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(54) Title: NEMATODE RESISTANT TRANSGENIC PLANTS

(57) Abstract: Compositions and methods for providing nematode resistance are provided. One aspect provides transgenic plants or cells comprising an inhibitory nucleic acid specific for one or more nematode esophageal polypeptides. Other aspects provide transgenic plants or cells resistant to at least two different root-knot nematode species.

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NEMATODE RESISTANT TRANSGENIC PLANTS

BACKGROUND

CROSS REFERENCE TO RELATED APPLICATION

This application claims benefit of and priority to US provisional application number 60/618,097 filed on October 13, 2004, and US provisional application number 60/704,560 filed on August 02, 2005, and where permissible each is incorporated by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

Aspects of the work disclosed herein were supported, in part, by Grant Number 2003-35302-13804 awarded by the United States Department of Agriculture. The US government may have certain rights in the claimed subject matter.

1. Technical Field

The present disclosure generally relates to compositions for controlling plant parasites and compositions for increasing root growth, more particularly to nucleic acid compositions for controlling nematode disease or increasing root growth.

2. Related Art

Nematodes are a very large group of invertebrate animals generally referred to as roundworms, threadworms, eelworms, or nema. Some nematodes are plant parasites and can feed on stems, buds, leaves, and in particular on roots. One important genus of plant parasitic nematodes is the root-knot nematode (*Meloidogyne spp.)*. These parasitic nematodes infect a wide range of important field, vegetable, fruit and ornamental plants. In 2001 the root-knot nematode was responsible for a loss of US$200.5 million in cotton alone.

Existing methods for treating or preventing root-knot nematode disease include the use of chemicals, pesticides, and fumigants. The use of pre-plant soil fumigants is highly effective in controlling root-knot and other plant-parasitic nematodes. However, the majority of the fumigant-type nematicides are no longer available and are also costly and difficult to apply properly under the prevailing conditions.

Crop rotation has also been used to control nematode disease. Rotating onion, carrot, or lettuce with a nonhost crop such as sweet corn and other grain crops, if economically possible, can be effective in controlling the northern root-knot nematode. Unfortunately, current crop rotations on organic soils are of limited value as most crops grown, including potatoes, beans, celery, lettuce, onion, and carrot are susceptible to disease.

The use of cover crops has also been attempted to control nematode disease. Cover crops grown between the main crops may provide an alternative management strategy. Ryegrain, barley, oats, sudangrass, tall fescue, annual ryegrass, and wheat have been shown to be non- or poor hosts to this nematode. Using cover crops, however, can be costly because the cover crops occupy space that could be used to grow more valuable crops.

Biological control organisms have also been used to try to control nematode disease in crops. Commercially available preparations of biological control organisms are limited in their use to regions that can support the growth of the control organism. Moreover, the outcome of using one organism to control another is unpredictable and subject to a variety of a factors such as weather and climate.

Additionally, the root-knot nematode (RKN) is a leading cause of crop loss due to plant parasitic nematodes. The most important species (*M. incognita, M. javanica, M. arenaria, M. hapla, M. chitwoodi*) have wide host ranges that limit nonhost rotation options. While several examples of host resistance genes in diverse crops exist, the availability of host plant resistance is substantially limited with appropriate resistance loci lacking for the majority of our crops (Roberts, P. A. 1992. Journal of Nematology

24:213-227). In addition, the resistance is limited to only a few RKN species or populations and some resistance genes are heat-sensitive and thus unsuitable for hot production areas. Another limitation of natural resistance genes is the durability of resistance since resistance-breaking populations of RKN can develop after continuous exposure to resistant cultivars, e.g. root-knot resistant tomatoes.

Accordingly, there is need for compositions and methods for controlling, preventing, or reducing nematode disease in plants.

Still other problems affecting crops relate to poorly developed root systems. Root systems of plants are an important part of a plant, and provide many functions that are vital to plant survival. For example, root systems store nutrients for the plant, filter out toxins, help regulate plant growth, provide an absorptive network for water and nutrients, and provide mechanical structures that support the plant and strengthen the soil. Plants with larger roots have increased growth and increased stress tolerance. Increased or enhanced root growth in crop plants would be particularly advantageous because the increased root growth would increase crop yield.

In perennial crops, increased root growth would increase the regrowth rate, increase the yield potential, and increase the likelihood that plants will survive winter. In annuals, increased root size would ensure yield potential under varying environmental conditions. In root crops, enhanced root growth would mean larger yields.

Existing root stimulators typically include fertilizers or plant hormones that must be mixed or formulated in specific concentrations when applied to the plant or soil near the plant. Over application of such stimulators can have adverse effects on the plants, and under application will not achieve the desired outcome. Additionally, application of plant hormones can have undesired consequences. For example, one plant hormone used as a root initiator is auxin or indole-3-acetic acid (IAA). IAA plays important roles in a number of plant activities, including: development of the embryo, leaf formation, phototropism, gravitropism, apical dominance, fruit development, abscission as well as root initiation.

Thus there is a need for new compositions and methods for stimulating or enhancing root growth or development.

SUMMARY

Aspects of the present disclosure generally provide nucleic acid constructs that inhibit the expression of proteins secreted by plant parasites. In some aspects, the proteins are secreted by a nematode and, optionally, modulate: gene expression of the plant or cell, formation of a giant cell, nematode migration through root tissue of the plant, cell metabolism of the plant, elicits signal transduction in the plant cell, or forms a feeding tube that enables the nematode to feed from giant-cells formed in the plant. One aspect provides inhibitory nucleic acids specific for esophageal gland cell proteins secreted by nematodes, in particular root knot nematodes. Other aspects provide transgenic cells or plants expressing or containing one or more inhibitory nucleic acids, for example inhibitory double or single stranded RNA, that inhibit or reduce the expression of nematode esophageal gland cell proteins.

Another aspect provides a transgenic plant that comprises inhibitory RNA that down regulates a target nematode parasitism gene transcript in 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more nematode species, for example RKN species. Thus, the present disclosure provides transgenic plants that are resistant to disease caused by multiple RKN species.

Representative esophageal gland cell proteins that are targeted by the disclosed inhibitory nucleic acids include one or more of the proteins encoded by SEQ ID NOs.1, 2, and 5-51. In certain aspects, one or more inhibitory nucleic acids are delivered to a parasitic nematode when the nematode enters the transgenic plant or transgenic plant cell, feeds on the transgenic plant or transgenic plant cell, or comes into physical contact with the transgenic plant or transgenic plant cell. Once the inhibitory nucleic acid is internalized by the parasitic nematode, the inhibitory nucleic acid interferes with, reduces, or inhibits the expression of a target esophageal gland cell

protein, for example, by directly or indirectly interfering, reducing, or inhibiting the translation of one or more mRNAs coding for one or more esophageal gland cell proteins.

Yet another aspect provides a plant cell transfected with heterologous nucleic acid encoding an inhibitory nucleic acid specific for one or more nematode esophageal gland cell proteins, wherein the heterologous nucleic acid is expressed in an amount sufficient to reduce or prevent nematode disease. In one aspect, the transgenic plant expresses the inhibitory nucleic acid, and the inhibitory nucleic acid is delivered to a nematode feeding or attempting to feed on the transgenic plant. Generally, the inhibitory nucleic acid is internalized by a nematode. Exemplary methods of internalizing the inhibitory nucleic acid include ingesting the nucleic acid or absorbing the nucleic acid.

Still another aspect provides a transgenic plant comprising an inhibitory nucleic acid specific for one or more nematode parasitism polypeptides, wherein the inhibitory nucleic acid provides resistance to two or more nematode species, for example two or more root-knot nematode species.

Further aspects provide compositions for stimulating, promoting, or enhancing root growth or development in plants or trees. Certain aspects provide nucleic acid constructs encoding proteins secreted by nematode esophageal gland cells, wherein the proteins or fragments thereof stimulate or enhance root development when delivered to or in contact with a plant. Other aspects provide compositions containing one more nematode esophageal gland cell proteins or fragments thereof that stimulate root growth when in contract with a plant or plant cell. Still other aspects provide transgenic plants comprising one or more nematode esophageal gland cell proteins or fragments thereof or nucleic acids encoding one more nematode esophageal gland cell proteins or fragments thereof sufficient to stimulate, enhance, or promote root growth compared to non-transgenic or control plants.

Representative nematode esophageal gland cell proteins (also referred to as esophageal proteins) include one or more of the proteins encoded by SEQ ID NOs.1, 2, and 5-51 or combinations thereof.

Yet another aspect provides a plant cell transfected with heterologous nucleic acid encoding one or more nematode esophageal gland cell proteins, wherein the heterologous nucleic acid is expressed in an amount sufficient to stimulate, enhance, or promote root growth or development.

The details of one or more embodiments are set forth in the accompanying drawings and the description below. Other features, objects, and advantages of the disclosure will be apparent from the description and drawings, and from the claims.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1A shows *A. thaliana* expressing 16D10 dsRNA inoculated with *M. incognita*. Note that no root knot disease (galls) on roots of *A. thaliana* expressing 16D10 dsRNA.

Figure 1B shows control plants inoculated with *M. incognita*.

Figure 2 shows a photograph of a transgenic *A. thaliana* plant expressing 16D10 and having enhanced root growth compared to a control plant (empty vector).

Figure 3 shows a bar graph indicating enhanced root growth of four transgenic *Arabidopsis* T_2 homozygous lines L7,L10, L11, L17 compared to control lines (L2, L3).

Figure 4 shows RT-PCR analysis of 16D10 dsRNA (RNA1 and RNA2) treated second-stage juveniles of root-knot nematode showing a significant reduction of transcripts of parasitism gene 16D10 in the treated nematodes. Resorcinol (Res) was used to help stimulate uptake of the dsRNA. No reduction of transcripts with dsRNA or res alone. Mi-act – internal transcript control.

Figure 5 shows a photograph of a gel indicating that RNAi directed to 8H11 or 31H06 down-regulates expression of parasitism genes 8H11 or 31H06 in nematodes.

Figure 6 shows DNA blot hybridization of restriction endonuclease-digested genomic DNA from four *Meloidogyne* species with a DIG-labeled 16D10 probe. *Mi*, *M. incognita*; *Mj*, *M. javanica*; *Ma*, *M. arenaria*; *Mh*, *M. hapla*. E, *Eco*RI; B, *Bam*HI. M, 80 ng DIG-labeled molecular weight marker in kb.

Figure 7 shows a bar graph indicating reproduction (eggs per gram root) of four *Meloidogyne* species (*Mi*, *M. incognita*; *Mj*, *M. javanica*; *Ma*, *M. arenaria*; *Mh*, *M. hapla*) on transgenic *A. thaliana* expressing 16D10 dsRNA was decreased compared with control plants.

DETAILED DESCRIPTION

1. Definitions

Before explaining the various embodiments of the disclosure, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description. Other embodiments can be practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

Throughout this disclosure, various publications, patents and published patent specifications are referenced. Where permissible, the disclosures of these publications, patents and published patent specifications are hereby incorporated by reference in their entirety into the present disclosure to more fully describe the state of the art. Unless otherwise indicated, the disclosure encompasses conventional techniques of plant breeding, immunology, molecular biology, microbiology, cell biology and recombinant DNA, which are within the skill of the art. See, e.g., Sambrook and Russell, Molecular Cloning: A Laboratory Manual, 3rd edition (2001); Current Protocols In Molecular Biology [(F. M. Ausubel, et al. eds., (1987)]; Plant Breeding: Principles and Prospects (Plant Breeding, Vol 1) M. D. Hayward, N. O. Bosemark, I. Romagosa; Chapman & Hall, (1993.); Coligan, Dunn, Ploegh, Speicher and Wingfeld, eds. (1995) CURRENT

Protocols in Protein Science (John Wiley & Sons, Inc.); the series Methods in Enzymology (Academic Press, Inc.): PCR 2: A Practical Approach (M. J. MacPherson, B. D. Hames and G. R. Taylor eds. (1995)], Harlow and Lane, eds. (1988) Antibodies, A Laboratory Manual, and Animal Cell Culture [R. I. Freshney, ed. (1987)].

Unless otherwise noted, technical terms are used according to conventional usage. Definitions of common terms in molecular biology may be found in Lewin, Genes VII, published by Oxford University Press, 2000; Kendrew et al. (eds.), The Encyclopedia of Molecular Biology, published by Wiley-Interscience., 1999; and Robert A. Meyers (ed.), Molecular Biology and Biotechnology, a Comprehensive Desk Reference, published by VCH Publishers, Inc., 1995; Ausubel et al. (1987) Current Protocols in Molecular Biology, Green Publishing; Sambrook and Russell. (2001) Molecular Cloning: A Laboratory Manual 3rd. edition.

In order to facilitate understanding of the disclosure, the following definitions are provided:

To "alter" the expression of a target gene in a plant cell means that the level of expression of the target gene in a plant cell after applying a method of the present invention is different from its expression in the cell before applying the method. To alter gene expression preferably means that the expression of the target gene in the plant is reduced, preferably strongly reduced, more preferably the expression of the gene is not detectable. The alteration of the expression of an essential gene may result in a knockout mutant phenotype in plant cells or plants derived therefrom. Alternatively, altered expression can included upregulating expression of plant genes.

"Antisense RNA" is an RNA strand having a sequence complementary to a target gene mRNA, and thought to induce RNAi by binding to the target gene mRNA. "Sense RNA" has a sequence complementary to the antisense RNA, and annealed to its complementary antisense RNA to form siRNA. These antisense and sense RNAs have been conventionally synthesized with an RNA synthesizer. In the present invention, these RNAs are intracellularly expressed from DNAs coding for

antisense and sense RNAs (antisense and sense code DNAs) respectively using the siRNA expression system.

The term "biological sample" refers to a body sample from any animal, such as a mammal, for example, a human. The biological sample can be obtained from vascular, diabetic, or cancer patients, for example. A biological sample can be biological fluids such as serum, plasma, vitreous fluid, lymph fluid, synovial fluid, follicular fluid, seminal fluid, amniotic fluid, milk, whole blood, urine, cerebro-spinal fluid, saliva, sputum, tears, perspiration, mucus, and tissue culture medium, as well as tissue extracts such as homogenized tissue, cellular extracts, or whole cells or tissue. The biological sample can be, for example, serum, plasma, or urine.

As used herein, "buffer" refers to a buffered solution that resists changes in pH by the action of its acid-base conjugate components.

When referring to expression, "control sequences" means DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. Control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, a ribosome binding site, and the like. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

The term "cell" refers to a membrane-bound biological unit capable of replication or division.

The term "construct" refers to a recombinant genetic molecule comprising one or more isolated polynucleotide sequences of the invention.

Genetic constructs used for transgene expression in a host organism comprise in the 5'-3' direction, a promoter sequence; a sequence encoding an inhibitory nucleic acid disclosed herein; and a termination sequence. The open reading frame may be orientated in either a sense or anti-sense direction. The construct may also comprise selectable marker gene(s) and other regulatory elements for expression.

As used herein, the term "control element" or "regulatory element" are used interchangably herein to mean sequences positioned within or adjacent to a promoter sequence so as to influence promoter activity. Control

elements may be positive or negative control elements. Positive control elements require binding of a regulatory element for initiation of transcription. Many such positive and negative control elements are known. Where heterologous control elements are added to promoters to alter promoter activity as described herein, they are positioned within or adjacent the promoter sequence so as to aid the promoter's regulated activity in expressing an operationally linked polynucleotide sequence.

The term "heterologous" refers to elements occurring where they are not normally found. For example, a promoter may be linked to a heterologous nucleic acid sequence, e.g., a sequence that is not normally found operably linked to the promoter. When used herein to describe a promoter element, heterologous means a promoter element that differs from that normally found in the native promoter, either in sequence, species, or number. For example, a heterologous control element in a promoter sequence may be a control/regulatory element of a different promoter added to enhance promoter control, or an additional control element of the same promoter.

As used herein, the term "homologues" is generic to "orthologues" and "paralogues".

The term "host plant" refers to a plant subject to nematode disease.

As used herein, the phrase "induce expression" means to increase the amount or rate of transcription and/or translation from specific genes by exposure of the cells containing such genes to an effector or inducer reagent or condition.

An "inducer" is a chemical or physical agent which, when applied to a population of cells, will increase the amount of transcription from specific genes. These are usually small molecules whose effects are specific to particular operons or groups of genes, and can include sugars, phosphate, alcohol, metal ions, hormones, heat, cold, and the like. For example, isopropyl (beta)-D-thiogalactopyranoside (IPTG) and lactose are inducers of the tacII promoter, and L-arabinose is a suitable inducer of the arabinose promoter.

The term "isolated," when used to describe the various compositions disclosed herein, means a substance that has been identified and separated and/or recovered from a component of its natural environment. For example an isolated polypeptide or polynucleotide is free of association with at least one component with which it is naturally associated. Contaminant components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide or polynucleotide and may include enzymes, and other proteinaceous or non-proteinaceous solutes. An isolated substance includes the substance *in situ* within recombinant cells. Ordinarily, however, an isolated substance will be prepared by at least one purification step.

An "isolated" nucleic acid molecule or polynucleotide is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the natural source. The isolated nucleic can be, for example, free of association with all components with which it is naturally associated. An isolated nucleic acid molecule is other than in the form or setting in which it is found in nature.

"IPTG" is the compound "isopropyl (beta)-D- thiogalactopyranoside", and is used herein as an inducer of *lac* operon. IPTG binds to a *lac* repressor effecting a conformational change in the *lac* repressor that results in dissociation of the *lac* repressor from the *lac* operator. With the *lac* repressor unbound, an operably linked promoter is activated and downstream genes are transcribed.

The term "*lac* operator" refers to a nucleic acid sequence that can be bound by a *lac* repressor, *lacI*, as described, for example, in Jacob *et al.,* 1961, *J. Mol. Biol.*, 3: 318-356. A promoter is not activated when the *lac* repressor is bound to the *lac* operator. When the *lac* repressor is induced to dissociate from the operator, the promoter is activated.

The term "leader sequence" refers to a nucleic acid sequence positioned upstream of a coding sequence of interest. Leader sequences described herein contain specific sequences known to bind efficiently to

ribosomes, thus delivering a greater efficiency of translation initiation of some polynucleotides.

As used herein, the term "mammal" refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. The mammal can be, for example, human.

The term "nematode esophageal glands or nematode esophageal gland cell" refers to three large, transcriptionally active gland cells, one dorsal and two subventral, located in the esophagus of a nematode and that are the principal sources of secretions (parasitism proteins) involved in infection and parasitism of plants by plant-parasitic nematodes in the orders Tylenchida and Aphelenchida.

A nucleic acid sequence or polynucleotide is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous and, in the case of a secretory leader, contiguous and in reading frame. Linking can be accomplished by ligation at convenient restriction sites. If such sites do not exist, synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

The term "orthologues" refers to separate occurrences of the same gene in multiple species. The separate occurrences have similar, albeit nonidentical, amino acid sequences, the degree of sequence similarity depending, in part, upon the evolutionary distance of the species from a common ancestor having the same gene.

As used herein, the term "paralogues" indicates separate occurrences of a gene in one species. The separate occurrences have similar, albeit

nonidentical, amino acid sequences, the degree of sequence similarity depending, in part, upon the evolutionary distance from the gene duplication event giving rise to the separate occurrences.

The term "parasitism proteins, parasitism polypeptides, esophageal polypeptides, or nematode esophageal gland cell secretory polypeptide" refers to the principal molecules involved in nematode parasitism of plants; products of parasitism genes expressed in plant-parasitic nematode esophageal gland cells and injected through their stylet into host tissues to mediate parasitism of plants.

"Percent (%) nucleic acid sequence identity" is defined as the percentage of nucleotides in a candidate sequence that are identical with the nucleotides in a reference nucleic acid sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent nucleic acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, ALIGN-2 or Megalign (DNASTAR) software. Appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full-length of the sequences being compared can be determined by known methods.

