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(54) **Title**: METHODS AND COMPOSITIONS FOR CONTROLLING NEMATODE PESTS

(57) **Abstract**: Transgenic plants expressing a Vip3 protein have been found to be efficacious against plant-infesting nematodes. Disclosed are methods of controlling nematode populations using transgenic plants expressing Vip3 protein.

METHODS AND COMPOSITIONS FOR CONTROLLING NEMATODE PESTS

FIELD OF THE INVENTION

[0001] The invention relates to methods for preventing or controlling nematode infestation of plants. More particularly, the invention relates to the control of nematode pests in plants using vegetative insecticidal proteins. The invention also relates to transgenic plants tolerant or resistant to nematode infestation.

BACKGROUND

[0002] Plant pests are a major factor in the loss of the world's important agricultural crops. About $8 billion are lost every year in the U.S. alone due to infestations of invertbrate pests including nematodes. In addition to losses in field crops, nematode pests are also a burden to vegetable and fruit growers, to producers of ornamental flowers, and to home gardeners. Plant-infesting nematodes, a majority of which are root feeders, are found in association with most plants. Some are endoparasitic, living and feeding within the tissue of the roots, tubers, buds, seeds, etc. Others are ectoparasitic, feeding externally through plant walls. A single endoparasitic nematode can kill a plant or reduce its productivity. Endoparasitic root feeders include such economically important pests as the root-knot nematodes (*Meloidogyne* species), the reniform nematodes (*Rotylenchulus* species), the cyst nematodes (*Heterodera* species), and the root-lesion nematodes (*Pratylenchus* species). Direct feeding by nematodes can drastically decrease a plant's uptake of nutrients and water. Nematodes have the greatest impact on crop productivity when they attack the roots of seedlings immediately after seed germination. Nematode feeding also creates open wounds that provide entry to a wide variety of plant-pathogenic fungi and bacteria. These microbial infections can be more economically damaging than the direct effects of nematode feeding.

[0003] Cyst nematodes are responsible for direct loss in soybean yield and indirect loss due to cost of pesticides and non-optimal use of land for rotation. Soybean cyst nematode (*Heterodera glycines*) has a negative economic impact that may exceed $1 billion per year in North America. Economically significant densities of cyst nematodes usually

cause stunting of crop plants. The stunted plants have smaller root systems, show symptoms of mineral deficiencies in their leaves, and wilt easily.

[0004] Traditional practices for managing nematode infestations include maintaining proper fertility and soil pH levels in nematode-infested land; controlling plant diseases that aid nematode invasion, as well as controlling insect and weed pests; using sanitation practices such as plowing, planting, and cultivating of nematode-infested fields only after working non-infested fields; cleaning equipment thoroughly after working in infested fields; not using seed from plants grown on infested land for planting non-infested fields unless the seed has been properly cleaned; rotating infested fields and alternating host crops with non-host crops, such as, corn, oat and alfalfa and planting resistant or tolerant plant varieties. While many of these can be effective they are time consuming and costly to implement. Nematodes are difficult pests to control without the use of chemical pesticides or fumigants (e.g., nematicides), and the availability of these nematicides is decreasing due to high toxicity to humans and the environment. Furthermore, under the Montreal Protocol of 1987, one of the main chemicals used to control nematode infestation, methyl bromide, has been phased out. Thus, there is currently no efficient and effective approach to control nematode infestation of plants.

[0005] Biological pest control agents, such as *Bacillus thuringiensis* strains expressing pesticidal toxins like delta-endotoxins (also called Cry proteins), have been applied to crop plants with satisfactory results, offering an alternative or compliment to chemical pesticides. Some Cry proteins, for example Cry1, Cry 5, Cry6, Cry11, Cry12, Cry13, Cry14, Cry21 and Cry22, have been shown to provide some activity against certain nematode species in laboratory bioassays. However, control of nematode pests by expression of Cry proteins in plants has not been demonstrated, particularly in field crops such as soybean or corn.

[0006] Other, non-endotoxin genes and the insecticidal proteins they encode have also been identified. Patents 5,877,012, 6,107,279, 6,137,033, and 6,291,156, as well as Estruch *et al.* (1996, Proc. Natl. Acad. Sci. 93:5389-5394) and Yu *et al.* (1997, Appl. Environ. Microbiol. 63:532-536), describe a new class of insecticidal proteins called. Vip3 (vegetative insecticidal protein 3). *Vip*3 genes encode approximately 88 kDa proteins that are produced and secreted by *Bacillus* during its vegetative stage of growth (vegetative insecticidal proteins, VIP). For example, one family of the Vip3 protein class,

called Vip3A, possesses insecticidal activity against a wide spectrum of lepidopteran pests, including, but not limited to, black cutworm (BCW, *Agrotis ipsilon*), fall armyworm (FAW, *Spodoptera frugiperda*), tobacco budworm (TBW, *Heliothis virescens*), and corn earworm (CEW, *Helicoverpa zea*). More recently, plants expressing the Vip3A protein have been found to be resistant to feeding damage caused by hemipteran insect pests (US Pantent No. 6,429,360). Thus, Vip3A proteins display a unique spectrum of insecticidal activities. Other disclosures, WO 98/18932, WO 98/33991, WO 98/00546, and WO 99/57282, have also now identified homologues of the Vip3 class of proteins. Proteins from the Vip3 class have heretofore not been shown to have any activity against non-insect pests such as nematodes.

[0007] Due to the above described limitations in the art, there remains a need to develop new methods for controlling nematode plant pests that provide an economic benefit to farmers and that are environmentally acceptable.

SUMMARY

[0008] The needs outlined above are met by the invention which, in various embodiments, provides new methods of controlling economically important nematode pests. In particular, transgenic plants and/or plant parts expressing the vegetative insecticidal protein, Vip3, were surprisingly found to be capable of inhibiting the ability of nematode pests to survive, grow and reproduce, or of limiting nematode-related damage or loss to crop plants. The invention is further drawn to transgenic nematode-resistant plants which express a Vip3 protein and to methods of using the transgenic plants alone or in combination with other nematode control strategies to confer maximal nematode control efficiency with minimal environmental impact. Plants and plant parts expressing a Vip3 protein are highly tolerant or resistant to nematode infestation. For example, the economically important nematode pest, soybean cyst nematode (*Heterodera glycines*) can be controlled by transgenic soybean plants which express a Vip3A protein.

[0009] According to one aspect, the invention provides a method of controlling a nematode pest comprising contacting the nematode pest with a Vip3 protein comprising SEQ ID NO: 19. In another aspect, the Vip3 protein can be a Vip3A protein, for example, SEQ ID NO:1 or a nematode-active Vip3A protein homologue having at least about 82% identity with SEQ ID NO: 1.

[0010] According to one aspect, the invention provides a method of controlling a nematode pest, comprising contacting the nematode pest with a transgenic plant or plant part comprising a heterologous nucleic acid molecule that directs expression of a Vip3 protein of the invention in the transgenic plant or plant part, wherein the transgenic plant or plant part controls the nematode pest compared to a plant or plant part of the same type that does not express the Vip3 protein.

[0011] In another aspect of the invention, the nematode pest is selected from the group consisting of *Criconemella, Ditylenchus, Globodera, Helicotylenchus, Heterodera, Longidorus, Meloidogyne, Paratrichodorus, Pratylenchus, Radolpholus, Rotelynchus, Rotylenchulus, Tylenchulus and Xiphinema*. Such nematode pests selected from these genera can be cyst-forming nematodes. In another aspect, the cyst-forming nematodes are in the genus *Heterodera*. In yet another aspect, the nematode pest is *Heterodera glycines* (soybean cyst nematode).

[0012] In another aspect of the invention, the transgenic plant or plant part is selected from the group consisting of alfalfa, apple, apricot, Arabidopsis, artichoke, asparagus, avocado, banana, barley, beans, beet, blackberry, blueberry, Brassica, broccoli, Brussels sprouts, cabbage, canola, carrot, cassaya, cauliflower, a cereal, celery, cherry, citrus, Clementine, coffee, corn, cotton, cucumber, eggplant, endive, eucalyptus, figs, grape, grapefruit, groundnuts, ground cherry, kiwifruit, lettuce, leek, lemon, lime, pine, maize, mango, melon, millet, mushroom, nut oat, okra, onion, orange, an ornamental plant or flower or tree, papaya, parsley, pea, peach, peanut, peat, pepper, persimmon, pineapple, plantain, plum, pomegranate, potato, pumpkin, radicchio, radish, rapeseed, raspberry, rice, rye, sorghum, soy, soybean, spinach, strawberry, sugar beet, sugarcane, sunflower, sweet potato, tangerine, tea, tobacco, tomato, a vine, watermelon, wheat, yams and zucchini. In still another aspect, the transgenic plant or plant part is a soybean plant or plant part.

[0013] In another aspect of the invention, the plant part is a root. In still another aspect, the root is a soybean root.

[0014] In yet another aspect of the invention, the Vip3 protein is a Vip3A protein. In still another aspect, the Vip3A protein comprises an amino acid sequence that is the translation product of a nucleotide sequence whose complement hybridizes to SEQ ID

NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15 or SEQ ID NO: 17 under high-stringency conditions.

[0015] In another aspect, the Vip3 protein of the invention is selected from the group consisting of Vip3Aa1 (AAC37036), Vip3Aa2 (AAC37037), Vip3Aa3 (US Patent 6137033), Vip3Aa4 (AAR81079), Vip3Aa5 (AAR81080), Vip3Aa6 (AAR81081), Vip3Aa7 (AAK95326), Vip3Aa8 (AAK97481), Vip3Aa9 (CAA76665), Vip3Aa10 (AAN60738), Vip3Aa11 (AAR36859), Vip3Aa12 (AAM22456), Vip3Aa13 (AAL69542), Vip3Aa14 (AAQ12340), Vip3Aa15 (AAP51131), Vip3Aa16 (AAW65132), Vip3Aa17 (US Patent 6603063), Vip3Aa18 (AAX49395), Vip3Aa19 (DQ241674), Vip3Aa19 (DQ539887), Vip3Aa20 (DQ539888), Vip3Aa21 (ABD84410), Vip3Aa22 (AAY41427), Vip3Aa23 (AAY41428), Vip3Aa24 (BI 880913), Vip3Aa25 (EF608501), Vip3Aa26 (EU294496), Vip3Aa27 (EU332167), Vip3Aa28 (FJ494817), Vip3Aa29 (FJ626674), Vip3Aa30 (FJ626675), Vip3Aa31 (FJ626676), Vip3Aa32 (FJ626677), Vip3Aa33 (GU073128), Vip3Aa34 (GU073129), Vip3Aa35 (GU733921), Vip3Aa36 (GU951510), Vip3Aa37 (HM132041), Vip3Aa38 (HM117632), Vip3Aa39 (HM117631), Vip3Aa40 (HM132042), Vip3Aa41 (HM132043), Vip3Aa42 (HQ587048), Vip3Aa43 (HQ594534), Vip3Aa44 (HQ650163), Vip3Ab1 (AAR40284), Vip3Ab2 (AAY88247), Vip3Ac1 (US Patent Application Publication 20040128716), Vip3Ad1 (US Patent Application Publication 20040128716), Vip3Ad2 (CAI43276), Vip3Ae1 (CAI43277), Vip3Af1 (CAI43275), Vip3Af2 (ADN08753), Vip3Af3 (HM117634), Vip3Ag1 (ADN08758),Vip3Ag2 (FJ556803),Vip3Ag3 (HM117633), Vip3Ag4 (HQ414237), Vip3Ag5 (HQ542193), and Vip3Ah1 (DQ832323).

[0016] In another aspect, a Vip3A protein of the invention comprises SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16, or a nematode-active homologue thereof having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99sequence identity with SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16. In another embodiment, the Vip3A protein comprises SEQ ID NO: 1 or a nematode-active homologue having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NO: 1.

[0017] In another aspect of the invention, a transgenic plant of the invention further comprises or expresses at least one additional pesticidal agent, for example without limitation, a patatin, a *Bacillus thuringiensis* insecticidal protein, a *Bacillus thuringiensis* nematicidal protein, a *Xenorhabdus* insecticidal protein, a *Photorhabdus* insecticidal protein, a *Bacillus laterosporous* insecticidal protein, a *Bacillus sphearicus* insecticidal protein and/or an RNAi molecule that targets a nematode pest.

[0018] In another another aspect, a *Bacillus thuringiensis* nematicidal protein is selected from the group consisting of a Cry1, a Cry3, a Cry11, a Cry12, a Cry13, a Cry14, a Cry21, and a Cry22.

[0019] According to another aspect, the invention provides a method of conferring nematode resistance to a plant and/or a plant part comprising inserting in the plant and/or a plant part a heterologous nucleic acid molecule encoding a Vip3 protein, wherein the plant and/or plant part expresses the Vip3 protein at a nematode-inhibiting level so as to confer nematode resistance to the plant and/or plant part compared to the same type of plant and/or plant part not expressing the Vip3 protein. Such insertion may occur via transformation or breeding.

[0020] According to another aspect, the invention provides a method of reducing nematode infectivity to a plant and/or plant part comprising contacting the nematode with a Vip3 protein, wherein nematode infectivity is reduced compared to infectivity of a plant and/or plant part by a nematode not contacted with a Vip3 protein.

[0021] In yet another aspect, the invention provides a transgenic soybean plant or plant part thereof comprising a heterologous nucleic acid molecule encoding a Vip3 protein, wherein the transgenic soybean plant or plant part is resistant to nematode infestation.

[0022] In another aspect, the invention provides a method of producing a soybean plant protected against nematode infestation, comprising transforming a soybean plant cell with a nucleic acid molecule encoding a Vip3 protein; and regenerating a transformed soybean plant from the soybean plant cell, wherein the transformed plant is protected against nematode infestation.

[0023] According to another aspect, the invention provides a method of producing a soybean plant protected against nematode infestation, comprising crossing a first parent soybean plant with a second parent soybean plant, wherein the first or second parent soybean plant comprises a heterologous nucleic acid molecule encoding a Vip3 protein,

thereby producing a plurality of progeny plants; and selecting from the plurality of progeny plants, a transgenic plant that is protected against nematode infestation.

[0024] In another aspect of the invention, a method of reducing nematode cyst development on roots of a plant infectable by a nematode is provided, comprising introducing into cells of the root a nucleic acid molecule capable of directing the expression of a Vip3 protein, thereby reducing nematode cyst development on roots of the plant.

[0025] In another aspect, the invention provides a method of controlling or preventing nematode growth comprising providing a nematode pest with plant material comprising a heterologous DNA capable of directing expression of a Vip3 protein, wherein said plant inhibits a nematode biological activity.

[0026] In yet another aspect of the invention a method of providing a grower with a means of controlling nematode pests is provided, the method comprising supplying seed to a grower, wherein the seed comprises a heterologous nucleic acid molecule that encodes a Vip3 protein and wherein the seed is capable of producing a plant that is resistant to nematode infestation.

[0027] In another aspect of the invention, a method of suppressing growth of a plant-pathogenic nematode population in a location capable of supporting growth of the nematode population is provided comprising growing in the location a population of transgenic soybean plants comprising a heterologous nucleic acid molecule capable of directing expression of a Vip3 protein, wherein growth of the plant-pathogenic nematode population is suppressed.

[0028] The invention also provides a method of controlling *Heterodera glycines* comprising providing a transgenic soybean plant comprising an expression cassette having SEQ ID NO: 1 operably linked to a promoter capable of driving expression of an encoded Vip3 protein to levels sufficient to inhibit nematodes, wherein the proliferation of *Heterodera glycines* feeding on the soybean plant is reduced compared to *Heterodera glycines* feeding on a non-transgenic soybean plant not comprising the expression cassette.

