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(54) **Title:** COMPOSITIONS AND METHODS USING RNA INTERFERENCE 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 plant gene, which is a 50657480 gene or a homolog thereof, and relates to the generation of plants that have increased resistance to parasitic nematodes.

COMPOSITIONS AND METHODS USING RNA INTERFERENCE FOR CONTROL OF NEMATODES

CROSS REFERENCE TO RELATED APPLICATIONS

5 **[Para 1]** This application claims the priority benefit of U.S. Provisional Application Serial No. 60/899739 filed February 06, 2007.

FILED OF THE INVENTION

[Para 2] The field of this invention is the control of nematodes, in particular the control of 10 soybean cyst nematodes. The invention also relates to the introduction of genetic material into plants that are susceptible to nematodes in order to increase resistance to nematodes.

BACKGROUND OF THE INVENTION

15 **[Para 3]** Nematodes are microscopic wormlike animals 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. One common type of nematode is the root-knot nematode (RKN), whose feeding causes the characteristic galls on roots. Other root-feeding nematodes are the cyst- and lesion-types, which are more host specific.

20 **[Para 4]** Nematodes are present throughout the United States, but are mostly a problem in warm, humid areas of the South and West, and in sandy soils. Soybean cyst nematode (SCN), Heterodera glycines, was first discovered in the United States in North Carolina in 1954. It is the most serious pest of soybean plants. Some areas are so heavily infested by SCN that soybean production is no longer economically possible without control measures. Although 25 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, nematodes, including SCN, can cause significant yield loss without obvious above-ground symptoms. In addition, roots infected with SCN are dwarfed 30 or stunted. Nematode infestation 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 35 as a year, or longer, to complete the life cycle. When temperature and moisture levels become adequate in the spring, worm-shaped juveniles hatch from eggs in the soil. These juveniles are the only life stage of the nematode that can infect soybean roots.

[Para 7] The life cycle of SCN has been the subject of many studies and therefore can be used as an example for understanding a nematode life cycle. After penetrating the soybean roots, SCN juveniles move through the root until they contact vascular tissue, where they stop and start to feed. The nematode injects secretions that modify certain root cells and transform
5 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 the nematodes feed, they swell and eventually female nematodes become so large that they break through the root tissue and are exposed on the
10 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 lemon-shaped 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, then
15 later within the body cavity. Eventually the entire body cavity of the adult female is filled with eggs, and the female 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.
20 Although many of the eggs may hatch within the first year, many also will survive within the 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
25 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.

30 **[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
35 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 PCT Publication WO 01/96584, WO 01/17654, 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 (742 nucleotides, 1033 nucleotides, 785 nucleotides, 531 nucleotides, 576 nucleotides, 651 nucleotides, 1015 nucleotides, 1033 nucleotides, 730 nucleotides, 830 nucleotides, see Table 1) 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] 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

5 **[Para 16]** The present inventors have discovered a novel plant target gene ("50657480") which is overexpressed in syncytia induced by infection of soybean roots by SCN. The inventors have further discovered that when expression of gene 50657480 is suppressed in a soybean root model system, the ability of nematodes to infect such roots is decreased.

[Para 17] In a first embodiment, therefore, the invention provides a double stranded RNA
10 (dsRNA) molecule comprising a) a first strand comprising a sequence substantially identical to a portion of a 50657480-like gene or a 50657480-homolog and b) a second strand comprising a sequence substantially complementary to the first strand.

[Para 18] 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
15 about 19 to 24 nucleotides, wherein said RNA molecules are derived from a polynucleotide being substantially identical to a portion of a 50657480-like gene or a 50657480-homolog.

[Para 19] In another embodiment, the invention provides a transgenic nematode-resistant plant capable of expressing a dsRNA that is substantially identical to a portion of a 50657480-like gene or a 50657480-homolog

20 **[Para 20]** 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 a 50657480-like gene or a 50657480-homolog.

25 **[Para 21]** 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 a 50657480-like gene or a 50657480-homolog 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 a 50657480-like gene or a 50657480-homolog, wherein the nucleic acid is able to
30 form a double-stranded transcript of a portion of a 50657480-like gene or a 50657480-homolog 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 22] The invention further provides a method of conferring nematode resistance to a
35 plant, said method comprising the steps of: a) preparing a nucleic acid having a region that is substantially identical to a portion of a 50657480-like gene or a 50657480-homolog, wherein the nucleic acid is able to form a double-stranded transcript of a portion of a 50657480-like gene or

a 50657480-homolog 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 23] The invention further provides an expression vector comprising a sequence substantially identical to a portion of a 50657480-like gene or a 50657480-homolog.

BRIEF DESCRIPTION OF THE DRAWINGS

[Para 24] Figure 1a-1c: Table describing primers used to generate the dsRNA construct RAW464 and the RACE fragments corresponding to 50657480/

[Para 25] Figure 2: DNA sequence alignment of RACE sequence variant A (SEQ ID NO:7) with 50657480 cDNA sequence (SEQ ID NO:1)

[Para 26] Figure 3: Contig consensus sequence (SEQ ID NO:8)of RACE variant A and 50657480 describing the open reading frame in bold letters.

[Para 27] Figure 4: Table showing representative homologs of the full length amino acid sequence of 50657480 described by SEQ ID NO:10. The table shows SEQ ID NO, sequence type, organism, and GenBank sequence Id for the representative homologs.

[Para 28] Figure 5a-5c: Amino acid sequence alignment of the representative homologs of SEQ ID NO:10.

[Para 29] Figure 6: Matrix table describing the global amino acid percent identity of the identified representative homologs.

[Para 30] Figure 7: Matrix table describing the global nucleotide percent identity of the DNA sequences of the identified representative homologs.

[Para 31] Figure 8a to 8i: shows various 21mers possible in SEQ ID NO:8 by nucleotide position. For example the 21mer could comprise nucleotides at position 1 to 21, nucleotides at position 2-22, nucleotides at position 3-23, etc. 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.

DETAILED DESCRIPTION OF THE INVENTION

[Para 32] 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. 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] In accordance with the invention, a plant is transformed with a nucleic acid or a dsRNA, which specifically inhibits expression of a 50657480 target gene, a 50657480-like gene, or a 50657480 homolog in the plant root 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 a preferred embodiment, inhibition of the 50657480 target gene, a 50657480-like gene, or a 50657480 homolog occurs using dsRNA capable of targeting said gene, which dsRNA has been transformed into an ancestor of the infected plant. Preferably, the nucleic acid sequence expressing the dsRNA is under the transcriptional control of a root specific promoter or a parasitic nematode feeding site-specific promoter or a nematode inducible promoter.

[Para 34] As used herein the terms “target gene”, “50657480 target gene”, “50657480-like gene” and “50657480 gene” refer to genes, which are at least about 50-60%, at least about 60-70%, or at least about 70-75%, 75-80%, 80-85%, 85-90%, or 90-95%, and may also be at least about 96%, 97%, 98%, 99%, or more identical to a polynucleotide comprising the sequence set forth in SEQ ID NO:1, nucleotides 7 to 483 of SEQ ID NO: 1, SEQ ID NO: 7, nucleotides 1 to 1096 of SEQ ID NO:7 or SEQ ID NO:8. Alternatively, suitable 50657480 target genes comprise a polynucleotide that hybridizes under stringent conditions to a polynucleotide comprising the sequence set forth in SEQ ID NO:1 nucleotides 7 to 483 of SEQ ID NO: 1, SEQ ID NO: 7, nucleotides 1 to 1096 of SEQ ID NO:7 or SEQ ID NO:8. The term “50657480 homolog” encompasses genes or sequences, which can be identified by using a part or the full length of any of the sequences disclosed herein, in particular SEQ ID NO: 8, 9, 17, 19, 21, 23, 25, 27, 29 or SEQ ID NO: 4, 5, 14 or 15.

[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 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 80%-90% identical to 20 or more contiguous nucleotides of the target gene, more preferably, at least 90-95%,identical to 20 or more contiguous nucleotides of the target gene, and most preferably at least 95%, 96%, 97%, 98% or 99% identical or absolutely identical to 20 or more contiguous nucleotides of the target gene. 20 or more contiguous nucleotides means a portion, being at least about 20, 21, 22, 23, 24, 25, 50, 100, 200, 300, 400, 500, 1000, 1500, or 2000 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 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, except for the gene responsible for the increased resistance to the nematode. 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 hamper, 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 2).

[Para 43] The dsRNA molecule of the invention comprises a first strand that is substantially identical to at least a portion of the 50657480 target gene, the 50657480-like gene, or 50657480 homolog. Preferably the portion of the gene is the full length of the 50657480 target gene as set forth in SEQ ID NO:8, or of the 50657480-like genes and 50657480 homologs as set forth in SEQ ID NOs:17, 19, 21, 23, 25, 27 or 29. More preferably, the dsRNA of the invention comprises a first strand that is substantially identical to from about 19 to about 477 consecutive nucleotides of a sequence selected from the group consisting of: a) a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, nucleotides 7 to 483 of SEQ ID NO: 1, SEQ ID NO: 7, nucleotides 1 to 1096 of SEQ ID NO:7 or SEQ ID NO:8; b) a polynucleotide comprising a sequence having at least 80% sequence identity to SEQ ID NO.1, nucleotides 7 to 483 of SEQ

ID NO: 1, SEQ ID NO: 7, nucleotides 1 to 1096 of SEQ ID NO:7 or SEQ ID NO:8; c) a polynucleotide that hybridizes under stringent conditions to a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, nucleotides 7 to 483 of SEQ ID NO: 1, SEQ ID NO: 7, nucleotides 1 to 1096 of SEQ ID NO:7 or SEQ ID NO:8 d) a polynucleotide being obtainable with primers having the sequence as set forth in SEQ ID NO: 4, 5, 14, or 15, e) a polynucleotide comprising a sequence having at least 50% sequence identity to a polynucleotide coding for a nucleotide sequence as set forth in SEQ ID NO: 9, 17, 19, 21, 23, 25, 27 or 29, f) a polynucleotide comprising a sequence having at least 40% sequence identity to a polynucleotide coding for an amino acid sequence as set forth in SEQ ID NO:10, 16, 18, 20, 22, 24, 26 or 28. The dsRNA of the invention further comprises a second strand that is substantially identical to the first strand. The dsRNA of the invention, can be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

[Para 44] Additional 50657480-like genes and 50657480 homologs can be identified with techniques known in the art, such like, but not excluding others, hybridization, RT-PCR, PCR, and the like. For example. 50657480-like genes and 50657480 homologs are obtainable with primers having the sequence as set forth in SEQ ID NO: 4, 5, 12, 13, 14, or 15. 50657480 homologs have at least 50%, 60%, 70, 80%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to a polynucleotide coding for a nucleotide sequence as set forth in SEQ ID NO: 9, 17, 19, 21, 23, 25, 27 or 29, or have at least 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to a polynucleotide coding for an amino acid sequence as set forth in SEQ ID NO:10, 16, 18, 20, 22, 24, 26 or 28. Preferably they have at least 50%, 60%, 70, 80%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to a polynucleotide coding for a nucleotide sequence as set forth in SEQ ID NO: 9, or have at least 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99% sequence identity to a polynucleotide coding for an amino acid sequence as set forth in SEQ ID NO:10. Also preferred are 50657480-like genes and 50657480 homologs having at least 90%, 95%, 96%, 97%, 98%, 99% sequence identity to a polynucleotide coding for a nucleotide sequence as set forth in SEQ ID NO: 9, 17, 19, 21, 23, 25, 27 or 29, or have at least 90%, 95%, 96%, 97%, 98%, 99% sequence identity to a polynucleotide coding for an amino acid sequence as set forth in SEQ ID NO:10, 16, 18, 20, 22, 24, 26 or 28. .

