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(54) **Title:** POLYNUCLEOTIDES ENCODING TRUNCATED SUCROSE ISOMERASE POLYPEPTIDES FOR CONTROL OF PARASITIC NEMATODES

(57) **Abstract:** The invention provides polynucleotides encoding N-terminal truncated forms of sucrose isomerase polypeptides which are capable of conferring increased nematode resistance in a plant. The invention also provides methods of producing transgenic plants with increased nematode resistance, seeds of such transgenic plants, and expression vectors, all of which comprise the polynucleotides of the invention.

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POLYNUCLEOTIDES ENCODING TRUNCATED SUCROSE ISOMERASE POLYPEPTIDES FOR CONTROL OF PARASITIC NEMATODES

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the priority benefit of U.S. Provisional Application Serial No.60/900,228 filed February 08, 2007.

FIELD OF THE INVENTION

[Para 1] The invention relates to the control of nematodes, in particular the control of soybean cyst nematodes. Disclosed herein are methods of producing transgenic plants with increased nematode resistance, expression vectors comprising polynucleotides encoding for functional proteins, and transgenic plants and seeds generated thereof.

BACKGROUND OF THE INVENTION

[Para 2] Nematodes are microscopic wormlike animals that feed on the roots, leaves, and stems of more than 2,000 vegetables, fruits, and ornamental plants, causing an estimated $100 billion crop loss worldwide. One common type of nematode is the root-knot nematode (RKN), whose feeding causes the characteristic galls on roots. Other root-feeding nematodes are the cyst- and lesion-types, which are more host specific.

[Para 3] Nematodes are present throughout the United States, but are mostly a problem in warm, humid areas of the South and West, and in sandy soils. Soybean cyst nematode (SCN), Heterodera glycines, was first discovered in the United States in North Carolina in 1954. It is the most serious pest of soybean plants. Some areas are so heavily infested by SCN that soybean production is no longer economically possible without control measures. Although soybean is the major economic crop attacked by SCN, SCN parasitizes some fifty hosts in total, including field crops, vegetables, ornamentals, and weeds.

[Para 4] Signs of nematode damage include stunting and yellowing of leaves, and wilting of the plants during hot periods. However, nematodes, including SCN, can cause significant yield loss without obvious above-ground symptoms. In addition, roots infected with SCN are dwarfed or stunted. Nematode infestation can decrease the number of nitrogen-fixing nodules on the roots, and may make the roots more susceptible to attacks by other soil-borne plant pathogens.

[Para 5] The nematode life cycle has three major stages: egg, juvenile, and adult. The life cycle varies between species of nematodes. For example, the SCN life cycle can usually be completed in 24 to 30 days under optimum conditions whereas other species can take as long as a year, or longer, to complete the life cycle. When temperature and moisture levels become adequate in the spring, worm-shaped juveniles hatch from eggs in the soil. These juveniles are the only life stage of the nematode that can infect soybean roots.

[Para 6] The life cycle of SCN has been the subject of many studies and therefore can be used as an example for understanding a nematode life cycle. After penetrating the soybean roots, SCN juveniles move through the root until they contact vascular tissue, where they stop and start to feed. The nematode injects secretions that modify certain root cells and transform them into specialized feeding sites. The root cells are morphologically transformed into large multinucleate syncytia (or giant cells in the case of RKN), which are used as a source of nutrients for the nematodes. The actively feeding nematodes thus steal essential nutrients from the plant resulting in yield loss. As the nematodes feed, they swell and eventually female nematodes become so large that they break through the root tissue and are exposed on the surface of the root.

[Para 7] Male SCN nematodes, which are not swollen as adults, migrate out of the root into the soil and fertilize the lemon-shaped adult females. The males then die, while the females remain attached to the root system and continue to feed. The eggs in the swollen females begin developing, initially in a mass or egg sac outside the body, then later within the body cavity. Eventually the entire body cavity of the adult female is filled with eggs, and the female nematode dies. It is the egg-filled body of the dead female that is referred to as the cyst. Cysts eventually dislodge and are found free in the soil. The walls of the cyst become very tough, providing excellent protection for the approximately 200 to 400 eggs contained within. SCN eggs survive within the cyst until proper hatching conditions occur. Although many of the eggs may hatch within the first year, many also will survive within the cysts for several years.

[Para 8] Nematodes can move through the soil only a few inches per year on its own power. However, nematode infestation can be spread substantial distances in a variety of ways. Anything that can move infested soil is capable of spreading the infestation, including farm machinery, vehicles and tools, wind, water, animals, and farm workers. Seed sized particles of soil often contaminate harvested seed. Consequently, nematode infestation can be spread when contaminated seed from infested fields is planted in non-infested fields. There is even evidence that certain nematode species can be spread by birds. Only some of these causes can be prevented.

[Para 9] Traditional practices for managing nematode infestation include: maintaining proper soil nutrients and soil pH levels in nematode-infested land; controlling other plant diseases, as well as insect and weed pests; using sanitation practices such as plowing, planting, and cultivating of nematode-infested fields only after working non-infested fields; cleaning equipment thoroughly with high pressure water or steam after working in infested fields; not using seed grown on infested land for planting non-infested fields unless the seed has been properly cleaned; rotating infested fields and alternating host crops with non-host crops; using nematicides; and planting resistant plant varieties.

[Para 10] Methods have been proposed for the genetic transformation of plants in order to confer increased resistance to plant parasitic nematodes. U.S. Patent Nos. 5,589,622 and 5,824,876 are directed to the identification of plant genes expressed specifically in or adjacent to the feeding site of the plant after attachment by the nematode. WO2004/005504 describes methods for generating nematode resistant plants by expressing a sucrose isomerase gene. Sucrose isomerase, which is produced in certain microbes, converts sucrose into isomaltulose (palatinose). (See, U.S. Patent Nos. 5,985,668 and 5,786,140).

SUMMARY OF THE INVENTION

[Para 11] The present inventors have surprisingly found that proteins similar to sucrose isomerase, but which do not have sucrose isomerase activity confer nematode resistance when expressed in transgenic plants. The present invention provides polynucleotides, transgenic plants and seeds, and methods to overcome, or at least alleviate, nematode infestation of valuable agricultural crops such as soybeans.

[Para 12] Thus the invention comprises an isolated polynucleotide encoding an N-terminal truncated form of a sucrose isomerase polypeptide that demonstrates anti-nematode activity when transformed into plants, wherein said polypeptide does not demonstrate sucrose isomerase enzymatic activity.

[Para 13] In another embodiment, the invention relates to an expression vector comprising a transcription regulatory element operably linked to a polynucleotide encoding an N-terminal truncated form of a sucrose isomerase, polypeptide that demonstrates anti-nematode activity when transformed into plants, but that does not have sucrose isomerase enzymatic activity.

[Para 14] In another embodiment, the invention provides a transgenic plant transformed with an expression vector comprising an isolated polynucleotide encoding an N-terminal truncated form of a sucrose isomerase, polypeptide that demonstrates anti-nematode activity when transformed into plants, but that does not have sucrose isomerase enzymatic activity. The transgenic plant of the invention demonstrates increased resistance to nematodes, as compared to a wild type variety of the plant.

[Para 15] Another embodiment of the invention provides a transgenic seed that is true breeding for an isolated polynucleotide encoding an N-terminal truncated form of a sucrose isomerase, polypeptide that demonstrates anti-nematode activity when transformed into plants, but that does not have sucrose isomerase enzymatic activity.

[Para 16] In yet another embodiment, the invention provides a method of producing a transgenic plant having increased nematode resistance, wherein the method comprises the steps of introducing into the plant an expression vector comprising a transcription regulatory element operably linked to an isolated polynucleotide encoding an N-terminal truncated form of a sucrose isomerase, polypeptide that demonstrates anti-nematode activity when transformed into plants, but that does not have sucrose isomerase enzymatic activity, and selecting transgenic plants for increased nematode resistance.

BRIEF DECRIPTION OF THE DRAWINGS

[Para 17] Figure 1a-1c shows the DNA sequence alignment of the truncated sucrose isomerase of the invention (SEQ ID NO:1) with full length sucrose isomerase from Erwinia rhapontici (Accession No AF279281; SEQ ID NO:3). The alignment is performed in VNTI using AlignX program (pairwise comparison, gap opnining penalty = 15, gap extension penalty = 6.66).

[Para 18] Figure 2 shows the global percent identity of the truncated Erwinia rhapontici amino acid sequence described by SEQ ID NO: 2 to the truncated amino acid sequence of the sucrose isomerase from Serratia plymuthica described by SEQ ID NO:5. PID = global percent identity

[Para 19] Figure 3a-3b shows the amino acid alignment of exemplary truncated homologs of the Erwinia truncated sucrose isomerase described by SEQ ID NO: 2, the homologs having

SEQ ID NOs:5, 14, 15, 16, 17, 18, 19 and 20. Vector NTI software suite (gap opening penalty = 15, gap extension penalty = 6.66, gap separation penalty = 8).

[Para 20] Figure 4 shows the global percent identity matrix table of exemplary truncated homologs of the Erwinia truncated sucrose isomerase.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[Para 21] The present invention may be understood more readily by reference to the following detailed description of the embodiments of the invention and the examples included herein. Unless otherwise noted, the terms used herein are to be understood according to conventional usage by those of ordinary skill in the relevant art.

[Para 22] Throughout this application, various patent and literature publications are referenced. The disclosures of all of these publications and those references cited within those publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains. Abbreviations and nomenclature, where employed, are deemed standard in the field and commonly used in professional journals such as those cited herein. As used herein and in the appended claims, the singular form “a”, “an”, or “the” includes plural reference unless the context clearly dictates otherwise. As used herein, the word “or” means any one member of a particular list and also includes any combination of members of that list.

[Para 23] The term “about” is used herein to mean approximately, roughly, around, or in the regions of. When the term “about” is used in conjunction with a numerical range, it modifies that range by extending the boundaries above and below the numerical values set forth. In general, the term “about” is used herein to modify a numerical value above and below the stated value by a variance of 10 percent, up or down (higher or lower).

[Para 24] As used herein, the word “nucleic acid”, “nucleotide”, or “polynucleotide” is intended to include DNA molecules (e.g., cDNA or genomic DNA), RNA molecules (e.g., mRNA), natural occurring, mutated, synthetic DNA or RNA molecules, and analogs of the DNA or RNA generated using nucleotide analogs. It can be single-stranded or double-stranded. Such nucleic acids or polynucleotides include, but are not limited to, coding sequences of structural genes, anti-sense sequences, and non-coding regulatory sequences that do not encode mRNAs or protein products. A polynucleotide may encode for an agronomically valuable or a phenotypic trait.

[Para 25] As used herein, an "isolated" polynucleotide is substantially free of other cellular materials or culture medium when produced by recombinant techniques, or substantially free of chemical precursors when chemically synthesized.

[Para 26] The term "gene" is used broadly to refer to any segment of nucleic acid associated with a biological function. Thus, genes include introns and exons as in genomic sequence, or just the coding sequences as in cDNAs and/or the regulatory sequences required for their expression. For example, gene refers to a nucleic acid fragment that expresses mRNA or functional RNA, or encodes a specific protein, and which includes regulatory sequences.