[0029] According to another aspect, the invention provides a method of improving plant yield in nematode infested fields, comprising expressing in the plant a Vip3 protein,

wherein plant yield is improved compared to yield of a plant of the same type not expressing a Vip3 protein.

[0030] In another aspect, the invention provides a method of increasing the vigor or yield in a transgenic soybean plant exposed to a population of nematodes comprising: introgressing a transgenic soybean event into a soybean plant resulting in a transgenic soybean plant, wherein the transgenic soybean event comprises a heterologous DNA sequence encoding a Vip3 protein that confers upon the transgenic soybean event resistance to nematodes; and growing the transgenic soybean plant or progeny thereof at a location where nematode infestation is yield limiting to a soybean plant not comprising the heterologous nucleic acid molecule encoding the Vip3 protein, whereby the transgenic soybean plant has increased vigor or yield compared to the control plant.

[0031] In another aspect, the invention provides a method of improving yield of a soybean field, comprising: introducing into a soybean plant a nucleic acid molecule capable of directing expression of a Vip3 protein, thus prodcing a transgenic plant; and cultivating a plurality of transgenic seeds from the transgenic plant in a filed producing a, resulting in a soybean field comprising a plurality of transgenic soybean plants having enhanced resistance to nematode infestation, thereby improving yield of the soybean field.

[0032] The present invention also provides a recombinant expression cassette comprising a heterologous promoter sequence operatively linked to a nucleic acid molecule encoding a Vip3 protein. Further, the present invention provides a recombinant vector comprising such an expression cassette. Still further, the present invention provides a transgenic host cell comprising such an expression cassette. A transgenic host cell according to this aspect of the invention may be a plant cell. Even further, the present invention provides a transgenic plant or plant part comprising such a plant cell.

[0033] The present invention also provides a nematicidal composition comprising an effective nematode-controlling amount of a Vip3 protein and an acceptable agricultural carrier. In another aspect, the agricultural carrier is a transgenic plant. In yet another aspect, the transgenic plant is a transgenic soybean plant and the Vip3 protein is a Vip3A protein having at least 82% sequence identity with SEQ ID NO: 1. In another aspect, the Vip3A protein comprises SEQ ID NO: 1.

[0034] In a further aspect, the present invention provides a method of producing a nematode-resistant transgenic plant, comprising introducing a nucleic acid molecule encoding a Vip3 protein into a plant cell therby making a transgenic plant cell; regenerating a transgenic plant from the transgenic plant cell, wherein the Vip3A protein is expressible in the transgenic plant in an effective amount to control nematodes. According to another aspect, the plant is a soybean plant. In another aspect, the Vip3 protein is a Vip3A protein having at least 82% sequence identity with SEQ ID NO: 1. In yet another aspect, the Vip3A protein comprises SEQ ID NO: 1. In still another aspect, the nematode is a cyst forming nematode. In another aspect, the cyst forming nematode is is the geneus Heterodera. In still another apsct the cyst nematode is soybean cyst nematode (*Heteroder a glycines*).

[0035] Other aspects and advantages of the invention will become apparent to those skilled in the art from a study of the following description of the invention and non-limiting examples.

BRIEF DESCRIPTION OF THE SEQUENCES IN THE SEQUENCE LISTING

SEQ ID NO: 1 is an amino acid sequence of a Vip3Aa20 protein.
SEQ ID NO: 2 is a nucleotide sequence encoding SEQ ID NO: 1.
SEQ ID NO: 3 is an amino acid sequence of a Vip3Aa1 protein.
SEQ ID NO: 4 is a nucleotide sequence encoding SEQ ID NO: 3.
SEQ ID NO: 5 is a nucleotide sequence encoding SEQ ID NO: 3.
SEQ ID NO: 6 is an amino acid sequence of a Vip3Aa2 protein.
SEQ ID NO: 7 is a nucleotide sequence encoding SEQ ID NO: 6.
SEQ ID NO: 8 is an amino aicd sequence of a Vip3Aa3 protein.
SEQ ID NO: 9 is a nucleotide sequenc encoding SEQ ID NO: 8.
SEQ ID NO: 10 is an amino acid sequence of a Vip3Af2 protein.
SEQ ID NO: 11 is a nucleotide sequence encoding SEQ ID NO: 10.
SEQ ID NO: 12 is an amino acid sequence of a Vip3Af4 protein.
SEQ ID NO: 13 is a nucleotide sequence encoding SEQ ID NO: 12.
SEQ ID NO: 14 is an amino acid sequence of a Vip3Af5 protein.
SEQ ID NO: 15 is a nucleotide sequence encoding SEQ ID NO: 14.
SEQ ID NO: 16 is an amino acid sequence of a Vip3Ag1 protein.

SEQ ID NO: 17 is a nucleotide sequence encoding SEQ ID NO: 16.

SEQ ID NO: 18 is the nucleotide sequence of the vector pKS214.

SEQ ID NO: 19 is a Vip3-identifying amino acid sequence.

DETAILED DESCRIPTION

[0036] 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.

[0037] 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. Flames and G. R. Taylor eds. (1995)], Harlow and Lane, eds. (1988) Antibodies, A Laboratory Manual, and Animal Cell Culture [R. I. Freshney, ed. (1987)].

[0038] 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.

[0039] In order to facilitate understanding of the disclosure, the following definitions are provided:

[0040] "Activity" of the Vip3 proteins of the invention is meant that the Vip3 proteins have a toxic effect on nematodes by disrupting or detering feeding, inhibiting the ability of nematode pests to survive, grow and reproduce which may or may not cause death of the nematode, or of limiting nematode-related damage or loss to crop plants.

[0041] As used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as lack of combinations when interpreted in the alternative (or).

[0042] "Associated with / operatively linked" refer to two nucleic acid sequences that are related physically or functionally. For example, a promoter or regulatory DNA sequence is said to be "associated with" a DNA sequence that codes for an RNA or a protein if the two sequences are operatively linked, or situated such that the regulator DNA sequence will affect the expression level of the coding or structural DNA sequence.

[0043] As used herein, the term “contacting” refers to a process by which a Vip3 protein of the invention or transgenic plant or plant part expressing the Vip3 protein of the invention are delivered or administered to target nematode pests or nematode pest populations. Contacting describes physical proximity of Vip3 proteins or transgenic plants or plant parts expessing a Vip3 protein and the target nematode so that they interact. The transgenic plants or plant parts may be contacted with a target nematode or nematode population by planting transgenic seed, transgenic seedlings, cuttings, plant runners, tubers, and the like in a location capable of supporting growth of a nematode pest or nematode pest population.

[0044] A "chimeric gene" is a recombinant nucleic acid sequence in which a promoter or regulatory nucleic acid sequence is operatively linked to, or associated with, a nucleic acid sequence that codes for an mRNA or which is expressed as a protein, such that the regulator nucleic acid sequence is able to regulate transcription or expression of the

associated nucleic acid sequence. The regulator nucleic acid sequence of the chimeric gene is not normally operatively linked to the associated nucleic acid sequence as found in nature.

[0045] A "coding sequence" is a nucleic acid sequence that is transcribed into RNA such as mRNA, rRNA, tRNA, snRNA, sense RNA or antisense RNA. Preferably the RNA is then translated in an organism to produce a protein.

[0046] As used herein the terms "to control" or “controlling” nemtodes means to inhibit, through a toxic effect, the ability of nemtode pests to survive, grow, feed, and/or reproduce, or to limit nematode-related damage or loss in crop plants. To "control" nematodes may or may not mean killing the nemtodes.

[0047] Corresponding to: in the context of the present invention, “corresponding to” or “corresponds to” means that when the nucleic acid coding sequences or amino acid sequences of different Vip3 genes or proteins are aligned with each other, the nucleic or amino acids that “correspond to” certain enumerated positions are those that align with these positions but that are not necessarily in these exact numerical positions relative to the particular Vip3’s respective nucleic acid coding sequence or amino acid sequence. Likewise, when the coding or amino acid sequence of a particular Vip3 (for example, Vip3Ag1) is aligned with the coding or amino acid sequence of a reference Vip3 (for example, Vip3Aa20), the nucleic acids or amino acids in the Vip3Ag1 sequence that “correspond to” certain enumerated positions of the Vip3Aa20 sequence are those that align with these positions of the Vip3Aa20 sequence, but are not necessarily in these exact numerical positions of the Vip3Ag1protein’s respective nucleic acid coding sequence or amino acid sequence.

[0048] To "deliver" a toxin means that the toxin comes in contact with a nematode or nematode population, resulting in a toxic effect and control of the nematode or nematode population. The toxin can be delivered in many recognized ways, e.g., orally by ingestion by the nematode or by contact with the nematode via transgenic plant expression, formulated protein composition(s), sprayable protein composition(s), a bait matrix, or any other art-recognized toxin delivery system.

[0049] The term “economic threshold” is defined as the pest nematode population that produces incremental damage equal to the cost of controlling or preventing that damage. It is the level of nematode population where the benefit of nematode control is equal to its

cost. In this regard, economic threshold may be defined as the nematode pest damage level where the value of incremental reduction in crop yield is equal to the cost of preventing its occurrence. In other words, economic threshold attempts to determine the point at which it becomes economically feasible to control a nematode pest population. Economic damage to the host crop normally is inflicted by the first generation progeny of nematodes and is prevented by transgenic plants expressing a Vip3 protein through lowering the concentration of progeny nematodes in the plant root zone.

[0050] A "nematode-controlling effective amount" as used herein refers to the concentration of a Vip3 toxin capable of inhibiting, through a toxic effect, the ability of nematodes to survive, grow, feed and/or reproduce, or of reducing or preventing nematode-related damage or loss in crop plants. "Nematode-controlling effective amount" may or may not mean killing the nematodes.

[0051] "Expression cassette" as used herein means a nucleic acid sequence capable of directing expression of a particular nucleotide sequence in an appropriate host cell, comprising a promoter operably linked to the nucleotide sequence of interest which is operably linked to termination signals. It also typically comprises sequences required for proper translation of the nucleotide sequence. The expression cassette comprising the nucleotide sequence of interest may be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components. The expression cassette may also be one that is naturally occurring but has been obtained in a recombinant form useful for heterologous expression. Typically, however, the expression cassette is heterologous with respect to the host, i.e., the particular nucleic acid sequence of the expression cassette does not occur naturally in the host cell and must have been introduced into the host cell or an ancestor of the host cell by a transformation event. The expression of the nucleotide sequence in the expression cassette may be under the control of a constitutive promoter or of an inducible promoter that initiates transcription only when the host cell is exposed to some particular external stimulus. In the case of a multicellular organism, such as a plant, the promoter can also be specific to a particular tissue, or organ, or stage of development.

[0052] A "gene" is a defined region that is located within a genome and that, besides the aforementioned coding nucleic acid sequence, comprises other, primarily regulatory, nucleic acid sequences responsible for the control of the expression, that is to say the

transcription and translation, of the coding portion. A gene may also comprise other 5' and 3' untranslated sequences and termination sequences. Further elements that may be present are, for example, introns.

[0053] A "heterologous" nucleic acid sequence is a nucleic acid sequence not naturally associated with a host cell into which it is introduced, including non- naturally occurring multiple copies of a naturally occurring nucleic acid sequence.

[0054] "Nematicidal" is defined as a toxic biological activity capable of controlling nematodes, preferably by killing them.

[0055] A nucleic acid sequence is "isocoding with" a reference nucleic acid sequence when the nucleic acid sequence encodes a polypeptide having the same amino acid sequence as the polypeptide encoded by the reference nucleic acid sequence. For example, a native coding sequence from Bacillus spp. that encodes a Vip3 protein is isocoding with a coding sequence codon optimized for expression in a plant that encodes the same Vip3 protein.

[0056] An "isolated" nucleic acid molecule or an isolated protein or toxin is a nucleic acid molecule or protein or toxin that, by the hand of man, exists apart from its native environment and is therefore not a product of nature. An isolated nucleic acid molecule or protein or toxin may exist in a purified form or may exist in a non-native environment such as, for example, a recombinant host cell or a transgenic plant.

[0057] The term “native” refers to a coding sequence or gene that is naturally present in the genome of a cell or plant.

[0058] The term "naturally occurring" is used herein to describe an object that can be found in nature as distinct from being artificially produced by man. For example, a protein or nucleotide sequence present in an organism (including a virus), which can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory, is naturally occurring.

[0059] A "plant" is any plant at any stage of development, particularly a seed plant.

[0060] A "plant cell" is a structural and physiological unit of a plant, comprising a protoplast and a cell wall. The plant cell may be in the form of an isolated single cell or a cultured cell, or as a part of higher organized unit such as, for example, plant tissue, a plant organ, or a whole plant.

[0061] "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.

[0062] A "plant organ" is a distinct and visibly structured and differentiated part of a plant such as a root, stem, leaf, flower bud, or embryo.

[0063] A “plant part” may be any part of a plant and include a plant cell, plant material, plant organ or plant tissue.

[0064] "Plant tissue" as used herein means a group of plant cells organized into a structural and functional unit. Any tissue of a plant in planta 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.

[0065] A "promoter" is an untranslated DNA sequence upstream of the coding region that contains the binding site for RNA polymerase 11 and initiates transcription of the DNA. The promoter region may also include other elements that act as regulators of gene expression.

[0066] “Regulatory elements" refer to sequences involved in controlling the expression of a nucleotide sequence. Regulatory elements comprise a promoter operably linked to the nucleotide sequence of interest and termination signals. They also typically encompass sequences required for proper translation of the nucleotide sequence.

[0067] As used herein, “resistant” or resistance means a transgenic soybean variety that prevents a majority of nematodes from surviving and/or reproducing upon their attempted infestation.

[0068] The term "substantially identical," in the context of two nucleic acid or protein sequences, refers to two or more sequences or subsequences that have at least 60%, preferably 80%, more preferably 90, even more preferably 95%, and most preferably at least 99% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection. Preferably, the substantial identity exists over a region of the sequences that is at least about 50 residues in length, more preferably over a

region of at least about 100 residues, and most preferably the sequences are substantially identical over at least about 150 residues. In an especially preferred embodiment, the sequences are substantially identical over the entire length of the coding regions. Furthermore, substantially identical nucleic acid or protein sequences perform substantially the same function.

[0069] For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0070] Optimal alignment of sequences for comparison can be conducted, e.g., by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2: 482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48: 443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (*see generally,* Ausubel *et al., infra).*

[0071] One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul *et al., J. Mol. Biol.* 215: 403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al.*, 1990). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences,

the parameters M (reward score for a pair of matching residues; always > 0) and N (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when the cumulative alignment score falls off by the quantity X from its maximum achieved value, the cumulative score goes to zero or below due to the accumulation of one or more negative-scoring residue alignments, or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an expectation (E) of 10, a cutoff of 100, M=5, N=-4, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix *(see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89: 10915 (1989)).

[0072] In addition to calculating percent sequence identity, the BLAST algorithm also performs a statistical analysis of the similarity between two sequences *(see, e.g.,* Karlin & Altschul, *Proc. Nat'l. Acad. Sci. USA* 90: 5873-5787 (1993)). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a test nucleic acid sequence is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid sequence to the reference nucleic acid sequence is less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

[0073] Another indication that two nucleic acid sequences are substantially identical is that the two molecules hybridize to each other under stringent conditions. The phrase "hybridizing specifically to" refers to the binding, duplexing, or hybridizing of a molecule only to a particular nucleotide sequence under stringent conditions when that sequence is present in a complex mixture (*e.g.,* total cellular) DNA or RNA. “Bind(s) substantially” refers to complementary hybridization between a probe nucleic acid and a target nucleic acid and embraces minor mismatches that can be accommodated by reducing the stringency of the hybridization media to achieve the desired detection of the target nucleic acid sequence.