[Para 45] For example, a nucleic acid molecule coding for a 50657480-like genes or 50657480 homolog can be isolated from a polynucleotide derived from a plant that hybridizes under stringent conditions to a nucleotide sequence of SEQ ID NO:1, SEQ ID NO: 7 or SEQ ID NO:8. Such a polynucleotide 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, SEQ ID NO:7 and SEQ ID NO:8. Nucleic acid molecules corresponding to the plant target genes of the invention 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. The nucleic acid sequences determined from the cloning of the genes from soybean allow for the generation of probes and primers designed for use in identifying and/or cloning 50657480-like genes and 50657480 homologs in other cell types and organisms, as well as homologs from other plant species. E.g. primers having the sequence as set forth in SEQ ID NO: 4, 5, 12, 13, 14, or 15 can be used in identifying and/or cloning 50657480-like genes and 50657480 homologs.

[Para 46] Such primers can also be used to clone variants of 50657480-like genes and 50657480 homologs. Variants are usually sequence variants having at least 95%, 96%, 97%, 98% or 99% sequence identity to a nucleotide sequence or an amino acid sequence as set forth in SEQ ID NO: 8, 9 or 10. Preferably such variants are obtained from plants of the familiy Fabaceae, in particular from the genus Glycine.

[Para 47] 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. Thus the dsRNA of the present invention may range in length from about 19 nucleotides up to the whole length of the 50657480-like gene or a 50657480-homolog . Preferably, the dsRNA of the invention has a length from about 21 nucleotides to about 600 nucleotides. More preferably, the dsRNA of the invention has a length from about 21 nucleotides to about 500 nucleotides, or from about 21 nucleotides to about 400 nucleotides.

[Para 48] When dsRNA of the invention has a length longer than about 21 nucleotides, for example from about 50 nucleotides to about 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 about 19mers to about 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 dsRNAs or siRNAs of the invention have sequences corresponding to fragments of about 19-24 contiguous nucleotides across the entire sequence of the 50657480-like gene or the 50657480-homolog. Figures 8a-8e set forth exemplary 21-mers derived from SEQ ID NO:8.

In a similar manner, 19-20, 22, 23, and 24-mers derived from SEQ ID NO:8 are encompassed by the present invention.

[Para 50] The invention is additionally embodied as a pool of dsRNA molecules derived from a 50657480 gene, a 50657480-like gene, or 50657480 homolog. For example, a pool of siRNA of the invention derived from the 50657480 gene as set forth in SEQ ID NO:1, SEQ ID NO: 7 or SEQ ID NO:8 may comprise a multiplicity of RNA molecules which are selected from the group consisting of oligonucleotides substantially identical to the 21mer nucleotides of SEQ ID NO:8 as disclosed in Figures 8a-8e or any 50657480-like gene or a 50657480-homolog. A pool of siRNA of the invention derived from the 50657480-like gene or the 50657480-homolog e.g. of SEQ ID NO:1, SEQ ID NO: 7 or SEQ ID NO:8 may also comprise any combination of the specific RNA molecules having any of the 21 contiguous nucleotide sequences derived from SEQ ID NO:8 as set forth in Figures 8a-8e. The table of Figures 8a-8e can also be used to calculate various 19, 20, 22, 23 or 24-mers or start and end of a portion of 50657480-like gene or a 50657480-homolog. Which 19, 20, 22, 23 or 24-mers or portion is the best to choose for a particular plant can be determined with the information given in Figures 5, 6 and 7. The 19, 20, 22, 23 or 24-mers or portion having the highest sequence identity to a particular 50657480-like gene or a 50657480-homolog of a particular plant or showing a high degree of sequence conservation in 50657480-like genes or a 50657480-homologs is the most preferred 19, 20, 22, 23 or 24-mer or portion.

[Para 51] A dsRNA comprising a nucleotide sequence identical to a portion of the 50657480 gene, 50657480-like gene or 50657480 homolog is preferred for inhibition. As disclosed herein, 100% sequence identity between the RNA and the 50657480 gene, 50657480-like gene or 50657480 homolog is preferred, but not required to practice the present invention. One of skill in the art will recognize that the siRNA can have a mismatch with the target gene of at least 1, 2, or more nucleotides. Further, these mismatches are intended to be included in the present invention. For example, it is contemplated in the present invention that the 21mer dsRNA sequences exemplified in Figures 8a-8e may contain an addition, deletion or substitution of 1, 2, or more nucleotides and the resulting sequence still interferes with the function of the 50657480 gene, 50657480-like gene or 50657480 homolog. Thus, the invention has the advantage of being able to tolerate sequence variations that might be expected due to gene manipulation or synthesis, genetic mutation, strain polymorphism, or evolutionary divergence.

[Para 52] The degree of sequence identity between the dsRNA and the 50657480 gene, 50657480-like gene or 50657480 homolog 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
5 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). The length of the portion or 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, or 2000 bases or up to the full length of the gene. In a preferred
10 embodiment, the length of the portion is approximately from about 19 to about 500 nucleotides in length. In another embodiment the portion is from about 50 to about 700 nucleotides in length, in a more preferred embodiment the portion if from about 100 to about 600 nucleotides in length, in an even more preferred embodiment the portion is from about 200 to 500 nucleotides in length. In a further embodiment the portion consists of from about 19 nucleotides
15 to 25% of the whole length of the target gene, more preferred from 25% to 50% even more preferred from 50% to 75% and most preferred 75% to 100% of the whole length of the target gene..

[Para 53] 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-
20 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
25 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 54] In another embodiment, the invention provides an isolated recombinant expression vector comprising a nucleic acid encoding a dsRNA molecule as described above, wherein
30 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
35 vector, 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, 5 “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 55] The recombinant expression vectors of the invention comprise a nucleic acid of the 10 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, 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 15 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 20 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 25 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.

30 **[Para 56]** In accordance with the invention, the recombinant expression vector comprises a regulatory sequence, e.g. a promoter, 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 35 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, 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 57] 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 on 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 58] 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. leafs, stems, flowers or seeds.

[Para 59] 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 feeding sites, e.g. syncytial cells or giant cells.

[Para 60] 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).

5 **[Para 61]** 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, but not limited to 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-
10 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
15 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 62] 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-
20 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
25 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).

30 **[Para 63]** 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.
35 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 64] 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 about 500, or up to the full length, consecutive nucleotides of SEQ ID NO:1, SEQ ID NO: 7 or SEQ ID NO:8; 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 about 3 to about 500 base pairs, and wherein after transcription, the RNA transcript folds on itself to form a hairpin.

[Para 65] 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 50657480-like gene or a 50657480-homolog 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 66] In another embodiment, the vector contains two promoters one mediating transcription of the sequence substantially identical to a portion of a 50657480-like gene or a 50657480-homolog and another promoter 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.
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 be 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 a 50657480 gene, while another promoter mediates tissue- or cell-specific transcription or pathogen inducible expression of the complementary nucleic acid.

[Para 67] The invention is also embodied in a transgenic plant capable of expressing the dsRNA of the invention and thereby inhibiting the 50657480-like genes or 50657480 homolog (target gene) in the roots, feeding site, syncytia and/or giant cell

[Para 68] 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,
5 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,
10 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,
15 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,
20 Browaalia, Phaseolus, Avena, and Allium. In one embodiment the plant is a monocotyledonous plant or a dicotyledonous plant.

[Para 69] Preferably 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.
25 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
30 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
35 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.

5 **[Para 70]** 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
10 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
15 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
20 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 Asteraceae, 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
25 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
30 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
35 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.

5 **[Para 71]** . 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 10 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 72] Transformation methods may include direct and indirect methods of transformation. Suitable direct methods include polyethylene glycol induced DNA uptake, 15 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, 20 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 73] Transformation can also be carried out by bacterial infection by means of 25 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 30 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 35 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 74] 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 75] 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 76] “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 77] In accordance with this embodiment, the transgenic plant of the invention is produced by a method comprising the steps of providing a preparing an expression cassette having a first region that is substantially identical to a portion of a 50657480 gene, a 50657480-like gene or a 50657480 homolog, 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 78] 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 79] 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 80] 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 81] 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 82] The present invention also provides a method for inhibiting expression of a 50657480 gene, a 50657480-like gene, or a 50657480 homolog. In accordance with this embodiment, the method comprises the step of administering to the plant a dsRNA of the invention.

[Para 83] 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 are within the ordinary level of skill in the art are intended to fall within the scope of the present invention.

EXAMPLE 1: CLONING OF 50657480 FROM SOYBEAN

Laser Excision of Syncytia

[Para 84] Glycine max cv. Williams 82 was germinated on agar plates for three days and then transferred to germination pouches. 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).

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 85] As demonstrated in Table 2, Soybean cDNA clone 50657480 was identified as being up-regulated in syncytia of SCN-infected soybean roots. The amino acid sequence of soybean cDNA clone 50657480 (SEQ ID NO:1) is described as SEQ ID NO: 3. The 50657480 cDNA sequence (SEQ ID NO:1) was determined not to be full-length as there no ATG start codon.

Table 2

Gene Name	Syncytia #1(N)¶	Syncytia #2 (N)	Non-Syncytia	Control Roots
50657480§	299±47 (4)	369±57 (5)	not detected	not detected

EXAMPLE 2 GENERATION OF TRANSGENIC SOYBEAN HAIRY-ROOT AND NEMATODE BIOASSAY

This exemplified method employs binary vectors containing fragments of the 50657480 target gene. The vector consists of an antisense fragment of the target 50657480 gene, a spacer, a sense fragment of the target gene and a vector backbone. The sequence of the 50657480 cDNA clone is described as SEQ ID NO:1. The target gene fragment described by SEQ ID NO:2 corresponding to nucleotides 7 to 483 of SEQ ID NO:1 was used to construct the binary vector RAW464. In RAW464 the dsRNA for the 50657480 target gene was expressed under a syncytia or root preferred promoter p-At5g05340 (US-provisional application No: 60/899,693 SEQ ID NO: 6), a peroxidase gene promoter. This promoter drives transgene expression preferentially in roots and/or syncytia or giant cells. The plant selectable marker in the binary vectors is a herbicide-resistant form of the acetohydroxy acid synthase (AHAS) gene from Arabidopsis thaliana driven by the native Arabidopsis AHAS promoter (Sathasivan et al.,

Plant Phys. 97:1044-50, 1991). ARSENAL (imazapyr, BASF Corp, Florham Park, NJ) was used as the selection agent.

