is a reverse PCR primer for the amplification of SEQ ID NO: 74.
SEQ ID NO: 96 is a forward PCR primer for the amplification of SEQ ID NO: 75.

SEQ ID NO: 97 is a reverse PCR primer for the amplification of SEQ ID NO: 75.

SEQ ID NO: 98 is a hybridization probe for detecting the RKN resistance locus of SEQ ID NO: 73.

SEQ ID NO: 99 is a second hybridization probe for detecting the RKN resistance locus of SEQ ID NO: 73.

SEQ ID NO: 100 is a hybridization probe for detecting the RKN resistance locus of SEQ ID NO: 74.

SEQ ID NO: 101 is a second hybridization probe for detecting the RKN resistance locus of SEQ ID NO: 74.

SEQ ID NO: 102 is a hybridization probe for detecting the RKN resistance locus of SEQ ID NO: 75.

SEQ ID NO: 103 is a second hybridization probe for detecting the RKN resistance locus of SEQ ID NO: 75.
DETAILED DESCRIPTION OF THE INVENTION


[0117] An “allele” refers to an alternative sequence at a particular locus; the length of an allele can be as small as 1 nucleotide base, but is typically larger.

[0118] A “locus” is a position on a genomic sequence that is usually found by a point of reference; e.g., a DNA sequence that is a gene, or part of a gene or intergenic region. The loci of this invention comprise one or more polymorphisms in a population; i.e., alternative alleles are present in some individuals.

[0119] As used herein, “polymorphism” means the presence of two or more variations of a nucleic acid sequence or nucleic acid feature at one or more loci in a population of one or more individuals. The variation may comprise but is not limited to one or more base changes, the insertion of one or more nucleotides or the deletion of one or more nucleotides. A polymorphism may arise from random processes in nucleic acid replication, through mutagenesis, as a result of mobile genomic elements, from copy number variation and during the process of meiosis, such as unequal crossing over, genome duplication and chromosome breaks and fusions. The variation can be commonly found or may exist at low frequency within a population, the former having greater utility in general plant breeding and the latter may be associated with rare but important phenotypic variation. Useful polymorphisms may include single nucleotide polymorphisms (SNPs), insertions or deletions in DNA sequence (Indels), simple sequence repeats of DNA sequence (SSRs), a restriction fragment length polymorphism, and a tag SNP. A genetic marker, a gene, a DNA-derived sequence, a haplotype, a RNA-derived sequence, a promoter, a 5’ untranslated region of a gene, a 3’ untranslated region of a gene, microRNA, siRNA, a QTL, a satellite marker, a transgene, mRNA, ds mRNA, a transcriptional profile, and a methylation pattern may also comprise polymorphisms. In addition, the presence, absence, or variation in copy number of the preceding may comprise polymorphisms.
As used herein, “marker” means a detectable characteristic that can be used to discriminate between organisms. Examples of such characteristics may include genetic markers, protein composition, protein levels, oil composition, oil levels, carbohydrate composition, carbohydrate levels, fatty acid composition, fatty acid levels, amino acid composition, amino acid levels, biopolymers, pharmaceuticals, starch composition, starch levels, fermentable starch, fermentation yield, fermentation efficiency, energy yield, secondary compounds, metabolites, morphological characteristics, and agronomic characteristics. As used herein, “genetic marker” means polymorphic nucleic acid sequence or nucleic acid feature. A genetic marker may be represented by one or more particular variant sequences, or by a consensus sequence. In another sense, a “genetic marker” is an isolated variant or consensus of such a sequence.

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

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

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

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

As used herein, “consensus sequence” refers to a constructed DNA sequence which identifies SNP and Indel polymorphisms in alleles at a locus. Consensus sequence can be based on either strand of DNA at the locus and states the nucleotide base of either one of each SNP in the locus and the nucleotide bases of all Indels in the locus. Thus, although a consensus sequence may not be a copy of an actual DNA sequence, a consensus sequence is useful for precisely designing primers and probes for actual polymorphisms in the locus.
[0126] As used herein, the term “single nucleotide polymorphism,” also referred to by the abbreviation “SNP,” means a polymorphism at a single site wherein the polymorphism constitutes a single base pair change, an insertion of one or more base pairs, or a deletion of one or more base pairs.

[0127] As used herein, the term “haplotype” means a chromosomal region within a haplotype window defined by at least one polymorphic molecular marker. The unique marker fingerprint combinations in each haplotype window define individual haplotypes for that window. Further, changes in a haplotype, brought about by recombination for example, may result in the modification of a haplotype so that it comprises only a portion of the original (parental) haplotype operably linked to the trait, for example, via physical linkage to a gene, QTL, or transgene. Any such change in a haplotype would be included in our definition of what constitutes a haplotype so long as the functional integrity of that genomic region is unchanged or improved.

[0128] As used herein, the term “haplotype window” means a chromosomal region that is established by statistical analyses known to those of skill in the art and is in linkage disequilibrium. Thus, identity by state between two inbred individuals (or two gametes) at one or more molecular marker loci located within this region is taken as evidence of identity-by-descent of the entire region. Each haplotype window includes at least one polymorphic molecular marker. Haplotype windows can be mapped along each chromosome in the genome. Haplotype windows are not fixed per se and, given the ever-increasing density of molecular markers, this invention anticipates the number and size of haplotype windows to evolve, with the number of windows increasing and their respective sizes decreasing, thus resulting in an ever-increasing degree confidence in ascertaining identity by descent based on the identity by state at the marker loci.

[0129] As used herein, “genotype” means the genetic component of the phenotype, and it can be indirectly characterized using markers or directly characterized by nucleic acid sequencing. Suitable markers include a phenotypic character, a metabolic profile, a genetic marker, or some other type of marker. A genotype may constitute an allele for at least one genetic marker locus or a haplotype for at least one haplotype window. In some embodiments, a genotype may represent a single locus and in others it may represent a genome-wide set of loci. In another embodiment, the genotype can reflect the sequence of a portion of a chromosome, an entire chromosome, a portion of the genome, and the entire genome.

[0130] As used herein, “genotyping” means the process of assaying the alleles present at one or more specific loci in an attempt to measure the genetic variation between members of a species. Current methods of genotyping include PCR, DNA sequencing, and probe hybridization. SNPs
are the most common type of genetic variation. A SNP is a single base pair mutation at a specific locus, usually consisting of two alleles

[0131] As used herein, “phenotype” means the detectable characteristics of a cell or organism which can be influenced by genotype.

[0132] As used herein, “linkage” refers to relative frequency at which types of gametes are produced in a cross. For example, if locus A has genes “A” or “a” and locus B has genes “B” or “b” and a cross between parent I with AABB and parent B with aabb will produce four possible gametes where the genes are segregated into AB, Ab, aB and ab. The null expectation is that there will be independent equal segregation into each of the four possible genotypes, i.e., with no linkage 1/4 of the gametes will of each genotype. Segregation of gametes into a genotypes differing from ¼ are attributed to linkage.

