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(54) **Title:** COMPOSITIONS AND METHODS OF USING RNA INTERFERENCE OF SCA1-LIKE GENES FOR CONTROL OF NEMATODES

(57) **Abstract:** The present invention concerns double stranded RNA compositions and transgenic plants capable of inhibiting expression of essential genes in parasitic nematodes, and methods associated therewith. Specifically, the invention relates to the use of RNA interference to inhibit expression of a target essential nematode gene, which is a nematode sca1-like gene, and relates to the generation of plants that have increased resistance to parasitic nematodes.

COMPOSITIONS AND METHODS OF USING RNA INTERFERENCE OF SCA1-LIKE GENES FOR CONTROL OF NEMATODES

CROSS REFERENCE TO RELATED APPLICATIONS

[Para 1] This application claims the priority benefit of U.S. Provisional Application Serial No.60/900,622 filed February 09, 2007.

Field of the Invention.

[Para 2] The field of this invention is the control of nematodes, in particular the control of 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

[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.

[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 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 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 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 begin to feed. The nematode injects secretions that modify certain root cells and transform them into specialized feeding sites. The root cells are morphologically transformed into large multinucleate syncytia (or giant cells in the case of RKN), which are used as a source of nutrients for the nematodes. The actively feeding nematodes thus steal essential nutrients from the plant resulting in yield loss. As the nematodes feed, they swell and eventually female nematodes become so large that they break through the root tissue and are exposed on the surface of the root.

[Para 8] 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 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. Although many of the eggs may hatch within the first year, many also will survive within the cysts for several years.

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

[Para 10] Traditional practices for managing nematode infestation include: maintaining proper soil nutrients and soil pH levels in nematode-infested land; controlling other plant diseases, as well as insect and weed pests; using sanitation practices such as plowing, planting, and cultivating of nematode-infested fields only after working non-infested fields; cleaning equipment thoroughly with high pressure water or steam after working in infested fields; not using seed grown on infested land for planting non-infested fields unless the seed has been

properly cleaned; rotating infested fields and alternating host crops with non-host crops; using nematicides; and planting resistant plant varieties.

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

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

[Para 13] Use of RNAi to target essential nematode genes has been proposed, for example, in 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 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

[Para 16] .The present inventors have discovered that down-regulation of the SCN gene CB377729, results in hindered development or death of SCN. The protein product of SCN gene CB377729 has highest homology to sarco-endoplasmic reticulum Ca^{++} ATPases, or sca1-like genes (also known as SERCA pumps). In C. elegans the sca1 gene encodes a sarco-endoplasmic reticulum Ca^{++} ATPase that is required for development and muscle function. Thus, the invention focuses on the elimination of plant parasitic nematodes using plant expressed dsRNAs that target plant parasitic nematode sca1 genes. The nucleic acids of the invention are capable of inhibiting expression of parasitic nematode target genes by RNA interference (RNAi). In accordance with the invention, the parasitic nematode target gene is a parasitic nematode sca1-like gene.

[Para 17] In one embodiment, the invention provides a dsRNA comprising (a) a first strand comprising a sequence substantially identical to a portion of a plant parasitic nematode sca1-like target gene; 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 about 19 to 24 nucleotides, wherein said RNA molecules are derived from a polynucleotide that is substantially identical to a portion of a plant parasitic nematode sca1-like gene.

[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 plant parasitic nematode sca1-like gene.

[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 plant parasitic nematode sca1-like gene.

[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 plant parasitic nematode sca1-like gene in a plant, said method comprising the steps of: a) preparing a nucleic acid having a region that is substantially identical to a portion of

the sca1-like gene, wherein the nucleic acid is able to form a double-stranded transcript of a portion of the sca1-like gene once expressed in the plant; b) transforming a recipient plant with said nucleic acid; c) producing one or more transgenic offspring of said recipient plant; and d) selecting the offspring for expression of said transcript.

[Para 22] The invention further provides a method of conferring nematode resistance to a plant, said method comprising the steps of: a) preparing a nucleic acid having a region that is substantially identical to a portion of a plant parasitic nematode sca1-like gene, wherein the nucleic acid is able to form a double-stranded transcript of a portion of the sca1-like gene once expressed in the plant; b) transforming a recipient plant with said nucleic acid; c) producing one or more transgenic offspring of said recipient plant; and d) selecting the offspring for nematode resistance.

[Para 23] The invention further provides an expression cassette and an expression vector comprising a sequence substantially identical to a portion of a plant parasitic nematode sca1-like gene.

BRIEF DESCRIPTION OF THE DRAWINGS

[Para 24] Figure 1a-1b shows the cDNA sequence of H. glycines sca1-like gene, which is identified as SEQ ID NO:1.

[Para 25] Figure 2 provides the sets of primers that were used to isolate the H. glycines sca1-like gene (SEQ ID NOs:2-7) and C. elegans homologs of the H. glycines sca1-like gene (SEQ ID NOs:8-9) by PCR. Figure 2 also shows a table containing the common primers that can be utilized in sequence isolation, including SL1 (SEQ ID NO: 13) and GeneRacer Oligo dT (SEQ ID NO: 12).

[Para 26] Figure 3 shows the sequence of the C. elegans sca1-like gene fragment (SEQ ID NO:10) used in the RNAi feeding assay of Example 2.

[Para 27] Figure 4 shows the sequence of the 499 nucleotide fragment (SEQ ID NO:11) used in the binary vector p(R)SA006 useful for transformation of soybean cells to produce the dsRNA of the invention in soybean plants, thereby inhibiting the H. glycines sca1-like target genes identified herein.

[Para 28] Figures 5a-5r show various 21mers possible in SEQ ID NO. 1 by nucleotide position.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[Para 29] The present invention may be understood more readily by reference to the following detailed description of the preferred embodiments of the invention and the examples included herein. Unless otherwise noted, the terms used herein are to be understood according to conventional usage by those of ordinary skill in the relevant art. In addition to the definitions of terms provided below, definitions of common terms in molecular biology may also be found in Rieger et al., 1991 Glossary of genetics: classical and molecular, 5th Ed., Berlin: Springer-Verlag; and in Current Protocols in Molecular Biology, F.M. Ausubel et al., Eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1998 Supplement). It is to be understood that as used in the specification and in the claims, "a" or "an" can mean one or more, depending upon the context in which it is used. Thus, for example, reference to "a cell" can mean that at least one cell can be utilized It is to be understood that the terminology used herein is for the purpose of describing specific embodiments only and is not intended to be limiting.

[Para 30] 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 31] A "plant parasitic nematode sca1-like gene" or "sca1-like gene" is defined herein as a gene having at least 70% sequence identity to a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, 10 or 11. Additional sca1-like genes (sca1-like gene homologs) may be isolated from nematodes other than SCN using the information provided herein and techniques known to those of skill in the art of biotechnology. For example, a nucleic acid molecule from a plant parasitic nematode that hybridizes under stringent conditions to the nucleic acid of SEQ ID NO:1 can be isolated from plant parasitic nematode cDNA libraries. Alternatively, mRNA can be isolated from nematodes (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. Nucleic acid molecules corresponding to the sca1-like target genes defined herein can be amplified using cDNA or, alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid molecules so amplified can be cloned into appropriate vectors and characterized by DNA sequence analysis.

[Para 32] As used herein, "RNAi" or "RNA interference" refers to the process of sequence-specific post-transcriptional gene silencing in nematodes, mediated by double-stranded RNA (dsRNA). As used herein, "dsRNA" refers to RNA that is partially or completely double stranded. Double stranded RNA is also referred to as small or short interfering RNA (siRNA), short interfering nucleic acid (siNA), short interfering RNA, micro-RNA (miRNA), and the like. In the RNAi process, dsRNA comprising a first strand that is substantially identical to a portion of a target gene, e.g. a sca1-like gene, and a second strand that is complementary to the first strand is introduced into a nematode, preferably by soaking and more preferably by feeding. After introduction into the nematode, the target gene-specific dsRNA is processed into relatively small fragments (siRNAs) and can subsequently become distributed throughout the nematode, 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 processed into relatively small fragments by a plant cell containing the RNAi processing machinery; and when the plant-processed small dsRNA is ingested by a parasitic nematode, the loss-of-function phenotype is obtained.

[Para 33] As used herein, taking into consideration the substitution of uracil for thymine when comparing RNA and DNA sequences, the term "substantially identical" as applied to dsRNA means that the nucleotide sequence of one strand of the dsRNA is at least about 80%-90% identical to 20 or more contiguous nucleotides of the target gene, more preferably, at least about 90-95% identical to 20 or more contiguous nucleotides of the target gene, and most preferably at least about 95%, 96%, 97%, 98% or 99% identical or absolutely identical to 20 or more contiguous nucleotides of the target gene. 20 or more nucleotides means a portion, being at least about 20, 21, 22, 23, 24, 25, 50, 100, 200, 300, 400, 500, 1000, 1500, consecutive bases or up to the full length of the target gene.

[Para 34] 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 at 80% of their nucleotides. Preferably, the two nucleic acid sequences are complementary over at least at 85%, 90%, 95%, 96%, 97%, 98%, 99% or more or all of their

nucleotides. Alternatively, "substantially complementary" means that two nucleic acid sequences can hybridize under high stringency conditions. As used herein, the term "substantially identical" or "corresponding to" means that two nucleic acid sequences have at least 80% sequence identity. Preferably, the two nucleic acid sequences have at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% of sequence identity.

[Para 35] 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 36] As used herein, the terms "contacting" and "administering" are used interchangeably, and refer to a process by which dsRNA of the present invention is delivered to a cell of a parasitic nematode, in order to inhibit expression of an essential target gene in the nematode. The dsRNA may be administered in a number of ways, including, but not limited to, direct introduction into a cell (i.e., intracellularly); or extracellular introduction into a cavity, interstitial space, or into the circulation of the nematode, oral introduction, the dsRNA may be introduced by bathing the nematode in a solution containing dsRNA, or the dsRNA may be present in food source. Methods for oral introduction include direct mixing of dsRNA with food of the nematode, as well as engineered approaches in which a species that is used as food is engineered to express a dsRNA, then fed to the organism to be affected. For example, the dsRNA may be sprayed onto a plant, or the dsRNA may be applied to soil in the vicinity of roots, taken up by the plant and/or the parasitic nematode, or a plant may be genetically engineered to express the dsRNA in an amount sufficient to kill some or all of the parasitic nematode to which the plant is exposed.

[Para 37] As used herein, the term "control," when used in the context of an infection, refers to the reduction or prevention of an infection. Reducing or preventing an infection by a nematode will cause a plant to have increased resistance to the nematode, however, such increased resistance does not imply that the plant necessarily has 100% resistance to infection. In preferred embodiments, the resistance to infection by a nematode in a resistant plant is greater than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% in comparison to a wild type plant that is not resistant to nematodes. Preferably the wild type plant is a plant of a

similar, more preferably identical genotype as the plant having increased resistance to the nematode, but does not comprise a dsRNA directed to the target gene. The plant's resistance to infection by the nematode may be due to the death, sterility, arrest in development, or impaired mobility of the nematode upon exposure to the dsRNA specific to an essential gene. 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 38] 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 39] 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 40] 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 parasitic nematode. 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, or such inhibition may delay or prevent entry into a particular developmental step (e.g., metamorphosis), if plant disease is associated with a particular stage of the parasitic nematode's life cycle. The consequences of inhibition can be confirmed by examination of the outward properties of the nematode (as presented below in the examples).

[Para 41] In accordance with the invention, a parasitic nematode is contacted with a dsRNA, which specifically inhibits expression of a sca1-like target gene that is essential for survival, metamorphosis, or reproduction of the nematode. Preferably, the parasitic nematode comes into contact with the dsRNA after entering a plant that expresses the dsRNA. In one embodiment, the dsRNA is encoded by a vector that has been transformed into an ancestor of the infected plant.

[Para 42] In one embodiment, the parasitic nematode target gene is a homolog of the C. elegans sca1 gene, sca1-like was identified in screens for essential genes and phenotypic analyses indicate that loss of sca1-like activity results in embryonic and larval lethality. Example 2 below shows that feeding C. elegans RNAi molecules specific for the sca1 gene results in sterile adults, i.e., animals do not produce any progeny. Preferably it is a homolog of the C. elegans sca1 gene derived from a plant parasitic nematode. In this embodiment of the present invention, the parasitic nematode sca1 target gene comprises a sequence selected from the group consisting of: (a) the sequence set forth in SEQ ID NO:1, (b) a polynucleotide having at least 80% sequence identity to SEQ ID NO:1, 10 or 11; and (c) a polynucleotide from a parasitic nematode that hybridizes under stringent conditions to the sequence set forth in SEQ ID NO:1, 10 or 11.