[Para 86] The binary vector RAW464 was transformed into Agrobacterium rhizogenes K599 strain by electroporation and transgenic hairy roots were generated using known methods. Several independent transgenic hairy root lines were generated from transformation. Non-transgenic hairy roots from soybean cultivar Williams 82 (SCN susceptible) and Jack (SCN resistant) were also generated by using non-transformed A. rhizogenes, to serve as controls for nematode growth in the assay. Hairy root cultures of each line were inoculated with SCN race 3 second stage juveniles (J2). Four weeks after nematode inoculation, the cyst number in each well was counted. For RAW464 transgenic root lines there were multiple lines demonstrating mean cyst counts around 6-7 and 11-18 as compared to a mean cyst count of 24 and 26 for the susceptible line Williams 82 (W82) and 1 and 1 for the known resistant line, Jack, respectively. These bioassay results indicate that the double stranded RNA expressed in RAW464 results in reduced cyst count.

Example 3 RACE to determine full transcribed sequence for 50657480 (SEQ ID NO:1)

[Para 87] Amplification of full-length transcript sequence corresponding to the cDNA sequence described by 50657480 (SEQ ID NO:1) was achieved using the GeneRacer Kit (L1502-01) from Invitrogen by following the manufacturers instructions. The primers used for the primary PCR reaction are described by SEQ ID NOs 12 and 14. The secondary nested PCR reaction primers are described by SEQ ID NOs 13 and 15.

[Para 88] As shown in Figure 2, SEQ ID NO:7 is the 5′ fragment of 50657480. Based on the alignment of SEQ ID NO:7 and SEQ ID NO:1 shown in Figure 2, a putative full length contig sequence was isolated and is described by SEQ ID NO:8. There is an open reading frame in SEQ ID NO:8 contig sequence that spans from bases 124 to 1440 as shown in Figure 3. The open reading frame sequence is described by SEQ ID NO:9. The amino acid sequence of the open reading frame described by SEQ ID NO:9 is shown as SEQ ID NO:10.

Example 4 Description of homologs (nucleotide and AA)

As disclosed in Example 3, the putative full length transcript sequence of the gene corresponding to SEQ ID NO:1 contains an open reading frame with the amino acid sequence disclosed as SEQ ID NO:10. The identification of gene homologs to the amino acid sequence described by SEQ ID NO:10 identifies additional sequences. A sample of genes with amino acid and DNA sequences homologous to SEQ ID NO:10 and SEQ ID NO:9, respectively, were identified and are described by SEQ ID NOs 16 to 29 and shown in Figure 4. The amino acid alignment of the identified truncated homologs to SEQ ID NO:10 is shown in Figure 5. A matrix table showing the amino acid percent identity of the identified homologs and SEQ ID NO:10 to

each other is shown in Figure 6. A matrix table showing the DNA sequence percent identity of the identified homologs and SEQ ID NO:9 to each other is shown in Figure 7.

[Para 89] Those skilled in the art will recognize, or will be able to ascertain using no more than
5 routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

What is claimed is:

1. A dsRNA molecule comprising a) a first strand comprising a sequence substantially identical to a portion of a a 50657480 gene, a 50657480-like gene or a 50657480-homolog and b) a second strand comprising a sequence substantially complementary to the first strand.

2. The dsRNA molecule of claim 1, wherein the portion of the 50657480 gene, 50657480-like gene or a 50657480-homolog is a sequence selected from the group consisting of:
a) a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, nucleotides 7 to 483 of SEQ ID NO: 1, SEQ ID NO: 7, nucleotides 1 to 1096 of SEQ ID NO:7 or SEQ ID NO:8;
b) a polynucleotide comprising a sequence having at least 80% sequence identity to SEQ ID NO.1, nucleotides 7 to 483 of SEQ ID NO: 1, SEQ ID NO: 7, nucleotides 1 to 1096 of SEQ ID NO:7 or SEQ ID NO:8;
c) a polynucleotide that hybridizes under stringent conditions to a polynucleotide comprising a sequence as set forth in SEQ ID NO:1 nucleotides 7 to 483 of SEQ ID NO: 1, SEQ ID NO: 7, nucleotides 1 to 1096 of SEQ ID NO:7 or SEQ ID NO:8,
d) a polynucleotide being obtainable with primers having the sequence as set forth in SEQ ID NO: 4, 5, 14, or 15,
e) a polynucleotide comprising a sequence having at least 50% sequence identity to a polynucleotide coding for a nucleotide sequence as set forth in SEQ ID NO: 9, 17, 19, 21, 23, 25, 27 or 29.
f) a polynucleotide comprising a sequence having at least 40% sequence identity to a polynucleotide coding for an amino acid sequence as set forth in SEQ ID NO:10, 16, 18, 20, 22, 24, 26 or 28.

3. The dsRNA molecule of claim 1, wherein the portion of the target gene is from about 19 to 500 nucleotides.

4. 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 a 50657480 gene, a 50657480-like gene or a 50657480-homolog.

5. A pool of dsRNA molecules as claimed in claim 4, 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, nucleotides 7 to 483 of SEQ ID NO: 1, SEQ ID NO: 7, nucleotides 1 to 1096 of SEQ ID NO:7 or SEQ ID NO:8;

b) a polynucleotide comprising a sequence having at least 80% sequence identity to SEQ ID NO.1, nucleotides 7 to 483 of SEQ ID NO: 1, SEQ ID NO: 7, nucleotides 1 to 1096 of SEQ ID NO:7 or SEQ ID NO:8;

c) a polynucleotide that hybridizes under stringent conditions to a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, nucleotides 7 to 483 of SEQ ID NO: 1, SEQ ID NO: 7, nucleotides 1 to 1096 of SEQ ID NO:7 or SEQ ID NO:8

d) a polynucleotide being obtainable with primers having the sequence as set forth in SEQ ID NO: 4, 5, 14, or 15,

e) a polynucleotide comprising a sequence having at least 50% sequence identity to a polynucleotide coding for a nucleotide sequence as set forth in SEQ ID NO: 9, 17, 19, 21, 23, 25, 27 or 29.

f) a polynucleotide comprising a sequence having at least 40% sequence identity to a polynucleotide coding for an amino acid sequence as set forth in SEQ ID NO:10, 16, 18, 20, 22, 24, 26 or 28.

6. A transgenic plant capable of expressing a dsRNA that is substantially identical to a portion of a 50657480-like gene or a 50657480-homolog.

7. The transgenic plant of claim 6, wherein the 50657480 gene,, 50657480-like gene or 50657480-homolog comprises a sequence selected from the group consisting of:

a) a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, nucleotides 7 to 483 of SEQ ID NO: 1, SEQ ID NO: 7, nucleotides 1 to 1096 of SEQ ID NO:7 or SEQ ID NO:8;

b) a polynucleotide comprising a sequence having at least 80% sequence identity to SEQ ID NO.1, nucleotides 7 to 483 of SEQ ID NO: 1, SEQ ID NO: 7, nucleotides 1 to 1096 of SEQ ID NO:7 or SEQ ID NO:8;

c) a polynucleotide that hybridizes under stringent conditions to a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, nucleotides 7 to 483 of SEQ ID NO: 1, SEQ ID NO: 7, nucleotides 1 to 1096 of SEQ ID NO:7 or SEQ ID NO:8,

d) a polynucleotide being obtainable with primers having the sequence as set forth in SEQ ID NO: 4, 5, 14, or 15,

e) a polynucleotide comprising a sequence having at least 50% sequence identity to a polynucleotide coding for a nucleotide sequence as set forth in SEQ ID NO: 9, 17, 19, 21, 23, 25, 27 or 29.

f) a polynucleotide comprising a sequence having at least 40% sequence identity to a polynucleotide coding for an amino acid sequence as set forth in SEQ ID NO:10, 16, 18, 20, 22, 24, 26 or 28.

5 8. A transgenic plant capable of expressing a pool of dsRNA molecules, wherein pool of RNA molecules each comprising a double stranded region having a length of about 19-24 nucleotides, , wherein the RNA molecules are derived from a polynucleotide substantially identical to a portion of a 50657480 gene, a 50657480-like gene or a 50657480-homolog.

10

9. The transgenic plant of claim 8, 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, nucleotides 7 to 483 of SEQ ID NO: 1, SEQ ID NO: 7, nucleotides 1 to 1096 of SEQ ID NO:7 or SEQ ID
15 NO:8;

b) a polynucleotide comprising a sequence having at least 80% sequence identity to SEQ ID NO.1, nucleotides 7 to 483 of SEQ ID NO: 1, SEQ ID NO: 7, nucleotides 1 to 1096 of SEQ ID NO:7 or SEQ ID NO:8;

c) a polynucleotide that hybridizes under stringent conditions to a polynucleotide
20 comprising a sequence as set forth in SEQ ID NO:1, nucleotides 7 to 483 of SEQ ID NO: 1, SEQ ID NO: 7, nucleotides 1 to 1096 of SEQ ID NO:7 or SEQ ID NO:8

d) a polynucleotide being obtainable with primers having the sequence as set forth in SEQ ID NO: 4, 5, 14, or 15,

e) a polynucleotide comprising a sequence having at least 50% sequence identity to a
25 polynucleotide coding for a nucleotide sequence as set forth in SEQ ID NO: 9, 17, 19, 21, 23, 25, 27 or 29.

f) a polynucleotide comprising a sequence having at least 40% sequence identity to a polynucleotide coding for an amino acid sequence as set forth in SEQ ID NO:10, 16, 18, 20, 22, 24, 26 or 28.

30

10. A method of making a transgenic plant capable of expressing a pool of dsRNA molecules that is substantially identical to a portion of a 50657480 gene, a 50657480-like gene or a 50657480-homolog in a plant, said method comprising the steps of:

a) preparing a nucleic acid sequence having a region that is substantially identical to a
35 portion of a 50657480 gene, a 50657480-like gene or a 50657480-homolog, wherein the nucleic acid is able to form a double-stranded transcript of a portion of a 50657480-like gene or a 50657480-homolog 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.

5 11. The method of claim 10, wherein the target gene comprises a sequence selected from the group consisting of:

a) a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, nucleotides 7 to 483 of SEQ ID NO: 1, SEQ ID NO: 7, nucleotides 1 to 1096 of SEQ ID NO:7 or SEQ ID NO:8;

10 b) a polynucleotide comprising a sequence having at least 80% sequence identity to SEQ ID NO.1, nucleotides 7 to 483 of SEQ ID NO: 1, SEQ ID NO: 7, nucleotides 1 to 1096 of SEQ ID NO:7 or SEQ ID NO:8;

c) a polynucleotide that hybridizes under stringent conditions to a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, nucleotides 7 to 483 of SEQ ID NO:
15 1, SEQ ID NO: 7, nucleotides 1 to 1096 of SEQ ID NO:7 or SEQ ID NO:8

d) a polynucleotide being obtainable with primers having the sequence as set forth in SEQ ID NO: 4, 5, 14, or 15,

e) a polynucleotide comprising a sequence having at least 50% sequence identity to a polynucleotide coding for a nucleotide sequence as set forth in SEQ ID NO: 9, 17, 19,
20 21, 23, 25, 27 or 29.

f) a polynucleotide comprising a sequence having at least 40% sequence identity to a polynucleotide coding for an amino acid sequence as set forth in SEQ ID NO:10, 16, 18, 20, 22, 24, 26 or 28.