[0074] "Stringent hybridization conditions" and "stringent hybridization wash conditions" in the context of nucleic acid hybridization experiments such as Southern and Northern

hybridizations are sequence dependent, and are different under different environmental parameters. Longer sequences hybridize specifically at higher temperatures. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology-Hybridization with Nucleic* Acid Probes part I chapter 2 "Overview of principles of hybridization and the strategy of nucleic acid probe assays" Elsevier, New York. Generally, highly stringent hybridization and wash conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. Typically, under "stringent conditions" a probe will hybridize to its target subsequence, but to no other sequences.

[0075] A nucleic acid molecule is said to be the "complement" of another nucleic acid molecule if they exhibit complete complementarity. As used herein, molecules are said to exhibit "complete complementarity" when every nucleotide of one of the molecules is complementary to a nucleotide of the other. Two molecules are said to be "minimally complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under at least conventional "low-stringency" conditions. Similarly, the molecules are said to be "complementary" if they can hybridize to one another with sufficient stability to permit them to remain annealed to one another under conventional "high-stringency" conditions. Conventional stringency conditions are described by Sambrook, et al., In: Molecular Cloning A Laboratory Manual, 2nd Edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989), and by Haymes, et al. In: Nucleic Acid Hybridization, A Practical Approach, IRL Press, Washington, D.C. (1985), herein incorporated by reference in its entirety.

[0076] The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the T_m for a particular probe. An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or northern blot is 50% formamide with 1 mg of heparin at 42°C, with the hybridization being carried out overnight. An example of highly stringent wash conditions is 0.1 5M NaCl at 72°C for about 15 minutes. An example of stringent wash conditions is a 0.2x SSC wash at 65°C for 15 minutes (*see,* Sambrook, *infra*, for a description of SSC buffer). Often, a high

stringency wash is preceded by a low stringency wash to remove background probe signal. An example medium stringency wash for a duplex of, e.g., more than 100 nucleotides, is 1x SSC at 45°C for 15 minutes. An example low stringency wash for a duplex of, *e.g.,* more than 100 nucleotides, is 4-6x SSC at 40°C for 15 minutes. For short probes (*e.g.,* about 10 to 50 nucleotides), stringent conditions typically involve salt concentrations of less than about 1.0 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3, and the temperature is typically at least about 30°C. Stringent conditions can also be achieved with the addition of destabilizing agents such as formamide. In general, a signal to noise ratio of 2x (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization. Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the proteins that they encode are substantially identical. This occurs, e.g., when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code.

[0077] The following are examples of sets of hybridization/wash conditions that may be used to clone homologous nucleotide sequences that are substantially identical to reference *vip3* nucleotide sequences of the present invention: a reference nucleotide sequence preferably hybridizes to the reference nucleotide sequence in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$, 1 mM EDTA at 50°C with washing in 2X SSC, 0.1% SDS at 50°C, more desirably in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$, 1 mM EDTA at 50°C with washing in 1X SSC, 0.1% SDS at 50°C, more desirably still in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$, 1 mM EDTA at 50°C with washing in 0.5X SSC, 0.1% SDS at 50°C, preferably in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$, 1 mM EDTA at 50°C with washing in 0.1X SSC, 0.1% SDS at 50°C, more preferably in 7% sodium dodecyl sulfate (SDS), 0.5 M $NaPO_4$, 1 mM EDTA at 50°C with washing in 0.1X SSC, 0.1% SDS at 65°C.

[0078] A further indication that two nucleic acid sequences or proteins are substantially identical is that the protein encoded by the first nucleic acid is immunologically cross reactive with, or specifically binds to, the protein encoded by the second nucleic acid. Thus, a protein is typically substantially identical to a second protein, for example, where the two proteins differ only by conservative substitutions.

[0079] "Synthetic" refers to a nucleotide sequence comprising structural characters that are not present in the natural sequence. For example, a vip3 coding sequence, naturally found in Bacillus, that resembles more closely the G+C content and the normal codon distribution of dicot and/or monocot genes is said to be synthetic.

[0080] "Transformation" is a process for introducing heterologous nucleic acid into a host cell or organism. In particular, "transformation" means the stable integration of a DNA molecule into the genome of an organism of interest.

[0081] "Transformed / transgenic / 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.

[0082] A "Vip3 protein" in the context of the invention means a vegetative insecticidal protein (VIP) that is a member of the Vip3 class including for example without limitation, Vip3Aa1, Vip3Aa2, Vip3Aa3, Vip3Aa19, Vip3Aa20, Vip3Af2, Vip3Ag1, and their homologues. Some structural features that identify a protein as being in the Vip3 class of proteins includes, 1) a size of about 80 kDa; and 2) a highly conserved N-terminal secretion signal that comprises the amino acid sequence IYGFATGIKDI (SEQ ID NO: 17) which is not processed during secretion in *Bacillus*. A nematode-active "homologue" as used herein means that the indicated protein or polypeptide is active against nematodes and bears a defined relationship to other members of the Vip3 class of proteins. This defined relationship may include but is not limited to, 1) proteins which are at least 70%, or least 80% , or at least 90% identical at the sequence level to another member of the Vip3 class of proteins while also retaining nematicidal activity, 2) proteins which are cross-reactive to antibodies which immunologically recognize another member of the Vip3 class of proteins, 3) proteins which are cross-reactive with a nematode receptor to another member of the Vip3 class of proteins and retain nematode activity when expressed in a transgenic plant, and 4) proteins which are at least 70%, or at least 80%, or

at least 90% identical at the sequence level to a toxic core region of another member of the Vip3 class of proteins while also retaining nematicidal activity. Non-limiting examples of members of the Vip3 class including those previously mentioned and their respective GenBank accession numbers or US Patent or patent publication number are Vip3Aa1 (AAC37036), Vip3Aa2 (AAC37037), Vip3Aa3 (US Patent 6137033), Vip3Aa4 (AAR81079), Vip3Aa5 (AAR81080), Vip3Aa6 (AAR81081), Vip3Aa7 (AAK95326), Vip3Aa8 (AAK97481), Vip3Aa9 (CAA76665), Vip3Aa10 (AAN60738), Vip3Aa11 (AAR36859), Vip3Aa12 (AAM22456), Vip3Aa13 (AAL69542), Vip3Aa14 (AAQ12340), Vip3Aa15 (AAP51131), Vip3Aa16 (AAW65132), Vip3Aa17 (US Patent 6603063), Vip3Aa18 (AAX49395), Vip3Aa19 (DQ241674), Vip3Aa19 (DQ539887), Vip3Aa20 (DQ539888), Vip3Aa21 (ABD84410), Vip3Aa22 (AAY41427), Vip3Aa23 (AAY41428), Vip3Aa24 (BI 880913), Vip3Aa25 (EF608501), Vip3Aa26 (EU294496), Vip3Aa27 (EU332167), Vip3Aa28 (FJ494817), Vip3Aa29 (FJ626674), Vip3Aa30 (FJ626675), Vip3Aa31 (FJ626676), Vip3Aa32 (FJ626677), Vip3Aa33 (GU073128), Vip3Aa34 (GU073129), Vip3Aa35 (GU733921), Vip3Aa36 (GU951510), Vip3Aa37 (HM132041), Vip3Aa38 (HM117632), Vip3Aa39 (HM117631), Vip3Aa40 (HM132042), Vip3Aa41 (HM132043), Vip3Aa42 (HQ587048), Vip3Aa43 (HQ594534), Vip3Aa44 (HQ650163), Vip3Ab1 (AAR40284), Vip3Ab2 (AAY88247), Vip3Ac1 (US Patent Application Publication 20040128716), Vip3Ad1 (US Patent Application Publication 20040128716), Vip3Ad2 (CAI43276), Vip3Ae1 (CAI43277), Vip3Af1 (CAI43275), Vip3Af2 (ADN08753), Vip3Af3 (HM117634), Vip3Ag1 (ADN08758),Vip3Ag2 (FJ556803),Vip3Ag3 (HM117633), Vip3Ag4 (HQ414237), Vip3Ag5 (HQ542193), and Vip3Ah1 (DQ832323).

[0083] As used herein, nucleotides are indicated by their bases by the following standard abbreviations: adenine (A), cytosine (C), thymine (T), and guanine (G). Amino acids are likewise indicated by the following standard abbreviations: alanine (Ala; A), arginine (Arg; R), asparagine (Asn; N), aspartic acid (Asp; D), cysteine (Cys; C), glutamine (Gln; Q), glutamic acid (Glu; E), glycine (Gly; G), histidine (His; H), isoleucine (Ile; 1), leucine (Leu; L), lysine (Lys; K), methionine (Met; M), phenylalanine (Phe; F), proline (Pro; P), serine (Ser; S), threonine (Thr; T), tryptophan (Trp; W), tyrosine (Tyr; Y), and valine (Val; V).

[0084] The materials and methods of the subject invention are useful for killing or controlling nematodes; retarding growth or reproduction of nematodes; reducing nematode populations; and/or reducing or retarding damage to plants caused by infestation of nematode pests. In particular, the invention provides methods of controlling nematode pests of crop plants such as soybean by using transgenic crop plants expressing a Vip3 protein.

[0085] The expression in transgenic plants or plant parts of the Vip3 proteins of the invention results in compositions that can be used to control nematode pests, for example, without limitation, *Meloidogyne spp.* (for example, *Meloidogyne incoginita* and *Meloidogyne javanica*, *Meloidogyne hapla*, *Meloidogyne arenari*), *Heterodera spp.* (for example, *Heterodera glycines*, *Heterodera carotae*, *Heterodera schachtii*, *Heterodora avenae* and *Heterodora trifolii*), *Globodera spp.* (for example, *Globodera rostochiensis*), *Radopholus spp.* (for example, *Radopholus similes*), *Rotylenchulus spp.*, *Pratylenchus spp.* (for example, *Pratylenchus neglectans* and *Pratylenchus penetrans*), *Aphelenchoides spp.*, *Helicotylenchus spp.*, *Hoplolaimus spp.*, *Paratrichodorus spp.*, *Longidorus spp.*, *Nacobbus spp.*, *Subanguina spp. Belonlaimus spp.*, *Criconemella spp.*, *Criconemoides spp. Ditylenchus spp.*, *Ditylenchus dipsaci*, *Dolichodorus spp.*, *Hemicriconemoides spp.*, *Hemicycliophora spp.*, *Hirschmaniella spp.*, *Hypsoperine spp.*, *Macroposthonia spp.*, *Melinius spp.*, *Punctodera spp.*, *Quinisulcius spp.*, *Scutellonema spp.*, *Xiphinema spp.*, and *Tylenchorhynchus spp.*

[0086] In one embodiment, the invention encompasses a method of controlling a nematode pest, comprising contacting the nematode pest with a Vip3 protein comprising SEQ ID NO: 19.

[0087] In another embodiment, the nematode is selected from the group consisting of Criconemella, Ditylenchus, Globodera, Helicotylenchus, Heterodera, Longidorus, Meloidogyne, Paratrichodorus, Pratylenchus, Radolpholus, Rotelynchus, Rotylenchulus, Tylenchulus and Xiphinema. In yet another embodiment, the nematode is a cyst forming nematode. In still another embodiment, the nematode is in the genus *Heterodera.* In a further embodiment, the nematode is *Heterodera glycines.*

[0088] In another embodiment, the contacting step is carried out with a plant or plant part transformed with at least one nucleic acid molecule encoding the Vip3 protein. In yet

another embodiment, the plant or plant part is a soybean plant or plant part. In still another embodiment, the soybean plant part is a soybean root.

[0089] In another embodiment, the Vip3 protein is a Vip3A protein. In yet another embodiment, the Vip3A protein comprises an amino acid sequence that is the translation product of a nucleotide sequence whose complement hybridizes to SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15 or SEQ ID NO: 17 under high-stringency conditions. In still another embodiment, the high-stringency conditions are 7% sodium dodecyl sulfate (SDS), 0.5 M NaP_{O4}, 1 mM EDTA at 50° C. with washing in 0.1XSSC, 0.1% SDS at 65°C. In a further embodiment, the Vip3A protein is selected from the group consisting of Vip3Aa1 (AAC37036), Vip3Aa2 (AAC37037), Vip3Aa3 (US Patent 6137033), Vip3Aa4 (AAR81079), Vip3Aa5 (AAR81080), Vip3Aa6 (AAR81081), Vip3Aa7 (AAK95326), Vip3Aa8 (AAK97481), Vip3Aa9 (CAA76665), Vip3Aa10 (AAN60738), Vip3Aa11 (AAR36859), Vip3Aa12 (AAM22456), Vip3Aa13 (AAL69542), Vip3Aa14 (AAQ12340), Vip3Aa15 (AAP51131), Vip3Aa16 (AAW65132), Vip3Aa17 (US Patent 6603063), Vip3Aa18 (AAX49395), Vip3Aa19 (DQ241674), Vip3Aa19 (DQ539887), Vip3Aa20 (DQ539888), Vip3Aa21 (ABD84410), Vip3Aa22 (AAY41427), Vip3Aa23 (AAY41428), Vip3Aa24 (BI 880913), Vip3Aa25 (EF608501), Vip3Aa26 (EU294496), Vip3Aa27 (EU332167), Vip3Aa28 (FJ494817), Vip3Aa29 (FJ626674), Vip3Aa30 (FJ626675), Vip3Aa31 (FJ626676), Vip3Aa32 (FJ626677), Vip3Aa33 (GU073128), Vip3Aa34 (GU073129), Vip3Aa35 (GU733921), Vip3Aa36 (GU951510), Vip3Aa37 (HM132041), Vip3Aa38 (HM117632), Vip3Aa39 (HM117631), Vip3Aa40 (HM132042), Vip3Aa41 (HM132043), Vip3Aa42 (HQ587048), Vip3Aa43 (HQ594534), Vip3Aa44 (HQ650163), Vip3Ab1 (AAR40284), Vip3Ab2 (AAY88247), Vip3Ac1 (US Patent Application Publication 20040128716), Vip3Ad1 (US Patent Application Publication 20040128716), Vip3Ad2 (CAI43276), Vip3Ae1 (CAI43277), Vip3Af1 (CAI43275), Vip3Af2 (ADN08753), Vip3Af3 (HM117634), Vip3Ag1 (ADN08758),Vip3Ag2 (FJ556803),Vip3Ag3 (HM117633), Vip3Ag4 (HQ414237), Vip3Ag5 (HQ542193), and Vip3Ah1 (DQ832323). In yet another embodiment, the Vip3A protein comprises SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16, or a nematode-active homologue thereof having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%,

94%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16. In another embodiment, the Vip3A protein comprises SEQ ID NO: 1 or a nematode-active homologue having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NO: 1.

[0090] In one embodiment, the invention encompasses a plant or plant part infestable by a nematode and which is protected from the nematode by being transformed with at least one nucleic acid molecule encoding a Vip3 protein. In another embodiment, the plant or plant part is a soybean plant or plant part. In another embodiment, the nematode is in the genus *Heterodera.* In still another embodiment, the nematode is *Heterodera glycines.*

[0091] In one embodiment, the invention encompasses a method of controlling a nematode pest, comprising contacting the nematode pest with a transgenic plant or plant part comprising a heterologous nucleic acid molecule that directs expression of a Vip3 protein in the transgenic plant, wherein the transgenic plant controls the nematode pest compared to a plant of the same type that does not express the Vip3 protein.