For purposes herein, the % nucleic acid sequence identity of a given nucleic acid sequence C to, with, or against a given nucleic acid sequence D (which can alternatively be phrased as a given nucleic acid sequence C that has or comprises a certain % nucleic acid sequence identity to, with, or against a given nucleic acid sequence D) is calculated as follows:

100 times the fraction W/Z,

where W is the number of nucleotides scored as identical matches by the sequence alignment program in that program's alignment of C and D, and where Z is the total number of nucleotides in D. It will he appreciated that where the length of nucleic acid sequence C is not equal to the length of

nucleic acid sequence D, the % nucleic acid sequence identity of C to D will not equal the % nucleic acid sequence identity of D to C.

The term "plant" is used in it broadest sense. It includes, but is not limited to, any species of woody, ornamental or decorative, crop or cereal, fruit or vegetable plant, and photosynthetic green algae (e.g., *Chlamydomonas reinhardtii*). It also refers to a plurality of plant cells that are largely differentiated into a structure that is present at any stage of a plant's development. Such structures include, but are not limited to, a fruit, shoot, stem, leaf, flower petal, etc. The term "plant tissue" includes differentiated and undifferentiated tissues of plants including those present in roots, shoots, leaves, pollen, seeds and tumors, as well as cells in culture (e.g., single cells, protoplasts, embryos, callus, etc.). Plant tissue may be in planta, in organ culture, tissue culture, or cell culture. The term "plant part" as used herein refers to a plant structure, a plant organ, or a plant tissue.

A non-naturally occurring plant refers to a plant that does not occur in nature without human intervention. Non-naturally occurring plants include transgenic plants and plants produced by non-transgenic means such as plant breeding.

The term "plant cell" refers to a structural and physiological unit of a plant, comprising a protoplast and a cell wall. The plant cell may be in form of an isolated single cell or a cultured cell, or as a part of higher organized unit such as, for example, a plant tissue, a plant organ, or a whole plant.

The term "plant cell culture" refers to cultures of plant units such as, for example, protoplasts, cell culture cells, cells in plant tissues, pollen, pollen tubes, ovules, embryo sacs, zygotes and embryos at various stages of development.

The term "plant material" refers to leaves, stems, roots, flowers or flower parts, fruits, pollen, egg cells, zygotes, seeds, cuttings, cell or tissue cultures, or any other part or product of a plant.

A "plant organ" refers to a distinct and visibly structured and differentiated part of a plant such as a root, stem, leaf, flower bud, or embryo.

"Plant tissue" refers to a group of plant cells organized into a structural and functional unit. Any tissue of a plant whether in a plant or in culture is included. This term includes, but is not limited to, whole plants, plant organs, plant seeds, tissue culture and any groups of plant cells organized into structural and/or functional units. The use of this term in conjunction with, or in the absence of, any specific type of plant tissue as listed above or otherwise embraced by this definition is not intended to be exclusive of any other type of plant tissue.

"Plasmids" are designated by a lower case "p" preceded and/or followed by capital letters and/or numbers. The starting plasmids herein are either commercially available, publicly available on an unrestricted basis, or can be constructed from available plasmids in accord with published procedures. In addition, equivalent plasmids to those described are known in the art and will be apparent to the ordinarily skilled artisan.

As used herein, "polypeptide" refers generally to peptides and proteins having more than about ten amino acids. The polypeptides can be "exogenous," meaning that they are "heterologous," i.e., foreign to the host cell being utilized, such as human polypeptide produced by a bacterial cell.

"Primate" is construed to mean any of an order of mammals comprising humans, apes, monkeys, and related forms, such as lemurs and tarsiers.

The term "promoter" refers to a regulatory nucleic acid sequence, typically located upstream (5') of a gene or protein coding sequence that, in conjunction with various elements, is responsible for regulating the expression of the gene or protein coding sequence. The promoters suitable for use in the constructs of this disclosure are functional in plants and in host organisms used for expressing the inventive polynucleotides. Many plant promoters are publicly known. These include constitutive promoters, inducible promoters, tissue- and cell-specific promoters and developmentally-regulated promoters. Exemplary promoters and fusion promoters are described, e.g., in U.S. Pat. No. 6,717,034, which is herein incorporated by reference in its entirety.

"Purifying" means increasing the degree of purity of a substance in a composition by removing (completely or partially) at least one contaminant from the composition. A "purification step" may be part of an overall purification process resulting in an "essentially pure" composition. An essentially pure composition contains at least about 90% by weight of the substance of interest, based on total weight of the composition, and can contain at least about 95% by weight.

The term "regulatory element" or "control element" refers to DNA sequences controlling initiation of transcription. Examples of control or regulatory elements include, but are not limited to, a TATA box, operators, enhancers, and the like. Regulatory or control elements include negative control elements and positive control elements. A negative control element is one that is removed for activation. Many such negative control elements are known, for example operator/repressor systems. For example, binding of IPTG to the *lac* repressor dissociates from the *lac* operator to activate and permit transcription. Other negative elements include the *E. coli* trp and lambda systems. A positive control element is one that is added for activation. Many such positive control elements are known.

Promoters naturally containing both positive and negative regulatory elements are rare. The metE promoter is one example. See, for example, Neidhardt, Ed., 1996, *Escherishia coli and Salmonella*, Second Ed., pages 1300-1309. Descriptions of known positive and negative control elements can be found, for example, in this reference. Positioning of a positive or negative control element within or adjacent to the promoter to achieve added regulation of the promoter is known, and is described, for example, in *Escherishia coli and Salmonella (Supra)* at pages 1232-1245.

Small RNA molecules are single stranded or double stranded RNA molecules generally less than 200 nucleotides in length. Such molecules are generally less than 100 nucleotides and usually vary from 10 to 100 nucleotides in length. In a preferred format, small RNA molecules have 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 nucleotides. Small RNAs include microRNAs (miRNA) and small interfering

RNAs (siRNAs). MiRNAs are produced by the cleavage of short stem-loop precursors by Dicer-like enzymes; whereas, siRNAs are produced by the cleavage of long double-stranded RNA molecules. MiRNAs are single-stranded, whereas siRNAs are double-stranded.

The term "siRNA" means a small interfering RNA that is a short-length double-stranded RNA that is not toxic. Generally, there is no particular limitation in the length of siRNA as long as it does not show toxicity. "siRNAs" can be, for example, 15 to 49 bp, preferably 15 to 35 bp, and more preferably 21 to 30 bp long. Alternatively, the double-stranded RNA portion of a final transcription product of siRNA to be expressed can be, for example, 15 to 49 bp, preferably 15 to 35 bp, and more preferably 21 to 30 bp long. The double-stranded RNA portions of siRNAs in which two RNA strands pair up are not limited to the completely paired ones, and may contain nonpairing portions due to mismatch (the corresponding nucleotides are not complementary), bulge (lacking in the corresponding complementary nucleotide on one strand), and the like. Nonpairing portions can be contained to the extent that they do not interfere with siRNA formation. The "bulge" used herein preferably comprise 1 to 2 nonpairing nucleotides, and the double-stranded RNA region of siRNAs in which two RNA strands pair up contains preferably 1 to 7, more preferably 1 to 5 bulges. In addition, the "mismatch" used herein is contained in the double-stranded RNA region of siRNAs in which two RNA strands pair up, preferably 1 to 7, more preferably 1 to 5, in number. In a preferable mismatch, one of the nucleotides is guanine, and the other is uracil. Such a mismatch is due to a mutation from C to T, G to A, or mixtures thereof in DNA coding for sense RNA, but not particularly limited to them. Furthermore, in the present invention, the double-stranded RNA region of siRNAs in which two RNA strands pair up may contain both bulge and mismatched, which sum up to, preferably 1 to 7, more preferably 1 to 5 in number.

The terminal structure of siRNA may be either blunt or cohesive (overhanging) as long as siRNA can silence, reduce, or inhibit the target gene expression due to its RNAi effect. The cohesive (overhanging) end

structure is not limited only to the 3' overhang, and the 5' overhanging structure may be included as long as it is capable of inducing the RNAi effect. In addition, the number of overhanging nucleotide is not limited to the already reported 2 or 3, but can be any numbers as long as the overhang is capable of inducing the RNAi effect. For example, the overhang consists of 1 to 8, preferably 2 to 4 nucleotides. Herein, the total length of siRNA having cohesive end structure is expressed as the sum of the length of the paired double-stranded portion and that of a pair comprising overhanging single-strands at both ends. For example, in the case of 19 bp double-stranded RNA portion with 4 nucleotide overhangs at both ends, the total length is expressed as 23 bp. Furthermore, since this overhanging sequence has low specificity to a target gene, it is not necessarily complementary (antisense) or identical (sense) to the target gene sequence. Furthermore, as long as siRNA is able to maintain its gene silencing effect on the target gene, siRNA may contain a low molecular weight RNA (which may be a natural RNA molecule such as tRNA, rRNA or viral RNA, or an artificial RNA molecule), for example, in the overhanging portion at its one end.

In addition, the terminal structure of the "siRNA" is not necessarily the cut off structure at both ends as described above, and may have a stem-loop structure in which ends of one side of double-stranded RNA are connected by a linker RNA. The length of the double-stranded RNA region (stem-loop portion) can be, for example, 15 to 49 bp, preferably 15 to 35 bp, and more preferably 21 to 30 bp long. Alternatively, the length of the double-stranded RNA region that is a final transcription product of siRNAs to be expressed is, for example, 15 to 49 bp, preferably 15 to 35 bp, and more preferably 21 to 30 bp long. Furthermore, there is no particular limitation in the length of the linker as long as it has a length so as not to hinder the pairing of the stem portion. For example, for stable pairing of the stem portion and suppression of the recombination between DNAs coding for the portion, the linker portion may have a clover-leaf tRNA structure. Even though the linker has a length that hinders pairing of the stem portion, it is possible, for example, to construct the linker portion to include introns so that the introns are excised

during processing of precursor RNA into mature RNA, thereby allowing pairing of the stem portion. In the case of a stem-loop siRNA, either end (head or tail) of RNA with no loop structure may have a low molecular weight RNA. As described above, this low molecular weight RNA may be a natural RNA molecule such as tRNA, rRNA or viral RNA, or an artificial RNA molecule.

"Signal peptide" refers to a short (15-60 amino acids long) peptide chain that directs the post translational transport of a protein; usually directs the peptide to the secretory pathway of the cell.

"Transformed," "transgenic," "transfected" and "recombinant" refer to a host organism such as a bacterium or a plant into which a heterologous nucleic acid molecule has been introduced. The nucleic acid molecule can be stably integrated into the genome of the host or the nucleic acid molecule can also be present as an extrachromosomal molecule. Such an extrachromosomal molecule can be auto-replicating. Transformed cells, tissues, or plants are understood to encompass not only the end product of a transformation process, but also transgenic progeny thereof. A "non-transformed," "non-transgenic," or "non-recombinant" host refers to a wild-type organism, e.g., a bacterium or plant, which does not contain the heterologous nucleic acid molecule.

A "transformed cell" refers to a cell into which has been introduced a nucleic acid molecule, for example by molecular biology techniques. As used herein, the term transformation encompasses all techniques by which a nucleic acid molecule might be introduced into such a cell, plant or animal cell, including transfection with viral vectors, transformation by Agrobacterium, with plasmid vectors, and introduction of naked DNA by electroporation, lipofection, and particle gun acceleration and includes transient as well as stable transformants.

The term "transgenic plant" refers to a plant or tree that contains recombinant genetic material not normally found in plants or trees of this type and which has been introduced into the plant in question (or into progenitors of the plant) by human manipulation. Thus, a plant that is grown

from a plant cell into which recombinant DNA is introduced by transformation is a transgenic plant, as are all offspring of that plant that contain the introduced transgene (whether produced sexually or asexually). It is understood that the term transgenic plant encompasses the entire plant or tree and parts of the plant or tree, for instance grains, seeds, flowers, leaves, roots, fruit, pollen, stems etc.

The term "translation initiation enhancer sequence", as used herein, refers to a nucleic acid sequence that can determining a site and efficiency of initiation of translation of a gene (See, for example, McCarthy et al., 1990, *Trends in Genetics*, 6: 78-85). A translation initiation enhancer sequence can extend to include sequences 5' and 3' to the ribosome binding site. The ribosome binding site is defined to include, minimally, the Shine-Dalgarno region and the start codon, in addition to any bases in between. In addition, the translation initiation enhancer sequence can include an untranslated leader or the end of an upstream cistron, and thus a translational stop codon. See, for example, US Patent No. 5,840,523.

The term "vector" refers to a nucleic acid molecule which is used to introduce a polynucleotide sequence into a host cell, thereby producing a transformed host cell. A "vector" may comprise genetic material in addition to the above-described genetic construct, e.g., one or more nucleic acid sequences that permit it to replicate in one or more host cells, such as origin(s) of replication, selectable marker genes and other genetic elements known in the art (e.g., sequences for integrating the genetic material into the genome of the host cell, and so on).

2. Exemplary Embodiments

Nematode Resistant Transgenic Plants

It has been discovered that interrupting the feeding cycle of nematodes by down-regulating one or more nematode parasitism genes is an effective method for reducing, preventing, or treating nematode disease in plants. Nematode parasitism genes refers to genes expressed in the esophageal gland cells encoding for secretory proteins exported from the

gland cell to be released through the nematode's stylet into host tissue. In particular, it has been discovered that interfering with the expression of proteins secreted by nematodes related to the formation of specialized feeding cells in host plants is an effective method for reducing, treating, or preventing nematode disease in plants. Representative parasitism genes encoding secreted proteins that can be targeted, for example with inhibitory RNA include, include but are not limited to those genes listed in Table 2, or a fragment thereof.

Nematode disease results in substantial losses of valuable crops. Root-knot nematodes, *Meloidogyne* species, are among nature's most successful parasites. They parasitize more than 2,000 plant species from diverse plant families and represent a tremendous threat to crop production world-wide. These biotrophic pathogens have evolved highly specialized and complex feeding relationships with their hosts. A successful nematode-host interaction requires molecular signals from the parasite to modify, directly or indirectly, plant root cells into elaborate feeding cells, called giant-cells, which are the sole source of nutrients needed for nematode development and reproduction. Plant-parasitic nematodes release proteinaceous secretions through a hollow protrusible stylet into plant cells when feeding. These secretions, collectively called the parasitome are encoded by parasitism genes expressed in large and transcriptionally active esophageal gland cells (Davis, E. L., R. Allen, and R. S. Hussey. 1994. Developmental expression of esophageal gland antigens and their detection in stylet secretions of *Meloidogyne incognita*. Fundam. Appl. Nematol. 17:255-262.; Hussey, R. S., E. L. Davis, and T. J. Baum. 2002. Secrets in secretions: genes that control nematode parasitism of plants. Braz. J. Plant Physiol. 14:183-194.). The profound cellular modifications induced by *Meloidogyne* species to form the giant-cells are the result of an alteration in host root cell gene expression and phenotype that is driven by the molecular signals secreted through the nematode's stylet.

One embodiment provides a plant or cell comprising one or more inhibitory RNAs specific for one or more mRNAs of one or more nematode

parasitism genes. For example, the present disclosure provides transgenic plants that express one or more inhibitory RNAs that down regulate nematode parasitism gene expression when the one or more inhibitory RNAs are absorbed or ingested by a nematode. The transgenic plant can be designed to express inhibitory RNA that down-regulates the target parasitism gene transcript in at least two different nematode species, for example two different RKN species. Another embodiment provides a transgenic plant that comprises inhibitory RNA that down regulates the target parasitism gene transcript in 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more nematode species, for example RKN species. Thus, the present disclosure provides transgenic plants that are resistant to disease caused by multiple RKN species.

Another embodiment, provides a transgenic plant comprising inhibitory RNA specific for one or more nematode parasitism genes in an amount effective to provide the plant with resistance to all RKN species, for example those RKN species referenced in Jepson, S. B. 1987. Identification of root-knot nematodes (*Meloidogyne* species). C. A. B. International, Oxford, United Kingdom. 1-265 pages, which, where permissible, is incorporated by reference in its entirety.

Another embodiment provides a transgenic plant or transgenic cell containing or expressing one or more inhibitory nucleic acids specific for at least a portion of a nucleic acid encoding one or more secretory polypeptides of a parasitic nematode. The inhibitory nucleic acid is typically a small inhibitory RNA or microRNA that is specific for mRNA encoding a nematode esophageal gland cell protein or polypeptide. It will be appreciated by one of skill in the art that the inhibitory nucleic acid can be RNA, DNA, or a combination thereof. Additionally, the inhibitory nucleic acid may be single or multi-stranded and may be anti-sense or enzymatic. In one embodiment, the inhibitory nucleic acid interferes, inhibits, or reduces the translation of a target mRNA. For example, the inhibitory nucleic acid can bind to a target mRNA and induce or promote the degradation of the target mRNA or physically prevent the cellular translational machinery from translating the

target mRNA into a functional protein. Inhibition of the secretory polypeptide can be compared to controls, for example plants or cells that do not contain or express the inhibitory nucleic acid. A "control" refers to a sample of material which is known to be identical to a sample containing the disclosed inhibitory nucleic acid in every regard, except that the control sample does not contain or express the inhibitory nucleic acid.

The term "esophageal gland cell protein or polypeptide" refers to a secretory polypeptide encoded by a nematode parasitism gene. In one embodiment, the esophageal gland cell protein or polypeptide to be down-regulated generally is a secreted protein that modulates expression of at least one host plant gene. Exemplary nematode polypeptides that are down-regulated in the disclosed compositions and methods include, but are not limited to polypeptides or fragments thereof encoded by SEQ ID NOs 1, 2, or 5-51, or fragments thereof. The secretory polypeptide can increase or decrease expression of host plant genes either directly or indirectly. For example, direct modulation can occur when the esophageal gland cell protein or polypeptide binds to a host plant nucleic acid, including genomic DNA, RNA, and mRNA. Indirect modulation can occur for example when the polypeptide binds with one or more other proteins or factors to form a complex. The complex can then bind to a host plant nucleic acid to either promote or suppress transcription or translation. Down-regulation of the secretory protein alleviates or reduces at least one symptom associated with nematode disease. Exemplary symptoms of nematode disease include, but are not limited to the formation of galls, giant cells, lesions, stunting, nutrient and water deficiencies, dieback, and numbers of nematodes infecting a plant. Levels of reduction or inhibition of nematode disease in transgenic plants or cells can be compared to levels of nematode disease in control plants or cells. In one embodiment, the inhibitory nucleic acid reduces, inhibits, alleviates, treats or prevents nematode disease.

In another embodiment, the esophageal gland cell protein or polypeptide to be down-regulated is encoded by a parasitism gene involved in the formation of a giant cell. In still other embodiments, the targeted

parasitism gene encodes a polypeptide or nucleic acid involved in nematode migration through root tissue, alters cell metabolism, elicits signal transduction in the recipient cell, or forms a feeding tube that enables the nematode to feed from the giant-cells. Additionally, the esophageal gland cell protein or polypeptide can cause cell wall modifications and potentially interact with signal transduction receptors in the extracellular space, influence cellular metabolism, cell cycle, selective protein degradation, localized defense response, and regulatory activity within the plant cell nucleus.

Exemplary plant genes that are modulated by the esophageal gland cell protein or polypeptide include, but are not limited to genes involved in the formation of specialized nematode feeding cells also known as giant cells. For example, nematode parasitism gene 16D10 encodes a protein that binds to a scarecrowlike transcription regulator. Representative plant genes that can be modulated by nematode esophageal gland cell polypeptides include, but are not limited to *WUN1*, *POX*, *CAT*, *GST*, *Mia-1*, *Mia-2*, *Mia-3*, *Mia-4*, *CHS1-CHS3*, *LOX*, Chitinase, Trypsin inhibitor, Miraculin, *HMGR*, *TSW12*, *LEA14*, *LEMMI9*, C6-19, C27-45, *TAS14*, *UBC* DB#103, *RPE*, *ISDGh*, *IPPP*, *LPPL*, *mUCp*, endomembrane protein, 20s proteasome, DAP decarboxylase, *GRP*, *ENOD40*, *ATAO1* or combinations thereof (Gheysen, G. and Fenoll, C. 2002. Annual Review of Phytopathology 40:191, which, where permissible, is incorporated by reference in its entirety). Generally, the plant gene is directly or indirectly involved in root cell growth, root cell division or the production of specific nutrients ingested by the parasitic nematode. The gene can be one expressed in a root cell or any other cell of the plant.