25 12. The method of claim 10, wherein the portion of the 50657480 gene, 50657480-like gene or 50657480-homolog e is from about 19 to about 500 nucleotides.

13. The method of claim 10, wherein the plant is selected from the group consisting of: soybean, potato, tomato, peanuts, cotton, cassava, coffee, coconut, pineapple, citrus
30 trees, banana, corn, rape, beet, sunflower, sorghum, wheat, oats, rye, barley, rice, green bean, lima bean, pea, and tobacco.

14. The method of claim 10 wherein the plant is a soybean plant.

35 15. A method of conferring nematode resistance to a plant, said method comprising the steps of:

a) preparing a nucleic acid sequence having a region that is substantially identical to a portion of a 50657480 gene, a 50657480-like gene or a 50657480-homolog, wherein the nucleic acid is able to form a double-stranded transcript of a portion of a 50657480-like gene or a 50657480-homolog once expressed in the plant;

5 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.

16. An expression vector comprising a sequence substantially identical to a portion of a
10 50657480 gene, a 50657480-like gene or a 50657480-homolog.

17. An expression as claimed in claim 16, comprising a second sequence substantially complementary to the first strand, capable of forming a dsRNA, when both sequences are transcribed.

15

18. An expression as claimed in claim 16, comprising a root-preferable promoter.

Figure 1 Primer table

Primer name	Primer sequence	SEQ ID NO:
RNAi50657480for	TTAATTAAGACGTCGTGCAGCCTGCTTCC	SEQ ID NO:4
RNAi50657480rev	CTCGAGGGCGCGCCGCTTACGAACAACTATTAC	SEQ ID NO:5
GeneRacer™ 5′ Primer	CGACTGGAGCACGAGGACACTGA	SEQ ID NO:12
GeneRacer™ 5′ Nested Primer	GGACACTGACATGGACTGAAGGAGTA	SEQ ID NO:13
50657480-5RACE	CCGAGGAACCATTTGAAGGGGTCCAAGC	SEQ ID NO:14
50657480-5RACE-nest	GCCCCTGCCATAAAGCCTGTTGGAAGAG	SEQ ID NO:15

Figure 2a Alignment of RACE sequence variant A with 50657480 seq.

```
                    1                                                  50
SEQ ID NO:1      (1) --------------------------------------------------
SEQ ID NO:7      (1) GATGTTCTTCTATTGATTCCTCAATTTCTCCAACTCAAAACTAAGTTTCG

                     51                                                100
SEQ ID NO:1      (1) --------------------------------------------------
SEQ ID NO:7     (51) TCTTTGTGATCTCTTGATTATATTATATCGATTTGTGAATTGTTTTTCCA

                     101                                               150
SEQ ID NO:1      (1) --------------------------------------------------
SEQ ID NO:7    (101) CAAAAAAAAAATTCATTCAAACCATGGCTTCATTATGCCGGCGGTTATCC

                     151                                               200
SEQ ID NO:1      (1) --------------------------------------------------
SEQ ID NO:7    (151) GCCGCCGCACCGGCCGGTCACTTCCGATTCCATCGGCCCGCCGGGATTTG

                     201                                               250
SEQ ID NO:1      (1) --------------------------------------------------
SEQ ID NO:7    (201) CGCGGTTCGATACGGTTATTCCGTCCGGCGGCCAATGCGGTTGGTTGCGG

                     251                                               300
SEQ ID NO:1      (1) --------------------------------------------------
SEQ ID NO:7    (251) CGGGGACCACCGTGGGCGGCGGCGCGTGGGTGACATCCGATGACGTCGGA

                     301                                               350
SEQ ID NO:1      (1) --------------------------------------------------
SEQ ID NO:7    (301) GGGAGGGAGGAGAAGGTGGGGCCCTGCTCCTACGCCGTGGAGGATCGGAG

                     351                                               400
SEQ ID NO:1      (1) --------------------------------------------------
SEQ ID NO:7    (351) GGTGGCGGAAGATGGCGGAAGCGATGCGGTGGTAGGAAGTAGGAACCGGG

                     401                                               450
SEQ ID NO:1      (1) --------------------------------------------------
SEQ ID NO:7    (401) TGGTGGAAGTGGCGGCGGCGGCGGTTGCAACGGTGGTGCTGGGTGTTGGG

                     451                                               500
SEQ ID NO:1      (1) --------------------------------------------------
SEQ ID NO:7    (451) AACCGGGTTCTGTATAAGTTGGCTTTGGTTCCGTTGAAGCACTACCCTTT

                     501                                               550
SEQ ID NO:1      (1) --------------------------------------------------
SEQ ID NO:7    (501) CTTCCTTGCTCAACTTGCCACTTTCGGATATGTAATAGTGTACTTTGCGA

                     551                                               600
SEQ ID NO:1      (1) --------------------------------------------------
SEQ ID NO:7    (551) TTTTGTATATTCGACACCATGCGGGCATTGTTACTGATGAGATGTTAGAT

                     601                                               650
SEQ ID NO:1      (1) --------------------------------------------------
SEQ ID NO:7    (601) GCTCCAAAGGCTCCATTTATAGTTGTTGGTCTATTGGAGGCTCTCGCTGC
```

Figure 2b

```
                        651                                                700
SEQ ID NO:1       (1)  --------------------------------------------------
SEQ ID NO:7     (651)  TGCCACTGGAATGGCCGCAGGAGCAATTCTCTCTGGAGCTTCGATTCCAA

                        701                                                750
SEQ ID NO:1       (1)  --------------------------------------------------
SEQ ID NO:7     (701)  TTTTATCTCAGACTTTTCTAGTGTGGCAAATACTCCTGTCAATTATTTTT

                        751                                                800
SEQ ID NO:1       (1)  --------------------------------------------------
SEQ ID NO:7     (751)  CTTGGGAGAAGATATAAAGTCAACCAATTACTTGGATGCTTTCTTGTAAC

                        801                                                850
SEQ ID NO:1       (1)  --------------------------------------------------
SEQ ID NO:7     (801)  CATTGGTGTAGTTGTTACTGTAGCAAGTGGAGCTGGTGCTGGGAATTTAT

                        851                                                900
SEQ ID NO:1       (1)  --------------------------------------------------
SEQ ID NO:7     (851)  TAAAGGAAGGTGGTATGTTTTGGAGTCTTTTGATGATAGTTTCATTTTTC

                        901                                                950
SEQ ID NO:1       (1)  --------------------------------------------------
SEQ ID NO:7     (901)  CTCCAAGCGGCTGATACCGTGCTGAAGGAAATTATCTTTTTGGATTCATC

                        951                                               1000
SEQ ID NO:1       (1)  --------------------------------------------------
SEQ ID NO:7     (951)  CCGAAAATTGAAGGGAGGTTGTTGTATGGACCTTTTTGTTGTCAATTCGT

                        1001                                              1050
SEQ ID NO:1       (1)  --------------------------------------------------
SEQ ID NO:7    (1001)  ACGGATCTGCTTTCCAAGCACTATTCGTGTGCCTTCTTCTCCCCTTCTTG

                        1051                                              1100
SEQ ID NO:1       (1)  ----------------------------------------------AAGA
SEQ ID NO:7    (1051)  TCAAAATTATGGGGCATTCCCTTCAGTCAACTACCAAACTACCTTAAAGA

                        1101                                              1150
SEQ ID NO:1       (5)  TGGTGCAGCCTGCTTCCTAAATTTTGGTACATTATCAAGTGGATGTGATG
SEQ ID NO:7    (1101)  TGGTGCAGCCTGCTTCCTAAATTTTGGTACATTATCAAGTGGATGTGATG

                        1151                                              1200
SEQ ID NO:1      (55)  GTGCCCCTCTGCTTCCTCTGCTGTTTATTATTGTAAACATAGGTTTCAAT
SEQ ID NO:7    (1151)  GTGCCCCTCTGCTTCCTCTGCTGTTTATTATTGTAAACATAGGTTTCAAT

                        1201                                              1250
SEQ ID NO:1     (105)  ATTGCATTGCTTCATCTCCTCAAGATCTCTTCAGCTGTTGTATCTTGTCT
SEQ ID NO:7    (1201)  ATTGCATTGCTTCATCTCCTCAAGATCTCTTCAGCTGTTGTATCTTGTCT

                        1251                                              1300
SEQ ID NO:1     (155)  TGCTTCCACATTTTCAGTCCCAATATCCATCTACGTGTTCACCATGCCAT
SEQ ID NO:7    (1251)  TGCTTCCACATTTTCAGTCCCAATATCCATCTACGTGTTCACCATGCCAT

                        1301                                              1350
SEQ ID NO:1     (205)  TGCCATACCTTGGTGTTGCCTCCTCTCTTCCAACAGGCTTTATGGCAGGG
SEQ ID NO:7    (1301)  TGCCATACCTTGGTGTTGCCTCCTCTCTTCCAACAGGCTTTATGGCAGGG
```

Figure 2c

```
                      1351                                              1400
SEQ ID NO:1    (255)  GCCATTATCCTCATTTTGGGCTTACTCATTTATGCTTGGACCCCTTCAAA
SEQ ID NO:7   (1351)  GC------------------------------------------------

                      1401                                              1450
SEQ ID NO:1    (305)  TGGTTCCTCGGGTGCTTCCTTCTCAACTTCCTCCACCTAGAGAGGCTAGA
SEQ ID NO:7   (1353)  --------------------------------------------------

                      1451                                              1500
SEQ ID NO:1    (355)  ATGAGTTGACATGTCATTGCAGATAGTACAACACCACAAGGAACTAATTC
SEQ ID NO:7   (1353)  --------------------------------------------------

                      1501                                              1550
SEQ ID NO:1    (405)  AGGTTCGCTTTTAGGAGACGGCTATAAGAAGGAGAAAGAAATAGGGCGTT
SEQ ID NO:7   (1353)  --------------------------------------------------

                      1551                                              1600
SEQ ID NO:1    (455)  CTTGTAAGTTGTAATAGTTGTTCGTAAGCATTTTTTATGAGCTAAGCTTA
SEQ ID NO:7   (1353)  --------------------------------------------------

                      1601                                              1650
SEQ ID NO:1    (505)  AGTAAGAAAGAGACTAGACTATAGATAGAACAGGTTCCAAGTTCAATTTT
SEQ ID NO:7   (1353)  --------------------------------------------------

                      1651                                              1700
SEQ ID NO:1    (555)  TATGTAAGCTAAGGAAAGTAAATAGAGAATAAAAGTCACTTTGTTGACAG
SEQ ID NO:7   (1353)  --------------------------------------------------

                      1701                                     1743
SEQ ID NO:1    (605)  AGGAAATGATATTGGACCATTTGGATGCAAAAAAAAAAAAAAA
SEQ ID NO:7   (1353)  -------------------------------------------
```