[Para 43] Complete cDNAs corresponding to the sca1-like target gene of the invention may be isolated from parasitic nematodes other than H. glycines using the information provided herein and techniques known to those of skill in the art of biotechnology. For example, a nucleic acid molecule from a parasitic nematode that hybridizes under stringent conditions to a nucleotide sequence of SEQ ID NO:1, 10 or 11 can be isolated from parasitic nematode cDNA libraries. Alternatively, mRNA can be isolated from parasitic nematode cells, and cDNA can be prepared using reverse transcriptase (e.g., Moloney MLV reverse transcriptase. Synthetic

oligonucleotide primers for polymerase chain reaction amplification can be designed based upon the nucleotide sequence shown in SEQ ID NO:1, 10 or 11. Examples for such primers are given by SEQ ID NO: 2, 3, 4, 5, 6, 7, 8, or 9. Nucleic acid molecules corresponding to the parasitic nematode 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.

[Para 44] Accordingly, the dsRNA of the invention comprises a first strand that is substantially identical to a portion of the sca1-like target gene of a plant parasitic nematode genome and a second strand that is substantially complementary to the first strand. In preferred embodiments, the target gene is selected from the group consisting of: (a) a polynucleotide having the sequence set forth in SEQ ID NO:1, 10 or 11; (b) a polynucleotide having at least 80% sequence identity to SEQ ID NO:1, 10 or 11; and (c) a polynucleotide from a parasitic nematode that hybridizes under stringent conditions to a polynucleotide having the sequence set forth in SEQ ID NO:1, 10 or 11.

[Para 45] As discussed above, fragments of dsRNA larger than about 19-24 nucleotides in length are cleaved intracellularly by nematodes and plants to siRNAs of about 19-24 nucleotides in length, and these siRNAs are the actual mediators of the RNAi phenomenon. The table in Figures 5a-5r sets forth exemplary 21-mers of the SCN sca1-like gene from SCN, SEQ ID NO:1. This table can also be used to calculate the 19, 20, 22, 23, or 24-mers by adding or subtracting the appropriate number of nucleotides from each 21mer. Thus the dsRNA of the present invention may range in length from about 19 nucleotides to about 500 consecutive nucleotides or up to the whole length of a sca1-like gene. Alternatively, the dsRNA of the invention has a length from about 21 nucleotides to about 600 consecutive nucleotides. Further, the dsRNA of the invention has a length from about 21 nucleotides to about 400 consecutive nucleotides, or from about 21 nucleotides to about 300 consecutive nucleotides.

[Para 46] As disclosed herein, 100% sequence identity between the dsRNA and the target gene is not required to practice the present invention. While a dsRNA comprising a nucleotide sequence identical to a portion of the sca1-like gene is preferred for inhibition, the invention can tolerate sequence variations that might be expected due to gene manipulation or synthesis, genetic mutation, strain polymorphism, or evolutionary divergence. Thus the dsRNAs of the invention also encompass dsRNAs comprising a mismatch with the target gene of at least 1, 2, or more nucleotides. For example, it is contemplated in the present invention that the 21mer dsRNA sequences exemplified in Figures 7a-7j may contain an addition, deletion or substitution

of 1, 2, or more nucleotides, so long as the resulting sequence still interferes with the sca1-like gene function.

[Para 47] Sequence identity between the dsRNAs of the invention and the sca1-like target genes may be optimized by sequence comparison and alignment algorithms known in the art (see Gribskov and Devereux, Sequence Analysis Primer, Stockton Press, 1991, and references cited therein) and calculating the percent difference between the nucleotide sequences by, for example, the Smith-Waterman algorithm as implemented in the BESTFIT software program using default parameters (e.g., University of Wisconsin Genetic Computing Group). Greater than 80 % sequence identity, 90% sequence identity, or even 100% sequence identity, between the inhibitory RNA and the portion of the target gene is preferred. Alternatively, the duplex region of the RNA may be defined functionally as a nucleotide sequence that is capable of hybridizing with a portion of the target gene transcript under stringent conditions (e.g., 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 60°C hybridization for 12-16 hours; followed by washing at 65°C with 0.1%SDS and 0.1% SSC for about 15-60 minutes).

[Para 48] When dsRNA of the invention has a length longer than about 21 nucleotides, for example from 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 21mer dsRNAs, derived from the longer dsRNA. This pool of 21mer dsRNAs is also encompassed within the scope of the present invention, whether generated intracellularly within the plant or nematode or synthetically using known methods of oligonucleotide synthesis.

[Para 49] The siRNAs of the invention have sequences corresponding to fragments of about 19-24 contiguous nucleotides across the entire sequence of the H. glycines sca1-like target gene. For example, a pool of siRNA of the invention derived from the H. glycines sca1-like gene as set forth in SEQ ID NO:1, 10 or 11 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:1, 10 or 11 found in Figures 5a-5r. One of skill in the art would 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 5a-5r may contain an addition, deletion or substitution of 1, 2, or more nucleotides and the resulting sequence still interferes with the sca1-like gene function. A pool of siRNA of the invention derived from the H. glycines sca1-like target gene of SEQ ID NO:1, 10 or 11 may also comprise any combination of the specific RNA molecules having any of the 21 contiguous nucleotide sequences derived from SEQ ID NO:1, 10 or 11 set forth in

Figures 5a-5r. Further, as multiple specialized Dicers in plants generate siRNAs typically ranging in size from 19nt to 24nt (See Henderson et al., 2006. Nature Genetics 38:721-725.), the siRNAs of the present invention can may range from about 19 contiguous nucleotide sequences to about 24 contiguous nucleotide sequences. Similarly, a pool of siRNA of the invention may comprise a multiplicity of RNA molecules having any 19, 20, 21, 22, 23, or 24 contiguous nucleotide sequences derived from SEQ ID NO:1, 10 or 11. Alternatively, the pool of siRNA of the invention may comprise a multiplicity of RNA molecules having a combination of any 19, 20, 21, 22, 23,and/or 24 contiguous nucleotide sequences derived from SEQ ID NO:1, 10 or 11.

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

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

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

[Para 53] In accordance with the invention, the recombinant expression vector comprises a regulatory sequence operatively linked to a nucleotide sequence that is a template for one or both strands of the dsRNA molecules of the invention. In one embodiment, the nucleic acid molecule further comprises a promoter flanking either end of the nucleic acid molecule, wherein the promoters drive expression of each individual DNA strand, thereby generating two complementary RNAs that hybridize and form the dsRNA. In another embodiment, the nucleic acid molecule comprises a nucleotide sequence that is transcribed into both strands of the dsRNA on one transcription unit, wherein the sense strand is transcribed from the 5' end of the transcription unit and the antisense strand is transcribed from the 3' end, wherein the two strands are separated by 3 to 500 base or more 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 54] According to the present invention, the introduced polynucleotide may be maintained in the plant cell stably if it is incorporated into a non-chromosomal autonomous replicon or integrated into the plant chromosomes. Alternatively, the introduced polynucleotide may be present on an extra-chromosomal non-replicating vector and be transiently expressed or transiently active. Whether present in an extra-chromosomal non-replicating vector or a vector that is integrated into a chromosome, the polynucleotide preferably resides in a plant expression cassette. A plant expression cassette preferably contains regulatory sequences capable of driving gene expression in plant cells that are operatively linked so that each sequence can fulfill its function, for example, termination of transcription by polyadenylation signals. Preferred polyadenylation signals are those originating from Agrobacterium tumefaciens t-DNA such as the gene 3 known as octopine synthase of the Ti-plasmid pTiACH5 (Gielen et al., 1984, EMBO J. 3:835) or functional equivalents thereof, but also all other terminators functionally active in plants are suitable. As plant gene expression is very often not limited 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 55] Plant gene expression should be operatively linked to an appropriate promoter conferring gene expression in a temporal-preferred, spatial-preferred, cell type-preferred, and/or tissue-preferred manner. Promoters useful in the expression cassettes of the invention include any promoter that is capable of initiating transcription in a plant cell present in the plant's roots. Such promoters include, but are not limited to those that can be obtained from plants, plant viruses and bacteria that contain genes that are expressed in plants, such as Agrobacterium and Rhizobium. Preferably, the expression cassette of the invention comprises a root-specific promoter, a pathogen inducible promoter, or a nematode inducible promoter. More preferably the nematode inducible promoter is or a parasitic nematode feeding site-specific promoter. A parasitic nematode feeding site-specific promoter may be specific for syncytial cells or giant cells or specific for both kinds of cells. A promoter is inducible, if its activity, measured on the amount of

RNA produced under control of the promoter, is at least 30%, 40%, 50% preferably at least 60%, 70%, 80%, 90% more preferred at least 100%, 200%, 300% higher in its induced state, than in its un-induced state. A promoter is cell-, tissue- or organ-specific, if its activity , measured on the amount of RNA produced under control of the promoter, is at least 30%, 40%, 50% preferably at least 60%, 70%, 80%, 90% more preferred at least 100%, 200%, 300% higher in a particular cell-type, tissue or organ, then in other cell-types or tissues of the same plant, preferably the other cell-types or tissues are cell types or tissues of the same plant organ, e.g. a root. In the case of organ specific promoters, the promoter activity has to be compared to the promoter activity in other plant organs, e.g. leaves, stems, flowers or seeds.

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

[Para 57] Inducible promoters are active under certain environmental conditions, such as the presence or absence of a nutrient or metabolite, heat or cold, light, pathogen attack, anaerobic conditions, and the like. For example, the promoters TobRB7, AtRPE, AtPyk10, Gemini19, and AtHMG1 have been shown to be induced by nematodes (for a review of nematode-inducible promoters, see Ann. Rev. Phytopathol. (2002) 40:191-219; see also U.S. Pat. No. 6,593,513). Method for isolating additional promoters, which are inducible by nematodes are set forth in U.S. Pat. Nos. 5,589,622 and 5,824,876. Other inducible promoters include the hsp80 promoter from Brassica, being inducible by heat shock; the PPDK promoter is induced by light; the PR-1 promoter from tobacco, Arabidopsis, and maize are inducible by infection with a

pathogen; and the Adh1 promoter is induced by hypoxia and cold stress. Plant gene expression can also be facilitated via an inducible promoter (For review, see Gatz, 1997, Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:89-108). Chemically inducible promoters are especially suitable if time-specific gene expression is desired. Non-limiting examples of such promoters are a salicylic acid inducible promoter (PCT Application No. WO 95/19443), a tetracycline inducible promoter (Gatz et al., 1992, Plant J. 2:397-404) and an ethanol inducible promoter (PCT Application No. WO 93/21334).

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

[Para 59] Other suitable tissue-preferred or organ-preferred promoters include, but are not limited to, the napin-gene promoter from rapeseed (U.S. Patent No. 5,608,152), the USP-promoter from Vicia faba (Baeumlein et al., 1991, Mol Gen Genet. 225(3):459-67), the oleosin-promoter from Arabidopsis (PCT Application No. WO 98/45461), the phaseolin-promoter from Phaseolus vulgaris (U.S. Patent No. 5,504,200), the Bce4-promoter from Brassica (PCT Application No. WO 91/13980), or the legumin B4 promoter (LeB4; Baeumlein et al., 1992, Plant Journal, 2(2):233-9), as well as promoters conferring seed specific expression in monocot plants like maize, barley, wheat, rye, rice, etc. Suitable promoters to note are the lpt2 or lpt1-gene promoter from barley (PCT Application No. WO 95/15389 and PCT Application No. WO 95/23230) or those described in PCT Application No. WO 99/16890 (promoters from the barley hordein-gene, rice glutelin gene, rice oryzin gene, rice prolamin gene, wheat gliadin gene, wheat glutelin gene, oat glutelin gene, Sorghum kasirin-gene, and rye secalin gene).

[Para 60] Other promoters useful in the expression cassettes of the invention include, but are not limited to, the major chlorophyll a/b binding protein promoter, histone promoters, the Ap3 promoter, the β-conglycin promoter, the napin promoter, the soybean lectin promoter, the

maize 15kD zein promoter, the 22kD zein promoter, the 27kD zein promoter, the g-zein promoter, the waxy, shrunken 1, shrunken 2, and bronze promoters, the Zm13 promoter (U.S. Patent No. 5,086,169), the maize polygalacturonase promoters (PG) (U.S. Patent Nos. 5,412,085 and 5,545,546), and the SGB6 promoter (U.S. Patent No. 5,470,359), as well as synthetic or other natural promoters.

[Para 61] In accordance with the present invention, the expression cassette comprises an expression control sequence operatively linked to a nucleotide sequence that is a template for one or both strands of the dsRNA. The dsRNA template comprises (a) a first stand having a sequence substantially identical to from about 19 to about 400–500, or up to the full length, consecutive nucleotides of SEQ ID NO:1; 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 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.

[Para 62] In another embodiment, the vector contains a bidirectional promoter, driving expression of two nucleic acid molecules, whereby one nucleic acid molecule codes for the sequence substantially identical to a portion of a sca1-like gene and the other nucleic acid molecule codes for a second sequence being substantially complementary to the first strand and capable of forming a dsRNA, when both sequences are transcribed.. A bidirectional promoter is a promoter capable of mediating expression in two directions.

[Para 63] In another embodiment, the vector contains two promoters one mediating transcription of the sequence substantially identical to a portion of a sca1-like gene 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.