[Para 27] The terms "polypeptide" and "protein" are used interchangeably herein to refer to a polymer of consecutive amino acid residues.

[Para 28] The term "operably linked" or "functionally linked" as used herein refers to the association of nucleic acid sequences on single nucleic acid fragment so that the function of one is affected by the other. For example, a regulatory DNA is said to be "operably linked to" a DNA that expresses an RNA or encodes a polypeptide if the two DNAs are situated such that the regulatory DNA affects the expression of the coding DNA.

[Para 29] The term "specific expression" as used herein refers to the expression of gene products that is limited to one or a few plant tissues (spatial limitation) and/or to one or a few plant developmental stages (temporal limitation). It is acknowledged that hardly a true specificity exists: promoters seem to be preferably switched on in some tissues, while in other tissues there can be no or only little activity. This phenomenon is known as leaky expression. However, with specific expression as defined herein is meant to encompass expression in one or a few plant tissues or specific sites in a plant.

[Para 30] The term "promoter" as used herein refers to a DNA sequence which, when ligated to a nucleotide sequence of interest, is capable of controlling the transcription of the nucleotide sequence of interest into mRNA. A promoter is typically, though not necessarily, located 5' (e.g., upstream) of a nucleotide of interest (e.g., proximal to the transcriptional start site of a structural gene) whose transcription into mRNA it controls, and provides a site for specific binding by RNA polymerase and other transcription factors for initiation of transcription.

[Para 31] The term "transcription regulatory element" as used herein refers to a polynucleotide that is capable of regulating the transcription of an operably linked polynucleotide. It includes, but not limited to, promoters, enhancers, introns, 5' UTRs, and 3' UTRs.

[Para 32] As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. A vector can be a binary vector or a T-DNA that comprises the left border and the right border and may include a gene of interest in between. The term "expression vector" as used herein means a vector capable of directing expression of a particular nucleotide in an appropriate host cell. An expression vector comprises a regulatory nucleic acid element operably linked to a nucleic acid of interest, which is – optionally – operably linked to a termination signal and/or other regulatory element.

[Para 33] The term "homologs" as used herein refers to a gene related to a second gene by descent from a common ancestral DNA sequence. The term "homologs" may apply to the relationship between genes separated by the event of speciation (e.g., orthologs) or to the relationship between genes separated by the event of genetic duplication (e.g., paralogs).

[Para 34] As used herein, the term "orthologs" refers to genes from different species, but that have evolved from a common ancestral gene by speciation. Orthologs retain the same function in the course of evolution. Orthologs encode proteins having the same or similar functions. As used herein, the term "paralogs" refers to genes that are related by duplication within a genome. Paralogs usually have different functions or new functions, but these functions may be related.

[Para 35] As used herein, the term "hybridizes under stringent conditions" is intended to describe conditions for hybridization and washing under which nucleotide sequences at least 60% similar or identical to each other typically remain hybridized to each other. In another embodiment, the conditions are such that sequences at least about 65%, or at least about 70%, or at least about 75% or more similar or identical to each other typically remain hybridized to each other. Such stringent conditions are known to those skilled in the art and described as below. A preferred, non-limiting example of stringent conditions are hybridization in 6X sodium chloride/sodium citrate (SSC) at about 45°C, followed by one or more washes in 0.2X SSC, 0.1% SDS at 50-65°C.

[Para 36] The term "sequence identity" or "identity" in the context of two nucleic acid or polypeptide sequences makes reference to the residues in the two sequences that are the

same when aligned for maximum correspondence over a specified comparison window, for example, either the entire sequence as in a global alignment or the region of similarity in a local alignment. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions that are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. When sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences that differ by such conservative substitutions are said to have "sequence similarity" or "similarity". Means for making this adjustment are well known to those of skilled in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage of sequence similarity.

[Para 37] As used herein, "percentage of sequence identity" or "sequence identity percentage" means the value determined by comparing two optimally aligned sequences over a comparison window, either globally or locally, wherein the portion of the sequence in the comparison window may comprise gaps for optimal alignment of the two sequences. In principle, the percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison, and multiplying the result by 100 to yield the percentage of sequence identity. "Percentage of sequence similarity" for protein sequences can be calculated using the same principle, wherein the conservative substitution is calculated as a partial rather than a complete mismatch. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions can be obtained from amino acid matrices known in the art, for example, Blosum or PAM matrices.

[Para 38] Methods of alignment of sequences for comparison are well known in the art. The determination of percent identity or percent similarity (for proteins) between two sequences can be accomplished using a mathematical algorithm. Preferred, non-limiting examples of such mathematical algorithms are, the algorithm of Myers and Miller (Bioinformatics, 4(1):11-17, 1988), the Needleman-Wunsch global alignment (J Mol Biol. 48(3):443-53, 1970), the Smith-Waterman local alignment (Journal of Molecular Biology, 147:195-197, 1981), the search-for-similarity-method of Pearson and Lipman (PNAS, 85(8): 2444-2448, 1988), the algorithm of Karlin and Altschul (J. Mol. Biol., 215(3):403-410, 1990; PNAS, 90:5873-5877,1993). Computer

implementations of these mathematical algorithms can be utilized for comparison of sequences to determine sequence identity or to identify homologs.

[Para 39] The term "conserved region" or "conserved domain" as used herein refers to a region in heterologous polynucleotide or polypeptide sequences where there is a relatively high degree of sequence identity between the distinct sequences. The "conserved region" can be identified, for example, from the multiple sequence alignment using the Clustal W algorithm.

[Para 40] The term "cell" or "plant cell" as used herein refers to single cell, and also includes a population of cells. The population may be a pure population comprising one cell type. Likewise, the population may comprise more than one cell type. A plant cell within the meaning of the invention may be isolated (e.g., in suspension culture) or comprised in a plant tissue, plant organ or plant at any developmental stage.

[Para 41] The term "tissue" with respect to a plant (or "plant tissue") means arrangement of multiple plant cells, including differentiated and undifferentiated tissues of plants. Plant tissues may constitute part of a plant organ (e.g., the epidermis of a plant leaf) but may also constitute tumor tissues (e.g., callus tissue) and various types of cells in culture (e.g., single cells, protoplasts, embryos, calli, protocorm-like bodies, etc.). Plant tissues may be in planta, in organ culture, tissue culture, or cell culture.

[Para 42] The term "organ" with respect to a plant (or "plant organ") means parts of a plant and may include, but not limited to, for example roots, fruits, shoots, stems, leaves, hypocotyls, cotyledons, anthers, sepals, petals, pollen, seeds, etc.

[Para 43] The term "plant" as used herein can, depending on context, be understood to refer to whole plants, plant cells, plant organs, plant seeds, and progeny of same. The word "plant" also refers to any plant, particularly, to seed plant, and may include, but not limited to, crop plants. Plant parts include, but are not limited to, stems, roots, shoots, fruits, ovules, stamens, leaves, embryos, meristematic regions, callus tissue, gametophytes, sporophytes, pollen, microspores, hypocotyls, cotyledons, anthers, sepals, petals, pollen, seeds and the like. The class of plants that can be used in the method of the invention is generally as broad as the class of higher and lower plants amenable to transformation techniques, including angiosperms (monocotyledonous and dicotyledonous plants), gymnosperms, ferns, horsetails, psilophytes, bryophytes, and multicellular algae.

[Para 44] The term "transgenic" as used herein is intended to refer to cells and/or plants which contain a transgene, or whose genome has been altered by the introduction of a transgene, or that have incorporated exogenous genes or polynucleotides. Transgenic cells, tissues, organs and plants may be produced by several methods including the introduction of a "transgene" comprising polynucleotide (usually DNA) into a target cell or integration of the transgene into a chromosome of a target cell by way of human intervention, such as by the methods described herein.

[Para 45] The term "true breeding" as used herein refers to a variety of plant for a particular trait if it is genetically homozygous for that trait to the extent that, when the true-breeding variety is self-pollinated, a significant amount of independent segregation of the trait among the progeny is not observed.

[Para 46] The term "control plant" or "wild type plant" as used herein refers to a plant cell, an explant, seed, plant component, plant tissue, plant organ, or whole plant used to compare against transgenic or genetically modified plant for the purpose of identifying an enhanced phenotype or a desirable trait in the transgenic or genetically modified plant. A "control plant" may in some cases be a transgenic plant line that comprises an empty vector or marker gene, but does not contain the recombinant polynucleotide of interest that is present in the transgenic or genetically modified plant being evaluated. A control plant may be a plant of the same line or variety as the transgenic or genetically modified plant being tested, or it may be another line or variety, such as a plant known to have a specific phenotype, characteristic, or known genotype. A suitable control plant would include a genetically unaltered or non-transgenic plant of the parental line used to generate a transgenic plant herein.

[Para 47] The term "resistant to nematode infection" or "a plant having nematode resistance" as used herein refers to the ability of a plant to avoid infection by nematodes, to kill nematodes or to hamper, reduce or stop the development, growth or multiplication of nematodes. This might be archieved by an active process, e.g. by producing a substance detrimental to the nematode, or by a passive process, like having a reduced nutritional value for the nematode or not developing structures induced by the nematode feeding site like syncytial or giant cells. The level of nematode resistance of a plant can be determined in various ways, e.g. by counting the nematodes being able to establish parasitism on that plant, or measuring development times of nematodes, proportion of male and female nematodes or the number of cysts or nematode eggs produced. A plant with increased resistance to nematode infection is a plant, which is more resistant to nematode infection in comparison to another plant having a similar or

preferably a identical genotype while lacking the gene or genes conferring increased resistance to nematodes, e.g, a control or wild type plant..

[Para 48] The term “feeding site” or “syncytia site” are used interchangeably and refer as used herein to the feeding site formed in plant roots after nematode infestation. The site is used as a source of nutrients for the nematodes. Syncytia is the feeding site for cyst nematodes and giant cells are the feeding sites of root knot nematodes.

[Para 49] As defined herein, an “N-terminal truncated form of a sucrose isomerase polypeptide” means a sucrose isomerase polypeptide that lacks at least about 5%, 10%, 15%, 18%, 20%, 21%, 22%, 23%, 24%, or 25% of the N-terminal amino acids found in the corresponding native sucrose isomerase polypeptide. An N-terminal truncated form of a sucrose isomerase polypeptide of the invention is a homolog of the polypeptide having the amino acid sequence set forth in SEQ ID NO:2. Additional N-terminal truncated forms of sucrose isomerase polypeptides may be isolated from orthologs and paralogs of full-length sucrose isomerase polypeptides.

