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(54) Titre : COMPOSITIONS ET METHODES UTILISANT L'ARN INTERFERENCE D'UN GENE DU TYPE OPR3 POUR LA LUTTE CONTRE LES NEMATODES

(54) Title: COMPOSITIONS AND METHODS USING RNA INTERFERENCE OF OPR3-LIKE GENE FOR CONTROL OF NEMATODES

(57) Abrégé/Abstract:
The present invention concerns double stranded RNA compositions and transgenic plants capable of inhibiting expression of genes essential to establishing or maintaining nematode infestation in a plant, and methods associated therewith. Specifically, the invention relates to the use of RNA interference to inhibit expression of a target OPR3-like plant gene, and relates to the generation of plants that have increased resistance to parasitic nematodes.

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(54) Title: COMPOSITIONS AND METHODS USING RNA INTERFERENCE OF OPR3-LIKE GENE FOR CONTROL OF NEMATODES

(57) Abstract: The present invention concerns double stranded RNA compositions and transgenic plants capable of inhibiting expression of genes essential to establishing or maintaining nematode infestation in a plant, and methods associated therewith. Specifically, the invention relates to the use of RNA interference to inhibit expression of a target OPR3-like plant gene, and relates to the generation of plants that have increased resistance to parasitic nematodes.

COMPOSITIONS AND METHODS USING RNA INTERFERENCE OF OPR3-LIKE GENE FOR CONTROL OF NEMATODES

CROSS REFERENCE TO RELATED APPLICATIONS

[Para 1] This application claims the priority benefit of U.S. Provisional Application Serial No.60/900,146 filed February 08, 2007.

FIELD OF THE INVENTION

[Para 2] The invention relates to the control of nematodes. Disclosed herein are methods of producing transgenic plants with increased nematode resistance, expression vectors comprising polynucleotides conferring nematode resistance, and transgenic plants and seeds generated thereof.

BACKGROUND OF THE INVENTION

[Para 3] Nematodes are microscopic roundworms that feed on the roots, leaves and stems of more than 2,000 row crops, vegetables, fruits, and ornamental plants, causing an estimated $100 billion crop loss worldwide. A variety of parasitic nematode species infect crop plants, including root-knot nematodes (RKN), cyst- and lesion-forming nematodes. Root-knot nematodes, which are characterized by causing root gall formation at feeding sites, have a relatively broad host range and are therefore pathogenic on a large number of crop species. The cyst- and lesion-forming nematode species have a more limited host range, but still cause considerable losses in susceptible crops.

[Para 4] Pathogenic nematodes are present throughout the United States, with the greatest concentrations occurring in the warm, humid regions of the South and West and in sandy soils. Soybean cyst nematode (Heterodera glycines), the most serious pest of soybean plants, was first discovered in the United States in North Carolina in 1954. Some areas are so heavily infested by soybean cyst nematode (SCN) that soybean production is no longer economically possible without control measures. Although soybean is the major economic crop attacked by SCN, SCN parasitizes some fifty hosts in total, including field crops, vegetables, ornamentals, and weeds.

[Para 5] Signs of nematode damage include stunting and yellowing of leaves, and wilting of the plants during hot periods. However, nematode infestation can cause significant yield losses without any obvious above-ground disease symptoms. The primary causes of yield reduction are due to root damage underground. Roots infected by SCN are dwarfed or stunted. Nematode infestation also can decrease the number of nitrogen-fixing nodules on the roots, and may make the roots more susceptible to attacks by other soil-borne plant pathogens.

[Para 6] The nematode life cycle has three major stages: egg, juvenile, and adult. The life cycle varies between species of nematodes. For example, the SCN life cycle can usually be

completed in 24 to 30 days under optimum conditions whereas other species can take as long as a year, or longer, to complete the life cycle. When temperature and moisture levels become favorable in the spring, worm-shaped juveniles hatch from eggs in the soil. Only nematodes in the juvenile developmental stage are capable of infecting soybean roots.

[Para 7] The life cycle of SCN has been the subject of many studies, and as such are a useful example for understanding the nematode life cycle. After penetrating soybean roots, SCN juveniles move through the root until they contact vascular tissue, at which time they stop migrating and begin to feed. With a stylet, the nematode injects secretions that modify certain root cells and transform them into specialized feeding sites. The root cells are morphologically transformed into large multinucleate syncytia (or giant cells in the case of RKN), which are used as a source of nutrients for the nematodes. The actively feeding nematodes thus steal essential nutrients from the plant resulting in yield loss. As female nematodes feed, they swell and eventually become so large that their bodies break through the root tissue and are exposed on the surface of the root.

[Para 8] After a period of feeding, male SCN nematodes, which are not swollen as adults, migrate out of the root into the soil and fertilize the enlarged adult females. The males then die, while the females remain attached to the root system and continue to feed. The eggs in the swollen females begin developing, initially in a mass or egg sac outside the body, and then later within the nematode body cavity. Eventually the entire adult female body cavity is filled with eggs, and the nematode dies. It is the egg-filled body of the dead female that is referred to as the cyst. Cysts eventually dislodge and are found free in the soil. The walls of the cyst become very tough, providing excellent protection for the approximately 200 to 400 eggs contained within. SCN eggs survive within the cyst until proper hatching conditions occur. Although many of the eggs may hatch within the first year, many also will survive within the protective cysts for several years.

[Para 9] A nematode can move through the soil only a few inches per year on its own power. However, nematode infestation can be spread substantial distances in a variety of ways. Anything that can move infested soil is capable of spreading the infestation, including farm machinery, vehicles and tools, wind, water, animals, and farm workers. Seed sized particles of soil often contaminate harvested seed. Consequently, nematode infestation can be spread when contaminated seed from infested fields is planted in non-infested fields. There is even evidence that certain nematode species can be spread by birds. Only some of these causes can be prevented.

[Para 10] Traditional practices for managing nematode infestation include: maintaining proper soil nutrients and soil pH levels in nematode-infested land; controlling other plant diseases, as

well as insect and weed pests; using sanitation practices such as plowing, planting, and cultivating of nematode-infested fields only after working non-infested fields; cleaning equipment thoroughly with high pressure water or steam after working in infested fields; not using seed grown on infested land for planting non-infested fields unless the seed has been properly cleaned; rotating infested fields and alternating host crops with non-host crops; using nematicides; and planting resistant plant varieties.

[Para 11] Methods have been proposed for the genetic transformation of plants in order to confer increased resistance to plant parasitic nematodes. U.S. Patent Nos. 5,589,622 and 5,824,876 are directed to the identification of plant genes expressed specifically in or adjacent to the feeding site of the plant after attachment by the nematode. The promoters of these plant target genes can then be used to direct the specific expression of detrimental proteins or enzymes, or the expression of antisense RNA to the target gene or to general cellular genes. The plant promoters may also be used to confer nematode resistance specifically at the feeding site by transforming the plant with a construct comprising the promoter of the plant target gene linked to a gene whose product induces lethality in the nematode after ingestion.

[Para 12] Recently, RNA interference (RNAi), also referred to as gene silencing, has been proposed as a method for controlling nematodes. When double-stranded RNA (dsRNA) corresponding essentially to the sequence of a target gene or mRNA is introduced into a cell, expression from the target gene is inhibited (See e.g., U.S. Patent No. 6,506,559). U.S. Patent No. 6,506,559 demonstrates the effectiveness of RNAi against known genes in Caenorhabditis elegans, but does not demonstrate the usefulness of RNAi for controlling plant parasitic nematodes.

[Para 13] Use of RNAi to target essential nematode genes has been proposed, for example, in WO 01/96584, WO 01/37654, US 2004/0098761, US 2005/0091713, US 2005/0188438, US 2006/0037101, US 2006/0080749, US 2007/0199100, and US 2007/0250947.

[Para 14] A number of models have been proposed for the action of RNAi. In mammalian systems, dsRNAs larger than 30 nucleotides trigger induction of interferon synthesis and a global shut-down of protein syntheses, in a non-sequence-specific manner. However, U.S. Patent No. 6,506,559 discloses that in nematodes, the length of the dsRNA corresponding to the target gene sequence may be at least 25, 50, 100, 200, 300, or 400 bases, and that even larger dsRNAs were also effective at inducing RNAi in C. elegans. It is known that when hairpin RNA constructs comprising double stranded regions ranging from 98 to 854 nucleotides were transformed into a number of plant species, the target plant genes were efficiently silenced. There is general agreement that in many organisms, including nematodes and plants,

large pieces of dsRNA are cleaved into about 19-24 nucleotide fragments (siRNA) within cells, and that these siRNAs are the actual mediators of the RNAi phenomenon.

[Para 15] The OPR3 enzyme (12-oxyphytodienoate reductase) is involved in jasmonic acid (JA) biosynthesis. The OPR3 enzyme converts 12-oxo-cis-10,15-phytodienoate (OPDA) to 3-oxo-2-cis(cis-2-pentenyl)-cyclopentane-1-octanoate, which undergoes 3 rounds of beta oxidation to generate (+)-7-isojasmonate (JA). Arabidopsis opr3 mutant plants are unable to accumulate JA and are male sterile (Stintzi and Browse, 2000, PNAS. 97:10625-10630). Treating Arabidopsis opr3 plants with exogenous OPDA up-regulated several genes and disclosed two distinct signal pathways, one through COI1 and one through an electrophile effect of the cyclopentones (Stintzi et al., 2001, PNAS 98:12317-12319). OPDA in concert with JA fine-tunes the expression of defense genes. Resistance to certain insects and fungi can occur in the absence of JA (Stintzi et al., 2001, PNAS 98:12837-42).

[Para 16] Although there have been numerous efforts to use RNAi to control plant parasitic nematodes, to date no transgenic nematode-resistant plant has been deregulated in any country. Accordingly, there continues to be a need to identify safe and effective compositions and methods for the controlling plant parasitic nematodes using RNAi, and for the production of plants having increased resistance to plant parasitic nematodes.

SUMMARY OF THE INVENTION

[Para 17] The present inventors have discovered that the soybean target gene, 12-oxyphytodienate reductase-like (OPR3-like) also designated as 45174942 (SEQ ID NO: 1), is up-regulated in SCN-induced syncytia compared to uninfected root tissue. The present inventors have demonstrated that inhibition of OPR3 levels using RNAi affects the ability of the plant to resist nematode infestation.

[Para 18] In a first embodiment, therefore, the invention provides a double stranded RNA (dsRNA) molecule comprising a) a first strand comprising a sequence substantially identical to a portion of an OPR3-like gene and b) a second strand comprising a sequence substantially complementary to the first strand.

[Para 19] The invention is further embodied in a pool of dsRNA molecules comprising a multiplicity of RNA molecules each comprising a double stranded region having a length of about 19 to 24 nucleotides, wherein said RNA molecules are derived from a polynucleotide being substantially identical to a portion of an OPR3-like gene.

[Para 20] In another embodiment, the invention provides a transgenic nematode-resistant plant capable of expressing a dsRNA that is substantially identical to a portion of an OPR3-like gene.

[Para 21] In another embodiment, the invention provides a transgenic plant capable of expressing a pool of dsRNA molecules, wherein each dsRNA molecule comprises a double stranded region having a length of about 19-24 nucleotides and wherein the RNA molecules are derived from a polynucleotide substantially identical to a portion of an OPR3-like gene.

[Para 22] In another embodiment, the invention provides a method of making a transgenic plant capable of expressing a pool of dsRNA molecules each of which is substantially identical to a portion of an OPR3-like gene in a plant, said method comprising the steps of: a) preparing a nucleic acid having a region that is substantially identical to a portion of an OPR3-like gene, wherein the nucleic acid is able to form a double-stranded transcript of a portion of an OPR3-like gene once expressed in the plant; b) transforming a recipient plant with said nucleic acid; c) producing one or more transgenic offspring of said recipient plant; and d) selecting the offspring for expression of said transcript.

[Para 23] The invention further provides a method of conferring nematode resistance to a plant, said method comprising the steps of: a) preparing a nucleic acid having a region that is substantially identical to a portion of an OPR3-like gene, wherein the nucleic acid is able to form a double-stranded transcript of a portion of a OPR3-like gene once expressed in the plant; b) transforming a recipient plant with said nucleic acid; c) producing one or more transgenic offspring of said recipient plant; and d) selecting the offspring for nematode resistance.

[Para 24] The invention further provides a expression cassette and an expression vector comprising a sequence substantially identical to a portion of an OPR3-like gene.

[Para 25] In another embodiment, the invention provides a method for controlling the infection of a plant by a parasitic nematode, comprising the steps of transforming the plant with a dsRNA molecule operably linked to a root-preferred, nematode inducible or feeding site-preferred promoter, whereby the dsRNA comprising one strand that is substantially identical to a portion of a target nucleic acid essential to the formation, development or support of the feeding site, in particular the formation, development or support of a syncytia or giant cell, thereby controlling the infection of the plant by the nematode by removing or functionally incapacitating the feeding site, syncytia or giant cell, wherein the target nucleic acid is an OPR3-like gene.

BRIEF DESCRIPTION OF THE DRAWINGS

[Para 26] Figure 1 shows the table of SEQ ID NOs assigned to corresponding sequences.

[Para 27] Figures 2a-2c show amino acid alignment of OPR3-like proteins: the full length GmOPR3-like protein (SEQ ID NO:30), Q9FEW9 from tomato (Seq ID NO: 8), the protein encoded by At2g06050 from Arabidopsis (SEQ ID NO: 10), AAY27752 from Hevea (SEQ ID NO: 12), EAZ42984 from rice (SEQ ID NO: 14), AAY26527 from maize (SEQ ID NO: 16), AAY26528

from maize (SEQ ID NO: 18), EG030595 from Arachis (SEQ ID NO: 20), the protein encoded by TA29350_4113 from Solanum (SEQ ID NO: 22), the protein encoded by TA4283_3760 from Prunus (SEQ ID NO: 24), the protein encoded by TA23750_3635 from Gossypium (SEQ ID NO: 26) and the protein encoded by TA7248_49390 from Coffea (SEQ ID NO:28). The alignment was performed in Vector NTI software suite (gap opening penalty = 10, gap extension penalty = 0.05, gap separation penalty = 8). The full length GmOPR3-like sequence was determined through 5' RACE PCR as described in Example 4.

[Para 28] Figure 3 shows the global amino acid percent identity of exemplary OPR3-like genes: the full-length GmOPR3-like protein (SEQ ID NO:30), Q9FEW9 from tomato (Seq ID NO: 8), the protein encoded by At2g06050 from Arabidopsis (SEQ ID NO: 10), AAY27752 from Hevea (SEQ ID NO: 12), EAZ42984 from rice (SEQ ID NO: 14), AAY26527 from maize (SEQ ID NO: 16), AAY26528 from maize (SEQ ID NO: 18), EG030595 from Arachis (SEQ ID NO: 20), the protein encoded by TA29350_4113 from Solanum (SEQ ID NO: 22), the protein encoded by TA4283_3760 from Prunus (SEQ ID NO: 24), the protein encoded by TA23750_3635 from Gossypium (SEQ ID NO: 26) and the protein encoded by TA7248_49390 from Coffea (SEQ ID NO:28). Pairwise alignments and percent identities were calculated using Needle of EMBOSS-4.0.0 (Needleman, S. B. and Wunsch, C. D. (1970) J. Mol. Biol. 48, 443-453).