[0092] In another embodiment, the nematode is selected from the group consisting of Criconemella, Ditylenchus, Globodera, Helicotylenchus, Heterodera, Longidorus, Meloidogyne, Paratrichodorus, Pratylenchus, Radolpholus, Rotelynchus, Rotylenchulus, Tylenchulus and Xiphinema. In yest another embodiment, the nematode is a cyst forming nematode. In still another embodiment, the nematode is in the genus *Heterodera.* In a further embodiment, the nematode is *Heterodera glycines.*

[0093] In another embodiment, the transgenic plant or plant part is selected from the group consisting of alfalfa, apple, apricot, Arabidopsis, artichoke, asparagus, avocado, banana, barley, beans, beet, blackberry, blueberry, Brassica, broccoli, Brussels sprouts, cabbage, canola, carrot, cassaya, cauliflower, a cereal, celery, cherry, citrus, Clementine, coffee, corn, cotton, cucumber, eggplant, endive, eucalyptus, figs, grape, grapefruit, groundnuts, ground cherry, kiwifruit, lettuce, leek, lemon, lime, pine, maize, mango, melon, millet, mushroom, nut oat, okra, onion, orange, an ornamental plant or flower or tree, papaya, parsley, pea, peach, peanut, peat, pepper, persimmon, pineapple, plantain, plum, pomegranate, potato, pumpkin, radicchio, radish, rapeseed, raspberry, rice, rye, sorghum, soy, soybean, spinach, strawberry, sugar beet, sugarcane, sunflower, sweet

potato, tangerine, tea, tobacco, tomato, a vine, watermelon, wheat, yams and zucchini. In yet another embodiment, the transgenic plant or plant pasrt is a soybean plant or plant part.

[0094] In another embodiment, the Vip3 protein is a Vip3A protein. In yet another embodiment, the Vip3A protein comprises an amino acid sequence that is the translation product of a nucleotide sequence whose complement hybridizes to SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15 or SEQ ID NO: 17 under high-stringency conditions. In still another embodiment, the high-stringency conditions are 7% sodium dodecyl sulfate (SDS), 0.5 M NaP_{O4}, 1 mM EDTA at 50° C. with washing in 0.1XSSC, 0.1% SDS at 65°C. In a further embodiment, the Vip3A protein is selected from the group consisting of Vip3Aa1 (AAC37036), Vip3Aa2 (AAC37037), Vip3Aa3 (US Patent 6137033), Vip3Aa4 (AAR81079), Vip3Aa5 (AAR81080), Vip3Aa6 (AAR81081), Vip3Aa7 (AAK95326), Vip3Aa8 (AAK97481), Vip3Aa9 (CAA76665), Vip3Aa10 (AAN60738), Vip3Aa11 (AAR36859), Vip3Aa12 (AAM22456), Vip3Aa13 (AAL69542), Vip3Aa14 (AAQ12340), Vip3Aa15 (AAP51131), Vip3Aa16 (AAW65132), Vip3Aa17 (US Patent 6603063), Vip3Aa18 (AAX49395), Vip3Aa19 (DQ241674), Vip3Aa19 (DQ539887), Vip3Aa20 (DQ539888), Vip3Aa21 (ABD84410), Vip3Aa22 (AAY41427), Vip3Aa23 (AAY41428), Vip3Aa24 (BI 880913), Vip3Aa25 (EF608501), Vip3Aa26 (EU294496), Vip3Aa27 (EU332167), Vip3Aa28 (FJ494817), Vip3Aa29 (FJ626674), Vip3Aa30 (FJ626675), Vip3Aa31 (FJ626676), Vip3Aa32 (FJ626677), Vip3Aa33 (GU073128), Vip3Aa34 (GU073129), Vip3Aa35 (GU733921), Vip3Aa36 (GU951510), Vip3Aa37 (HM132041), Vip3Aa38 (HM117632), Vip3Aa39 (HM117631), Vip3Aa40 (HM132042), Vip3Aa41 (HM132043), Vip3Aa42 (HQ587048), Vip3Aa43 (HQ594534), Vip3Aa44 (HQ650163), Vip3Ab1 (AAR40284), Vip3Ab2 (AAY88247), Vip3Ac1 (US Patent Application Publication 20040128716), Vip3Ad1 (US Patent Application Publication 20040128716), Vip3Ad2 (CAI43276), Vip3Ae1 (CAI43277), Vip3Af1 (CAI43275), Vip3Af2 (ADN08753), Vip3Af3 (HM117634), Vip3Ag1 (ADN08758),Vip3Ag2 (FJ556803),Vip3Ag3 (HM117633), Vip3Ag4 (HQ414237), Vip3Ag5 (HQ542193), and Vip3Ah1 (DQ832323). In still a further embodiment, the Vip3A protein comprises SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16, or a nematode-active homologue thereof having

at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16. In another embodiment, the Vip3A protein comprises SEQ ID NO: 1 or a nematode-active homologue having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NO: 1.

[0095] In another embodiment, the the transgenic plant further comprises or expresses at least one additional pesticidal agent selected from the group consisting of a patatin, a *Bacillus thuringiensis* insecticidal protein, a *Bacillus thuringiensis* nematicidal protein, a *Xenorhabdus* insecticidal protein, a *Photorhabdus* insecticidal protein, a *Bacillus laterosporous* insecticidal protein, and a *Bacillus sphearicus* insecticidal protein. In yet another embodiment, the *Bacillus thuringiensis* nematicidal protein is selected from the group consisting of a Cry1, Cry3, Cry11, Cry12, Cry13, Cry14, Cry21, and Cry22.

[0096] In one embodiment, the invention encompasses a method of conferring nematode resistance to a plant or plant part comprising inserting into the plant or plant part a heterologous nucleic acid molecule encoding a Vip3 protein, wherein the plant or plant part expresses the Vip3 protein at a nematode-inhibiting level so as to confer nematode resistance to the plant or plant part compared to the same type of plant or plant part not expressing the Vip3 protein.

[0097] In another embodiment, the nematode is selected from the group consisting of Criconemella, Ditylenchus, Globodera, Helicotylenchus, Heterodera, Longidorus, Meloidogyne, Paratrichodorus, Pratylenchus, Radolpholus, Rotelynchus, Rotylenchulus, Tylenchulus and Xiphinema. In yet another embodiment, the nematode is a cyst forming nematode. In still another embodiment, the nematode is in the genus *Heterodera.* In a further embodiment, the nematode is *Heterodera glycines.*

[0098] In another embodiment, the plant or plant part is selected from the group consisting of alfalfa, apple, apricot, Arabidopsis, artichoke, asparagus, avocado, banana, barley, beans, beet, blackberry, blueberry, Brassica, broccoli, Brussels sprouts, cabbage, canola, carrot, cassaya, cauliflower, a cereal, celery, cherry, citrus, Clementine, coffee, corn, cotton, cucumber, eggplant, endive, eucalyptus, figs, grape, grapefruit, groundnuts, ground cherry, kiwifruit, lettuce, leek, lemon, lime, pine, maize, mango, melon, millet,

mushroom, nut oat, okra, onion, orange, an ornamental plant or flower or tree, papaya, parsley, pea, peach, peanut, peat, pepper, persimmon, pineapple, plantain, plum, pomegranate, potato, pumpkin, radicchio, radish, rapeseed, raspberry, rice, rye, sorghum, soy, soybean, spinach, strawberry, sugar beet, sugarcane, sunflower, sweet potato, tangerine, tea, tobacco, tomato, a vine, watermelon, wheat, yams and zucchini. In yet another embodiment, the plant or plant part is a soybean plant or plant part.

[0099] In another embodiment, the Vip3 protein is a Vip3A protein. In yet another embodiment, the Vip3A protein comprises an amino acid sequence that is the translation product of a nucleotide sequence whose complement hybridizes to SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15 or SEQ ID NO: 17 under high-stringency conditions. In still another embodiment, the high-stringency conditions are 7% sodium dodecyl sulfate (SDS), 0.5 M NaP_{O4}, 1 mM EDTA at 50° C. with washing in 0.1XSSC, 0.1% SDS at 65°C. In a further embodiment, the Vip3A protein is selected from the group consisting of Vip3Aa1 (AAC37036), Vip3Aa2 (AAC37037), Vip3Aa3 (US Patent 6137033), Vip3Aa4 (AAR81079), Vip3Aa5 (AAR81080), Vip3Aa6 (AAR81081), Vip3Aa7 (AAK95326), Vip3Aa8 (AAK97481), Vip3Aa9 (CAA76665), Vip3Aa10 (AAN60738), Vip3Aa11 (AAR36859), Vip3Aa12 (AAM22456), Vip3Aa13 (AAL69542), Vip3Aa14 (AAQ12340), Vip3Aa15 (AAP51131), Vip3Aa16 (AAW65132), Vip3Aa17 (US Patent 6603063), Vip3Aa18 (AAX49395), Vip3Aa19 (DQ241674), Vip3Aa19 (DQ539887), Vip3Aa20 (DQ539888), Vip3Aa21 (ABD84410), Vip3Aa22 (AAY41427), Vip3Aa23 (AAY41428), Vip3Aa24 (BI 880913), Vip3Aa25 (EF608501), Vip3Aa26 (EU294496), Vip3Aa27 (EU332167), Vip3Aa28 (FJ494817), Vip3Aa29 (FJ626674), Vip3Aa30 (FJ626675), Vip3Aa31 (FJ626676), Vip3Aa32 (FJ626677), Vip3Aa33 (GU073128), Vip3Aa34 (GU073129), Vip3Aa35 (GU733921), Vip3Aa36 (GU951510), Vip3Aa37 (HM132041), Vip3Aa38 (HM117632), Vip3Aa39 (HM117631), Vip3Aa40 (HM132042), Vip3Aa41 (HM132043), Vip3Aa42 (HQ587048), Vip3Aa43 (HQ594534), Vip3Aa44 (HQ650163), Vip3Ab1 (AAR40284), Vip3Ab2 (AAY88247), Vip3Ac1 (US Patent Application Publication 20040128716), Vip3Ad1 (US Patent Application Publication 20040128716), Vip3Ad2 (CAI43276), Vip3Ae1 (CAI43277), Vip3Af1 (CAI43275), Vip3Af2 (ADN08753), Vip3Af3 (HM117634), Vip3Ag1 (ADN08758),Vip3Ag2 (FJ556803),Vip3Ag3 (HM117633), Vip3Ag4 (HQ414237), Vip3Ag5 (HQ542193), and

Vip3Ah1 (DQ832323). In still a further embodiment, the Vip3A protein comprises SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16, or a nematode-active homologue thereof having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16. In another embodiment, the Vip3A protein comprises SEQ ID NO: 1 or a nematode-active homologue having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NO: 1.

[00100] In another embodiment, transgenic plant or plant part further comprises or expresses at least one additional pesticidal agent selected from the group consisting of a patatin, a *Bacillus thuringiensis* insecticidal protein, a *Bacillus thuringiensis* nematicidal protein, a *Xenorhabdus* insecticidal protein, a *Photorhabdus* insecticidal protein, a *Bacillus laterosporous* insecticidal protein, and a *Bacillus sphearicus* insecticidal protein. In yet another embodiment, the *Bacillus thuringiensis* nematicidal protein is selected from the group consisting of a Cry1, Cry3, Cry11, Cry12, Cry13, Cry14, Cry21, and Cry22.

[00101] In one embodiment, the invention encompasses a method of reducing nematode infectivity to a plant comprising contacting the nematode with a Vip3 protein, wherein nematode infectivity is reduced compared to nematode infectivity of plant not contacted with a Vip3 protein. In another embodiment, the contacting step comprises planting a transgenic seed capable of producing a transgenic plant that expresses a Vip3 protein, wherein the nematode feeds on the transgenic plant. In yet another embodiment, the transgenic plant is soybean (*Glycine max*). In still another embodiment, the nematode is soybean cyst nematode (*Heterodera glycines*).

[00102] In one embodiment, the invention encompasses method of improving plant yield in nematode infested fields, comprising expressing in the plant a Vip3 protein, wherein plant yield is improved compared to yield of a plant of the same type not expressing a Vip3 protein.

[00103] In another embodiment, the invention encompasses a method of producing a soybean plant protected against nematode infestation comprising transforming a soybean

plant cell with a nucleic acid molecule encoding a Vip3 protein and regenerating a transformed soybean plant from the soybean plant cell.

[00104] In another embodiment, the invention encompasses a method of producing a soybean plant protected against nematode infestation comprising crossing a first parent soybean plant with a second parent soybean plant, wherein said first or second parent soybean plant comprises a heterologous nucleic acid molecule encoding a Vip3 protein, thereby producing a plurality of progeny plants; and selecting from the plurality of progeny plants, a transgenic plant that is protected against nematode infestation.

[00105] In still another embodiment, the invention encompasses a method of providing a grower with a means of controlling nematode pests comprising supplying seed to a grower, wherein the seed comprises a heterologous nucleic acid molecule that encodes a Vip3 protein and wherein the seed is capable of producing a plant that is resistant to nematode damage.

[00106] In one embodiment, the invention encompasses a transgenic soybean plant or plant part comprising a heterologous nucleic acid molecule encoding a Vip3 protein, wherein said transgenic plant or plant part has improved resistance to at least one plant pathogenic nematode, as compared to a control plant or plant part not expressing theVip3 protein. In another embodiment, the Vip3 protein comprises SEQ ID NO: 19. In still another embodiment, the Vip3 protein is a Vip3A protein. In a further embodiment, the Vip3A protein having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NO: 1. In yet another embodiment, the Vip3A protein comprises SEQ ID NO: 1. In another embodiment, the cyst forking nematode is a soybean cyst nematode (*Heterodera glycines*).

[00107] In another embodiment, the invention encompasses transgenic seed of a transgenic plant of the invention, wherein the transgenic seed comprises a heterologous nucleic acid molecule encoding a Vip3 protein of the invention.

[00108] In yet another embodiment, the invention encompasses a method of controlling *Heterodera glycines* comprising providing a transgenic soybean plant or plant part comprising an expression cassette having SEQ ID NO: 2 operably linked to a promoter capable of driving expression of an encoded Vip3 protein to levels sufficient to inhibit nematodes, wherein the proliferation of *Heterodera glycines* cysts on said plant or plant

part is reduced compared to *Heterodera glycines* cysts on a soybean plant or plant part not expressing the Vip3 protein. In another embodiment, the promoter is selected from the group consisting of: a) a constitutive promoter; b) a tissue-specific promoter; and c) an inducible promoter. In yet another embodiment, the promoter is an actin2 promoter.

[00109] In one embodiment, the invention encompasses a method of increasing the vigor or yield in a transgenic soybean plant exposed to a population of nematodes comprising introgressing a transgenic soybean event into a soybean plant resulting in a transgenic soybean plant, wherein the transgenic soybean event comprises a heterologous nucleic acid molecule encoding a Vip3 protein that confers upon the transgenic soybean event resistance to nematodes; and growing the transgenic soybean plant or progeny thereof at a location where nematode infestation is yield limiting to a soybean plant not comprising the heterologous nucleic acid molecule encoding the Vip3 protein, whereby the transgenic soybean plant has increased vigor or yield compared to the control plant.