In one embodiment, expression of a targeted nematode secretory protein is reduced, inhibited, or blocked, as compared to a control, when the inhibitory nucleic acid is delivered to the nematode. Delivery of the inhibitory nucleic acid can be achieved, for example, when the nematode comes into contact with the inhibitory nucleic acid as the nematode feeds on the transgenic plant or cell. The nematode can ingest the inhibitory nucleic acid

during feeding, or the nucleic acid can be transported across a cellular membrane of the nematode by active transport or passive diffusion. It will be appreciated that the inhibitory nucleic acid can be delivered to the nematode in combination or alternation with an agent that induces or promotes the uptake of the inhibitory nucleic acid by the nematode. An exemplary inducing agent includes, but is not limited to resorcinol (3-hydroxyphenol).

In one embodiment, the transgenic plant or transgenic cell expresses the inhibitory nucleic in an amount effective to modulate the expression of a nematode esophageal gland cell polypeptide or protein in a nematode when the inhibitory nucleic acid is delivered to the nematode. Levels of expression of the inhibitory nucleic acid in a transgenic plant or cell can be controlled using methods known in the art, for example using vectors with strong promoters or constitutively active promoters, high copy number vectors, etc. The plant or cell can be stably or transiently transfected.

An exemplary parasitic nematode includes, but is not limited to members of *Meloidogyne spp.* also referred to as root-knot nematodes. Representative species include, but are not limited to *M. arenaria*, *M. incognita*, *M. javanica*, *M. hapla*, *M. chitwoodi* and *M. naasi*.

Representative phylogenetic families of host plants include *Acanthaceae*, *Aceraceae*, *Actinidiaceae*, *Agavaceae*, *Aizoaceae*, *Amaranthaceae*, *Annonaceae*, *Apiaceae*, *Apocynaceae*, *Araceae*, *Araliaceae*, *Arecaceae*, *Aristolochiaceae*, *Balsaminaceae*, *Barringtoniaceae*, *Basellaceae*, *Berberidaceae*, *Betulaceae*, *Bignoniaceae*, *Bixaceae*, *Bombacaceae*, *Boraginaceae*, *Buxaceae*, *Byttneriaceae*, *Cactaceae*, *Caesalpiniaceae*, *Cannaceae*, *Capparaceae*, *Caprifoliaceae*, *Caricaceae*, *Caryophyllaceae*, *Casuarinaceae*, *Casuarinaceae*, *Celastraceae*, *Chenopodiaceae*, *Chenopodiaceae*, *Chloranthaceae*, *Commelinaceae*, *Convolvulaceae*, *Cornaceae*, *Corylaceae*, *Crassulaceae*, *Cucurbitaceae*, *Cupressaceae*, *Cyatheaceae*, *Cyperaceae*, *Datiscaceae*, *Dilleniaceae*, *Dioscoreaceae*, *Dipsacaceae*, *Ebenaceae*, *Ericaceae*, *Euphorbiaceae*, *Fabaceae*, *Flacourtiaceae*, *Fumariaceae*, *Gentianaceae*, *Geraniaceae*, *Gesneriaceae*, *Ginkgoaceae*, *Goodeniaceae*, *Guttiferae*, *Haemodoraceae*,

Hamamelidaceae, *Heliconiaceae*, *Hydrophyllaceae*, *Hypericaceae*, *Iridaceae*, *Juglandaceae*, *Juncaceae*, *Labiatae*, *Lamiaceae*, *Lauraceae*, *Liliaceae*, *Linaceae*, *Lobeliaceae*, *Loganiaceae*, *Lythraceae*, *Magnoliaceae*, *Malpighiaceae*, *Malvaceae*, *Marantaceae*, *Melastomataceae*, *Meliaceae*, *Menispermaceae*, *Mimosaceae*, *Moraceae*, *Musaceae*, *Myoporaceae*, *Myricaceae*, *Myristicaceae*, *Myrtaceae*, *Nyctaginaceae*, *Oleaceae*, *Onagraceae*, *Orchidaceae*, *Othnaceae*, *Oxalidaceae*, *Paeoniaceae*, *Pandanaceae*, *Papaveraceae*, *Pedaliaceae*, *Phytolaccaceae*, *Pinaceae*, *Piperaceae*, *Pittosporaceae*, *Plantaginaceae*, *Platanaceae*, *Plumbaginaceae*, *Poaceae*, *Podostemaceae*, *Polemoniaceae*, *Polygalaceae*, *Portulacaceae*, *Primulaceae*, *Proteaceae*, *Punicaceae*, *Ranunculaceae*, *Resedaceae*, *Rhamnaceae*, *Rosaceae*, *Rubiaceae*, *Rutaceae*, *Salicaceae*, *Santalaceae*, *Sapindaceae*, *Sarraceniaceae*, *Saxifragaceae*, *Scrophulariaceae*, *Smilacaceae*, *Solanaceae*, *Sterculiaceae*, *Styracaceae*, *Tamaricaceae*, *Taxodiaceae*, *Tetragoniaceae*, *Theaceae*, *Theophrastaceae*, *Thymelaeaceae*, *Tiliaceae*, *Tropaeolaceae*, *Turneraceae*, *Typhaceae*, *Ulmaceae*, *Urticaceae*, *Valerianaceae*, *Verbenaceae*, *Violaceae*, *Vitaceae*, *Zamiaceae*, *Zingiberaceae*, or *Zygophyllaceae*.

Common names of host plants that can be transfected with an inhibitory nucleic acid according the present disclosure include, but are not limited to tomato, eggplant, potato, melon, cucumber, carrot, lettuce, artichoke, celery, cucurbits (melon, watermelon, etc.), barley, corn, peanut, soybean, sugar beet, cotton, cowpea, beans, alfalfa, tobacco, citrus, clover, pepper, grape, coffee, olive, or tea.

It will be appreciated by one of skill in the art that the present disclosure encompasses any of the fifty or more known root-knot nematode species.

Another embodiment provides a composition having an inhibitory nucleic acid specific for an mRNA or fragment thereof encoding a polypeptide encoded by one or more of SEQ ID NOs. 1, 2 or 5-51 or a fragment or homologues thereof, in an amount sufficient to inhibit expression of the polypeptide encoded by one or more of SEQ ID NOs 1, 2 or 5-51 or

homologues thereof when delivered to a nematode, for example when the nematode is feeding on a plant or cell expressing or containing the inhibitory nucleic acid. The composition can contain one or more nematicides, pesticides, fungicides, or combinations thereof. Representative nematicides include, but are not limited to chloropicrin, methyl bromide, 1,3-dichloropropene, sodium methyl dithiocarbamate, sodium tetrathiocarbonate; and carbamates such as 2-methyl-2-(methylthio)propionaldehyde O-methylcarbamoyloxime (aldicarb), 2,3-Dihydro-2,2-dimethyl-7-benzofuranol methylcarbamate (carbofuran), methyl 2-(dimethylamino)-N-[[(methylamino)carbonyl]oxy]-2-oxoethanimidothioate (oxamyl), 2-methyl-2-(methylsulfonyl)propanal O-[(methylamino)carbonyl]oxime (aldoxycarb), *O,O*-diethyl *O*-[4-(methylsulfinyl)phenyl] phosphorothioate (fensulfothion), O-Ethyl S,S-dipropylphosphorodithioate (ethoprop), and Ethyl-3-methyl-4-(methylthio)phenyl(1-methylethyl)phosphoramidate (phenamiphos).

Another embodiment provides a cell containing a nucleic acid encoding an inhibitory nucleic acid specific for an mRNA or fragment thereof, wherein the mRNA encodes a esophageal gland cell protein or polypeptide that directly or indirectly modulates: root cell gene expression, nematode migration through root tissue, cell metabolism, signal transduction, or is involved in the formation of a feeding tube that enables the nematode to feed from the giant-cells of at least one plant gene involved in the formation of a giant cell. Additionally, the esophageal gland cell protein or polypeptide or esophageal polypeptide can cause cell wall modifications and potentially interact with signal transduction receptors in the extracellular space, influence cellular metabolism, cell cycle, selective protein degradation, localized defense response, and regulatory activity within the plant cell nucleus. The cell can be prokaryotic or eukaryotic, and generally is a plant cell, particularly a root cell.

Still another embodiment provides a method for providing nematode resistance to a plant by contacting the plant with one or more inhibitory nucleic acids specific for one or more nematode esophageal gland cell proteins in an amount sufficient to reduce nematode disease, wherein the

one or more nematode esophageal gland cell proteins modulate: gene expression of the plant or cell, formation of a giant cell, nematode migration through root tissue of the plant, cell metabolism of the plant, elicits signal transduction in the plant cell, or forms a feeding tube that enables the nematode to feed from giant-cells formed in the plant. One aspect provides inhibitory nucleic acids specific for esophageal gland cell proteins secreted by nematodes, in particular root knot nematodes. The inhibitory nucleic acid can be sprayed onto the plant or otherwise delivered to the plant so that the inhibitory nucleic acid comes into contact with a parasitic nematode.

Yet another embodiment provides transgenic plants or plant cells containing an inhibitory nucleic acid, for example siRNA or microRNA, that down regulates root-knot nematode esophageal gland cell proteins when delivered to a nematode feeding on the plant or plant cell. RNA interference is known in the art. See for example, Kreutzer et al., International PCT Publication No. WO 00/44895; Zernicka-Goetz et al, International PCT Publication No. WO 01/36646; Fire, International PCT Publication No. WO 99/32619; Plaetinck et al., International PCT Publication No. WO 00/01846; Mello and Fire, International PCT Publication No. WO 01/29058; Deschamps-Depaillette, International PCT Publication No. WO 99/07409; Li et al., International PCT Publication No. WO 00/44914; and Trick et al., US20040098761.

In one embodiment, the nematode is not a soybean cyst nematode.

In another embodiment, the inhibitory nucleic acid is not directly lethal to embryonic or adult nematodes or is not involved in nematode fertility, but instead inhibits the ability of the nematode to feed on or obtain nutrients from the transgenic plant or plant cell.

In some embodiments, inhibitory double stranded RNA (dsRNA) is derived from an "exogenous template". Such a template may be all or part of a plant or nematode nucleotide sequence; it may be a DNA gene sequence or a cDNA produced from an mRNA isolated from a parasitic nematode, for example by reverse transcriptase. When the template is all or a part of a DNA gene sequence, it is preferred if it is from one or more or all

exons of the gene. While the dsRNA is derived from an endogenous or exogenous template, there is no limitation on the manner in which it could be synthesized. For example, the siRNA can be chemically synthesized, produced by in vitro transcription; produced by digestion of long dsRNA by an RNase III family enzyme (e.g., Dicer, RNase III); expressed in cells from an siRNA expression plasmid or viral vector; or expressed in cells from a PCR-derived siRNA expression cassette

SiRNA prepared in vitro is then introduced directly into cells by transfection, electroporation, or by another method. Alternatively, transfection of DNA-based vectors and cassettes that express siRNAs within the cells can be used. RNAi may be synthesized in vitro or in vivo, using manual and/or automated procedures. In vitro synthesis may be chemical or enzymatic, for example using cloned RNA polymerase (e.g., T3, T7, SP6) for transcription of the endogenous DNA (or cDNA) template, or a mixture of both.

In vivo, the dsRNA may be synthesised using recombinant techniques well known in the art (see e.g., Sambrook, et al., Molecular Cloning; A Laboratory Manual, Third Edition (2001). For example, bacterial cells can be transformed with an expression vector which comprises the DNA template from which the dsRNA is to be derived. Alternatively, the cells, of a plant for example, in which inhibition of gene expression is required may be transformed with an expression vector or by other means. Bidirectional transcription of one or more copies of the template may be by endogenous RNA polymerase of the transformed cell or by a cloned RNA polymerase (e.g., T3, T7, SP6) coded for by the expression vector or a different expression vector. The use and production of an expression construct are known in the art (see WO98/32016; U.S. Pat. Nos. 5,593,874, 5,698,425, 5712,135, 5,789,214, and 5,804,693). Inhibition of gene expression may be targeted by specific transcription in an organ, tissue, or cell type; an environmental condition (e.g. temperature, chemical); and/or engineering transcription at a developmental stage or age, especially when the dsRNA is synthesized in vivo in the plant cell for example. dsRNA may also be

delivered to specific tissues or cell types using known gene delivery systems. Components of these systems include the seed-specific lectin promoter and the flower specific promoter from APETALA3. These vectors are listed solely by way of illustration of the many commercially available and well known vectors that are available to those of skill in the art.

If synthesized outside the cell, the RNA may be purified prior to introduction into the cell. Purification may be by extraction with a solvent (such as phenol/chloroform) or resin, precipitation (for example in ethanol), electrophoresis, chromatography, or a combination thereof. However, purification may result in loss of dsRNA and may therefore be minimal or not carried out at all. The RNA may be dried for storage or dissolved in an aqueous solution, which may contain buffers or salts to promote annealing, and/or stabilization of the RNA strands.

Suitable dsRNA can also contain one or more modified bases, or have a modified a backbone to increase stability or for other reasons. For example, the phosphodiester linkages of natural RNA may be modified to include at least one of a nitrogen or sulfur heteroatom. Moreover, dsRNA comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, can be used. It will be appreciated that a great variety of modifications have been made to RNA that serve many useful purposes known to those of skill in the art. The term dsRNA as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of dsRNA, provided that it is derived from an endogenous template.

The double-stranded structure may be formed by a single self-complementary RNA strand or two separate complementary RNA strands. RNA duplex formation may be initiated either inside or outside the plant cell.

The sequence of at least one strand of the dsRNA contains a region complementary to at least a part of the target mRNA sufficient for the dsRNA to specifically hybridize to the target mRNA. In one embodiment, the siRNA is substantially identical to at least a portion of the target mRNA. "Identity", as known in the art, is the relationship between two or more polynucleotide

(or polypeptide) sequences, as determined by comparing the sequences. In the art, identity also means the degree of sequence relatedness between polynucleotide sequences, as determined by the match between strings of such sequences. Identity can be readily calculated (Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, N.J., 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991). While there exist a number of methods to measure identity between two polynucleotide sequences, the term is well known to skilled artisans (Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM J. Applied Math., 48: 1073 (1988). Methods commonly employed to determine identity between sequences include, but are not limited to those disclosed in Carillo, H., and Lipman, D., SIAM J. Applied Math., 48:1073 (1988). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity are codified in computer programs. Computer program methods to determine identity between two sequences include, but are not limited to, GCG program package (Devereux, J., et al., Nucleic Acids Research 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Atschul, S. F. et al., J. Molec. Biol. 215: 403 (1990)). Another software package well known in the art for carrying out this procedure is the CLUSTAL program. It compares the sequences of two polynucleotides and finds the optimal alignment by inserting spaces in either sequence as appropriate. The identity for an optimal alignment can also be calculated using a software package such as BLASTx. This program aligns the largest stretch of similar sequence and assigns a value to the fit. For any one pattern comparison several regions of similarity may be found, each having a different score.

One skilled in the art will appreciate that two polynucleotides of different lengths may be compared over the entire length of the longer fragment. Alternatively small regions may be compared. Normally sequences of the same length are compared for a useful comparison to be made.

In one embodiment, the inhibitory nucleic acid has 100% sequence identity with at least a part of the target mRNA. However, inhibitory nucleic acids having 70%, 80% or greater than 90% or 95% sequence identity may be used. Thus sequence variations that might be expected due to genetic mutation, strain polymorphism, or evolutionary divergence can be tolerated.

The duplex region of the RNA may have a nucleotide sequence that is capable of hybridizing with a portion of the target gene transcript (e.g., 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50° C or 70° C hybridization for 12-16 hours; followed by washing).

While the optimum length of the dsRNA may vary according to the target gene and experimental conditions, the duplex region of the RNA may be at least 19, 20, 21-23, 25, 50, 100, 200, 300, 400 or more bases long.

Target genes are nematode genes encoding secreted proteins, in particular secreted proteins that modulate: gene expression of the plant or cell, formation of a giant cell, nematode migration through root tissue of the plant, cell metabolism of the plant, elicits signal transduction in the plant cell, or forms a feeding tube that enables the nematode to feed from giant-cells formed in the plant. One aspect provides inhibitory nucleic acids specific for esophageal gland cell proteins secreted by nematodes, in particular root knot nematodes. Typically, the dsRNA or inhibitory nucleic acid is substantially identical to the whole of the target gene, i.e. the coding portion of the gene. However, the dsRNA or inhibitory nucleic acid can be substantially identical to a part of the target gene. The size of this part depends on the particular target gene and can be determined by those skilled in the art by varying the size of the dsRNA and observing whether expression of the gene has been inhibited.

Plants with Enhanced Root Growth

One embodiment provides a transgenic plant or transgenic cell containing or expressing one or more nucleic acids encoding one or more nematode esophageal gland cell polypeptides or fragments thereof of a parasitic nematode. Expression of the one or more nematode esophageal gland cell polypeptides or fragments thereof in a plant or plant cell promotes, stimulates, or enhances root growth of the transgenic plant compared to non-transgenic plants or control plants. A root includes a seminal root, adventitious root, first order lateral root, second order laterals, etc., feeder roots primary roots, secondary roots, and coarse roots.

The nematode esophageal gland cell polypeptides or fragments used with the disclosed embodiments can increase the size of roots, the number of roots, the surface area of roots, and the overall quality of a root system. Root crops can be produced with the disclosed compositions and methods that are larger than root crops produced in the absence of the disclosed compositions and methods. Other crops produced using the disclosed compositions and methods can be resistant to drought, erosion, or increased environmental stress. Environmental stress includes changes in climate such as rainfall, temperature, and humidity.

Exemplary nematode esophageal gland cell polypeptides or fragments thereof include, but are not limited to polypeptides encoded by SEQ ID NOs 1, 2, or 5-51, fragments thereof, or combinations thereof. The nematode esophageal gland cell polypeptides or fragments can increase, stimulate, or enhance root growth directly or indirectly. For example, direct modulation can occur when the nematode secretory polypeptide binds to a host plant nucleic acid, including genomic DNA, RNA, and mRNA. Indirect modulation can occur for example when the polypeptide binds with one or more other proteins or factors to form a complex. The complex can then bind to a host plant nucleic acid to either promote or suppress transcription or translation.

In one embodiment, the transgenic plant or transgenic cell expresses the nematode esophageal gland cell polypeptide or fragment thereof in an

amount effective to stimulate, enhance or promote root growth or development. Alternatively, the nematode esophageal gland cell polypeptide or fragment thereof can be delivered directly to the plant. Levels of nematode esophageal gland cell polypeptide or fragment thereof expression in a transgenic plant or cell can be controlled using methods known in the art, for example using vectors with strong promoters or constitutively active promoters, high copy number vectors, etc. The plant or cell can be stably or transiently transfected.