[Para 29] Figure 4 shows the global nucleotide percent identity of exemplary OPR3-like genes: the full-length GmOPR3-like DNA (SEQ ID NO:29), Q9FEW9 DNA from tomato (Seq ID NO: 7), At2g06050 DNA from Arabidopsis (SEQ ID NO: 9), AAY27752 DNA from Hevea (SEQ ID NO: 11), EAZ42984 DNA from rice (SEQ ID NO: 13), AAY26527 DNA from maize (SEQ ID NO: 15), AAY26528 DNA from maize (SEQ ID NO: 17), EG030595 DNA from Arachis (SEQ ID NO: 19), TA29350_4113 DNA from Solanum (SEQ ID NO: 21), TA4283_3760 DNA from Prunus (SEQ ID NO: 23), TA23750_3635 DNA from Gossypium (SEQ ID NO: 25) and TA7248_49390 DNA from Coffea (SEQ ID NO:27). Pairwise alignments and percent identities were calculated using Needle of EMBOSS-4.0.0.

[Para 30] Figures 5a-5j show various 21mers by nucleotide position possible for exemplary OPR3-like encoding polynucleotide of SEQ ID NO:1, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, or a polynucleotide sequence encoding an OPR3-like homolog.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[Para 31] The present invention may be understood more readily by reference to the following detailed description of the preferred embodiments of the invention and the examples included herein. Unless otherwise noted, the terms used herein are to be understood according to conventional usage by those of ordinary skill in the relevant art. In addition to the definitions

of terms provided below, definitions of common terms in molecular biology may also be found in Rieger et al., 1991 Glossary of genetics: classical and molecular, 5th Ed., Berlin: Springer-Verlag; and in Current Protocols in Molecular Biology, F.M. Ausubel et al., Eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1998 Supplement). It is to be understood that as used in the specification and in the claims, "a" or "an" can mean one or more, depending upon the context in which it is used. Thus, for example, reference to "a cell" can mean that at least one cell can be utilized. It is to be understood that the terminology used herein is for the purpose of describing specific embodiments only and is not intended to be limiting.

[Para 32] Throughout this application, various patent and literature publications are referenced. The disclosures of all of these publications and those references cited within those publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

[Para 33] A plant "OPR3-like gene" is defined herein as a gene having at least 60% sequence identity to a the 45174942 polynucleotide having the sequence as set forth in SEQ ID NO:1, which is the G. max OPR3-like gene. In accordance with the invention, OPR3-like genes include genes having sequences such as those set forth in SEQ ID NOs:2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29, which are homologs of the G. max OPR3-like gene of SEQ ID NO:1. The OPR3-like genes defined herein encode polypeptides having at least 60% sequence identity to the G. max OPR3-like polypeptide having the sequence as set forth in SEQ ID NO:30. Such polypeptides include OPR3-like polypeptides having sequences as set forth in SEQ ID NOs: 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, and 28

[Para 34] Additional OPR3-like genes (OPR3-like gene homologs) may be isolated from plants other than soybean using the information provided herein and techniques known to those of skill in the art of biotechnology. For example, a nucleic acid molecule from a plant that hybridizes under stringent conditions to the nucleic acid of SEQ ID NO:1 can be isolated from plant tissue cDNA libraries. Alternatively, mRNA can be isolated from plant cells (e.g., by the guanidinium-thiocyanate extraction procedure of Chirgwin et al., 1979, Biochemistry 18:5294-5299), and cDNA can be prepared using reverse transcriptase (e.g., Moloney MLV reverse transcriptase, available from Gibco/BRL, Bethesda, MD; or AMV reverse transcriptase, available from Seikagaku America, Inc., St. Petersburg, FL). Synthetic oligonucleotide primers for polymerase chain reaction amplification can be designed based upon the nucleotide sequence shown in SEQ ID NO:1. Additional oligonucleotide primers may be designed that are based on the sequences of the OPR3-like genes having the sequences as set forth in SEQ ID NOs: 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, and 29. Nucleic acid molecules corresponding to the OPR3-like target genes

defined herein can be amplified using cDNA or, alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid molecules so amplified can be cloned into appropriate vectors and characterized by DNA sequence analysis.

[Para 35] As used herein, “RNAi” or “RNA interference” refers to the process of sequence-specific post-transcriptional gene silencing in plants, mediated by double-stranded RNA (dsRNA). As used herein, “dsRNA” refers to RNA that is partially or completely double stranded. Double stranded RNA is also referred to as small or short interfering RNA (siRNA), short interfering nucleic acid (siNA), short interfering RNA, micro-RNA (miRNA), and the like. In the RNAi process, dsRNA comprising a first strand that is substantially identical to a portion of a target gene, e.g. an OPR3-like gene, and a second strand that is complementary to the first strand is introduced into a plant. After introduction into the plant, the target gene-specific dsRNA is processed into relatively small fragments (siRNAs) and can subsequently become distributed throughout the plant, leading to a loss-of-function mutation having a phenotype that, over the period of a generation, may come to closely resemble the phenotype arising from a complete or partial deletion of the target gene. Alternatively, the target gene-specific dsRNA is operably associated with a regulatory element or promoter that results in expression of the dsRNA in a tissue, temporal, spatial or inducible manner and may further be processed into relatively small fragments by a plant cell containing the RNAi processing machinery, and the loss-of-function phenotype is obtained. Also, the regulatory element or promoter may direct expression preferentially to the roots or syncytia or giant cell where the dsRNA may be expressed either constitutively in those tissues or upon induction by the feeding of the nematode or juvenile nematode, such as J2 nematodes.

[Para 36] As used herein, taking into consideration the substitution of uracil for thymine when comparing RNA and DNA sequences, the term “substantially identical” as applied to dsRNA means that the nucleotide sequence of one strand of the dsRNA is at least about 80%-90% identical to 20 or more contiguous nucleotides of the target gene, more preferably, at least about 90-95% identical to 20 or more contiguous nucleotides of the target gene, and most preferably at least about 95%, 96%, 97%, 98% or 99% identical or absolutely identical to 20 or more contiguous nucleotides of the target gene. 20 or more nucleotides means a portion, being at least about 20, 21, 22, 23, 24, 25, 50, 100, 200, 300, 400, 500, 1000, 1500, or 2000 consecutive bases or up to the full length of the target gene.

[Para 37] As used herein, “complementary” polynucleotides are those that are capable of base pairing according to the standard Watson-Crick complementarity rules. Specifically, purines will base pair with pyrimidines to form a combination of guanine paired with cytosine

(G:C) and adenine paired with either thymine (A:T) in the case of DNA, or adenine paired with uracil (A:U) in the case of RNA. It is understood that two polynucleotides may hybridize to each other even if they are not completely complementary to each other, provided that each has at least one region that is substantially complementary to the other. As used herein, the term “substantially complementary” means that two nucleic acid sequences are complementary over at least 80% of their nucleotides. Preferably, the two nucleic acid sequences are complementary over at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or more or all of their nucleotides. Alternatively, “substantially complementary” means that two nucleic acid sequences can hybridize under high stringency conditions. As used herein, the term “substantially identical” or “corresponding to” means that two nucleic acid sequences have at least 80% sequence identity. Preferably, the two nucleic acid sequences have at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% of sequence identity.

[Para 38] Also as used herein, the terms “nucleic acid” and “polynucleotide” refer to RNA or DNA that is linear or branched, single or double stranded, or a hybrid thereof. The term also encompasses RNA/DNA hybrids. When dsRNA is produced synthetically, less common bases, such as inosine, 5-methylcytosine, 6-methyladenine, hypoxanthine and others can also be used for antisense, dsRNA, and ribozyme pairing. For example, polynucleotides that contain C-5 propyne analogues of uridine and cytidine have been shown to bind RNA with high affinity and to be potent antisense inhibitors of gene expression. Other modifications, such as modification to the phosphodiester backbone, or the 2’-hydroxy in the ribose sugar group of the RNA can also be made.

[Para 39] As used herein, the term “control,” when used in the context of an infection, refers to the reduction or prevention of an infection. Reducing or preventing an infection by a nematode will cause a plant to have increased resistance to the nematode; however, such increased resistance does not imply that the plant necessarily has 100% resistance to infection. In preferred embodiments, the resistance to infection by a nematode in a resistant plant is greater than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% in comparison to a wild type plant that is not resistant to nematodes. Preferably the wild type plant is a plant of a similar, more preferably identical genotype as the plant having increased resistance to the nematode, but does not comprise a dsRNA directed to the target gene. The plant’s resistance to infection by the nematode may be due to the death, sterility, arrest in development, or impaired mobility of the nematode upon exposure to the plant comprising dsRNA specific to a gene essential for development or maintenance of a functional feeding site, syncytia, or giant cell. The term “resistant to nematode infection” or “a plant having nematode resistance” as used herein refers to the ability of a plant, as compared to a wild type plant, to avoid infection by nematodes, to kill nematodes or to ham-

per, reduce or stop the development, growth or multiplication of nematodes. This might be achieved by an active process, e.g. by producing a substance detrimental to the nematode, or by a passive process, like having a reduced nutritional value for the nematode or not developing structures induced by the nematode feeding site like syncytia or giant cells. The level of nematode resistance of a plant can be determined in various ways, e.g. by counting the nematodes being able to establish parasitism on that plant, or measuring development times of nematodes, proportion of male and female nematodes or, for cyst nematodes, counting the number of cysts or nematode eggs produced on roots of an infected plant or plant assay system.

[Para 40] The term “plant” is intended to encompass plants at any stage of maturity or development, as well as any tissues or organs (plant parts) taken or derived from any such plant unless otherwise clearly indicated by context. Plant parts include, but are not limited to, stems, roots, flowers, ovules, stamens, seeds, leaves, embryos, meristematic regions, callus tissue, anther cultures, gametophytes, sporophytes, pollen, microspores, protoplasts, hairy root cultures, and the like. The present invention also includes seeds produced by the plants of the present invention. In one embodiment, the seeds are true breeding for an increased resistance to nematode infection as compared to a wild-type variety of the plant seed. As used herein, a “plant cell” includes, but is not limited to, a protoplast, gamete producing cell, and a cell that regenerates into a whole plant. Tissue culture of various tissues of plants and regeneration of plants therefrom is well known in the art and is widely published.

[Para 41] As used herein, the term “transgenic” refers to any plant, plant cell, callus, plant tissue, or plant part that contains all or part of at least one recombinant polynucleotide. In many cases, all or part of the recombinant polynucleotide is stably integrated into a chromosome or stable extra-chromosomal element, so that it is passed on to successive generations. For the purposes of the invention, the term “recombinant polynucleotide” refers to a polynucleotide that has been altered, rearranged, or modified by genetic engineering. Examples include any cloned polynucleotide, or polynucleotides, that are linked or joined to heterologous sequences. The term “recombinant” does not refer to alterations of polynucleotides that result from naturally occurring events, such as spontaneous mutations, or from non-spontaneous mutagenesis followed by selective breeding.

[Para 42] As used herein, the term “amount sufficient to inhibit expression” refers to a concentration or amount of the dsRNA that is sufficient to reduce levels or stability of mRNA or protein produced from a target gene in a plant. As used herein, “inhibiting expression” refers to the absence or observable decrease in the level of protein and/or mRNA product from a target gene. Inhibition of target gene expression may be lethal to the parasitic nematode either directly or indirectly through modification or eradication of the feeding site, syncytia, or giant cell,

or such inhibition may delay or prevent entry into a particular developmental step (e.g., metamorphosis), if access to a fully functional feeding site, syncytia, or giant cell is associated with a particular stage of the parasitic nematode's life cycle. The consequences of inhibition can be confirmed by examination of the plant root for reduction or elimination of cysts or other properties of the nematode or nematode infestation (as presented below in Example 3).

[Para 43] In accordance with the invention, a plant is transformed with a nucleic acid or a dsRNA, which specifically inhibits expression of OPR3-like gene in the plant that is essential for the development or maintenance of a feeding site, syncytia, or giant cell; ultimately affecting the survival, metamorphosis, or reproduction of the nematode. In one embodiment, the dsRNA is encoded by a vector that has been transformed into an ancestor of the infected plant. Preferably, the nucleic acid sequence expressing said dsRNA is under the transcriptional control of a root specific promoter or a parasitic nematode feeding cell-specific promoter or a nematode inducible promoter.

[Para 44] Accordingly, the dsRNA of the invention comprises a first strand that is substantially identical to a portion of the OPR3-like target gene of a plant genome, and a second strand that is substantially complementary to the first strand. In preferred embodiments, the target gene is selected from the group consisting of: a) a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29; b) a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30; and c) a polynucleotide having 70% sequence identity to a polynucleotide having a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29. The length of the substantially identical double-stranded nucleotide sequences may be at least about 19, 20, 21, 22, 23, 24, 25, 50, 100, 200, 300, 400, 500, 1000, 1500, consecutive bases or up to the whole length of the OPR3-like gene. In a preferred embodiment, the length of the double-stranded nucleotide sequence is from approximately from about 19 to about 200-500 consecutive nucleotides in length. In another preferred embodiment, the dsRNA of the invention is substantially identical or is identical to bases 1 to 229 of SEQ ID NO: 2.

[Para 45] As discussed above, fragments of dsRNA larger than about 19-24 nucleotides in length are cleaved intracellularly by nematodes and plants to siRNAs of about 19-24 nucleotides in length, and these siRNAs are the actual mediators of the RNAi phenomenon. The table in Figures 5a-5j sets forth exemplary 21-mers of the OPR3-like genes defined herein. This table can also be used to calculate the 19, 20, 22, 23 or 24-mers by adding or subtracting the appropriate number of nucleotides from each 21mer. Thus the dsRNA of the present invention may range in length from about 21 nucleotides to 200 nucleotides. Preferably, the dsRNA of the invention has a length from about 21 nucleotides to 600 consecutive nucleotides or up to

the whole length of the OPR3-like gene. More preferably, the dsRNA of the invention has a length from about 21 nucleotides to 500 consecutive nucleotides, or from about 21 nucleotides to about 200 consecutive nucleotides.

[Para 46] As disclosed herein, 100% sequence identity between the RNA and the target gene is not required to practice the present invention. While a dsRNA comprising a nucleotide sequence identical to a portion of the OPR3-like gene is preferred for inhibition, the invention can tolerate sequence variations that might be expected due to gene manipulation or synthesis, genetic mutation, strain polymorphism, or evolutionary divergence. Thus the dsRNAs of the invention also encompass dsRNAs comprising a mismatch with the target gene of at least 1, 2, or more nucleotides. For example, it is contemplated in the present invention that the 21mer dsRNA sequences exemplified in Figures 5a-5j may contain an addition, deletion or substitution of 1, 2, or more nucleotides, so long as the resulting sequence still interferes with the OPR3-like gene function.

[Para 47] Sequence identity between the dsRNAs of the invention and the OPR3-like target genes may be optimized by sequence comparison and alignment algorithms known in the art (see Gribskov and Devereux, Sequence Analysis Primer, Stockton Press, 1991, and references cited therein) and calculating the percent difference between the nucleotide sequences by, for example, the Smith-Waterman algorithm as implemented in the BESTFIT software program using default parameters (e.g., University of Wisconsin Genetic Computing Group). Greater than 80 % sequence identity, 90% sequence identity, or even 100% sequence identity, between the inhibitory RNA and the portion of the target gene is preferred. Alternatively, the duplex region of the RNA may be defined functionally as a nucleotide sequence that is capable of hybridizing with a portion of the target gene transcript under stringent conditions (e.g., 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 60°C hybridization for 12-16 hours; followed by washing at 65°C with 0.1%SDS and 0.1% SSC for about 15-60 minutes).