[Para 50] In one embodiment, the invention provides an isolated polynucleotide encoding an N-terminal truncated form of a sucrose isomerase polypeptide that does not demonstrate sucrose isomerase activity. In accordance with the invention, the polynucleotide sequence of any full-length sucrose isomerase polypeptide may be employed to identify polynucleotides encoding N-terminal truncated forms of sucrose isomerase polypeptides that do not demonstrate sucrose isomerase activity. Assays to determine the presence or absence of sucrose isomerase activity in N-terminal truncated forms of sucrose isomerase polypeptides are disclosed in the examples below. In exemplary embodiments, the polynucleotide is selected from the group consisting of: a polynucleotide having the sequence as defined in SEQ ID NO: 1, 3, 4, 6, 21, 22, 23, 24, 25, 26 or 27; a polynucleotide encoding a polypeptide having the sequence as defined in SEQ ID NO: 2, 5, 14, 15, 16, 17, 18, 19 or 20; a polynucleotide having 70% sequence identity to a polynucleotide having the sequence as defined in SEQ ID NO: 1, 3, 4, 6, 21, 22, 23, 24, 25, 26 or 27; a polynucleotide encoding a polypeptide having 70% sequence identity to a polypeptide having the sequence as defined in SEQ ID NO: 2, 5, 14, 15, 16, 17, 18, 19 or 20; a polynucleotide that hybridizes under stringent conditions to a polynucleotide having the sequence as defined in SEQ ID NO: 1, 3, 4, 6, 21, 22, 23, 24, 25, 26 or 27; and a polynucleotide that hybridizes under stringent conditions to a polynucleotide encoding a polypeptide having the sequences defined in SEQ ID NOs: 2, 5, 14, 15, 16, 17, 18, 19 or 20.

[Para 51] The invention is also embodied in isolated polynucleotides having at least about 50-60%, or at least about 60-70%, or at least about 70-80%, 80-85%, 85-90%, 90-95%, or at least about 95%, 96%, 97%, 98%, 99% or more identical or similar to a polynucleotide having the sequence as defined in SEQ ID NO: 1, 3, 4, 6, 21, 22, 23, 24, 25, 26 or 27. In yet another embodiment, a polynucleotide of the invention comprises a polynucleotide encoding a polypeptide which is at least about 50-60%, or at least about 60-70%, or at least about 70-80%, 80-85%, 85-90%, 90-95%, or at least about 95%, 96%, 97%, 98%, 99% or more identical or similar to any of the polypeptides having the sequences defined in SEQ ID NOs: 2, 5, 14, 15, 16, 17, 18, 19 or 20.

[Para 52] Also encompassed in the isolated polynucleotides of the invention are allelic variants of full-length sucrose isomerase polypeptides that do not demonstrate sucrose isomerase activity. As used herein, the term “allelic variant” refers to a polynucleotide containing polymorphisms that lead to changes in the amino acid sequences of a protein encoded by the nucleotide and that exist within a natural population (e.g., a plant species or variety). Such natural allelic variations can typically result in 1-5% variance in a polynucleotide encoding a protein, or 1-5% variance in the encoded protein. Allelic variants can be identified by sequencing the nucleic acid of interest in a number of different plants, which can be readily carried out by using, for example, hybridization probes to identify the same gene genetic locus in those plants. Any and all such nucleic acid variations in a polynucleotide and resulting amino acid polymorphisms or variations of a protein that are the result of natural allelic variation and that do not alter the functional activity of the encoded protein, are intended to be within the scope of the invention.

[Para 53] The invention is also embodied in a transgenic plant transformed with an expression vector comprising an isolated polynucleotide encoding an N-terminal truncated form of a sucrose isomerase polypeptide that does not demonstrate sucrose isomerase activity, wherein expression of the polynucleotide confers increased nematode resistance to the plant. In one exemplary embodiment, the transgenic plant of the invention comprises a polynucleotide having the sequence as defined in SEQ ID NO: 1, 3, 4, 6, 21, 22, 23, 24, 25, 26 or 27. In another exemplary embodiment, the transgenic plant comprises a polynucleotide encoding a polypeptide having the sequence as defined in SEQ ID NO: 2, 5, 14, 15, 16, 17, 18, 19 or 20. In yet another exemplary embodiment, a transgenic plant of the invention comprises a polynucleotide which is at least about 50-60%, or at least about 60-70%, or at least about 70-80%, 80-85%, 85-90%, 90-95%, or at least about 95%, 96%, 97%, 98%, 99% or more identical or similar to a polynucleotide having the sequence as defined in SEQ ID NO: 1, 3, 4, 6, 21, 22, 23, 24, 25, 26 or 27. In yet another exemplary embodiment, a transgenic plant of the invention

comprises a polynucleotide encoding a polypeptide which is at least about 50-60%, or at least about 60-70%, or at least about 70-80%, 80-85%, 85-90%, 90-95%, or at least about 95%, 96%, 97%, 98%, 99% or more identical or similar to the polypeptide having the sequence as defined in SEQ ID NO: 2, 5, 14, 15, 16, 17, 18, 19 or 20.

[Para 54] The present invention also provides a transgenic seed that is true-breeding for a polynucleotide encoding an N-terminal truncated form of a sucrose isomerase polypeptide that does not demonstrate sucrose isomerase activity, and progeny plants from such a seed, including hybrids and inbreds. The invention also provides a method of plant breeding, e.g., to prepare a crossed fertile transgenic plant. The method comprises crossing a fertile transgenic plant comprising a particular expression vector of the invention with itself or with a second plant, e.g., one lacking the particular expression vector, to prepare the seed of a crossed fertile transgenic plant comprising the particular expression vector. The seed is then planted to obtain a crossed fertile transgenic plant. The plant may be a monocot or dicot. The crossed fertile transgenic plant may have the particular expression vector inherited through a female parent or through a male parent. The second plant may be an inbred plant. The crossed fertile transgenic may be a hybrid. Also included within the present invention are seeds of any of these crossed fertile transgenic plants.

[Para 55] Another embodiment of the invention relates to an expression vector comprising one or more transcription regulatory elements operably linked to one or more polynucleotides of the invention, wherein expression of the polynucleotide confers increased nematode resistance to a transgenic plant. In one embodiment, the transcription regulatory element is a promoter capable of regulating constitutive expression of an operably linked polynucleotide. A “constitutive promoter” refers to a promoter that is able to express the open reading frame or the regulatory element that it controls in all or nearly all of the plant tissues during all or nearly all developmental stages of the plant. Constitutive promoters include, but not limited to, the 35S CaMV promoter from plant viruses (Franck et al., Cell 21:285-294, 1980), the Nos promoter, the ubiquitin promoter (Christensen et al., Plant Mol. Biol. 12:619-632, 1992 and 18:581-8, 1991), the MAS promoter (Velten et al., EMBO J. 3:2723-30, 1984), the maize H3 histone promoter (Lepetit et al., Mol Gen. Genet 231:276-85, 1992), the ALS promoter (WO96/30530), the 19S CaMV promoter (US 5,352,605), the super-promoter (US 5,955,646), the figwort mosaic virus promoter (US 6,051,753), the rice actin promoter (US 5,641,876), and the Rubisco small subunit promoter (US 4,962,028).

[Para 56] In another embodiment, the transcription regulatory element is a regulated promoter. A “regulated promoter” refers to a promoter that directs gene expression not constitutively, but

in a temporally and/or spatially manner, and includes both tissue-specific and inducible promoters. Different promoters may direct the expression of a gene or regulatory element in different tissues or cell types, or at different stages of development, or in response to different environmental conditions.

[Para 57] A "tissue-specific promoter" refers to a regulated promoter that is not expressed in all plant cells but only in one or more cell types in specific organs (such as leaves or seeds), specific tissues (such as embryo or cotyledon), or specific cell types (such as leaf parenchyma or seed storage cells). These also include promoters that are temporally regulated, such as in early or late embryogenesis, during fruit ripening in developing seeds or fruit, in fully differentiated leaf, or at the onset of sequence. Suitable promoters include the napin-gene promoter from rapeseed (US 5,608,152), the USP-promoter from Vicia faba (Baeumlein et al., 1991 Mol Gen Genet. 225(3):459-67), the oleosin-promoter from Arabidopsis (WO 98/45461), the phaseolin-promoter from Phaseolus vulgaris (US 5,504,200), the Bce4-promoter from Brassica (WO 91/13980) or the legumin B4 promoter (LeB4; Baeumlein et al., 1992 Plant Journal, 2(2):233-9) as well as promoters conferring seed specific expression in monocot plants like maize, barley, wheat, rye, rice, etc. Suitable promoters to note are the lpt2 or lpt1-gene promoter from barley (WO 95/15389 and WO 95/23230) or those described in WO 99/16890 (promoters from the barley hordein-gene, rice glutelin gene, rice oryzin gene, rice prolamin gene, wheat gliadin gene, wheat glutelin gene, maize zein gene, oat glutelin gene, Sorghum kasirin-gene and rye secalin gene). Promoters suitable for preferential expression in plant root tissues include, for example, the promoter derived from corn nicotianamine synthase gene (US 20030131377) and rice RCC3 promoter (US 11/075,113). Suitable promoter for preferential expression in plant green tissues include the promoters from genes such as maize aldolase gene FDA (US 20040216189), aldolase and pyruvate orthophosphate dikinase (PPDK) (Taniguchi et. al., Plant Cell Physiol. 41(1):42-48, 2000).

[Para 58] "Inducible promoters" refer to those regulated promoters that can be turned on in one or more cell types by an external stimulus, for example, a chemical, light, hormone, stress, or a pathogen such as nematodes. Chemically inducible promoters are especially suitable if gene expression is wanted to occur in a time specific manner. Examples of such promoters are a salicylic acid inducible promoter (WO 95/19443), a tetracycline inducible promoter (Gatz et al., 1992 Plant J. 2:397-404), the light-inducible promoter from the small subunit of Ribulose-1,5-bis-phosphate carboxylase (ssRUBISCO), and an ethanol inducible promoter (WO 93/21334). Also, suitable promoters responding to biotic or abiotic stress conditions are those such as the pathogen inducible PRP1-gene promoter (Ward et al., 1993 Plant. Mol. Biol. 22:361-366), the heat inducible hsp80-promoter from tomato (US 5187267), cold inducible alpha-amylase

promoter from potato (WO 96/12814), the drought-inducible promoter of maize (Busk et. al., Plant J. 11:1285-1295, 1997), the cold, drought, and high salt inducible promoter from potato (Kirch, Plant Mol. Biol. 33:897-909, 1997) or the RD29A promoter from Arabidopsis (Yamaguchi-Shinozalei et. al. Mol. Gen. Genet. 236:331-340, 1993), many cold inducible promoters such as cor15a promoter from Arabidopsis (Genbank Accession No U01377), blt101 and blt4.8 from barley (Genbank Accession Nos AJ310994 and U63993), wcs120 from wheat (Genbank Accession No AF031235), mlip15 from corn (Genbank Accession No D26563), bn115 from Brassica (Genbank Accession No U01377), and the wound-inducible pinII-promoter (European Patent No. 375091).

[Para 59] Preferred promoters are root-specific, feeding site-specific, pathogen inducible or nematode inducible promoters.

[Para 60] Yet another embodiment of the invention relates to a method of producing a transgenic plant comprising a polynucleotide encoding an N-terminal truncated form of a sucrose isomerase polypeptide that does not demonstrate sucrose isomerase activity, wherein the method comprises the steps of: introducing into the plant the expression vector comprising the polynucleotide of the invention; and selecting transgenic plants for increased nematode resistance.