An exemplary nematode includes, but is not limited to members of *Meloidogyne spp.* also referred to as root-knot nematodes. Representative species include, but are not limited to *M. arenaria*, *M. incognita*, *M. javanica*, *M. hapla*, and *M. naasi.*

Representative phylogenetic families of host plants include *Acanthaceae*, *Aceraceae*, *Actinidiaceae*, *Agavaceae*, *Aizoaceae*, *Amaranthaceae*, *Annonaceae*, *Apiaceae*, *Apocynaceae*, *Araceae*, *Araliaceae*, *Arecaceae*, *Aristolochiaceae*, *Balsaminaceae*, *Barringtoniaceae*, *Basellaceae*, *Berberidaceae*, *Betulaceae*, *Bignoniaceae*, *Bixaceae*, *Bombacaceae*, *Boraginaceae*, *Buxaceae*, *Byttneriaceae*, *Cactaceae*, *Caesalpiniaceae*, *Cannaceae*, *Capparaceae*, *Caprifoliaceae*, *Caricaceae*, *Caryophyllaceae*, *Casuarinaceae*, *Casuarinaceae*, *Celastraceae*, *Chenopodiaceae*, *Chenopodiaceae*, *Chloranthaceae*, *Commelinaceae*, *Convolvulaceae*, *Cornaceae*, *Corylaceae*, *Crassulaceae*, *Cucurbitaceae*, *Cupressaceae*, *Cyatheaceae*, *Cyperaceae*, *Datiscaceae*, *Dilleniaceae*, *Dioscoreaceae*, *Dipsacaceae*, *Ebenaceae*, *Ericaceae*, *Euphorbiaceae*, *Fabaceae*, *Flacourtiaceae*, *Fumariaceae*, *Gentianaceae*, *Geraniaceae*, *Gesneriaceae*, *Ginkgoaceae*, *Goodeniaceae*, *Guttiferae*, *Haemodoraceae*, *Hamamelidaceae*, *Heliconiaceae*, *Hydrophyllaceae*, *Hypericaceae*, *Iridaceae*, *Juglandaceae*, *Juncaceae*, *Labiatae*, *Lamiaceae*, *Lauraceae*, *Liliaceae*, *Linaceae*, *Lobeliaceae*, *Loganiaceae*, *Lythraceae*, *Magnoliaceae*, *Malpighiaceae*, *Malvaceae*, *Marantaceae*, *Melastomataceae*, *Meliaceae*, *Menispermaceae*, *Mimosaceae*, *Moraceae*, *Musaceae*, *Myoporaceae*, *Myricaceae*, *Myristicaceae*, *Myrtaceae*, *Nyctaginaceae*, *Oleaceae*,

Onagraceae, *Orchidaceae*, *Othnaceae*, *Oxalidaceae*, *Paeoniaceae*, *Pandanaceae*, *Papaveraceae*, *Pedaliaceae*, *Phytolaccaceae*, *Pinaceae*, *Piperaceae*, *Pittosporaceae*, *Plantaginaceae*, *Platanaceae*, *Plumbaginaceae*, *Poaceae*, *Podostemaceae*, *Polemoniaceae*, *Polygalaceae*, *Portulacaceae*, *Primulaceae*, *Proteaceae*, *Punicaceae*, *Ranunculaceae*, *Resedaceae*, *Rhamnaceae*, *Rosaceae*, *Rubiaceae*, *Rutaceae*, *Salicaceae*, *Santalaceae*, *Sapindaceae*, *Sarraceniaceae*, *Saxifragaceae*, *Scrophulariaceae*, *Smilacaceae*, *Solanaceae*, *Sterculiaceae*, *Styracaceae*, *Tamaricaceae*, *Taxodiaceae*, *Tetragoniaceae*, *Theaceae*, *Theophrastaceae*, *Thymelaeaceae*, *Tiliaceae*, *Tropaeolaceae*, *Turneraceae*, *Typhaceae*, *Ulmaceae*, *Urticaceae*, *Valerianaceae*, *Verbenaceae*, *Violaceae*, *Vitaceae*, *Zamiaceae*, *Zingiberaceae*, or *Zygophyllaceae*.

Common names of host plants that can be transfected with nucleic acid encoding a RKN esophageal gland cell secretory polypeptide according the present disclosure include, but are not limited to tomato, eggplant, potato, melon, cucumber, carrot, lettuce, artichoke, celery, cucurbits (melon, watermelon, etc.), barley, corn, peanut, soybean, sugar beet, cotton, cowpea, beans, alfalfa, tobacco, citrus, clover, pepper, grape, coffee, olive, or tea.

It will be appreciated by one of skill in the art that the present disclosure encompasses any nematode that secretes a protein that alters the expression of a host gene. For example, one embodiment provides a transgenic plant or cell containing a nucleic acid encoding a protein secreted by a member of *Meloidogyne spp.*, wherein the secreted protein stimulates, enhances, or promotes root growth or development.

Another embodiment provides a composition comprising a nucleic acid having a sequence of SEQ ID NOs. 1, 2 or 5-51 or a fragment or homologues thereof. The composition stimulates, promotes, or enhances root growth or development when delivered to a plant or plant cell.

Still another embodiment provides a composition comprising a one or more polypeptides or fragments thereof encoded by SEQ ID NOs 1, 2 or 5-51 or homologues thereof when delivered to a plant or plant cell.

Root stimulating compositions disclosed herein can optionally contain a growth enhancer, fertilizer, one or more nemiticides, pesticides, fungicides, or combinations thereof. Representative nematicides include, but are not limited to chloropicrin, methyl bromide, 1,3-dichloropropene, sodium methyl dithiocarbamate, sodium tetrathiocarbonate; and carbamates such as 2-methyl-2-(methylthio)propionaldehyde O-methylcarbamoyloxime (aldicarb), 2,3-Dihydro-2,2-dimethyl-7-benzofuranol methylcarbamate (carbofuran), methyl 2-(dimethylamino)-N-[[(methylamino)carbonyl]oxy]-2-oxoethanimidothioate (oxamyl), 2-methyl-2-(methylsulfonyl)propanal O-[(methylamino)carbonyl]oxime (aldoxycarb), *O,O*-diethyl *O*-[4-(methylsulfinyl)phenyl] phosphorothioate (fensulfothion), O-Ethyl S,S-dipropylphosphorodithioate (ethoprop), and Ethyl-3-methyl-4-(methylthio)phenyl(1-methylethyl)phosphoramidate (phenamiphos).

Another embodiment provides a cell, for example a plant cell, containing one or more nucleic acids encoding a nematode secretory polypeptide or fragment thereof wherein the nematode secretory polypeptides directly or indirectly stimulate, enhance or promote root growth or development. The cell can be prokaryotic or eukaryotic, and generally is a plant cell, particularly a root cell.

Still another embodiment provides a method for providing drought resistance to a plant by contacting the plant with one or more nematode esophageal proteins or nucleic acids encoding nematode esophageal proteins in an amount sufficient to stimulate, promote, or enhance root development. The composition can be sprayed onto the plant, applied to the soil surrounding the plant or otherwise delivered to the plant so that the composition contacts the plant.

Plant Transformation Technology

DNA molecules and RNA molecules of the present disclosure are incorporated in plant or bacterial cells using conventional recombinant DNA technology. Generally, a DNA or an RNA molecule of the present disclosure is comprised in a transformation vector. A large number of such vector

systems known in the art may be used, such as plasmids. The components of the expression system are also modified, e.g., to increase expression of the introduced RNA fragments. For example, truncated sequences, nucleotide substitutions or other modifications may be employed. Expression systems known in the art may be used to transform virtually any plant cell under suitable conditions. A transgene comprising a DNA molecule of the present invention is preferably stably transformed and integrated into the genome of the host cells. Transformed cells are preferably regenerated into whole plants. Detailed description of transformation techniques are within the knowledge of those skilled in the art.

Reporter genes or selectable marker genes may be included in the expression cassette. Examples of suitable reporter genes known in the art can be found in, for example, Jefferson et al. (1991) in Plant Molecular Biology Manual, ed. Gelvin et al. (Kluwer Academic Publishers), pp. 1-33; DeWet et al. (1987) Mol. Cell. Biol. 7:725-737; Goff et al. (1990) EMBO J. 9:2517-2522; Kain et al. (1995) Bio Techniques 19:650-655; and Chiu et al. (1996) Current Biology 6:325-330.

Selectable marker genes for selection of transformed cells or tissues can include genes that confer antibiotic resistance or resistance to herbicides. Examples of suitable selectable marker genes include, but are not limited to, genes encoding resistance to chloramphenicol (Herrera Estrella et al. (1983) EMBO J. 2:987-992); methotrexate (Herrera Estrella et al. (1983) Nature 303:209-213; Meijer et al. (1991) Plant Mol. Biol. 16:807-820); hygromycin (Waldron et al. (1985) Plant Mol. Biol. 5:103-108; Zhijian et al. (1995) Plant Science 108:219-227); streptomycin (Jones et al. (1987) Mol. Gen. Genet 210:86-91); spectinomycin (Bretagne-Sagnard et al. (1996) Transgenic Res. 5:131-137); bleomycin (Hille et al. (1990) Plant Mol. Biol 7:171-176); sulfonamide (Guerineau et al. 1990) Plant Mol. Biol. 15:127-136); bromoxynil (Stalker et al. (1988) Science 242:41 9423); glyphosate (Shaw et al. (1986) Science 233:478481); phosphinothricin (DeBlock et al. (1987) EMBO J. 6:2513-2518).

Other genes that could serve utility in the recovery of transgenic events but might not be required in the final product would include, but are not limited to, examples such as GUS (b-glucoronidase; Jefferson (1987) Plant Mol. Biol. Rep. 5:387), GFP (green florescence protein; Chalfie et al. (1994) Science 263:802), luciferase (Riggs et al. (1987) Nucleic Acids Res. 15(19):8115 and Luehrsen et al. (1992) Methods Enzymol. 216:397-414) and the maize genes encoding for anthocyanin production (Ludwig et al. (1990) Science 247:449).

The expression cassette comprising a promoter sequence operably linked to a heterologous nucleotide sequence of interest can be used to transform any plant. In this manner, genetically modified plants, plant cells, plant tissue, seed, and the like can be obtained.

Transformation protocols as well as protocols for introducing nucleotide sequences into plants may vary depending on the type of plant or plant cell, i.e., monocot or dicot, targeted for transformation. Suitable methods of introducing nucleotide sequences into plant cells and subsequent insertion into the plant genome include microinjection (Crossway et al. (1986) Biotechniques 4:320-334), electroporation (Riggs et al. (1986) Proc. Natl. Acad. Sci. USA 83:5602-5606, Agrobacterium-mediated transformation (Townsend et al., U.S. Pat. No. 5,563,055; Zhao et al. WO US98/01268), direct gene transfer (Paszkowski et al. (1984) EMBO J. 3:2717-2722), and ballistic particle acceleration (see, for example, Sanford et al., U.S. Pat. No. 4,945,050; Tomes et al. (1995) "Direct DNA Transfer into Intact Plant Cells via Microprojectile Bombardment," in Plant Cell, Tissue, and Organ Culture: Fundamental Methods, ed. Gamborg and Phillips (Springer-Verlag, Berlin); and McCabe et al. (1988) Biotechnology 6:923-926). Also see Weissinger et al. (1988) Ann. Rev. Genet. 22:421-477; Sanford et al. (1987) Particulate Science and Technology 5:27-37 (onion); Christou et al. (1988) Plant Physiol. 87:671-674 (soybean); McCabe et al. (1988) Bio/Technology 6:923-926 (soybean); Finer and McMullen (1991) In Vitro Cell Dev. Biol. 27P:175-182 (soybean); Singh et al. (1998) Theor. Appl. Genet. 96:319-324 (soybean); Dafta et al. (1990) Biotechnology 8:736-740

(rice); Klein et al. (1988) Proc. Natl. Acad. Sci. USA 85:4305-4309 (maize); Klein et al. (1988) Biotechnology 6:559-563 (maize); Tomes, U.S. Pat. No. 5,240,855; Buising et al., U.S. Pat. Nos. 5,322,783 and 5,324,646; Tomes et al. (1995) "Direct DNA Transfer into Intact Plant Cells via Microprojectile Bombardment," in Plant Cell, Tissue, and Organ Culture: Fundamental Methods, ed. Gamborg (Springer-Verlag, Berlin) (maize); Klein et al. (1988) Plant Physiol. 91:440-444 (maize); Fromm et al. (1990) Biotechnology 8:833-839 (maize); Hooykaas-Van Slogteren et al. (1984) Nature (London) 311:763-764; Bowen et al., U.S. Pat. No. 5,736,369 (cereals); Bytebier et al. (1987) Proc. Natl. Acad. Sci. USA 84:5345-5349 (Liliaceae); De Wet et al. (1985) in The Experimental Manipulation of Ovule Tissues, ed. Chapman et al. (Longman, N.Y.), pp. 197-209 (pollen); Kaeppler et al. (1990) Plant Cell Reports 9:415-418 and Kaeppler et al. (1992) Theor. Appl. Genet. 84:560-566 (whisker-mediated transformation); D'Halluin et al. (1992) Plant Cell 4:1495-1505 (electroporation); Li et al. (1993) Plant Cell Reports 12:250-255 and Christou and Ford (1995) Annals of Botany 75:407-413 (rice); Osjoda et al. (1996) Nature Biotechnology 14:745-750 (maize via Agrobacterium tumefaciens); all of which are herein incorporated by reference in their entirety.

The cells that have been transformed may be grown into plants in accordance with conventional techniques. See, for example, McCormick et al. (1986) Plant Cell Reports 5:81-84. These plants may then be grown, and either pollinated with the same transformed strain or different strains, and the resulting hybrid having constitutive expression of the desired phenotypic characteristic identified. Two or more generations may be grown to ensure that constitutive expression of the desired phenotypic characteristic is stably maintained and inherited and then seeds harvested to ensure constitutive expression of the desired phenotypic characteristic has been achieved.

Chemical-regulated promoters can be used to modulate the expression of a gene in a plant through the application of an exogenous chemical regulator. Depending upon the objective, the promoter may be a chemical-inducible promoter, where application of the chemical induces

gene expression, or a chemical-repressible promoter, where application of the chemical represses gene expression. Chemical-inducible promoters are known in the art and include, but are not limited to, the maize 1n2-2 promoter, which is activated by benzenesulfonamide herbicide safeners, the maize GST promoter, which is activated by hydrophobic electrophilic compounds that are used as pre-emergent herbicides, and the tobacco PR-1 a promoter, which is activated by salicylic acid. Other chemical-regulated promoters of interest include steroid-responsive promoters (see, for example, the glucocorticoid-inducible promoter in Schena et al. (1991) Proc. Natl. Acad. Sci. USA 88:10421-10425 and McNellis et al. (1998) Plant J. 14(2):247-257) and tetracycline-inducible and tetracycline-repressible promoters (see, for example, Gatz et al. (1991) Mol. Gen. Genet. 227:229-237, and U.S. Pat. Nos. 5,814,618 and 5,789,156), herein incorporated by reference in their entirety.

Constitutive promoters include, for example, the core promoter of the Rsyn7 promoter and other constitutive promoters disclosed in WO 99/43838 and U.S. Pat. No. 6,072,050; the core CAMV 35S promoter (Odell et al. (1985) Nature 313:810-812); rice actin (McElroy et al. (1990) Plant Cell 2:163-171); ubiquitin (Christensen et al. (1989) Plant Mol. Biol. 12:619-632 and Christensen et al. (1992) Plant Mol. Biol. 18:675-689); pEMU (Last et al. (1991) Theor. Appl. Genet. 81:581-588); MAS (Velten et al. (1984) EMBO J. 3:2723-2730); ALS promoter (U.S. Pat. No. 5,659,026), and the like. Other constitutive promoters include, for example, U.S. Pat. Nos. 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; 5,608,142.

Where low level expression is desired, weak promoters may be used. Generally, by "weak promoter" is intended a promoter that drives expression of a coding sequence at a low level. By low level is intended at levels of about 1/1000 transcripts to about 1/100,000 transcripts to about 1/500,000 transcripts. Alternatively, it is recognized that weak promoters also encompasses promoters that are expressed in only a few cells and not in others to give a total low level of expression. Where a promoter is expressed

at unacceptably high levels, portions of the promoter sequence can be deleted or modified to decrease expression levels.

Such weak constitutive promoters include, for example, the core promoter of the Rsyn7 promoter (WO 99/43838 and U.S. Pat. No. 6,072,050), the core 35S CaMV promoter, and the like. Other constitutive promoters include, for example, U.S. Pat. Nos. 5,608,149; 5,608,144; 5,604,121; 5,569,597; 5,466,785; 5,399,680; 5,268,463; and 5,608,142.

"Tissue-preferred" promoters can be used to target a gene expression within a particular tissue. Tissue-preferred promoters include Yamamoto et al. (1997) Plant J. 12(2)255-265; Kawamata et al. (1997) Plant Cell Physiol. 38(7):792-803; Hansen et al (1997) Mol. Gen. Genet. 254(3):337-343; Russell et al. (1997) Transgenic Res. 6(2):157-168; Rinehart et al. (1996) Plant Physiol. 112(3):1331-1341; Van Camp et al (1996) Plant Physiol. 112(2):525-535; Canevascini et al. (1996) Plant Physiol. 112(2):513-524; Yamamoto et al. (1994) Plant Cell Physiol. 35(5):773-778; Lam (1994) Results Probl. Cell Differ. 20:181-196; Orozco et al. (1993) Plant Mol. Biol. 23(6):1129-1138; Matsuoka et al. (1993) Proc Natl. Acad. Sci. USA 90(20):9586-9590; and Guevara-Garcia et al. (1993) Plant J. 4(3):495-505. Such promoters can be modified, if necessary, for weak expression.

"Seed-preferred" promoters include both "seed-specific" promoters (those promoters active during seed development such as promoters of seed storage proteins) as well as "seed-germinating" promoters (those promoters active during seed germination). See Thompson et al. (1989) BioEssays 10:108, herein incorporated by reference. Such seed-preferred promoters include, but are not limited to, Cim1 (cytokinin-induced message); cZ19B1 (maize 19 kDa zein); milps (myo-inositol-1-phosphate synthase); and ce1A (cellulose synthase). Gama-zein is a preferred endosperm-specific promoter. Glob-1 is a preferred embryo-specific promoter. For dicots, seed-specific promoters include, but are not limited to, bean.beta.-phaseolin, napin,.beta.-conglycinin, soybean lectin, cruciferin, and the like. For monocots, seed-specific promoters include, but are not limited to, maize 15 kDa zein, 22 kDa zein, 27 kDa zein, g-zein, waxy, shrunken 1, shrunken 2, globulin 1, etc.

Leaf-specific promoters are known in the art. See, for example, Yamamoto et al. (1997) Plant J. 12(2):255-265; Kwon et al. (1994) Plant Physiol. 105:357-67; Yamamoto et al. (1994) Plant Cell Physiol. 35(5):773-778; Gotor et al. (1993) Plant J. 3:509-18; Orozco et al. (1993) Plant Mol. Biol. 23(6):1129-1138; and Matsuoka et al. (1993) Proc. Natl. Acad. Sci. USA 90(20):9586-9590.

Root-preferred promoters are known and may be selected from the many available from the literature or isolated de novo from various compatible species. See, for example, Hire et al. (1992) Plant Mol. Biol. 20(2): 207-218 (soybean root-specific glutamine synthetase gene); Keller and Baumgartner (1991) Plant Cell 3(10):1051-1061 (root-specific control element in the GRP 1.8 gene of French bean); Sanger et al. (1990) Plant Mol. Biol. 14(3):433-443 (root-specific promoter of the mannopine synthase (MAS) gene of Agrobacterium tumefaciens); and Miao et al. (1991) Plant Cell 3(1):l 1'-22 (full-length cDNA clone encoding cytosolic glutamine synthetase (GS), which is expressed in roots and root nodules of soybean). See also U.S. Pat. Nos. 5,837,876; 5,750,386; 5,633,363; 5,459,252; 5,401,836; 5,110,732; and 5,023,179.

Chloroplast targeting sequences are known in the art and include the chloroplast small subunit of ribulose-1,5-bisphosphate carboxylase (Rubisco) (de Castro Silva Filho et al. (1996) Plant Mol. Biol. 30:769-780; Schnell et al. (1991) J. Biol. Chem. 266(5):3335-3342); 5-(enolpyruvyl)shikimate-3-phosphate synthase (EPSPS) (Archer et al. (1990) J. Bioenerg. Biomemb. 22(6):789-810); tryptophan synthase (Zhao et al. (1995) J. Biol. Chem. 270(11):6081-6087); plastocyanin (Lawrence et al. (1997) J. Biol. Chem. 272(33):20357-20363); chorismate synthase (Schmidt et al. (1993) J. Biol. Chem. 268(36):27447-27457); and the light harvesting chlorophyll a/b binding protein (LHBP) (Lamppa et al. (1988) J. Biol. Chem. 263:14996-14999). See also Von Heijne et al. (1991) Plant Mol. Biol. Rep. 9:104-126; Clark et al. (1989) J. Biol. Chem. 264:17544-17550; Della-Cioppa et al. (1987) Plant Physiol. 84:965-968; Romer et al. (1993) Biochem. Biophys.

Res. Commun. 196:1414-1421; and Shah et al. (1986) Science 233:478-481.