[Para 48] When dsRNA of the invention has a length longer than about 21 nucleotides, for example from 50 nucleotides to 1000 nucleotides, it will be cleaved randomly to dsRNAs of about 21 nucleotides within the plant or parasitic nematode cell, the siRNAs. The cleavage of a longer dsRNA of the invention will yield a pool of about 21mer dsRNAs (ranging from 19mers to 24mers), derived from the longer dsRNA. This pool of about 21mer dsRNAs is also encompassed within the scope of the present invention, whether generated intracellularly within the plant or nematode or synthetically using known methods of oligonucleotide synthesis.

[Para 49] The siRNAs of the invention have sequences corresponding to fragments of 19-24 contiguous nucleotides across the entire sequence of an OPR3-like target gene. For example, a

pool of siRNA of the invention derived from the OPR3-like genes as set forth in SEQ ID NO: 1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29may comprise a multiplicity of RNA molecules which are selected from the group consisting of oligonucleotides comprising one strand which is substantially identical to the 21mer nucleotides of SEQ ID NO: 1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29 found in Figures 5a-5j. A pool of siRNA of the invention derived from OPR3-like genes described by SEQ ID NO: 1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29 may also comprise any combination of the specific RNA molecules having any of the 21 contiguous nucleotide sequences derived from SEQ ID NO: 1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29 set forth in Figures 5a-5j. Further, as noted above, multiple specialized Dicers in plants generate siRNAs typically ranging in size from 19nt to 24nt (See Henderson et al., 2006. Nature Genetics 38:721-725.). The siRNAs of the present invention may range from about 19 contiguous nucleotide sequences to about 24 contiguous nucleotide sequences. Similarly, a pool of siRNA of the invention may comprise a multiplicity of RNA molecules having any of about 19, 20, 21, 22, 23, or 24 contiguous nucleotide sequences derived from SEQ ID NO: 1, 2, 5, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24 or 26. Alternatively, the pool of siRNA of the invention may comprise a multiplicity of RNA molecules having a combination of any of about 19, 20, 21, 22, 23, and/or 24 contiguous nucleotide sequences derived from SEQ ID NO: 1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29.

[Para 50] The dsRNA of the invention may optionally comprise a single stranded overhang at either or both ends. The double-stranded structure may be formed by a single self-complementary RNA strand (i.e. forming a hairpin loop) or two complementary RNA strands. RNA duplex formation may be initiated either inside or outside the cell. When the dsRNA of the invention forms a hairpin loop, it may optionally comprise an intron, as set forth in US 2003/0180945A1 or a nucleotide spacer, which is a stretch of sequence between the complementary RNA strands to stabilize the hairpin transgene in cells. Methods for making various dsRNA molecules are set forth, for example, in WO 99/53050 and in U.S. Pat. No. 6,506,559. The RNA may be introduced in an amount that allows delivery of at least one copy per cell. Higher doses of double-stranded material may yield more effective inhibition.

[Para 51] In another embodiment, the invention provides an isolated recombinant expression vector comprising a nucleic acid encoding a dsRNA molecule as described above, wherein expression of the vector in a host plant cell results in increased resistance to a parasitic nematode as compared to a wild-type variety of the host plant cell. As used herein, the term “vector” refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a “plasmid,” which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vec-

tor, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host plant cell into which they are introduced. Other vectors are integrated into the genome of a host plant cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as “expression vectors.” In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, “plasmid” and “vector” can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., potato virus X, tobacco rattle virus, and Geminivirus), which serve equivalent functions.

[Para 52] The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host plant cell, which means that the recombinant expression vector includes one or more regulatory sequences, e.g. promoters, selected on the basis of the host plant cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. With respect to a recombinant expression vector, the terms “operatively linked” and “in operative association” are interchangeable and are intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in a host plant cell when the vector is introduced into the host plant cell). The term “regulatory sequence” is intended to include promoters, enhancers, and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990) and Gruber and Crosby, in: Methods in Plant Molecular Biology and Biotechnology, Eds. Glick and Thompson, Chapter 7, 89-108, CRC Press: Boca Raton, Florida, including the references therein. Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cells and those that direct expression of the nucleotide sequence only in certain host cells or under certain conditions. It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of dsRNA desired, etc. The expression vectors of the invention can be introduced into plant host cells to thereby produce dsRNA molecules of the invention encoded by nucleic acids as described herein.

[Para 53] In accordance with the invention, the recombinant expression vector comprises a regulatory sequence operatively linked to a nucleotide sequence that is a template for one or

both strands of the dsRNA molecules of the invention. In one embodiment, the nucleic acid molecule further comprises a promoter flanking either end of the nucleic acid molecule, wherein the promoters drive expression of each individual DNA strand, thereby generating two complementary RNAs that hybridize and form the dsRNA. In another embodiment, the nucleic acid molecule comprises a nucleotide sequence that is transcribed into both strands of the dsRNA on one transcription unit, wherein the sense strand is transcribed from the 5' end of the transcription unit and the antisense strand is transcribed from the 3' end, wherein the two strands are separated by about 3 to about 500 base pairs or more, and wherein after transcription, the RNA transcript folds on itself to form a hairpin. In accordance with the invention, the spacer region in the hairpin transcript may be any DNA fragment.

[Para 54] According to the present invention, the introduced polynucleotide may be maintained in the plant cell stably if it is incorporated into a non-chromosomal autonomous replicon or integrated into the plant chromosomes. Alternatively, the introduced polynucleotide may be present on an extra-chromosomal non-replicating vector and be transiently expressed or transiently active. Whether present in an extra-chromosomal non-replicating vector or a vector that is integrated into a chromosome, the polynucleotide preferably resides in a plant expression cassette. A plant expression cassette preferably contains regulatory sequences capable of driving gene expression in plant cells that are operatively linked so that each sequence can fulfill its function, for example, termination of transcription by polyadenylation signals. Preferred polyadenylation signals are those originating from Agrobacterium tumefaciens t-DNA such as the gene 3 known as octopine synthase of the Ti-plasmid pTiACH5 (Gielen et al., 1984, EMBO J. 3:835) or functional equivalents thereof, but also all other terminators functionally active in plants are suitable. As plant gene expression is very often not limited at transcriptional levels, a plant expression cassette preferably contains other operatively linked sequences like translational enhancers such as the overdrive-sequence containing the 5'-untranslated leader sequence from tobacco mosaic virus enhancing the polypeptide per RNA ratio (Gallie et al., 1987, Nucl. Acids Research 15:8693-8711). Examples of plant expression vectors include those detailed in: Becker, D. et al., 1992, New plant binary vectors with selectable markers located proximal to the left border, Plant Mol. Biol. 20:1195-1197; Bevan, M.W., 1984, Binary Agrobacterium vectors for plant transformation, Nucl. Acid. Res. 12:8711-8721; and Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol. 1, Engineering and Utilization, eds.: Kung and R. Wu, Academic Press, 1993, S. 15-38.

[Para 55] Plant gene expression should be operatively linked to an appropriate promoter conferring gene expression in a temporal-preferred, spatial-preferred, cell type-preferred, and/or tissue-preferred manner. Promoters useful in the expression cassettes of the invention include

any promoter that is capable of initiating transcription in a plant cell present in the plant's roots. Such promoters include, but are not limited to those that can be obtained from plants, plant viruses and bacteria that contain genes that are expressed in plants, such as Agrobacterium and Rhizobium. Preferably, the expression cassette of the invention comprises a root-specific promoter, a pathogen inducible promoter, or a nematode inducible promoter. More preferably the nematode inducible promoter is a parasitic nematode feeding site-specific promoter. A parasitic nematode feeding site-specific promoter may be specific for syncytial cells or giant cells or specific for both kinds of cells. A promoter is inducible, if its activity, measured on the amount of RNA produced under control of the promoter, is at least 30%, 40%, 50% preferably at least 60%, 70%, 80%, 90% more preferred at least 100%, 200%, 300% higher in its induced state, than in its un-induced state. A promoter is cell-, tissue- or organ-specific, if its activity , measured on the amount of RNA produced under control of the promoter, is at least 30%, 40%, 50% preferably at least 60%, 70%, 80%, 90% more preferred at least 100%, 200%, 300% higher in a particular cell-type, tissue or organ, then in other cell-types or tissues of the same plant, preferably the other cell-types or tissues are cell types or tissues of the same plant organ, e.g. a root. In the case of organ specific promoters, the promoter activity has to be compared to the promoter activity in other plant organs, e.g. leaves, stems, flowers or seeds.

[Para 56] The promoter may be constitutive, inducible, developmental stage-preferred, cell type-preferred, tissue-preferred or organ-preferred. Constitutive promoters are active under most conditions. Non-limiting examples of constitutive promoters include the CaMV 19S and 35S promoters (Odell et al., 1985, Nature 313:810-812), the sX CaMV 35S promoter (Kay et al., 1987, Science 236:1299-1302), the Sep1 promoter, the rice actin promoter (McElroy et al., 1990, Plant Cell 2:163-171), the Arabidopsis actin promoter, the ubiquitin promoter (Christensen et al., 1989, Plant Molec. Biol. 18:675-689); pEmu (Last et al., 1991, Theor. Appl. Genet. 81:581-588), the figwort mosaic virus 35S promoter, the Smas promoter (Velten et al., 1984, EMBO J. 3:2723-2730), the GRP1-8 promoter, the cinnamyl alcohol dehydrogenase promoter (U.S. Patent No. 5,683,439), promoters from the T-DNA of Agrobacterium, such as mannopine synthase, nopaline synthase, and octopine synthase, the small subunit of ribulose biphosphate carboxylase (ssuRUBISCO) promoter, and the like. Promoters that express the dsRNA in a cell that is contacted by parasitic nematodes are preferred. Alternatively, the promoter may drive expression of the dsRNA in a plant tissue remote from the site of contact with the nematode, and the dsRNA may then be transported by the plant to a cell that is contacted by the parasitic nematode in particular cells of, or close by nematode feeding sites, e.g. syncytial cells or giant cells.

[Para 57] Inducible promoters are active under certain environmental conditions, such as the presence or absence of a nutrient or metabolite, heat or cold, light, pathogen attack, anaerobic conditions, and the like. For example, the promoters TobRB7, AtRPE, AtPyk10, Gemini19, and AtHMG1 have been shown to be induced by nematodes (for a review of nematode-inducible promoters, see Ann. Rev. Phytopathol. (2002) 40:191-219; see also U.S. Pat. No. 6,593,513). Method for isolating additional promoters, which are inducible by nematodes are set forth in U.S. Pat. Nos. 5,589,622 and 5,824,876. Other inducible promoters include the hsp80 promoter from Brassica, being inducible by heat shock; the PPDK promoter is induced by light; the PR-1 promoter from tobacco, Arabidopsis, and maize are inducible by infection with a pathogen; and the Adh1 promoter is induced by hypoxia and cold stress. Plant gene expression can also be facilitated via an inducible promoter (For review, see Gatz, 1997, Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:89-108). Chemically inducible promoters are especially suitable if time-specific gene expression is desired. Non-limiting examples of such promoters are a salicylic acid inducible promoter (PCT Application No. WO 95/19443), a tetracycline inducible promoter (Gatz et al., 1992, Plant J. 2:397-404) and an ethanol inducible promoter (PCT Application No. WO 93/21334).

[Para 58] Developmental stage-preferred promoters are preferentially expressed at certain stages of development. Tissue and organ preferred promoters include those that are preferentially expressed in certain tissues or organs, such as leaves, roots, seeds, or xylem. Examples of tissue preferred and organ preferred promoters include, but are not limited to fruit-preferred, ovule-preferred, male tissue-preferred, seed-preferred, integument-preferred, tuber-preferred, stalk-preferred, pericarp-preferred, and leaf-preferred, stigma-preferred, pollen-preferred, anther-preferred, a petal-preferred, sepal-preferred, pedicel-preferred, silique-preferred, stem-preferred, root-preferred promoters and the like. Seed preferred promoters are preferentially expressed during seed development and/or germination. For example, seed preferred promoters can be embryo-preferred, endosperm preferred and seed coat-preferred. See Thompson et al., 1989, BioEssays 10:108. Examples of seed preferred promoters include, but are not limited to cellulose synthase (celA), Cim1, gamma-zein, globulin-1, maize 19 kD zein (cZ19B1) and the like.

[Para 59] Other suitable tissue-preferred or organ-preferred promoters include, but are not limited to, the napin-gene promoter from rapeseed (U.S. Patent No. 5,608,152), the USP-promoter from Vicia faba (Baeumlein et al., 1991, Mol Gen Genet. 225(3):459-67), the oleosin-promoter from Arabidopsis (PCT Application No. WO 98/45461), the phaseolin-promoter from Phaseolus vulgaris (U.S. Patent No. 5,504,200), the Bce4-promoter from Brassica (PCT Application No. WO 91/13980), or the legumin B4 promoter (LeB4; Baeumlein et al., 1992, Plant

Journal, 2(2):233-9), as well as promoters conferring seed specific expression in monocot plants like maize, barley, wheat, rye, rice, etc. Suitable promoters to note are the lpt2 or lpt1-gene promoter from barley (PCT Application No. WO 95/15389 and PCT Application No. WO 95/23230) or those described in PCT Application No. WO 99/16890 (promoters from the barley hordein-gene, rice glutelin gene, rice oryzin gene, rice prolamin gene, wheat gliadin gene, wheat glutelin gene, oat glutelin gene, Sorghum kasirin-gene, and rye secalin gene).

[Para 60] Other promoters useful in the expression cassettes of the invention include, but are not limited to, the major chlorophyll a/b binding protein promoter, histone promoters, the Ap3 promoter, the β-conglycin promoter, the napin promoter, the soybean lectin promoter, the maize 15kD zein promoter, the 22kD zein promoter, the 27kD zein promoter, the g-zein promoter, the waxy, shrunken 1, shrunken 2, and bronze promoters, the Zm13 promoter (U.S. Patent No. 5,086,169), the maize polygalacturonase promoters (PG) (U.S. Patent Nos. 5,412,085 and 5,545,546), and the SGB6 promoter (U.S. Patent No. 5,470,359), as well as synthetic or other natural promoters.

[Para 61] In accordance with the present invention, the expression cassette comprises an expression control sequence operatively linked to a nucleotide sequence that is a template for one or both strands of the dsRNA. The dsRNA template comprises (a) a first stand having a sequence substantially identical to from about 19 to 500, or up to the full length, consecutive nucleotides of an OPR3-like gene; and (b) a second strand having a sequence substantially complementary to the first strand. In further embodiments, a promoter flanks either end of the template nucleotide sequence, wherein the promoters drive expression of each individual DNA strand, thereby generating two complementary RNAs that hybridize and form the dsRNA. In alternative embodiments, the nucleotide sequence is transcribed into both strands of the dsRNA on one transcription unit, wherein the sense strand is transcribed from the 5' end of the transcription unit and the anti-sense strand is transcribed from the 3' end, wherein the two strands are separated by 3 to 500 base pairs, and wherein after transcription, the RNA transcript folds on itself to form a hairpin.

[Para 62] In another embodiment, the vector contains a bidirectional promoter, driving expression of two nucleic acid molecules, whereby one nucleic acid molecule codes for the sequence substantially identical to a portion of a OPR3-like gene and the other nucleic acid molecule codes for a second sequence being substantially complementary to the first strand and capable of forming a dsRNA, when both sequences are transcribed.. A bidirectional promoter is a promoter capable of mediating expression in two directions.

[Para 63] In another embodiment, the vector contains two promoters one mediating transcription of the sequence substantially identical to a portion of a OPR3-like gene and another pro-

moter mediating transcription of a second sequence being substantially complementary to the first strand and capable of forming a dsRNA, when both sequences are transcribed. The second promoter might be a different promoter.