[Para 64] A different promoter means a promoter having a different activity in regard to cell or tissue specificity, or showing expression on different inducers for example, pathogens, abiotic stress or chemicals. For example, one promoter might by constitutive or tissue specific and another might be tissue specific or inducible by pathogens. In one embodiment one promoter mediates the transcription of one nucleic acid molecule suitable for over expression of a sca1-like

gene, while another promoter mediates tissue- or cell-specific transcription or pathogen inducible expression of the complementary nucleic acid.

[Para 65] The invention is also embodied in a transgenic plant capable of expressing the dsRNA of the invention and thereby inhibiting the sca1-like genes in parasitic nematodes. The plant or transgenic plant may be any plant, such like, but not limited to trees, cut flowers, ornamentals, vegetables or crop plants. The plant may be from a genus selected from the group consisting of Medicago, Lycopersicon, Brassica, Cucumis, Solanum, Juglans, Gossypium, Malus, Vitis, Antirrhinum, Populus, Fragaria, Arabidopsis, Picea, Capsicum, Chenopodium, Dendranthema, Pharbitis, Pinus, Pisum, Oryza, Zea, Triticum, Triticale, Secale, Lolium, Hordeum, Glycine, Pseudotsuga, Kalanchoe, Beta, Helianthus, Nicotiana, Cucurbita, Rosa, Fragaria, Lotus, Medicago, Onobrychis, trifolium, Trigonella, Vigna, Citrus, Linum, Geranium, Manihot, Daucus, Raphanus, Sinapis, Atropa, Datura, Hyoscyamus, Nicotiana, Petunia, Digitalis, Majorana, Ciahorium, Lactuca, Bromus, Asparagus, Antirrhinum, Heterocallis, Nemesis, Pelargonium, Panieum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Browaalia, Phaseolus, Avena, and Allium, or the plant may be selected from a genus selected from the group consisting of Arabidopsis, Medicago, Lycopersicon, Brassica, Cucumis, Solanum, Juglans, Gossypium, Malus, Vitis, Antirrhinum, Brachipodium, Populus, Fragaria, Arabidopsis, Picea, Capsicum, Chenopodium, Dendranthema, Pharbitis, Pinus, Pisum, Oryza, Zea, Triticum, Triticale, Secale, Lolium, Hordeum, Glycine, Pseudotsuga, Kalanchoe, Beta, Helianthus, Nicotiana, Cucurbita, Rosa, Fragaria, Lotus, Medicago, Onobrychis, trifolium, Trigonella, Vigna, Citrus, Linum, Geranium, Manihot, Daucus, Raphanus, Sinapis, Atropa, Datura, Hyoscyamus, Nicotiana, Petunia, Digitalis, Majorana, Ciahorium, Lactuca, Bromus, Asparagus, Antirrhinum, Heterocallis, Nemesis, Pelargonium, Panicum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Browaalia, Phaseolus, Avena, and Allium. In one embodiment the plant is a monocotyledonous plant or a dicotyledonous plant.

[Para 66] 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. Accordingly, in yet another embodiment the plant is a Poaceae plant of the genus Zea, Triticum, Oryza, Hordeum, Secale, Avena, Saccharum, Sorghum, Pennisetum, Setaria, Panicum, Eleusine, Miscanthus, Brachypodium, Festuca or Lolium. When the plant is of the genus Zea, the preferred species is Z. mays. When the plant is of the genus Triticum, the preferred species is T. aestivum, T. speltae or T. durum. When the plant is of the genus Oryza, the preferred species is O. sativa. When the plant is of the genus Hordeum, the preferred species is H. vulgare. When the plant is of the genus Secale, the preferred species S. cereale. When the plant is of

the genus Avena, the preferred species is A. sativa. When the plant is of the genus Saccarum, the preferred species is S. officinarum. When the plant is of the genus Sorghum, the preferred species is S. vulgare, S. bicolor or S. sudanense. When the plant is of the genus Pennisetum, the preferred species is P. glaucum. When the plant is of the genus Setaria, the preferred species is S. italica. When the plant is of the genus Panicum, the preferred species is P. miliaceum or P. virgatum. When the plant is of the genus Eleusine, the preferred species is E. coracana. When the plant is of the genus Miscanthus, the preferred species is M. sinensis. When the plant is a plant of the genus Festuca, the preferred species is F. arundinaria, F. rubra or F. pratensis. When the plant is of the genus Lolium, the preferred species is L. perenne or L. multiflorum. Alternatively, the plant may be Triticosecale.

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

is I. batatas. When the plant is of the family Rosaceae, the preferred genus is Rosa, Malus, Pyrus, Prunus, Rubus, Ribes, Vaccinium or Fragaria and the preferred species is the hybrid Fragaria x ananassa. When the plant is of the family Cucurbitaceae, the preferred genus is Cucumis, Citrullus or Cucurbita and the preferred species is Cucumis sativus, Citrullus lanatus or Cucurbita pepo. When the plant is of the family Theaceae, the preferred genus is Camellia and the preferred species is C. sinensis. When the plant is of the family Rubiaceae, the preferred genus is Coffea and the preferred species is C. arabica or C. canephora. When the plant is of the family Sterculiaceae, the preferred genus is Theobroma and the preferred species is T. cacao. When the plant is of the genus Citrus, the preferred species is C. sinensis, C. limon, C. reticulata, C. maxima and hybrids of Citrus species, or the like. In a preferred embodiment of the invention, the plant is a soybean, a potato or a corn plant

[Para 68] Suitable methods for transforming or transfecting host cells including plant cells are well known in the art of plant biotechnology. Any method may be used to transform the recombinant expression vector into plant cells to yield the transgenic plants of the invention. General methods for transforming dicotyledenous plants are disclosed, for example, in U.S. Pat. Nos. 4,940,838; 5,464,763, and the like. Methods for transforming specific dicotyledenous plants, for example, cotton, are set forth in U.S. Pat. Nos. 5,004,863; 5,159,135; and 5,846,797. Soybean transformation methods are set forth in U.S. Pat. Nos. 4,992,375; 5,416,011; 5,569,834; 5,824,877; 6,384,301 and in EP 0301749B1 may be used. Transformation methods may include direct and indirect methods of transformation. Suitable direct methods include polyethylene glycol induced DNA uptake, liposome-mediated transformation (US 4,536,475), biolistic methods using the gene gun (Fromm ME et al., Bio/Technology. 8(9):833-9, 1990; Gordon-Kamm et al. Plant Cell 2:603, 1990), electroporation, incubation of dry embryos in DNA-comprising solution, and microinjection. In the case of these direct transformation methods, the plasmids used need not meet any particular requirements. Simple plasmids, such as those of the pUC series, pBR322, M13mp series, pACYC184 and the like can be used. If intact plants are to be regenerated from the transformed cells, an additional selectable marker gene is preferably located on the plasmid. The direct transformation techniques are equally suitable for dicotyledonous and monocotyledonous plants.

[Para 69] Transformation can also be carried out by bacterial infection by means of Agrobacterium (for example EP 0 116 718), viral infection by means of viral vectors (EP 0 067 553; US 4,407,956; WO 95/34668; WO 93/03161) or by means of pollen (EP 0 270 356; WO 85/01856; US 4,684,611). Agrobacterium based transformation techniques (especially for dicotyledonous plants) are well known in the art. The Agrobacterium strain (e.g., Agrobacterium tumefaciens or Agrobacterium rhizogenes) comprises a plasmid (Ti or Ri plasmid) and a T-DNA

element which is transferred to the plant following infection with Agrobacterium. The T-DNA (transferred DNA) is integrated into the genome of the plant cell. The T-DNA may be localized on the Ri- or Ti-plasmid or is separately comprised in a so-called binary vector. Methods for the Agrobacterium-mediated transformation are described, for example, in Horsch RB et al. (1985) Science 225:1229. The Agrobacterium-mediated transformation is best suited to dicotyledonous plants but has also been adapted to monocotyledonous plants. The transformation of plants by Agrobacteria is described in, for example, White FF, Vectors for Gene Transfer in Higher Plants, Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press, 1993, pp. 15 - 38; Jenes B et al. Techniques for Gene Transfer, Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press, 1993, pp. 128-143; Potrykus (1991) Annu Rev Plant Physiol Plant Molec Biol 42:205- 225. 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 70] 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 71] “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 72] In accordance with this embodiment, the transgenic plant of the invention is produced by a method comprising the steps of providing a parasitic nematode sca1-like target gene, preparing an expression cassette having a first region that is substantially identical to a portion of the selected sca1-like gene and a second region which is complementary to the first region, transforming the expression cassette into a plant, and selecting progeny of the transformed plant which express the dsRNA construct of the invention.

[Para 73] As increased resistance to nematode infection is a general trait wished to be inherited into a wide variety of plants. Increased resistance to nematode infection is a general trait wished to be inherited into a wide variety of plants. The present invention may be used to reduce crop destruction by any plant parasitic nematode. Preferably, the parasitic nematodes belong to nematode families inducing giant or syncytial cells. Nematodes inducing giant or syncytial cells are found in the families Longidoridae, Trichodoridae, Heterodidae, Meloidogynidae, Pratylenchidae or Tylenchulidae. In particular in the families Heterodidae and Meloidogynidae.

[Para 74] 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 75] 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. 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 76] The following examples are not intended to limit the scope of the claims to the invention, but are rather intended to be exemplary of certain embodiments. Any variations in the exemplified methods that occur to the skilled artisan are intended to fall within the scope of the present invention.

EXAMPLE 1: IDENTIFICATION AND ISOLATION OF H. GLYCINES SCA1-LIKE TARGET GENE.

[Para 77] Using total RNA isolated from SCN J2 stage, RT-PCR was used to isolate cDNA fragments that were approximately 400-500 bp in length. The PCR products were cloned into TOPO pCR2.1 vector (Invitrogen, Carlsbad, CA) and inserts were confirmed by sequencing. RT-PCR was performed using primer sets (SEQ ID NOs:2 and 3). Briefly, total RNA was isolated from SCN J2 (race 3) using standard TRIzol method (e.g., TriReagent, Molecular Research Center, Inc., Cincinnati, OH). RT-PCR reactions contained SCN J2 total RNA. A gene fragment represented by nucleotides 1-499 of SEQ ID NO:1 was isolated using this method, and determined to be a homolog of C. elegans sca1.

[Para 78] In order to obtain full-length cDNA for H. glycines sca1-like, an RT-PCT method, based on highly conserved spliced leader sequence (SL1) present in many nematode species, is used. The reactions are conducted using Supercript One-Step kit (Invitrogen, Carlsbad, Calif., catalog no. 10928-034) and a primer set. The forward primer is a 22-mer SL1 sequence (SEQ ID NO:13) and reverse primers will be gene specific and are located in the previously cloned cDNA region. PCR products will be cloned into Pcr4-topo VECTOR (Invitrogen, Carlsbad, Calif.) and sequenced.

[Para 79] 3'cDNA ends were amplified using the GeneRacer Kit (Invitrogen, Carlsbad, CA, catalog No. L1500-01). The first-strand cDNAs were generated through reverse transcription using total RNA and the GeneRacer Oligo dT Primer (SEQ ID NO:12). The 3' RACE PCR was performed with the GeneRacer 3' Primer (SEQ ID NO:5) and a gene-specific forward primer (SEQ ID NO:4). The nested PCR reactions were subsequently conducted using GeneRacer 3' Nested Primer (SEQ ID NO:7) and a gene-specific forward primer (SEQ ID NO:6). PCR products were cloned into pCR4-TOPO (Invitrogen, Carlsbad, CA) and sequenced.

[Para 80] The sequences of the sca1-like PCR fragments isolated above were assembled into cDNA corresponding to the gene designated H. glycines sca1-like, and this sequence is set forth as SEQ ID NO: 1 in Figure 1.

EXAMPLE 2: DEMONSTRATION OF ESSENTIALITY OF C. ELEGANS TARGET GENE AND ISOLATION OF HOMOLOGS FROM SCN.

[Para 81] The homolog of the SCN target gene identified in Example 1 was isolated from C. elegans using PCR primers (SEQ ID NOs: 8 and 9 in Figure 2) and C. elegans genomic DNA as a template. (see K11D9.2, Genbank, National Center for Biotechnology Information, Bethesda, MD) The PCR products (~1 kb in length) were cloned into the multiple cloning site of pLitmus28i (New England Biolabs, Beverly, MA), so that C. elegans gene fragments were flanked by two T7 promoters in a head-to-head configuration. The DNA sequences of C. elegans gene fragment used in RNAi assay are shown in Fig. 3 (SEQ ID NO:10).

[Para 82] The pLitmus28i vectors with the target genes were then transformed into E. coli strain HT115(DE3). This strain is deficient in RNase III—an enzyme that degrades dsRNA. Therefore, dsRNA produced in HT115(DE3) is expected to be more stable. Upon IPTG (Isopropyl β-D-Thiogalactopyranoside) induction, T7 RNA polymerase, was expressed and transcribed dsRNA. The production of dsRNA in E. coli was confirmed by total RNA extraction using RiboPure-Bacteria Kit (Ambion, Austin, TX, cat no 1925) and subsequent S1 nuclease treatment.