Figure 3

Contig consensus sequence of RACE variant A and 50657480 describing orf

1 GATGTTCTTC TATTGATTCC TCAATTTCTC CAACTCAAAA CTAAGTTTCG
51 TCTTTGTGAT CTCTTGATTA TATTATATCG ATTTGTGAAT TGTTTTTCCA
101 CAAAAAAAAA ATTCATTCAA ACC**ATGGCTT CATTATGCCG GCGGTTATCC**
151 **GCCGCCGCAC CGGCCGGTCA CTTCCGATTC CATCGGCCCG CCGGGATTTG**
201 **CGCGGTTCGA TACGGTTATT CCGTCCGGCG GCCAATGCGG TTGGTTGCGG**
251 **CGGGGACCAC CGTGGGCGGC GGCGCGTGGG TGACATCCGA TGACGTCGGA**
301 **GGGAGGGAGG AGAAGGTGGG GCCCTGCTCC TACGCCGTGG AGGATCGGAG**
351 **GGTGGCGGAA GATGGCGGAA GCGATGCGGT GGTAGGAAGT AGGAACCGGG**
401 **TGGTGGAAGT GGCGGCGGCG GCGGTTGCAA CGGTGGTGCT GGGTGTTGGG**
451 **AACCGGGTTC TGTATAAGTT GGCTTTGGTT CCGTTGAAGC ACTACCCTTT**
501 **CTTCCTTGCT CAACTTGCCA CTTTCGGATA TGTAATAGTG TACTTTGCGA**
551 **TTTTGTATAT TCGACACCAT GCGGGCATTG TTACTGATGA GATGTTAGAT**
601 **GCTCCAAAGG CTCCATTTAT AGTTGTTGGT CTATTGGAGG CTCTCGCTGC**
651 **TGCCACTGGA ATGGCCGCAG GAGCAATTCT CTCTGGAGCT TCGATTCCAA**
701 **TTTTATCTCA GACTTTTCTA GTGTGGCAAA TACTCCTGTC AATTATTTTT**
751 **CTTGGGAGAA GATATAAAGT CAACCAATTA CTTGGATGCT TTCTTGTAAC**
801 **CATTGGTGTA GTTGTTACTG TAGCAAGTGG AGCTGGTGCT GGGAATTTAT**
851 **TAAAGGAAGG TGGTATGTTT TGGAGTCTTT TGATGATAGT TTCATTTTTC**
901 **CTCCAAGCGG CTGATACCGT GCTGAAGGAA ATTATCTTTT TGGATTCATC**
951 **CCGAAAATTG AAGGGAGGTT GTTGTATGGA CCTTTTTGTT GTCAATTCGT**
1001 **ACGGATCTGC TTTCCAAGCA CTATTCGTGT GCCTTCTTCT CCCCTTCTTG**
1051 **TCAAAATTAT GGGGCATTCC CTTCAGTCAA CTACCAAACT ACCTTAAAGA**
1101 **TGGTGCAGCC TGCTTCCTAA ATTTTGGTAC ATTATCAAGT GGATGTGATG**
1151 **GTGCCCCTCT GCTTCCTCTG CTGTTTATTA TTGTAAACAT AGGTTTCAAT**
1201 **ATTGCATTGC TTCATCTCCT CAAGATCTCT TCAGCTGTTG TATCTTGTCT**
1251 **TGCTTCCACA TTTTCAGTCC CAATATCCAT CTACGTGTTC ACCATGCCAT**
1301 **TGCCATACCT TGGTGTTGCC TCCTCTCTTC CAACAGGCTT TATGGCAGGG**
1351 **GCCATTATCC TCATTTTGGG CTTACTCATT TATGCTTGGA CCCCTTCAAA**
1401 **TGGTTCCTCG GGTGCTTCCT TCTCAACTTC CTCCACCTAG** AGAGGCTAGA
1451 ATGAGTTGAC ATGTCATTGC AGATAGTACA ACACCACAAG GAACTAATTC
1501 AGGTTCGCTT TTAGGAGACG GCTATAAGAA GGAGAAAGAA ATAGGGCGTT
1551 CTTGTAAGTT GTAATAGTTG TTCGTAAGCA TTTTTTATGA GCTAAGCTTA
1601 AGTAAGAAAG AGACTAGACT ATAGATAGAA CAGGTTCCAA GTTCAATTTT
1651 TATGTAAGCT AAGGAAAGTA AATAGAGAAT AAAAGTCACT TTGTTGACAG
1701 AGGAAATGAT ATTGGACCAT TTGGATGCAA AAAAAAAAAA AAA

Open reading frame is shown in bold from bases 124 to 1440

Figure 4: Table of homologs showing GenBank Id for AA and DNA seq.

SEQ ID NO:	Sequence type	Organism	Genbank ID
16	amino acid	*Vitis vinifera*	CAO16866
17	dna	*Vitis vinifera*	CU459242
18	amino acid	*Vitis vinifera*	CAO61583
19	dna	*Vitis vinifera*	CU459218
20	amino acid	*Oryza sativa*	NP_001066868
21	dna	*Oryza sativa*	NM_001073400
22	amino acid	*Oryza sativa*	NP_001045437
23	dna	*Oryza sativa*	NM_001051972
24	amino acid	*Oryza sativa*	EAY77278
25	dna	*Oryza sativa*	CM000126
26	amino acid	*Arabidopsis thaliana*	BAB10035
27	dna	*Arabidopsis thaliana*	AB007727
28	amino acid	*Arabidopsis thaliana*	AAM62809
29	dna	*Arabidopsis thaliana*	AY085588

Figure 5a: AA Alignment of homologs

```
                            1                                                50
SEQ ID NO:10       (1)  -MASLCRRLSAAAPA--------GHFRFHRPAGICAVRYGYSVRRPMRLV
SEQ ID NO:16       (1)  -MSASCRRLTAGVRVPMVARQVLGASEISRLCGVRMNRRKGIVLRGRRLV
SEQ ID NO:18       (1)  ------------MAFSCAVSCVRFHVIPPKPLTLHTYTAHLSPFSPLISM
SEQ ID NO:20       (1)  --------------------------------------------------
SEQ ID NO:22       (1)  -----------------------------------MELLPTVRRREAVRA
SEQ ID NO:24       (1)  -MASSTTTAPPVSCRPSTARGRLLRLLPANGPAAAMELLPTVRRREAVRA
SEQ ID NO:26       (1)  --------------------------------------------------
SEQ ID NO:28       (1)  MATTSSDRLIAGLTASIGSIESRYANPAQSVSLICRNQINGAPPIVLRSS

                        51                                              100
SEQ ID NO:10      (42)  AAGTTVGGGAWVTSDDVGGREEKVGPCSYAVEDRR--------VAEDGGS
SEQ ID NO:16      (50)  VAEAEAMGRGGVRVRSDGGGEERVEKWSYGSEDRRRGDLVVVEEKEDVGC
SEQ ID NO:18      (39)  HISQNPRYHLHFPSRSPLHGVSSHNSPKTPNFRVR------------ASA
SEQ ID NO:20       (1)  --------------------------------------------------
SEQ ID NO:22      (16)  AHVRRIEAAAWLGARRATRREDAAARCAAAGEVVG---------------
SEQ ID NO:24      (50)  AHVRRIEAAAWLGARRATRREDAAARCAAAGEVVG---------------
SEQ ID NO:26       (1)  --------------------------------------------------
SEQ ID NO:28      (51)  RRSRLWLIEAIPPAKSWNGSNDGDEDIKKSDTRNY--------AIGGTGG

                        101                                             150
SEQ ID NO:10      (84)  DAVVGSRNRVVEVAAAAVATVVLGVGNRVLYKLALVPLKHYPFFLAQLAT
SEQ ID NO:16     (100)  AGGGCEGDRRMKVVIAAAFTVVLGVGNRVLYKLALVPLKHYPFFLAQLAT
SEQ ID NO:18      (77)  DNSQTSSSNTGLVIVCSAITVILAVVNRVFYKLALVPLKQYPFFLAQFTT
SEQ ID NO:20       (1)  ------MSSSPAIAAASAAVVALAVANRVLYKLALVPLKQYPFFLAQLTT
SEQ ID NO:22      (51)  SAAGVGRSAGMEVAIATAAVVAMGTGNRVLYKLALVPLRDYPFFLAQLAT
SEQ ID NO:24      (85)  SAAGVGRSAGMEVAIATAAVVAMGTGNRVLYKLALVPLRDYPFFLAQLAT
SEQ ID NO:26       (1)  --------------------------------------------------
SEQ ID NO:28      (93)  HAVAGKDDRTMEIVIAAATTAALGVGNRVLYKLALIPLKQYPFFLAQLST

                        151                                             200
SEQ ID NO:10     (134)  FGYVIVYFAILYIRHHAGIVTDEMLDAP----KAPFIVVGLLEALAAATG
SEQ ID NO:16     (150)  VGYVLVYFSILSLRYNAGIVTDEMLSLP----KTPYVAVGLLEALGAATG
SEQ ID NO:18     (127)  FGYAAIYFSILYIRYRAGIVTDEMIALP----KSRFMAIGILEALGVASG
SEQ ID NO:20      (45)  FGYVAVYFSILYARYRAGVVTGDMLALP----KRRLAAIGLLEALGLAAG
SEQ ID NO:22     (101)  FG--LCDGCLVLGVHLSGRITGRDFLLVNISIPVRLCAFFLVGGRARELA
SEQ ID NO:24     (135)  FGYVVVYFSILYLRHQAGIVTDEMLSLP----QKPFLAVGLLEALSAASG
SEQ ID NO:26       (1)  -----------------------MLSVP----KSPFLIVGILEALAAAAG
SEQ ID NO:28     (143)  FGYVAVYFSILYFRYRAGIVTKEMLSVP----KLPFLIVGVLESLALAAG

                        201                                             250
SEQ ID NO:10     (180)  MAAGAILSGASIPILSQTFLVWQILLSIIFLGRRYKVNQLLGCFLVTIGV
SEQ ID NO:16     (196)  MAAGAILSGASIPILSQSFLVWQLLLSAIFLGRRYKVNQLLGCFLVAIGV
SEQ ID NO:18     (173)  MASAAMLPGPAIPLLNQTFLVWQLALSTLILGRKYSFNQILGCFLVAAGV
SEQ ID NO:20      (91)  MSAGAMLPGPAIPILSQSFLVWQLIFSALLLGRTYSMRQIIGCFLVASGV
SEQ ID NO:22     (149)  FQASWRLYQQHQGWLLETYLVWQLLLSAIFLKRRYRINEITGCFLVTVGV
SEQ ID NO:24     (181)  MAAGAVLSGASIPILSQTYLVWQLLLSAIFLKRRYRINEITGCFLVTVGV
SEQ ID NO:26      (24)  MAAAANLSGPSTTVLSQ------RKPNTRMYSCSSRCNRQCGKVRSAF--
SEQ ID NO:28     (189)  MAAASNLSGPSTTVLSQTFLVWQILFSIIFLGRRYRINQILGCTLVAFGV
```