[Para 61] A variety of methods for introducing polynucleotides into the genome of plants and for the regeneration of plants from plant tissues or plant cells are known in, for example, Plant Molecular Biology and Biotechnology (CRC Press, Boca Raton, Florida), chapter 6/7, pp. 71-119 (1993); White FF (1993) Vectors for Gene Transfer in Higher Plants; Transgenic Plants, vol. 1, Engineering and Utilization, Ed.: Kung and Wu R, Academic Press, 15-38; Jenes B et al. (1993) Techniques for Gene Transfer; Transgenic Plants, vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, pp. 128-143; Potrykus (1991) Annu Rev Plant Physiol Plant Molec Biol 42:205-225; Halford NG, Shewry PR (2000) Br Med Bull 56(1):62-73.

[Para 62] Transformation methods may include direct and indirect methods of transformation. Suitable direct methods include polyethylene glycol induced DNA uptake, liposome-mediated transformation (US 4,536,475), biolistic methods using the gene gun (Fromm ME et al. (1990) Bio/Technology. 8(9):833-9; Gordon-Kamm et al. (1990) Plant Cell 2:603), electroporation, incubation of dry embryos in DNA-comprising solution, and microinjection. In the case of these direct transformation methods, the plasmid used need not meet any particular requirements. Simple plasmids, such as those of the pUC series, pBR322, M13mp series, pACYC184 and the like can be used. If intact plants are to be regenerated from the transformed cells, an additional

selectable marker gene is preferably located on the plasmid. The direct transformation techniques are equally suitable for dicotyledonous and monocotyledonous plants.

[Para 63] Transformation can also be carried out by bacterial infection by means of Agrobacterium (for example EP 0 116 718), viral infection by means of viral vectors (EP 0 067 553; US 4,407,956; WO 95/34668; WO 93/03161) or by means of pollen (EP 0 270 356; WO 85/01856; US 4,684,611). Agrobacterium based transformation techniques (especially for dicotyledonous plants) are well known in the art. The Agrobacterium strain (e.g., Agrobacterium tumefaciens or Agrobacterium rhizogenes) comprises a plasmid (Ti or Ri plasmid) and a T-DNA element which is transferred to the plant following infection with Agrobacterium. The T-DNA (transferred DNA) is integrated into the genome of the plant cell. The T-DNA may be localized on the Ri- or Ti-plasmid or is separately comprised in a so-called binary vector. Methods for the Agrobacterium-mediated transformation are described, for example, in Horsch RB et al. (1985) Science 225:1229f. The Agrobacterium-mediated transformation is best suited to dicotyledonous plants but has also been adopted to monocotyledonous plants. The transformation of plants by Agrobacteria is described in, for example, White FF, Vectors for Gene Transfer in Higher Plants, Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press, 1993, pp. 15 - 38; Jenes B et al. Techniques for Gene Transfer, Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press, 1993, pp. 128-143; Potrykus (1991) Annu Rev Plant Physiol Plant Mol Biol 42:205- 225.

[Para 64] Transformation may result in transient or stable transformation and expression. Although a polynucleotide of the present invention can be inserted into any plant and plant cell falling within these broad classes, it is particularly useful in crop plant cells.

[Para 65] The polynucleotides of the present invention can be directly transformed into the plastid genome. Plastid expression, in which genes are inserted by homologous recombination into 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 high expression levels. In one embodiment, the nucleotides are inserted into a plastid targeting vector and transformed into the plastid genome of a desired plant host. Plants homoplasmic for plastid genomes containing the nucleotide sequences are obtained, and are preferentially capable of high expression of the nucleotides.

[Para 66] Plastid transformation technology is for example extensively described in U.S. Pat. Nos. 5,451,513, 5,545,817, 5,545,818, and 5,877,462 in WO 95/16783 and WO 97/32977, and

in McBride et al. (1994) Proc. Natl. Acad. Sci. USA 91, 7301-7305, all incorporated herein by reference in their entirety. The basic technique for plastid transformation involves introducing regions of cloned plastid DNA flanking a selectable marker together with the nucleotide sequence into a suitable target tissue, e.g., using biolistic 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 et al. (1990) PNAS 87, 8526-8530; Staub et al. (1992) Plant Cell 4, 39-45). The presence of cloning sites between these markers allows creation of a plastid targeting vector for introduction of foreign genes (Staub et al. (1993) EMBO J. 12, 601-606). Substantial increases in transformation frequency are obtained by replacement of the recessive rRNA or r-protein antibiotic resistance genes with a dominant selectable marker, the bacterial aadA gene encoding the spectinomycin-detoxifying enzyme aminoglycoside-3'-adenyltransferase (Svab et al. (1993) PNAS 90, 913-917). Other selectable markers useful for plastid transformation are known in the art and encompassed within the scope of the invention.

[Para 67] The transgenic plant of the invention may be any plant, such as, but not limited to trees, cut flowers, ornamentals, vegetables or crop plants. The plant may be from a genus selected from the group consisting of Medicago, Lycopersicon, Brassica, Cucumis, Solanum, Juglans, Gossypium, Malus, Vitis, Antirrhinum, Populus, Fragaria, Arabidopsis, Picea, Capsicum, Chenopodium, Dendranthema, Pharbitis, Pinus, Pisum, Oryza, Zea, Triticum, Triticale, Secale, Lolium, Hordeum, Glycine, Pseudotsuga, Kalanchoe, Beta, Helianthus, Nicotiana, Cucurbita, Rosa, Fragaria, Lotus, Medicago, Onobrychis, trifolium, Trigonella, Vigna, Citrus, Linum, Geranium, Manihot, Daucus, Raphanus, Sinapis, Atropa, Datura, Hyoscyamus, Nicotiana, Petunia, Digitalis, Majorana, Ciahorium, Lactuca, Bromus, Asparagus, Antirrhinum, Heterocallis, Nemesis, Pelargonium, Panieum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Browaalia, Phaseolus, Avena, and Allium, or the plant may be selected from the group consisting of cereals including wheat, barley, sorghum, rye, triticale, maize, rice, sugarcane, and trees including apple, pear, quince, plum, cherry, peach, nectarine, apricot, papaya, mango, poplar, pine, sequoia, cedar, and oak. The term “plant” as used herein can be dicotyledonous crop plants, such as pea, alfalfa, soybean, carrot, celery, tomato, potato, cotton, tobacco, pepper, oilseed rape, beet, cabbage, cauliflower, broccoli, lettuce and Arabidopsis thaliana.,. In one embodiment the plant is a monocotyledonous plant or a dicotyledonous plant.

[Para 68] Preferably the plant is a crop plant. Crop plants are all plants, used in agriculture. Accordingly in one embodiment the plant is a monocotyledonous plant, preferably a plant of the family Poaceae, Musaceae, Liliaceae or Bromeliaceae, preferably of the family Poaceae. Accordingly, in yet another embodiment the plant is a Poaceae plant of the genus Zea, Triticum, Oryza, Hordeum, Secale, Avena, Saccharum, Sorghum, Pennisetum, Setaria, Panicum, Eleusine, Miscanthus, Brachypodium, Festuca or Lolium. When the plant is of the genus Zea, the preferred species is Z. mays. When the plant is of the genus Triticum, the preferred species is T. aestivum, T. speltae or T. durum. When the plant is of the genus Oryza, the preferred species is O. sativa. When the plant is of the genus Hordeum, the preferred species is H. vulgare. When the plant is of the genus Secale, the preferred species S. cereale. When the plant is of the genus Avena, the preferred species is A. sativa. When the plant is of the genus Saccarum, the preferred species is S. officinarum. When the plant is of the genus Sorghum, the preferred species is S. vulgare, S. bicolor or S. sudanense. When the plant is of the genus Pennisetum, the preferred species is P. glaucum. When the plant is of the genus Setaria, the preferred species is S. italica. When the plant is of the genus Panicum, the preferred species is P. miliaceum or P. virgatum. When the plant is of the genus Eleusine, the preferred species is E. coracana. When the plant is of the genus Miscanthus, the preferred species is M. sinensis. When the plant is a plant of the genus Festuca, the preferred species is F. arundinaria, F. rubra or F. pratensis. When the plant is of the genus Lolium, the preferred species is L. perenne or L. multiflorum. Alternatively, the plant may be Triticosecale.

[Para 69] Alternatively, in one embodiment the plant is a dicotyledonous plant, preferably a plant of the family Fabaceae, Solanaceae, Brassicaceae, Chenopodiaceae, Asteraceae, Malvaceae, Linacea, Euphorbiaceae, Convolvulaceae Rosaceae, Cucurbitaceae, Theaceae, Rubiaceae, Sterculiaceae or Citrus. In one embodiment the plant is a plant of the family Fabaceae, Solanaceae or Brassicaceae. Accordingly, in one embodiment the plant is of the family Fabaceae, preferably of the genus Glycine, Pisum, Arachis, Cicer, Vicia, Phaseolus, Lupinus, Medicago or Lens. Preferred species of the family Fabaceae are M. truncatula, M, sativa, G. max, P. sativum, A. hypogea, C. arietinum, V. faba, P. vulgaris, Lupinus albus, Lupinus luteus, Lupinus angustifolius or Lens culinaris. More preferred are the species G. max A. hypogea and M. sativa. Most preferred is the species G. max. When the plant is of the family Solanaceae, the preferred genus is Solanum, Lycopersicon, Nicotiana or Capsicum. Preferred species of the family Solanaceae are S. tuberosum, L. esculentum, N. tabaccum or C. chinense. More preferred is S. tuberosum. Accordingly, in one embodiment the plant is of the family Brassicaceae, preferably of the genus Brassica or Raphanus. Preferred species of the family Brassicaceae are the species B. napus, B. oleracea, B. juncea or B. rapa. More preferred is the species B. napus. When the plant is of the family Chenopodiaceae, the preferred genus is Beta and the preferred species is the B. vulgaris. When the plant is of the family Asteraceae, the

preferred genus is Helianthus and the preferred species is H. annuus. When the plant is of the family Malvaceae, the preferred genus is Gossypium or Abelmoschus. When the genus is Gossypium, the preferred species is G. hirsutum or G. barbadense and the most preferred species is G. hirsutum. A preferred species of the genus Abelmoschus is the species A. esculentus. When the plant is of the family Linacea, the preferred genus is Linum and the preferred species is L. usitatissimum. When the plant is of the family Euphorbiaceae, the preferred genus is Manihot, Jatropa or Rhizinus and the preferred species are M. esculenta, J. curcas or R. comunis. When the plant is of the family Convolvulaceae, the preferred genus is Ipomea and the preferred species is I. batatas. When the plant is of the family Rosaceae, the preferred genus is Rosa, Malus, Pyrus, Prunus, Rubus, Ribes, Vaccinium or Fragaria and the preferred species is the hybrid Fragaria x ananassa. When the plant is of the family Cucurbitaceae, the preferred genus is Cucumis, Citrullus or Cucurbita and the preferred species is Cucumis sativus, Citrullus lanatus or Cucurbita pepo. When the plant is of the family Theaceae, the preferred genus is Camellia and the preferred species is C. sinensis. When the plant is of the family Rubiaceae, the preferred genus is Coffea and the preferred species is C. arabica or C. canephora. When the plant is of the family Sterculiaceae, the preferred genus is Theobroma and the preferred species is T. cacao. When the plant is of the genus Citrus, the preferred species is C. sinensis, C. limon, C. reticulata, C. maxima and hybrids of Citrus species, or the like. In a preferred embodiment of the invention, the plant is a soybean, a potato or a corn plant

[Para 70] The transgenic plants of the invention may be used in a method of controlling infestation of a crop by a plant parasitic nematode, which comprises the step of growing said crop from seeds comprising an expression cassette comprising a transcription regulatory element operably linked to a polynucleotide of the invention wherein the expression cassette is stably integrated into the genomes of the seeds. .