Methods for transformation of chloroplasts are known in the art. See, for example, Svab et al. (1990) Proc. Natl. Acad. Sci. USA 87:8526-8530; Svab and Maliga (1993) Proc. Natl. Acad. Sci. USA 90:913-917; Svab and Maliga (1993) EMBO J. 12:601-606. The method relies on particle gun delivery of DNA containing a selectable marker and targeting of the DNA to the plastid genome through homologous recombination. Additionally, plastid transformation may be accomplished by transactivation of a silent plastid-bome transgene by tissue-preferred expression of a nuclear-encoded and plastid-directed RNA polymerase. Such a system has been reported in McBride et al. (1994) Proc. Natl. Acad. Sci. USA 91:7301-7305.

The nucleic acids of interest to be targeted to the chloroplast may be optimized for expression in the chloroplast to account for differences in codon usage between the plant nucleus and this organelle. In this manner, the nucleic acids of interest may be synthesized using chloroplast-preferred codons. See, for example, U.S. Pat. No. 5,380,831, herein incorporated by reference.

Plants transformed in accordance with the present disclosure may be monocots or dicots and include, but are not limited to, any nematode host plant.

Requirements for Construction of Plant Expression Cassettes

Nucleic acid sequences intended for expression in transgenic plants are first assembled in expression cassettes behind a suitable promoter expressible in plants. The expression cassettes may also comprise any further sequences required or selected for the expression of the transgene. Such sequences include, but are not restricted to, transcription terminators, extraneous sequences to enhance expression such as introns, vital sequences, and sequences intended for the targeting of the gene product to specific organelles and cell compartments. These expression cassettes can then be easily transferred to the plant transformation vectors described infra.

The following is a description of various components of typical expression cassettes.

Promoters

The selection of the promoter used in expression cassettes determine the spatial and temporal expression pattern of the transgene in the transgenic plant. Selected promoters express transgenes in specific cell types (such as leaf epidermal cells, mesophyll cells, root cortex cells) or in specific tissues or organs (roots, leaves or flowers, for example) and the selection reflects the desired location of accumulation of the gene product. Alternatively, the selected promoter drives expression of the gene under various inducing conditions.

Promoters vary in their strength, i.e., ability to promote transcription. Depending upon the host cell system utilized, any one of a number of suitable promoters known in the art may be used. For example, for constitutive expression, the CaMV 35S promoter, the rice actin promoter, or the ubiquitin promoter may be used. For example, for regulatable expression, the chemically inducible PR-1 promoter from tobacco or Arabidopsis may be used (see, e.g., U.S. Pat. No. 5,689,044).

A suitable category of promoters is that which is wound inducible. Numerous promoters have been described which are expressed at wound sites. Preferred promoters of this kind include those described by Stanford et al. Mol. Gen. Genet. 215: 200-208 (1989), Xu et al. Plant Molec. Biol. 22: 573-588 (1993), Logemann et al. Plant Cell 1: 151-158 (1989), Rohrmeier & Lehle, Plant Molec. Biol. 22: 783-792 (1993), Firek et al. Plant Molec. Biol. 22: 129-142 (1993), and Warner et al. Plant J. 3: 191-201 (1993).

Suitable tissue specific expression patterns include green tissue specific, root specific, stem specific, and flower specific. Promoters suitable for expression in green tissue include many which regulate genes involved in photosynthesis, and many of these have been cloned from both monocotyledons and dicotyledons. A suitable promoter is the maize PEPC promoter from the phosphoenol carboxylase gene (Hudspeth & Grula, Plant

Molec.Biol. 12: 579-589 (1989)). A suitable promoter for root specific expression is that described by de Framond (FEBS 290: 103-106 (1991); EP 0 452 269 and a root-specific promoter is that from the T-1 gene. A suitable stem specific promoter is that described in U.S. Pat. No. 5,625,136 and which drives expression of the maize trpA gene.

Transcriptional Terminators

A variety of transcriptional terminators are available for use in expression cassettes. These are responsible for the termination of transcription beyond the transgene and its correct polyadenylation. Appropriate transcriptional terminators are those that are known to function in plants and include the CaMV 35S terminator, the tm1 terminator, the nopaline synthase terminator and the pea rbcS E9 terminator. These are used in both monocotyledonous and dicotyledonous plants.

Sequences for the Enhancement or Regulation of Expression

Numerous sequences have been found to enhance gene expression from within the transcriptional unit and these sequences can be used in conjunction with the genes to increase their expression in transgenic plants. For example, various intron sequences such as introns of the maize Adh1 gene have been shown to enhance expression, particularly in monocotyledonous cells. In addition, a number of non-translated leader sequences derived from viruses are also known to enhance expression, and these are particularly effective in dicotyledonous cells.

Coding Sequence Optimization

The coding sequence of the selected gene may be genetically engineered by altering the coding sequence for optimal expression in the crop species of interest. Methods for modifying coding sequences to achieve optimal expression in a particular crop species are well known (see, e.g. Perlak et al., Proc. Natl. Acad. Sci. USA 88: 3324 (1991); and Koziel et al, Bio/technol. 11: 194 (1993)).

Another embodiment provides an RNA molecule directly transformed into the plastid genome. Plastid transformation technology is extensively described in U.S. Pat. Nos. 5,451,513,5,545,817, and 5,545,818, in PCT application no. WO 95/16783, and in McBride et al. (1994) Proc. Natl. Acad. Sci. USA 91,7301-7305. The basic technique for chloroplast transformation involves introducing regions of cloned plastid DNA flanking a selectable marker together with the gene of interest into a suitable target tissue, e.g., using biolistics or protoplast transformation (e.g., calcium chloride or PEG mediated transformation). The 1 to 1.5 kb flanking regions, termed targeting sequences, facilitate homologous recombination with the plastid genome and thus allow the replacement or modification of specific regions of the plastome. Initially, point mutations in the chloroplast 16S rRNA and rps12 genes conferring resistance to spectinomycin and/or streptomycin are utilized as selectable markers for transformation (Svab, Z., Hajdukiewicz, P., and Maliga, P. (1990) Proc. Natl. Acad. Sci. USA 87,8526-8530; Staub, J. M., and Maliga, P. (1992) Plant Cell 4,39-45). The presence of cloning sites between these markers allowed creation of a plastid targeting vector for introduction of foreign DNA molecules (Staub, J. M., and Maliga, P. (1993) EMBO J. 12,601-606). Substantial increases in transformation frequency are obtained by replacement of the recessive rRNA or r-protein antibiotic resistance genes with a dominant selectable marker, the bacterial aadA gene encoding the spectinomycin-detoxifying enzyme aminoglycoside-3'-adenyltransferase (Svab, Z., and Maliga, P. (1993) Proc. Natl. Acad. Sci. USA 90,913-917). Previously, this marker had been used successfully for high-frequency transformation of the plastid genome of the green alga Chlamydomonas reinhardtii (Goldschmidt-Clermont, M. (1991) Nucl. Acids Res. 19: 4083-4089). Other selectable markers useful for plastid transformation are known in the art and are encompassed within the scope of the invention.

Construction of Plant Transformation Vectors

Numerous transformation vectors available for plant transformation are known to those of ordinary skill in the plant transformation arts, and the genes pertinent to this disclosure can be used in conjunction with any such vectors. The selection of vector depends upon the selected transformation technique and the target species for transformation. For certain target species, different antibiotic or herbicide selection markers are preferred. Selection markers used routinely in transformation include the npt11 gene, which confers resistance to kanamycin and related antibiotics (Messing & Vierra. Gene 19: 259-268 (1982); Bevan et al., Nature 304: 184-187 (1983)), the bar gene, which confers resistance to the herbicide phosphinothricin (White et al., Nucl. Acids Res 18: 1062 (1990), Spencer et al. Theor. Appl. Genet 79: 625-631 (1990)), the hph gene, which confers resistance to the antibiotic hygromycin (Blochinger & Diggelmann, Mol Cell Biol 4: 2929-2931), the manA gene, which allows for positive selection in the presence of mannose (Miles and Guest (1984) Gene, 32: 41-48; U.S. Patent No. 5,767,378), and the dhfr gene, which confers resistance to methotrexate (Bourouis et al., EMBO J. 2 (7): 1099-1104 (1983)), and the EPSPS gene, which confers resistance to glyphosate (U.S. Pat. Nos. 4,940,935 and 5,188,642).

Many vectors are available for transformation using Agrobacterium tumefaciens. These typically carry at least one T-DNA border sequence and include vectors such as pBIN19 (Bevan, Nucl. Acids Res. (1984). Typical vectors suitable for Agrobacterium transformation include the binary vectors pCIB200 and pCIB2001, as well as the binary vector pCIB 10 and hygromycin selection derivatives thereof. (See, for example, U.S. Pat. No. 5,639,949).

Transformation without the use of Agrobacterium tumefaciens circumvents the requirement for T-DNA sequences in the chosen transformation vector and consequently vectors lacking these sequences are utilized in addition to vectors such as the ones described above which contain T-DNA sequences. Transformation techniques that do not rely on

Agrobacterium include transformation via particle bombardment, protoplast uptake (e.g. PEG and electroporation) and microinjection. The choice of vector depends largely on the preferred selection for the species being transformed. Typical vectors suitable for non-Agrobacterium transformation include pCIB3064, pSOG 19, and pSOG35. (See, for example, U.S. Pat. No. 5,639,949).

Transformation Techniques

Once the DNA sequence of interest is cloned into an expression system, it is transformed into a plant cell. Methods for transformation and regeneration of plants are well known in the art. For example, Ti plasmid vectors have been utilized for the delivery of foreign DNA, as well as direct DNA uptake, liposomes, electroporation, micro-injection, and microprojectiles. In addition, bacteria from the genus Agrobacterium can be utilized to transform plant cells.

Transformation techniques for dicotyledons are well known in the art and include Agrobacterium-based techniques and techniques that do not require Agrobacterium. Non Agrobacterium techniques involve the uptake of exogenous genetic material directly by protoplasts or cells. This is accomplished by PEG or electroporation mediated uptake, particle bombardment-mediated delivery, or microinjection. In each case the transformed cells may be regenerated to whole plants using standard techniques known in the art.

Transformation of most monocotyledon species has now become somewhat routine. Preferred techniques include direct gene transfer into protoplasts using PEG or electroporation techniques, particle bombardment into callus tissue or organized structures, as well as Agrobacterium-mediated transformation.

Plants from transformation events are grown, propagated and bred to yield progeny with the desired trait, and seeds are obtained with the desired trait, using processes well known in the art. The methods can result in plant cells comprising the RNA fragments of the present invention, wherein the

expression of said target gene in said plant cell is altered by said RNA fragments, a plant and the progeny thereof derived from the plant cell, and seeds derived from the plant.

The disclosed inhibitory nucleic acids or RKN esophageal gland cell secretory polypeptides may be used alone or as a component of a kit having at least one of the reagents necessary to carry out the in vitro or in vivo introduction of RNA to subjects. Suitable components are the dsRNA and a vehicle that promotes introduction of the dsRNA. Such a kit may also include instructions to allow a user of the kit to practice the invention.

Another embodiment provides a method for providing resistance to nematode disease by introducing into a nematode host plant cell an RNA comprising a double stranded structure having a nucleotide sequence which is complementary to at least a part of the target mRNA; and optionally verifying inhibition of expression of the target mRNA.

One embodiment provides a method for treating or preventing nematode disease in a plant by contacting a parasitic nematode in or on the plant with a with dsRNA having a sequence which is complementary to at least a part of a mRNA encoding a nematode secretory protein, for example an esophageal gland cell protein; wherein the secretory protein modulates gene expression of plant.

Still another embodiment provides a plant cell, for example, containing an expression construct, the construct coding for an RNA which forms a double stranded structure having a nucleotide sequence which is complementary to at least a part of a target mRNA that encodes a nematode secretory protein, for example an esophageal gland cell protein, as well as a transgenic plant containing such a cell.

In another embodiment, the RNA fragments are comprised in two different RNA molecules. In this case, the RNA fragments are mixed before being introduced into said cell, e.g. under conditions allowing them to form a double-stranded RNA molecule. In another embodiment, the RNA fragments are introduced into said cell sequentially. Preferably, the time interval

between the introduction of each of the RNA molecules is short, preferably less than one hour.

In still another embodiment, the RNA fragments are comprised in one RNA molecule. By using one single RNA molecule, the two complementary RNA fragments are in close proximity such that pairing and double strand formation is favored. In such case, the RNA molecule is preferably capable of folding such that said RNA fragments comprised therein form a double-stranded region. In this case, the complementary parts of the RNA fragments recognize one another, pair with each other and form the double-stranded RNA molecule. In another embodiment, the RNA fragments are incubated under conditions allowing them to form a double-stranded RNA molecule prior to introduction into the cell. In yet another embodiment, the RNA molecule comprises a linker between the sense RNA fragment and the antisense RNA fragment. The linker preferably comprises a RNA sequence encoded by an expression cassette comprising a functional gene, e.g. a selectable marker gene. In another embodiment, the linker comprises a RNA sequence encoded by regulatory sequences, which e.g. comprise intron processing signals.

Another embodiment provides a dsRNA construct having a promoter operably linked to said dsRNA and might further comprise said dsRNA molecule. The promoter can be a heterologous promoter, for example a tissue specific promoter, a developmentally regulated promoter, a constitutive promoter, divergent or an inducible promoter. Termination signal are also optionally included in the DNA molecules.

The single RNA molecule or the two distinct RNA molecules are preferably capable of forming a double-stranded region, in which the complementary parts of the RNA fragments recognize one another, pair with each other and form the double-stranded RNA molecule.

Another embodiment provides the disclosed transgenic plant material in the form of feedstock, pellets, granules, flakes and the like. The inhibitory nucleic acids disclosed here can be in seeds and seed products derived from the transgenic plants described above. Another embodiment provides

a composition comprising the disclosed inhibitory nucleic acids that can be coated on seeds. The coating can be formulated so that the inhibitory nucleic acids remain able to inhibit nematode secretory proteins as the seed matures and develops roots.

A further embodiment provides provides chimeric or fusion proteins containing the disclosed nematode esophageal gland cell proteins or fragments thereof. As used herein, a "chimeric protein" or "fusion protein" includes a nematode esophageal gland cell protein or fragment thereof linked to a foreign polypeptide. A "foreign polypeptide" is polypeptide that is not substantially homologous to a nematode esophageal gland cell protein or fragment thereof. The foreign polypeptide can be fused to the N-terminus or C-terminus of the nematode esophageal gland cell protein or fragment thereof.

The fusion protein can include a moiety which has a high affinity for a ligand. For example, the fusion protein can be a GST fusion protein in which a nematode esophageal gland cell protein or fragment thereof is fused to the C-terminus of GST. Such fusion proteins can facilitate the purification of the polypeptide. Alternatively, the fusion protein can contain a heterologous signal sequence at its N-terminus. In certain host cells, expression, secretion or transport of a protein can be increased through use of a heterologous signal sequence. For example, in a plant cell, a polypeptide of the invention may be fused with a chloroplast transit peptide. The chloroplast transit peptide allows the polypeptide to be transported from the cytoplasm of the plant cell into the chloroplast, thereby increase root growth. Expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). A nucleic acid encoding a nematode esophageal gland cell protein or fragment thereof can be cloned into such an expression vector so that the fusion moiety is linked in-frame to the polypeptide.

The following are only exemplary examples. It should be understood that the invention is not limited to these examples. Other important applications of disclosure would be readily recognized by those of ordinary

skills in the art. Other uses which are potentially recognizable by those of ordinary skills in the art are also part of the disclosure.

The references mentioned herein are incorporated in their entirety to the fullest extent permitted by applicable law.

EXAMPLES

Example 1: Nematodes and Plants

Meloidogyne species were propagated on roots of greenhouse-grown tomato (*Lycopersicon esculentum* cv. Marion or Better-Boy). *Meloidogyne* eggs were collected as described (Hussey and Barker, 1973). Pre-parasitic second-stage juveniles (pre-J2) were collected *via* hatching eggs on 25-μm-pore sieves in deionized water in plastic bowls. The different parasitic stages of *M. incognita* were collected by root blending and sieving (Ding et al. 1998). Mixed parasitic stages (MS) of *M. incognita* for *in situ* hybridizations were collected 13-15 days after inoculation of eggs as described in De Boer et al. (1998). Similarly, pre-J2 and MS of *Heterogera glycines* were collected from infected soybean (*Glycine max*) roots. *Caenorhabditis elegans* was cultured on OP50 of *E. coli* (Brenner, 1974). One-month-old host plant leaves were collected from growth-chamber grown *Nicotiana tabacum* cv. Petite Havana SR1, and *Arabidopsis thaliana* ecotype Col-0.

Example 2: Nucleic Acid Manipulation

Pre-J2 of packed nematodes were frozen in 1.5-ml microcentrifuge tubes with liquid nitrogen and ground with a smooth-end metal bar. The frozen nematode fragments were mixed with 0.5ml extraction solution (100 mM NaCl, 100 mM Tris-HCl [pH8.0], 50 mM EDTA, 1% sodium dodecyl sulfate, 4 mg/ml proteinase K and 10 μg/ml RNase) and incubated at 37°C for 1 hr. DNA was extracted with phenol/chloroform and then precipitated with isopropanol (Sambrook et al., 1989). The DNA was re-suspended in H_2O. Tobacco and *Arabidopsis* genomic DNA was extracted using standard techniques (Dellaporta 1993).

mRNAs were extracted and purified from ground plant tissues using Dynabeads mRNA DIRECT kit (Dynal, Lake Success, NY), eluted with 10μl diethylpyrocarbonate (DEPC)-treated water, and converted into first-strand cDNA by reverse transcription (RT)-PCR SMART PCR cDNA Synthesis kit (BD Biosciences, Palo Alto, CA), following the manufacturer's instructions. RT-PCR reactions contained the following components: 4.0 μl of 5× first-strand buffer, 2.0μl of 20mM DTT, 2.0μl of 10mM 50× dNTP, 1μl of 10μM 3'-CDS primer, 10μl of isolated mRNA and 1μl of Superscript II reverse transcriptase (200 units/μl, Gibco BRL, Rockville, MD). The reaction was incubated at 42°C for 1hr.

Example 3: Isolation of *16D10* cDNA Clone

Clone 16D10 encoding a secretory signaling peptide was identified during random sequencing of a gland-cell specific cDNA library of *M. incognita* (Huang et al., 2003) and designated as *16D10*. The full-length double-strand cDNA sequences of *16D10* in pGEM-T Easy vector were obtained by using T7 and SP6 primers in sequencing reactions. The longest open reading frame of the 16D10 cDNA (364 bp) encoded a deduced protein of 43 aa including a 30 aa N-terminal hydrophobic signal peptide as predicted by Signal P (Nielsen *et al*, 1997). While the mature 16D10 peptide of 13 aa (GKKPSGPNPGGNN, M_r 1,223 Da)(SEQ ID NO:52) provided no significant BLASTX similarity, it did contain 8 aa (K---PSGPNP--N) (SEQ ID NO:53) of the conserved C-terminal 13 aa motif (KRLVPSGPNPLHN)(SEQ ID NO:54) of the functional domain of *Arabidopsis* CLV3-like proteins (Cock and McCormick, 2001) as well as a cAMP/cGMP-dependent protein kinase phosphorylation site [KKpS] as predicted by PROSITE (Hofmann *et al*, 1999).

Example 4: Genomic Clones in *Meloidogyne* Species

One pair of the gene-specific primers 16D10GF (5'-GAGAAAATAAAATATAAATTATTCCTC-3') (SEQ ID NO:55) and 16D10GR (5'-CAGATATAATTTTATTCAG-3') (SEQ ID NO:56) designed from the most

extreme 5'- and 3'-ends of the cDNA sequence of *M. incognita 16D10*, were used to amplify the corresponding genomic sequences (or the highest homologues) from 200ng of *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* genomic DNA. The PCR products were cut from a 1.2% agarose gel, and purified with a QIAquick gel extraction kit (Qiagen, Valencia, CA). The purified products were cloned into pGEM-T Easy vector (Promega, Madison, WI) for sequencing. The 16D10 homologues from the *Meloidogyne* species shared over 95% identity at the nucleotide level and the deduced proteins encoded by putative cDNAs were identical to that of *M. incognita* 16D10.