[Para 64] A different promoter means a promoter having a different activity in regard to cell or tissue specificity, or showing expression on different inducers for example, pathogens, abiotic stress or chemicals. For example, one promoter might by constitutive or tissue specific and another might be tissue specific or inducible by pathogens. In one embodiment one promoter mediates the transcription of one nucleic acid molecule suitable for overexpression of an OPR3-like gene, while another promoter mediates tissue- or cell-specific transcription or pathogen inducible expression of the complementary nucleic acid.

[Para 65] The invention is also embodied in a transgenic plant capable of expressing the dsRNA of the invention and thereby inhibiting the target genes e.g. in the roots, feeding site, syncytia and/or giant cell. The plant or transgenic plant may be any plant, such like, but not limited to trees, cut flowers, ornamentals, vegetables or crop plants. The plant may be from a genus selected from the group consisting of Medicago, Lycopersicon, Brassica, Cucumis, Solanum, Juglans, Gossypium, Malus, Vitis, Antirrhinum, Populus, Fragaria, Arabidopsis, Picea, Capsicum, Chenopodium, Dendranthema, Pharbitis, Pinus, Pisum, Oryza, Zea, Triticum, Triticale, Secale, Lolium, Hordeum, Glycine, Pseudotsuga, Kalanchoe, Beta, Helianthus, Nicotiana, Cucurbita, Rosa, Fragaria, Lotus, Medicago, Onobrychis, trifolium, Trigonella, Vigna, Citrus, Linum, Geranium, Manihot, Daucus, Raphanus, Sinapis, Atropa, Datura, Hyoscyamus, Nicotiana, Petunia, Digitalis, Majorana, Ciahorium, Lactuca, Bromus, Asparagus, Antirrhinum, Heterocallis, Nemesis, Pelargonium, Panieum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Browaalia, Phaseolus, Avena, and Allium, or the plant may be selected from a genus selected from the group consisting of Arabidopsis, Medicago, Lycopersicon, Brassica, Cucumis, Solanum, Juglans, Gossypium, Malus, Vitis, Antirrhinum, Brachipodium, Populus, Fragaria, Arabidopsis, Picea, Capsicum, Chenopodium, Dendranthema, Pharbitis, Pinus, Pisum, Oryza, Zea, Triticum, Triticale, Secale, Lolium, Hordeum, Glycine, Pseudotsuga, Kalanchoe, Beta, Helianthus, Nicotiana, Cucurbita, Rosa, Fragaria, Lotus, Medicago, Onobrychis, trifolium, Trigonella, Vigna, Citrus, Linum, Geranium, Manihot, Daucus, Raphanus, Sinapis, Atropa, Datura, Hyoscyamus, Nicotiana, Petunia, Digitalis, Majorana, Ciahorium, Lactuca, Bromus, Asparagus, Antirrhinum, Heterocallis, Nemesis, Pelargonium, Panicum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Browaalia, Phaseolus, Avena, and Allium. In one embodiment the plant is a monocotyledonous plant or a dicotyledonous plant.

[Para 66] In another embodiment the plant is a crop plant. Crop plants are all plants, used in agriculture. Accordingly in one embodiment the plant is a monocotyledonous plant, preferably a

plant of the family Poaceae, Musaceae, Liliaceae or Bromeliaceae, preferably of the family Poaceae. Accordingly, in yet another embodiment the plant is a Poaceae plant of the genus Zea, Triticum, Oryza, Hordeum, Secale, Avena, Saccharum, Sorghum, Pennisetum, Setaria, Panicum, Eleusine, Miscanthus, Brachypodium, Festuca or Lolium. When the plant is of the genus Zea, the preferred species is Z. mays. When the plant is of the genus Triticum, the preferred species is T. aestivum, T. speltae or T. durum. When the plant is of the genus Oryza, the preferred species is O. sativa. When the plant is of the genus Hordeum, the preferred species is H. vulgare. When the plant is of the genus Secale, the preferred species S. cereale. When the plant is of the genus Avena, the preferred species is A. sativa. When the plant is of the genus Saccarum, the preferred species is S. officinarum. When the plant is of the genus Sorghum, the preferred species is S. vulgare, S. bicolor or S. sudanense. When the plant is of the genus Pennisetum, the preferred species is P. glaucum. When the plant is of the genus Setaria, the preferred species is S. italica. When the plant is of the genus Panicum, the preferred species is P. miliaceum or P. virgatum. When the plant is of the genus Eleusine, the preferred species is E. coracana. When the plant is of the genus Miscanthus, the preferred species is M. sinensis. When the plant is a plant of the genus Festuca, the preferred species is F. arundinaria, F. rubra or F. pratensis. When the plant is of the genus Lolium, the preferred species is L. perenne or L. multiflorum. Alternatively, the plant may be Triticosecale.

[Para 67] Alternatively, in one embodiment the plant is a dicotyledonous plant, preferably a plant of the family Fabaceae, Solanaceae, Brassicaceae, Chenopodiaceae, Asteraceae, Malvaceae, Linacea, Euphorbiaceae, Convolvulaceae Rosaceae, Cucurbitaceae, Theaceae, Rubiaceae, Sterculiaceae or Citrus. In one embodiment the plant is a plant of the family Fabaceae, Solanaceae or Brassicaceae. Accordingly, in one embodiment the plant is of the family Fabaceae, preferably of the genus Glycine, Pisum, Arachis, Cicer, Vicia, Phaseolus, Lupinus, Medicago or Lens. Preferred species of the family Fabaceae are M. truncatula, M, sativa, G. max, P. sativum, A. hypogea, C. arietinum, V. faba, P. vulgaris, Lupinus albus, Lupinus luteus, Lupinus angustifolius or Lens culinaris. More preferred are the species G. max A. hypogea and M. sativa. Most preferred is the species G. max. When the plant is of the family Solanaceae, the preferred genus is Solanum, Lycopersicon, Nicotiana or Capsicum. Preferred species of the family Solanaceae are S. tuberosum, L. esculentum, N. tabaccum or C. chinense. More preferred is S. tuberosum. Accordingly, in one embodiment the plant is of the family Brassicaceae, preferably of the genus Brassica or Raphanus. Preferred species of the family Brassicaceae are the species B. napus, B. oleracea, B. juncea or B. rapa. More preferred is the species B. napus. When the plant is of the family Chenopodiaceae, the preferred genus is Beta and the preferred species is the B. vulgaris. When the plant is of the family As-

teraceae, the preferred genus is Helianthus and the preferred species is H. annuus. When the plant is of the family Malvaceae, the preferred genus is Gossypium or Abelmoschus. When the genus is Gossypium, the preferred species is G. hirsutum or G. barbadense and the most preferred species is G. hirsutum. A preferred species of the genus Abelmoschus is the species A. esculentus. When the plant is of the family Linacea, the preferred genus is Linum and the preferred species is L. usitatissimum. When the plant is of the family Euphorbiaceae, the preferred genus is Manihot, Jatropa or Rhizinus and the preferred species are M. esculenta, J. curcas or R. comunis. When the plant is of the family Convolvulaceae, the preferred genus is Ipomea and the preferred species is I. batatas. When the plant is of the family Rosaceae, the preferred genus is Rosa, Malus, Pyrus, Prunus, Rubus, Ribes, Vaccinium or Fragaria and the preferred species is the hybrid Fragaria x ananassa. When the plant is of the family Cucurbitaceae, the preferred genus is Cucumis, Citrullus or Cucurbita and the preferred species is Cucumis sativus, Citrullus lanatus or Cucurbita pepo. When the plant is of the family Theaceae, the preferred genus is Camellia and the preferred species is C. sinensis. When the plant is of the family Rubiaceae, the preferred genus is Coffea and the preferred species is C. arabica or C. canephora. When the plant is of the family Sterculiaceae, the preferred genus is Theobroma and the preferred species is T. cacao. When the plant is of the genus Citrus, the preferred species is C. sinensis, C. limon, C. reticulata, C. maxima and hybrids of Citrus species, or the like. In a preferred embodiment of the invention, the plant is a soybean, a potato or a corn plant. In one embodiment the plant is a Fabaceae plant and the target gene is substantially similar to SEQ ID NO: 1, 2, 19 or 29. In a further embodiment the plant is a Brassicaceae plant and the target gene is substantially identical to SEQ ID NO: 9. In an alternative embodiment the plant is a Solanaceae plant and the target gene is substantially identical to SEQ ID NO: 7 or 21. In a further embodiment the plant is a Poaceae plant and the target gene is substantially identical to SEQ ID NO: 13, 15 or 17. In one embodiment the plant is a Malvaceae plant and the target gene is substantially identical to SEQ ID NO: 25.

[Para 68] Suitable methods for transforming or transfecting host cells including plant cells are well known in the art of plant biotechnology. Any method may be used to transform the recombinant expression vector into plant cells to yield the transgenic plants of the invention. General methods for transforming dicotyledenous plants are disclosed, for example, in U.S. Pat. Nos. 4,940,838; 5,464,763, and the like. Methods for transforming specific dicotyledenous plants, for example, cotton, are set forth in U.S. Pat. Nos. 5,004,863; 5,159,135; and 5,846,797. Soybean transformation methods are set forth in U.S. Pat. Nos. 4,992,375; 5,416,011; 5,569,834; 5,824,877; 6,384,301 and in EP 0301749B1 may be used.

[Para 69] Transformation methods may include direct and indirect methods of transformation. Suitable direct methods include polyethylene glycol induced DNA uptake, liposome-mediated transformation (US 4,536,475), biolistic methods using the gene gun (Fromm ME et al., Bio/Technology. 8(9):833-9, 1990; Gordon-Kamm et al. Plant Cell 2:603, 1990), electroporation, incubation of dry embryos in DNA-comprising solution, and microinjection. In the case of these direct transformation methods, the plasmids used need not meet any particular requirements. Simple plasmids, such as those of the pUC series, pBR322, M13mp series, pACYC184 and the like can be used. If intact plants are to be regenerated from the transformed cells, an additional selectable marker gene is preferably located on the plasmid. The direct transformation techniques are equally suitable for dicotyledonous and monocotyledonous plants.

[Para 70] Transformation can also be carried out by bacterial infection by means of Agrobacterium (for example EP 0 116 718), viral infection by means of viral vectors (EP 0 067 553; US 4,407,956; WO 95/34668; WO 93/03161) or by means of pollen (EP 0 270 356; WO 85/01856; US 4,684,611). Agrobacterium based transformation techniques (especially for dicotyledonous plants) are well known in the art. The Agrobacterium strain (e.g., Agrobacterium tumefaciens or Agrobacterium rhizogenes) comprises a plasmid (Ti or Ri plasmid) and a T-DNA element which is transferred to the plant following infection with Agrobacterium. The T-DNA (transferred DNA) is integrated into the genome of the plant cell. The T-DNA may be localized on the Ri- or Ti-plasmid or is separately comprised in a so-called binary vector. Methods for the Agrobacterium-mediated transformation are described, for example, in Horsch RB et al. (1985) Science 225:1229. The Agrobacterium-mediated transformation is best suited to dicotyledonous plants but has also been adapted to monocotyledonous plants. The transformation of plants by Agrobacteria is described in, for example, White FF, Vectors for Gene Transfer in Higher Plants, Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press, 1993, pp. 15 - 38; Jenes B et al. Techniques for Gene Transfer, Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press, 1993, pp. 128-143; Potrykus (1991) Annu Rev Plant Physiol Plant Molec Biol 42:205- 225.

[Para 71] Transformation may result in transient or stable transformation and expression. Although a nucleotide sequence of the present invention can be inserted into any plant and plant cell falling within these broad classes, it is particularly useful in crop plant cells.

[Para 72] Various tissues are suitable as starting material (explant) for the Agrobacterium-mediated transformation process including but not limited to callus (US 5,591,616; EP-A1 604 662), immature embryos (EP-A1 672 752), pollen (US 54,929,300), shoot apex (US 5,164,310), or in planta transformation (US 5,994,624). The method and material described herein can be combined with virtually all Agrobacterium mediated transformation methods

known in the art. The transgenic plants of the invention may be crossed with similar transgenic plants or with transgenic plants lacking the nucleic acids of the invention or with non-transgenic plants, using known methods of plant breeding, to prepare seeds. Further, the transgenic plant of the present invention may comprise, and/or be crossed to another transgenic plant that comprises one or more nucleic acids, thus creating a "stack" of transgenes in the plant and/or its progeny. The seed is then planted to obtain a crossed fertile transgenic plant comprising the nucleic acid of the invention. The crossed fertile transgenic plant may have the particular expression cassette inherited through a female parent or through a male parent. The second plant may be an inbred plant. The crossed fertile transgenic may be a hybrid. Also included within the present invention are seeds of any of these crossed fertile transgenic plants. The seeds of this invention can be harvested from fertile transgenic plants and be used to grow progeny generations of transformed plants of this invention including hybrid plant lines comprising the DNA construct.

[Para 73] "Gene stacking" can also be accomplished by transferring two or more genes into the cell nucleus by plant transformation. Multiple genes may be introduced into the cell nucleus during transformation either sequentially or in unison. Multiple genes in plants or target pathogen species can be down-regulated by gene silencing mechanisms, specifically RNAi, by using a single transgene targeting multiple linked partial sequences of interest. Stacked, multiple genes under the control of individual promoters can also be over-expressed to attain a desired single or multiple phenotype. Constructs containing gene stacks of both over-expressed genes and silenced targets can also be introduced into plants yielding single or multiple agronomically important phenotypes. In certain embodiments the nucleic acid sequences of the present invention can be stacked with any combination of polynucleotide sequences of interest to create desired phenotypes. The combinations can produce plants with a variety of trait combinations including but not limited to disease resistance, herbicide tolerance, yield enhancement, cold and drought tolerance. These stacked combinations can be created by any method including but not limited to cross breeding plants by conventional methods or by genetic transformation. If the traits are stacked by genetic transformation, the polynucleotide sequences of interest can be combined sequentially or simultaneously in any order. For example if two genes are to be introduced, the two sequences can be contained in separate transformation cassettes or on the same transformation cassette. The expression of the sequences can be driven by the same or different promoters.

[Para 74] In accordance with this embodiment, the transgenic plant of the invention is produced by a method comprising the steps of providing an OPR3-like target gene, preparing an expression cassette having a first region that is substantially identical to a portion of the se-

lected OPR3-like gene and a second region which is complementary to the first region, transforming the expression cassette into a plant, and selecting progeny of the transformed plant which express the dsRNA construct of the invention.

[Para 75] Increased resistance to nematode infection is a general trait wished to be inherited into a wide variety of plants. The present invention may be used to reduce crop destruction by any plant parasitic nematode. Preferably, the parasitic nematodes belong to nematode families inducing giant or syncytial cells. Nematodes inducing giant or syncytial cells are found in the families Longidoridae, Trichodoridae, Heterodidae, Meloidogynidae, Pratylenchidae or Tylenchulidae. In particular in the families Heterodidae and Meloidogynidae.

[Para 76] Accordingly, parasitic nematodes targeted by the present invention belong to one or more genus selected from the group of Naccobus, Cactodera, Dolichodera, Globodera, Heterodera, Punctodera, Longidorus or Meloidogyne. In a preferred embodiment the parasitic nematodes belong to one or more genus selected from the group of Naccobus, Cactodera, Dolichodera, Globodera, Heterodera, Punctodera or Meloidogyne. In a more preferred embodiment the parasitic nematodes belong to one or more genus selected from the group of Globodera, Heterodera, or Meloidogyne. In an even more preferred embodiment the parasitic nematodes belong to one or both genus selected from the group of Globodera or Heterodera. In another embodiment the parasitic nematodes belong to the genus Meloidogyne.