[Para 83] The C. elegans RNAi feeding assay consisted growing the HT115(DE3) cultures overnight and adding 50 µl of the E. coli cultures to each well of a 96 well microtiter plate, Approximately 3 µl of L1 larvae (10 to 15 L1s) were then added to each well, and the plate was incubated at approximately 25°C for 5 days. Each culture was triplicated, so a total of six wells were used for each C. elegans gene tested in the assay. The bacteria transformed with pLitmus28i alone (no inserts) was used as the control. The assay was examined and RNAi phenotypes of the C. elegans were analyzed.

[Para 84] By Day 5, in the control (pLitmus28i alone), L1 larvae developed into gravid adults and produced many progeny. The administration by feeding dsRNA substantially identical to the C. elegans target gene resulted in arrest in development of nematodes, and the worms in all six wells for the gene showed consistent RNAi phenotypes. A dsRNA substantially identical to the C. elegans sca1 gene (SEQ ID NO:10), the homolog of H. glycines sca1-like (SEQ ID NO:1)), caused mortality of the adult as evidenced by a phenotype of a rigid, non-moving straight body type rather than the living plant, moving s-shaped body type.. These data demonstrated that C. elegans homologue of the sca1-like target gene candidate identified in Example 1 is essential for C. elegans development. This further indicated that the selected target gene indeed plays a key role for nematode survival in both plant parasitic nematodes and C. elegans.

EXAMPLE 3: BINARY VECTOR CONSTRUCTION FOR SOYBEAN TRANSFORMATION.

[Para 85] This exemplified method employs a binary vector containing the sca1-like target gene. The vector consists of an antisense fragment (SEQ ID NO:11) of the target sca1-like gene, a spacer, a sense fragment of the target gene and a vector backbone. The sequence of the sca1-like gene (SEQ ID NO.1) is set forth in Figure 1. The target gene fragment (SEQ ID NO:11) corresponding to nucleotides 1-499 of SEQ ID NO:1 was used to construct the binary vector RSA006 (pSA006). In this vector, dsRNA for the sca1-like target gene was expressed under a constitutive promoter, Super Promoter (see US 5955,646, incorporated herein by reference). The selection marker for transformation was a mutated AHAS gene from Arabidopsis thaliana that conferred resistance to the herbicide ARSENAL (imazepyr, BASF Corporation, Mount Olive, NJ). The expression of mutated AHAS was driven by a ubiquitin promoter. (See Plesch, G. and Ebneth, M., "Method for the stable expression of nucleic acids in transgenic plants, controlled by a parsley ubiquitin promoter", WO 03/102198, hereby incorporated by reference.)

Example 4 Bioassay of dsRNA targeted to H. glycines sca1 target gene

[Para 86] The rooted explant assay was employed to demonstrate dsRNA expression and the resulting nematode resistance. This assay can be found in co-pending application USSN 12/001,234, the contents of which are incorporated herein by reference.

[Para 87] Clean soybean seeds from soybean cultivar were surface sterilized and germinated. Three days before inoculation, an overnight liquid culture of the disarmed Agrobacterium culture, for example, the disarmed A. rhizogenes strain K599 containing the binary vector RSA006, was initiated. The next day the culture was spread onto an LB agar plate containing kanamycin as a selection agent. The plates were incubated at 28°C for two days. One plate was prepared for every 50 explants to be inoculated. Cotyledons containing the proximal end from its connection with the seedlings were used as the explant for transformation. After removing the cotyledons the surface was scraped with a scalpel around the cut site. The cut and scraped cotyledon was the target for Agrobacterium inoculation. The prepared explants were dipped onto the disarmed thick A. rhizogenes colonies prepared above so that the colonies were visible on the cut and scraped surface. The explants were then placed onto 1% agar in Petri dishes for co-cultivation under light for 6-8 days.

[Para 88] After the transformation and co-cultivation soybean explants were transferred to rooting induction medium with a selection agent, for example S-B5-708 for the mutated acetohydroxy acid synthase (AHAS) gene (Sathasivan et al., Plant Phys. 97:1044-50, 1991). Cultures were maintained in the same condition as in the co-cultivation step. The S-B5-708 medium comprises: 0.5X B5 salts, 3mM MES, 2% sucrose, 1X B5 vitamins, 400µg/ml Timentin, 0.8% Noble agar, and 1 µM Imazapyr (selection agent for AHAS gene) (BASF Corporation, Florham Park, NJ) at pH5.8.

[Para 89] Two to three weeks after the selection and root induction, transformed roots were formed on the cut ends of the explants. Explants were transferred to the same selection medium (S-B5-708 medium) for further selection. Transgenic roots proliferated well within one week in the medium and were ready to be subcultured.

[Para 90] Strong and white soybean roots were excised from the rooted explants and cultured in root growth medium supplemented with 200 mg/l Timentin (S-MS-606 medium) in six-well plates. Cultures were maintained at room temperature under the dark condition. The S-MS-606 medium comprises: 0.2X MS salts and B5 vitamins, 2% sucrose, and 200mg/l Timentin at pH5.8.

[Para 91] One to five days after subculturing, the roots were inoculated with surface sterilized nematode juveniles in multi-well plates for either gene of interest or promoter construct assay. As a control, soybean cultivar Williams 82 control vector and Jack control vector roots

were used. The root cultures of each line that occupied at least half of the well were inoculated with surface-decontaminated race 3 of soybean cyst nematode (SCN) second stage juveniles (J2) at the level of 500 J2/well. The plates were then sealed and put back into the incubator at 25°C in darkness. Several independent root lines were generated from each binary vector transformation and the lines were used for bioassay. Four weeks after nematode inoculation, the cysts in each well were counted. Bioassay results for construct RSA006 show a statistically significant reduction (p-value <0.05) in cyst count over multiple transgenic lines and a general trend of reduced cyst count in the majority of transgenic lines tested.

[Para 92] Those skilled in the art will recognize, or will be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

1. A dsRNA molecule comprising a) a first strand comprising a sequence substantially identical to a portion of a sca1-like target gene of a parasitic nematode, and b) a second strand comprising a sequence substantially complementary to the first strand, wherein the target gene is a parasitic nematode sca1-like gene.

2. The dsRNA molecule of claim 1, wherein the portion of the target gene is of a sequence selected from the group consisting of: a) a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, 10 or 11; b) a polynucleotide comprising a sequence having at least 80% sequence identity to SEQ ID NO.1, 10 or 11; and c) a polynucleotide from a nematode that hybridizes under stringent conditions to a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, 10 or 11.

3. 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 selected from the group consisting of: a) a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, 10 or 11; b) a polynucleotide comprising a sequence having at least 80% sequence identity to SEQ ID NO.1, 10 or 11; and c) a polynucleotide from a nematode that hybridizes under stringent conditions to a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, 10 or 11.

4. A transgenic plant capable of expressing a dsRNA that is substantially identical to a portion of a parasitic nematode sca1-like target gene.

5. The transgenic plant of claim 4, 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, 10 or 11; b) a polynucleotide comprising a sequence having at least 80% sequence identity to SEQ ID NO.1, 10 or 11; and c) a polynucleotide from a parasitic nematode that hybridizes under stringent conditions to a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, 10 or 11.

6. The transgenic plant of claim 4, wherein the dsRNA comprises a multiplicity of RNA molecules each comprising a double stranded region having a length of about 19-24 nucleotides, wherein said RNA molecules are derived a polynucleotide selected from the group consisting of: a) a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, 10 or 11; b) a polynucleotide comprising a sequence having at least 80% sequence identity to SEQ ID NO.1, 10 or 11; and c) a polynucleotide from a parasitic nematode that hybridizes under stringent conditions to a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, 10 or 11.

7. The transgenic plant of claim 4, wherein the plant is selected from the group consisting of: soybean, potato, tomato, peanuts, cotton, cassava, coffee, coconut, pineapple, citrus trees, banana, corn, rape, beet, sunflower, sorghum, wheat, oats, rye, barley, rice, green bean, lima bean, pea, and tobacco.

8. The transgenic plant of claim 4 wherein the plant is a soybean plant.

9. A method for controlling the infection of a plant by a parasitic nematode, comprising the steps of exposing the nematode to a dsRNA molecule that is substantially identical to a portion of a target gene essential to the nematode, thereby controlling the infection of the plant by the nematode, wherein the target gene a parasitic nematode sca1-like gene.

10. The method of claim 9, 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, 10 or 11; b) a polynucleotide comprising a sequence having at least 80% sequence identity to SEQ ID NO.1, 10 or 11; and c) a polynucleotide from a parasitic nematode that hybridizes under stringent conditions to a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, 10 or 11.

11. A method of making a transgenic plant capable of expressing a sca1-like dsRNA that is substantially identical to a portion of a target gene in a parasitic nematode, said method comprising the steps of: a) preparing a nucleic acid sequence having a region that is substantially identical to a portion of a parasitic nematode sca1-like target gene, wherein the nucleic acid is able to form a sca1-like double-stranded transcript 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.

12. The method of claim 11, 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, 10 or 11; b) a polynucleotide comprising a sequence having at least 80% sequence identity to SEQ ID NO.1, 10 or 11; and c) a polynucleotide from a parasitic nematode that hybridizes under stringent conditions to a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, 10 or 11.

13. The method of claim 11, wherein the portion of the target gene is from about 19 to about 400 nucleotides of a sequence selected from the group consisting of: a) a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, 10 or 11; and b) a polynucleotide from a parasitic nematode that hybridizes under stringent conditions to a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, 10 or 11.

14. The method of claim 11, wherein the plant is selected from the group consisting of: soybean, potato, tomato, peanuts, cotton, cassava, coffee, coconut, pineapple, citrus trees, ba-

nana, corn, rape, beet, sunflower, sorghum, wheat, oats, rye, barley, rice, green bean, lima bean, pea, and tobacco.

15. The method of claim 11 wherein the plant is a soybean plant.

FIGURE 1a

SEQ ID NO: 1

ATGGAGAACGCTCATACGAAAAGTGAAGACGAATTGTTTCGGTTTTTCGGCA
CAGGGCCAGACGGACTGACAGAGGAACAAGCAGACGAATTGCGGGATAAAT
ATGGCTATAATGAAATGCCCGCGGAGGAGGGGAAAAAGCTGTGGGAATTGAT
TCTCGAGCAGTTCGATGATCTCCTTGTAAAAATTTTACTCCTAGCCGCAATAA
TTTCTTTTGTCCTTGCCTTGTTTGAGGAGCACGATGACCAGACGAGTGCAGTC
ACTGCGTTTGTGGAACCTTTTGTTATTCTCCTAATTCTCATTGCGAATGCCAC
GGTCGGAGTTTGGCAGGAGAGAAATGCGGAAAGTGCAATTGAAGCGCTGAA
GGAATACGAACCGGAAATGGCAAAAGTCATCCGAGCGGGCAAACACGGCAT
TCAGATGATCCGTGCAAAGGAACTCGTCCCGGGCGATCTCGTCGAAGTTTCG
GTTGGAGATAAAATTCCGGCCGATTTGCGACTTGTCAAAATTTATTCGACGAC
CATTCGCATTGACCAATCCATTCTGACGGGAGAGTCTGTGTCGGTCATTAAG
AATTTGGACGTGGTGCCCGACCCGAGGGCGGTCAACCAGGACAAGAAGAAC
TGCCTTTTCTCTGGCACAAATGTTGCGTCAGGCAAAGCCCGGGGAATTGTTT
TTGGCACCGGACTAAGTACGGAAATTGGCAAAATCCGCACGGAAATGGCGG
AAACCGAATCGGACAAAACGCCGCTGCAACAGAAGTTGGACGAGTTCAGCG
AGCAGTTGTCCAAAGTCATTTCCATAATTTGTGTCGCGGTGTGGGCCATCAAC
ATCGGCCACTTCAACGACCCGGCCCATGGCGGCTCGTGGCTAAAGGGTGCC
ATTTACTACTTCAAAATTGCGGTTGCCCTTGCCGTGGCAGCCATTCCCGAGG
GTTTGCCGGCCGTGATCACCACTTGTTTGGCATTGGGCACCCGTCGGATGG
CCAAAAAGAATGCCATTGTCCGCTCGTTGCCTTCCGTTGAGACATTGGGCTG
CACTTCGGTGATTTGTTCGGACAAGACTGGAACATTAACCACCAATCAAATGT
CTGTGTCCAAAATGTTTGTTGTTGAACATGCGCACGGCGACCAAATCACTTTC
GGCGAATTCACAATCTCTGGCTCCACCTATGAGCCGACTGGGCAAATCATGT
ACAACGGAGTCCAAATAAACTGTGCAACCGACCAACACAAAGCATTGACAGA
ATTAGCCACCATTTGTTCACTGTGCAACGACTCTTCCGTGGATTACAACGAAA
TGAAACACGCTTATGAAAAAGTTGGGGAGGCCACTGAAACAGCATTGGTTGT
GTTGGCTGAGAAAATGAATGTGTACGACACGCCGAAACACAATGGACTGAGT
CCGCGCGAGTTGGGCAGCGTGTGCAACCGTGTGATCCAACTCAAGTGGAAA
AAGGAGTTCACGCTGGAATTTTCACGTGATAGGAAAGCGATGTCGGTGTATT
GTAAGCCGTCAGCGGACAGAACGGGGGCCGGTGCCAAAATGTTTGTGAAAG
GAGCGCCCGAAGGGGTGCTCTCCCGGTGCAC