[00110] The invention also encompasses a method of improving soybean yield comprising introducing into a soybean plant a nucleic acid molecule capable of directing expression of a Vip3 protein; and cultivating a plurality of transgenic seeds from the plant of step (a), resulting in a plurality of transgenic plants having enhanced resistance to nematode infestation, thereby improving soybean yield.

[00111] The invention further encompasses a nematicidal composition comprising a Vip3 protein and an acceptable agricultural carrier.

[00112] In another embodiment, the Vip3 protein comprises SEQ ID NO: 19. In still another embodiment the Vip3 protein is a Vip3A protein. In yet another embodiment, the Vip3A protein comprises an amino acid sequence that is the translation product of a nucleotide sequence whose complement hybridizes to SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15 or SEQ ID NO: 17 under high-stringency conditions. In still another embodiment, the high-stringency conditions are 7% sodium dodecyl sulfate (SDS), 0.5 M NaP_{O4}, 1 mM EDTA at 50° C. with washing in 0.1XSSC, 0.1% SDS at 65°C. In a further embodiment, the Vip3A protein is selected from the group consisting of Vip3Aa1 (AAC37036), Vip3Aa2 (AAC37037), Vip3Aa3 (US Patent 6137033), Vip3Aa4 (AAR81079), Vip3Aa5 (AAR81080), Vip3Aa6 (AAR81081), Vip3Aa7 (AAK95326), Vip3Aa8 (AAK97481), Vip3Aa9 (CAA76665), Vip3Aa10 (AAN60738), Vip3Aa11

(AAR36859), Vip3Aa12 (AAM22456), Vip3Aa13 (AAL69542), Vip3Aa14 (AAQ12340), Vip3Aa15 (AAP51131), Vip3Aa16 (AAW65132), Vip3Aa17 (US Patent 6603063), Vip3Aa18 (AAX49395), Vip3Aa19 (DQ241674), Vip3Aa19 (DQ539887), Vip3Aa20 (DQ539888), Vip3Aa21 (ABD84410), Vip3Aa22 (AAY41427), Vip3Aa23 (AAY41428), Vip3Aa24 (BI 880913), Vip3Aa25 (EF608501), Vip3Aa26 (EU294496), Vip3Aa27 (EU332167), Vip3Aa28 (FJ494817), Vip3Aa29 (FJ626674), Vip3Aa30 (FJ626675), Vip3Aa31 (FJ626676), Vip3Aa32 (FJ626677), Vip3Aa33 (GU073128), Vip3Aa34 (GU073129), Vip3Aa35 (GU733921), Vip3Aa36 (GU951510), Vip3Aa37 (HM132041), Vip3Aa38 (HM117632), Vip3Aa39 (HM117631), Vip3Aa40 (HM132042), Vip3Aa41 (HM132043), Vip3Aa42 (HQ587048), Vip3Aa43 (HQ594534), Vip3Aa44 (HQ650163), Vip3Ab1 (AAR40284), Vip3Ab2 (AAY88247), Vip3Ac1 (US Patent Application Publication 20040128716), Vip3Ad1 (US Patent Application Publication 20040128716), Vip3Ad2 (CAI43276), Vip3Ae1 (CAI43277), Vip3Af1 (CAI43275), Vip3Af2 (ADN08753), Vip3Af3 (HM117634), Vip3Ag1 (ADN08758),Vip3Ag2 (FJ556803),Vip3Ag3 (HM117633), Vip3Ag4 (HQ414237), Vip3Ag5 (HQ542193), and Vip3Ah1 (DQ832323). In still a further embodiment, the Vip3A protein comprises SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16, or a nematode-active homologue thereof having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16. In another embodiment, the Vip3A protein comprises SEQ ID NO: 1 or a nematode-active homologue having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NO: 1.

[00113] In another embodiment, the agricultural carrier is a transgenic plant or plant part. In yet another embodiment, the transgenic plant or plant part is a soybean plant or plant part.

[00114] In one embodiment, the invention encompasses a method of producing a nematode-resistant plant or plant part comprising introducing a nucleic acid molecule encoding a Vip3 protein into the plant or plant part thereby producing a transgenic plant

or plant part, wherein the nucleic acid molecule causes the expression of the Vip3 protein in an amount that makes the transgenic plant or plant part resistant to nematodes.

[00115] In still another embodiment, the invention encompasses a method of reducing nematode cyst development on roots of a plant infected by a nematode, comprising introducing into cells of the plant a nucleic acid molecule capable of directing the expression of a Vip3 protein, thereby reducing nematode cyst development on roots of the plant.

[00116] The invention also encompasses a method for controlling or preventing nematode growth comprising providing a nematode pest with plant material comprising a heterologous nucleic acid molecule capable of directing expression of a Vip3 protein, wherein the plant material inhibits a nematode biological activity. In another embodiment, the biological activity is an ability to produce cysts on roots of plants. In yet another embodiment, the plant material is a soybean plant or plant part.

[00117] In another embodiment, the invention encompasses a method of suppressing the growth of a plant-pathogenic nematode population in a location capable of supporting said growth comprising growing in the location a population of transgenic soybean plants comprising a heterologous nucleic acid molecule capable of directing expression of a Vip3 protein, wherein the plant-pathogenic nematode population is suppressed.

[00118] The present invention also encompasses recombinant vectors and expression cassettes comprising the *vip3* nucleic acid sequences of the invention. In such vectors, the nucleic acid sequences are preferably comprised in expression cassettes comprising regulatory elements for expression of the *vip3* nucleotide sequences in a transgenic host cell capable of expressing the nucleotide sequences. Such regulatory elements usually comprise promoter and termination signals and preferably also comprise elements allowing efficient translation of polypeptides encoded by the nucleic acid sequences of the present invention. Vectors comprising the nucleic acid sequences are usually capable of replication in particular host cells, preferably as extrachromosomal molecules, and are therefore used to amplify the nucleic acid sequences of this invention in the host cells. In one embodiment, host cells for such vectors are microorganisms, such as bacteria, in particular *E. coli*. In another embodiment, host cells for such recombinant vectors are endophytes or epiphytes. One example of a host cell for such vectors is a eukaryotic cell, such as a plant cell. Suh plant cells may be soybean cells or maize cells. In another

embodiment, such vectors are viral vectors and are used for replication of the nucleotide sequences in particular host cells, e.g. insect cells or plant cells. Recombinant vectors are also used for transformation of the nucleotide sequences of this invention into transgenic host cells, whereby the nucleotide sequences are stably integrated into the DNA of such transgenic host cells. In one, such transgenic host cells are prokaryotic cells. In another embodiment, such transgenic host cells are eukaryotic cells, such as yeast cells, insect cells, or plant cells. In still another embodiment, the transgenic host cells are plant cells, such as soybean cells or maize cells.

[00119] The pesent invention further encompasses an expression cassette comprising an actin promoter operably linked to a *vip3* coding sequence operably linked to a terminator, wherein the expression cassette functions in a transgenic plant to drive expression of a Vip3 protein at a nematode-controlling effective amount. In anoter embodiment, the actin promoter is an actin2 promoter and the terminator is a NOS terminator. In yet another embodiment, the *vip3* coding sequence comprises a nucleotide sequence whose complement hybridizes to SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15 or SEQ ID NO: 17 under high-stringency conditions. In a further embodiment, the high-stringency conditions are 7% sodium dodecyl sulfate (SDS), 0.5 M NaP_{O4}, 1 mM EDTA at 50° C. with washing in 0.1XSSC, 0.1% SDS at 65°C. In another embodiment, the Vip3 protein comprises SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16, or a nematode-active homologue thereof having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16. In another embodiment, the Vip3A protein comprises SEQ ID NO: 1 or a nematode-active homologue having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with SEQ ID NO: 1.

[00120] In further embodiments, the *vip3* nucleotide sequences of the invention can be modified by incorporation of random mutations in a technique known as *in vitro* recombination or DNA shuffling to increase nematode activity for example. This technique is described in Stemmer *et al.*, Nature 370:389-391 (1994) and U.S. Patent

5,605,793, which are incorporated herein by reference. Millions of mutant copies of a nucleotide sequence are produced based on an original nucleotide sequence of this invention and variants with improved properties, such as increased nematicidal activity, enhanced stability, or different specificity or range of target nematode pests are recovered. The method encompasses forming a mutagenized double-stranded polynucleotide from a template double-stranded polynucleotide comprising a nucleotide sequence of this invention, wherein the template double-stranded polynucleotide has been cleaved into double-stranded-random fragments of a desired size, and comprises the steps of adding to the resultant population of double-stranded random fragments one or more single or double-stranded oligonucleotides, wherein said oligonucleotides comprise an area of identity and an area of heterology to the double-stranded template polynucleotide; denaturing the resultant mixture of double-stranded random fragments and oligonucleotides into single-stranded fragments; incubating the resultant population of single-stranded fragments with a polymerase under conditions which result in the annealing of said single- stranded fragments at said areas of identity to form pairs of annealed fragments, said areas of identity being sufficient for one member of a pair to prime replication of the other, thereby forming a mutagenized double-stranded polynucleotide; and repeating the second and third steps for at least two further cycles, wherein the resultant mixture in the second step of a further cycle includes the mutagenized double-stranded polynucleotide from the third step of the previous cycle, and the further cycle forms a further mutagenized double-stranded polynucleotide. In a preferred embodiment, the concentration of a single species of double- stranded random fragment in the population of double-stranded random fragments is less than 1% by weight of the total DNA. In a further embodiment, the template double-stranded polynucleotide comprises at least about 100 species of polynucleotides. In another preferred embodiment, the size of the double-stranded random fragments is from about 5 bp to 5 kb. In another embodiment, the fourth step of the method comprises repeating the second and the third steps for at least 10 cycles.

[00121] In another embodiment, at least one of the *vip3* nucleotide sequences of the invention is inserted into an appropriate expression cassette, comprising a promoter and termination signals. Expression of the nucleotide sequence is constitutive, or an inducible promoter responding to various types of stimuli to initiate transcription is used. In a

preferred embodiment, the cell in which the toxin is expressed is a microorganism, such as a virus, a bacteria, or a fungus. In a preferred embodiment, a virus, such as a baculovirus, contains a nucleotide sequence of the invention in its genome and expresses large amounts of the corresponding insecticidal toxin after infection of appropriate eukaryotic cells that are suitable for virus replication and expression of the nucleotide sequence. The insecticidal toxin thus produced is used as an insecticidal agent. Alternatively, baculoviruses engineered to include the nucleotide sequence are used to infect insects *in vivo* and kill them either by expression of the insecticidal toxin or by a combination of viral infection and expression of the insecticidal toxin.

[00122] Bacterial cells are also hosts for the expression of the nucleotide sequences of the invention. In a preferred embodiment, non-pathogenic symbiotic bacteria, which are able to live and replicate within plant tissues, so-called endophytes, or non- pathogenic symbiotic bacteria, which are capable of colonizing the phyllosphere or the rhizosphere, so-called epiphytes, are used. Such bacteria include bacteria of the genera *Agrobacterium, Alcaligenes, Azospirillum, Azotobacter, Bacillus, Clavibacter, Enterobacter, Erwinia, Flavobacter, Klebsiella, Pseudomonas, Rhizobium, Serratia, Streptomyces* and *Xanthomonas*. Symbiotic fungi, such as *Trichoderma* and *Gliocladium* are also possible hosts for expression of the inventive nucleotide sequences for the same purpose.

[00123] Techniques for these genetic manipulations are specific for the different available hosts and are known in the art. For example, the expression vectors pKK223-3 and pKK223-2 can be used to express heterologous genes in *E. coli*, either in transcriptional or translational fusion, behind the tac or trc promoter. For the expression of operons encoding multiple ORFs, the simplest procedure is to insert the operon into a vector such as pKK223- 3 in transcriptional fusion, allowing the cognate ribosome binding site of the heterologous genes to be used. Techniques for overexpression in gram-positive species such as *Bacillus* are also known in the art and can be used in the context of this invention (Quax et al. In:Industrial Microorganisms:Basic and Applied Molecular Genetics, Eds. Baltz et al., American Society for Microbiology, Washington (1993)). Alternate systems for overexpression rely for example, on yeast vectors and include the use of Pichia, Saccharomyces and Kluyveromyces (Sreekrishna, In:Industrial microorganisms:basic and applied molecular genetics, Baltz, Hegeman, and Skatrud eds., American Society for

Microbiology, Washington (1993); Dequin & Barre, Biotechnology L2:173- 177 (1994); van den Berg et al., Biotechnology 8:135-139 (1990)).

[00124] In one embodiment, at least one Vip3 protein of the invention is expressed in a higher organism, e.g., a plant. In this case, transgenic plants expressing effective amounts of the toxins protect themselves from nematode pests. When the nematode starts feeding on such a transgenic plant, it also ingests the expressed Vip3 toxin. This may deter the nematode from further feeding in the plant tissue, may harm or kill the nematode or may reduce the nematodes ability to reproduce. A nucleotide sequence of the present invention is inserted into an expression cassette, which is then preferably stably integrated in the genome of the plant. Plants transformed in accordance with the invention may be monocots or dicots and include, but are not limited to, maize, wheat, barley, rye, sweet potato, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish, spinach, asparagus, onion, garlic, pepper, celery, squash, pumpkin, hemp, zucchini, apple, pear, quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, pineapple, avocado, papaya, mango, banana, soybean, tomato, sorghum, sugarcane, sugar beet, sunflower, rapeseed, clover, tobacco, carrot, cotton, alfalfa, rice, potato, eggplant, cucumber, Arabidopsis, and woody plants such as coniferous and deciduous trees.

[00125] Once a desired nucleotide sequence has been transformed into a particular plant species, it may be propagated in that species or moved into other varieties of the same species, particularly including commercial varieties, using traditional breeding techniques.

[00126] A nucleotide sequence of the invention is expressed in transgenic plants, thus causing the biosynthesis of the corresponding toxin in the transgenic plants. In this way, transgenic plants with enhanced resistance to nematodes are generated. For their expression in transgenic plants, the nucleotide sequences of the invention may require modification and optimization. Although in many cases genes from microbial organisms can be expressed in plants at high levels without modification, low expression in transgenic plants may result from microbial nucleotide sequences having codons that are not preferred in plants. It is known in the art that all organisms have specific preferences for codon usage, and the codons of the nucleotide sequences described in this invention can be changed to conform with plant preferences, while maintaining the amino acids

encoded thereby. Furthermore, high expression in plants is best achieved from coding sequences that have at least about 35% GC content, preferably more than about 45%, more preferably more than about 50%, and most preferably more than about 60%. Although preferred gene sequences may be adequately expressed in both monocotyledonous and dicotyledonous plant species, sequences can be modified to account for the specific codon preferences and GC content preferences of monocotyledons or dicotyledons as these preferences have been shown to differ (Murray *et al.* Nucl. Acids Res. 17:477-498 (1989)). In addition, the nucleotide sequences are screened for the existence of illegitimate splice sites that may cause message truncation. All changes required to be made within the nucleotide sequences such as those described above are made using well known techniques of site directed mutagenesis, PCR, and synthetic gene construction using the methods known in the art.

[00127] In one embodiment of the invention synthetic genes are made according to the procedure disclosed in U.S. Patent 5,625,136, herein incorporated by reference. In this procedure, maize preferred codons, i.e., the single codon that most frequently encodes that amino acid in maize, are used. The maize preferred codon for a particular amino acid can be derived, for example, from known gene sequences from maize. Maize codon usage for 28 genes from maize plants is found in Murray *et al.*, Nucleic Acids Research 17:477-498 (1989), the disclosure of which is incorporated herein by reference. A specifically exemplified synthetic sequence of the invention made with maize optimized codons is set forth in SEQ ID NO:2.