[Para 77] When the parasitic nematodes are of the genus Globodera, the species are preferably from the group consisting of G. achilleae, G. artemisiae, G. hypolysi, G. mexicana, G. millefolii, G. mali, G. pallida, G. rostochiensis, G. tabacum, and G. virginiae. In another preferred embodiment the parasitic Globodera nematodes includes at least one of the species G. pallida, G. tabacum, or G. rostochiensis. When the parasitic nematodes are of the genus Heterodera, the species may be preferably from the group consisting of H. avenae, H. carotae, H. ciceri, H. cruciferae, H. delvii, H. elachista, H. filipjevi, H. gambiensis, H. glycines, H. goettingiana, H. graduni, H. humuli, H. hordecalis, H. latipons, H. major, H. medicaginis, H. oryzicola, H. pakistanensis, H. rosii, H. sacchari, H. schachtii, H. sorghi, H. trifolii, H. urticae, H. vigni and H. zeae. In another preferred embodiment the parasitic Heterodera nematodes include at least one of the species H. glycines, H. avenae, H. cajani, H. gottingiana, H. trifolii, H. zeae or H. schachtii. In a more preferred embodiment the parasitic nematodes includes at least one of the species H. glycines or H. schachtii. In a most preferred embodiment the parasitic nematode is the species H. glycines.

[Para 78] When the parasitic nematodes are of the genus Meloidogyne, the parasitic nematode may be selected from the group consisting of M. acronea, M. arabica, M. arenaria, M. artiellia, M. brevicauda, M. camelliae, M. chitwoodi, M. cofeicola, M. esigua, M. graminicola, M.

hapla, M. incognita, M. indica, M. inornata, M. javanica, M. lini, M. mali, M. microcephala, M. microtyla, M. naasi, M. salasi and M. thamesi. In a preferred embodiment the parasitic nematodes includes at least one of the species M. javanica, M. incognita, M. hapla, M. arenaria or M. chitwoodi.

[Para 79] The present invention also provides a method for inhibiting expression of a OPR3-like gene. In accordance with this embodiment, the method comprises the step of administering to the plant a dsRNA of the invention.

[Para 80] The following examples are not intended to limit the scope of the claims to the invention, but are rather intended to be exemplary of certain embodiments. Any variations in the exemplified methods that occur to the skilled artisan are intended to fall within the scope of the present invention.

EXAMPLES

Example 1: Cloning of OPR3-Like Gene from Soybean

[Para 81] Glycine max cv. Williams 82 was germinated and one day later, each seedling was inoculated with second stage juveniles (J2) of H. glycines race 3. Six days after inoculation, new root tissue was sliced into 1 cm long pieces, fixed, embedded in a cryomold, and sectioned using known methods. Syncytia cells were identified by their unique morphology of enlarged cell size, thickened cell wall, and dense cytoplasm and dissected into RNA extraction buffer using a PALM microscope (P.A.L.M. Microlaser Technologies GmbH, Bernried, Germany).

[Para 82] Total cellular RNA was extracted, amplified, and fluorescently labeled using known methods. As controls, total RNA was isolated from both “non-syncytia” and untreated control roots subjected to the same RNA amplification process. The amplified RNA was hybridized to proprietary soybean cDNA arrays.

[Para 83] Soybean cDNA clone 45174942 was identified as being up-regulated in syncytia of SCN-infected soybean roots. The 45174942 cDNA sequence (SEQ ID NO:1) was determined not to be full-length as there is no ATG start codon. The remaining residues were identified in the full length sequence (SEQ ID NO: 29) corresponding to 45174942 through 5’ RACE PCR as described in Example 4.

Table 2

Gene Name	Syncytia #1(N)¶	Syncytia #2 (N)	Non-Syncytia	Control Roots
45174942§	311± 54(4)	194±46(5)	not detected	not detected

Example 2. GENERATION OF TRANSGENIC SOYBEAN ROOTS AND NEMATODE BIOASSAY

[Para 84] This exemplified method employs binary vectors containing the 45174942 target gene. The vector consists of a sense fragment (SEQ ID NO:2) of the target 45174942 gene, a spacer, antisense fragment of the target gene and a vector backbone. The target gene fragment (SEQ ID NO:2) corresponding to nucleotides 556 to 784 of SEQ ID NO:1 was used to construct the binary vectors RCB564, RCB573 and RCB582. In these vectors, dsRNA for the OPR3-like target gene was expressed under a syncytia or root preferred promoter, TPP promoter (SEQ ID NO: 4) in RCB564, A. thaliana promoter of locus At5g12170 (SEQ ID NO: 5), in RCB573 or MtN3-like promoter (SEQ ID NO: 6) in RCB582. These promoters drive transgene

expression preferentially in roots and/or syncytia or giant cells. The selection marker for transformation was the mutated form of the acetohydroxy acid synthase (AHAS) selection gene (also referred to as AHAS2) from Arabidopsis thaliana (Sathasivan et al., Plant Phys. 97:1044-50, 1991), conferring resistance to the herbicide ARSENAL (imazapyr, BASF Corporation, Mount Olive, NJ). The expression of AHAS2 was driven by a ubiquitin promoter from parsley (WO 03/102198).

Example 3. Rooted Explant Assays

[Para 85] The rooted explant assay was employed to demonstrate dsRNA expression and the resulting nematode resistance. This assay can be found in co-pending application 12/001,234, the contents of which are incorporated herein by reference.

[Para 86] Clean soybean seeds from soybean cultivar were surface sterilized and germinated. Three days before inoculation, an overnight liquid culture of the disarmed Agrobacterium culture, for example, the disarmed A. rhizogenes strain K599 containing the binary vector RCB564, RCB573 or RCB582, was initiated. The next day the culture was spread onto an LB agar plate containing kanamycin as a selection agent. The plates were incubated at 28°C for two days. One plate was prepared for every 50 explants to be inoculated. Cotyledons containing the proximal end from its connection with the seedlings were used as the explant for transformation. After removing the cotyledons the surface was scraped with a scalpel around the cut site. The cut and scraped cotyledon was the target for Agrobacterium inoculation. The prepared explants were dipped onto the disarmed thick A. rhizogenes colonies prepared above so that the colonies were visible on the cut and scraped surface. The explants were then placed onto 1% agar in Petri dishes for co-cultivation under light for 6-8 days.

[Para 87] After the transformation and co-cultivation, soybean explants were transferred to rooting induction medium with a selection agent, for example S-B5-708 for the mutated acetohydroxy acid synthase (AHAS) gene (Sathasivan et al., Plant Phys. 97:1044-50, 1991). Cultures were maintained in the same condition as in the co-cultivation step. The S-B5-708 medium comprises: 0.5X B5 salts, 3mM MES, 2% sucrose, 1X B5 vitamins, 400µg/ml Timentin, 0.8% Noble agar, and 1 µM Imazapyr (selection agent for AHAS gene) (BASF Corporation, Florham Park, NJ) at pH5.8.

[Para 88] Two to three weeks after the selection and root induction, transformed roots were formed on the cut ends of the explants. Explants were transferred to the same selection medium (S-B5-708 medium) for further selection. Transgenic roots proliferated well within one week in the medium and were ready to be subcultured.

[Para 89] Strong and white soybean roots were excised from the rooted explants and cultured in root growth medium supplemented with 200 mg/l Timentin (S-MS-606 medium) in six-well plates. Cultures were maintained at room temperature under the dark condition. The S-MS-606 medium comprises: 0.2X MS salts and B5 vitamins, 2% sucrose, and 200mg/l Timentin at pH5.8.

[Para 90] One to five days after sub-culturing, the roots were inoculated with surface sterilized nematode juveniles in multi-well plates for the gene of interest construct assay. As a control, soybean cultivar Williams 82 control vector and Jack control vector roots were used. The root cultures of each line that occupied at least half of the well were inoculated with surface-decontaminated race 3 of soybean cyst nematode (SCN) second stage juveniles (J2) at the level of 500 J2/well. The plates were then sealed and put back into the incubator at 25C in darkness. Several independent root lines were generated from each binary vector transformation and the lines were used for bioassay. Four weeks after nematode inoculation, the cysts in each well were counted.. For each transformed line, the average number of cysts per line, the female index and the standard error values were determined across several replicated wells (Female index = average number of SCN cysts developing on the transgenic roots expressed as percentage of the average number of cysts developing on the W82 wild type susceptible control roots). Multiple independent, biologically replicated experiments were run for each expression construct. Rooted explant cultures transformed with constructs RCB564, RCB573 and RCB582 exhibited a general trend of reduced cyst numbers and female index relative to the known susceptible variety, Williams82.

Example 4. RACE PCR to Clone Full-Length 45174942 Coding Region

[Para 91] A full length transcript sequence with 100% homology to the partial cDNA clone 45174942 (SEQ ID NO: 1) was isolated using the GeneRacer Kit (L1502-01) from Invitrogen by following the manufacturers instructions. Total RNA from soybean roots harvested 6 days after infection with SCN was prepared according to the Invitrogen GeneRacer Kit protocol. The prepared RNA was reverse transcribed according to the GeneRacer Kit protocol and used as the RACE library template for PCR to isolate 5' cDNA ends using primary and secondary (nested) PCR reactions according to the GeneRacer Kit protocol. Nested PCR reactions were performed according to manufacturer's instructions to obtain the desired amplification product.

[Para 92] Specific products from secondary PCR reaction were separated by gel electrophoresis. Fragments were purified from agarose gel and cloned into pCR4-TOPO vectors (Invitrogen) following manufacturers instructions. Resulting colonies were miniprepped and se-

quenced. One of the full length fragments described as SEQ ID NO:29 (Full length GmOPR3-like DNA) had 100% percent identity with the overlapping region of SEQ ID NO:1 (45174942 DNA sequence). The alignment between proteins encoded by the full length Glycine max GmOPR3-like sequence and OPR3-like genes from other plant species is shown in Figures 2a-2c.

[Para 93] Those skilled in the art will recognize, or will be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

1. A dsRNA molecule comprising i) a first strand comprising a sequence substantially identical to a portion of a OPR3-like gene, and ii) a second strand comprising a sequence substantially complementary to the first strand, wherein the portion of the OPR3-like gene is from a polynucleotide selected from the group consisting of:

a) a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29;

b)a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30;

c) a polynucleotide having at least 70% sequence identity to a polynucleotide having a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29;

d) a polynucleotide encoding a polypeptide having at least 70% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30;

e) a polynucleotide comprising a fragment of at least 19 consecutive nucleotides, or at least 50 consecutive nucleotides, or at least 100 consecutive nucleotides, or at least 200 consecutive nucleotides of a polynucleotide having a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29;

f) a polynucleotide comprising a fragment encoding a biologically active portion of a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30;

g) a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising at least 19 consecutive nucleotides, or at least 50 consecutive nucleotides, or at least 100 consecutive nucleotides, or at least 200 consecutive nucleotides of a polynucleotide having a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29; and

h) a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising at least 19 consecutive nucleotides, or at least 50 consecutive nucleotides, or at least 100 consecutive nucleotides, or at least 200 consecutive nucleotides of a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30.

2. The dsRNA of claim 1, wherein the polynucleotide comprises a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29.

3. The dsRNA of claim 1, wherein the polynucleotide has at least 70% sequence identity to a polynucleotide having a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29.

4. The dsRNA of claim 1, wherein the polynucleotide encodes a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30.

5. The dsRNA of claim 1, wherein the polynucleotide encodes a polypeptide having at least 70% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30.

6. The dsRNA of claim 1, wherein the a polynucleotide hybridizes under stringent conditions to a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29.

7. The dsRNA of claim 1, wherein the polynucleotide comprises a fragment encoding a biologically active portion of a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30.

8. A pool of dsRNA molecules comprising a multiplicity of RNA molecules each comprising a double stranded region having a length of about 19 to 24 nucleotides, wherein said dsRNA molecules are derived from a portion of a polynucleotide selected from the group consisting of:

a) a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29;

b) a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30;

c) a polynucleotide having at least 90% sequence identity to a polynucleotide having a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29; and

d) a polynucleotide encoding a polypeptide having 90% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30.

9. The pool of dsRNA of claim 8, wherein the polynucleotide comprises a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29.

10. The pool of dsRNA of claim 8, wherein the polynucleotide has at least 90% sequence identity to a polynucleotide having a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29.

11. The pool of dsRNA of claim 8, wherein the polynucleotide encodes a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30.

12. The pool of dsRNA of claim 8, wherein the polynucleotide encodes a polypeptide having at leat 90% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30.

13. A transgenic plant capable of expressing a dsRNA that is substantially identical to a portion of an OPR3-like gene, wherein the portion of the OPR3-like gene is from a polynucleotide selected from the group consisting of:

a) a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29;

b) a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30;

c) a polynucleotide having at least 70% sequence identity to a polynucleotide having a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29;

d) a polynucleotide encoding a polypeptide having at least 70% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30;

e) a polynucleotide comprising a fragment of at least 19 consecutive nucleotides, or at least 50 consecutive nucleotides, or at least 100 consecutive nucleotides, or at least 200 consecutive nucleotides of a polynucleotide having a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29;

f) a polynucleotide comprising a fragment encoding a biologically active portion of a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30;

g) a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising at least 19 consecutive nucleotides, or at least 50 consecutive nucleotides, or at least 100 consecutive nucleotides, or at least 200 consecutive nucleotides of a polynucleotide having a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29; and

h) a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising at least 19 consecutive nucleotides, or at least 50 consecutive nucleotides, or at least 100 consecutive nucleotides, or at least 200 consecutive nucleotides of a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30.

14. The transgenic plant of claim 13, wherein the dsRNA comprises a multiplicity of RNA molecules each comprising a double stranded region having a length of about 19 to 24 nucleotides, wherein said RNA molecules are derived from a portion of a polynucleotide selected from the group consisting of:

a) a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29;

b) a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30;

c) a polynucleotide having at least 90% sequence identity to a polynucleotide having a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29; and

d) a polynucleotide encoding a polypeptide having at least 90% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30.

15.The transgenic plant of claim 13, wherein the plant is selected from the group consisting of maize, wheat, barley, sorghum, rye, triticale, rice, sugarcane, citrus trees, pineapple, coconut, banana, coffee, tea, tobacco, sunflower, pea, alfalfa, soybean, carrot, celery, tomato, potato, cotton, tobacco, eggplant, pepper, oilseed rape, canola, beet, cabbage, cauliflower, broccoli, lettuce, Lotus sp., Medicago truncatula, prerennial grass, ryegrass, and Arabidopsis thaliana.

16. The transgenic plant of claim 13, wherein the plant is soybean.

17. The transgenic plant of claim 13, wherein the polynucleotide comprises a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29.

18. The transgenic plant of claim 13, wherein the polynucleotide has at least 70% sequence identity to a polynucleotide having a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29.

19. The transgenic plant of claim 13, wherein the polynucleotide encodes a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30.

20. The transgenic plant of claim 13, wherein the polynucleotide encodes a polypeptide having at least 70% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30.