FIGURE 1b

CCACGTGCGCATCGGGGACCAAAAGGTGCCACTGACTCAGGCGATGACCCA
ACGCATTGTGCAGCAGTGCGTCAAATACGGCACCGGACGCGACACTTTGCG
TTGTCTCGCGCTTGGCACCATCGACGAGCCGCCAAGCCCCGAAAACATGAA
CCTCGAGGACTCCACCAAATTCGGCGAGTACGAACAGAACATTACTTTTGTC
GGCGTCGTCGGCATGTTGGACCCGCCCCGTACCGAAGTTGCGACGTCCATC
CGCGAGTGCTATCACGCGGGCATCCGAGTGATAATGATCACTGGGGATAACA
AAAACACTGCCGAAGCAATTGGCCGACGCATTGGACTGTTTGGCGAAAATGA
GGACACCGCCGGACTTTCGTACACCGGCCGTGAGTTTGACGACTTGCCGCC
CCAACAGCAAAGCGACGCGTGCCGTCGTGCCAAATTATTCGCTCGCGTTGAA
CCCGCGCACAAATCGAAGATTGTCGAATATTTGCAATCGCATGGCGAAATCA
CTGCGATGACCGGCGACGGAGTGAACGATGCGCCGGCACTGAAAAAGGCC
GAAATTGGCATTGCTATGGGCAGTGGCACGGCGGTGGCAAAAAGTGCCGCG
GAAATGGTGTTGGCGGATGACAATTTCTCAACAATTGTGGCAGCGGTGGAGG
AAGGCCGTGCCATTTACAACAACATGAAACAATTCATTCGCTATCTCATCTCG
TCAAACATTGGTGAAGTCGTCTCCATTTTCCTTGTCGCTGCGCTTGGCATTCC
CGAAGCTCTGATCCCCGTCCAATTGCTTTGGGTCAATTTGGTCACCGATGGT
CTTCCCGCCACTGCGCTCGGCTTCAATCCGCCCGACTTGGACATTATGGACC
GACTGCCGCGTTCCGCCTCCGAATCGCTCATTTCCAAATGGCTTTTCTTCAGA
TACATGGCAATCGGAACTTACGTCGGCGTCGCCACTGTTGCCGCTTCGATGT
GGTGGTTTTTGATTTACGAGGACGGCCCGCAAGTGTCTTATTACCAGCTGAC
CCATTGGATGCGCTGTGAAATTGAGCCGGAGAACTTTGAGGATTTGGACTGT
GCCGTTTTTGTTGACAACCATCCAAACGCAATGGCATTGTCAGTGCTCGTCAC
AATCGAAATGCTGAATGCGATCAACAGTTTGTCCGAGAATCAGTCCATTCTGA
AGATGCCCCCGTGGACAAACATTTGGCTTTGCGCGGCCATCGCTCTGTCCAT
GTCGCTGCACTTTCTCATCCTTTACGTGGACATCATGGCCACCATCTTCCAAA
TTACTCCCCTCAACTTCACCGAATGGATGGCCGTGCTCAAATTCTCTATCCCT
GTCATTTTGTTGGATGAAATTCTCAAATTTGTCGCCCGACGGATGGAAGCACA
TGGCGAAGATGAATTATTGACTGCGAAGAAATTGAAGTGA

FIGURE 2

Gene	**SEQ ID NO**	**Forward Primer**	**SEQ ID NO**	**Reverse Primer**
H. glycines *sca1-like* middle	2	CGCCTCCGAATCGCTCATTT	3	TCGGGCGACAAATTTGAGAA
H. glycines *sca1-like* 3'	4	GCTGACCCATTGGATGCGCCATGA	5	GCTGTCAACGATACGCTACGTAACG
H. glycines *sca1-like* 3' nested	6	ACGCAATGGCATTGTCAGTGCTCGTCA	7	CGCTACGTAACGGCATGACAGTG
Ce K11D9.2	8	GGAACCTTTTGTCCGTTAACTCT	9	CCGGAGAATCTGTGTCTGTTATC

Common Primers	SEQ ID NO	
SL1	13	GGTTTAATTACCCAAGTTTGA
GeneRacer 3' Primer	5	GCTGTCAACGATACGCTACGTAACG
GeneRacer 3' Nested Primer	7	CGCTACGTAACGGCATGACAGTG
GeneRacer Oligo dT	12	GCTGTCAACGATACGCTACGTAACG GCATGACAGTG$(T)_{24}$

FIGURE 3

SEQ ID NO: 10

GGAACCTTTTGTCCGTTAACTCTGACGTGGGTGCATCTTCCGAGAACTCCTTC
TGGGGCTCCCTTCACGAACATCTTGGCTCCAGATCCTCCGGAAGCTGGGAA
GCAGTAGGCGGACATGGATTTACGATCACGGGAGAACTCGAGTGTGAACTC
CTTCTTCCATTTTTGTTGGATGACACGGTTGCAAACTCCTCCGAGCTCCTTTG
GTGAAAGTCCGGCTTTCGAGGTTCCGAAAACATTCATCTTCTCAGCAAGAAC
GATAAGAGCAGTTTCAGTGGCTTCTCCGACTTTCTCGTAGATCTTCTTGGTCT
CATTGTAATCAACAGATGAATCATTGCACATAGCGCAGATCATGGCCAACTCG
GTGAGTGATTCGAATTCTCCAGCAGCTGGGTTGATTTCACGTCCATTGGTGG
AAACCTTTCCGACTGGCTCGTAGGTGGATCCGGAGATGGCGAACTCGGTGA
AGTTGATGTTGTCTCCAGAAGCTTGTCCAGCGATGAACATCTTTGACACAGAC
ATCTGGTTGGTGGTGAGAGTTCCAGTCTTGTCAGAGCAGATAACAGATGTGC
ATCCAAGAGTTTCGACGGATGGAAGGGATCTTACAATAGCGTTCTTCTTGGC
CATACGGCGAGTTCCGAGGGCAAGGCACGTGGTGATGACAGCTGGAAGTCC
TTCTGGAATAGCAGCGACGGCAAGAGCAACGGCGATTTTGAAGTAGTAGATT
GCTCCCTTAACCCATGATCCACCGTGAGCTGGATCGTTGAAATGTCCAATGTT
GATAGCCCAAACAGCAACGCAAATAACAGAGATAACCTTGGAAAGTTGCTCT
CCGAATTCGTCCAACTTCTGTTGAAGTGGTGTCTTCTCATTCTCGGTCTCAGC
CATTTCGGTACGGATCTTTCCGATTTCAGTGGTCAATCCGGTTCCGAAGACG
ATTCCACGAGCCTTTCCAGATGCGACATTGGTTCCCGAGAACAGACAATTCTT
CTTGTCCTGGTTAACAGCGCGTGGATCTGGCACAGAGTCGGTGTGCTTGATA
ACAGACACAGATTCTCCGG

FIGURE 4

SEQ ID NO: 11

CGCCTCCGAATCGCTCATTTCCAAATGGCCTTTCTTCAGATACATGGCAATCG
GAACTTACGTCGGCGTCGCCACTGTTGCCGCTTCGATGTGGTGGTTTTTGAT
TTACGAGGACGGCCCGCAAGTGTCTTATTACCAGCTGACCCATTGGATGCGC
CATGAAATTGAGCCGGAGAACTTTGAGGATTTGGACTGTGCCGTTTTTGTTGA
CAACCATCCAAACGCAATGGCATTGTCAGTGCTCGTCACAATCGAAATGCTG
AATGCGATCAACAGTTTGTCCGAGAATCAGTCCATTCTGAAGATGCCCCCGT
GGACAAACATTTGGCTTTGCGCGGCCATCGCTCTGTCCATGTCGCTGCACTT
TCTCATCCTTTACGTGGACATCATGGCCACCATCTTCCAAATTACTCCCCTCA
ACTTCACCGAATGGATGGCCGTGCTCAAATTCTCTATCCCTGTCATTTTGTTG
GATGAAATTCTCAAATTTGTCGCCCGA

Figure 5a

nucleotide positions of 21mers of sca1-like genes									
e.g. SEQ ID NO: 1, 10 or 11									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
1	21	37	57	73	93	109	129	145	165
2	22	38	58	74	94	110	130	146	166
3	23	39	59	75	95	111	131	147	167
4	24	40	60	76	96	112	132	148	168
5	25	41	61	77	97	113	133	149	169
6	26	42	62	78	98	114	134	150	170
7	27	43	63	79	99	115	135	151	171
8	28	44	64	80	100	116	136	152	172
9	29	45	65	81	101	117	137	153	173
10	30	46	66	82	102	118	138	154	174
11	31	47	67	83	103	119	139	155	175
12	32	48	68	84	104	120	140	156	176
13	33	49	69	85	105	121	141	157	177
14	34	50	70	86	106	122	142	158	178
15	35	51	71	87	107	123	143	159	179
16	36	52	72	88	108	124	144	160	180
17	37	53	73	89	109	125	145	161	181
18	38	54	74	90	110	126	146	162	182
19	39	55	75	91	111	127	147	163	183
20	40	56	76	92	112	128	148	164	184
21	41	57	77	93	113	129	149	165	185
22	42	58	78	94	114	130	150	166	186
23	43	59	79	95	115	131	151	167	187
24	44	60	80	96	116	132	152	168	188
25	45	61	81	97	117	133	153	169	189
26	46	62	82	98	118	134	154	170	190
27	47	63	83	99	119	135	155	171	191
28	48	64	84	100	120	136	156	172	192
29	49	65	85	101	121	137	157	173	193
30	50	66	86	102	122	138	158	174	194
31	51	67	87	103	123	139	159	175	195
32	52	68	88	104	124	140	160	176	196
33	53	69	89	105	125	141	161	177	197
34	54	70	90	106	126	142	162	178	198
35	55	71	91	107	127	143	163	179	199
36	56	72	92	108	128	144	164	180	200

Figure 5b

nucleotide positions of 21mers of sca1-like genes									
e.g. SEQ ID NO: 1, 10 or 11									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
181	201	217	237	253	273	289	309	325	345
182	202	218	238	254	274	290	310	326	346
183	203	219	239	255	275	291	311	327	347
184	204	220	240	256	276	292	312	328	348
185	205	221	241	257	277	293	313	329	349
186	206	222	242	258	278	294	314	330	350
187	207	223	243	259	279	295	315	331	351
188	208	224	244	260	280	296	316	332	352
189	209	225	245	261	281	297	317	333	353
190	210	226	246	262	282	298	318	334	354
191	211	227	247	263	283	299	319	335	355
192	212	228	248	264	284	300	320	336	356
193	213	229	249	265	285	301	321	337	357
194	214	230	250	266	286	302	322	338	358
195	215	231	251	267	287	303	323	339	359
196	216	232	252	268	288	304	324	340	360
197	217	233	253	269	289	305	325	341	361
198	218	234	254	270	290	306	326	342	362
199	219	235	255	271	291	307	327	343	363
200	220	236	256	272	292	308	328	344	364
201	221	237	257	273	293	309	329	345	365
202	222	238	258	274	294	310	330	346	366
203	223	239	259	275	295	311	331	347	367
204	224	240	260	276	296	312	332	348	368
205	225	241	261	277	297	313	333	349	369
206	226	242	262	278	298	314	334	350	370
207	227	243	263	279	299	315	335	351	371
208	228	244	264	280	300	316	336	352	372
209	229	245	265	281	301	317	337	353	373
210	230	246	266	282	302	318	338	354	374
211	231	247	267	283	303	319	339	355	375
212	232	248	268	284	304	320	340	356	376
213	233	249	269	285	305	321	341	357	377
214	234	250	270	286	306	322	342	358	378
215	235	251	271	287	307	323	343	359	379
216	236	252	272	288	308	324	344	360	380