[0133] As used herein, “linkage disequilibrium” is defined in the context of the relative frequency of gamete types in a population of many individuals in a single generation. If the frequency of allele A is p, a is p’, B is q and b is q’, then the expected frequency (with no linkage disequilibrium) of genotype AB is pq, Ab is pq’, aB is p’q and ab is p’q’. Any deviation from the expected frequency is called linkage disequilibrium. Two loci are said to be “genetically linked” when they are in linkage disequilibrium.

[0134] As used herein, “chromosomal position” means a linear designation of sites within a chromosome or genome, based upon the various frequencies of recombination between genetic markers

[0135] As used herein, “quantitative trait locus (QTL)” means a locus that controls to some degree numerically representable traits that are usually continuously distributed.

[0136] As used herein, “resistance allele” means the isolated nucleic acid sequence that includes the polymorphic allele associated with resistance to root knot nematode.

[0137] As used herein, “cotton” means *Gossypium hirsutum* and includes all plant varieties that can be bred with cotton, including wild cotton species. More specifically, cotton plants from the species *Gossypium hirsutum* and the subspecies *Gossypium hirsutum* L. can be genotyped using these compositions and methods. In an additional aspect, the cotton plant is from the group *Gossypium arboreum* L., otherwise known as tree cotton. In another aspect, the cotton plant is from the group *Gossypium barbadense* L., otherwise known as American pima or Egyptian cotton. In another aspect, the cotton plant is from the group *Gossypium herbaceum* L., otherwise known as levant cotton. *Gossypium* or cotton plants can include hybrids, inbreds, partial inbreds, or members of defined or undefined populations.

[0138] As used herein, the term “comprising” means “including but not limited to”.

[0139] As used herein, the term “elite line” means any line that has resulted from breeding and selection for superior agronomic performance. Non-limiting examples of elite lines that are

[0140] In the present invention, an RKN resistant locus is located on Chromosome A11 (RKN-1). SNP markers used to monitor the introgression of RKN-1 include those selected from the group consisting of SEQ ID NOs: 1-38. Illustrative RKN-1 SNP marker DNA sequence SEQ ID NO: 33 can be amplified using the primers indicated as SEQ ID NOs: 39 through 40 and detected with probes indicated as SEQ ID NOs: 45 through 46. Illustrative RKN-1 SNP marker DNA sequence SEQ ID NO: 36 can be amplified using the primers indicated as SEQ ID NOs: 41 through 42 and detected with probes indicated as SEQ ID NOs: 47 through 48. Illustrative RKN-1 SNP marker DNA sequence SEQ ID NO: 9 can be amplified using the primers indicated as SEQ ID NOs: 43 through 44 and detected with probes indicated as SEQ ID NOs: 49 through 50.

[0141] In the present invention an RKN resistant locus is located on Chromosome A07 (RKN-2). SNP markers used to monitor the introgression of RKN-2 include those selected from the group consisting of SEQ ID NOs: 63-91. Illustrative RKN-2 SNP marker DNA sequence SEQ ID NO: 73 can be amplified using the primers indicated as SEQ ID NOs: 92 through 93 and detected with probes indicated as SEQ ID NOs: 98 through 99. Illustrative RKN-2 SNP marker DNA sequence SEQ ID NO: 74 can be amplified using the primers indicated as SEQ ID NOs: 94 through 95 and detected with probes indicated as SEQ ID NOs: 100 through 101. Illustrative RKN-2 SNP marker DNA sequence SEQ ID NO: 75 can be amplified using the primers indicated as SEQ ID NOs: 96 through 97 and detected with probes indicated as SEQ ID NOs: 102 through 103.

[0142] The present invention also provides a cotton plant comprising a nucleic acid molecule selected from the group consisting of SEQ ID NOs: 1-38, fragments thereof, and complements of both. The present invention also provides a cotton plant comprising a nucleic acid molecule selected from the group consisting of SEQ ID NOs: 39 through 50, fragments thereof, and complements of both.

[0143] The present invention also provides a cotton plant comprising at least one RKN resistance loci. In one aspect, a cotton plant is provided comprising an RKN resistant locus of chromosome A11 (RKN-1). In an additional aspect, a cotton plant is provided comprising an RKN resistant locus of chromosome A07 (RKN-2). In a further aspect, a cotton plant is provided comprising both resistant alleles, RKN-1 and RKN-2, respectively. In all aspects such alleles may be homozygous or heterozygous.
As used herein, RKN refers to any RKN variant or isolate. A cotton plant of the present invention can be resistant to one or more nematodes capable of causing or inducing galls similar to RKN. In one aspect, the present invention provides plants resistant to RKN as well as methods and compositions for screening cotton plants for resistance or susceptibility to RKN, caused by the genus *Meloidogyne*. In a preferred aspect, the present invention provides methods and compositions for screening cotton plants for resistance or susceptibility to *Meloidogyne incognita*.

In one aspect, the plant is selected from the genus *Gossypium*. In another aspect, the plant is selected from the species *Gossypium hirsutum*. In a further aspect, the plant is selected from the subspecies *Gossypium hirsutum* L. In an additional aspect, the plant is from the group *Gossypium arboreum* L., otherwise known as tree cotton. In another aspect, the plant is from the group *Gossypium barbadense* L., otherwise known as American pima or Egyptian cotton. In another aspect, cotton plant is from the group *Gossypium herbaceum* L., otherwise known as levant cotton. *Gossypium* or cotton plants can include hybrids, inbreds, partial inbreds, or members of defined or undefined populations.

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

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

A galling index scale is used to rate plants as resistant or susceptible to RKN. Roots of plants are examined for number and size of galls and rated according to a 0 (no galls) to 5 (100% roots with galls) scale. The detailed description of indexing is as follows: 0 (no visible galls, healthy root system); 1 (1-2 galls, healthy root system); 2 (3-12 galls, small size galls more visible); 3 (13-30 galls, large size galls more visible on tap root); 4 (31-60 galls, severe galling with large gall size); 5 (over 60 galls, >75% roots with large galls, root system non-functional). In this aspect, the plants with a rating below 2 were considered as resistant plants.

In another aspect, the cotton plant can show a comparative resistance compared to a non-resistant control cotton plant. In this aspect, a control cotton plant will preferably be genetically similar except for the RKN resistance allele or alleles in question. Such plants can be grown under similar conditions with equivalent or near equivalent exposure to the pathogen. In this aspect, the resistant plant or plants has less than 25%, 15%, 10%, 5%, 2% or 1% of leaf area infected.
[0150] A disease resistance QTL of the present invention may be introduced into an elite cotton inbred line. An “elite line” is any line that has resulted from breeding and selection for superior agronomic performance.

[0151] An RKN resistance QTL of the present invention may also be introduced into an elite cotton plant comprising one or more transgenes conferring 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. In one aspect, the herbicide tolerance is selected from the group consisting of glyphosate, dicamba, glufosinate, sulfonylurea, bromoxynil and norflurazon herbicides. These traits can be provided by methods of plant biotechnology as transgenes in cotton.