21. A method of making a transgenic plant capable of expressing a dsRNA that inhibits expression of an OPR3-like target gene in the plant, said method comprises the steps of i) preparing a nucleic acid having a region that is substantially identical to a portion of the OPR3-like gene, wherein the nucleic acid is able to form a double-stranded transcript once expressed in the plant; ii) transforming a recipient plant with said nucleic acid; iii) producing one or more transgenic offspring of said recipient plant; and iv) selecting the offspring for expression of said transcript,

wherein the portion of the target gene is from 19 to 500 nucleotides of a polynucleotide selected from the group consisting of:

a) a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29;

b) a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30;

c) a polynucleotide having at least 70% sequence identity to a polynucleotide having a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29;

d) a polynucleotide encoding a polypeptide having at least 70% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30;

e) a polynucleotide comprising a fragment of at least 19 consecutive nucleotides, or at least 50 consecutive nucleotides, or at least 100 consecutive nucleotides, or at least 200 consecutive nucleotides of a polynucleotide having a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29;

f) a polynucleotide comprising a fragment encoding a biologically active portion of a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30;

g) a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising at least 19 consecutive nucleotides, or at least 50 consecutive nucleotides, or at least 100 consecutive nucleotides, or at least 200 consecutive nucleotides of a polynucleotide having a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29; and

h) a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising at least 19 consecutive nucleotides, or at least 50 consecutive nucleotides, or at least 100 consecutive nucleotides, or at least 200 consecutive nucleotides of a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30.

22. The method of claim 21, wherein the dsRNA comprises a multiplicity of RNA molecules each comprising a double stranded region having a length of about 19 to 24 nucleotides, wherein said RNA molecules are derived from a polynucleotide selected from the group consisting of:

a) a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29;

b) a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30;

c) a polynucleotide having at least 90% sequence identity to a polynucleotide having a sequence as set forth in SEQ ID NO:1, 2, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, or 29; and

d) a polynucleotide encoding a polypeptide having at least 90% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO:3, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, or 30.

23. The method of claim 21, wherein the plant is selected from the group consisting of soybean, potato, tomato, peanuts, cotton, cassava, coffee, coconut, pineapple, citrus trees, banana, corn, rape, beet, sunflower, sorghum, wheat, oats, rye, barley, rice, green bean, lima bean, pea, and tobacco.

24. The method of claim 21, wherein the plant is soybean.

25. The method of claim 21, wherein the dsRNA is expressed in plant roots or nematode feeding sites.

Figure 1

Gene Name	Species	SEQ ID NO:
45174942 partial cDNA	Glycine max	1
45174942 sense fragment	Glycine max	2
translation of 45174942 partial DNA	Glycine max	3
TPP-like promoter	Arabidopsis thaliana	4
At5g12170 promoter	Arabidopsis thaliana	5
MtN3-like promoter	Glycine max	6
Q9FEW9 tomato DNA	Lycopersicon esculentum	7
Q9FEW9 tomato protein	Lycopersicon esculentum	8
At2g06050 Arabidopsis DNA	Arabidopsis thaliana	9
At2g06050 Arabidopsis protein	Arabidopsis thaliana	10
AAY27752 Hevea DNA	Hevea brasiliensis	11
AAY27752 Hevea protein	Hevea brasiliensis	12
EAZ42984 Oryza DNA	Oryza sativa	13
EAZ42984 Oryza protein	Oryza sativa	14
AAY26527 Zea DNA	Zea mays	15
AAY26527 Zea protein	Zea mays	16
AAY26528 Zea DNA	Zea mays	17
AAY26528 Zea protein	Zea mays	18
EG030595 Arachis DNA	Arachis hypogaea	19
EG030595 Arachis protein	Arachis hypogaea	20
TA29350_4113 potato DNA	Solanum tuberosum	21
TA29350_4113 potato protein	Solanum tuberosum	22
TA4283_3760 Prunus DNA	Prunus persica	23
TA4283_3760 Prunus protein	Prunus persica	24
TA23750_3635 Gossypium DNA	Gossypium hirsutum	25
TA23750_3635 Gossypium protein	Gossypium hirsutum	26
TA7248_49390 Coffea DNA	Coffea canephora	27
TA7248_49390 Coffea protein	Coffea canephora	28
Full length GmOPR3-like DNA	Glycine max	29
Full length GmOPR3-like protein	Glycine max	30

Figure 2a

```
GmOPR3-like                (1) ------------MADN-SISLFSPYNKMGKFNLSHRVVLAPMTRCRALNG
EG030595_Arachis           (1) ------------MADNESSSLFSAY-KMAKFSLSHRVVLAPMTRCRALNG
AAY27752_Hevea             (1) -------MAETGTEGTGITTLFSPY-KMGKFSLSHRVVLAPMTRCRALNG
At2g06050_Arabidopsis      (1) ---------MTAAQGNSNETLFSSY-KMGRFDLSHRVVLAPMTRCRALNG
Q9FEW9_tomato              (1) ---------MASSAQDGNNPLFSPY-KMGKFNLSHRVVLAPMTRCRALNN
TA29350_4113_potato        (1) -----MAKTTSSSAQDGSNPLFSPY-KMAKFNLSHRIVLAPMTRCRALNN
TA23750_3635_Gossypium     (1) ---MEHGEKVKMADSQETPTLFSPY-KMGKFNLSHRVVLAPMTRCRALNG
TA4283_3760_Prunus         (1) ----------MAEASSQGPTLFSPF-KMGKFNLSHRVVLAPMTRCRALNG
TA7248_49390_Coffea        (1) --------MAETKSDQGSPSLFSPY-KMGKFNLSHRVVLAPMTRCRAINS
AAY26528_Zea               (1) MASTDRSAPAEDQQQPQRPSLFSPY-QMPHFRLAHRVVLAPMTRCRAPDA
AAY26527_Zea               (1) MASTDRSTPAEDEQQQKRPSLFSPY-QMPRFRLAHRVVLAPMTRCRAPDA
EAZ42984_Oryza             (1) -----MDRPPPDQQRQKQAPLFSPY-QMPRFRLNHRVVLAPMTRCRAIGG

GmOPR3-like               (38) TPLAAHAEYYAQRSTPGGFLITEGTLISPTSSGFPHVPGIYSDEQVEAWR
EG030595_Arachis          (38) IPRAAHAEYYAQRSTPGGFLITEGTLISPTAPGFPHVPGIYSEEQVEAWR
AAY27752_Hevea            (43) IPNAALVDYYTQRSTPGGFLITEGTLVSPTAPGFPHVPGIYTEEQAEAWK
At2g06050_Arabidopsis     (41) VPNAALAEYYAQRTTPGGFLISEGTMVSPGSAGFPHVPGIYSDEQVEAWK
Q9FEW9_tomato             (41) IPQAALGEYYEQRATAGGFLITEGTMISPTSAGFPHVPGIFTKEQVREWK
TA29350_4113_potato       (45) IPSAALGEYYEQRATAGGFLITEGTMISPTSAGFPHVPGIFTKEQVEEWK
TA23750_3635_Gossypium    (47) IPRPALAEYYTQRSTPGGFLITEGTLISDTGAGFPHVPGIYNEEQVEAWK
TA4283_3760_Prunus        (40) LPQPALAEYYTQRSTNGGFLITEGTLVSDTGAGFPHVPGIYNDEQVEAWK
TA7248_49390_Coffea       (42) IPQPAMAEYYAQRATNGGFLITEGTMISPSAAGFPHVPGIFTKEQVEAWK
AAY26528_Zea              (50) LPGPALAEYYAQRSTEGGLLISEGTIISPAGPGFPRVPGIYNQEQTDAWK
AAY26527_Zea              (50) VPGPALAEYYAQRSTDGGLLISEGTIISPSGPGFPRVPGIYNQEQTDAWR
EAZ42984_Oryza            (45) VPGPALAEYYAQRTTQGGLLISEGTVVSPAGPGFPHVPGIYNQEQTDAWK

GmOPR3-like               (88) NVVDAVHANGSFIFCQLWHVGRASHPVYQPGGALPSSSTSKPIS-DKWKI
EG030595_Arachis          (88) NVVDAVHAKGSFIFCQLWHAGRASHPVYQPGAAPPISSTNKAIS-SRWRI
AAY27752_Hevea            (93) RVVDAVHAKGSIIFCQLWHVGRASHQVYQPNGAAPISSTGKAIS-NRWRI
At2g06050_Arabidopsis     (91) QVVEAVHAKGGFIFCQLWHVGRASHAVYQPNGGSPISSTNKPISENRWRV
Q9FEW9_tomato             (91) KIVDVVHAKGAVIFCQLWHVGRASHEVYQPAGAAPISSTEKPIS-NRWRI
TA29350_4113_potato       (95) KIVDVVHAKGAVIFCQLWHVGRASHEVYQPAGAAPISSTEKPIS-KRWRI
TA23750_3635_Gossypium    (97) MIVDAVHAKGGIIFCQLWHVGRASHTVYQPGGVAPISSTNKPIS-KRWRI
TA4283_3760_Prunus        (90) KVVDAVHAKGAIIFCQLWHVGRASHEVYQPGGGSPISSTDVPIS-RRWRI
TA7248_49390_Coffea       (92) QVVDAVHAKGAIIFCQLWHVGRASHEVYQPGGGAPISSTGKPIS-KRWRI
AAY26528_Zea             (100) KVVDAVHAKGAIFFCQLWHVGRASHQVYQPGGSAPISSTDKPIS-SRWRI
AAY26527_Zea             (100) KVVDAVHAKGAIFFCQLWHVGRASHQVYQPGAAAPISSTDKPIS-SRWRI
EAZ42984_Oryza            (95) KVVDAVHAKGGIFFCQLWHVGRASHQVYQPNGAAPISSTDKPIS-ARWRI
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Figure 2b

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GmOPR3-like              (137) LMPDGSHGIYPEPRALTTS-EISEIVHHYRQAAINAIRAGFDGIEIHGAH
EG030595_Arachis         (137) LLPDQSYGVYPEPRPLDSS-EIPQIVDHYRQSAVNAIRAGFDGIEIHGAH
AAY27752_Hevea           (142) LMPDGSYGKYPTPRPLETP-EILEVVKNYRQSALNAIRAGFDGIEVHGAH
At2g06050_Arabidopsis    (141) LLPDGSHVKYPKPRALEAS-EIPRVVEDYCLSALNAIRAGFDGIEIHGAH
Q9FEW9_tomato            (140) LMPDGTHGIYPKPRAIGTY-EISQVVEDYRRSALNAIEAGFDGIEIHGAH
TA29350_4113_potato      (144) LMPDGTHGIYPKPRAIGTY-EISQVVEDYCRSALNAIEAGFDGIEIHGAH
TA23750_3635_Gossypium   (146) LMPDGSYGIYPKPRPLETS-EIQEVVEHYRKAALNAIRAGFDGIEIHGAH
TA4283_3760_Prunus       (139) LLPDASHATYPKPRRLETP-EILQVVEHYRQAALNAIRAGFDGIEIHGAH
TA7248_49390_Coffea      (141) LMPDGSHGIYPKPRPLTTAHEIAQVVEDYRQSALNAIEAGFDGIEIHGAH
AAY26528_Zea             (149) LMPDGSYGKYPTPRRLATS-EIPEIVEQYRQAAINAIKAGFDGIEIHGAH
AAY26527_Zea             (149) LMPDGSYGKYPTPRRLATS-EIPEIVEQYRQAAVNAIKAGFDGIEIHGAH
EAZ42984_Oryza           (144) LMPDGSYGKYPKPRRLAAS-EIPEIVEQYRQAAINAIEAGFDGIEIHGAH

GmOPR3-like              (186) GYLIDQFLKDAINDRTDEYGGPLENRCRFLMEVVEAVVSAIGAERVAIRI
EG030595_Arachis         (186) GYLIDQFLKDGINERRDEYGGSISNRCRFLMQVVKAVVSAIGAERVGVRI
AAY27752_Hevea           (191) GYLIDQFLKDGINDRTDEYGGSINNRCRFLMQVIQAVVAAIGADRVGFRM
At2g06050_Arabidopsis    (190) GYLIDQFLKDGINDRTDQYGGSIANRCRFLKQVVEGVVSAIGASKVGVRV
Q9FEW9_tomato            (189) GYLIDQFLKDGINDRTDEYGGSLANRCKFITQVVQAVVSAIGADRVGVRV
TA29350_4113_potato      (193) GYLIDQFLKDGINDRTDEYGGSLANRCKFITQVVQAVISAIGADRVGVRV
TA23750_3635_Gossypium   (195) GYLIDQFLKDGINDRTDEYGGSLANRCKFLMQIVQAVASAIGIDRVAVRM
TA4283_3760_Prunus       (188) GYLIDQFLKDGINDRTDEYGGSLANRCKFLLQVVQAVVGAVGADRVGVRI
TA7248_49390_Coffea      (191) GYLIDQFLKDGINDRTDEYGGSVANRCKFIVQVVQAVVSAIGADRVGVRI
AAY26528_Zea             (198) GYLIDQFLKDGINDRADEYGGSLSNRCRFLLEVTRAVVSAIGADRVAVRV
AAY26527_Zea             (198) GYLIDQFLKGGINDRTDEYGGSLSNRCRFLLEVTRAVVSAIGADRVAVRV
EAZ42984_Oryza           (193) GYIIDQFLKDGINDRTDEYGGSLSNRCRFLLEVTRAVVSAIGADRVAVRI

GmOPR3-like              (236) SPAIDFNDAFDSDPLGLGLAVIERLNNLQKQVGTKLAYLHVTQPRFTLLA
EG030595_Arachis         (236) SPAIDHLDAMDSDPL--------------------------------
AAY27752_Hevea           (241) SPAIDHLDAIDSDPLNLGLAVIERLNKLQLNLGSKLTYLHVTQPRYTAYG
At2g06050_Arabidopsis    (240) SPAIDHLDATDSDPLSLGLAVVGMLNKLQGVNGSKLAYLHVTQPRYHAYG
Q9FEW9_tomato            (239) SPAIDHLDAMDSNPLSLGLAVVERLNKIQLHSGSKLAYLHVTQPRYVAYG
TA29350_4113_potato      (243) SPAIDHLDAMDSNPLSLGLAVVERLNKIQLHSGSKLAYLHVTQPRYVAYG
TA23750_3635_Gossypium   (245) SPAIDHLDATDSNPLNLGLAVIERLNKLQLQLGSKLAYLHVTQPRYHAYG
TA4283_3760_Prunus       (238) SPAIDHLDAVDSAPLTLSLGVIERLNKLQQDWGSKLTYLHVTQPRYAAYG
TA7248_49390_Coffea      (241) SPAIDHLDAMDSDPLSLGLAVIERLNELQLNSGSKLTYLHVTQPRYTAYG
AAY26528_Zea             (248) SPAIDHLDAYDSNPLQLGLAVVDRLNALQEETG-RLAYLHVTQPRYTAYG
AAY26527_Zea             (248) SPAIDHLDAYDSNPLQLGLAVVERLNALQQEAG-RLAYLHVTQPRYTAYG
EAZ42984_Oryza           (243) SPAIDHLDAYDSDPIKLGMAVVERLNALQQQSG-RLAYLHVTQPRYTAYG
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Figure 2c

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GmOPR3-like              (286) QTESV---SEKEEAHFMQKWREAYEGTFMCSGAFTRDSGMEAVAEGHADL
EG030595_Arachis         (251) ----------------------------------------------
AAY27752_Hevea           (291) QTESGRHGTEEEEARLMRTWRRAYKGTFICSGGFTRELGMEAIAQDDADL
At2g06050_Arabidopsis    (290) QTESGRQGSDEEEAKLMKSLRMAYNGTFMSSGGFNKELGMQAVQQGDADL
Q9FEW9_tomato            (289) QTEAGRLGSEEEEARLMRTLRNAYQGTFICSGGYTRELGIEAVAQGDADL
TA29350_4113_potato      (293) QTEAGRLGSEEEEAHLMRTLRNAYQGTFICSGGYTRELGIEAVAQGDADL
TA23750_3635_Gossypium   (295) QTESGKHGNEDEEAYLLRALKRTYHGTFMCSGGFNRELGMQAVAEGDADL
TA4283_3760_Prunus       (288) QTESGKPGSDEEEAVFMRTLRNAYRGTFVASGGYTRELGIHAVASRDADL
TA7248_49390_Coffea      (291) QTEAGRQGSEEEEAQLVRTLRKAYQGTFISSGGFTRELGVEAVAQGDADL
AAY26528_Zea             (297) QTESGQHGSAEEESRLMRALRGAYRGTFMCSGGYTRELGVEAVESWDADL
AAY26527_Zea             (297) QTESGQHGSAEEESRLMRAVRGAYRGTFMCSGGYTRELGVEAIESGDADL
EAZ42984_Oryza           (292) QTESGQHGSAEEESRLMRTLRGTYQGTFMCSGGYTRELGLEAVESGDADL

GmOPR3-like              (333) VSYGRLFISNPDLVLRLKLNAPLTKYNRNTFYTQDPVIGYTDYPFFNGTT
EG030595_Arachis         (251) ----------------------------------------------
AAY27752_Hevea           (341) VSYGRLFISNPDLVLRFKLNAPLNKYVRKTFYTQDPVVGYTDYPFFRKVD
At2g06050_Arabidopsis    (340) VSYGRLFIANPDLVSRFKIDGELNKYNRKTFYTQDPVVGYTDYPFLAPF-
Q9FEW9_tomato            (339) VSYGRLFISNPDLVMRIKLNAPLNKYNRKTFYTQDPVVGYTDYPFLQGNG
TA29350_4113_potato      (343) VSYGRLFISNPDLVMRIKLNAPLNKYNRKTFYTQDPVVGYTDYPFLQGNG
TA23750_3635_Gossypium   (345) VSYGRLFISNPDLVFRLKVNAPLNRYIRKTFYTHDPVVGYTDYPFLNEEK
TA4283_3760_Prunus       (338) VSYGRLFISNPDLVLRLKLNAPLTRYNRKTFYTQDPVVGYTDYPFLSNAN
TA7248_49390_Coffea      (341) VSYGRLFISNPDLVLRFKLNAPLIRYNRSTFYTHDPVVGYTDYPFLSNGT
AAY26528_Zea             (347) VSYGRLFIANPDLVERFRRDAPLNRYVRKTFYTPDPVVGYTDYPFLGQ--
AAY26527_Zea             (347) VSYGRLFIANPDLVERFRRDAPLNKYVRKTFYTPDPVVGYTDYTFLGQ--
EAZ42984_Oryza           (342) VSYGRLFISNPDLVERFRLNAGLNKYVRKTFYTPDPVVGYTDYPFLGQ--

GmOPR3-like              (383) ET--KLSN--------------
EG030595_Arachis         (251) ----------------------
AAY27752_Hevea           (391) GSQEPRSRL-------------
At2g06050_Arabidopsis    (389) ------SRL-------------
Q9FEW9_tomato            (389) SNG-PLSRL-------------
TA29350_4113_potato      (393) SNG-PLSRL-------------
TA23750_3635_Gossypium   (395) GRQV-LSRL-------------
TA4283_3760_Prunus       (388) GKEEPLSRL-------------
TA7248_49390_Coffea      (391) SGNVPQSRL-------------
AAY26528_Zea             (395) ----PKARM-------------
AAY26527_Zea             (395) ----PKARM-------------
EAZ42984_Oryza           (390) ----PKSRM-------------
```