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Figure 5c

nucleotide positions of 21mers of sca1-like genes									
e.g. SEQ ID NO: 1, 10 or 11									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
361	381	397	417	433	453	469	489	505	525
362	382	398	418	434	454	470	490	506	526
363	383	399	419	435	455	471	491	507	527
364	384	400	420	436	456	472	492	508	528
365	385	401	421	437	457	473	493	509	529
366	386	402	422	438	458	474	494	510	530
367	387	403	423	439	459	475	495	511	531
368	388	404	424	440	460	476	496	512	532
369	389	405	425	441	461	477	497	513	533
370	390	406	426	442	462	478	498	514	534
371	391	407	427	443	463	479	499	515	535
372	392	408	428	444	464	480	500	516	536
373	393	409	429	445	465	481	501	517	537
374	394	410	430	446	466	482	502	518	538
375	395	411	431	447	467	483	503	519	539
376	396	412	432	448	468	484	504	520	540
377	397	413	433	449	469	485	505	521	541
378	398	414	434	450	470	486	506	522	542
379	399	415	435	451	471	487	507	523	543
380	400	416	436	452	472	488	508	524	544
381	401	417	437	453	473	489	509	525	545
382	402	418	438	454	474	490	510	526	546
383	403	419	439	455	475	491	511	527	547
384	404	420	440	456	476	492	512	528	548
385	405	421	441	457	477	493	513	529	549
386	406	422	442	458	478	494	514	530	550
387	407	423	443	459	479	495	515	531	551
388	408	424	444	460	480	496	516	532	552
389	409	425	445	461	481	497	517	533	553
390	410	426	446	462	482	498	518	534	554
391	411	427	447	463	483	499	519	535	555
392	412	428	448	464	484	500	520	536	556
393	413	429	449	465	485	501	521	537	557
394	414	430	450	466	486	502	522	538	558
395	415	431	451	467	487	503	523	539	559
396	416	432	452	468	488	504	524	540	560

Figure 5d

nucleotide positions of 21mers of sca1-like genes									
e.g. SEQ ID NO: 1, 10 or 11									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
541	561	577	597	613	633	649	669	685	705
542	562	578	598	614	634	650	670	686	706
543	563	579	599	615	635	651	671	687	707
544	564	580	600	616	636	652	672	688	708
545	565	581	601	617	637	653	673	689	709
546	566	582	602	618	638	654	674	690	710
547	567	583	603	619	639	655	675	691	711
548	568	584	604	620	640	656	676	692	712
549	569	585	605	621	641	657	677	693	713
550	570	586	606	622	642	658	678	694	714
551	571	587	607	623	643	659	679	695	715
552	572	588	608	624	644	660	680	696	716
553	573	589	609	625	645	661	681	697	717
554	574	590	610	626	646	662	682	698	718
555	575	591	611	627	647	663	683	699	719
556	576	592	612	628	648	664	684	700	720
557	577	593	613	629	649	665	685	701	721
558	578	594	614	630	650	666	686	702	722
559	579	595	615	631	651	667	687	703	723
560	580	596	616	632	652	668	688	704	724
561	581	597	617	633	653	669	689	705	725
562	582	598	618	634	654	670	690	706	726
563	583	599	619	635	655	671	691	707	727
564	584	600	620	636	656	672	692	708	728
565	585	601	621	637	657	673	693	709	729
566	586	602	622	638	658	674	694	710	730
567	587	603	623	639	659	675	695	711	731
568	588	604	624	640	660	676	696	712	732
569	589	605	625	641	661	677	697	713	733
570	590	606	626	642	662	678	698	714	734
571	591	607	627	643	663	679	699	715	735
572	592	608	628	644	664	680	700	716	736
573	593	609	629	645	665	681	701	717	737
574	594	610	630	646	666	682	702	718	738
575	595	611	631	647	667	683	703	719	739
576	596	612	632	648	668	684	704	720	740

Figure 5e

nucleotide positions of 21mers of sca1-like genes									
e.g. SEQ ID NO: 1, 10 or 11									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
721	741	757	777	793	813	829	849	865	885
722	742	758	778	794	814	830	850	866	886
723	743	759	779	795	815	831	851	867	887
724	744	760	780	796	816	832	852	868	888
725	745	761	781	797	817	833	853	869	889
726	746	762	782	798	818	834	854	870	890
727	747	763	783	799	819	835	855	871	891
728	748	764	784	800	820	836	856	872	892
729	749	765	785	801	821	837	857	873	893
730	750	766	786	802	822	838	858	874	894
731	751	767	787	803	823	839	859	875	895
732	752	768	788	804	824	840	860	876	896
733	753	769	789	805	825	841	861	877	897
734	754	770	790	806	826	842	862	878	898
735	755	771	791	807	827	843	863	879	899
736	756	772	792	808	828	844	864	880	900
737	757	773	793	809	829	845	865	881	901
738	758	774	794	810	830	846	866	882	902
739	759	775	795	811	831	847	867	883	903
740	760	776	796	812	832	848	868	884	904
741	761	777	797	813	833	849	869	885	905
742	762	778	798	814	834	850	870	886	906
743	763	779	799	815	835	851	871	887	907
744	764	780	800	816	836	852	872	888	908
745	765	781	801	817	837	853	873	889	909
746	766	782	802	818	838	854	874	890	910
747	767	783	803	819	839	855	875	891	911
748	768	784	804	820	840	856	876	892	912
749	769	785	805	821	841	857	877	893	913
750	770	786	806	822	842	858	878	894	914
751	771	787	807	823	843	859	879	895	915
752	772	788	808	824	844	860	880	896	916
753	773	789	809	825	845	861	881	897	917
754	774	790	810	826	846	862	882	898	918
755	775	791	811	827	847	863	883	899	919
756	776	792	812	828	848	864	884	900	920

Figure 5f

nucleotide positions of 21mers of sca1-like genes									
e.g. SEQ ID NO: 1, 10 or 11									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
901	922	937	958	973	994	1009	1030	1045	1066
902	923	938	959	974	995	1010	1031	1046	1067
903	924	939	960	975	996	1011	1032	1047	1068
904	925	940	961	976	997	1012	1033	1048	1069
905	926	941	962	977	998	1013	1034	1049	1070
906	927	942	963	978	999	1014	1035	1050	1071
907	928	943	964	979	1000	1015	1036	1051	1072
908	929	944	965	980	1001	1016	1037	1052	1073
909	930	945	966	981	1002	1017	1038	1053	1074
910	931	946	967	982	1003	1018	1039	1054	1075
911	932	947	968	983	1004	1019	1040	1055	1076
912	933	948	969	984	1005	1020	1041	1056	1077
913	934	949	970	985	1006	1021	1042	1057	1078
914	935	950	971	986	1007	1022	1043	1058	1079
915	936	951	972	987	1008	1023	1044	1059	1080
916	937	952	973	988	1009	1024	1045	1060	1081
917	938	953	974	989	1010	1025	1046	1061	1082
918	939	954	975	990	1011	1026	1047	1062	1083
919	940	955	976	991	1012	1027	1048	1063	1084
920	941	956	977	992	1013	1028	1049	1064	1085
921	942	957	978	993	1014	1029	1050	1065	1086
922	943	958	979	994	1015	1030	1051	1066	1087
923	944	959	980	995	1016	1031	1052	1067	1088
924	945	960	981	996	1017	1032	1053	1068	1089
925	946	961	982	997	1018	1033	1054	1069	1090
926	947	962	983	998	1019	1034	1055	1070	1091
927	948	963	984	999	1020	1035	1056	1071	1092
928	949	964	985	1000	1021	1036	1057	1072	1093
929	950	965	986	1001	1022	1037	1058	1073	1094
930	951	966	987	1002	1023	1038	1059	1074	1095
931	952	967	988	1003	1024	1039	1060	1075	1096
932	953	968	989	1004	1025	1040	1061	1076	1097
933	954	969	990	1005	1026	1041	1062	1077	1098
934	955	970	991	1006	1027	1042	1063	1078	1099
935	956	971	992	1007	1028	1043	1064	1079	1100
936	957	972	993	1008	1029	1044	1065	1080	1101

FIGURE 5g

nucleotide positions of 21mers of sca1-like genes									
e.g. SEQ ID NO: 1, 10 or 11									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
1081	1102	1117	1138	1153	1174	1189	1210	1225	1246
1082	1103	1118	1139	1154	1175	1190	1211	1226	1247
1083	1104	1119	1140	1155	1176	1191	1212	1227	1248
1084	1105	1120	1141	1156	1177	1192	1213	1228	1249
1085	1106	1121	1142	1157	1178	1193	1214	1229	1250
1086	1107	1122	1143	1158	1179	1194	1215	1230	1251
1087	1108	1123	1144	1159	1180	1195	1216	1231	1252
1088	1109	1124	1145	1160	1181	1196	1217	1232	1253
1089	1110	1125	1146	1161	1182	1197	1218	1233	1254
1090	1111	1126	1147	1162	1183	1198	1219	1234	1255
1091	1112	1127	1148	1163	1184	1199	1220	1235	1256
1092	1113	1128	1149	1164	1185	1200	1221	1236	1257
1093	1114	1129	1150	1165	1186	1201	1222	1237	1258
1094	1115	1130	1151	1166	1187	1202	1223	1238	1259
1095	1116	1131	1152	1167	1188	1203	1224	1239	1260
1096	1117	1132	1153	1168	1189	1204	1225	1240	1261
1097	1118	1133	1154	1169	1190	1205	1226	1241	1262
1098	1119	1134	1155	1170	1191	1206	1227	1242	1263
1099	1120	1135	1156	1171	1192	1207	1228	1243	1264
1100	1121	1136	1157	1172	1193	1208	1229	1244	1265
1101	1122	1137	1158	1173	1194	1209	1230	1245	1266
1102	1123	1138	1159	1174	1195	1210	1231	1246	1267
1103	1124	1139	1160	1175	1196	1211	1232	1247	1268
1104	1125	1140	1161	1176	1197	1212	1233	1248	1269
1105	1126	1141	1162	1177	1198	1213	1234	1249	1270
1106	1127	1142	1163	1178	1199	1214	1235	1250	1271
1107	1128	1143	1164	1179	1200	1215	1236	1251	1272
1108	1129	1144	1165	1180	1201	1216	1237	1252	1273
1109	1130	1145	1166	1181	1202	1217	1238	1253	1274
1110	1131	1146	1167	1182	1203	1218	1239	1254	1275
1111	1132	1147	1168	1183	1204	1219	1240	1255	1276
1112	1133	1148	1169	1184	1205	1220	1241	1256	1277
1113	1134	1149	1170	1185	1206	1221	1242	1257	1278
1114	1135	1150	1171	1186	1207	1222	1243	1258	1279
1115	1136	1151	1172	1187	1208	1223	1244	1259	1280
1116	1137	1152	1173	1188	1209	1224	1245	1260	1281

FIGURE 5h

nucleotide positions of 21mers of sca1-like genes									
e.g. SEQ ID NO: 1, 10 or 11									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
1261	1282	1297	1318	1333	1354	1369	1390	1405	1426
1262	1283	1298	1319	1334	1355	1370	1391	1406	1427
1263	1284	1299	1320	1335	1356	1371	1392	1407	1428
1264	1285	1300	1321	1336	1357	1372	1393	1408	1429
1265	1286	1301	1322	1337	1358	1373	1394	1409	1430
1266	1287	1302	1323	1338	1359	1374	1395	1410	1431
1267	1288	1303	1324	1339	1360	1375	1396	1411	1432
1268	1289	1304	1325	1340	1361	1376	1397	1412	1433
1269	1290	1305	1326	1341	1362	1377	1398	1413	1434
1270	1291	1306	1327	1342	1363	1378	1399	1414	1435
1271	1292	1307	1328	1343	1364	1379	1400	1415	1436
1272	1293	1308	1329	1344	1365	1380	1401	1416	1437
1273	1294	1309	1330	1345	1366	1381	1402	1417	1438
1274	1295	1310	1331	1346	1367	1382	1403	1418	1439
1275	1296	1311	1332	1347	1368	1383	1404	1419	1440
1276	1297	1312	1333	1348	1369	1384	1405	1420	1441
1277	1298	1313	1334	1349	1370	1385	1406	1421	1442
1278	1299	1314	1335	1350	1371	1386	1407	1422	1443
1279	1300	1315	1336	1351	1372	1387	1408	1423	1444
1280	1301	1316	1337	1352	1373	1388	1409	1424	1445
1281	1302	1317	1338	1353	1374	1389	1410	1425	1446
1282	1303	1318	1339	1354	1375	1390	1411	1426	1447
1283	1304	1319	1340	1355	1376	1391	1412	1427	1448
1284	1305	1320	1341	1356	1377	1392	1413	1428	1449
1285	1306	1321	1342	1357	1378	1393	1414	1429	1450
1286	1307	1322	1343	1358	1379	1394	1415	1430	1451
1287	1308	1323	1344	1359	1380	1395	1416	1431	1452
1288	1309	1324	1345	1360	1381	1396	1417	1432	1453
1289	1310	1325	1346	1361	1382	1397	1418	1433	1454
1290	1311	1326	1347	1362	1383	1398	1419	1434	1455
1291	1312	1327	1348	1363	1384	1399	1420	1435	1456
1292	1313	1328	1349	1364	1385	1400	1421	1436	1457
1293	1314	1329	1350	1365	1386	1401	1422	1437	1458
1294	1315	1330	1351	1366	1387	1402	1423	1438	1459
1295	1316	1331	1352	1367	1388	1403	1424	1439	1460
1296	1317	1332	1353	1368	1389	1404	1425	1440	1461