[Para 71] Accordingly the invention comprises a method of conferring nematode resistance to a plant, said method comprising the steps of: preparing an expression cassette comprising a polynucleotide of the invention operably linked to a promoter; transforming a recipient plant with said expression cassette; producing one or more transgenic offspring of said recipient plant; and selecting the offspring for nematode resistance. Preferably the promoter is a root-preferred or nematode inducible promoter or a promoter mediating expression in nematode feeding sites, e.g. syncytia or giant cells.

[Para 72] The present invention may be used to reduce crop destruction by plant parasitic nematodes or to confer nematode resistance to a plant. The nematode may be any plant

parasitic nematode, like nematodes of the families Longidoridae, Trichodoridae, Aphelenchoidida, Anguinidae, Belonolaimidae, Criconematidae, Heterodidae, Hoplolaimidae, Meloidogynidae, Paratylenchidae, Pratylenchidae, Tylenchulidae, Tylenchidae, or the like. Preferably, the parasitic nematodes belong to nematode families inducing giant or syncytial cells. Nematodes inducing giant or syncytial cells are found in the families Longidoridae, Trichodoridae, Heterodidae, Meloidogynidae, Pratylenchidae or Tylenchulidae. In particular in the families Heterodidae and Meloidogynidae.

[Para 73] Accordingly, parasitic nematodes targeted by the present invention belong to one or more genus selected from the group of Naccobus, Cactodera, Dolichodera, Globodera, Heterodera, Punctodera, Longidorus or Meloidogyne. In a preferred embodiment the parasitic nematodes belong to one or more genus selected from the group of Naccobus, Cactodera, Dolichodera, Globodera, Heterodera, Punctodera or Meloidogyne. In a more preferred embodiment the parasitic nematodes belong to one or more genus selected from the group of Globodera, Heterodera, or Meloidogyne. In an even more preferred embodiment the parasitic nematodes belong to one or both genus selected from the group of Globodera or Heterodera. In another embodiment the parasitic nematodes belong to the genus Meloidogyne.

[Para 74] When the parasitic nematodes are of the genus Globodera, the species are preferably from the group consisting of G. achilleae, G. artemisiae, G. hypolysi, G. mexicana, G. millefolii, G. mali, G. pallida, G. rostochiensis, G. tabacum, and G. virginiae. In another preferred embodiment the parasitic Globodera nematodes includes at least one of the species G. pallida, G. tabacum, or G. rostochiensis. When the parasitic nematodes are of the genus Heterodera, the species may be preferably from the group consisting of H. avenae, H. carotae, H. ciceri, H. cruciferae, H. delvii, H. elachista, H. filipjevi, H. gambiensis, H. glycines, H. goettingiana, H. graduni, H. humuli, H. hordecalis, H. latipons, H. major, H. medicaginis, H. oryzicola, H. pakistanensis, H. rosii, H. sacchari, H. schachtii, H. sorghi, H. trifolii, H. urticae, H. vigni and H. zeae. In another preferred embodiment the parasitic Heterodera nematodes include at least one of the species H. glycines, H. avenae, H. cajani, H. gottingiana, H. trifolii, H. zeae or H. schachtii. In a more preferred embodiment the parasitic nematodes includes at least one of the species H. glycines or H. schachtii. In a most preferred embodiment the parasitic nematode is the species H. glycines.

[Para 75] When the parasitic nematodes are of the genus Meloidogyne, the parasitic nematode may be selected from the group consisting of M. acronea, M. arabica, M. arenaria, M. artiellia, M. brevicauda, M. camelliae, M. chitwoodi, M. cofeicola, M. esigua, M. graminicola, M. hapla, M. incognita, M. indica, M. inornata, M. javanica, M. lini, M. mali, M. microcephala, M.

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

[Para 76] While the compositions and methods of this invention have been described in terms of certain embodiments, it will be apparent to those of skilled in the art that variations may be applied to the composition, methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention.

EXAMPLES

Example 1: Cloning a polynucleotide encoding an N-terminal truncated form of a sucrose isomerase

[Para 77] Approximately 0.1 µg of plasmid DNA containing the Erwinia sucrose isomerase AF279281 sequence was used as the DNA template in the PCR reaction. The primers used for PCR amplification of the truncated sucrose isomerase sequence are shown in Table 1 and were designed based on AF279281 sequence. The primer sequences described by SEQ ID NO:12 contains the AscI restriction site for ease of cloning. The primer sequences described by SEQ ID NO:13 contains the XhoI site for ease of cloning. Primer sequences described by SEQ ID NO:12 and SEQ ID NO:13 were used to amplify the truncated sucrose isomerase sequence.

[Para 78] The amplified DNA fragment size for was verified by standard agarose gel electrophoresis and the DNA extracted from gel The purified fragments were TOPO cloned into pCR2.1 using the TOPO TA cloning kit following the manufacturer's instructions (Invitrogen). The cloned fragments were sequenced using an Applied Biosystem 373A (Applied Biosystems, Foster City, California, US) automated sequencer and verified to be the expected sequence by using the sequence alignment ClustalW (European Bioinformatics Institute, Cambridge, UK) from the sequence analysis tool Vector NTI (Invitrogen, Carlsbad, California, USA). The polynucleotide encoding an N-terminal truncated form of the Erwinia sucrose isomerase is described by SEQ ID NO:1. The restriction sites introduced in the primers for facilitating cloning are not included in the sequence.

Table 1: Primers used to amplify polynucleotide of SEQ ID NO:1

Primer name	Sequence	Purpose	SEQ ID NO:

JT28 primer	GGCGCGCCACCATGAAAGAATACGGTACGATGGAAGAC	5' primer	12
JT59 primer	CTCGAGCTACGGATTAAGTTTATAAATGCCCGACTG	3' primer	13

Example 2: Vector construction for transformation

[Para 79] To evaluate the function of the cloned Erwinia polynucleotide encoding an N-terminal truncated form of the sucrose isomerase encoding gene, a gene fragment corresponding to nucleotides of 1-1464 of SEQ ID NO:1 was cloned downstream of a promoter using the restriction enzymes AscI and XhoI to create the expression vectors described in Table 2 operably linked to the described promoter sequences. The syncytia preferred promoters included a soybean MTN3 promoter SEQ ID NO:7 (p-47116125) (USSN 60/899,714), Arabidopsis peroxidase POX promoter SEQ ID NO:8 (p-At5g05340) (USSN 60/876,416), Arabidopsis TPP trehalose-6-phosphate phosphatase promoter SEQ ID NO:9 (p-At1g35910) (USSN 60/874,375), MTN21 promoter SEQ ID NO:10 (p-At1g21890) (USSN 60/743,341), and At5g12170-like promoter SEQ ID NO:11 (USSN 60/899,693). The plant selectable marker in the binary vectors described in Table 2 is a herbicide-resistant form of the acetohydroxy acid synthase (AHAS) gene from Arabidopsis thaliana (Sathasivan et al., Plant Phys. 97:1044-50, 1991). ARSENAL (imazapyr, BASF Corp, Florham Park, NJ) was used as the selection agent.

Table 2. expression vectors comprising SEQ ID NO:1 fragment

vector	Composition of the expression cassette (promoter::NCP encoding gene)
RJT21	Super promoter::SEQ ID NO:1
RJT22	p-At1g21890::SEQ ID NO:1
RJT23	p-47116125::SEQ ID NO:1
RJT51	p-At5g05340::SEQ ID NO:1
RJT52	p-At5g12170::SEQ ID NO:1
RJT53	p-At1g35910::SEQ ID NO:1

Example 3: Generation of transgenic soybean hairy-root and nematode bioassay

[Para 80] Binary vectors RJT21, RJT22, RJT23, RJT51, RJT52, and RJT53 were transformed into A. rhizogenes K599 strain by electroporation. The transformed strains of Agrobacterium were used to induce soybean hairy-root formation using known methods. Non-transgenic hairy roots from soybean cultivar Williams 82 (SCN susceptible) and Jack (SCN resistant) were also generated by using non-transformed A. rhizogenes, to serve as controls for nematode growth in the assay.

[Para 81] A bioassay to assess nematode resistance was performed on the transgenic hairy-root transformed with the vectors and on non-transgenic hairy roots from Williams 82 and Jack as controls. Several independent hairy root lines were generated from each binary vector transformation for bioassay. For each transformation line, several replicated wells were inoculated with SCN according to the procedure outlined above. Four weeks after nematode inoculation, the cyst number in each well was counted and the female index determined. The female index is a relationship where numbers of cysts are compared to the susceptible cultivar Williams82.

[Para 82] For each transformation line, the number of replicated wells (n), the average number of cysts per well (MEAN), and the standard error (SE) values are determined. The results indicate that five of the six constructs tested show a significant reduction in cyst count over multiple transgenic lines. Bioassay results for constructs RJT21, RJT22, RJT23, RJT52, and RJT53 show a statistically significant reduction (p-value <0.05) in cyst count over multiple transgenic lines and a general trend of reduced cyst count in the majority of transgenic lines assayed. Bioassay results for construct RJT51 did not show a noticeable effect on cyst count.

Example 4: Sucrose isomerase assay of SEQ ID NO:1 sucrose isomerase fragment

[Para 83] The N-terminal truncated form of a sucrose isomerase polynucleotide represented by SEQ ID NO:1 is a truncated form of sucrose isomerase from Erwinia rhapontici (accession number AF279281 sequence represented by SEQ ID NO:3). The DNA sequence alignment of SEQ ID NO:1 and SEQ ID NO:3 is shown in Figure 1. The polypeptide (SEQ ID NO:2) encoded by the truncated NCP DNA sequence described by SEQ ID NO:1 results in an N-terminal truncation. The polypeptide described by SEQ ID NO:2 did not have sucrose isomerase activity based on experimental data.

[Para 84] Two sets of experiments (assays A and B below) were performed to demonstrate that the truncated version of the NCP did not function as a sucrose isomerase (i.e. that the truncated protein could not catalyze the isomerization of sucrose into palatinose).

Assay A. Sucrose isomerase activity assay using transgenic soybean roots

[Para 85] Analysis of transgenic soybean roots transformed with RJT51 and RJT53 was done. Sugars were extracted from root samples, and triplicated aliquots were run on HPLC. Control samples consisted of W82, Jack, and W82 supplemented with external palatinose (W82 + palatinose). No palatinose was detected in any of the samples analyzed, with the exception of the positive control (W82 + palatinose).