[0152] A disease resistant QTL allele or alleles can be introduced from any plant that contains that allele (donor) to any recipient cotton plant. In one aspect, the recipient cotton plant can contain additional RKN resistant loci. In another aspect, the recipient cotton plant can contain a transgene. In another aspect, while maintaining the introduced QTL, the genetic contribution of the plant providing the disease resistant QTL can be reduced by back-crossing or other suitable approaches. In one aspect, the nuclear genetic material derived from the donor material in the cotton plant can be less than or about 50%, less than or about 25%, less than or about 13%, less than or about 5%, 3%, 2% or 1%, but that genetic material contains the cotton resistant locus or loci of interest.

[0153] It is further understood that a cotton plant of the present invention may exhibit the characteristics of any relative maturity group. In an aspect, the maturity group is selected from the group consisting of early maturing varieties, mid season maturing varieties, and full season varieties.

[0154] An allele of a QTL can, of course, comprise multiple genes or other genetic factors even within a contiguous genomic region or linkage group, such as a haplotype. As used herein, an allele of a disease resistance locus can therefore encompass more than one gene or other genetic factor where each individual gene or genetic component is also capable of exhibiting allelic variation and where each gene or genetic factor is also capable of eliciting a phenotypic effect on the quantitative trait in question. In an aspect of the present invention the 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 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 the polymorphisms indicate identity by descent. A haplotype within that window can be defined by the unique fingerprint of alleles at each marker. As used herein, an allele is one of several alternative forms of a gene occupying a given locus on a chromosome. When all the alleles present at a given locus on a chromosome are the same, that plant is homozygous at that locus. If the alleles present at a given locus on a chromosome differ, that plant is heterozygous at that locus. Plants of the present invention may be homozygous or heterozygous at any particular RKN locus or for a particular polymorphic marker.

[0155] The present invention also provides for parts of the plants of the present invention. Plant parts, without limitation, include seed, endosperm, ovule and pollen. In a particularly preferred aspect of the present invention, the plant part is a seed.

[0156] The present invention also provides a container of cotton in which greater than 50%, 60%, 70%, 80%, 90%, 95%, or 99% of the seeds comprising RKN resistance loci.

[0157] The container of cotton seeds can contain any number, weight, or volume of seeds. For example, a container can contain at least, or greater than, about 10, 25, 50, 100, 200, 300, 400, 500, 600, 700, 80, 90, 1000, 1500, 2000, 2500, 3000, 3500, 4000 or more seeds. In another aspect, a container can contain about, or greater than about, 1 gram, 5 grams, 10 grams, 15 grams, 20 grams, 25 grams, 50 grams, 100 grams, 250 grams, 500 grams, or 1000 grams of seeds. Alternatively, the container can contain at least, or greater than, about 0 ounces, 1 ounce, 5 ounces, 10 ounces, 1 pound, 2 pounds, 3 pounds, 4 pounds, 5 pounds, 10 pounds, 15 pounds, 20 pounds, 25 pounds, or 50 pounds or more seeds.

[0158] Containers of cotton seeds can be any container available in the art. For example, a container can be a box, a bag, a can, a packet, a pouch, a tape roll, a bail, or a tube.

[0159] In another aspect, the seeds contained in the containers of cotton seeds can be treated or untreated cotton seeds. In one aspect, the seeds can be treated to improve germination, for example, by priming the seeds, or by disinfection to protect against seed-born pathogens. In another aspect, seeds can be coated with any available coating to improve, for example, plantability, seed emergence, and protection against seed-born pathogens. Seed coating can be any form of seed coating including, but not limited to, pelleting, film coating, and encrustments.

[0160] Various patent and non-patent publications are cited herein, the disclosures of each of which are incorporated herein by reference in their entireties.

[0161] As various modifications could be made in the constructions and methods herein described and illustrated without departing from the scope of the invention, it is intended that all
matter contained in the foregoing description or shown in the accompanying drawings shall be interpreted as illustrative rather than limiting. The breadth and scope of the present invention should not be limited by any of the above-described exemplary embodiments, but should be defined only in accordance with the following claims appended hereto and their equivalents.
EXAMPLES

[0162] The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

Example 1: Phenotypic Rating Scale

[0163] To assess the reaction of plants to RKN, cotton plants were grown in growth chambers and artificially inoculated with 2,500 nematode eggs approximately 7 days after emergence. Plant roots were examined for galling 45-50 days after inoculation. A galling index of 0 (no galls) to 5 (100% roots with galls) was used to rate the plants. Table 1 provides the phenotypic rating scale used to identify RKN reaction in cotton plants.

[0164] Table 1: Phenotypic Rating Scale Used for RKN Reaction

<table>
<thead>
<tr>
<th>Rating</th>
<th>Phenotypic Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No visible galls; healthy root system</td>
</tr>
<tr>
<td>1</td>
<td>1-2 galls; healthy root system</td>
</tr>
<tr>
<td>2</td>
<td>3-12 galls; small gall size</td>
</tr>
<tr>
<td>3</td>
<td>13-30 galls; large size galls more visible on tap root</td>
</tr>
<tr>
<td>4</td>
<td>31-60 galls; severe galling with large gall size</td>
</tr>
<tr>
<td>5</td>
<td>&gt;60 galls; severe galling with &gt; 75% roots with large galls; root system non-functional</td>
</tr>
</tbody>
</table>
Example 2: Identification of SNP Markers Associated with RKN Resistance

A mapping population was developed from the cross of the RKN resistant parent M240 with RKN susceptible parent 33B. A total of 250 near-isogenic lines (NILs) were developed for the mapping population. Ten replicates of each line were evaluated for reaction to RKN as described in Example 1.

Eleven SNP markers located on Chromosome A11 were used to screen the NIL mapping population. Of these, SNP marker NG0204877 was found to be highly associated with RKN resistance. Of 248 lines screened, the mean galling index of lines with the TT genotype was significantly lower than those with the AA genotype. Table 2 provides the mean galling index for lines with the genotype AA, AT, and TT. A t-test analysis was performed and the p-value for no mean difference between AA and TT genotypes on the galling index was 3.84 x 10^{-82}. The marker NG0204877 is on Chromosome A11 at position 181.1.

Table 2: Marker NG0204877 (SEQ ID NO. 31) is Associated with RKN Resistance in Cotton.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Mean Galling Index</th>
<th>Number of Lines</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>4.5</td>
<td>78</td>
</tr>
<tr>
<td>AT</td>
<td>3.0</td>
<td>37</td>
</tr>
<tr>
<td>TT</td>
<td>1.1</td>
<td>133</td>
</tr>
</tbody>
</table>

Example 3: Use of SNP markers for Monitoring RKN Resistance

Additional SNP markers are located on Chromosome A11. Table 3 provides the marker names, chromosome position, and the position of the polymorphism in the marker, and alleles.

In a breeding program, one or more markers provided in Table 3 can be used to select for and to introgress RKN resistance into a cotton plant. A cotton breeder can select one or more markers which are polymorphic between parents in a breeding cross to select progeny with the genotype of the RKN resistant parent.

Table 3: SNP Markers on Chromosome A11 for Detecting RKN Resistance.