Figure 5b

```
                          251                                                300
SEQ ID NO:10   (230) VVTVASGAGAGNLLKEGGMFWSLLMIVSFFLQAADTVLKEIIFLDSSRKL
SEQ ID NO:16   (246) IITVASGSSAGASLKGAGIFWSLLMMVSFLFQAADTVLKERIFLKAAERL
SEQ ID NO:18   (223) VTAVASGSNGDQMLSGIEFIWPALMIASSAFQAGASIIKEFVFVDAATRL
SEQ ID NO:20   (141) ILAVASGANEGQFLSEVKFIWLALMVASSAFQAGASILKESVFIDGAKRL
SEQ ID NO:22   (199) IITVASGSSAGASLKGTGILWPLLMIISFFLQAADTVLKEIIFLNAAKKL
SEQ ID NO:24   (231) IITVASGSSAGASLKGTGILWPLLMIISFFLQAADTVLKEIIFLNAAKKL
SEQ ID NO:26    (66) -VLIFCGSGAAHSLNEAGVLWILLMVLSFLLQGAGTVLKEVIFIDSQRRL
SEQ ID NO:28   (239) IVSVASGSGAAHSFKDTGILWSLLMVFSFLLQGADTVMKEVIFLDSKKRL

                          301                                                350
SEQ ID NO:10   (280) KGGCCMDLFVVNSYGSAFQALFVCLLLPFLSKLWGIPFSQLPNYLKDGAA
SEQ ID NO:16   (296) KGGS-VDLFVVNSYGSAFQALFICLLLPFLSKLWGVPFSHLPNYLKDGAA
SEQ ID NO:18   (273) KGKL-LDIFVVNSFGSGFQALFVLLLLPLLSNFRGIPFPQLPSYLKAGAG
SEQ ID NO:20   (191) KGRR-PDIFVVNSFGSGFQALFVFLLLPLLSNLKGIKFAELPAYLNGGAE
SEQ ID NO:22   (249) KGGS-VDLFVVNSYGSAYQALFMCLLLPFLSKLWGVPFHQLPTYIRDGTA
SEQ ID NO:24   (281) KGGS-VDLFVVNSYGSAYQALFMCLLLPFLSKLWGVPFHQLPTYIRDGTA
SEQ ID NO:26   (115) KGAS-LDLFIVNSYGSAFQAICIALLLPFLSKLWGIPFNQLGTYLKDGAV
SEQ ID NO:28   (289) KGAS-LDLFVVNSYGSIFQVICIALLLPFLSKLWGIPFNQLPSYIRDGGA

                          351                                                400
SEQ ID NO:10   (330) CFLNFGTLSSGCDGAPLLPLLFIIVNIGFNIALLHLLKISSAVVSCLAST
SEQ ID NO:16   (345) CFLNIGSLSSGCDGAPLLPLLFVVVNMGFNISLLHLLKISSAVVSCLAST
SEQ ID NO:18   (322) CFLNIGSNIPGCDGAPLLPLLYLATNIAFNISLLNLVKISSAVVSTLAAM
SEQ ID NO:20   (240) CFLNVDDSLIDCGGAPFLPLLFILVNMAFNIALLNLVKLSSALVASLTAT
SEQ ID NO:22   (298) CFLNMGSLSSGCEGAPLLPLLFVLVNMGFNISLLHLLKISSAVVSSLAST
SEQ ID NO:24   (330) CFLNMGSLSSGCEGAPLLPLLFVLVNMGFNISLLHLLKISSAVVSSLAST
SEQ ID NO:26   (164) CFLNNGTITKGCDGAPFLPLLFVIMNIGYNIALLRLLKISSAVVSCLAST
SEQ ID NO:28   (338) CFLNIGSXITGCEGAPLLPVMFVMMNMAYNISLLRLIKISSAVVSSLAST

                          401                                                450
SEQ ID NO:10   (380) FSVPISIYVFTMPLPYLGVASSLPTGFMAGAIILILGLLIYAWTPS----
SEQ ID NO:16   (395) FSVPIAVYMFTLPLPYLGVASSLPPAFVTGAIILLVGLMIYAWTPP----
SEQ ID NO:18   (372) ASVPISIYVLSLPLPYLPQGASLSPFFLFGGVILLLGLLLYNIPQP----
SEQ ID NO:20   (290) SAVPISIYILSLPLPYIPHGAELSSSFILGGVVLLMGLIIYNLPQS----
SEQ ID NO:22   (348) FSVPLSIYAFTLPLPYIGVASTLPPGFVAGAMFLDGTRTREKRRSIITTG
SEQ ID NO:24   (380) FSVPLSIYAFTLPLPYIGVASTLPPGFVAGAVLGDASTVSRRDK---NQG
SEQ ID NO:26   (214) VSVPIAVFLFTMPLPYLGVASSLPKGFMGGTIILVLGMILYSWTPHGANS
SEQ ID NO:28   (388) VSVPIAVYCFTLPLPYLGVASTLPRGFVAGTIILVVGMLLYAWTPS---T

                          451                                                500
SEQ ID NO:10   (426) NGSSG----------------ASFSTSST---------------------
SEQ ID NO:16   (441) SMDLN----------------SSSSPSIH---------------------
SEQ ID NO:18   (418) ---------------------AKQAP------------------------
SEQ ID NO:20   (336) ---------------------SKKQSKIE---------------------
SEQ ID NO:22   (398) MKSKNGGGGGSGGNTNGSHRRITAAAAINIIRTLLSILASPAAVDWTASS
SEQ ID NO:24   (427) EKKE--------HHHDGDEEQEMAAAAVAAATPIGEPQGGSPPPPPSTSS
SEQ ID NO:26   (264) SHTDS----------------VIPSPPPT---------------------
SEQ ID NO:28   (435) NTSDS----------------IIPSPXST---------------------
```

Figure 5c

```
                         501                                                550
SEQ ID NO:10   (439) --------------------------------------------------
SEQ ID NO:16   (454) --------------------------------------------------
SEQ ID NO:18   (423) --------------------------------------------------
SEQ ID NO:20   (344) --------------------------------------------------
SEQ ID NO:22   (448) GR----------------RLTGCSTLRCSSVDLWVPSLGRRGFRWSPGVR
SEQ ID NO:24   (469) GRSCPSWPPLQLLTGLPPPAGGSPVAPLSGVHPSTCGSLLWEVEDSGGPR
SEQ ID NO:26   (277) --------------------------------------------------
SEQ ID NO:28   (448) --------------------------------------------------

                         551         564
SEQ ID NO:10   (439) --------------
SEQ ID NO:16   (454) --------------
SEQ ID NO:18   (423) --------------
SEQ ID NO:20   (344) --------------
SEQ ID NO:22   (482) QGGHDPDKHRVMGI
SEQ ID NO:24   (519) GCGREAMTPTSIG-
SEQ ID NO:26   (277) --------------
SEQ ID NO:28   (448) --------------
```

Figure 6: Matrix table of AA homologs

	SEQ ID NO:10	SEQ ID NO:16	SEQ ID NO:18	SEQ ID NO:20	SEQ ID NO:22	SEQ ID NO:24	SEQ ID NO:26	SEQ ID NO:28
SEQ ID NO:10	100	69	49	54	54	60	62	56
SEQ ID NO:16		100	48	56	55	61	60	56
SEQ ID NO:18			100	67	39	44	46	46
SEQ ID NO:20				100	45	53	41	51
SEQ ID NO:22					100	72	43	49
SEQ ID NO:24						100	52	52
SEQ ID NO:26							100	70
SEQ ID NO:28								100

Figure 7: Matrix table of nt of homologs

	SEQ ID NO:17	SEQ ID NO:9	SEQ ID NO:19	SEQ ID NO:21	SEQ ID NO:23	SEQ ID NO:25	SEQ ID NO:27	SEQ ID NO:29
SEQ ID NO:17	100	75	53	59	62	63	66	63
SEQ ID NO:9		100	54	58	60	63	65	63
SEQ ID NO:19			100	67	50	52	54	52
SEQ ID NO:21				100	54	58	53	57
SEQ ID NO:23					100	93	55	57
SEQ ID NO:25						100	58	59
SEQ ID NO:27							100	75
SEQ ID NO:29								100

FIGURE 8a

nucleotide positions of 21mers of SEQ ID NO: 8									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
1	22	44	65	87	108	130	151	173	194
2	23	45	66	88	109	131	152	174	195
3	24	46	67	89	110	132	153	175	196
4	25	47	68	90	111	133	154	176	197
5	26	48	69	91	112	134	155	177	198
6	27	49	70	92	113	135	156	178	199
7	28	50	71	93	114	136	157	179	200
8	29	51	72	94	115	137	158	180	201
9	30	52	73	95	116	138	159	181	202
10	31	53	74	96	117	139	160	182	203
11	32	54	75	97	118	140	161	183	204
12	33	55	76	98	119	141	162	184	205
13	34	56	77	99	120	142	163	185	206
14	35	57	78	100	121	143	164	186	207
15	36	58	79	101	122	144	165	187	208
16	37	59	80	102	123	145	166	188	209
17	38	60	81	103	124	146	167	189	210
18	39	61	82	104	125	147	168	190	211
19	40	62	83	105	126	148	169	191	212
20	41	63	84	106	127	149	170	192	213
21	42	64	85	107	128	150	171	193	214
22	43	65	86	108	129	151	172	194	215
23	44	66	87	109	130	152	173	195	216
24	45	67	88	110	131	153	174	196	217
25	46	68	89	111	132	154	175	197	218
26	47	69	90	112	133	155	176	198	219
27	48	70	91	113	134	156	177	199	220
28	49	71	92	114	135	157	178	200	221
29	50	72	93	115	136	158	179	201	222
30	51	73	94	116	137	159	180	202	223
31	52	74	95	117	138	160	181	203	224
32	53	75	96	118	139	161	182	204	225
33	54	76	97	119	140	162	183	205	226
34	55	77	98	120	141	163	184	206	227
35	56	78	99	121	142	164	185	207	228
36	57	79	100	122	143	165	186	208	229
37	58	80	101	123	144	166	187	209	230
38	59	81	102	124	145	167	188	210	231
39	60	82	103	125	146	168	189	211	232
40	61	83	104	126	147	169	190	212	233
41	62	84	105	127	148	170	191	213	234
42	63	85	106	128	149	171	192	214	235
43	64	86	107	129	150	172	193	215	236