Assay B. Sucrose isomerase activity assay using E.coli

[Para 86] Constructs containing the full length (SEQ ID NO:3) and truncated (SEQ ID NO:1) Erwinia sucrose isomerase genes for expression in bacteria were generated. In addition, constructs containing full length (SEQ ID NO:6) and truncated (SEQ ID NO:4) Serratia plymuthica sucrose isomerase genes (accession number CQ765997) for expression in bacteria were generated. The amino acid global percent identity of the truncated Serratia sucrose isomerase amino acid sequence described by SEQ ID NO:5 and the truncated Erwinia sucrose isomerase sequence described by SEQ ID NO:2 is shown in Figure 2. The four constructs contained the designated full length and truncated sucrose isomerase genes from Erwinia and Serratia under the control of an IPTG inducible promoter. The four constructs were transformed into E. coli and sucrose isomerase expression was either not induced with IPTG (sample a) or was induced by adding IPTG (sample b). After the addition of IPTG, crude extracts from transgenic bacteria were incubated with 90 mM sucrose. Samples were taken at zero minutes and 60 minutes after addition of sucrose. At the designated time point, the reactions were stopped and an aliquot of the mix was injected into the HPLC to determine sugar content. It was observed that the addition of IPTG did not have a major effect on the experimental outcome, meaning that the IPTG inducible promoter used in this experiment was somewhat active without the addition of IPTG. The result showed that both full-length gene versions (from Erwinia and Serratia) had sucrose isomerase activity, since both produced a significant amount of palatinose after 60 minutes incubation while sucrose was totally depleted. In contrast, both truncated gene forms failed to produce any detectable palatinose under the same experimental conditions, and the sucrose peak remained unchanged. The results are shown in Table 3.

Table 3. HPLC assay to determine sugar content for constructs expressed in E.coli

Sample name	Sucrose (nmol)	Palatinose (nmol)	Trehalulose (nmol)
SRS73-5a T0	1870.0	n.a.	n.a.
SRS73-5a T60	2239.1	n.a.	n.a.
SRS73-5b T0	1918.5	n.a.	n.a.
SRS73-5b T60	2277.7	n.a.	n.a.
SRS74-4a T0	1944.2	239.2	1.7
SRS74-4a T60	17.2	1911.0	189.8
SRS74-4b T0	1186.4	137.2	n.a.
SRS74-4b T60	9.3	3254.6	315.7
SRS75-2a T0	1834.0	n.a.	n.a.
SRS75-2a T60	2024.7	n.a.	n.a.
SRS75-2b T0	1907.3	n.a.	n.a.
SRS75-2b T60	1700.5	n.a.	n.a.
SRS76-3a T0	1952.0	8.2	n.a.
SRS76-3a T60	414.6	1093.8	38.9
SRS76-3b T0	2315.8	10.9	n.a.
SRS76-3b T60	96.8	3238.4	144.0

SRS73-5 NCP truncated Erwinia sucrose isomerase (SEQ ID NO:1)

SRS74-4 full length Erwinia sucrose isomerase (SEQ ID NO:3)

SRS75-2 truncated Serratia sucrose isomerase (SEQ ID NO:4)

SRS76-3 full length Serratia sucrose isomerase (SEQ ID NO:6)

In the table: “a” sample: without IPTG; “b” sample: with IPTG.

Example 5: Additional N-terminal truncated forms of sucrose isomerase polypeptides

[Para 87] As disclosed in Example 3, the truncated version of the Erwinia sucrose isomerase NCP gene described by SEQ ID NO:1 results in reduced cyst count when operably linked with constitutive and nematode-inducible promoters and expressed in soybean roots. As disclosed in Example 4, it has been shown that the truncated version of the Erwinia sucrose isomerase gene does not have sucrose isomerase activity. In addition, a truncated version of a homologous sucrose isomerase gene from Serratia does not have sucrose isomerase activity as shown in Example 4.

[Para 88] The truncated Erwinia sucrose isomerase amino acid sequence described by SEQ ID NO:2 was used to identify homologous genes using the BLAST algorithm. The truncated

versions of several exemplary sucrose isomerase genes homologous to the N-terminal truncated form of the Erwinia sucrose isomerase polypeptide described by SEQ ID NO:2 were identified and are described by SEQ ID NO:5 and SEQ ID NOs 14-20. The described homologs represent a range of homology to the Erwinia truncated sucrose isomerase NCP gene described by SEQ ID NO:2. The amino acid alignment of the identified truncated homologs to the Erwinia truncated sucrose isomerase described by SEQ ID NO:2 is shown in Figure 3. A matrix table showing the percent identity of the identified homologs and SEQ ID NO:2 to each other is shown in Figure 4.

Example 6: Vector construction of homologs

[Para 89] The nucleotide sequences corresponding to the amino acid sequences described by SEQ ID NO:5 and SEQ ID NOs 14-20 encoding truncated versions of genes homologous to the Erwinia truncated sucrose isomerase described by SEQ ID NO:2 is cloned into plant binary vectors. The truncated homolog DNA sequences are described by SEQ ID NO:4 and SEQ ID NOs 21-27. The described nucleotide sequences are operably linked to the nematode inducible promoter p-At1g35910 described by SEQ ID NO:9 using standard cloning techniques. The plant selection marker in the binary vectors results in resistance to the herbicide Imazapyr.

Example 7 Bioassay and cyst count.

[Para 90] A bioassay to assess nematode resistance conferred by homologs of the truncated Erwinia sucrose isomerase of SEQ ID NO:1 is performed using a rooted plant assay system disclosed in commonly owned copending USSN 12/001,234. Transgenic roots are generated after transformation with the binary vectors described in Example 6. Multiple transgenic root lines are sub-cultured and inoculated with surface-decontaminated race 3 SCN second stage juveniles (J2) at the level of about 500 J2/well. Four weeks after nematode inoculation, the cyst number in each well is counted. For each transformation construct, the number of cysts per line is calculated to determine the average cyst count and standard error for the construct. The cyst count values for each transformation construct is compared to the cyst count values of an empty vector control tested in parallel to determine if the construct tested results in a reduction in cyst count.

[Para 91] Those skilled in the art will recognize, or will be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

1. An isolated polynucleotide encoding an N-terminal truncated form of a sucrose isomerase polypeptide that demonstrates anti-nematode activity when transformed into plants, wherein said polypeptide does not demonstrate sucrose isomerase enzymatic activity.

2. The isolated polynucleotide of claim 1, selected from the group consisting of:
   a. a polynucleotide having the sequence as defined in SEQ ID NO: 1, 3, 4, 6, 21, 22, 23, 24, 25, 26 or 27;
   b. a polynucleotide encoding a polypeptide having the sequence as defined in SEQ ID NO: 2, 5, 14, 15, 16, 17, 18, 19 or 20;
   c. a polynucleotide having 70% sequence identity to a polynucleotide having the sequence as defined in SEQ ID NO: 1, 3, 4, 6, 21, 22, 23, 24, 25, 26 or 27;
   d. a polynucleotide encoding a polypeptide having 70% sequence identity to a polypeptide having the sequence as defined in SEQ ID NO: 2, 5, 14, 15, 16, 17, 18, 19 or 20;
   e. a polynucleotide that hybridizes under stringent conditions to a polynucleotide having the sequence as defined in SEQ ID NO: 1, 3, 4, 6, 21, 22, 23, 24, 25, 26 or 27; and
   f. a polynucleotide that hybridizes under stringent conditions to a polynucleotide encoding a polypeptide having the sequence as defined in SEQ ID NO: 2, 5, 14, 15, 16, 17, 18, 19 or 20.

3. The isolated polynucleotide of claim 2, wherein the polynucleotide has the sequence as defined in SEQ ID NO: 1, 3, 4, 6, 21, 22, 23, 24, 25, 26 or 27

4. The isolated polynucleotide of claim 2, wherein the polynucleotide encodes a polypeptide having the sequence as defined in SEQ ID NO: 2, 5, 14, 15, 16, 17, 18, 19 or 20,.

5. A transgenic plant transformed with an expression vector comprising an isolated polynucleotide encoding an N-terminal truncated form of a sucrose isomerase polypeptide that demonstrates anti-nematode activity when transformed into plants, wherein said polypeptide does not demonstrate sucrose isomerase enzymatic activity.

6. The transgenic plant of claim 5, wherein the isolated polynucleotide is selected from the group consisting of:
   a) a polynucleotide having the sequence as defined in SEQ ID NO: 1, 3, 4, 6, 21, 22, 23, 24, 25, 26 or 27;

b) a polynucleotide encoding a polypeptide having the sequence as defined in SEQ ID NO: 2, 5, 14, 15, 16, 17, 18, 19 or 20,;

c) a polynucleotide having 70% sequence identity to a polynucleotide having the sequence as defined in SEQ ID NO: 1, 3, 4, 6, 21, 22, 23, 24, 25, 26 or 27;

d) a polynucleotide encoding a polypeptide having 70% sequence identity to a polypeptide having the sequence as defined in SEQ ID NO: 2, 5, 14, 15, 16, 17, 18, 19 or 20,;

e) a polynucleotide that hybridizes under stringent conditions to a polynucleotide having the sequence as defined in SEQ ID NO: 1, 3, 4, 6, 21, 22, 23, 24, 25, 26 or 27; and

f) a polynucleotide that hybridizes under stringent conditions to a polynucleotide encoding a polypeptide having the sequence as defined in SEQ ID NO: 2, 5, 14, 15, 16, 17, 18, 19 or 20.

7. The plant of claim 6, wherein the polynucleotide has the sequence as defined in SEQ ID NO: 1, 3, 4, 6, 21, 22, 23, 24, 25, 26 or 27.

8. The plant of claim 6, wherein the polynucleotide encodes a polypeptide having the sequence as defined in SEQ ID NO: 2, 5, 14, 15, 16, 17, 18, 19 or 20.

9. The plant of claim 5, further defined as a monocot.

10. The plant of claim 9, wherein the plant is selected from the group consisting of maize, wheat, rice, barley, oat, rye, sorghum, banana, and ryegrass.

11. The plant of claim 5, further defined as a dicot.

12. The plant of claim 11, wherein the plant is selected from the group consisting of pea, alfalfa, soybean, carrot, celery, tomato, potato, cotton, tobacco, pepper, oilseed rape, beet, cabbage, cauliflower, broccoli, lettuce and Arabidopsis thaliana.

13. The plant of claim 12, wherein the plant is soybean.

14. An expression vector comprising a promoter operably linked to a polynucleotide encoding an N-terminal truncated form of a sucrose isomerase polypeptide that demonstrates anti-nematode activity when transformed into plants, wherein said polypeptide does not demonstrate sucrose isomerase enzymatic activity.