<table>
<thead>
<tr>
<th>Marker</th>
<th>SEQ ID NO:</th>
<th>Chromosome Position</th>
<th>SNP Position</th>
<th>Allele1</th>
<th>Allele 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG0204212</td>
<td>1</td>
<td>142.5</td>
<td>303</td>
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<td>C</td>
</tr>
<tr>
<td>NG0204865</td>
<td>2</td>
<td>143</td>
<td>367</td>
<td>A</td>
<td>G</td>
</tr>
<tr>
<td>NG0203354</td>
<td>3</td>
<td>145</td>
<td>253</td>
<td>A</td>
<td>G</td>
</tr>
<tr>
<td>SEQ ID NO.</td>
<td>Position</td>
<td>SNP Type</td>
<td>Position</td>
<td>Allele 1</td>
<td>Allele 2</td>
</tr>
<tr>
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<td>----------</td>
<td>----------</td>
</tr>
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<td>T</td>
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<td></td>
<td>147</td>
<td>A</td>
<td>G</td>
</tr>
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<td></td>
<td>150.7</td>
<td>C</td>
<td>T</td>
</tr>
<tr>
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<td>150.7</td>
<td>A</td>
<td>G</td>
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<td>C</td>
</tr>
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<td>A</td>
<td>G</td>
</tr>
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<td>10</td>
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<td>152.2</td>
<td>G</td>
<td>T</td>
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<td>C</td>
<td>G</td>
</tr>
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<td>160.1</td>
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<td>NG0210596</td>
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<td>NG0207455</td>
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<td>NG0203802</td>
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<tr>
<td>NG0207423</td>
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<td>NG0206483</td>
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<td>G</td>
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<td>C</td>
<td>G</td>
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<td>T</td>
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<td>183.5</td>
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<td>G</td>
</tr>
</tbody>
</table>

**Indicates a single nucleotide Deletion**

1 SNP Position: refers to the position of the SNP polymorphism in the indicated SEQ ID NO.
Table 3A: SNP Markers on Chromosome A07 for Detecting RKN Resistance.

<table>
<thead>
<tr>
<th>Marker</th>
<th>SEQ ID NO:</th>
<th>Chromosome Position</th>
<th>SNP Position¹</th>
<th>Allele 1</th>
<th>Allele 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>NG0203799</td>
<td>63</td>
<td>32.2</td>
<td>268</td>
<td>T</td>
<td>A</td>
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<tr>
<td>NG0210921</td>
<td>64</td>
<td>33.1</td>
<td>190</td>
<td>G</td>
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<tr>
<td>NG0210441</td>
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<td>34.7</td>
<td>356</td>
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<td>50</td>
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<td>C</td>
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¹SNP Position: refers to the position of the SNP polymorphism in the indicated SEQ ID NO.

Example 4: Exemplary Marker Assays for Detecting RKN Resistance

In one embodiment, the detection of polymorphic sites in a sample of DNA, RNA, or cDNA may be facilitated through the use of nucleic acid amplification methods. Such methods specifically increase the concentration of polynucleotides that span the polymorphic site, or include that site and sequences located either distal or proximal to it. Such amplified molecules can be readily detected by gel electrophoresis, fluorescence detection methods, or other means. Exemplary primers and probes for amplifying and detecting genomic regions associated with cotton RKN resistance are given in Table 4.
[0173] **Table 4: Exemplary Assays for Detecting RKN Resistance**

<table>
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<th>Marker</th>
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**Example 5: Oligonucleotide Probes Useful for Detecting Cotton Plants with RKN Resistance Loci by Single Base Extension Methods**

[0174] Oligonucleotides can also be used to detect or type the polymorphisms associated with RKN resistance disclosed herein by single base extension (SBE)-based SNP detection methods. Exemplary oligonucleotides for use in SBE-based SNP detection are provided in Table 5. SBE methods are based on extension of a nucleotide primer that is hybridized to sequences adjacent to a polymorphism to incorporate a detectable nucleotide residue upon extension of the primer. It is also anticipated that the SBE method can use three synthetic oligonucleotides. Two of the oligonucleotides serve as PCR primers and are complementary to the sequence of the locus which flanks a region containing the polymorphism to be assayed. Exemplary PCR primers that can be used to type polymorphisms disclosed in this invention are provided in Table 4 in the columns labeled “Forward Primer SEQ ID” and “Reverse Primer SEQ ID”. Following amplification of the region containing the polymorphism, the PCR product is hybridized with an extension primer which anneals to the amplified DNA adjacent to the polymorphism. DNA polymerase and two differentially labeled dideoxynucleoside triphosphates are then provided. If the polymorphism is present on the template, one of the labeled dideoxynucleoside triphosphates can be added to the primer in a single base chain extension. The allele present is then inferred by determining which of the two differential labels was added to the extension primer. Homozygous samples will result in only one of the two labeled bases being incorporated and thus only one of the two labels will be detected. Heterozygous samples have both alleles present, and will thus direct incorporation of both labels (into different molecules of the extension primer) and thus both labels will be detected. Exemplary forward and reverse SBE probes are provided in Table 5.
Table 5. Probes (Extension Primers) for Single Base Extension (SBE) Assays

<table>
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<th>Marker SEQID NO.</th>
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<th>Probe (SBE)</th>
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Example 6: Oligonucleotide Hybridization Probes Useful for Detecting Cotton Plants with RKN Resistance Loci

Oligonucleotides can also be used to detect or type the polymorphisms associated with RKN resistance disclosed herein by hybridization-based SNP detection methods. Oligonucleotides capable of hybridizing to isolated nucleic acid sequences which include the polymorphism are provided. It is within the skill of the art to design assays with experimentally determined stringency to discriminate between the allelic states of the polymorphisms presented herein. Exemplary assays include Southern blots, Northern blots, microarrays, in situ hybridization, and other methods of polymorphism detection based on hybridization. Exemplary oligonucleotides for use in hybridization-based SNP detection are provided in Table 6. These oligonucleotides can be detectably labeled with radioactive labels, fluorophores, or other chemiluminescent means to facilitate detection of hybridization to samples of genomic or amplified nucleic acids derived from one or more cotton plants using methods known in the art.

Table 6. Oligonucleotide hybridization probes

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Example 7: Prophetic Example of Introgression of RKN Resistance Using SNP Markers

[0178] A plant breeder can use SNP markers to facilitate the introgression of the RKN resistance locus on Chromosome A11 and to select for lines carrying the favorable alleles for one or more of said SNP markers. In this example, the cotton line M240 is used as a donor of RKN resistance. The SNP marker NG0204877 (SEQ ID NO: 31) is used to monitor the introgression of the RKN resistance locus. A plant breeder can select the favorable genotype as provided in Table 2 to select plants for RKN resistance arising from the donor while selecting for the recipient genome in adjacent chromosome regions. In practice, this reduces the amount of linkage drag from the donor genome that maybe associated with undesirable agronomic or fiber quality properties.

[0179] The introgression of one or more resistance loci is achieved via repeated backcrossing to a recurrent parent accompanied by selection to retain one or more RKN resistance loci from the donor parent. This backcrossing procedure is implemented at any stage in line development and occurs in conjunction with breeding for superior agronomic characteristics or one or more traits of interest, including transgenic and nontransgenic traits.