Figure 3: Global Amino Acid Percent Identity Between Exemplary OPR3-like Genes

	GmOPR3-like	EG030595 Arachis	AAY27752 Hevea	At2g06050 Arabidopsis	Q9FEW9 tomato	TA29350_4113 potato	TA23750_3635 Gossypium	TA4283_3760 Prunus	TA7248_49390 Coffea	AAY26528 Zea	AAY26527 Zea	EAZ42984 Oryza
GmOPR3-like	100	82	73	70	72	72	74	72	73	69	70	70
EG030595 Arachis		100	79	76	73	73	78	75	78	73	74	72
AAY27752 Hevea			100	74	77	75	78	77	78	72	73	74
At2g06050 Arabidop.				100	75	74	74	74	74	70	70	72
Q9FEW9 tomato					100	97	77	77	83	72	72	73
TA29350_4113 potato						100	76	76	83	70	70	72
TA23750_3635 Gos.							100	79	78	73	72	73
TA4283_3760 Prunus								100	79	74	72	73
TA7248_49390 Cof.									100	74	73	74
AAY26528 Zea										100	94	87
AAY26527 Zea											100	87
EAZ42984 Oryza												100

Figure 4: Global Nucleotide Percent Identity Between Exemplary OPR3-like Genes

	GmOPR3-like	EG030595 Arachis	AAY27752 Hevea	AT2G06050 Arabidopsis	AJ278332 tomato	TA29350_4113 Solanum	TA23750_3635 Gossypium	TA4283_3760 Prunus	TA7248_49390 Coffea	AAY26527 Zea	AAY26528 Zea	EAZ42984 rice
GmOPR3-like	100	79	72	68	72	71	73	71	70	63	62	65
EG030595 Arachis		100	75	70	72	71	74	72	70	69	68	68
AAY27752 Hevea			100	70	74	73	77	76	74	66	65	68
AT2G06050 Arabi.				100	70	69	71	70	69	63	63	66
AJ278332 tomato					100	97	74	74	77	65	64	66
TA29350_4113 So.						100	73	73	77	64	64	65
TA23750_3635 Gos.							100	76	74	66	66	68
TA4283_3760 Prun.								100	75	66	66	68
TA7248_49390 Cof.									100	66	65	65
AAY26527 Zea										100	94	82
AAY26528 Zea											100	82
EAZ42984 rice												100

Figure 5a

nucleotide positions of 21mers of an OPR3-like encoding polynucleotide of				
SEQ ID NO:1, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, or				
a polynucleotide sequence encoding an OPR3-like homolog				
1 to 21	31 to 51	61 to 81	91 to 111	121 to 141
2 to 22	32 to 52	62 to 82	92 to 112	122 to 142
3 to 23	33 to 53	63 to 83	93 to 113	123 to 143
4 to 24	34 to 54	64 to 84	94 to 114	124 to 144
5 to 25	35 to 55	65 to 85	95 to 115	125 to 145
6 to 26	36 to 56	66 to 86	96 to 116	126 to 146
7 to 27	37 to 57	67 to 87	97 to 117	127 to 147
8 to 28	38 to 58	68 to 88	98 to 118	128 to 148
9 to 29	39 to 59	69 to 89	99 to 119	129 to 149
10 to 30	40 to 60	70 to 90	100 to 120	130 to 150
11 to 31	41 to 61	71 to 91	101 to 121	131 to 151
12 to 32	42 to 62	72 to 92	102 to 122	132 to 152
13 to 33	43 to 63	73 to 93	103 to 123	133 to 153
14 to 34	44 to 64	74 to 94	104 to 124	134 to 154
15 to 35	45 to 65	75 to 95	105 to 125	135 to 155
16 to 36	46 to 66	76 to 96	106 to 126	136 to 156
17 to 37	47 to 67	77 to 97	107 to 127	137 to 157
18 to 38	48 to 68	78 to 98	108 to 128	138 to 158
19 to 39	49 to 69	79 to 99	109 to 129	139 to 159
20 to 40	50 to 70	80 to 100	110 to 130	140 to 160
21 to 41	51 to 71	81 to 101	111 to 131	141 to 161
22 to 42	52 to 72	82 to 102	112 to 132	142 to 162
23 to 43	53 to 73	83 to 103	113 to 133	143 to 163
24 to 44	54 to 74	84 to 104	114 to 134	144 to 164
25 to 45	55 to 75	85 to 105	115 to 135	145 to 165
26 to 46	56 to 76	86 to 106	116 to 136	146 to 166
27 to 47	57 to 77	87 to 107	117 to 137	147 to 167
28 to 48	58 to 78	88 to 108	118 to 138	148 to 168
29 to 49	59 to 79	89 to 109	119 to 139	149 to 169
30 to 50	60 to 80	90 to 110	120 to 140	150 to 170

Figure 5b

nucleotide positions of 21mers of an OPR3-like encoding polynucleotide of SEQ ID NO:1, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, or a polynucleotide sequence encoding an OPR3-like homolog				
151 to 171	181 to 201	211 to 231	241 to 261	271 to 291
152 to 172	182 to 202	212 to 232	242 to 262	272 to 292
153 to 173	183 to 203	213 to 233	243 to 263	273 to 293
154 to 174	184 to 204	214 to 234	244 to 264	274 to 294
155 to 175	185 to 205	215 to 235	245 to 265	275 to 295
156 to 176	186 to 206	216 to 236	246 to 266	276 to 296
157 to 177	187 to 207	217 to 237	247 to 267	277 to 297
158 to 178	188 to 208	218 to 238	248 to 268	278 to 298
159 to 179	189 to 209	219 to 239	249 to 269	279 to 299
160 to 180	190 to 210	220 to 240	250 to 270	280 to 300
161 to 181	191 to 211	221 to 241	251 to 271	281 to 301
162 to 182	192 to 212	222 to 242	252 to 272	282 to 302
163 to 183	193 to 213	223 to 243	253 to 273	283 to 303
164 to 184	194 to 214	224 to 244	254 to 274	284 to 304
165 to 185	195 to 215	225 to 245	255 to 275	285 to 305
166 to 186	196 to 216	226 to 246	256 to 276	286 to 306
167 to 187	197 to 217	227 to 247	257 to 277	287 to 307
168 to 188	198 to 218	228 to 248	258 to 278	288 to 308
169 to 189	199 to 219	229 to 249	259 to 279	289 to 309
170 to 190	200 to 220	230 to 250	260 to 280	290 to 310
171 to 191	201 to 221	231 to 251	261 to 281	291 to 311
172 to 192	202 to 222	232 to 252	262 to 282	292 to 312
173 to 193	203 to 223	233 to 253	263 to 283	293 to 313
174 to 194	204 to 224	234 to 254	264 to 284	294 to 314
175 to 195	205 to 225	235 to 255	265 to 285	295 to 315
176 to 196	206 to 226	236 to 256	266 to 286	296 to 316
177 to 197	207 to 227	237 to 257	267 to 287	297 to 317
178 to 198	208 to 228	238 to 258	268 to 288	298 to 318
179 to 199	209 to 229	239 to 259	269 to 289	299 to 319
180 to 200	210 to 230	240 to 260	270 to 290	300 to 320

Figure 5c

nucleotide positions of 21mers of an OPR3-like encoding polynucleotide of				
SEQ ID NO:1, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, or				
a polynucleotide sequence encoding an OPR3-like homolog				
301 to 321	331 to 351	361 to 381	391 to 411	421 to 441
302 to 322	332 to 352	362 to 382	392 to 412	422 to 442
303 to 323	333 to 353	363 to 383	393 to 413	423 to 443
304 to 324	334 to 354	364 to 384	394 to 414	424 to 444
305 to 325	335 to 355	365 to 385	395 to 415	425 to 445
306 to 326	336 to 356	366 to 386	396 to 416	426 to 446
307 to 327	337 to 357	367 to 387	397 to 417	427 to 447
308 to 328	338 to 358	368 to 388	398 to 418	428 to 448
309 to 329	339 to 359	369 to 389	399 to 419	429 to 449
310 to 330	340 to 360	370 to 390	400 to 420	430 to 450
311 to 331	341 to 361	371 to 391	401 to 421	431 to 451
312 to 332	342 to 362	372 to 392	402 to 422	432 to 452
313 to 333	343 to 363	373 to 393	403 to 423	433 to 453
314 to 334	344 to 364	374 to 394	404 to 424	434 to 454
315 to 335	345 to 365	375 to 395	405 to 425	435 to 455
316 to 336	346 to 366	376 to 396	406 to 426	436 to 456
317 to 337	347 to 367	377 to 397	407 to 427	437 to 457
318 to 338	348 to 368	378 to 398	408 to 428	438 to 458
319 to 339	349 to 369	379 to 399	409 to 429	439 to 459
320 to 340	350 to 370	380 to 400	410 to 430	440 to 460
321 to 341	351 to 371	381 to 401	411 to 431	441 to 461
322 to 342	352 to 372	382 to 402	412 to 432	442 to 462
323 to 343	353 to 373	383 to 403	413 to 433	443 to 463
324 to 344	354 to 374	384 to 404	414 to 434	444 to 464
325 to 345	355 to 375	385 to 405	415 to 435	445 to 465
326 to 346	356 to 376	386 to 406	416 to 436	446 to 466
327 to 347	357 to 377	387 to 407	417 to 437	447 to 467
328 to 348	358 to 378	388 to 408	418 to 438	448 to 468
329 to 349	359 to 379	389 to 409	419 to 439	449 to 469
330 to 350	360 to 380	390 to 410	420 to 440	450 to 470

Figure 5d

nucleotide positions of 21mers of an OPR3-like encoding polynucleotide of				
SEQ ID NO:1, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, or				
a polynucleotide sequence encoding an OPR3-like homolog				
451 to 471	481 to 501	511 to 531	541 to 561	571 to 591
452 to 472	482 to 502	512 to 532	542 to 562	572 to 592
453 to 473	483 to 503	513 to 533	543 to 563	573 to 593
454 to 474	484 to 504	514 to 534	544 to 564	574 to 594
455 to 475	485 to 505	515 to 535	545 to 565	575 to 595
456 to 476	486 to 506	516 to 536	546 to 566	576 to 596
457 to 477	487 to 507	517 to 537	547 to 567	577 to 597
458 to 478	488 to 508	518 to 538	548 to 568	578 to 598
459 to 479	489 to 509	519 to 539	549 to 569	579 to 599
460 to 480	490 to 510	520 to 540	550 to 570	580 to 600
461 to 481	491 to 511	521 to 541	551 to 571	581 to 601
462 to 482	492 to 512	522 to 542	552 to 572	582 to 602
463 to 483	493 to 513	523 to 543	553 to 573	583 to 603
464 to 484	494 to 514	524 to 544	554 to 574	584 to 604
465 to 485	495 to 515	525 to 545	555 to 575	585 to 605
466 to 486	496 to 516	526 to 546	556 to 576	586 to 606
467 to 487	497 to 517	527 to 547	557 to 577	587 to 607
468 to 488	498 to 518	528 to 548	558 to 578	588 to 608
469 to 489	499 to 519	529 to 549	559 to 579	589 to 609
470 to 490	500 to 520	530 to 550	560 to 580	590 to 610
471 to 491	501 to 521	531 to 551	561 to 581	591 to 611
472 to 492	502 to 522	532 to 552	562 to 582	592 to 612
473 to 493	503 to 523	533 to 553	563 to 583	593 to 613
474 to 494	504 to 524	534 to 554	564 to 584	594 to 614
475 to 495	505 to 525	535 to 555	565 to 585	595 to 615
476 to 496	506 to 526	536 to 556	566 to 586	596 to 616
477 to 497	507 to 527	537 to 557	567 to 587	597 to 617
478 to 498	508 to 528	538 to 558	568 to 588	598 to 618
479 to 499	509 to 529	539 to 559	569 to 589	599 to 619
480 to 500	510 to 530	540 to 560	570 to 590	600 to 620