[00128] In this manner, the nucleotide sequences can be optimized for expression in any plant. It is recognized that all or any part of the gene sequence may be optimized or synthetic. That is, synthetic or partially optimized sequences may also be used.

[00129] For efficient initiation of translation, sequences adjacent to the initiating methionine may require modification. For example, they can be modified by the inclusion of sequences known to be effective in plants. Joshi has suggested an appropriate consensus for plants (NAR 15:6643-6653 (1987)) and Clonetech suggests a further consensus translation initiator (1993/1994 catalog, page 210). These consensuses are suitable for use with the nucleotide sequences of this invention. The sequences are incorporated into constructions comprising the nucleotide sequences, up to and including the ATG (whilst leaving the second amino acid unmodified), or alternatively up to and

including the GTC subsequent to the ATG (with the possibility of modifying the second amino acid of the transgene).

[00130] The *vip*3 toxin genes of the invention, either as their native sequence or as optimized synthetic sequences as described above, can be operably fused to a variety of promoters for expression in plants including constitutive, inducible, temporally regulated, developmentally regulated, chemically regulated, tissue-preferred and tissue-specific promoters to prepare recombinant DNA molecules, i.e., chimeric genes. The choice of promoter will vary depending on the temporal and spatial requirements for expression. Thus, expression of the nucleotide sequences encoding Vip3 proteins of the invention in leaves, in stalks or stems, in ears, in inflorescences (e.g. spikes, panicles, cobs, etc.), in roots, and/or seedlings can be achieved, but particularly preferred for control of nematodes is expression in roots. In many cases, however, protection against more than one type of nematode pest is sought, and thus expression in multiple tissues is desirable. Although many promoters from dicotyledons have been shown to be operational in monocotyledons and vice versa, ideally dicotyledonous promoters are selected for expression in dicotyledons, and monocotyledonous promoters for expression in monocotyledons. However, there is no restriction to the provenance of selected promoters; it is sufficient that they are operational in driving the expression of the nucleotide sequences of the invention in the desired cell.

[00131] Constitutive promoters include for example the Actin 2 promoter (An *et al.* (1996) Plant J 10(1):107-21). Additionally, a promoter useful in the present inventioncould be derived from any one of several of the actin genes, which are expressed in most cell types. The promoter expression cassettes described by McElroy *et al.* (Mol. Gen. Genet. 231: 150-160 (1991)) can be modified for the expression of the novel toxin gene and are particularly suitable for use in monocotyledonous hosts.

[00132] Yet another constitutive promoter is derived from ubiquitin, which is another gene product known to accumulate in many cell types. A ubiquitin promoter has been cloned from several species for use in transgenic plants, for example, sunflower (Binet *et al.*, 1991. Plant Science 79: 87-94), maize (Christensen *et al.*, 1989. Plant Molec. Biol. 12: 619-632), and arabidopsis (Norris et al. 1993. Plant Molec. Biol. 21:895-906). The maize ubiquitin promoter has been developed in transgenic monocot systems and its sequence and vectors constructed for monocot transformation are disclosed in the patent

publication EP 0 342 926. The ubiquitin promoter is suitable for the expression of the novel toxin gene in transgenic plants, especially monocotyledons.

[00133] Tissue-specific or tissue-preferential promoters useful for the expression of the novel toxin genes of the invention in plants, particularly maize, are those that direct expression in root, pith, leaf or pollen. Such promoters are disclosed in WO 93/07278, herein incorporated by reference in its entirety. Other tissue specific promoters useful in the present invention include the cotton rubisco promoter disclosed in US Patent 6,040,504; the rice sucrose synthase promoter disclosed in US Patent 5,604,121; and the cestrum yellow leaf curling virus promoter disclosed in WO 01/73087, all incorporated by reference. Chemically inducible promoters useful for directing the expression of the novel toxin gene in plants are disclosed in US Patent 5,614,395 herein incorporated by reference in its entirety.

[00134] The nucleotide sequences of the invention can also be expressed under the regulation of promoters that are chemically regulated. This enables the Vip3 toxins to be synthesized only when the crop plants are treated with the inducing chemicals. One example of a promoter for chemical induction is the PR-1a promoter detailed in the U.S. Patent 5,614,395, incorporated herein by reference.

[00135] Another category of promoters useful in the invention is that which is wound inducible. Numerous promoters have been described which are expressed at wound sites and also at the sites of phytopathogen infection. Ideally, such a promoter should only be active locally at the sites of infection, and in this way the insecticidal toxins only accumulate in cells that need to synthesize the insecticidal toxins to kill the invading insect pest. Examples of 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).

[00136] Tissue specific expression patterns include green tissue specific, root specific, stem specific, and flower specific. Promoters suitable for expression in green tissue include many that regulate genes involved in photosynthesis and many of these have been cloned from both monocotyledons and dicotyledons. A preferred promoter is the maize PEPC promoter from the phosphoenol carboxylase gene (Hudspeth & Grula, Plant Molec.

Biol. 12:579-589 (1989)). A preferred promoter for root specific expression is that described by de Framond (FEBS 290:103-106 (1991); EP 0 452 269 to Ciba- Geigy). A preferred stem specific promoter is that described in U.S. Patent 5,625,136 (to Ciba-Geigy) and which drives expression of the maize trpA gene.

[00137] Further embodiments of the invention are transgenic plants expressing the nucleotide sequences in a wound-inducible or nematode-inducible manner.

[00138] In addition to the selection of a suitable promoter, constructions for expression of an insecticidal toxin in plants require an appropriate transcription terminator to be attached downstream of the heterologous nucleotide sequence. Several such terminators are available and known in the art (e.g. tml from CaMV, E9 from rbcS). Any available terminator known to function in plants can be used in the context of this invention.

[00139] Numerous other sequences can be incorporated into expression cassettes described in this invention. These include sequences that have been shown to enhance expression such as intron sequences (e.g. from Adhl and bronzel) and viral leader sequences (e.g. from TMV, MCMV and AMV).

[00140] It may be useful to target expression of the nucleotide sequences of the invention to different cellular localizations in the plant. In some cases, localization in the cytosol may be desirable, whereas in other cases, localization in some subcellular organelle may be preferred. Subcellular localization of transgene-encoded enzymes is undertaken using techniques well known in the art. Typically, the DNA encoding the target peptide from a known organelle-targeted gene product is manipulated and fused upstream of the nucleotide sequence. Many such target sequences are known for the chloroplast and their functioning in heterologous constructions has been shown. The expression of the nucleotide sequences of the present invention is also targeted to the endoplasmic reticulum or to the vacuoles of the host cells. Techniques to achieve this are well known in the art.

[00141] Numerous transformation vectors available for plant transformation are known to those of ordinary skill in the plant transformation art, and the nucleic acid molecules of the invention can be used in conjunction with any such vectors. The selection of vector will depend upon the preferred transformation technique and the target plant species for transformation. For certain target species, different antibiotic or herbicide selection markers may be preferred. Selection markers used routinely in transformation include the

nptII gene, which confers resistance to kanamycin and related antibiotics (Messing & Vierra., 1982. Gene 19: 259-268; and Bevan *et al.*, 1983. Nature 304:184-187), the *bar* gene, which confers resistance to the herbicide phosphinothricin (White *et al.*, 1990. Nucl. Acids Res 18: 1062, and Spencer *et al.*, 1990. Theor. Appl. Genet 79: 625-631), the *hph* gene, which confers resistance to the antibiotic hygromycin (Blochinger & Diggelmann, Mol Cell Biol 4: 2929-2931), and the *dhfr* gene, which confers resistance to methatrexate (Bourouis *et al.*, 1983. EMBO J. 2(7): 1099-1104), the EPSPS gene, which confers resistance to glyphosate (U.S. Patent Nos. 4,940,935 and 5,188,642), and the mannose-6-phosphate isomerase gene, which provides the ability to metabolize mannose (U.S. Patent Nos. 5,767,378 and 5,994,629). The choice of selectable marker is not, however, critical to the invention.

[00142] In another embodiment, a nucleotide sequence of the invention is directly transformed into the plastid genome. A major advantage of plastid transformation is that plastids are generally capable of expressing bacterial genes without substantial modification, and plastids are capable of expressing multiple open reading frames under control of a single promoter. Plastid transformation technology is extensively described in U.S. Patent 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. Nati. 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. Nati. Acad. Sci. USA 87, 8526-8530; Staub, J. M., and Maliga, P. (1992) Plant Cell 4, 39-45). This resulted in stable homoplasmic transformants at a frequency of approximately one per 100 bombardments of target leaves. The presence of cloning sites between these markers allowed creation of a plastid targeting vector for introduction of foreign genes (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-cletoxifying enzyme aminoglycoside- 3'- adenyltransf erase (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 encompassed within the scope of the invention. Typically, approximately 15-20 cell division cycles following transformation are required to reach a homoplastidic state. Plastid expression, in which genes are inserted by homologous recombination into all of the several thousand copies of the circular plastid genome present in each plant cell, takes advantage of the enormous copy number advantage over nuclear- expressed genes to permit expression levels that can readily exceed 10% of the total soluble plant protein. In a preferred embodiment, a nucleotide sequence of the present invention is inserted into a plastid-targeting vector and transformed into the plastid genome of a desired plant host. Plants homoplastic for plastid genomes containing a nucleotide sequence of the present invention are obtained, and are preferentially capable of high expression of the nucleotide sequence.

[00143] It will be apparent to the skilled person that the Vip3 proteins of the invention can be used in combination with other nematicidal agents such as proteins, chemicals, other natural products and the like. Non-limiting examples of such chemicals and natural products include abamectin, carbamate nematicides selected from aldicarb, carbofuran, carbosulfan, oxamyl, aldoxycarb, ethoprop benomyl, and alanycarb; organophosphorus nematicides selected from phenamiphos, fenamiphos, fensulfothion, terbufos, fosthiazate, phosphocarb, dichlofenthion, isamidofos, fosthietan, isazofos, ethoprophos, cadusafos, chlorpyrifos, heterophos, isamidofos, mecarphon, phorate, thionazin, triazophos, diamidafos, and phosphamidon; methyl bromide, methyl iodide, carbon disulfide, 1,3-dichloropropene, chloropicrin, cytokinins, dazomet, DCIP, ethylene dibromide, GY-81, metam, methyl isocyanate, myrothecium verrucaria composition, and flupyrazofos, benchlothiaz, [2-cyanoimino-3-ethylimidazolidin-1-yl]phosphonothioic acid O-ethyl S-propyl ester.

[00144] Avermectins and derivatives of avermectins for use in the invention are known. Abamectin and abamectin seed treatment formulations for nematode control that are particularly useful in the invention are disclosed, e.g., in U.S. Pat. No. 6,875,727. Agrochemically compatible salts are, for example, acid addition salts of inorganic and organic acids, in particular of hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, perchloric acid, phosphoric acid, formic acid, acetic acid, tri-fluoroacetic acid, oxalic acid, malonic acid, toluenesulfonic acid or benzoic acid. Examples of formulations of avermectin compounds that can be used in the method according to the invention, i.e., solutions, granules, dusts, sprayable powders, emulsion concentrates, coated granules and suspension concentrates, have been described, e.g., in EP-A-580 553.

[00145] Derivatives of avermectin or abamectin can be obtained via conventional chemical syntheses. For example, in some embodiments emamectin, which is 4"-De-oxy-4"-epi-N-methylamino avermectin B.sub.1b/B.sub.1a known from U.S. Pat. No. 4,874,749, can be used. Agrochemically useful salts of emamectin are additionally described, e.g., in U.S. Pat. No. 5,288,710.

[00146] The amount of a nematicide present on (or adhered to) the seed varies, for example, according to type of crop, and type of plant propagation material. However, the amount is such that the at least one nematicide is an effective amount to provide the desired enhanced action and can be determined by routine experimentation and field trials. In the event the nematicide is abamectin, the amount of active abamectin ingredient present in the seed coating is in the range of from 0.002 to 1.2 mg/seed, typically at least 0.1 mg/seed, often at least 0.2 mg/seed. Frequently, the abamectin is present at a level of 0.3 mg or more per seed.

[00147] Bacterial parasites can also be used as nematode antagonistic biocontrol agents. These include, e.g., Pasteuria species, e.g., P. penetrans, P. nishizawae, P. thornei, Candidatus Pasteuria usgae sp. nov., Myrothecium verrucaria, Candidatus Pasteuria sp. strain HG, and other species. These parasites can attach to the cuticle of nematodes.

[00148] The nematicidal Vip3 toxins of the invention can be used in combination with Bt Cry toxins or other pesticidal principles to increase pest target range. Such Bt Cry toxins include for example Cry1, Cry 5, Cry6, Cry11, Cry12, Cry13, Cry14, Cry21 and Cry22. Furthermore, the use of the nematicidal Vip3 toxins of the invention in combination with

Bt δ-endotoxins or other pesticidal principles of a distinct nature has particular utility for the prevention and/or management of nematode resistance.

EXAMPLES

[00149] The invention will be further described by reference to the following detailed examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified. Standard recombinant DNA and molecular cloning techniques used here are well known in the art and are described by Ausubel (ed.), Current Protocols in Molecular Biology, John Wiley and Sons, Inc. (1994); J. Sambrook, *et al.*, Molecular Cloning: *A Laboratory Manual, 3d Ed.,* Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press (2001); and by T.J. Silhavy, M.L. Berman, and L.W. Enquist, Experiments with Gene Fusions, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1984).

Example 1: Construction of expression cassettes

[00150] A *vip3A* coding sequence (SEQ ID NO: 2) was cloned into a act2:vip3A:tNOS expression cassette. The expression cassette with the *vip3A* coding sequence was then cloned into a binary vector to create the vector pKS214 (SEQ ID NO: 18).

Example 2: Expression Vip3 in Transgenic Soybean Roots.

[00151] The pKS214 binary expression vector containing a *vip3A* gene and an empty vector (without *vip3A* coding sequence) were transformed into soybean roots to test pKS214's ability to reduce soybean cyst nematode (SCN) cysts as transgenes. Soybean cultivar Williams 82 was used as the germplasm for the hairy root transformation. Seeds of soybean seeds were germinated on 1% agar containing 0.5% sucrose in Petri dishes at 27 °C for 5 days. The cotyledons were then cut off the seedlings, and the wounded surface was inoculated with cultures of the *Agrobacterium rhizogenes* carrying the binary vector. The cotyledons were placed on 1% agar for 6 days and then transferred onto selection media. In about two weeks, independent transgenic hairy root events induced

from the cotyledons were harvested and transferred onto culture media, and cultured in the darkness at 27 °C. Narayanan *et al.* indicated that SCN resistance phenotypes in the whole plant were preserved in transgenic hairy roots, therefore transgenic hairy root system is useful for evaluating candidate SCN resistance genes. Narayanan *et al.* (1999) *Crop Science* 39, 1680-1686.

[00152] Approximately two weeks after transfer onto the culture plates, the transformed hairy roots were inoculated with surface-sterilized J2 stage soybean cyst nematodes (SCN J2) and the roots were grown in darkness at 27 °C, which allowed cyst formation on the hairy root events. One month after nematode inoculation, the number of cysts was determined for both the roots expressing Vip3 protein and the roots expressing the empty vector (as a negative control).

[00153] The experiment was repeated five (5) times. Table 1 and Figure 1 show the summary of the comparison of mean cyst number. ANOVA test indicated that the average number of cysts formed on the transgenic soybean roots expressing Vip3 protein is significantly lower than on the transgenic soybean roots comprising the empty vector control ($p<0.05$).