[0180] Alternatively, a forward breeding approach is employed wherein one or more RKN resistance loci can be monitored for successful introgression following a cross with a susceptible parent with subsequent generations genotyped for one or more RKN resistance loci and for one or more additional traits of interest, including transgenic and nontransgenic traits.

Example 8: Introgression of RKN-1 and RKN-2 Using SNP markers to Produce a Cotton Plant Resistant to Root Knot Nematode

[0181] A plant breeder can use SNP markers to facilitate the introgression of the RKN-1 resistant locus on Chromosome A11 and the RKN-2 resistant locus on Chromosome A07 to select for lines carrying the favorable alleles for one or more of said SNP markers. In this example, the cotton line M-315 is used as a donor of RKN resistance. The SNP marker NG0204877 (SEQ ID NO: 31) was used to monitor the introgression of the RKN-1 resistant locus and the SNP markers NG0206957 (SEQ ID NO: 73), NG0207837 (SEQ ID NO: 74), and NG0207518 (SEQ ID NO: 75) were used to monitor the introgression of the RKN-2 resistance locus. A plant breeder can select the favorable polymorphic genotype as provided in to select plants for RKN resistance arising from the donor while selecting for the recipient genome in adjacent chromosome regions. In practice, this reduces the amount of linkage drag from the donor genome that maybe associated with undesirable agronomic or fiber quality properties.
[0182] The introgression of one or more resistance loci is achieved via repeated backcrossing to a recurrent parent accompanied by selection to retain one or more RKN resistance loci from the donor parent. This backcrossing procedure is implemented at any stage in line development and occurs in conjunction with breeding for superior agronomic characteristics or one or more traits of interest, including transgenic and nontransgenic traits.

[0183] Alternatively, a forward breeding approach is employed wherein one or more RKN resistance loci can be monitored for successful introgression following a cross with a susceptible parent with subsequent generations genotyped for one or more RKN resistance loci and for one or more additional traits of interest, including transgenic and nontransgenic traits.

[0184] In view of the foregoing, it will be seen that the several advantages of the invention are achieved and attained. The embodiments were chosen and described in order to best explain the principles of the invention and its practical application to thereby enable others skilled in the art to best utilize the invention in various embodiments and with various modifications as are suited to the particular use contemplated.

[0185] Various patent and non-patent publications are cited herein, the disclosures of each of which are, to the extent necessary, incorporated herein by reference in their entireties. As various modifications could be made in the constructions and methods herein described and illustrated without departing from the scope of the invention, it is intended that all matter contained in the foregoing description or shown in the accompanying drawings shall be interpreted as illustrative rather than limiting. The breadth and scope of the present invention should not be limited by any of the above-described exemplary embodiments, but should be defined only in accordance with the following claims appended hereto and their equivalents.

[0186] A reference herein to a patent document or non-patent document which is given as prior art is not taken as an admission that that document or prior art was part of common general knowledge at the priority date of any of the claims.
CLAIMS:

1. A method for selecting a cotton plant comprising in its genome the root knot nematode resistance loci RKN-1 and RKN-2, comprising the steps of:
   a. providing a population of cotton plants;
   b. genotyping at least one cotton plant from said population with respect to a RKN-1 locus using at least one SNP marker selected from Table 3 and with respect to a RKN-2 locus using at least one SNP marker selected from Table 3A; and
   c. selecting a cotton plant comprising a desirable genotype at each of the RKN-1 locus and the RKN-2 locus, wherein said desirable genotype confers resistance to root knot nematode in said identified cotton plant.

2. The method of claim 1, wherein the population is derived by crossing at least one root knot nematode resistant cotton plant with at least one other cotton plant to form a population.

3. The method of claim 1, further comprising exposing the selected cotton plant to a root knot nematode inducing pathogen.

4. The method of claim 3, wherein the selected cotton plant exhibits a root knot nematode resistance reaction rating of no worse than about 2.0.

5. The method of claim 1, wherein said at least one SNP marker selected from Table 3 is NG0204877.

6. The method of claim 1, wherein said at least one SNP marker selected from Table 3A are NG0206957, NG0207837, and NG0207518.

7. The method of claim 1, wherein the population of cotton plants exhibits a transgenic trait.

8. The method of claim 7, wherein the transgenic trait is selected from the group consisting of 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/or seedling growth control, enhanced animal and human nutrition, low raffinose, environmental stress resistance, increased digestibility, improved processing traits, improved flavor, nitrogen fixation, hybrid seed production, and reduced allergenicity.

9. The method of claim 1, wherein said genotyping comprises detection of a locus comprising a nucleic acid molecule comprising the sequence of SEQ ID NOs: 6, 8, 9, 12, 13, 15-17, 22, 25, 28-32, 34-36, 64-66, 69, 71, 72, 76-78, 80-83, 85 or 89.

10. A cotton plant comprising in its genome the root knot nematode resistance loci RKN-1 and RKN-2, produced by a method comprising the steps of:
   a. crossing at least one root knot nematode resistant cotton plant with at least one other cotton plant in order to form a population segregating for resistance to root knot nematode;
   b. genotyping at least one cotton plant from said population with respect to a RKN-1 locus using at least one SNP marker selected from Table 3 and with respect to a RKN-2 locus using at least one SNP marker selected from Table 3A; and
   c. identifying a cotton plant comprising a desirable genotype at each of the RKN-1 locus and the RKN-2 locus, wherein said desirable genotype confers resistance to root knot nematode in said identified cotton plant.

11. The cotton plant of claim 10, wherein said at least one SNP marker selected from Table 3 is NG0204877.

12. The cotton plant of claim 10, wherein said at least one SNP marker selected from Table 3A are NG0206957, NG0207837, and NG0207518.

13. The cotton plant of claim 10, wherein the cotton plant exhibits a transgenic trait.
14. The cotton plant of claim 13, wherein the transgenic trait is selected from the group consisting of 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/or seedling growth control, enhanced animal and human nutrition, low raffinose, environmental stress resistance, increased digestibility, improved processing traits, improved flavor, nitrogen fixation, hybrid seed production, and reduced allergenicity.