15. The expression vector of claim 14, wherein the polynucleotide is selected from the group consisting of:
    a) a polynucleotide having the sequence as defined in SEQ ID NO: 1, 3, 4, 6, 21, 22, 23, 24, 25, 26 or 27;
    b) a polynucleotide encoding a polypeptide having the sequence as defined in SEQ ID NO: 2, 5, 14, 15, 16, 17, 18, 19 or 20,;
    c) a polynucleotide having 70% sequence identity to a polynucleotide having the sequence as defined in SEQ ID NO: 1, 3, 4, 6, 21, 22, 23, 24, 25, 26 or 27;
    d) a polynucleotide encoding a polypeptide having the sequence as defined in SEQ ID NO: 2, 5, 14, 15, 16, 17, 18, 19 or 20;
    e) a polynucleotide that hybridizes under stringent conditions to a polynucleotide having the sequence as defined in SEQ ID NO: 1, 3, 4, 6, 21, 22, 23, 24, 25, 26 or 27; and
    f) a polynucleotide that hybridizes under stringent conditions to a polynucleotide encoding a polypeptide having the sequence as defined in SEQ ID NO: 2, 5, 14, 15, 16, 17, 18, 19 or 20.

16. The expression vector of claim 14, wherein the promoter is selected from the groups consisting of a constitutive promoter, root-specific promoter, and a syncytia-specific promoter.

17. The expression vector of claim 14, wherein the polynucleotide has the sequence as defined in SEQ ID NO: 1, 3, 4, 6, 21, 22, 23, 24, 25, 26 or 27

18. The expression vector of claim 14, wherein the polynucleotide encodes a polypeptide having the sequence as defined in SEQ ID NO: 2, 5, 14, 15, 16, 17, 18, 19 or 20,.

19. A method of producing a transgenic plant having increased nematode resistance, wherein the method comprises the steps of:
    a) introducing into the plant an expression vector comprising a promoter operably linked to a polynucleotide encoding an N-terminal truncated form of a sucrose isomerase polypeptide that demonstrates anti-nematode activity when transformed into plants, wherein said polypeptide does not demonstrate sucrose isomerase enzymatic activity t; and
    b) selecting transgenic plants with increased nematode resistance.

20. The method of claim 19, wherein the polynucleotide is selected form the group consisting of:

a) a polynucleotide having a sequence as defined in SEQ ID NO: 1, 3, 4, 6, 21, 22, 23, 24, 25, 26 or 27;

b) a polynucleotide encoding a polypeptide having the sequence as defined in SEQ ID NO: 2, 5, 14, 15, 16, 17, 18, 19 or 20;

c) a polynucleotide having 70% sequence identity to a polynucleotide having the sequence as defined in SEQ ID NO: 1, 3, 4, 6, 21, 22, 23, 24, 25, 26 or 27;

d) a polynucleotide encoding a polypeptide having 70% sequence identity to a polypeptide having the sequence as defined in SEQ ID NO: 2, 5, 14, 15, 16, 17, 18, 19 or 20

e) a polynucleotide that hybridizes under stringent conditions to a polynucleotide having the sequence as defined in SEQ ID NO: 1, 3, 4, 6, 21, 22, 23, 24, 25, 26 or 27; and

f) a polynucleotide that hybridizes under stringent conditions to a polynucleotide encoding a polypeptide having the sequence as defined in SEQ ID NO: 2, 5, 14, 15, 16, 17, 18, 19 or 20,.

Figure 1a

```
                    1                                                   50
SEQ ID NO:3    (1) ATGTCCTCTCAAGGATTGAAAACGGCTGTCGCTATTTTTCTTGCAACCAC
SEQ ID NO:1    (1) --------------------------------------------------

                   51                                                  100
SEQ ID NO:3   (51) TTTTTCTGCCACATCCTATCAGGCCTGCAGTGCCGGGCCAGATACCGCCC
SEQ ID NO:1    (1) --------------------------------------------------

                  101                                                  150
SEQ ID NO:3 (101) CCTCACTCACCGTTCAGCAATCAAATGCCCTGCCCACATGGTGGAAGCAG
SEQ ID NO:1    (1) --------------------------------------------------

                  151                                                  200
SEQ ID NO:3 (151) GCTGTTTTTTATCAGGTATATCCACGCTCATTTAAAGATACGAATGGGGA
SEQ ID NO:1    (1) --------------------------------------------------

                  201                                                  250
SEQ ID NO:3 (201) TGGCATTGGGGATTTAAACGGTATTATTGAGAATTTAGACTATCTGAAGA
SEQ ID NO:1    (1) --------------------------------------------------

                  251                                                  300
SEQ ID NO:3 (251) AACTGGGTATTGATGCGATTTGGATCAATCCACATTACGATTCGCCGAAT
SEQ ID NO:1    (1) --------------------------------------------------

                  301                                                  350
SEQ ID NO:3 (301) ACGGATAATGGTTATGACATCCGGGATTACCGTAAGATAATGAAAGAATA
SEQ ID NO:1    (1) ----------------------------------------ATGAAAGAATA

                  351                                                  400
SEQ ID NO:3 (351) CGGTACGATGGAAGACTTTGACCGTCTTATTTCAGAAATGAAGAAACGCA
SEQ ID NO:1   (12) CGGTACGATGGAAGACTTTGACCGTCTTATTTCAGAAATGAAGAAACGCA

                  401                                                  450
SEQ ID NO:3 (401) ATATGCGTTTGATGATTGATATTGTTATCAACCACACCAGCGATCAGCAT
SEQ ID NO:1   (62) ATATGCGTTTGATGATTGATATTGTTATCAACCACACCAGCGATCAGCAT

                  451                                                  500
SEQ ID NO:3 (451) GCCTGGTTTGTTCAGAGCAAATCGGGTAAGAACAACCCCTACAGGGACTA
SEQ ID NO:1 (112) GCCTGGTTTGTTCAGAGCAAATCGGGTAAGAACAACCCCTACAGGGACTA

                  501                                                  550
SEQ ID NO:3 (501) TTACTTCTGGCGTGACGGTAAGGATGGCCATGCCCCCAATAACTATCCCT
SEQ ID NO:1 (162) TTACTTCTGGCGTGACGGTAAGGATGGCCATGCCCCCAATAACTATCCCT

                  551                                                  600
SEQ ID NO:3 (551) CCTTCTTCGGTGGCTCAGCCTGGGAAAAAGACGATAAATCAGGCCAGTAT
SEQ ID NO:1 (212) CCTTCTTCGGTGGCTCAGCCTGGGAAAAAGACGATAAATCAGGCCAGTAT

                  601                                                  650
SEQ ID NO:3 (601) TACCTCCATTACTTTGCCAAACAGCAACCCGACCTCAACTGGGACAATCC
SEQ ID NO:1 (262) TACCTCCATTACTTTGCCAAACAGCAACCCGACCTCAACTGGGACAATCC
```

Figure 1b

```
                    651                                                 700
SEQ ID NO:3 (651) CAAAGTCCGTCAAGACCTGTATGACATGCTCCGCTTCTGGTTAGATAAAG
SEQ ID NO:1 (312) CAAAGTCCGTCAAGACCTGTATGACATGCTCCGCTTCTGGTTAGATAAAG

                    701                                                 750
SEQ ID NO:3 (701) GCGTTTCTGGTTTACGCTTTGATACCGTTGCCACCTACTCGAAAATCCCG
SEQ ID NO:1 (362) GCGTTTCTGGTTTACGCTTTGATACCGTTGCCACCTACTCGAAAATCCCG

                    751                                                 800
SEQ ID NO:3 (751) AACTTCCCTGACCTTAGCCAACAGCAGTTAAAAAATTTCGCCGAGGAATA
SEQ ID NO:1 (412) AACTTCCCTGACCTTAGCCAACAGCAGTTAAAAAATTTCGCCGAGGAATA

                    801                                                 850
SEQ ID NO:3 (801) TACTAAAGGTCCTAAAATTCACGACTACGTGAATGAAATGAACAGAGAAG
SEQ ID NO:1 (462) TACTAAAGGTCCTAAAATTCACGACTACGTGAATGAAATGAACAGAGAAG

                    851                                                 900
SEQ ID NO:3 (851) TATTATCCCACTATGATATCGCCACTGCGGGGGAAATATTTGGGGTTCCT
SEQ ID NO:1 (512) TATTATCCCACTATGATATCGCCACTGCGGGGGAAATATTTGGGGTTCCT

                    901                                                 950
SEQ ID NO:3 (901) CTGGATAAATCGATTAAGTTTTTCGATCGCCGTAGAAATGAATTAAATAT
SEQ ID NO:1 (562) CTGGATAAATCGATTAAGTTTTTCGATCGCCGTAGAAATGAATTAAATAT

                    951                                                1000
SEQ ID NO:3 (951) AGCGTTTACGTTTGATCTGATCAGGCTCGATCGTGATGCTGATGAAAGAT
SEQ ID NO:1 (612) AGCGTTTACGTTTGATCTGATCAGGCTCGATCGTGATGCTGATGAAAGAT

                    1001                                               1050
SEQ ID NO:3(1001) GGCGGCGAAAAGACTGGACCCTTTCGCAGTTCCGAAAAATTGTCGATAAG
SEQ ID NO:1 (662) GGCGGCGAAAAGACTGGACCCTTTCGCAGTTCCGAAAAATTGTCGATAAG

                    1051                                               1100
SEQ ID NO:3(1051) GTTGACCAAACGGCAGGAGAGTATGGGTGGAATGCCTTTTTCTTAGACAA
SEQ ID NO:1 (712) GTTGACCAAACGGCAGGAGAGTATGGGTGGAATGCCTTTTTCTTAGACAA

                    1101                                               1150
SEQ ID NO:3(1101) TCACGACAATCCCCGCGCGGTTTCTCACTTTGGTGATGATCGACCACAAT
SEQ ID NO:1 (762) TCACGACAATCCCCGCGCGGTTTCTCACTTTGGTGATGATCGACCACAAT

                    1151                                               1200
SEQ ID NO:3(1151) GGCGCGAGCATGCGGCGAAAGCACTGGCAACATTGACGCTGACCCAGCGT
SEQ ID NO:1 (812) GGCGCGAGCATGCGGCGAAAGCACTGGCAACATTGACGCTGACCCAGCGT

                    1201                                               1250
SEQ ID NO:3(1201) GCAACGCCGTTTATCTATCAGGGTTCAGAACTCGGTATGACCAATTATCC
SEQ ID NO:1 (862) GCAACGCCGTTTATCTATCAGGGTTCAGAACTCGGTATGACCAATTATCC

                    1251                                               1300
SEQ ID NO:3(1251) CTTTAAAAAAATCGATGATTTCGATGATGTAGAGGTGAAAGGTTTTTGGC
SEQ ID NO:1 (912) CTTTAAAAAAATCGATGATTTCGATGATGTAGAGGTGAAAGGTTTTTGGC
```