Table 1. In vivo transgenic root-SCN assay

Plasmid ID	Gene of Interest	Avg. Cysts	*n*	Standard error
Empty Vector	None (Negative Control)	60	5	3.8
pKS104 (SEQ ID NO: 16)	*vip3Aa20*	28	5	1.1

Example 2: Production of Transgenic Soybean Expressing Vip3 Protein

[00154] Transformation of soybean to produce transgenic soybean plants is accomplished using immature seed targets of variety Williams 82 via *A. tumefaciens*-mediated transformation. Explant materials and media recipes were essentially as described in Hwang *et al.* (PCT International Publication No. WO 08/112044) and Que et al. (PCT International Publication No. WO 08/112267), with some variations as noted below. Using this method, genetic elements within the left and right border regions of the transformation plasmid are efficiently transferred and integrated into the genome of the

plant cell, while genetic elements outside these border regions are generally not transferred.

[00155] Maturing soybean pods are harvested from greenhouse-grown plants, sterilized with diluted bleach solution, and rinsed with sterile water. Immature seeds are then excised from seedpods and rinsed briefly with sterile water. Explants are prepared from sterilized immature seeds as described in Hwang et al. (PCT International Publication No. WO 08/112044) and infected with *A. tumefaciens* strain EHA101 harboring the transformation binary vector 18963 and allowed to incubate for an additional 30 to 240 minutes. Excess *A. tumefaciens* suspension is removed by aspiration and the explants are moved to plates containing a non-selective co-culture medium. The explants are co-cultured with the remaining *A. tumefaciens* at 23 °C for 4 days in the dark and then transferred to recovery and regeneration medium supplemented with an antibiotics mixture consisting of ticarcillin (75 mg/L), cefotaxime (75 mg/L) and vancomycin (75 mg/L) where they are incubated in the dark for seven days.

[00156] The explants are then transferred to regeneration medium containing hygromycin B (3 to 6 mg/L) and a mixture of antibiotics consisting of ticarcillin (75 mg/L), cefotaxime (75 mg/L) and vancomycin (75 mg/L) to inhibit and kill *A. tumefaciens*. Shoot elongation and regeneration is carried out in elongation media containing 2–4 mg/L of hygromycin B. The hygromycin phosphor-transferase (HPT) gene was used as a selectable marker during the transformation process. Regenerated plantlets are transplanted in soil as described (PCT International Publication No. WO 08/112267) and tested for the presence of HPT and CMP promoter sequences using TaqMan PCR analyses. Ingham et al. (2001) *Biotech* 31, 132-140. This screen allows for the selection of transgenic events that carry the T-DNA and are free of vector DNA. Plants positive for HPT gene and CMP sequences and negative for the spectinomycin (spec) gene are transferred to the greenhouse for analysis of miRNA expression and seed setting.

[00157] When the roots are about 2–3 inches, they are then transplanted into 1-gallon pots using Fafard #3 soil and 30 grams of incorporated Osmocote Plus 15–9–12. They are watered in thoroughly and placed in the cubicle under florescent lighting set to a 16-hour day. The temperatures are 85 °F (29.4 °C) during the day and 70 °F (21 °C) at night. Plants are watered once daily.

[00158] The plants remain in the cubicle until secondary Taqman sampling has been performed, typically 1–2 weeks. The plants are then placed on an automatic drip watering system and watered twice daily. A cage is placed over the plant, and it may be pruned very lightly if needed. The lighting is a combination of Metal Halide and Sodium Vapor fixtures with 400- and 1000-watt bulbs with a 10-hour day period. The outside wall is darkened to keep out light that would extend the day length. Temperatures are set at 79 °F (26 °C) during the day and 70 °F (21 °C) at night. The humidity is ambient.

[00159] The plants are maintained in this manner until pods reach maturity, approximately 100 days based on the date of the Taqman selection. The pods are then harvested, placed in a paper bag, air-dried for 2-days, and then machine dried at 80 °F (27 °C) for 2-additional days. The pods are shelled and the T1 seeds are harvested and stored at 4 °C until further testing.

Example 3: Analysis of Transgenic Soybean Plants

[00160] Soybean plants were transformed with one of three binary vectors, 1) vector 19993 comprising an expression cassette with an *Arabidopsis* actin 2 promoter operatively linked to a *vip3A* coding sequence which is operatively linked to a nopaline synthetase terminator (prAct2:vip3A:tNOS); or 2) vector 20048 comprising an expression cassette with a *Medicago truncatula* Mt51186 promoter operatively linked to a *vip3A* coding sequence which is operatively linked a nopaline synthetase terminator (prMt51186:vip3A:tNOS); or 3) a vector comprising an expression cassette with a cestrum virus promoter operatively linked to a *vip3A* coding sequence which is operatively linked a nopaline synthetase terminator (cmp:vip3A:tNOS). T1 transgenic soybean plants and control plants are inoculated with J2-stage soybean cyst nematodes (SCN J2). Briefly, 1-3 week old seedlings of the transgenic T1 generation soybean that are either homozygous (Hom) or heterozygous (Het) for the *vip3* gene or null segregants (i.e. do not comprise a *vip3* gene; Null) were grown in germination pouches and inoculated with a suspension of J2 stage soybean cyst nematodes at the level of 750 J2 per plant. Approximately one month after nematode inoculation, the number of cysts is determined for the transgenic plants comprising the *vip3* expression cassette and for the null segregants from the same T0 parents.

[00161] Results shown in Table 2 demonstrate that transgenic T1 soybean expressing Vip3 protein have reduced number of SCN cysts compared to the null segregant (negative control). Transformed plants comprsing the cmp:vip3A:nos expression cassette did not have a reduced number of cysts compared to the null segregant.

Table 2. Efficacy of transgenic soybean expressing Vip3 against SCN

Binary Vector	Promoter	Soybean Event	Zygosity	N	No. of Cysts	Standard Error
19993	Act2	9358	Het	6	82.0	9.3
			Hom	3	79.3	11.1
			NULL	3	99.0	12.3
		9363	Het	1	120.0	
			Hom	22	115.0	9.8
			NULL	5	137.0	6.4
		0052	Het	7	91.9	8.0
			Hom	12	86.5	6.0
			NULL	10	120.5	10.4
		2600	Het	16	110.7	6.9
			Hom	6	82.7	18.1
			NULL	5	117.0	12.0
20048	Mt51186	6861	Het	5	82.2	15.6
			HOM	3	123.3	16.6
			NULL	4	138.8	15.5

[00162] All publications and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art that this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

[00163] It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims.

CLAIMS

1. A method of controlling a nematode pest, comprising contacting the nematode pest with a Vip3 protein comprising SEQ ID NO: 19.

2. The method of claim 1, wherein the nematode is selected from the group consisting of Criconemella, Ditylenchus, Globodera, Helicotylenchus, Heterodera, Longidorus, Meloidogyne, Paratrichodorus, Pratylenchus, Radolpholus, Rotelynchus, Rotylenchulus, Tylenchulus and Xiphinema.

3. The method of claim 2, wherein the nematode is a cyst forming nematode.

4. The method of claim 3, wherein the nematode is in the genus *Heterodera*.

5. The method of claim 4, wherein the nematode is *Heterodera glycines*.

6. The method of claim 1, wherein the contacting step is carried out with a plant or plant part transformed with at least one nucleic acid molecule encoding the Vip3 protein.

7. The method of claim 6, wherein the plant or plant part is a soybean plant or plant part.

8. The method of claim 7, wherein the soybean plant part is a soybean root.

9. The method of any one of claims 1 to 8, wherein the Vip3 protein is a Vip3A protein.

10. The method of claim 9, wherein the Vip3A protein comprises an amino acid sequence that is the translation product of a nucleotide sequence whose complement hybridizes to SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15 or SEQ ID NO: 17 under high-stringency conditions.

11. The method of claim 10, wherein the high-stringency conditions are 7% sodium dodecyl sulfate (SDS), 0.5 M NaP_{O4}, 1 mM EDTA at 50° C. with washing in 0.1XSSC, 0.1% SDS at 65°C.

12. The method of claim 9, wherein the Vip3A protein is selected from the group consisting of Vip3Aa1 (AAC37036), Vip3Aa2 (AAC37037), Vip3Aa3 (US Patent 6137033), Vip3Aa4 (AAR81079), Vip3Aa5 (AAR81080), Vip3Aa6 (AAR81081), Vip3Aa7 (AAK95326), Vip3Aa8 (AAK97481), Vip3Aa9 (CAA76665), Vip3Aa10 (AAN60738), Vip3Aa11 (AAR36859), Vip3Aa12 (AAM22456), Vip3Aa13 (AAL69542), Vip3Aa14 (AAQ12340), Vip3Aa15 (AAP51131), Vip3Aa16 (AAW65132), Vip3Aa17 (US Patent 6603063), Vip3Aa18 (AAX49395), Vip3Aa19 (DQ241674), Vip3Aa19 (DQ539887), Vip3Aa20 (DQ539888), Vip3Aa21 (ABD84410), Vip3Aa22 (AAY41427), Vip3Aa23 (AAY41428), Vip3Aa24 (BI 880913), Vip3Aa25 (EF608501), Vip3Aa26 (EU294496), Vip3Aa27 (EU332167), Vip3Aa28 (FJ494817), Vip3Aa29 (FJ626674), Vip3Aa30 (FJ626675), Vip3Aa31 (FJ626676), Vip3Aa32 (FJ626677), Vip3Aa33 (GU073128), Vip3Aa34 (GU073129), Vip3Aa35 (GU733921), Vip3Aa36 (GU951510), Vip3Aa37 (HM132041), Vip3Aa38 (HM117632), Vip3Aa39 (HM117631), Vip3Aa40 (HM132042), Vip3Aa41 (HM132043), Vip3Aa42 (HQ587048), Vip3Aa43 (HQ594534), Vip3Aa44 (HQ650163), Vip3Ab1 (AAR40284), Vip3Ab2 (AAY88247), Vip3Ac1 (US Patent Application Publication 20040128716), Vip3Ad1 (US Patent Application Publication 20040128716), Vip3Ad2 (CAI43276), Vip3Ae1 (CAI43277), Vip3Af1 (CAI43275), Vip3Af2 (ADN08753), Vip3Af3 (HM117634), Vip3Ag1 (ADN08758),Vip3Ag2 (FJ556803),Vip3Ag3 (HM117633), Vip3Ag4 (HQ414237), Vip3Ag5 (HQ542193), and Vip3Ah1 (DQ832323).

13. The method of claim 12, wherein the Vip3A protein comprises SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16, or a nematode-active homologue thereof having at least 82% sequence identity with SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16.

14. A plant or plant part infestable by a nematode and which is protected from the nematode by being transformed with at least one nucleic acid molecule encoding a Vip3 protein of claim 13.

15. The plant of claim 14, wherein the plant is a soybean plant.

16. The plant of claim 15, wherein the nematode is in the genus *Heterodera*.

17. The plant of claim 16, wherein the nematode is *Heterodera glycines*.

18. A method of controlling a nematode pest, comprising contacting the nematode pest with a transgenic plant or plant part thereof comprising a heterologous nucleic acid molecule that directs expression of a Vip3 protein in the transgenic plant or plant part, wherein the transgenic plant or plant part controls the nematode pest compared to a plant or plant part of the same type that does not express the Vip3 protein.

19. The method of claim 18, wherein the nematode is selected from the group consisting of Criconemella, Ditylenchus, Globodera, Helicotylenchus, Heterodera, Longidorus, Meloidogyne, Paratrichodorus, Pratylenchus, Radolpholus, Rotelynchus, Rotylenchulus, Tylenchulus and Xiphinema.

20. The method of claim 19, wherein the nematode is a cyst forming nematode.

21. The method of claim 20, wherein the nematode is in the genus *Heterodera*.

22. The method of claim 21, wherein the nematode is *Heterodera glycines*.

23. The method of claim 18, wherein the transgenic plant is selected from the group consisting of alfalfa, apple, apricot, Arabidopsis, artichoke, asparagus, avocado, banana, barley, beans, beet, blackberry, blueberry, Brassica, broccoli, Brussels sprouts, cabbage, canola, carrot, cassaya, cauliflower, a cereal, celery, cherry, citrus, Clementine, coffee, corn, cotton, cucumber, eggplant, endive, eucalyptus, figs, grape, grapefruit, groundnuts, ground cherry, kiwifruit, lettuce, leek, lemon, lime, pine,

maize, mango, melon, millet, mushroom, nut oat, okra, onion, orange, an ornamental plant or flower or tree, papaya, parsley, pea, peach, peanut, peat, pepper, persimmon, pineapple, plantain, plum, pomegranate, potato, pumpkin, radicchio, radish, rapeseed, raspberry, rice, rye, sorghum, soy, soybean, spinach, strawberry, sugar beet, sugarcane, sunflower, sweet potato, tangerine, tea, tobacco, tomato, a vine, watermelon, wheat, yams and zucchini.

24. The method of claim 23, wherein the transgenic plant is soybean.

25. The method of any one of claims 18 to 24, wherein the Vip3 protein is a Vip3A protein.

26. The method of claim 25, wherein the Vip3A protein comprises an amino acid sequence that is the translation product of a nucleotide sequence whose complement hybridizes to SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15 or SEQ ID NO: 17 under high-stringency conditions.

27. The method of claim 26, wherein the high-stringency conditions are 7% sodium dodecyl sulfate (SDS), 0.5 M NaP_{O4}, 1 mM EDTA at 50° C. with washing in 0.1XSSC, 0.1% SDS at 65°C.

28. The method of claim 25, wherein the Vip3A protein is selected from the group consisting of Vip3Aa1 (AAC37036), Vip3Aa2 (AAC37037), Vip3Aa3 (US Patent 6137033), Vip3Aa4 (AAR81079), Vip3Aa5 (AAR81080), Vip3Aa6 (AAR81081), Vip3Aa7 (AAK95326), Vip3Aa8 (AAK97481), Vip3Aa9 (CAA76665), Vip3Aa10 (AAN60738), Vip3Aa11 (AAR36859), Vip3Aa12 (AAM22456), Vip3Aa13 (AAL69542), Vip3Aa14 (AAQ12340), Vip3Aa15 (AAP51131), Vip3Aa16 (AAW65132), Vip3Aa17 (US Patent 6603063), Vip3Aa18 (AAX49395), Vip3Aa19 (DQ241674), Vip3Aa19 (DQ539887), Vip3Aa20 (DQ539888), Vip3Aa21 (ABD84410), Vip3Aa22 (AAY41427), Vip3Aa23 (AAY41428), Vip3Aa24 (BI

880913), Vip3Aa25 (EF608501), Vip3Aa26 (EU294496), Vip3Aa27 (EU332167), Vip3Aa28 (FJ494817), Vip3Aa29 (FJ626674), Vip3Aa30 (FJ626675), Vip3Aa31 (FJ626676), Vip3Aa32 (FJ626677), Vip3Aa33 (GU073128), Vip3Aa34 (GU073129), Vip3Aa35 (GU733921), Vip3Aa36 (GU951510), Vip3Aa37 (HM132041), Vip3Aa38 (HM117632), Vip3Aa39 (HM117631), Vip3Aa40 (HM132042), Vip3Aa41 (HM132043), Vip3Aa42 (HQ587048), Vip3Aa43 (HQ594534), Vip3Aa44 (HQ650163), Vip3Ab1 (AAR40284), Vip3Ab2 (AAY88247), Vip3Ac1 (US Patent Application Publication 20040128716), Vip3Ad1 (US Patent Application Publication 20040128716), Vip3Ad2 (CAI43276), Vip3Ae1 (CAI43277), Vip3Af1 (CAI43275), Vip3Af2 (ADN08753), Vip3Af3 (HM117634), Vip3Ag1 (ADN08758),Vip3Ag2 (FJ556803),Vip3Ag3 (HM117633), Vip3Ag4 (HQ414237), Vip3Ag5 (HQ542193), and Vip3Ah1 (DQ832323).