15. The cotton plant of claim 10, wherein said genotyping comprises detection of a locus comprising a nucleic acid molecule comprising the sequence of SEQ ID NOs: 6, 8, 9, 12, 13, 15-17, 22, 25, 28-32, 34-36, 64-66, 69, 71, 72, 76-78, 80-83, 85 or 89.
Xiao, Jinhua  
Bhatti, Muhammad  
Cantrell, Roy

Methods and compositions to select cotton plants resistant to 
cotton root knot nematode

US 61/092,649  
2008-08-28

103

DNA  
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n = a, t, c, or g

n = a, t, c, or g

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2

DNA  
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n = a, t, c, or g

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<210> 11
<211> 635
<212> DNA
<213> Gossypium hirsutum
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<210> 12
<211> 598
<212> DNA
<213> Gossypium hirsutum
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aacatatatt tgtatcattt gaattgtgtt cagattttagc taataatttg ttaacagcaac 180
agtcgatctg acgatactcg attcagcccc aattcaagtg tgcttagcat aatcatacat 240
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<210> 13
<211> 585
<212> DNA
<213> Gossypium hirsutum
<220>
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<222> (1)..<(585)
<223> n = a, t, c, or g
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<210> 14
<211> DNA
<212> Gossypium hirsutum
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cctcaatttt attatgggaa aaagaaaaag tggtggatag atagagatag tgctttagaa 300
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<220>
<221> n = a, t, c, or g
<222> (1) .. (699)
<223> n = a, t, c, or g
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tagttctaa caacaaaaaagt taatttaag taacaaaaat agcagcattg 180
tctcaacgt cgacactgct ggtctgggca gttgtttctct tctttttgcct gctctcatgtg 240
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tgagctttaa tttgtgagtt atttgtctct cctaggaata ctggctttct tgaatgtcga 480
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gctaagattt tccttagcca antggcttg ggtaaacttc ctgcagctgc cacatatattc 600

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gaaccaagag taaaaacata tcctgaggta ctcttcattg 699

<210> 16
<211> 560
<212> DNA
<213> Gossypium hirsutum

<220>
<221> n = a, t, c, or g
<222> (1)..(560)
<223> n = a, t, c, or g

<400> 16

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aaacctttta aaagtaaaan aaaaaaaca aacccctttgt tccccctccc gatctcagaa 180
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<210> 17
<211> 738
<212> DNA
<213> Gossypium hirsutum

<220>
<221> n = a, t, c, or g
<222> (1)..(738)
<223> n = a, t, c, or g

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<210> 18
<211> 557
<212> DNA
<213> Gossypium hirsutum

<400> 18

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taaaaaaag aaaaaaagca aaaaaactaa aaaaaaaaa tttttaccct ttttctgaaaa tccggccacc 180
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ttttctaaa tttttttgtct ttatgaaatga cgcgctttttt cattataaacacgata 360
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gaatccgacgt tgttgctgtac ggaagggcta attgcaacct tggccctttc gctttttatt 480
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<210> 19
<211> 450
<212> DNA
<213> Gossypium hirsutum

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cgagattaa aaggctaacta catggccaga caagtaggg ttattctccg gtacaattgc 180
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<210> 20
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<212> DNA
<213> Gossypium hirsutum
pa_53664

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tccgtaaa 548

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pa_53664

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<211> 645
<212> DNA
<213> Gossypium hirsutum
<400> 23

<210> 24
<211> 611
<212> DNA
<213> Gossypium hirsutum
<400> 24

<210> 25
<211> 611
<212> DNA
<213> Gossypium hirsutum
<400> 25
DNA

Gossypium hirsutum

25

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26

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gatgcaaaga gggttgcgc ac tggaaaaacct 631

27

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<210>  28
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<212>  DNA
<213>  Gossypium hirsutum

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<210>  29
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<212>  DNA
<213>  Gossypium hirsutum

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<210> 30
Gossypium hirsutum

30

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31

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32

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<400>  34
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cagtgtttttg aagaaacata ttcggtttttttgttgattttgct tgtttttttatt 180
tgattattaa aagacactcattc tctctctctg agttttaaat tcctctctct ttcagatttt 240
tttgaatatt gtagcccaagct tcatgggcct ctaacagcttt gcggagggc catctttaagc 300
acaatagcct taacacgaacc tccctgtttt gsgagagcag agaaagaaagc teggggagaga 360
gttttgcatc gtttttttttttt gactttctctc aaaccttttat aaaaatccttcc cgggcttttt 420
tttctttttt cgttttataag ttcggttttct cggtcacctg gatgctgacat ccactcagctt 480
Page 15
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<210>  35
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<213>  Gossypium hirsutum

<220>
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<222>  (1)...(789)
<223>  n = a, t, c, or g
<br>400>  35

<210>  36
<211>  687
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<213>  Gossypium hirsutum
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<213>  Gossypium hirsutum
<400>  37

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aatagtcctg tcgtgggtac gtttaggtgta actgtacact gcgctgaaga tgtaccaag 180
gcattgcaaa aaactcgc aaaaagtcc aaaaagttt tagtattctc tcttagtatgt 240
cctaaatact accttattga aatagatatg atgtatatat gacattttgtg tgaactcgcga 300
tgaattgagta agatacacttg gccagttgtat gcaaaaaagg gagcaatttc tgtgaaagggt 360
gaaactctgctt cacaaaccata cttccccagct acatgggaag cattggaagc acctcagat 420
tccggtaaggct ctaaaactat gttgatctgg caaaaagctc caggagacta 480
tgtaagttcg aagcatcgcgc gcgctcctgc aactagttgg aacactccaccc tcttatggcag 540
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gcgcggctcaggtctcatca 620

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<213>  Gossypium hirsutum
<400>  38

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aaccaccactaa aagggcgtt caaaacggccc ccaaaaagcaca cgtatacgcg cattagaacc 180
ggcgctttttaa gatgcatagct ctcggaagttg gctgacccag tttcagtactgc catcactatc 240
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cacctttcaac agatactatt tattccccaga cttttttttttt cttatagcttt 420
taatctggtttt ccccttaacc caaagtaattg aacaacactct gcgcgctacag ccattatatatatc 480
atatcatcat cttacctgaga aactagaaa ttttagatctt tcatctaatc aacaccttt 540
aaacatcatatatctctctatcc aatacactgtg caccatctcag cccctgactg 600
aatttagtcagtc aataaggctca atctggtggtt acttggaactt cccctggctga ggccttggagt 660

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tatacgcttgc

<210> 39
<211> 30
<212> DNA
<213> Artificial Sequence

<220> Description of artificial sequence: synthetic primer
<223>
<400> 39
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<210> 40
<211> 25
<212> DNA
<213> Artificial Sequence

<220> Description of artificial sequence: synthetic primer
<223>
<400> 40
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<210> 41
<211> 34
<212> DNA
<213> Artificial Sequence

<220> Description of artificial sequence: synthetic primer
<223>
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<210> 42
<211> 32
<212> DNA
<213> Artificial Sequence

<220> Description of artificial sequence: synthetic primer
<223>
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<210> 43
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<212> DNA
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<210> 45
<211> 18
<212> DNA
<213> Artificial Sequence
<220>
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<400> 45
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<210> 46
<211> 16
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<213> Artificial Sequence
<220>
<223> Description of artificial sequence: synthetic probe
<400> 46
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<210> 47
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<220>
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<400> 47
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<210> 48
<211> 15
<212> DNA
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<220>
<223> Description of artificial sequence: synthetic probe
<400> 48
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<210> 49
<211> 18
Description of artificial sequence: synthetic probe

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Description of artificial sequence: synthetic probe
cctttgtag ctttttt

Description of artificial sequence: synthetic probe
tgataacggg atttatt

Description of artificial sequence: synthetic probe
gatcaatccg atgaaca

Description of artificial sequence: synthetic probe
attaatgaa aaacggg
Description of artificial sequence: synthetic probe