FIGURE 8b

nucleotide positions of 21mers of SEQ ID NO: 8									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
216	237	259	280	302	323	345	366	387	408
217	238	260	281	303	324	346	367	388	409
218	239	261	282	304	325	347	368	389	410
219	240	262	283	305	326	348	369	390	411
220	241	263	284	306	327	349	370	391	412
221	242	264	285	307	328	350	371	392	413
222	243	265	286	308	329	351	372	393	414
223	244	266	287	309	330	352	373	394	415
224	245	267	288	310	331	353	374	395	416
225	246	268	289	311	332	354	375	396	417
226	247	269	290	312	333	355	376	397	418
227	248	270	291	313	334	356	377	398	419
228	249	271	292	314	335	357	378	399	420
229	250	272	293	315	336	358	379	400	421
230	251	273	294	316	337	359	380	401	422
231	252	274	295	317	338	360	381	402	423
232	253	275	296	318	339	361	382	403	424
233	254	276	297	319	340	362	383	404	425
234	255	277	298	320	341	363	384	405	426
235	256	278	299	321	342	364	385	406	427
236	257	279	300	322	343	365	386	407	428
237	258	280	301	323	344	366	387	408	429
238	259	281	302	324	345	367	388	409	430
239	260	282	303	325	346	368	389	410	431
240	261	283	304	326	347	369	390	411	432
241	262	284	305	327	348	370	391	412	433
242	263	285	306	328	349	371	392	413	434
243	264	286	307	329	350	372	393	414	435
244	265	287	308	330	351	373	394	415	436
245	266	288	309	331	352	374	395	416	437
246	267	289	310	332	353	375	396	417	438
247	268	290	311	333	354	376	397	418	439
248	269	291	312	334	355	377	398	419	440
249	270	292	313	335	356	378	399	420	441
250	271	293	314	336	357	379	400	421	442
251	272	294	315	337	358	380	401	422	443
252	273	295	316	338	359	381	402	423	444
253	274	296	317	339	360	382	403	424	445
254	275	297	318	340	361	383	404	425	446
255	276	298	319	341	362	384	405	426	447
256	277	299	320	342	363	385	406	427	448
257	278	300	321	343	364	386	407	428	449
258	279	301	322	344	365	387	408	429	450

FIGURE 8c

nucleotide positions of 21mers of SEQ ID NO: 8									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
430	451	473	494	516	537	559	580	602	623
431	452	474	495	517	538	560	581	603	624
432	453	475	496	518	539	561	582	604	625
433	454	476	497	519	540	562	583	605	626
434	455	477	498	520	541	563	584	606	627
435	456	478	499	521	542	564	585	607	628
436	457	479	500	522	543	565	586	608	629
437	458	480	501	523	544	566	587	609	630
438	459	481	502	524	545	567	588	610	631
439	460	482	503	525	546	568	589	611	632
440	461	483	504	526	547	569	590	612	633
441	462	484	505	527	548	570	591	613	634
442	463	485	506	528	549	571	592	614	635
443	464	486	507	529	550	572	593	615	636
444	465	487	508	530	551	573	594	616	637
445	466	488	509	531	552	574	595	617	638
446	467	489	510	532	553	575	596	618	639
447	468	490	511	533	554	576	597	619	640
448	469	491	512	534	555	577	598	620	641
449	470	492	513	535	556	578	599	621	642
450	471	493	514	536	557	579	600	622	643
451	472	494	515	537	558	580	601	623	644
452	473	495	516	538	559	581	602	624	645
453	474	496	517	539	560	582	603	625	646
454	475	497	518	540	561	583	604	626	647
455	476	498	519	541	562	584	605	627	648
456	477	499	520	542	563	585	606	628	649
457	478	500	521	543	564	586	607	629	650
458	479	501	522	544	565	587	608	630	651
459	480	502	523	545	566	588	609	631	652
460	481	503	524	546	567	589	610	632	653
461	482	504	525	547	568	590	611	633	654
462	483	505	526	548	569	591	612	634	655
463	484	506	527	549	570	592	613	635	656
464	485	507	528	550	571	593	614	636	657
465	486	508	529	551	572	594	615	637	658
466	487	509	530	552	573	595	616	638	659
467	488	510	531	553	574	596	617	639	660
468	489	511	532	554	575	597	618	640	661
469	490	512	533	555	576	598	619	641	662
470	491	513	534	556	577	599	620	642	663
471	492	514	535	557	578	600	621	643	664
472	493	515	536	558	579	601	622	644	665

FIGURE 8d

nucleotide positions of 21mers of SEQ ID NO: 8									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
645	666	688	709	731	752	774	795	817	838
646	667	689	710	732	753	775	796	818	839
647	668	690	711	733	754	776	797	819	840
648	669	691	712	734	755	777	798	820	841
649	670	692	713	735	756	778	799	821	842
650	671	693	714	736	757	779	800	822	843
651	672	694	715	737	758	780	801	823	844
652	673	695	716	738	759	781	802	824	845
653	674	696	717	739	760	782	803	825	846
654	675	697	718	740	761	783	804	826	847
655	676	698	719	741	762	784	805	827	848
656	677	699	720	742	763	785	806	828	849
657	678	700	721	743	764	786	807	829	850
658	679	701	722	744	765	787	808	830	851
659	680	702	723	745	766	788	809	831	852
660	681	703	724	746	767	789	810	832	853
661	682	704	725	747	768	790	811	833	854
662	683	705	726	748	769	791	812	834	855
663	684	706	727	749	770	792	813	835	856
664	685	707	728	750	771	793	814	836	857
665	686	708	729	751	772	794	815	837	858
666	687	709	730	752	773	795	816	838	859
667	688	710	731	753	774	796	817	839	860
668	689	711	732	754	775	797	818	840	861
669	690	712	733	755	776	798	819	841	862
670	691	713	734	756	777	799	820	842	863
671	692	714	735	757	778	800	821	843	864
672	693	715	736	758	779	801	822	844	865
673	694	716	737	759	780	802	823	845	866
674	695	717	738	760	781	803	824	846	867
675	696	718	739	761	782	804	825	847	868
676	697	719	740	762	783	805	826	848	869
677	698	720	741	763	784	806	827	849	870
678	699	721	742	764	785	807	828	850	871
679	700	722	743	765	786	808	829	851	872
680	701	723	744	766	787	809	830	852	873
681	702	724	745	767	788	810	831	853	874
682	703	725	746	768	789	811	832	854	875
683	704	726	747	769	790	812	833	855	876
684	705	727	748	770	791	813	834	856	877
685	706	728	749	771	792	814	835	857	878
686	707	729	750	772	793	815	836	858	879
687	708	730	751	773	794	816	837	859	880

FIGURE 8e

nucleotide positions of 21mers of SEQ ID NO: 8									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
881	902	924	945	967	988	1010	1031	1053	1074
882	903	925	946	968	989	1011	1032	1054	1075
883	904	926	947	969	990	1012	1033	1055	1076
884	905	927	948	970	991	1013	1034	1056	1077
885	906	928	949	971	992	1014	1035	1057	1078
886	907	929	950	972	993	1015	1036	1058	1079
887	908	930	951	973	994	1016	1037	1059	1080
888	909	931	952	974	995	1017	1038	1060	1081
889	910	932	953	975	996	1018	1039	1061	1082
890	911	933	954	976	997	1019	1040	1062	1083
891	912	934	955	977	998	1020	1041	1063	1084
892	913	935	956	978	999	1021	1042	1064	1085
893	914	936	957	979	1000	1022	1043	1065	1086
894	915	937	958	980	1001	1023	1044	1066	1087
895	916	938	959	981	1002	1024	1045	1067	1088
896	917	939	960	982	1003	1025	1046	1068	1089
897	918	940	961	983	1004	1026	1047	1069	1090
898	919	941	962	984	1005	1027	1048	1070	1091
899	920	942	963	985	1006	1028	1049	1071	1092
900	921	943	964	986	1007	1029	1050	1072	1093
901	922	944	965	987	1008	1030	1051	1073	1094
902	923	945	966	988	1009	1031	1052	1074	1095
903	924	946	967	989	1010	1032	1053	1075	1096
904	925	947	968	990	1011	1033	1054	1076	1097
905	926	948	969	991	1012	1034	1055	1077	1098
906	927	949	970	992	1013	1035	1056	1078	1099
907	928	950	971	993	1014	1036	1057	1079	1100
908	929	951	972	994	1015	1037	1058	1080	1101
909	930	952	973	995	1016	1038	1059	1081	1102
910	931	953	974	996	1017	1039	1060	1082	1103
911	932	954	975	997	1018	1040	1061	1083	1104
912	933	955	976	998	1019	1041	1062	1084	1105
913	934	956	977	999	1020	1042	1063	1085	1106
914	935	957	978	1000	1021	1043	1064	1086	1107
915	936	958	979	1001	1022	1044	1065	1087	1108
916	937	959	980	1002	1023	1045	1066	1088	1109
917	938	960	981	1003	1024	1046	1067	1089	1110
918	939	961	982	1004	1025	1047	1068	1090	1111
919	940	962	983	1005	1026	1048	1069	1091	1112
920	941	963	984	1006	1027	1049	1070	1092	1113
921	942	964	985	1007	1028	1050	1071	1093	1114
922	943	965	986	1008	1029	1051	1072	1094	1115
923	944	966	987	1009	1030	1052	1073	1095	1116

FIGURE 8f

nucleotide positions of 21mers of SEQ ID NO: 8									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
1096	1117	1139	1160	1182	1203	1225	1246	1268	1289
1097	1118	1140	1161	1183	1204	1226	1247	1269	1290
1098	1119	1141	1162	1184	1205	1227	1248	1270	1291
1099	1120	1142	1163	1185	1206	1228	1249	1271	1292
1100	1121	1143	1164	1186	1207	1229	1250	1272	1293
1101	1122	1144	1165	1187	1208	1230	1251	1273	1294
1102	1123	1145	1166	1188	1209	1231	1252	1274	1295
1103	1124	1146	1167	1189	1210	1232	1253	1275	1296
1104	1125	1147	1168	1190	1211	1233	1254	1276	1297
1105	1126	1148	1169	1191	1212	1234	1255	1277	1298
1106	1127	1149	1170	1192	1213	1235	1256	1278	1299
1107	1128	1150	1171	1193	1214	1236	1257	1279	1300
1108	1129	1151	1172	1194	1215	1237	1258	1280	1301
1109	1130	1152	1173	1195	1216	1238	1259	1281	1302
1110	1131	1153	1174	1196	1217	1239	1260	1282	1303
1111	1132	1154	1175	1197	1218	1240	1261	1283	1304
1112	1133	1155	1176	1198	1219	1241	1262	1284	1305
1113	1134	1156	1177	1199	1220	1242	1263	1285	1306
1114	1135	1157	1178	1200	1221	1243	1264	1286	1307
1115	1136	1158	1179	1201	1222	1244	1265	1287	1308
1116	1137	1159	1180	1202	1223	1245	1266	1288	1309
1117	1138	1160	1181	1203	1224	1246	1267	1289	1310
1118	1139	1161	1182	1204	1225	1247	1268	1290	1311
1119	1140	1162	1183	1205	1226	1248	1269	1291	1312
1120	1141	1163	1184	1206	1227	1249	1270	1292	1313
1121	1142	1164	1185	1207	1228	1250	1271	1293	1314
1122	1143	1165	1186	1208	1229	1251	1272	1294	1315
1123	1144	1166	1187	1209	1230	1252	1273	1295	1316
1124	1145	1167	1188	1210	1231	1253	1274	1296	1317
1125	1146	1168	1189	1211	1232	1254	1275	1297	1318
1126	1147	1169	1190	1212	1233	1255	1276	1298	1319
1127	1148	1170	1191	1213	1234	1256	1277	1299	1320
1128	1149	1171	1192	1214	1235	1257	1278	1300	1321
1129	1150	1172	1193	1215	1236	1258	1279	1301	1322
1130	1151	1173	1194	1216	1237	1259	1280	1302	1323
1131	1152	1174	1195	1217	1238	1260	1281	1303	1324
1132	1153	1175	1196	1218	1239	1261	1282	1304	1325
1133	1154	1176	1197	1219	1240	1262	1283	1305	1326
1134	1155	1177	1198	1220	1241	1263	1284	1306	1327
1135	1156	1178	1199	1221	1242	1264	1285	1307	1328
1136	1157	1179	1200	1222	1243	1265	1286	1308	1329
1137	1158	1180	1201	1223	1244	1266	1287	1309	1330
1138	1159	1181	1202	1224	1245	1267	1288	1310	1331