Figure 5e

nucleotide positions of 21mers of an OPR3-like encoding polynucleotide of				
SEQ ID NO:1, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, or				
a polynucleotide sequence encoding an OPR3-like homolog				
601 to 621	631 to 651	661 to 681	691 to 711	721 to 741
602 to 622	632 to 652	662 to 682	692 to 712	722 to 742
603 to 623	633 to 653	663 to 683	693 to 713	723 to 743
604 to 624	634 to 654	664 to 684	694 to 714	724 to 744
605 to 625	635 to 655	665 to 685	695 to 715	725 to 745
606 to 626	636 to 656	666 to 686	696 to 716	726 to 746
607 to 627	637 to 657	667 to 687	697 to 717	727 to 747
608 to 628	638 to 658	668 to 688	698 to 718	728 to 748
609 to 629	639 to 659	669 to 689	699 to 719	729 to 749
610 to 630	640 to 660	670 to 690	700 to 720	730 to 750
611 to 631	641 to 661	671 to 691	701 to 721	731 to 751
612 to 632	642 to 662	672 to 692	702 to 722	732 to 752
613 to 633	643 to 663	673 to 693	703 to 723	733 to 753
614 to 634	644 to 664	674 to 694	704 to 724	734 to 754
615 to 635	645 to 665	675 to 695	705 to 725	735 to 755
616 to 636	646 to 666	676 to 696	706 to 726	736 to 756
617 to 637	647 to 667	677 to 697	707 to 727	737 to 757
618 to 638	648 to 668	678 to 698	708 to 728	738 to 758
619 to 639	649 to 669	679 to 699	709 to 729	739 to 759
620 to 640	650 to 670	680 to 700	710 to 730	740 to 760
621 to 641	651 to 671	681 to 701	711 to 731	741 to 761
622 to 642	652 to 672	682 to 702	712 to 732	742 to 762
623 to 643	653 to 673	683 to 703	713 to 733	743 to 763
624 to 644	654 to 674	684 to 704	714 to 734	744 to 764
625 to 645	655 to 675	685 to 705	715 to 735	745 to 765
626 to 646	656 to 676	686 to 706	716 to 736	746 to 766
627 to 647	657 to 677	687 to 707	717 to 737	747 to 767
628 to 648	658 to 678	688 to 708	718 to 738	748 to 768
629 to 649	659 to 679	689 to 709	719 to 739	749 to 769
630 to 650	660 to 680	690 to 710	720 to 740	750 to 770

Figure 5f

nucleotide positions of 21mers of an OPR3-like encoding polynucleotide of SEQ ID NO:1, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, or a polynucleotide sequence encoding an OPR3-like homolog				
751 to 771	781 to 801	811 to 831	841 to 861	871 to 891
752 to 772	782 to 802	812 to 832	842 to 862	872 to 892
753 to 773	783 to 803	813 to 833	843 to 863	873 to 893
754 to 774	784 to 804	814 to 834	844 to 864	874 to 894
755 to 775	785 to 805	815 to 835	845 to 865	875 to 895
756 to 776	786 to 806	816 to 836	846 to 866	876 to 896
757 to 777	787 to 807	817 to 837	847 to 867	877 to 897
758 to 778	788 to 808	818 to 838	848 to 868	878 to 898
759 to 779	789 to 809	819 to 839	849 to 869	879 to 899
760 to 780	790 to 810	820 to 840	850 to 870	880 to 900
761 to 781	791 to 811	821 to 841	851 to 871	881 to 901
762 to 782	792 to 812	822 to 842	852 to 872	882 to 902
763 to 783	793 to 813	823 to 843	853 to 873	883 to 903
764 to 784	794 to 814	824 to 844	854 to 874	884 to 904
765 to 785	795 to 815	825 to 845	855 to 875	885 to 905
766 to 786	796 to 816	826 to 846	856 to 876	886 to 906
767 to 787	797 to 817	827 to 847	857 to 877	887 to 907
768 to 788	798 to 818	828 to 848	858 to 878	888 to 908
769 to 789	799 to 819	829 to 849	859 to 879	889 to 909
770 to 790	800 to 820	830 to 850	860 to 880	890 to 910
771 to 791	801 to 821	831 to 851	861 to 881	891 to 911
772 to 792	802 to 822	832 to 852	862 to 882	892 to 912
773 to 793	803 to 823	833 to 853	863 to 883	893 to 913
774 to 794	804 to 824	834 to 854	864 to 884	894 to 914
775 to 795	805 to 825	835 to 855	865 to 885	895 to 915
776 to 796	806 to 826	836 to 856	866 to 886	896 to 916
777 to 797	807 to 827	837 to 857	867 to 887	897 to 917
778 to 798	808 to 828	838 to 858	868 to 888	898 to 918
779 to 799	809 to 829	839 to 859	869 to 889	899 to 919
780 to 800	810 to 830	840 to 860	870 to 890	900 to 920

Figure 5g

nucleotide positions of 21mers of an OPR3-like encoding polynucleotide of SEQ ID NO:1, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, or a polynucleotide sequence encoding an OPR3-like homolog				
901 to 921	931 to 951	961 to 981	991 to 1011	1021 to 1041
902 to 922	932 to 952	962 to 982	992 to 1012	1022 to 1042
903 to 923	933 to 953	963 to 983	993 to 1013	1023 to 1043
904 to 924	934 to 954	964 to 984	994 to 1014	1024 to 1044
905 to 925	935 to 955	965 to 985	995 to 1015	1025 to 1045
906 to 926	936 to 956	966 to 986	996 to 1016	1026 to 1046
907 to 927	937 to 957	967 to 987	997 to 1017	1027 to 1047
908 to 928	938 to 958	968 to 988	998 to 1018	1028 to 1048
909 to 929	939 to 959	969 to 989	999 to 1019	1029 to 1049
910 to 930	940 to 960	970 to 990	1000 to 1020	1030 to 1050
911 to 931	941 to 961	971 to 991	1001 to 1021	1031 to 1051
912 to 932	942 to 962	972 to 992	1002 to 1022	1032 to 1052
913 to 933	943 to 963	973 to 993	1003 to 1023	1033 to 1053
914 to 934	944 to 964	974 to 994	1004 to 1024	1034 to 1054
915 to 935	945 to 965	975 to 995	1005 to 1025	1035 to 1055
916 to 936	946 to 966	976 to 996	1006 to 1026	1036 to 1056
917 to 937	947 to 967	977 to 997	1007 to 1027	1037 to 1057
918 to 938	948 to 968	978 to 998	1008 to 1028	1038 to 1058
919 to 939	949 to 969	979 to 999	1009 to 1029	1039 to 1059
920 to 940	950 to 970	980 to 1000	1010 to 1030	1040 to 1060
921 to 941	951 to 971	981 to 1001	1011 to 1031	1041 to 1061
922 to 942	952 to 972	982 to 1002	1012 to 1032	1042 to 1062
923 to 943	953 to 973	983 to 1003	1013 to 1033	1043 to 1063
924 to 944	954 to 974	984 to 1004	1014 to 1034	1044 to 1064
925 to 945	955 to 975	985 to 1005	1015 to 1035	1045 to 1065
926 to 946	956 to 976	986 to 1006	1016 to 1036	1046 to 1066
927 to 947	957 to 977	987 to 1007	1017 to 1037	1047 to 1067
928 to 948	958 to 978	988 to 1008	1018 to 1038	1048 to 1068
929 to 949	959 to 979	989 to 1009	1019 to 1039	1049 to 1069
930 to 950	960 to 980	990 to 1010	1020 to 1040	1050 to 1070

Figure 5h

nucleotide positions of 21mers of an OPR3-like encoding polynucleotide of				
SEQ ID NO:1, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, or				
a polynucleotide sequence encoding an OPR3-like homolog				
1051 to 1071	1081 to 1101	1111 to 1131	1141 to 1161	1171 to 1191
1052 to 1072	1082 to 1102	1112 to 1132	1142 to 1162	1172 to 1192
1053 to 1073	1083 to 1103	1113 to 1133	1143 to 1163	1173 to 1193
1054 to 1074	1084 to 1104	1114 to 1134	1144 to 1164	1174 to 1194
1055 to 1075	1085 to 1105	1115 to 1135	1145 to 1165	1175 to 1195
1056 to 1076	1086 to 1106	1116 to 1136	1146 to 1166	1176 to 1196
1057 to 1077	1087 to 1107	1117 to 1137	1147 to 1167	1177 to 1197
1058 to 1078	1088 to 1108	1118 to 1138	1148 to 1168	1178 to 1198
1059 to 1079	1089 to 1109	1119 to 1139	1149 to 1169	1179 to 1199
1060 to 1080	1090 to 1110	1120 to 1140	1150 to 1170	1180 to 1200
1061 to 1081	1091 to 1111	1121 to 1141	1151 to 1171	1181 to 1201
1062 to 1082	1092 to 1112	1122 to 1142	1152 to 1172	1182 to 1202
1063 to 1083	1093 to 1113	1123 to 1143	1153 to 1173	1183 to 1203
1064 to 1084	1094 to 1114	1124 to 1144	1154 to 1174	1184 to 1204
1065 to 1085	1095 to 1115	1125 to 1145	1155 to 1175	1185 to 1205
1066 to 1086	1096 to 1116	1126 to 1146	1156 to 1176	1186 to 1206
1067 to 1087	1097 to 1117	1127 to 1147	1157 to 1177	1187 to 1207
1068 to 1088	1098 to 1118	1128 to 1148	1158 to 1178	1188 to 1208
1069 to 1089	1099 to 1119	1129 to 1149	1159 to 1179	1189 to 1209
1070 to 1090	1100 to 1120	1130 to 1150	1160 to 1180	1190 to 1210
1071 to 1091	1101 to 1121	1131 to 1151	1161 to 1181	1191 to 1211
1072 to 1092	1102 to 1122	1132 to 1152	1162 to 1182	1192 to 1212
1073 to 1093	1103 to 1123	1133 to 1153	1163 to 1183	1193 to 1213
1074 to 1094	1104 to 1124	1134 to 1154	1164 to 1184	1194 to 1214
1075 to 1095	1105 to 1125	1135 to 1155	1165 to 1185	1195 to 1215
1076 to 1096	1106 to 1126	1136 to 1156	1166 to 1186	1196 to 1216
1077 to 1097	1107 to 1127	1137 to 1157	1167 to 1187	1197 to 1217
1078 to 1098	1108 to 1128	1138 to 1158	1168 to 1188	1198 to 1218
1079 to 1099	1109 to 1129	1139 to 1159	1169 to 1189	1199 to 1219
1080 to 1100	1110 to 1130	1140 to 1160	1170 to 1190	1200 to 1220

Figure 5i

nucleotide positions of 21mers of an OPR3-like encoding polynucleotide of SEQ ID NO:1, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, or a polynucleotide sequence encoding an OPR3-like homolog				
1201 to 1221	1231 to 1251	1261 to 1281	1291 to 1311	1321 to 1341
1202 to 1222	1232 to 1252	1262 to 1282	1292 to 1312	1322 to 1342
1203 to 1223	1233 to 1253	1263 to 1283	1293 to 1313	1323 to 1343
1204 to 1224	1234 to 1254	1264 to 1284	1294 to 1314	1324 to 1344
1205 to 1225	1235 to 1255	1265 to 1285	1295 to 1315	1325 to 1345
1206 to 1226	1236 to 1256	1266 to 1286	1296 to 1316	1326 to 1346
1207 to 1227	1237 to 1257	1267 to 1287	1297 to 1317	1327 to 1347
1208 to 1228	1238 to 1258	1268 to 1288	1298 to 1318	1328 to 1348
1209 to 1229	1239 to 1259	1269 to 1289	1299 to 1319	1329 to 1349
1210 to 1230	1240 to 1260	1270 to 1290	1300 to 1320	1330 to 1350
1211 to 1231	1241 to 1261	1271 to 1291	1301 to 1321	1331 to 1351
1212 to 1232	1242 to 1262	1272 to 1292	1302 to 1322	1332 to 1352
1213 to 1233	1243 to 1263	1273 to 1293	1303 to 1323	1333 to 1353
1214 to 1234	1244 to 1264	1274 to 1294	1304 to 1324	1334 to 1354
1215 to 1235	1245 to 1265	1275 to 1295	1305 to 1325	1335 to 1355
1216 to 1236	1246 to 1266	1276 to 1296	1306 to 1326	1336 to 1356
1217 to 1237	1247 to 1267	1277 to 1297	1307 to 1327	1337 to 1357
1218 to 1238	1248 to 1268	1278 to 1298	1308 to 1328	1338 to 1358
1219 to 1239	1249 to 1269	1279 to 1299	1309 to 1329	1339 to 1359
1220 to 1240	1250 to 1270	1280 to 1300	1310 to 1330	1340 to 1360
1221 to 1241	1251 to 1271	1281 to 1301	1311 to 1331	1341 to 1361
1222 to 1242	1252 to 1272	1282 to 1302	1312 to 1332	1342 to 1362
1223 to 1243	1253 to 1273	1283 to 1303	1313 to 1333	1343 to 1363
1224 to 1244	1254 to 1274	1284 to 1304	1314 to 1334	1344 to 1364
1225 to 1245	1255 to 1275	1285 to 1305	1315 to 1335	1345 to 1365
1226 to 1246	1256 to 1276	1286 to 1306	1316 to 1336	1346 to 1366
1227 to 1247	1257 to 1277	1287 to 1307	1317 to 1337	1347 to 1367
1228 to 1248	1258 to 1278	1288 to 1308	1318 to 1338	1348 to 1368
1229 to 1249	1259 to 1279	1289 to 1309	1319 to 1339	1349 to 1369
1230 to 1250	1260 to 1280	1290 to 1310	1320 to 1340	1350 to 1370

Figure 5j

nucleotide positions of 21mers of an OPR3-like encoding polynucleotide of	
SEQ ID NO:1, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, or	
a polynucleotide sequence encoding an OPR3-like homolog	
1351 to 1371	1381 to 1401
1352 to 1372	1382 to 1402
1353 to 1373	1383 to 1403
1354 to 1374	1384 to 1404
1355 to 1375	1385 to 1405
1356 to 1376	1386 to 1406
1357 to 1377	1387 to 1407
1358 to 1378	1388 to 1408
1359 to 1379	1389 to 1409
1360 to 1380	1390 to 1410
1361 to 1381	1391 to 1411
1362 to 1382	1392 to 1412
1363 to 1383	1393 to 1413
1364 to 1384	1394 to 1414
1365 to 1385	…..
1366 to 1386	…..
1367 to 1387	n-5 to n+15
1368 to 1388	n-4 to n+16
1369 to 1389	n-3 to n+17
1370 to 1390	n-2 to n+18
1371 to 1391	n-1 to n+19
1372 to 1392	n to n+20
1373 to 1393	
1374 to 1394	
1375 to 1395	
1376 to 1396	
1377 to 1397	
1378 to 1398	
1379 to 1399	
1380 to 1400	

n = total number of nucleotides of the entire length of an OPR3-like encoding polynucleotide – 20.

For example:
n = 1390 (=1410-20) for SEQ ID NO:1; n =1171 (=1191-20) for SEQ ID NO:7;
n = 1156 (=1176-20) for SEQ ID NO:9; n =1180 (=1200-20) for SEQ ID NOs:11,15,17&27;
n = 1165 (=1185-20) for SEQ ID NO:13; n =742 (=762-20) for SEQ ID NO:19;
n = 1183 (=1203-20) for SEQ ID NO:21; n =1171(=1191-20) for SEQ ID NO:23;
n = 1189 (=1209-20) for SEQ ID NO:25; n =1394 (=1414-20) for SEQ ID NO:29