54

gaggttttat tacaaca

55
56
DNA
Artificial Sequence

55
56
tactgtctcc ttgtaga

56
57
16
DNA
Artificial Sequence

57
58
16
DNA
Artificial Sequence

58
59
tttagggcaat taaagaa

59
tttattagtg ttcatc

58
tttattttagt ttcac

59
Description of artificial sequence: synthetic probe

DNA Artificial Sequence

Description of artificial sequence: synthetic probe

DNA Artificial Sequence

Description of artificial sequence: synthetic probe

Gossypium hirsutum

n = a, t, c, or g

n = a, t, c, or g

attgagcttc acaatccaa acaaggacat aatcatgttaa acaaaccaca cttatgtcaat
actgatctct atctatgtcc acagccaagt cactactaa aacttaacaac ctatgtgcaa
taacataaga tgacaatlcc tactaagcttc ataatgcata tttggtcna ttacagncat
tgcttattt gctnattttc aagttggtgcc taatgtttga ttcatataaa gttcaagtcag
ttgcttagat ggataacac aagcaatncg gatcaacaca taacagaaa taatgtctata
tgaatagac attatagcag gctgatccac tatttttgtca acaaaccaca atgatggagc
aatgtatgt atctttaatg catcaacaa cttgtatgtt gtcataaat ctcctccac 420
cngaagttg atgatctatt taatgtcaaa taatagtttc atcaataata ccaatgagaa 480
taatagttc taagggtaa ttttccagg tgcaggttg aagtagttga agaaacaaaa 540
ccttcacgc gacaccacca ccaagcgcag cttatggcgt cttgggaatt ctatcttacg 600
gatgaccttg caagccctgga taatagacgc gntccacctg cccttgataat ttgataag 658

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<n212> DNA
<n213> Gossypium hirsutum

<n220>
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<n222> (1) .. (802)
<n223> n = a, t, c, or g

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aggcaactga cctgactttta tattgagcctt aatttaggca acaactgaat aatgtcataa 180
aacgcaatgn ctgtaatngc ccaaatagtg cattataagc ttgtacggaatttgcatctc 240
atgtattgct ataggttttgta taagtattga ttagtgactt ggtgtgggac atagatagag 300
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gggatttgaat ataacctttgc tctgcttcacgc tctcgagttgga gacactttagga 480
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ggttttaaat gaccgcctcta ttgatttttg gtttattggtg cttctctggaag 600
tctgttcttc attttaggtg gatcttaggc cccccgcgttaa taattggaatttgctttttg 660
aatccccatca atggggggat tgttttttaa atggttttac aggggattaa 720
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<n222> (1) .. (624)
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pa_53664
gagaGCTAATA CATTCAAAA ACATGCATCT ACTATGAGT TCTTTAAATA TCGCCCTAT 180
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gatgttctctt CAGGGAAAT CATTGATGTT AATGCCTGA TACCTGCAAA 300
agctttctgc gcaaatgtgaa acgggacatt cctgtgtagc tgaGCTTCCA TAGCCNTAAG 360	
ttcctctgcag cagcccacaag ccgcagtct cgtagcctat tagagttcc ttttatcaaa 420
agttgcatgc aactgatagt ctactttgtt ataaacctcc tgaGCTCCTG AACTGCTACT 480
cgtatttaag gtacctnctc tttccccgca ccccccaagt ttggctagtgt acttttgagac 540
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<210> 66
<211> 774
<212> DNA
<213> Gossypium hirsutum

<220>
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<222> (1) .. (774)
<223> n = a, t, c, or g

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gaagagaaag attccacgng cctattggtg aatgttaacc taatttgagat atttatagtga 180
aagagatttt taattatcgt atctctttgtc tgtctgtctat attttatatg tgtttttctt 240		
tcctactgtc gctttattcg tctgttaact tctgttgagaa ggagaatttt ttttaagct 300
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<210> 67
<211> 170
<212> DNA
<213> Gossypium hirsutum

<220>
<221> n = a, t, c, or g
<222> (1) .. (170)
<223> n = a, t, c, or g

Page 24
<400> 67
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    tcatctctc cagtctctct nagtcttttt ctttggtdct tcatcttttt tttgaaaaa  120
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<210> 68
<211> 551
<212> DNA
<213> Gossypium hirsutum

<220> n = a, t, c, or g
<221> (1) .. (551)
<223> n = a, t, c, or g

<400> 68
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    tttggactat cgtttatgg gcncgggtct aaaatttggc cctacaacta taatgcataat  180
    tttattatat taaaaacccac aaataaatat aataaatat ttattgttat taataatatatat  240
    tttaatatg tttacttgg ggttcgatta tttattggat natttttttt aagttttgga  300
    taatcgattt aattgtttat ggataaataa ttaatatatat ataatttttt aacataatatata  360
    attaatatatattttttttt aacataatatattttgcgata gttttgggct aaaaaatcttct  420
    tacccaatgc tcggctcata taaaaatgg tcccaaattt tacaaaaaaa tttttttttttta  480
    gattttgtat ttattcacaac cccctttttatttttaggttaa attttttaaatttccagat  540
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<210> 69
<211> 635
<212> DNA
<213> Gossypium hirsutum

<220> n = a, t, c, or g
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<223> n = a, t, c, or g

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    ttactaactc tttcatgccc tttaaactct ttggtgctatt gtagaatog tggaatcaca  180
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    accaccacag ctttggtata gcacatccttg tcctttgcctttcttaatgta ttcaagggtta  360
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tgggtgtatat ttcattgtgtg tntgaagcca ttactttggt tgtcttttg tggagaaat 480
cacccaaacct tcgagcggat aacaataaag ccaagttga tggcatcagc 540
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<210>  70
<211>  583
<212>  DNA
<213>  Gossypium hirsutum

<220>  n = a, t, c, or g
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<223>  n = a, t, c, or g
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gctcttcggc ttcttaagtt gtgaatttc ccgtaacga tttccnagac agttgtaagc 180
ttagtagatt gganggcaga gttttccagg aagaaacangc ttctttcttc tggagagctt 240
tgcctctctt ccccatcttc atctcttatg ttcttcaaac cagctttggtt 300
attctttgca atgtagntnc acaatagagct gcaaggatta attacaataa ggttcaattt 360
attttacttg aataaacaag angaatgtca tttatatttag ttctcttaga agttgaaatc 420
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<210>  71
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<213>  Gossypium hirsutum

<220>  n = a, t, c, or g
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<223>  n = a, t, c, or g
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caataatcc acctcaccatc tgaacccctcc ttccttaaaa tccaggttca ccagcgagggt 180
cctcccgccct gtacacttaaaa tccggtcttaa atacacactc tctcgttctctt cccccacaga 240
gatcttttcga cggtagatcg ggaataaggg tgtacgtgcag aatgcticaattccttccc 300
tagctcagg agaattggga gttaaggcaatt gcttttggag cttcgaagcc gatcttataa 360
tgcctcaagac cttcgttcat gcagaagatc tgggaagact aatcaagccca tcatgaaaaa 420
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<210>  
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<213>  Gossypium hirsutum
<220>  
<221>  n = a, t, c, or g
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<223>  n = a, t, c, or g
<400>  72