29. The method of claim 28, wherein the Vip3A protein comprises SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16, or a nematode-active homologue thereof having at least 82% sequence identity with SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16.

30. The method of claim 18, wherein the transgenic plant further comprises or expresses at least one additional pesticidal agent selected from the group consisting of a patatin, a lectin, a *Bacillus thuringiensis* insecticidal protein, a *Bacillus thuringiensis* nematicidal protein, a *Xenorhabdus* insecticidal protein, a *Photorhabdus* insecticidal protein, a *Bacillus laterosporous* insecticidal protein, a *Bacillus sphearicus* insecticidal protein, and an RNAi that targets a nemtode.

31. The method of claim 30, wherein the *Bacillus thuringiensis* nematicidal protein is selected from the group consisting of a Cry1, Cry3, Cry11, Cry12, Cry13, Cry14, Cry21, and Cry22.

32. A method of conferring nematode resistance to a plant or plant part comprising inserting in the plant or plant part a heterologous nucleic acid molecule encoding a

Vip3 protein, wherein the plant or plant part expresses the Vip3 protein at a nematode-inhibiting level so as to confer nematode resistance to the plant or plant part compared to the same type of plant or plant part not expressing the Vip3 protein.

33. The method of claim 32, wherein the nematode is selected from the group consisting of Criconemella, Ditylenchus, Globodera, Helicotylenchus, Heterodera, Longidorus, Meloidogyne, Paratrichodorus, Pratylenchus, Radolpholus, Rotelynchus, Rotylenchulus, Tylenchulus and Xiphinema.

34. The method of claim 33, wherein the nematode is a cyst forming nematode.

35. The method of claim 34, wherein the nematode is in the genus *Heterodera*.

36. The method of claim 35, wherein the nematode is *Heterodera glycines*.

37. The method of claim 32, wherein the transgenic plant or plant part is selected from the group consisting of alfalfa, apple, apricot, Arabidopsis, artichoke, asparagus, avocado, banana, barley, beans, beet, blackberry, blueberry, Brassica, broccoli, Brussels sprouts, cabbage, canola, carrot, cassaya, cauliflower, a cereal, celery, cherry, citrus, Clementine, coffee, corn, cotton, cucumber, eggplant, endive, eucalyptus, figs, grape, grapefruit, groundnuts, ground cherry, kiwifruit, lettuce, leek, lemon, lime, pine, maize, mango, melon, millet, mushroom, nut oat, okra, onion, orange, an ornamental plant or flower or tree, papaya, parsley, pea, peach, peanut, peat, pepper, persimmon, pineapple, plantain, plum, pomegranate, potato, pumpkin, radicchio, radish, rapeseed, raspberry, rice, rye, sorghum, soy, soybean, spinach, strawberry, sugar beet, sugarcane, sunflower, sweet potato, tangerine, tea, tobacco, tomato, a vine, watermelon, wheat, yams and zucchini.

38. The method of claim 37, wherein the transgenic plantor plant part is a soybean plant or plant part.

39. The method of any one of claims 32 to 38, wherein the Vip3 protein is a Vip3A protein.

40. The method of claim 39, wherein the Vip3A protein comprises an amino acid sequence that is the translation product of a nucleotide sequence whose complement hybridizes to SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15 or SEQ ID NO: 17 under high-stringency conditions.

41. The method of claim 40, wherein the high-stringency conditions are 7% sodium dodecyl sulfate (SDS), 0.5 M NaP_{O4}, 1 mM EDTA at 50° C. with washing in 0.1XSSC, 0.1% SDS at 65°C.

42. The method of claim 39, wherein the Vip3A protein is selected from the group consisting of Vip3Aa1 (AAC37036), Vip3Aa2 (AAC37037), Vip3Aa3 (US Patent 6137033), Vip3Aa4 (AAR81079), Vip3Aa5 (AAR81080), Vip3Aa6 (AAR81081), Vip3Aa7 (AAK95326), Vip3Aa8 (AAK97481), Vip3Aa9 (CAA76665), Vip3Aa10 (AAN60738), Vip3Aa11 (AAR36859), Vip3Aa12 (AAM22456), Vip3Aa13 (AAL69542), Vip3Aa14 (AAQ12340), Vip3Aa15 (AAP51131), Vip3Aa16 (AAW65132), Vip3Aa17 (US Patent 6603063), Vip3Aa18 (AAX49395), Vip3Aa19 (DQ241674), Vip3Aa19 (DQ539887), Vip3Aa20 (DQ539888), Vip3Aa21 (ABD84410), Vip3Aa22 (AAY41427), Vip3Aa23 (AAY41428), Vip3Aa24 (BI 880913), Vip3Aa25 (EF608501), Vip3Aa26 (EU294496), Vip3Aa27 (EU332167), Vip3Aa28 (FJ494817), Vip3Aa29 (FJ626674), Vip3Aa30 (FJ626675), Vip3Aa31 (FJ626676), Vip3Aa32 (FJ626677), Vip3Aa33 (GU073128), Vip3Aa34 (GU073129), Vip3Aa35 (GU733921), Vip3Aa36 (GU951510), Vip3Aa37 (HM132041), Vip3Aa38 (HM117632), Vip3Aa39 (HM117631), Vip3Aa40 (HM132042), Vip3Aa41 (HM132043), Vip3Aa42 (HQ587048), Vip3Aa43 (HQ594534), Vip3Aa44 (HQ650163), Vip3Ab1 (AAR40284), Vip3Ab2 (AAY88247), Vip3Ac1 (US Patent Application Publication 20040128716), Vip3Ad1 (US Patent Application Publication 20040128716), Vip3Ad2 (CAI43276), Vip3Ae1 (CAI43277), Vip3Af1 (CAI43275), Vip3Af2 (ADN08753), Vip3Af3 (HM117634), Vip3Ag1 (ADN08758),Vip3Ag2

(FJ556803),Vip3Ag3 (HM117633), Vip3Ag4 (HQ414237), Vip3Ag5 (HQ542193), and Vip3Ah1 (DQ832323).

43. The method of claim 39, wherein the Vip3A protein comprises SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, or a nematode-active homologue thereof having at least 82% sequence identity with SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14 or SEQ ID NO: 16.

44. The method of claim 32, wherein the transgenic plant further comprises or expresses at least one additional pesticidal agent selected from the group consisting of a patatin, a lectin, a *Bacillus thuringiensis* insecticidal protein, a *Bacillus thuringiensis* nematicidal protein, a *Xenorhabdus* insecticidal protein, a *Photorhabdus* insecticidal protein, a *Bacillus laterosporous* insecticidal protein, a *Bacillus sphearicus* insecticidal protein, and an RNAi that targets a nemtode.

45. The method of claim 44, wherein the *Bacillus thuringiensis* nematicidal protein is selected from the group consisting of a Cry1, Cry3, Cry11, Cry12, Cry13, Cry14, Cry21, and Cry22.

46. A method of reducing nematode infectivity to a plant or plant part comprising contacting the nematode with a Vip3 protein, wherein nematode infectivity is reduced compared to nematode infectivity of plant or plant part not contacted with a Vip3 protein.

47. The method of claim 46, wherein the contacting comprises planting a transgenic seed capable of producing a transgenic plant that expresses a Vip3 protein, wherein the nematode feeds on the transgenic plant.

48. The method of claim 47, wherein the transgenic plant is *Glycine max*.

49. The method of claim 48, wherein the nematode is soybean cyst nematode (*Heterodera glycines*).

50. A method of improving plant yield in nematode infested fields, comprising expressing in the plant a Vip3 protein, wherein plant yield is improved compared to yield of a plant of the same type not expressing a Vip3 protein.

51. A transgenic soybean plant or plant part thereof comprising a heterologous nucleic acid molecule encoding a Vip3 protein, wherein the transgenic soybean plant is resistant to nematode infestation.

52. A method of producing a soybean plant protected against nematode infestation, comprising:
    a. transforming a soybean plant cell with a nucleic acid molecule encoding a Vip3 protein;
    b. regenerating a transformed soybean plant from the soybean plant cell.

53. A method of producing a soybean plant protected against nematode infestation, comprising:
    a. crossing a first parent soybean plant with a second parent soybean plant, wherein said first or second parent soybean plant comprises a heterologous nucleic acid molecule encoding a Vip3 protein, thereby producing a plurality of progeny plants;
    b. selecting from the plurality of progeny plants, a transgenic plant that is protected against nematode infestation.

54. A method of providing a grower with a means of controlling nematode pests comprising supplying seed to a grower, wherein the seed comprises a heterologous nucleic acid molecule that encodes a Vip3 protein and wherein the seed is capable of producing a plant that is resistant to nematode damage.

55. A transgenic soybean plant or plant part comprising a heterologous nucleic acid molecule encoding a Vip3 protein, wherein said transgenic plant or plant part has improved resistance to at least one plant pathogenic nematode, as compared to a control plant or plant part not expressing theVip3 protein.

56. The plant of claim 55, wherein the Vip3 protein comprises SEQ ID NO: 19.

57. The plant of claim 56, wherein the Vip3 protein is a Vip3A protein having at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, or at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identity with SEQ ID NO: 1.

58. The plant of claim 57, wherein the Vip3A protein has the amino acid sequence set forth in SEQ ID NO: 1.

59. The plant of claim 55, wherein the nematode is cyst forming nematode.

60. The plant of claim 59, wherein the cyst forming nematode is in the order *Heterodera*.

61. The plant of claim 60, wherein the cyst forming nematode is a soybean cyst nematode, *Heterodera glycines*.

62. Transgenic seed of the plant of any of claims 55 to 61, wherein the seed comprises the heterologous nucleic acid molecule.

63. A method of controlling *Heterodera glycines* comprising providing a transgenic soybean plant or plant part comprising an expression cassette having SEQ ID NO: 1 operably linked to a promoter capable of driving expression of an encoded Vip3 protein to levels sufficient to inhibit nematodes, wherein the proliferation of *Heterodera glycines* cysts on said plant or plant part is reduced compared to *Heterodera glycines* cysts on a soybean plant or plant part not expressing the Vip3 protein.

64. The method of claim 63, wherein the promoter is selected from the group consisting of: a) a constitutive promoter; b) a tissue-specific promoter; and c) an inducible promoter.

65. The method of claim 64, wherein the promoter is an actin 2 promoter.

66. A method of increasing the vigor or yield in a transgenic soybean plant exposed to a population of nematodes comprising:
    a. introgressing a transgenic soybean event into a soybean plant resulting in a transgenic soybean plant, wherein the transgenic soybean event comprises a heterologous nucleic acid molecule encoding a Vip3 protein that confers upon the transgenic soybean event resistance to nematodes; and
    b. growing the transgenic soybean plant or progeny thereof at a location where nematode infestation is yield limiting to a soybean plant not comprising the heterologous nucleic acid molecule encoding the Vip3 protein, whereby the transgenic soybean plant has increased vigor or yield compared to the control plant.

67. A nematicidal composition comprising a Vip3 protein and an acceptable agricultural carrier.

68. The nematicidal composition of claim 67, wherein the agricultural carrier is a transgenic soybean plant or plant part.

69. A method of producing a nematode-resistant plant, comprising introducing a nucleic acid molecule encoding a Vip3 protein into the plant thereby producing a transgenic plant, wherein the nucleic acid molecule causes the expression of the Vip3 protein in an amount that makes the transgenic plant resistant to nematodes.

70. A method of reducing nematode cyst development on roots of a plant infected by a nematode, comprising introducing into cells of the plant a nucleic acid molecule capable of directing the expression of a Vip3 protein, thereby reducing nematode cyst

development on roots of the plant.

71. A method for controlling or preventing nematode growth comprising providing a nematode pest with plant material comprising a heterologous nucleic acid molecule capable of directing expression of a Vip3 protein, wherein said plant inhibits a nematode biological activity.

72. The method of claim 71, wherein the biological activity is an ability to produce cysts on roots of the plant.

73. A method of improving soybean yield, comprising:
    a. introducing into a soybean plant a nucleic acid molecule capable of directing expression of a Vip3 protein; and
    b. cultivating a plurality of transgenic seeds from the plant of step (a), resulting in a plurality of transgenic plants having enhanced resistance to nematode infestation, thereby improving soybean yield.

74. A method of suppressing the growth of a plant-pathogenic nematode population in a location capable of supporting said growth comprising growing in the location a population of transgenic soybean plants comprising a heterologous DNA capable of directing expression of a Vip3 protein, wherein the plant-pathogenic nematode population is reduced.

75. A method for nematode control by delivering to the situs of a nematode infection an effective amount of a Vip3 protein, wherein the Vip3 protein controls the nematode.

76. A method of controlling a nematode pest population comprising contacting the nematode pest population with a transgenic plant or part thereof comprising a heterologous nucleic acid molecule encoding a Vip3 protein.

77. An expression cassette comprising an actin promoter operably linked to a *vip3* coding sequence operably linked to a terminator, wherein the expression cassette functions in a transgenic plant to drive expression of a Vip3 protein at a nematode-controlling effective amount.

78. The expression cassette of claim 77, wherein the actin promoter is an actin2 promoter.

79. The expression cassette of claim 77, wherein the *vip3* coding sequence comprises a nucleotide sequence whose complement hybridizes to SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15 or SEQ ID NO: 17 under high-stringency conditions.

80. The method of claim 79, wherein the high-stringency conditions are 7% sodium dodecyl sulfate (SDS), 0.5 M NaP_{O4}, 1 mM EDTA at 50° C. with washing in 0.1XSSC, 0.1% SDS at 65°C.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/046239

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/82 A01N63/02 A01H5/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A01N A01H

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	ESTRUCH J J ET AL: "VIP3A, A NOVEL BACILLUS THURINGIENSIS VEGETATIVE INSECTICIDAL PROTEIN WITH A WIDE SPECTRUM OF ACTIVITIES AGAINST LEPIDOPTERAN INSECTS", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, NATIONAL ACADEMY OF SCIENCES, US, vol. 93, 1 May 1996 (1996-05-01), pages 5389-5394, XP002071759, ISSN: 0027-8424, DOI: 10.1073/PNAS.93.11.5389 the whole document -----	67,68
X	WO 98/44137 A2 (NOVARTIS AG [CH]; NOVARTIS ERFIND VERWALT GMBH [AT]; ESTRUCH JUAN JOSE) 8 October 1998 (1998-10-08) the whole document -----	67,68

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

☒ See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 October 2012	11/01/2013

Name and mailing address of the ISA/	Authorized officer
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/046239

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

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

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

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

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

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

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

see additional sheet

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

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

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

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

1-13, 18-49, 54, 63-65, 67, 68, 70, 71, 74-76

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2012/046239

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-13, 18-49, 54, 63-65, 67, 68, 70, 71, 74-76

   Control of nematodes using Vip3 - methods etc relating thereto

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2. claims: 50, 66, 73

   Method of improving plant yield

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3. claims: 14-17, 51-53, 55-62, 69

   Provision of plants expressing Vip3

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4. claims: 77-80

   Expression cassettes for Vip3 expression