Example 5: Southern Blot Analysis

For each sample, 10 μg of genomic DNA was completely digested with 50 units of *Eco*RI or *Bam*HI (New England Biolabs, Beverly, MA), separated on a 0.7% (w/v) agarose gel, transferred onto a Hybond-N Nylon membranes (Amersham Pharmacia Biotech, Piscataway, NJ) and blotting using a standard protocol (Sambrook et al., 1989). *16D10* probe was generated by amplification of the corresponding full-length cDNA from insert in pGEM-T Easy vector with T7 and SP6 primers. Gel-purified PCR products were labeled by PCR with a PCR-DIG probe synthesis system (Roche Applied Science, Indianapolis, IN). About 15ng of DIG-labeled probe per ml was used for each-hybridization. Hybridizations were performed in DIG Easy Hyb solution (Roche Applied Science, Indianapolis, IN) at 40°C for16 h followed by two 5-min washes in 2×SSC/0.1% SDS solution at RT. The membranes were then washed twice at 68°C with 0.5× SSC/0.1% SDS solution for 30 min. After incubating the membrane in 1% blocking reagent for 1 hr, the membranes were incubated with a 1:10,000 dilution of sheep anti-DIG alkaline phosphatase (AP) conjugate for 30 min. Unbound antibody was removed by two 15-min washes with maleic acid washing buffer (100 mM maleic acid, 150 mM NaCl, pH7.5, and 0.3% Tween 20). The membrane was incubated in AP detection buffer (100 mM Tris-HCl, pH9.5, 100 mM NaCl, and 50 mM $MgCl_2$) for 10 min followed by a 1:50 dilution of the chemiluminescent substrate disodium 3-(4-methoxyspiro{1,2-dioxetane-3,2'-

(5'-chloro)tricyclo[3.3.1.1^{3,7}]decan}-4-yl)pheryl phosphate (CSPD) (Roche Applied Science) before sealing the membrane in two sheets of transparency film and exposing it to X-ray film for 1.5 hr. A blot containing genomic DNA from *M. incognita*, *M. javanica*, *M. arenaria* and *M. hapla* hybridized with a 16D10 cDNA probe showed that 16D10 was present in each of the four agriculturally important *Meloidogyne* species with 3-4 copies or homologues (Figure 6). No hybridization was detected with genomic DNAs from the soybean cyst nematode *H. glycines*, the non-parasitic free-living nematode *Caenorhabditis elegans*, and plants (tobacco and *Arabidopsis*).

Example 6: Sequence Analyses

Sequence similarity searches were carried out using the BLAST programs PSI-BLASTP and BLASTX at the National Center for Biotechnology Information (NCBI) (Altschul et al., 1998). Multiple sequence alignments of *Meloidogyne* 16D10 genomic DNA sequences were generated using ClustalW1.8 (Jeanmougin et al., 1994). Prediction of a signal peptide for secretion and the cleavage site was performed *via* the SignalP program (Nielsen et al., 1997).

Example 7: *In situ* Hybridization

Specific forward and reverse primers for 16D10 cDNA clone were used to synthesize digoxigenin (DIG)-labeled sense and antisense cDNA probes (Roche Applied Science, Indianapolis, IN) by asymmetric PCR (Huang et al., 2003). *In situ* hybridization was performed using formalin-fixed, permeabilized pre-parasitic juveniles and mixed parasitic stages of *M. incognita* (De Boer et al., 1998; Huang et al., 2003). cDNA probes that hybridized within the nematode were detected with alkaline phosphatase-conjugated anti-DIG antibody and substrate, and specimens were observed with a compound light microscope (De Boer et al., 1998). *In situ* mRNA hybridization revealed that 16D10 was strongly expressed in the two

subventral esophageal gland cells of *M. incognita* at the early parasitic stages.

Example 8: Immunofluorescence Assay

The purified 16D10 polyclonal antiserum was used to localize 16D10 expression in sections of pre-parasitic J2, mixed parasitic stages of *M. incognita* with indirect immunofluorescence as described previously by Goverse et al. (1994). Following fixation in freshly prepared 2% paraformaldehyde in PBS buffer (80 mM Na_2HPO_4, 20 mM NaH_2PO_4, 100 mM NaCl, pH7.4) for 5 days at 4°C, the nematodes were washed three times in PBS buffer and once in deionized water. The fixed nematodes were cut into sections and incubated in 0.6mg of proteinase K (Roche Applied Science, Indianapolis, IN) per ml in phosphate buffered saline (PBS) buffer at 37°C for 1 hr. After washed once with PBS, the partially digested nematodes were placed in a –80°C freezer for 20 min, incubated in dry-ice cold methanol for 3min, and then incubated in dry-ice cold acetone for 15min. The nematodes were washed once with blocking solution (10% goat serum, 0.02% NaN_3, 1mM phenylmethylsulfonyl fluoride, 1× PBS) amended with protease inhibitors as previously described (Goverse et al., 1994), incubated at 4°C for 3 days and then used immediately for immunofluorescence. The blocked nematodes were aliquoted to wells of a 96-well MultiScreen plate (Millipore, Bedford, MA), and agitated in a 1:250 dilution of the 16D10 purified polyclonal antibody in ELISA diluent (0.05% Tween, 0.02% NaN_3, 1% BSA, 1× PBS) in a moisture chamber overnight at RT. Nematode sections were washed three times for 5 min each with PBST (1× PBS, 0.5% Triton X-100) and agitated in a 1:1000 dilution of fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG (Sigma-Aldrich, St. Louis, MO) in Tris-Saline-BSA (0.15M NaCl, 0.01M Tris, pH7.2, 0.2% Triton X-100, 3% BSA) in the dark for 3h at RT. Sections were washed twice in PBST and once with distilled water. Treated sections were transferred in a 15-µl drop of water to individual wells on Multitest slides (ICN-Flow, Horsham, PA) that previously coated with 5µl of 0.1% poly-L-lysine (Sigma

Chemical). Sections were airy dried on slides, covered with a 3µl drop of antiquenching agent (0.02mg/ml phenylenediamine in 500mM carbonate buffer, pH8.6, mixed with nonfluorescent glycerol), and a coverslip was applied. Specimens were observed on an Olympus fluorescence microscope. Negative control consisted of pre-immune rabbit serum. The purified 16D10 antiserum bound to secretory granules within the subventral gland cells of pre-parasitic and parasitic J2 and their cytoplasmic extensions and expanded ampullae, which are located posterior to the pump chamber at the metacarpus. No specific labeling with the rabbit preimmune serum was observed in any nematode specimens.

Example 9: Protein Extraction

Nematode proteins were extracted by grinding pre-parasitic J2 and mixed parasitic stages of *M. incognita* and *H. glycines* in 200µl of extraction buffer [100mM Tris-HCl, pH7.0, 150mM NaCl and 1× complete protease inhibitors (Roche Applied Science, Indianapolis, IN)] in microcentrifuge tubes in liquid nitrogen. Plant proteins (0.5 g) were extracted by grinding transgenic seedlings or root tissues in 200 µl of extraction buffer [50 mM Tris-HCl, pH7.0, 150 mM NaCl, 1× complete protease inhibitors (Roche Applied Science)] in microcentrifuge tubes in liquid nitrogen. Supernatant was recovered from homogenates after centrifugation at 13,000 rpm for 10 min. All protein concentrations were estimated (with a Bio-Rad Protein Assay Kit II) with BSA as a standard. As the positive control, the 16D10 peptide (GKKPSGPNPGGNN, >95% purity)(SEQ ID NO:52) was synthesized from Sigma-Genosys, TX for immunodetection assays (see examples 12-13).

Example 10: Collection of Stylet Secretions

Stylet secretions from *M. incognita* J2 were produced and collected *in vitro* as described by Davis et al. (1994). Pre-parasitic J2 were incubated in 0.4% resorcinol (Sigma-Aldrich, St. Louis, MO) for 6 hr at room temperature in a humid chamber. Stylet secretions were solubilized *via* adding an equal volume of 0.1M Tris-NaOH, pH11.0. Solubilized stylet secretions were

concentrated with StrataClean (Stratagene, La Jolla, CA). Briefly, soluble secretory proteins were trapped *via* suspending 1.5ml of beads in the supernatant of induction mixture (460ml) and incubating it for 1 hr under constant mixing. The beads were centrifuged, re-suspended in 2× SDS-PAGE sample buffer, and boiled for 3 min to release the absorbed proteins. The concentrated stylet secretions were used in enzyme-linked immunosorbent assay (ELISA) and immunoblotting analyses using the purified 16D10 antiserum (see Examples 11-13). Both assays identified 16D10 peptide in the stylet secretions as well as total extracts of J2 and mixed parasitic stages of *M. incognita.*

Example 11: Production of Antisera

Polyclonal antiserum to 16D10 was produced by immunizing two rabbits with a synthetic mature (i.e., without the N-terminal signal peptide) 16D10 peptide (GKKPSGPNPGGNN)(SEQ ID NO:52) from Eurogentec, Inc. (Herstal, Belgium). The antiserum was affinity-purified from 15 ml of last crude sera with the peptide antigen. Peptide affinity-purified 16D10 polyclonal antiserum was used to localize 16D10 expression in specimens of *M. incognita* using immunofluorescence microscopy (Goverse *et al*, 1994), and for immunodetection of 16D10 in stylet secretions and transgenic plant-expressed or *in vitro* translated 16D10.

Example 12: Western Dot-blot Analysis

Protein samples (2μl) were spotted onto Hybond ECL nitrocellulose. The nitrocellulose membrane was allowed to air dry for 20 min. The membrane was incubated in a blocking solution (2% nonfat dry milk, 1× Tris-buffered-saline-Tween [TBS-T: 20 mM Tris-HCl, pH7.4, 0.8% NaCl, 0.1% Tween 20] overnight at 37°C and then treated with the purified 16D10 polyclonal antiserum (1:2,000), followed by anti-rabbit IgG (whole molecule) alkaline phosphatase conjugate (1:30,000) (Sigma). The membrane was washed three times in 1× TBS-T buffer at room temperature, and incubated in the substrate solution (45μl of nitroblue tetrazolium [NBT] solution and

35µl of 5-bromo-4-chloro-3-indolyl-phosphate toluidinium [BCIP] solution in 10 ml of AP buffer [100 mM Tris-HCl, pH9.5, 100 mM NaCl, 5 mM MgCl]) at room temperature until color develops.

Example 13: ELISA Assay

ELISA was modified from Pratt et al. (1986). Dynatech Immulon plate wells were coated overnight at 4°C with proteins diluted in borate saline (0.2M sodium borate, 75mM NaCl, pH8.5) from the following sources: 2µl of 1000× concentrated stylet secretions of *M. incognita* J2, 10µg of total extracted proteins of pre-J2 of *M. incognita*, MS of *M. incognita*, pre-J2 and MS of *H. glycines*, or 10µg of BSA (Sigma Chemical) as a negative control. As a positive control, wells were coated with 100ng of synthetic 16D10 peptide (>95% purity, Sigma-Genosys, TX). Wells were rinsed three times with wash buffers (10mM Tris.HCl, pH8.0, 0.5M NaCl) and blocked with 1% BSA in PBS (32.9mM Na_2HPO_4, 1.77mM NaH_2PO_4, 0.14M NaCl, pH7.4) for 30 min at room temperature. After being rinsed once with wash buffer, each coated well was incubated with 16D10 purified polyclonal antisera diluted 1:1,000 with 0.5% BSA in PBS for 1 hr at room temperature. Negative controls included omitting incubation with the primary polyclonal antibody, and incubation with the rabbit pre-immune serum. The wells were washed three times, incubated with alkaline phosphatase-conjugated goat anti-rabbit antibody (Sigma Chemical) at 1:5,000 dilution for 1 hr at room temperature, washed three times before phosphate colorimetric substrate was added. The substrate, *p*-nitrophenyl phosphate was prepared according to manufacturer's directions in alkaline phosphatase buffer (1M diethanolamine, 0.5mM $MgCl_2$, pH 9.8) and incubated in the treated wells 30 min at room temperature before the reaction was stopped with 3 N NaOH. Absorbance was measured at 405 nm and 490 nm on an ELISA reader.

Example 14: Plasmid Construction

The coding regions of 16D10 with or without a signal peptide sequence were amplified from the full-length cDNA clone with primers

16D10SF (5'-CGGGGTACC*TAG*ATGTTTACTAATTCAATTAA-3') (SEQ ID NO:57) or 16D10F (5'-CGGGGTACC*TAGATG*GGCAAAAAGCCTAGTG-3') (SEQ ID NO:58) and 16D10R (5'-GCTCTAGATCAATTATTTCCTCCAGG-3') (SEQ ID NO:59) that introduced *Kpn*I or *Xba*I restriction sites (underlined) and the stop/start codons (in italics), cloned into the *Kpn*I and *Xba*I sites of binary vector pBIX under the control of CaMV 35S promoter to generate pBIX(16D10S) and pBIX(16D10), respectively, and confirmed by sequencing. pBIX was derived from pBI101 (BD Biosciences, Palo Alto, CA) and contains a *nos* promoter-*nptII*-nos terminator cassette, a 35S promoter-*gusA-nos* terminator, and a second 35S promoter with a polylinker having *Kpn*I and *Xba*I sites. The hybrid expressed sequence of *clv3* and *16D10* was generated by PCR amplifications from *Arabidopsis* genomic DNA using primers C3K (5'-GGGGTACCATGGATTCTAAAAGCTTTG-3') (SEQ ID NO:60) that introduced *Kpn*I restriction site (underlined) and C3R (5'-CCACTAGGCTTTTTGCCAAGGAACAAGAAGCAG-3') (SEQ ID NO:61) for signal sequence, and from 16D10 cDNA using primers C3F (5'-CTTCTGCTTCTTGTTCCTTGGCAAAAAGCCTAGTGG-3') (SEQ ID NO:62) and 16D10X (5'-GCTCTAGATCAATTATTTCCTCCAGG-3') (SEQ ID NO:63) that introduced *Xba*I restriction site (underlined) for mature peptide coding sequence using Vent polymerase (New England Biolabs, Beverly, MA). The two products were then used to prime each other in a fusion PCR reaction. The resulting fragment was cloned into pBIX to generate pBIX(C3S-16D10) and verified by sequencing.

Example 15: Tobacco Hairy-root Transformation

The plasmids pBIX(16D10), pBIX(16D10S) and the empty vector pBIX as a control were transferred into *Agrobacterium rhizogenes* ATCC 15834 by electroporation (Shen and Forde, 1989) and transformed into tobacco (*Nicotiana tabacum* cv Petite Havana SR1) using the *A. rhyzogenes*-mediated cotyledon transformation (Christey, 1997). Transformed hairy roots were generated from inoculated tobacco cotyledons on Gamborg's B-5 plates containing 0.8% Noble agar with 100 mg/L

kanamycin and timentins (230.8 mg/L ticarcillin disodium plus 7.69 mg/L clavulanate potassium). Individual hairy root tips (about 0.5 cm) were cultured for 3 weeks at 24°C in the dark, and 2 to 3 roots from individual hairy root system were subjected to GUS-staining (Jefferson *et al*, 1987). The kanamycin-resistant and GUS-positive root lines with no bacterial contamination, confirmed by PCR analyses, were used to establish hairy root lines. The root-tips were sub-cultured for root growth assay on Gamborg's B-5 plates without hormones every 2 weeks and the cut roots were kept in culture on the old plates at 24°C in the dark for assays. For root-growth assays, plates were cultured horizontally in the dark and 5 hairy roots from each transgenic line in each of the three repeats were investigated. Relative RT-PCR and immunoblotting analyses of transgenic hairy roots or calli with a single transgenic copy identified as described (Does *et al*, 1991) were carried out using the same procedures as in those of transgenic *Arabidopsis*. Expression of 16D10 in the cytoplasm of hairy root cells increased root growth at the rate of approximately 65% [mean root length after 2 weeks of 5.20 ± 0.61 cm (n = 90) in 16D10 transgenic lines, compared to 3.15 ± 0.34 cm (n = 90) in control lines], generated extensive lateral roots and led to the formation of calli where roots were cut for subculturing at 5 weeks. RT-PCR analysis of 16D10 expression showed that the steady-state mRNA levels in calli were higher than in the hairy roots. Immunoblotting analysis with the purified 16D10 antiserum revealed that 16D10 was produced in both hairy roots and calli. No expression of 16D10 was detected in the control vector-transformed hairy roots.

Example 16: *Arabidopsis* Floral-dip Transformation

The plasmids pBIX(16D10), pBIX(C3S-16D10) and the empty vector pBIX as a control were introduced into *Agrobacterium tumefaciens* C58C1 by electroporation (Shen and Forde, 1989) and transformed into *A. thaliana* wild-type Col-0 plants by the floral dip method (Clough and Bent, 1998). Segregation of kanamycin resistance, GUS-straining (Jefferson *et al*, 1987), and 16D10 expression coupled to PCR analyses confirmed generation of the

transgenic homogenous T_2 lines. Inverse PCR (Does *et al*, 1991) identified the homogenous lines with a single transgenic copy in the genome for molecular and root growth assays. Thirty plants from each transgenic line in each of the three repeats were *in vitro* cultured on MS plates with 3% sucrose with 16 h light (24°C)/8 h dark (20°C) cycles and the plates were kept vertically for root growth assay.

Four transgenic *Arabidopsis* T_2 homozygous lines containing a single-copy of 16D10 without a signal peptide under the control of the 35S promoter were generated. Two transgenic lines originating from the blank transformation vector were also generated as controls. RT-PCR and immunoblotting analyses confirmed that 16D10 was expressed in all of the 16D10 transgenic lines, but not in the control lines. Compared to controls, expression of 16D10 in the cytoplasm of *Arabidopsis* cells increased the length of primary roots 85% [mean 54.01 ± 8.75 mm in four 16D10 transgenic lines (n = 90/line), and 29.20 ± 4.50 mm in 2 control lines (n = 90/line)] and the number of lateral branches and adventitious roots increased 1.4-fold and 2.08-fold, respectively (Figure 2 and Figure 3). Increased primary root growth was closely correlated with increased lateral root number and increased adventitious root number. Measurements of the root tip growth rate over 3 days revealed an increase (20%) in length only in the meristematic zone of 16D10 roots, indicating increase in cell number and not cell size contributed to the enhanced root growth.

Example 17: Complementation Tests

Since the mature 16D10 peptide of 13 aa (GKKPSGPNPGGNN) (SEQ ID NO:52) contained 8 aa (K---PSGPNP--N) (SEQ ID NO:53) of the conserved C-terminal 13 aa motif (KRLVPSGPNPLHN) (SEQ ID NO:54) of the functional domain of *Arabidopsis* CLV3-like proteins (Cock and McCormick, 2001), the plasmid pBIX(Clv3S-16D10) encoding *M. incognita* 16D10 with *A. thaliana* CLAVATA3 signal peptide was transferred into the *A. thaliana clv3* mutants *clv3-1* (intermediate), *clv3-2* and *clv3-6 via A. tumefaciens* C58C1-mediated floral-dip transformation (Clough and Bent,

1998) for functional complementation tests. As controls, the plasmids pBIX(16D10) and pBIX were also introduced into the *clv3* mutants. Three transgenic T_2 homozygous lines for each construct were also generated. The phenotypes (flower and shoot apical meristem) of 16D10-transformed *clv3* lines were investigated and compared with those of vector-transformed lines, *A. thaliana* wild-type ecotype Col-0 and the *clv3* mutant progeny as described in Fletcher et al. (1999). While 16D10 contained the functional domain of *Arabidopsis* CLV3-like proteins, expression of 16D10 in the apoplast or cytoplasm of *Arabidopsis clv3* mutants did not restore wild type phenotype, indicating 16D10 does not function as CLV3-like proteins.

Example 18: Histological Analysis

Primary root tissues of *A. thaliana* were fixed and dehydrated (Dolan *et al*, 1993), and embedded in Spurrs resin using Low Viscosity Embedding kit (Electron Microscopy Sciences, Hatfield, PA) according to the manufacturer's instructions. Thin sections (0.4 μM) were made on a Reichert-Jung Ultracut E and stained with 1% toluidine blue. Transverse root sections in and above the root meristem and longitudinal sections at the root-tip revealed that the average cell-size and number of cell types and cell-layer did not differ in the transgenic lines, compared to wild type. Root morphology was also not altered in our transgenic plants, and increased growth was accompanied by accelerated development of the root system. Thus ectopic 16D10 expression enhanced root growth rate and induced lateral root initiation, possibly by stimulation of cell division in meristems, increasing the rate of cell production without altering meristem organization.