FIGURE 5i

nucleotide positions of 21mers of sca1-like genes									
e.g. SEQ ID NO: 1, 10 or 11									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
1441	1462	1477	1498	1513	1534	1549	1570	1585	1606
1442	1463	1478	1499	1514	1535	1550	1571	1586	1607
1443	1464	1479	1500	1515	1536	1551	1572	1587	1608
1444	1465	1480	1501	1516	1537	1552	1573	1588	1609
1445	1466	1481	1502	1517	1538	1553	1574	1589	1610
1446	1467	1482	1503	1518	1539	1554	1575	1590	1611
1447	1468	1483	1504	1519	1540	1555	1576	1591	1612
1448	1469	1484	1505	1520	1541	1556	1577	1592	1613
1449	1470	1485	1506	1521	1542	1557	1578	1593	1614
1450	1471	1486	1507	1522	1543	1558	1579	1594	1615
1451	1472	1487	1508	1523	1544	1559	1580	1595	1616
1452	1473	1488	1509	1524	1545	1560	1581	1596	1617
1453	1474	1489	1510	1525	1546	1561	1582	1597	1618
1454	1475	1490	1511	1526	1547	1562	1583	1598	1619
1455	1476	1491	1512	1527	1548	1563	1584	1599	1620
1456	1477	1492	1513	1528	1549	1564	1585	1600	1621
1457	1478	1493	1514	1529	1550	1565	1586	1601	1622
1458	1479	1494	1515	1530	1551	1566	1587	1602	1623
1459	1480	1495	1516	1531	1552	1567	1588	1603	1624
1460	1481	1496	1517	1532	1553	1568	1589	1604	1625
1461	1482	1497	1518	1533	1554	1569	1590	1605	1626
1462	1483	1498	1519	1534	1555	1570	1591	1606	1627
1463	1484	1499	1520	1535	1556	1571	1592	1607	1628
1464	1485	1500	1521	1536	1557	1572	1593	1608	1629
1465	1486	1501	1522	1537	1558	1573	1594	1609	1630
1466	1487	1502	1523	1538	1559	1574	1595	1610	1631
1467	1488	1503	1524	1539	1560	1575	1596	1611	1632
1468	1489	1504	1525	1540	1561	1576	1597	1612	1633
1469	1490	1505	1526	1541	1562	1577	1598	1613	1634
1470	1491	1506	1527	1542	1563	1578	1599	1614	1635
1471	1492	1507	1528	1543	1564	1579	1600	1615	1636
1472	1493	1508	1529	1544	1565	1580	1601	1616	1637
1473	1494	1509	1530	1545	1566	1581	1602	1617	1638
1474	1495	1510	1531	1546	1567	1582	1603	1618	1639
1475	1496	1511	1532	1547	1568	1583	1604	1619	1640
1476	1497	1512	1533	1548	1569	1584	1605	1620	1641

FIGURE 5J

nucleotide positions of 21mers of sca1-like genes									
e.g. SEQ ID NO: 1, 10 or 11									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
1621	1642	1656	1677	1692	1713	1728	1749	1764	1785
1622	1643	1657	1678	1693	1714	1729	1750	1765	1786
1623	1644	1658	1679	1694	1715	1730	1751	1766	1787
1624	1645	1659	1680	1695	1716	1731	1752	1767	1788
1625	1646	1660	1681	1696	1717	1732	1753	1768	1789
1626	1647	1661	1682	1697	1718	1733	1754	1769	1790
1627	1648	1662	1683	1698	1719	1734	1755	1770	1791
1628	1649	1663	1684	1699	1720	1735	1756	1771	1792
1629	1650	1664	1685	1700	1721	1736	1757	1772	1793
1630	1651	1665	1686	1701	1722	1737	1758	1773	1794
1631	1652	1666	1687	1702	1723	1738	1759	1774	1795
1632	1653	1667	1688	1703	1724	1739	1760	1775	1796
1633	1654	1668	1689	1704	1725	1740	1761	1776	1797
1634	1655	1669	1690	1705	1726	1741	1762	1777	1798
1635	1656	1670	1691	1706	1727	1742	1763	1778	1799
1636	1657	1671	1692	1707	1728	1743	1764	1779	1800
1637	1658	1672	1693	1708	1729	1744	1765	1780	1801
1638	1659	1673	1694	1709	1730	1745	1766	1781	1802
1639	1660	1674	1695	1710	1731	1746	1767	1782	1803
1640	1661	1675	1696	1711	1732	1747	1768	1783	1804
1641	1662	1676	1697	1712	1733	1748	1769	1784	1805
1642	1663	1677	1698	1713	1734	1749	1770	1785	1806
1643	1664	1678	1699	1714	1735	1750	1771	1786	1807
1644	1665	1679	1700	1715	1736	1751	1772	1787	1808
1645	1666	1680	1701	1716	1737	1752	1773	1788	1809
1646	1667	1681	1702	1717	1738	1753	1774	1789	1810
1647	1668	1682	1703	1718	1739	1754	1775	1790	1811
1648	1669	1683	1704	1719	1740	1755	1776	1791	1812
1649	1670	1684	1705	1720	1741	1756	1777	1792	1813
1650	1671	1685	1706	1721	1742	1757	1778	1793	1814
1651	1672	1686	1707	1722	1743	1758	1779	1794	1815
1652	1673	1687	1708	1723	1744	1759	1780	1795	1816
1653	1674	1688	1709	1724	1745	1760	1781	1796	1817
1654	1675	1689	1710	1725	1746	1761	1782	1797	1818
1655	1676	1690	1711	1726	1747	1762	1783	1798	1819
1656	1677	1691	1712	1727	1748	1763	1784	1799	1820

FIGURE 5k

nucleotide positions of 21mers of sca1-like genes									
e.g. SEQ ID NO: 1, 10 or 11									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
1800	1821	1836	1857	1872	1893	1908	1929	1944	1965
1801	1822	1837	1858	1873	1894	1909	1930	1945	1966
1802	1823	1838	1859	1874	1895	1910	1931	1946	1967
1803	1824	1839	1860	1875	1896	1911	1932	1947	1968
1804	1825	1840	1861	1876	1897	1912	1933	1948	1969
1805	1826	1841	1862	1877	1898	1913	1934	1949	1970
1806	1827	1842	1863	1878	1899	1914	1935	1950	1971
1807	1828	1843	1864	1879	1900	1915	1936	1951	1972
1808	1829	1844	1865	1880	1901	1916	1937	1952	1973
1809	1830	1845	1866	1881	1902	1917	1938	1953	1974
1810	1831	1846	1867	1882	1903	1918	1939	1954	1975
1811	1832	1847	1868	1883	1904	1919	1940	1955	1976
1812	1833	1848	1869	1884	1905	1920	1941	1956	1977
1813	1834	1849	1870	1885	1906	1921	1942	1957	1978
1814	1835	1850	1871	1886	1907	1922	1943	1958	1979
1815	1836	1851	1872	1887	1908	1923	1944	1959	1980
1816	1837	1852	1873	1888	1909	1924	1945	1960	1981
1817	1838	1853	1874	1889	1910	1925	1946	1961	1982
1818	1839	1854	1875	1890	1911	1926	1947	1962	1983
1819	1840	1855	1876	1891	1912	1927	1948	1963	1984
1820	1841	1856	1877	1892	1913	1928	1949	1964	1985
1821	1842	1857	1878	1893	1914	1929	1950	1965	1986
1822	1843	1858	1879	1894	1915	1930	1951	1966	1987
1823	1844	1859	1880	1895	1916	1931	1952	1967	1988
1824	1845	1860	1881	1896	1917	1932	1953	1968	1989
1825	1846	1861	1882	1897	1918	1933	1954	1969	1990
1826	1847	1862	1883	1898	1919	1934	1955	1970	1991
1827	1848	1863	1884	1899	1920	1935	1956	1971	1992
1828	1849	1864	1885	1900	1921	1936	1957	1972	1993
1829	1850	1865	1886	1901	1922	1937	1958	1973	1994
1830	1851	1866	1887	1902	1923	1938	1959	1974	1995
1831	1852	1867	1888	1903	1924	1939	1960	1975	1996
1832	1853	1868	1889	1904	1925	1940	1961	1976	1997
1833	1854	1869	1890	1905	1926	1941	1962	1977	1998
1834	1855	1870	1891	1906	1927	1942	1963	1978	1999
1835	1856	1871	1892	1907	1928	1943	1964	1979	2000

FIGURE 5I

nucleotide positions of 21mers of sca1-like genes									
e.g. SEQ ID NO: 1, 10 or 11									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
1980	2001	2016	2037	2052	2073	2088	2109	2124	2145
1981	2002	2017	2038	2053	2074	2089	2110	2125	2146
1982	2003	2018	2039	2054	2075	2090	2111	2126	2147
1983	2004	2019	2040	2055	2076	2091	2112	2127	2148
1984	2005	2020	2041	2056	2077	2092	2113	2128	2149
1985	2006	2021	2042	2057	2078	2093	2114	2129	2150
1986	2007	2022	2043	2058	2079	2094	2115	2130	2151
1987	2008	2023	2044	2059	2080	2095	2116	2131	2152
1988	2009	2024	2045	2060	2081	2096	2117	2132	2153
1989	2010	2025	2046	2061	2082	2097	2118	2133	2154
1990	2011	2026	2047	2062	2083	2098	2119	2134	2155
1991	2012	2027	2048	2063	2084	2099	2120	2135	2156
1992	2013	2028	2049	2064	2085	2100	2121	2136	2157
1993	2014	2029	2050	2065	2086	2101	2122	2137	2158
1994	2015	2030	2051	2066	2087	2102	2123	2138	2159
1995	2016	2031	2052	2067	2088	2103	2124	2139	2160
1996	2017	2032	2053	2068	2089	2104	2125	2140	2161
1997	2018	2033	2054	2069	2090	2105	2126	2141	2162
1998	2019	2034	2055	2070	2091	2106	2127	2142	2163
1999	2020	2035	2056	2071	2092	2107	2128	2143	2164
2000	2021	2036	2057	2072	2093	2108	2129	2144	2165
2001	2022	2037	2058	2073	2094	2109	2130	2145	2166
2002	2023	2038	2059	2074	2095	2110	2131	2146	2167
2003	2024	2039	2060	2075	2096	2111	2132	2147	2168
2004	2025	2040	2061	2076	2097	2112	2133	2148	2169
2005	2026	2041	2062	2077	2098	2113	2134	2149	2170
2006	2027	2042	2063	2078	2099	2114	2135	2150	2171
2007	2028	2043	2064	2079	2100	2115	2136	2151	2172
2008	2029	2044	2065	2080	2101	2116	2137	2152	2173
2009	2030	2045	2066	2081	2102	2117	2138	2153	2174
2010	2031	2046	2067	2082	2103	2118	2139	2154	2175
2011	2032	2047	2068	2083	2104	2119	2140	2155	2176
2012	2033	2048	2069	2084	2105	2120	2141	2156	2177
2013	2034	2049	2070	2085	2106	2121	2142	2157	2178
2014	2035	2050	2071	2086	2107	2122	2143	2158	2179
2015	2036	2051	2072	2087	2108	2123	2144	2159	2180

FIGURE 5m

nucleotide positions of 21mers of sca1-like genes									
e.g. SEQ ID NO: 1, 10 or 11									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
2160	2181	2196	2217	2232	2253	2268	2289	2304	2325
2161	2182	2197	2218	2233	2254	2269	2290	2305	2326
2162	2183	2198	2219	2234	2255	2270	2291	2306	2327
2163	2184	2199	2220	2235	2256	2271	2292	2307	2328
2164	2185	2200	2221	2236	2257	2272	2293	2308	2329
2165	2186	2201	2222	2237	2258	2273	2294	2309	2330
2166	2187	2202	2223	2238	2259	2274	2295	2310	2331
2167	2188	2203	2224	2239	2260	2275	2296	2311	2332
2168	2189	2204	2225	2240	2261	2276	2297	2312	2333
2169	2190	2205	2226	2241	2262	2277	2298	2313	2334
2170	2191	2206	2227	2242	2263	2278	2299	2314	2335
2171	2192	2207	2228	2243	2264	2279	2300	2315	2336
2172	2193	2208	2229	2244	2265	2280	2301	2316	2337
2173	2194	2209	2230	2245	2266	2281	2302	2317	2338
2174	2195	2210	2231	2246	2267	2282	2303	2318	2339
2175	2196	2211	2232	2247	2268	2283	2304	2319	2340
2176	2197	2212	2233	2248	2269	2284	2305	2320	2341
2177	2198	2213	2234	2249	2270	2285	2306	2321	2342
2178	2199	2214	2235	2250	2271	2286	2307	2322	2343
2179	2200	2215	2236	2251	2272	2287	2308	2323	2344
2180	2201	2216	2237	2252	2273	2288	2309	2324	2345
2181	2202	2217	2238	2253	2274	2289	2310	2325	2346
2182	2203	2218	2239	2254	2275	2290	2311	2326	2347
2183	2204	2219	2240	2255	2276	2291	2312	2327	2348
2184	2205	2220	2241	2256	2277	2292	2313	2328	2349
2185	2206	2221	2242	2257	2278	2293	2314	2329	2350
2186	2207	2222	2243	2258	2279	2294	2315	2330	2351
2187	2208	2223	2244	2259	2280	2295	2316	2331	2352
2188	2209	2224	2245	2260	2281	2296	2317	2332	2353
2189	2210	2225	2246	2261	2282	2297	2318	2333	2354
2190	2211	2226	2247	2262	2283	2298	2319	2334	2355
2191	2212	2227	2248	2263	2284	2299	2320	2335	2356
2192	2213	2228	2249	2264	2285	2300	2321	2336	2357
2193	2214	2229	2250	2265	2286	2301	2322	2337	2358
2194	2215	2230	2251	2266	2287	2302	2323	2338	2359
2195	2216	2231	2252	2267	2288	2303	2324	2339	2360