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ggcaagtta acacccaagcg tcaacctccag cactgtaata aagggcaaga agcggtgtctt 180
gctgagccag atttgntggttt attacaacct agtagagggaa agagtggggttt ccccggttgtg 240
gtctccgga aacctcaatgac taccaattgttt gcaaggtttt tgtggtgtgac 300
atcggccgta attcggtcag cattgatctt atgtcagaaga agggaggt attctctgct 360
aaaccattgc ttgctatgtg aagggcgcgg tttggcacccc cttgtctactc gtttcttggtg 420
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<210>  
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<213>  Gossypium hirsutum
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<221>  n = a, t, c, or g
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<223>  n = a, t, c, or g
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cacaaccact acacccctga taataactccat tcaatntatct tgtacccctta gtgtattgtat 240
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tccgatcaca cttgcattct cttgctttgcact agaatnttcttc actttcaatnt atttcttttg 360
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aacaccaacat atccaaaatttt ttcagaggttg gtttccctct aaaaataattt catgtcattaga 540
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a 661

<210>  74
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<222>  (1)..<(472)
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gagtacttgat tttcacaacca taaactgctgg ctaaatagtt gctttngaat tgggtcagc 240
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gttcgcatttc aatctttactt tcanggctct tgccttacagc gattgatacct tatttttttc 360
gcaatcaacc aatttattaat gtttaacattt caataataact cttcaaaaaatt tactgacctc 420
accattgtac aaagactttaa tccatttcttc attaagcttc tgcctcaaaaa aa 472

<210>  75
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<212>  DNA
<213>  Gossypium hirsutum
<220>
<221>  n = a, t, c, or g
<222>  (1)..<(662)
<223>  n = a, t, c, or g 
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ttgtaaatttg tgaatagctt gggtttctttat gacataatag gatattttgctt gatcttttaa 240
tngtggtaaaa ttgcaatgat ttctattttnn cagccttaaa gacttaaatttg taaagaagtt 300
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gaataaagat taagatttgt aaattttgatt atatagatca agaagaagcaa cgagtacataat 420
tagatcagg aaaaagataaa gtattttggtt gaacgatatg ttttttcgatt ttttttcttgc 480
ggttaagttcg tgttaattttaa tttcggntttt gtaatgatgttgaattagatta tctgatattgaa 540
tttgtaaattt attttgtata attatcaagc atataacccga cgacgtacga aagatatactga 600
gccccctttg aacccatgga attctagtgga tataaataac attgtcattag ggttaccgat 660

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<210>  76  
<211>  711  
<212> DNA  
<213> Gossypium hirsutum  
<220>  
<221>  n = a, t, c, or g  
<222> (1)..<(711)  
<223>  n = a, t, c, or g  
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aagtgtcgt gatatcaatg gggttagtgt atggcaacaa gaaacaaggg aagggccaag 180  
gcggagagta agggagact gcaaggggtg tggcagaca aatgagttag gattctctttt 240  
tctataccac tgcagagagaggataacgc atggagacgt gcggctcacc ccggccgctt 300  
aggtgtgtct cagggacgct cttgaaaaag ataggggtta aaccttttcat cccctttctt 360  
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<210>  77  
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<213> Gossypium hirsutum  
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<223>  n = a, t, c, or g  
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aaagtgattg ctcctcaat atctttgtcc gcgctgaggt aggctgtgag cggggagagc 180  
tgctgaaatc accatcattc gtttgacgag tggcagaca aatgagttag gattctctttt 240  
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gaggtgtcct cttttaatgt tgggttaacc gcaacaaatt aagtaaaatg cttcagaca 360  
tcattacacc atagctaatc aaatatatag agaatatact caaccttctct gttgggataa 420  
tctgagaagat cgtcgctgag atgcagagtt ccacgctgag cactcccaa ccataattat ggttttttttt 480
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<210>  78  
<211>  610  
<212> DNA  
<213> Gossypium hirsutum  

<220>  
<221> n = a, t, c, or g  
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<400>  78  

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acgtctttca aattnctnca aatgtctttac acggnngttt cttttggaaac ngttatatattt  
gaaaaaccg ttgctcttca aattccggttt tttatcgtca ctatgctaga aaccaaattt  
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<210>  79  
<211>  608  
<212> DNA  
<213> Gossypium hirsutum  

<220>  
<221> n = a, t, c, or g  
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<223> n = a, t, c, or g  

<400>  79  

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gtaagagaag cacgtnaac cagaagtagttt gaaaaaaacg tggatatggttttattagaga  
caacctttt ttgtaaaaaa taaaataaca aataacttaaa tagttaaca caaaaatcaca  

Page 30
acataactta aaccaaaatt cgaataaaac caattattct ttaacacaca tgaggattca 540
aacctgagac cngaaggtaa actaacacac atccaaccc cgaaccaaca atctcattcc 600
gacattag

<210>  80
<211>  655
<212>  DNA
<213>  Gossypium hirsutum

<220>  n = a, t, c, or g
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<222>  n = a, t, c, or g
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atatgttctt atagatattct cacactttgtg atctgattat gttgaggac cttgtaatttc 600
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<210>  81
<211>  549
<212>  DNA
<213>  Gossypium hirsutum

<220>  n = a, t, c, or g
<221>  (1)...(549)
<222>  n = a, t, c, or g
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<210> 82
<211> 634
<212> DNA
<213> Gossypium hirsutum
<220>
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<222> (1)..(634)
<223> n = a, t, c, or g
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catgtgttta aagttccata aagaattctta tgtgtaaggt gtctctattgg aatattagggaa 480
tttaattgaa taatattgcaa aacctggatt ctagaagttta tgttatgaa attgcttttgag 540
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<210> 83
<211> 681
<212> DNA
<213> Gossypium hirsutum
<220>
<221> n = a, t, c, or g
<222> (1)..(681)
<223> n = a, t, c, or g
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gatgtgttta aacccgcatct atctgtgtcat tgtcagaaaaacgtgtgatgttta tgtcagacgag 180
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gctgttggg gatgttccctt ggaagtacgt atagttttac tcttttacat 480
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atnangannn cttctgcgggct 681

<210> 84
<211> 667
<212> DNA
<213> Gossypium hirsutum

<220>
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<222> (1) .. (667)
<223> n = a, t, c, or g

<400> 84

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<210> 85
<211> 648
<212> DNA
<213> Gossypium hirsutum

<220>
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<222> (1) .. (648)
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360
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480
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<210> 86
<211> 617
<212> DNA
<213> Gossypium hirsutum
<220>
<221> n = a, t, c, or g
<222> (1) .. (617)
<223> n = a, t, c, or g
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<211> 607
<212> DNA
<213> Gossypium hirsutum
<220>
<221> n = a, t, c, or g
<222> (1) .. (607)
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taactagcataagagaga atgggaagtttctagcagagccccagtgagtcagagcc 180
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pa_53664

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Description of artificial sequence: synthetic primer

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96

DNA

Artificial Sequence

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Artificial Sequence

98
dna

Artificial Sequence

99
dna

Artificial Sequence

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Description of artificial sequence: synthetic probe

DNA Artificial Sequence

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Description of artificial sequence: synthetic probe

DNA Artificial Sequence

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