Example 19: Relative RT-PCR

Reverse transcription (RT)-PCR was conducted on mRNA extracted from equivalent amounts of plant tissue. The 16D10 gene-specific primers 16D10F and 16D10R as described above were used in subsequent PCR amplifications. In controls, the primers UBQ1 (5'-GATCTTTGCCGGAAAACAATTGGAGGATGGT-3') (SEQ ID NO:64) and

UBQ2 (5'-CGACTTGTCATTAGAAAGAAAGAGATAACAGG-3') (SEQ ID NO:65) designed from the uniformly expressed UBQ10 gene (GenBank accession no. NM_202787) of *A. thaliana* wild-type ecotype Col-0, were used to amplify a 483bp unique sequence of UBQ10 from transgenic *Arabidopsis* lines. The primers ActF (5'-CCGGTCGTGGTCTTACTGAT-3') (SEQ ID NO:66) and ActR (5'-GCACCGATTGTGATGACTTG-3') (SEQ ID NO:67) designed from the uniformly expressed actin gene (GenBank accession no. U60494) of *N. tabacum* cv Petite Havana SR1 were used to amplify a 271bp unique sequence of the tobacco actin (Tob104) gene from transgenic tobacco hairy roots. PCRs containing the following components: 5μl of 10× BD Advantage 2 PCR buffer, 1.0μl of 10mM dNTP mix, 1.5μl of 5' primer, 1.5μl of 3' primer, 2μl of cDNA, 38μl of water, and 1.0μl of 50× BD Advantage 2 Polymerase Mix (BD Biosciences, Palo Alto, CA). PCR cycles consisted of an initial denaturation step at 94°C for 2 min, followed by 35 cycles of 94°C for 1 min, 55°C for 30 seconds, 72°C for 40 seconds, and a final 10-min elongation step at 72°C. Ten-microliter aliquots of each RT-PCR reaction were electrophoresed on a 2% agarose gel, transferred to nylon membranes, and hybridized with corresponding DIG-labeled DNA probes. RT-PCR analysis revealed that 16D10 transcripts were steadily present in the16D10 transgenic tobacco hairy roots and *Arabidopsis* lines, but absent in the vector-transformed control lines.

Example 20: Yeast Two-hybrid Screens

The MATCHMAKER yeast two-hybrid system II (BD Biosciences, Palo Alto, CA) was used in the yeast two-hybrid screening. The cDNA encoding the mature peptide of 16D10 was cloned in frame into the GAL4-binding domain (BD) of pGBKT7 to generate pGBKT7(16D10) and expressed as bait to screen a tomato root cDNA library constructed from mRNA from tomato root tissues in the GAL4 activation domain (AD) of pGADT7. Twelve full-length SCL-encoding cDNAs (*AtSCL*1, *AtSCL*3, *AtSCL*5, *AtSCL*6, *AtSCL*9, *AtSCL*13, *AtSCL*14, *AtSCL*21, *AtSCR*, *AtSHR*, *AtRGA*, *AtGAI*) were amplified from a root cDNA pool made from mRNA

from *A. thaliana* root tissues with specific primers of each gene based on the corresponding sequences in GenBank databases (Bolle, 2004), and cloned in frame into pGADT7. Each of the constructs was introduced with pGBKT7(16D10) into the yeast strain AH109. cDNAs encoding the specific regions of AtSCL6 and AtSCL21 were cloned into pGADT7, and then co-transformed with pGBKT7(16D10) into the strain AH109. All procedures including cDNA library screening, selection of positive clones and the assay of β-galactosidase activity, were performed by following the protocol of MATCHMAKER yeast two-hybrid system II (BD Biosciences, Palo Alto, CA). Two *Arabidopsis* SCL proteins, AtSCL6 and AtSCL21, interacted with 16D10 in yeast. Domain analysis revealed the specific interaction of 16D10 with the SAW domain of AtSCL6 and AtSCL21, and no interaction of 16D10 with the rest of the domains of the SCL proteins, and indicated that the SCL transcription factor(s) was a putative target of the secreted 16D10 during RKN parasitism of plants.

Example 21: RNAi by Soaking

Forty-two bp and 271bp sequences of 16D10 were respectively amplified from the full-length cDNA clone using the primers 16D10T7F1 (5'-TAATACGACTCACTATAGGGCCTCAAAAATACCATAAAG-3')(SEQ ID NO:68) and 16D10T7R1 (5'-TAATACGACTCACTATAGGGGAAATTAACAAAGGAAACC-3') (SEQ ID NO:69), and 16D10T7F2 (5'-TAATACGACTCACTATAGGGGGCAAAAAGCCTAGTGGGC-3) (SEQ ID NO:70) and 16D10T7R2 (5'-TAATACGACTCACTATAGGGTCAATTATTTCCTCCAGG-3') (SEQ ID NO:71) each of that incorporates the RNA primer site T7 (underlined). The gel-purified PCR products were used as templates for synthesis of sense and antisense 16D10 RNAs in a single reaction *in vitro* using the MEGAscript RNAi kit (Ambion, Austin, TX) according to manufacturer's instructions, except that the reactions were incubated for 16 hr to increase RNA yield. The amount and quality of generated double-strand (ds) RNA

were estimated and quantitated by standard procedures (Sambrook et al., 1989). The dsRNA products were ethanol precipitated and re-suspended in nuclease-free water to 10-15µg/µl.

Approximately 10,000 freshly hatched J2s of *M. incognita* were soaked in ¼ M9 buffer (10.9mM Na_2HPO_4, 5mM KH_2PO_4, 4.7mM NH_4Cl, and 2.2mM NaCl) containing 1mg/ml of 16D10 dsRNA, 1% resorcinol, 0.13mg/ml FITC isomer I, 0.05% gelatin and 3mM spermidine, and incubated for 4 hr in the dark at RT on a rotator. Resorcinol (Res) was used to help stimulate uptake of the dsRNA. Control samples were incubated in the same solution but without resorcinol or dsRNA. After soaking, nematodes were thoroughly washed five times with nuclease-free water by centrifugation and about 100% of treated nematodes were observed with an Olympus fluorescence microscope to take up FITC, a marker for uptake of dsRNA. The FITC-labeled transgenic J2 were assayed to determine silencing of the 16D10 transcripts by relative RT-PCR analysis, using first-strand cDNAs synthesized from mRNA of equivalent number of treated J2 as templates and a 284bp amplified fragment of the *M. incognita* constitutively expressed actin gene (GenBank accession no. BE225475) as a control. The ingestion of short or full-length 16D10 dsRNA by second-stage juveniles of root-knot nematode caused a significant reduction of 16D10 transcripts in the treated nematodes (Figure 4), providing direct evidence for *in vivo* targeting of 16D10 in root-knot nematodes by RNAi.

J2s of *M. incognita* were also soaked as described above with 1 mg/ml of dsRNA specific for 8H11 (SEQ ID NO:17) or 31H06 (SEQ ID NO:33). Relative RT-PCR analysis revealed that ingestion of 8H11 and 31H06 dsRNA by second-stage juveniles of root-knot nematode caused a significant reduction of transcripts of these two additional parasitism genes in the treated nematodes (Figure 5).

Example 22: *In planta* Delivery of RNAi

The sense and anti-sense cDNAs (42bp or 271bp) of 16D10 were amplified from the full-length cDNA clone with the gene-specific primers

16D10Xho1 (5'-CCGCTCGAGGGCAAAAAGCCTAGTGGGC-3') (SEQ ID NO:72) and 16D10Kpn1 (5'-CGGGGTACCTCAATTATTTCCTCCAGG-3') (SEQ ID NO:73), 16D10Cla1 (5'-CCATCGATTCAATTATTTCCTCCAGG-3') (SEQ ID NO:74) and 16D10Xba1 (5'-GCTCTAGAGGCAAAAAGCCTAGTGGGC-3') (SEQ ID NO:75), 16D10Xho3 (5'-CCGCTCGAGCCTCAAAAATACCATAAAG-3'(SEQ ID NO:76) and 16D10Kpn2 (5'-CGGGGTACCGAAATTAACAAAGGAAACC-3') (SEQ ID NO:77), 16D10Cla2 (5'-CCATCGATGAAATTAACAAAGGAAACC-3') (SEQ ID NO:78) and 16D10Xba3 (5'GCTCTAGACCTCAAAAATACCATAAAG-3') (SEQ ID NO:79) that introduced *Xho*I, *Kpn*I, *Cla*I or *Xba*I restriction sites (underlined), respectively. The PCR products were gel-purified, and digested with the restriction enzymes *Xho*I and *Kpn*I, or *Cla*I and *Xba*I, respectively. The digested-PCR products were cloned into the *Xho-Kpn*I sites, and the *Cla*I-*Xba*I sites of pHANNIBAL to generate pHANNIBAL(16D10#1) and pHANNIBAL(16D10#2), respectively. The sense and antisense 16D10 cDNAs of pHANNIBAL-derived plasmids were subcloned as *Not*I fragments into the binary vector pART27 (Gleave, 1992) to produce highly effective intron-containing "hairpin" RNA (ihpRNA) silencing constructs (Wesley et al., 2001). The pART27-derived constructs were electroporation transformed into *A. tumefaciens* C58C1. The transformants were selected on LB media containing rifampicin (50mg/L), gentamycin (25mg/L) and spectinomycin (100 mg/L), and then introduced into *A. thaliana* ecotype Col-0 *via* floral-dip transformation as described above. Transgenic homologous T2 lines constitutively transcribing the specific ihpRNA of 16D10 under the CaMV35S promoter were generated for resistance assays to the root-knot nematodes, *Meloidogyne* species.

Example 23: Resistance Assays

Seeds from the *A. thaliana* transgenic lines generated from transformation of pART27-derived constructs were surface sterilized in 70% (v/v) ethanol for 1 min and 3% (v/v) sodium hypochloride for 5 min, and then rinsed 5 times in sterile distilled water. The sterilized seeds were geminated and grown on

Gamborg's B-5 medium for 3 weeks. *M. incognita* eggs were sterilized and then inoculated about 500 eggs for each plant near to the roots as described (Sijmons et al., 1991). The number and size of galls on the infected roots were analyzed after inoculation of 3 weeks, and the infected roots were stained red with acid fuschin as described (Hwang et al., 2000) and assayed by the number of eggs per gram of roots after inoculation of 8 weeks. Transgenic *Arabidopsis* lines expressing 16D10 dsRNA were resistant to the four major *Meloidogyne* species - *M. incognita, M. javanica, M. arenaria,* and *M. hapla*. Root galling assay showed a 63-90% reduction in the number (and size) of galls on the 16D10 dsRNA transgenic *Arabidopsis* lines, compared to galls on the vector-transformed line (Figures 1A, 1B and Table 1). Nematode reproduction assay revealed a 70-97% reduction in the number of RKN eggs per gram root in the 16D10 dsRNA transgenic lines when compared to the control plants (Figure 7).

Table 1

Gall production on transgenic *A. thaliana* expressing 16D10 dsRNA and inoculated with four *Meloidogyne* species (*M. incognita, M. javanica, M. arenaria, M. hapla*) compared with control plants.

Galling No. (Mean value)	CK				16D10i-1				16D10i-2			
	T	L	M	S	T	L	M	S	T	L	M	S
M. incognita	13.50	4.83	7.67	1.00	1.50	0	0.25	1.25	3.40	0	0.80	2.60
M. javanica	14.38	7.41	5.47	1.50	3.29	0.14	1.00	2.15	2.50	0.13	1.25	1.12
M. araneria	11.75	6.75	3.25	1.75	3.00	0.17	1.50	1.33	3.50	0.25	0.75	2.50
M. hapla	10.21	3.46	6.25	0.50	3.63	0.25	2.33	1.05	3.78	0.50	1.67	1.61
T: total L: large (>2 mm) M: medium (1-2 mm) S: small (<1 mm) n: 8-16												

Example 24: 16d10 Sequence Data

16D10 Genomic DNA sequence (840bp)

GAGAAAATAAAATATAAATTATTCCTCAAAAATACCATAAAG**GTTAGCCA**
ATATTAATTCTTTTGAAATTTTCTTTGCTTCCATAAATTAAAAAAAATTG

TTTTTAAGTGAGGGAATGTGGATTAAGCATCTTTCTTATTTTTAAAATTT
TTGATAGAGTGTAGCGACAGTCAATCAAAATATTTTGATTTTTTTAAAG
TTAAAAATTAAGGATGATAAAGAAGTTTAAAATGTAGGTGGAAATATA
AGTATACCGAAAAACATCTTTTATTTTTAAGTTTAAACAAGCAGTAAAA
CTTTGTCTGGTTTTATCACCGGGCAACTGTAAGGGAAGCTTTAATAAA
AATTTTGTAAGATACGAAAATCATTGTCCCCAGTAGCTTGAGTGATCG
AAGCGCCTGGTTGCCATTAAGTTTTTTGCTTGAGACTTATATAACAAGT
ATATATCAAACCGGATTATAAAGTTAAAGAACAGAAAAAATTTCACGG
AATAAATATTGGCTAACCACTCAATTTATTTAATTATTCTTCAATCAAAA
AATGTTTACTAATTCAATTAAAAATTTAATTATTTATTTAATGCCTTTAATG
GTTACTTTAATGCTTTTGTCTGTCTCATTTGTGGATGCAGGCAAAAAGCC
TAGTGGGCCAAATCCTGGAGGAAATAATTGAAGAAAAATGATTGAAGAA
AAACGTTTAAATTAAACGATAAATGGGAAATAATGGAATTTAAATTAAGC
TAATTTTGATGGTTTCCTTTGTTAATTTCAACATAAAATTAATTGAATTTA
CTGAATAAAATTATATCTGAAAAAAA (SEQ ID NO:1)
(One 476-bp intron sequence is bolded).

16D10 cDNA sequence (364bp)
GAGAAAATAAAATATAAATTATTCCTCAAAAATACCATAAAGTTAATTATT
CTTCAATCAAAAAAATGTTTACTAATTCAATTAAAAATTTAATTATTTATTT
AATGCCTTTAATGGTTACTTTAATGCTTTTGTCTGTCTCATTTGTGGATG
CAGGCAAAAAGCCTAGTGGGCCAAATCCTGGAGGAAATAATTGAAGAA
AAATGATTGAAGAAAAACGTTTAAATTAAACGATAAATGGGAAATAATGG
AATTTAAATTAAGCTAATTTTGATGGTTTCCTTTGTTAATTTCAACATAAA
ATTAATTGAATTTACTGAATAAAATTATATCTGAAAAAAAAAAAAAAAAA
AAAAAAAAAAA (SEQ ID NO:2)

16D10 cDNA sequence region used for making 16D10 RNAi constructs

GAGAAAATAAAATATAAATTATTCCTCAAAAATACCATAAAGTTAATTATT
CTTCAATCAAAAAAA*TG*TTTACTAATTCAATTAAAAATTTAATTATTTATTT

AATGCCTTTAATGGTTACTTTAATGCTTTTGTCTGTCTCATTTGTGGATG
CA**GGCAAAAAGCCTAGTGGGCCAAATCCTGGAGGAAATAATTGA**AGA
AAAATGATTGAAGAAAAACGTTTAAATTAAACGATAAATGGGAAATAATG
GAATTTAAATTAAGCTAATTTTGATGGTTTCCTTTGTTAATTTCAACATAA
AATTAATTGAATTTACTGAATAAAATTATATCTGAAAAAAAAAAAAAAAA
AAAAAAAAAAAA (SEQ ID NO:2) [The bold 42-bp sequence was used for constructing pHANNIBAL(16D10#1), and the underlined 271-bp sequence was used for constructing pHANNIBAL(16D10#2)]

pHANNIBAL(16D10#1):

(a) Construct: (*Xho*I + 42bp 16D10 sense-strand-sequence + *Kpn*I = 54bp) + Pdk intron + (*Cla*I + 42bp 16D10 antisense-strand-sequence + *Xba*I = 54bp)

*Xho*I
CTCGAG**GGCAAAAAGCCTAGTGGGCCAAATCCTGGAGGAAATAATTGA**GGTACC------
----
*Kpn*I
----------Pdk intron----------
----
*Cla*I
ATCGAT**TCAATTATTTCCTCCAGGATTTGGCCCACTAGGCTTTTTGCC**TCTAGA
*Xba*I

SEQ ID NO:3)

(b) PCR detection: primers H1F1 & H1R1 (234bp PCR product)
Primers H1F2 & H1R2 (273bp PCR product)

pHANNIBAL(16D10#2)

(1). Construct #2: (*Xho*I + 271bp 16D10 sense-strand-sequence + *Kpn*I = 283bp) + Pdk intron + (*Cla*I + 271bp 16D10 antisense-strand-sequence + *Xba*I = 283bp)

*Xho*I

CTCGAGCCTCAAAAATACCATAAAGTTAATTATTCTTCAATCAAAAAA
ATGTTTACTAATTCAATTAAAAATTTAATTATTTATTTAATGCCTTTAAT
GGTTACTTTAATGCTTTTGTCTGTCTCATTTGTGGATGCAGGCAAAAAG
CCTAGTGGGCCAAATCCTGGAGGAAATAATTGAAGAAAAATGATTGA
AGAAAAACGTTTAAATTAAACGATAAATGGGAAATAATGGAATTTAAA
TTAAGCTAATTTTGATGGTTTCCTTTGTTAATTTCGGTACC

*Kpn*I ----------Pdk intron----------

---

*Cla*I

ATCGATGAAATTAACAAAGGAAACCATCAAAATTAGCTTAATTTAAATT
CCATTATTTCCCATTTATCGTTTAATTTAAACGTTTTTCTTCAATCATTTT
TCTTCAATTATTTCCTCCAGGATTTGGCCCACTAGGCTTTTTGCCTGCA
TCCACAAATGAGACAGACAAAAGCATTAAAGTAACCATTAAAGGCAT
TAAATAAATAATTAAATTTTTAATTGAATTAGTAAACATTTTTTTGATTG
AAGAATAATTAACTTTATGGTATTTTTGAGGTCTAGA (SEQ ID NO:4)

XaI

Table 2:

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>6F06>msp4>>bankit482257>>>AF531163

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>6G07>msp5>>bankit482261>>>AF531164

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>7A01>msp6>>bankit482263>>>AF531165

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>7E12>msp7>>bankit478534>>>AF531166

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>7H08>msp8>>bankit482285>>>AF531168

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>8D05>msp9>>bankit478548>>>AF531169

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>8H11>msp10>>bankit478550>>>AF531170

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>9H10>msp11>>bankit478544>>>AF531167

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>10A08>msp34>>bankit487923>>>AY142117
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>10G02>msp29>>bankit479222>>>AY135365

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>11A01>msp12>>bankit478790>>>AY134431

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>12H03>msp13>>bankit482577>>>AY134432

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>14E06>msp15>>bankit478812>>>AY134434

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>16D10>msp16>>bankit478814>>>AY134435

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>16E05>msp17>>bankit482587>>>AY134436

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>1C05B>msp36>>AY422829

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1D08B>msp37>>AY422830

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2B02B>pel2>>AY327873

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2G06B>cm1>>AY422834

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4F05B>msp38>>AY422831
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5A12B>eng3>>AY422836

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5C03B>msp39>>AY422832

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6D09B>cm2>>AY422835

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8E08B>eng4>>AY422837

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8E10B>msp40>>AY422833

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AAAAAAAAAAAAAAAAA (SEQ ID NO:51)

What is claimed is:

1. A transgenic plant or cell comprising:

an inhibitory nucleic acid specific for at least a portion of a nucleic acid encoding a nematode esophageal gland cell secretory polypeptide.

2. The transgenic plant or cell of claim 1, wherein the plant or cell is resistant to nematode disease caused directly or indirectly by at least two different nematode species.

3. The transgenic plant or cell of claim 2, wherein the plant or cell is resistant to nematode disease caused directly or indirectly by *M. incognita*.