FIGURE 5n

nucleotide positions of 21mers of sca1-like genes									
e.g. SEQ ID NO: 1, 10 or 11									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
2340	2361	2376	2397	2412	2433	2448	2469	2484	2505
2341	2362	2377	2398	2413	2434	2449	2470	2485	2506
2342	2363	2378	2399	2414	2435	2450	2471	2486	2507
2343	2364	2379	2400	2415	2436	2451	2472	2487	2508
2344	2365	2380	2401	2416	2437	2452	2473	2488	2509
2345	2366	2381	2402	2417	2438	2453	2474	2489	2510
2346	2367	2382	2403	2418	2439	2454	2475	2490	2511
2347	2368	2383	2404	2419	2440	2455	2476	2491	2512
2348	2369	2384	2405	2420	2441	2456	2477	2492	2513
2349	2370	2385	2406	2421	2442	2457	2478	2493	2514
2350	2371	2386	2407	2422	2443	2458	2479	2494	2515
2351	2372	2387	2408	2423	2444	2459	2480	2495	2516
2352	2373	2388	2409	2424	2445	2460	2481	2496	2517
2353	2374	2389	2410	2425	2446	2461	2482	2497	2518
2354	2375	2390	2411	2426	2447	2462	2483	2498	2519
2355	2376	2391	2412	2427	2448	2463	2484	2499	2520
2356	2377	2392	2413	2428	2449	2464	2485	2500	2521
2357	2378	2393	2414	2429	2450	2465	2486	2501	2522
2358	2379	2394	2415	2430	2451	2466	2487	2502	2523
2359	2380	2395	2416	2431	2452	2467	2488	2503	2524
2360	2381	2396	2417	2432	2453	2468	2489	2504	2525
2361	2382	2397	2418	2433	2454	2469	2490	2505	2526
2362	2383	2398	2419	2434	2455	2470	2491	2506	2527
2363	2384	2399	2420	2435	2456	2471	2492	2507	2528
2364	2385	2400	2421	2436	2457	2472	2493	2508	2529
2365	2386	2401	2422	2437	2458	2473	2494	2509	2530
2366	2387	2402	2423	2438	2459	2474	2495	2510	2531
2367	2388	2403	2424	2439	2460	2475	2496	2511	2532
2368	2389	2404	2425	2440	2461	2476	2497	2512	2533
2369	2390	2405	2426	2441	2462	2477	2498	2513	2534
2370	2391	2406	2427	2442	2463	2478	2499	2514	2535
2371	2392	2407	2428	2443	2464	2479	2500	2515	2536
2372	2393	2408	2429	2444	2465	2480	2501	2516	2537
2373	2394	2409	2430	2445	2466	2481	2502	2517	2538
2374	2395	2410	2431	2446	2467	2482	2503	2518	2539
2375	2396	2411	2432	2447	2468	2483	2504	2519	2540

FIGURE 5o

nucleotide positions of 21mers of sca1-like genes									
e.g. SEQ ID NO: 1, 10 or 11									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
2520	2541	2556	2577	2592	2613	2628	2649	2664	2685
2521	2542	2557	2578	2593	2614	2629	2650	2665	2686
2522	2543	2558	2579	2594	2615	2630	2651	2666	2687
2523	2544	2559	2580	2595	2616	2631	2652	2667	2688
2524	2545	2560	2581	2596	2617	2632	2653	2668	2689
2525	2546	2561	2582	2597	2618	2633	2654	2669	2690
2526	2547	2562	2583	2598	2619	2634	2655	2670	2691
2527	2548	2563	2584	2599	2620	2635	2656	2671	2692
2528	2549	2564	2585	2600	2621	2636	2657	2672	2693
2529	2550	2565	2586	2601	2622	2637	2658	2673	2694
2530	2551	2566	2587	2602	2623	2638	2659	2674	2695
2531	2552	2567	2588	2603	2624	2639	2660	2675	2696
2532	2553	2568	2589	2604	2625	2640	2661	2676	2697
2533	2554	2569	2590	2605	2626	2641	2662	2677	2698
2534	2555	2570	2591	2606	2627	2642	2663	2678	2699
2535	2556	2571	2592	2607	2628	2643	2664	2679	2700
2536	2557	2572	2593	2608	2629	2644	2665	2680	2701
2537	2558	2573	2594	2609	2630	2645	2666	2681	2702
2538	2559	2574	2595	2610	2631	2646	2667	2682	2703
2539	2560	2575	2596	2611	2632	2647	2668	2683	2704
2540	2561	2576	2597	2612	2633	2648	2669	2684	2705
2541	2562	2577	2598	2613	2634	2649	2670	2685	2706
2542	2563	2578	2599	2614	2635	2650	2671	2686	2707
2543	2564	2579	2600	2615	2636	2651	2672	2687	2708
2544	2565	2580	2601	2616	2637	2652	2673	2688	2709
2545	2566	2581	2602	2617	2638	2653	2674	2689	2710
2546	2567	2582	2603	2618	2639	2654	2675	2690	2711
2547	2568	2583	2604	2619	2640	2655	2676	2691	2712
2548	2569	2584	2605	2620	2641	2656	2677	2692	2713
2549	2570	2585	2606	2621	2642	2657	2678	2693	2714
2550	2571	2586	2607	2622	2643	2658	2679	2694	2715
2551	2572	2587	2608	2623	2644	2659	2680	2695	2716
2552	2573	2588	2609	2624	2645	2660	2681	2696	2717
2553	2574	2589	2610	2625	2646	2661	2682	2697	2718
2554	2575	2590	2611	2626	2647	2662	2683	2698	2719
2555	2576	2591	2612	2627	2648	2663	2684	2699	2720

FIGURE 5p

nucleotide positions of 21mers of sca1-like genes									
e.g. SEQ ID NO: 1, 10 or 11									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
2700	2721	2736	2757	2772	2793	2808	2829	2844	2865
2701	2722	2737	2758	2773	2794	2809	2830	2845	2866
2702	2723	2738	2759	2774	2795	2810	2831	2846	2867
2703	2724	2739	2760	2775	2796	2811	2832	2847	2868
2704	2725	2740	2761	2776	2797	2812	2833	2848	2869
2705	2726	2741	2762	2777	2798	2813	2834	2849	2870
2706	2727	2742	2763	2778	2799	2814	2835	2850	2871
2707	2728	2743	2764	2779	2800	2815	2836	2851	2872
2708	2729	2744	2765	2780	2801	2816	2837	2852	2873
2709	2730	2745	2766	2781	2802	2817	2838	2853	2874
2710	2731	2746	2767	2782	2803	2818	2839	2854	2875
2711	2732	2747	2768	2783	2804	2819	2840	2855	2876
2712	2733	2748	2769	2784	2805	2820	2841	2856	2877
2713	2734	2749	2770	2785	2806	2821	2842	2857	2878
2714	2735	2750	2771	2786	2807	2822	2843	2858	2879
2715	2736	2751	2772	2787	2808	2823	2844	2859	2880
2716	2737	2752	2773	2788	2809	2824	2845	2860	2881
2717	2738	2753	2774	2789	2810	2825	2846	2861	2882
2718	2739	2754	2775	2790	2811	2826	2847	2862	2883
2719	2740	2755	2776	2791	2812	2827	2848	2863	2884
2720	2741	2756	2777	2792	2813	2828	2849	2864	2885
2721	2742	2757	2778	2793	2814	2829	2850	2865	2886
2722	2743	2758	2779	2794	2815	2830	2851	2866	2887
2723	2744	2759	2780	2795	2816	2831	2852	2867	2888
2724	2745	2760	2781	2796	2817	2832	2853	2868	2889
2725	2746	2761	2782	2797	2818	2833	2854	2869	2890
2726	2747	2762	2783	2798	2819	2834	2855	2870	2891
2727	2748	2763	2784	2799	2820	2835	2856	2871	2892
2728	2749	2764	2785	2800	2821	2836	2857	2872	2893
2729	2750	2765	2786	2801	2822	2837	2858	2873	2894
2730	2751	2766	2787	2802	2823	2838	2859	2874	2895
2731	2752	2767	2788	2803	2824	2839	2860	2875	2896
2732	2753	2768	2789	2804	2825	2840	2861	2876	2897
2733	2754	2769	2790	2805	2826	2841	2862	2877	2898
2734	2755	2770	2791	2806	2827	2842	2863	2878	2899
2735	2756	2771	2792	2807	2828	2843	2864	2879	2900

FIGURE 5q

nucleotide positions of 21mers of sca1-like genes									
e.g. SEQ ID NO: 1, 10 or 11									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
2880	2901	2916	2937	2952	2973	2988	3009		
2881	2902	2917	2938	2953	2974	2989	3010		
2882	2903	2918	2939	2954	2975	2990	3011		
2883	2904	2919	2940	2955	2976	2991	3012		
2884	2905	2920	2941	2956	2977	2992	3013		
2885	2906	2921	2942	2957	2978	2993	3014		
2886	2907	2922	2943	2958	2979	2994	3015		
2887	2908	2923	2944	2959	2980	2995	3016		
2888	2909	2924	2945	2960	2981	2996	3017		
2889	2910	2925	2946	2961	2982	2997	3018		
2890	2911	2926	2947	2962	2983	2998	3019		
2891	2912	2927	2948	2963	2984	2999	3020		
2892	2913	2928	2949	2964	2985	3000	3021		
2893	2914	2929	2950	2965	2986	3001	3022		
2894	2915	2930	2951	2966	2987	3002	3023		
2895	2916	2931	2952	2967	2988	3003	3024		
2896	2917	2932	2953	2968	2989	3004	3025		
2897	2918	2933	2954	2969	2990	3005	3026		
2898	2919	2934	2955	2970	2991	3006	3027		
2899	2920	2935	2956	2971	2992	3007	3028		
2900	2921	2936	2957	2972	2993	3008	3029		
2901	2922	2937	2958	2973	2994	3009	3030		
2902	2923	2938	2959	2974	2995	3010	3031		
2903	2924	2939	2960	2975	2996	3011	3032		
2904	2925	2940	2961	2976	2997	3012	3033		
2905	2926	2941	2962	2977	2998				
2906	2927	2942	2963	2978	2999				
2907	2928	2943	2964	2979	3000	.....	.....		
2908	2929	2944	2965	2980	3001	.....	.....		
2909	2930	2945	2966	2981	3002	n-5	n+15		
2910	2931	2946	2967	2982	3003	n-4	n+16		
2911	2932	2947	2968	2983	3004	n-3	n+17		
2912	2933	2948	2969	2984	3005	n-2	n+18		
2913	2934	2949	2970	2985	3006	n-1	n+19		
2914	2935	2950	2971	2986	3007	n	n+20		
2915	2936	2951	2972	2987	3008				

FIGURE 5r

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

For example:

n = 1025 (=3033-20) for SEQ ID NO:1;

n =300 (=1063-20) for SEQ ID NO:10;

n = 1991 (=499-20) for SEQ ID NO:11;

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2008/051480

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.
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) example 4 page 54, paragraph 2	1-15
A	WO 02/33405 A (DEVGEN NV [BE]; ZWAAL RICHARD [BE]; KALETTA TITUS [BE]; DEN CRAEN MARC) 25 April 2002 (2002-04-25) SEQ ID NOs: 10, 16 page 12, paragraph 4 - page 13, paragraph 2	1-15

☐ Further documents are listed in the continuation of Box C.

☒ 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
14 April 2008	12/06/2008

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Barnas, Christoph

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2008/051480

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.:

claims 1-15 (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: claims 1-15 (partially)

The subject matter of claims 1-15 as far as relating to the polynucleotides comprising a sequence as set forth in SEQ ID NOs: 1 and 11.

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2. claims: 1-15 (partially)

The subject matter of claims 1-15 as far as relating to the polynucleotides comprising a sequence as set forth in SEQ ID NO: 10.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2008/051480

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004005485 A	15-01-2004	AU 2003247951 A1	23-01-2004
		BR PI0312580 A	10-10-2006
		US 2004098761 A1	20-05-2004
WO 0233405 A	25-04-2002	AU 1845702 A	29-04-2002