FIGURE 8g

nucleotide positions of 21mers of SEQ ID NO: 8									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
1312	1333	1355	1376	1398	1419	1441	1462	1484	1505
1313	1334	1356	1377	1399	1420	1442	1463	1485	1506
1314	1335	1357	1378	1400	1421	1443	1464	1486	1507
1315	1336	1358	1379	1401	1422	1444	1465	1487	1508
1316	1337	1359	1380	1402	1423	1445	1466	1488	1509
1317	1338	1360	1381	1403	1424	1446	1467	1489	1510
1318	1339	1361	1382	1404	1425	1447	1468	1490	1511
1319	1340	1362	1383	1405	1426	1448	1469	1491	1512
1320	1341	1363	1384	1406	1427	1449	1470	1492	1513
1321	1342	1364	1385	1407	1428	1450	1471	1493	1514
1322	1343	1365	1386	1408	1429	1451	1472	1494	1515
1323	1344	1366	1387	1409	1430	1452	1473	1495	1516
1324	1345	1367	1388	1410	1431	1453	1474	1496	1517
1325	1346	1368	1389	1411	1432	1454	1475	1497	1518
1326	1347	1369	1390	1412	1433	1455	1476	1498	1519
1327	1348	1370	1391	1413	1434	1456	1477	1499	1520
1328	1349	1371	1392	1414	1435	1457	1478	1500	1521
1329	1350	1372	1393	1415	1436	1458	1479	1501	1522
1330	1351	1373	1394	1416	1437	1459	1480	1502	1523
1331	1352	1374	1395	1417	1438	1460	1481	1503	1524
1332	1353	1375	1396	1418	1439	1461	1482	1504	1525
1333	1354	1376	1397	1419	1440	1462	1483	1505	1526
1334	1355	1377	1398	1420	1441	1463	1484	1506	1527
1335	1356	1378	1399	1421	1442	1464	1485	1507	1528
1336	1357	1379	1400	1422	1443	1465	1486	1508	1529
1337	1358	1380	1401	1423	1444	1466	1487	1509	1530
1338	1359	1381	1402	1424	1445	1467	1488	1510	1531
1339	1360	1382	1403	1425	1446	1468	1489	1511	1532
1340	1361	1383	1404	1426	1447	1469	1490	1512	1533
1341	1362	1384	1405	1427	1448	1470	1491	1513	1534
1342	1363	1385	1406	1428	1449	1471	1492	1514	1535
1343	1364	1386	1407	1429	1450	1472	1493	1515	1536
1344	1365	1387	1408	1430	1451	1473	1494	1516	1537
1345	1366	1388	1409	1431	1452	1474	1495	1517	1538
1346	1367	1389	1410	1432	1453	1475	1496	1518	1539
1347	1368	1390	1411	1433	1454	1476	1497	1519	1540
1348	1369	1391	1412	1434	1455	1477	1498	1520	1541
1349	1370	1392	1413	1435	1456	1478	1499	1521	1542
1350	1371	1393	1414	1436	1457	1479	1500	1522	1543
1351	1372	1394	1415	1437	1458	1480	1501	1523	1544
1352	1373	1395	1416	1438	1459	1481	1502	1524	1545
1353	1374	1396	1417	1439	1460	1482	1503	1525	1546
1354	1375	1397	1418	1440	1461	1483	1504	1526	1547

FIGURE 8h

nucleotide positions of 21mers of SEQ ID NO: 8									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
1527	1548	1570	1591	1613	1634	1656	1677	1699	1720
1528	1549	1571	1592	1614	1635	1657	1678	1700	1721
1529	1550	1572	1593	1615	1636	1658	1679	1701	1722
1530	1551	1573	1594	1616	1637	1659	1680	1702	1723
1531	1552	1574	1595	1617	1638	1660	1681	1703	1724
1532	1553	1575	1596	1618	1639	1661	1682	1704	1725
1533	1554	1576	1597	1619	1640	1662	1683	1705	1726
1534	1555	1577	1598	1620	1641	1663	1684	1706	1727
1535	1556	1578	1599	1621	1642	1664	1685	1707	1728
1536	1557	1579	1600	1622	1643	1665	1686	1708	1729
1537	1558	1580	1601	1623	1644	1666	1687	1709	1730
1538	1559	1581	1602	1624	1645	1667	1688	1710	1731
1539	1560	1582	1603	1625	1646	1668	1689	1711	1732
1540	1561	1583	1604	1626	1647	1669	1690	1712	1733
1541	1562	1584	1605	1627	1648	1670	1691	1713	1734
1542	1563	1585	1606	1628	1649	1671	1692	1714	1735
1543	1564	1586	1607	1629	1650	1672	1693	1715	1736
1544	1565	1587	1608	1630	1651	1673	1694	1716	1737
1545	1566	1588	1609	1631	1652	1674	1695	1717	1738
1546	1567	1589	1610	1632	1653	1675	1696	1718	1739
1547	1568	1590	1611	1633	1654	1676	1697	1719	1740
1548	1569	1591	1612	1634	1655	1677	1698	1720	1741
1549	1570	1592	1613	1635	1656	1678	1699	1721	1742
1550	1571	1593	1614	1636	1657	1679	1700	1722	1743
1551	1572	1594	1615	1637	1658	1680	1701		
1552	1573	1595	1616	1638	1659	1681	1702		
1553	1574	1596	1617	1639	1660	1682	1703	.....	.....
1554	1575	1597	1618	1640	1661	1683	1704	.....	.....
1555	1576	1598	1619	1641	1662	1684	1705	n-5	n+15
1556	1577	1599	1620	1642	1663	1685	1706	n-4	n+16
1557	1578	1600	1621	1643	1664	1686	1707	n-3	n+17
1558	1579	1601	1622	1644	1665	1687	1708	n-2	n+18
1559	1580	1602	1623	1645	1666	1688	1709	n-1	n+19
1560	1581	1603	1624	1646	1667	1689	1710	n	n+20
1561	1582	1604	1625	1647	1668	1690	1711		
1562	1583	1605	1626	1648	1669	1691	1712		
1563	1584	1606	1627	1649	1670	1692	1713		
1564	1585	1607	1628	1650	1671	1693	1714		
1565	1586	1608	1629	1651	1672	1694	1715		
1566	1587	1609	1630	1652	1673	1695	1716		
1567	1588	1610	1631	1653	1674	1696	1717		
1568	1589	1611	1632	1654	1675	1697	1718		
1569	1590	1612	1633	1655	1676	1698	1719		

FIGURE 8i

n = total number of nucleotides of the entire length of a 50657480-like gene or a 50657480-homolog encoding polynucleotide – 20.

For example:

5 n = 627 (647-20) for SEQ ID NO:1

n = 1335 (1352-20) for SEQ ID NO:7;

n = 1297 (1317-20) for SEQ ID NO:9;

n = 1342 (1362-20) for SEQ ID NO:17;

n = 1249 (1269-20) for SEQ ID NO:19;

10 n = 1012 (1032-20) for SEQ ID NO:21;

n = 1468 (1488-20) for SEQ ID NO:23;

n = 1576 (1596-20) for SEQ ID NO:25;

n = 811 (831-20) for SEQ ID NO:27;

n = 1324 (1344-20) for SEQ ID NO:29;

15

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2008/051326

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/11 C12N15/82 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2004/031072 A1 (LA ROSA THOMAS J [US] ET AL) 12 February 2004 (2004-02-12) SEQ ID NOs: 54069, 92113 paragraph [0031] paragraph [0086] paragraph [0089]	1-18
P,X	-& DATABASE EMBL 18 October 1007 (1007-10-18), XP002477888 Database accession no. AF062890 abstract	1-18
P,X	-& DATABASE EMBL 18 October 2007 (2007-10-18), XP002477889 Database accession no. AFP00935 abstract	1-18

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 April 2008	11/06/2008

Name and mailing address of the ISA/
European Patent Office, P.B. 5818 Patentlaan 2
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2008/051326

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2004/005485 A (UNIV KANSAS STATE [US]; TRICK HAROLD N [US]; ROE JUDITH L [US]; TODD T) 15 January 2004 (2004-01-15) page 28, paragraph 2 page 54, paragraph 2 -----	1-18
A	US 2004/034888 A1 (LIU JINGDONG [US] ET AL) 19 February 2004 (2004-02-19) SEQ ID NO: 11561 paragraph [0031] paragraph [0089]	1-18
A	-& DATABASE EMBL 21 April 2005 (2005-04-21), XP002477890 Database accession no. ADX28741 abstract -----	1-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2008/051326

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-18 partially

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the *payment of a protest fee.*

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-18 (part)

   The subject matter of claims 1-18 as far as relating to a dsRNA for a polynucleotide comprising SEQ ID NOs: 1, 7, 8, 9.

   ---

2. claims: 1-18 (part)

   The subject matter of claims 1-18 as far as relating to a dsRNA for a polynucleotide comprising SEQ ID NO: 17.

   ---

3. claims: 1-18 (part)

   The subject matter of claims 1-18 as far as relating to a dsRNA for a polynucleotide comprising SEQ ID NO: 19.

   ---

4. claims: 1-18 (part)

   The subject matter of claims 1-18 as far as relating to a dsRNA for a polynucleotide comprising SEQ ID NO: 21.

   ---

5. claims: 1-18 (part)

   The subject matter of claims 1-18 as far as relating to a dsRNA for a polynucleotide comprising SEQ ID NO: 23.

   ---

6. claims: 1-18 (part)

   The subject matter of claims 1-18 as far as relating to a dsRNA for a polynucleotide comprising SEQ ID NO: 25.

   ---

7. claims: 1-18 (part)

   The subject matter of claims 1-18 as far as relating to a dsRNA for a polynucleotide comprising SEQ ID NO: 27.

   ---

8. claims: 1-18 (part)

   The subject matter of claims 1-18 as far as relating to a dsRNA for a polynucleotide comprising SEQ ID NO: 29.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2008/051326

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2004031072 A1	12-02-2004	US 2004034888 A1	19-02-2004
		US 2006236419 A1	19-10-2006
WO 2004005485 A	15-01-2004	AU 2003247951 A1	23-01-2004
		BR PI0312580 A	10-10-2006
		US 2004098761 A1	20-05-2004
US 2004034888 A1	19-02-2004	US 2004031072 A1	12-02-2004
		US 2006236419 A1	19-10-2006