4. The transgenic plant or cell of claim 3, wherein the plant or cell is also resistant to nematode disease caused directly or indirectly by *M. javanica,*

5. The transgenic plant or cell of claim 4, wherein the plant or cell is also resistant to nematode disease caused directly or indirectly by *M. arenaria*.

6. The transgenic plant or cell of claim 5, wherein the plant or cell is also resistant to nematode disease caused directly or indirectly by *M. hapla* or *M. chitwoodi*.

7. The transgenic plant or cell of claim 1, wherein the inhibitory nucleic acid is in an amount effective to reduce, inhibit, or prevent expression of the nematode esophageal polypeptide by a parasitic nematode feeding on the transgenic plant or cell compared to a control plant or cell.

8. The transgenic plant or cell of claim 1, wherein the inhibitory nucleic acid is in an amount effective to reduce, inhibit, or prevent nematode disease compared to a control plant or cell.

9. The transgenic plant or cell of claim 1, wherein the inhibitory nucleic acid is in an amount effective to reduce, inhibit, or prevent expression of the nematode esophageal polypeptide by at least two root-knot nematode species parasitic nematode feeding on the transgenic plant or cell compared to a control plant or cell.

10. The transgenic plant or cell of claim 1, wherein the inhibitory nucleic acid is in an amount effective to reduce, inhibit, or prevent nematode disease caused by at least two different root-knot nematode species compared to a control plant or cell.

11. The transgenic plant or cell of claim 1, wherein the nematode esophageal polypeptide or fragment thereof modulates: gene expression of the plant or cell, formation of a giant cell, nematode migration through root tissue of the plant, cell metabolism of the plant, elicits signal transduction in the plant cell, or forms a feeding tube that enables the nematode to feed from giant-cells formed in the plant.

12. The transgenic plant or cell of claim 11, wherein the gene expression of the plant or cell is modulated directly or indirectly by the nematode esophageal gland cell polypeptide.

13. The transgenic plant or cell of claim 12, wherein the modulation of gene expression occurs in a root cell.

14. The transgenic plant or cell of claim 1, wherein the nematode is a member of *Meloidogyne spp.*

15. The transgenic plant or cell of claim 1, wherein the transgenic plant or cell is a monocot or dicot.

16. The transgenic plant or cell of claim 1, wherein the transgenic plant or cell is a member of the phylogenic family selected from the group consisting of *Rosaceae*, *Fabaceae*, *Passifloraceae*, *Cucurbitaceae*, *Malvaceae*, *Euphorbiaceae*, *Vitaceae*, *Solanaceae*, *Convolvulaceae*, *Rubiaceae*, *Leguminosae*, and *Brassicaceae*.

17. The transgenic plant or cell of claim 16, wherein the plant is a tomato, eggplant, potato, melon, cucumber, carrot, lettuce, artichoke, celery, cucurbits, barley, corn, peanut, soybean, sugar beet, cotton, cowpea, beans, alfalfa, tobacco, citrus, clover, pepper, grape, coffee, olive, or tea.

18. The transgenic plant or cell of claim 1, wherein the inhibitory nucleic acid comprises at least a portion complementary to part or all of mRNA encoding a protein encoded by one or more of SEQ ID NOs 1, 2, or 5-51.

19. The transgenic plant or cell of claim 1, wherein the inhibitory nucleic acid comprises small interfering RNA.

20. The transgenic plant or cell of claim 1, wherein the inhibitory nucleic acid comprises microRNA.

21. The transgenic plant or cell of claim 1, wherein the inhibitory nucleic acid comprises antisense DNA.

22. The transgenic plant or cell of claim 1, wherein the inhibitory nucleic acid inhibits or interferes with the translation of mRNA encoding the nematode esophageal gland cell polypeptide.

23. The transgenic plant or cell of claim 1, wherein the inhibitory nucleic acid induces or promotes the degradation or mRNA encoding the nematode esophageal gland cell polypeptide.

24. The transgenic plant or cell of claim 1, wherein the transgenic plant or cell comprises two or more inhibitory nucleic acids specific for different nematode esophageal gland cell polypeptides.

25. The transgenic plant or cell of claim 1, wherein the inhibitory nucleic acid comprises non-natural or natural nucleotides.

26. The transgenic plant or cell of claim 1, wherein the inhibitory nucleic acid comprises at least one modified internucleotide linkage.

27. A composition comprising:

an inhibitory nucleic acid specific for an mRNA or fragment thereof encoding a polypeptide encoded by one or more of SEQ ID NOs. 1, 2 or 5-51 or a fragment or homologue thereof, in an amount sufficient to inhibit expression of the polypeptide encoded by one or more of SEQ ID NOs 1, 2 or 5-51 or homologue thereof when delivered to a nematode.

28. The composition of claim 27, wherein the polypeptide or fragment thereof encoded by one or more of SEQ ID NOs. 1, 2 or 5-51 modulates: gene expression of the plant or cell, formation of a giant cell, nematode migration through root tissue of the plant, cell metabolism of the plant, elicits signal transduction in the plant cell, or forms a feeding tube that enables the nematode to feed from giant-cells formed in the plant.

29. The composition of claim 28, wherein the gene expression of the plant or cell is modulated directly or indirectly by the polypeptide.

30. The composition of claim 29, wherein the modulation of gene expression occurs in a root cell.

31. The composition of claim 27, wherein the nematode is a member of *Meloidogyne spp.*

32. The composition of claim 27, wherein the transgenic plant or cell is a monocot or dicot.

33. The composition of claim 27, wherein the plant or cell is a member of the family selected from the group consisting of *Rosaceae*, *Fabaceae*, *Passifloraceae*, *Cucurbitaceae*, *Malvaceae*, *Euphorbiaceae*, *Vitaceae*, *Solanaceae*, *Convolvulaceae*, *Rubiaceae*, *Leguminosae*, and *Brassicaceae*.

34. The composition of claim 33, wherein the plant or cell is a tomato, eggplant, potato, melon, cucumber, carrot, lettuce, artichoke, celery, cucurbits, barley, corn, peanut, soybean, sugar beet, cotton, cowpea, beans, alfalfa, tobacco, citrus, clover, pepper, grape, coffee, olive, or tea.

35. A cell comprising a nucleic acid encoding an inhibitory nucleic acid specific for an mRNA or fragment thereof encoding a nematode esophageal gland cell polypeptide that modulates: gene expression of the plant or cell, formation of a giant cell, nematode migration through root tissue of the plant, cell metabolism of the plant, elicits signal transduction in the plant cell, or forms a feeding tube that enables the nematode to feed from giant-cells formed in the plant.

36. A vector comprising a promoter operably linked to a nucleic acid encoding an inhibitory nucleic acid specific for an mRNA encoding a nematode esophageal gland cell polypeptide that modulates: gene expression of the plant or cell, formation of a giant cell, nematode migration through root tissue of the plant, cell metabolism of the plant, elicits signal transduction in the plant cell, or forms a feeding tube that enables the nematode to feed from giant-cells formed in the plant.

37. The vector of claim 36, wherein the nematode esophageal gland cell protein is encoded by one or more of SEQ ID NOs.1, 2, or 5-51.

38. A method for providing nematode resistance to a plant comprising:

expressing in the plant an inhibitory nucleic acid specific for a nematode esophageal gland cell protein.

39. The method of claim 38, wherein the nematode is a member of *Meloidogyne spp*.

40. A method for providing nematode resistance to a plant comprising:

contacting the plant with one or more inhibitory nucleic acids specific for one or more nematode esophageal gland cell proteins in an amount sufficient to reduce nematode disease.

41. The method of claim 40, wherein the one or more nematode esophageal gland cell proteins indirectly or directly modulates: gene expression of the plant or cell, formation of a giant cell, nematode migration through root tissue of the plant, cell metabolism of the plant, signal transduction in the plant cell, or forms a feeding tube that enables the nematode to feed from giant-cells formed in the plant.

42. The method of claim 41, wherein the nematode is a member of *Meloidogyne spp*.

43. The method of claim 40, wherein the plant is resistant to nematode disease of at least two different species of root-knot nematode.

44. The method of claim 40, wherein the plant is resistant to nematode disease caused by *M. incognita, M. javanica, M. arenaria,* and *M. hapla*.

45. A transgenic plant or cell comprising:

at least a portion of a nucleic acid encoding an esophageal gland cell secretory polypeptide of a parasitic nematode.

46. The transgenic plant or cell of claim 45, wherein the esophageal gland cell secretory polypeptide enhances root growth of the transgenic plant compared to a control plant or cell.

47. The transgenic plant or cell of claim 45, wherein the esophageal gland cell secretory polypeptide is expressed in an amount effective to enhance root growth of the transgenic plant or cell compared to a control plant or cell.

48. The transgenic plant or cell of claim 45, wherein the esophageal gland cell secretory polypeptide is expressed in a root cell.

49. The transgenic plant or cell of claim 45, wherein the nematode is a member of *Meloidogyne spp.*

50. The transgenic plant or cell of claim 45, wherein the nematode is a not a member of *Heterodera spp.*

51. The transgenic plant or cell of claim 45, wherein the transgenic plant or cell is a monocot or dicot.

52. The transgenic plant or cell of claim 45, wherein the transgenic plant or cell is a member of the family selected from the group consisting of *Rosaceae*, *Fabaceae*, *Passifloraceae*, *Cucurbitaceae*, *Malvaceae*, *Euphorbiaceae*, *Vitaceae*, *Solanaceae*, *Convolvulaceae*, *Rubiaceae*, *Leguminosae*, and *Brassicaceae*.

53. The transgenic plant or cell of claim 52, wherein the plant is a tomato, eggplant, potato, melon, cucumber, carrot, lettuce, artichoke, celery, cucurbits, barley, corn, peanut, soybean, sugar beet, cotton, cowpea, beans, alfalfa, tobacco, citrus, clover, pepper, grape, coffee, olive, or tea.

54. The transgenic plant or cell of claim 45, wherein the nucleic acid comprises at least a portion of SEQ ID NOs 1, 2, or 5-51.

55. The transgenic plant or cell of claim 45, wherein the transgenic plant or cell comprises two or more nucleic acids encoding different nematode secretory polypeptides.

56. A composition comprising:

a nucleic acid comprising SEQ ID NOs. 1, 2 or 5-51, a complement, a fragment, or homologue thereof, in an amount sufficient to enhance or promote root growth when expressed in a plant.

57. A composition comprising:

a polypeptide encoded by SEQ ID NOs. 1, 2 or 5-51, a complement, a fragment, or homologue thereof, in an amount sufficient to enhance or promote root growth.

58. The composition of claim 57, wherein the nematode is a member of *Meloidogyne spp.*

59. The composition of claim 57, wherein the nematode is a member of *Heterodera spp.*

60. The composition of claim 57, wherein the transgenic plant or cell is a monocot or dicot.

61. The composition of claim 57, wherein the plant or cell is a member of the family selected from the group consisting of *Rosaceae*, *Fabaceae*, *Passifloraceae*, *Cucurbitaceae*, *Malvaceae*, *Euphorbiaceae*, *Vitaceae*, *Solanaceae*, *Convolvulaceae*, *Rubiaceae*, *Leguminosae*, and *Brassicaceae*.

62. The composition of claim 61, wherein the plant or cell is a tomato, eggplant, potato, melon, cucumber, carrot, lettuce, artichoke, celery, cucurbits, barley, corn, peanut, soybean, sugar beet, cotton, cowpea, beans, alfalfa, tobacco, citrus, clover, pepper, grape, coffee, olive, or tea.

63. A cell comprising a nucleic acid encoding a nematode esophageal gland cell secretory polypeptide that stimulates root growth.

64. A vector comprising a promoter operably linked to a nucleic acid encoding a nematode esophageal gland cell secretory protein that stimulates plant root growth.

65. The vector of claim 64, wherein the nematode esophageal gland cell secretory protein is encoded by one or more of SEQ ID NOs.1, 2, or 5-51.

66. A method for stimulating root growth or for enhancing the formation of lateral or adventitious roots comprising delivering a nematode esophageal gland cell secretory polypeptide to the interior of a root cell.

67. A method for the producing an altered plant, plant cell or plant tissue comprising introducing one or more nematode esophageal gland cell secretory polypeptides into a cell, tissue or organ of said plant.

68. A seed produced by the transgenic plant or cell of claim 45.

69. A recombinant plant exhibiting increased root growth as compared to the corresponding wild-type plant, wherein said recombinant plant comprises a recombinant nucleic acid encoding a nematode esophageal gland cell secretory protein or fragment thereof operably associated with a regulatory sequence.

70. A recombinant plant of claim 69, wherein the nematode esophageal gland cell secretory protein is encoded by one or more of SEQ ID NOs.1, 2, or 5-51.

71. A fusion protein comprising a polypeptide encoded by SEQ ID NOs.1, 2, or 5-51 or fragments thereof.

72. The fusion protein of claim 71, comprising an auxin or fragment thereof.

Figure 1A

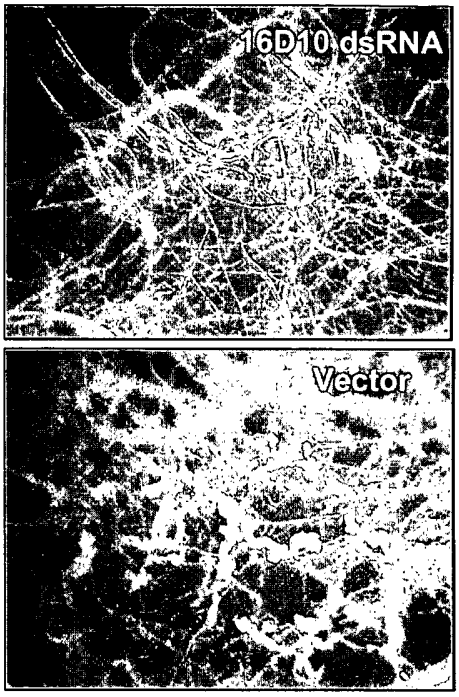


Figure 1B

35S::16D10 Vector

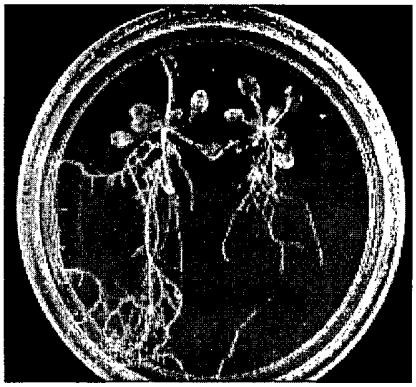


Figure 2

Figure 3

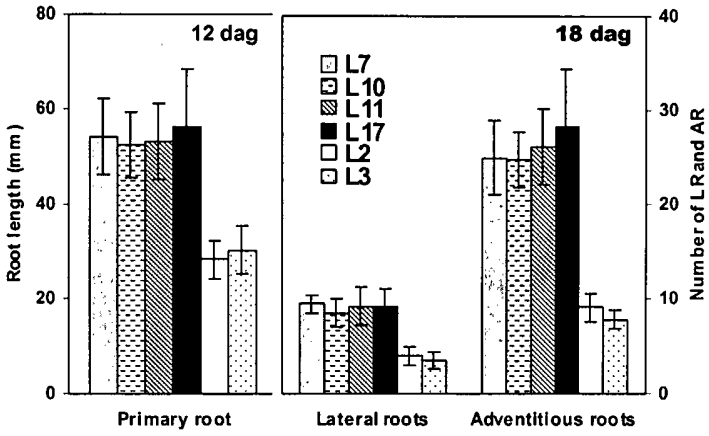

12 dag
18 dag
L7
L10
L11
L17
L2
L3
Root length (mm)
Number of LR and AR
0
20
40
60
80
0
10
20
30
40
Primary root
Lateral roots
Adventitious roots

Figure 4

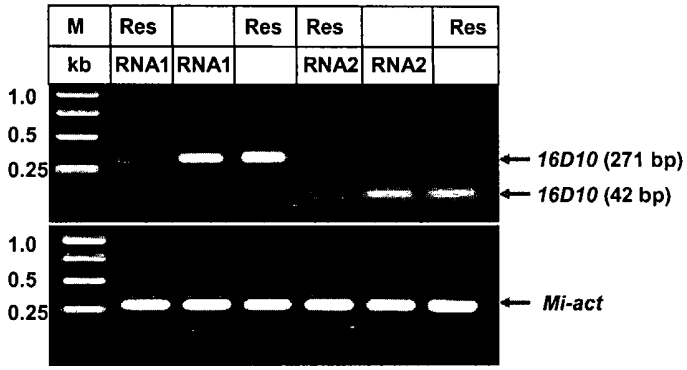

M
Res
Res
Res
Res
kb
RNA1
RNA1
RNA2
RNA2
1.0
0.5
0.25
16D10 (271 bp)
16D10 (42 bp)
1.0
0.5
0.25
Mi-act

Figure 5

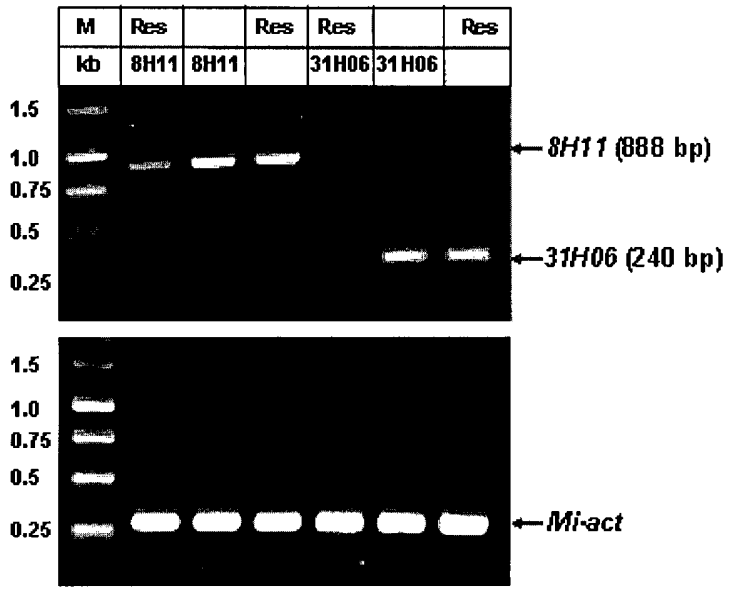

M
Res
Res
Res
Res
kb
8H11
8H11
31H06
31H06
1.5
1.0
0.75
0.5
0.25
8H11 (888 bp)
31H06 (240 bp)
1.5
1.0
0.75
0.5
0.25
Mi-act

Figure 6

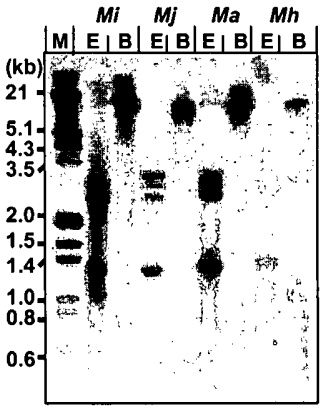
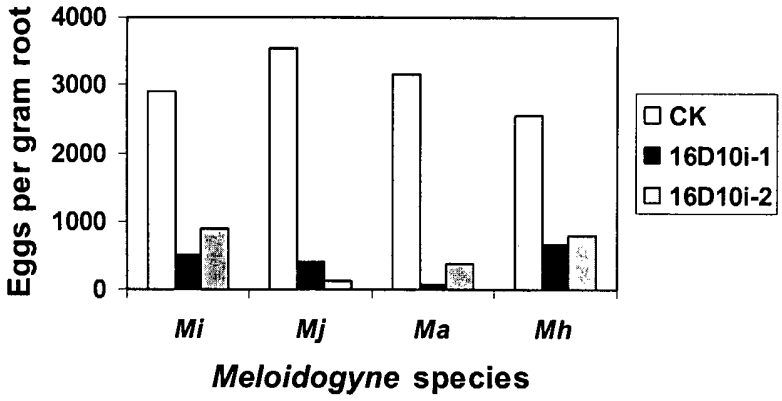


Figure 7


Eggs per gram root
4000
3000
2000
1000
0
Mi
Mj
Ma
Mh
Meloidogyne species
CK
16D10i-1
16D10i-2