Figure 1c

```
                    1301                                                1350
SEQ ID NO:3(1301) AAGACTACGTTGAAACAGGCAAAGTGAAAGCTGAGGAATTCCTTCAAAAC
SEQ ID NO:1 (962) AAGACTACGTTGAAACAGGCAAAGTGAAAGCTGAGGAATTCCTTCAAAAC

                    1351                                                1400
SEQ ID NO:3(1351) GTACGCCAAACCAGCCGTGATAACAGCAGAACCCCCTTCCAGTGGGATGC
SEQ ID NO:1(1012) GTACGCCAAACCAGCCGTGATAACAGCAGAACCCCCTTCCAGTGGGATGC

                    1401                                                1450
SEQ ID NO:3(1401) AAGCAAAAACGCGGGCTTTACCAGTGGAACCCCCTGGTTAAAAATCAATC
SEQ ID NO:1(1062) AAGCAAAAACGCGGGCTTTACCAGTGGAACCCCCTGGTTAAAAATCAATC

                    1451                                                1500
SEQ ID NO:3(1451) CCAATTATAAAGAAATCAACAGCGCAGATCAGATTAATAATCCAAATTCC
SEQ ID NO:1(1112) CCAATTATAAAGAAATCAACAGCGCAGATCAGATTAATAATCCAAATTCC

                    1501                                                1550
SEQ ID NO:3(1501) GTATTTAACTATTATAGAAAGCTGATTAACATTCGCCATGACATCCCTGC
SEQ ID NO:1(1162) GTATTTAACTATTATAGAAAGCTGATTAACATTCGCCATGACATCCCTGC

                    1551                                                1600
SEQ ID NO:3(1551) CTTGACCTACGGCAGTTATATTGATTTAGACCCTGACAACAATTCAGTCT
SEQ ID NO:1(1212) CTTGACCTACGGCAGTTATATTGATTTAGACCCTGACAACAATTCAGTCT

                    1601                                                1650
SEQ ID NO:3(1601) ATGCTTACACCCGAACGCTCGGCGCTGAAAAATATCTTGTGGTCATTAAT
SEQ ID NO:1(1262) ATGCTTACACCCGAACGCTCGGCGCTGAAAAATATCTTGTGGTCATTAAT

                    1651                                                1700
SEQ ID NO:3(1651) TTTAAAGAAGAAGTGATGCACTACACCCTGCCCGGGGATTTATCCATCAA
SEQ ID NO:1(1312) TTTAAAGAAGAAGTGATGCACTACACCCTGCCCGGGGATTTATCCATCAA

                    1701                                                1750
SEQ ID NO:3(1701) TAAGGTGATTACTGAAAACAACAGTCACACTATTGTGAATAAAAATGACA
SEQ ID NO:1(1362) TAAGGTGATTACTGAAAACAACAGTCACACTATTGTGAATAAAAATGACA

                    1751                                                1800
SEQ ID NO:3(1751) GGCAACTCCGTCTTGAACCCTGGCAGTCGGGCATTTATAAACTTAATCCG
SEQ ID NO:1(1412) GGCAACTCCGTCTTGAACCCTGGCAGTCGGGCATTTATAAACTTAATCCG

                    1801
SEQ ID NO:3(1801) TAG
SEQ ID NO:1(1462) TAG
```

Figure 2

Query (NCP)	Hit	Query Length	Hit Length	Hit gene name and source	Global PID* over query length
SEQ ID NO:2	SEQ ID NO:5	487	487	Truncated protein of Serratia plymuthica sucrose isomerase	83.41

*PID = percent identity

Figure 3a Amino Acid alignment of truncated homologs of SEQ ID NO:2

```
                        1                                                  50
SEQ ID NO:14      (1) MKEYGTMEDFDSLVAEMKKRNMRLMIDVVINHTSDQHPWFIQSKSDKNNP
SEQ ID NO:15      (1) MKEYGTMEDFDNLVAEMKKRNMRLMIDVVINHTSDQHPWFIQSKSDKNNP
 SEQ ID NO:5      (1) MKEYGTMEDFDRLISEMKKRNMRLMIDVVINHTSDQNEWFVKSKSSKDNP
 SEQ ID NO:2      (1) MKEYGTMEDFDRLISEMKKRNMRLMIDIVINHTSDQHAWFVQSKSGKNNP
SEQ ID NO:16      (1) MKEYGSMADFDRLVAEMNKRGMRLMIDIVINHTSDRHRWFVQSRSGKDNP
SEQ ID NO:17      (1) MKEYGTMEDFDRLMAELKKRGMRLMVDVVINHSSDQHEWFKSSRASKDNP
SEQ ID NO:18      (1) MKEYGTMDDFDRLIAEMKKRDMRLMIDVVVNHTSDEHEWFVESKKSKDNP
SEQ ID NO:19      (1) MSEFGDMDDFERLLAGMNKRGMRLIIDLVVNHSSDEHRWFVESRRSKDNP
SEQ ID NO:20      (1) MTQFGTMADFDAMLAGMTARGMRLIIDLVVNHSSDEHAWFVKSRKGRENP

                        51                                                 100
SEQ ID NO:14     (51) YRDYYFWRDGKDNQPPNNYPSFFGGSAWQKDAKSGQYYLHYFARQQPDLN
SEQ ID NO:15     (51) YRDYYFWRDGKDNQPPNNYPSFFGGSAWQKDAKSGQYYLHYFARQQPDLN
 SEQ ID NO:5     (51) YRGYYFWKDAKEGQAPNNYPSFFGGSAWQKDEKTNQYYLHYFAKQQPDLN
 SEQ ID NO:2     (51) YRDYYFWRDGKDGHAPNNYPSFFGGSAWEKDDKSGQYYLHYFAKQQPDLN
SEQ ID NO:16     (51) YRDYYFWRDGKQGQAPNNYPSFFGGSAWQLDKQTDQYYLHYFAPQQPDLN
SEQ ID NO:17     (51) YRDYYFWRDGKDGHEPNNYPSFFGGSAWEKDPVTGQYYLHYFGRQQPDLN
SEQ ID NO:18     (51) YRDYYIWRDGKDGTQPNNYPSFFGGSAWQKDNATQQYYLHYFGVQQPDLN
SEQ ID NO:19     (51) YRDYYTWRDGKDGAAPNNYPSFFGGSAWKKDEATGQYYLHYFAGKQPDLN
SEQ ID NO:20     (51) YRDYYIWRDGKDGGPPNNYSAFFGGPAWTFDAVTDQYYLHYFAAKQPDLN

                        101                                                150
SEQ ID NO:14    (101) WDNPKVREDLYAMLRFWLDKGVSGMRFDTVATYSKIPGFPNLTPEQQKNF
SEQ ID NO:15    (101) WDNPKVREDLYAMLRFWLDKGVSSMRFDTVATYSKIPGFPNLTPEQQKNF
 SEQ ID NO:5    (101) WDNPKVRQDLYAMLRFWLDKGVSGLRFDTVATYSKIPDFPNLTQQQLKNF
 SEQ ID NO:2    (101) WDNPKVRQDLYDMLRFWLDKGVSGLRFDTVATYSKIPNFPDLSQQQLKNF
SEQ ID NO:16    (101) WDNPKVRAELYDILRFWLDKGVSGLRFDTVATFSKIPGFPDLSKAQLKNF
SEQ ID NO:17    (101) WDTPKLREELYAMLRFWLDKGVSGMRFDTVATYSKTPGFPDLTPEQMKNF
SEQ ID NO:18    (101) WDNPKVREEVYDMLRFWIDKGVSGLRMDTVATFSKNPAFPDLTPKQLQNF
SEQ ID NO:19    (101) WENPEVRAEVHDIMRFWLDKGVSGFRMDVIPFISKQDGLPDLPAQALAHP
SEQ ID NO:20    (101) WENPKVRAEVHDLMRFWLDKGVSGFRMDVIPFISKPPGLPDLTPQERRAP

                        151                                                200
SEQ ID NO:14    (151) AEQYTMGPNIHRYIQEMNRKVLSRYDVATAGEIFGVPLDRSSQFFDRRRH
SEQ ID NO:15    (151) AEQYTMGPNIHRYIQEMNRKVLSRYDVATAGEIFGVPLDRSSQFFDPRRH
 SEQ ID NO:5    (151) AAEYTKGPNIHRYVNEMNREVLSHYDIATAGEIFGVPLDQSIKFFDRRRD
 SEQ ID NO:2    (151) AEEYTKGPKIHDYVNEMNREVLSHYDIATAGEIFGVPLDKSIKFFDRRRN
SEQ ID NO:16    (151) AEAYTEGPNIHKYIHEMNRQVLSKYNVATAGEIFGVPVSAMPDYFDRRRE
SEQ ID NO:17    (151) AEAYTQGPNLHRYLQEMHEKVFDHYDAVTAGEIFGAPLNQVPLFIDSRRK
SEQ ID NO:18    (151) AYTYTQGPNLHRYIQEMHQKVLAKYDVVSAGEIFGVPLEEAAPFIDQRRK
SEQ ID NO:19    (151) EFVYANGPRIHEYLQEMNREVLSRYDTMTVGEAFGITFEQAPLFTDARRH
SEQ ID NO:20    (151) QFVYAADPKLHDYLREMRREVLDHYDTMTVGEAFGVTPDAARDLIDSRRG

                        201                                                250
SEQ ID NO:14    (201) ELNMAFMFDLIRLDRDSNERWRHKSWSLSQFRQIISKMDVTVGKYGWNTF
SEQ ID NO:15    (201) ELNMAFMFDLIRLDRDSNERWRHKSWSLSQFRQIISKMDVTVGKYGWNTF
 SEQ ID NO:5    (201) ELNIAFTFDLIRLDRDSDQRWRRKEWKLSQFRQVIDNVDRTAGEYGWNAF
 SEQ ID NO:2    (201) ELNIAFTFDLIRLDRDADERWRRKDWTLSQFRKIVDKVDQTAGEYGWNAF
SEQ ID NO:16    (201) ELNIAFTFDLIRLDRYPDQRWRRKPWTLSQFRQVISQTDRAAGEFGWNAF
SEQ ID NO:17    (201) ELDMAFTFDLIRYDRALD-RWHTIPRTLADFRQTIDKVDAIAGEYGWNTF
SEQ ID NO:18    (201) ELDMAFSFDLIRLDRAVEERWRRNDWTLSQFRQIINNRLVDMAGQHGWNTF
SEQ ID NO:19    (201) ELNMIFHFDLVRLDRDG---WRKKDWTLPELKATYARIDRTGGDHGWNTS
SEQ ID NO:20    (201) ELDLVFNFDIVRMDIDG---WRKTSWTLPRLKALYTQLDQAAGPFGWNTQ
```

Figure 3b

```
                    251                                                 300
SEQ ID NO:14  (251) FLDNHDNPRAVSHFGDDRPQWREASAKALATITLTQRATPFIYQGSELGM
SEQ ID NO:15  (251) FLDNHDNPRAVSHFGDDRPQWREASAKALATITLTQRATPFIYQGSELGM
 SEQ ID NO:5  (251) FLDNHDNPRAVSHFGDDRPQWREPSAKALATLTLTQRATPFIYQGSELGM
 SEQ ID NO:2  (251) FLDNHDNPRAVSHFGDDRPQWREHAAKALATLTLTQRATPFIYQGSELGM
SEQ ID NO:16  (251) FLDNHDNPRQVSHFGDDSPQWRERSAKALATLLLTQRATPFIFQGAELGM
SEQ ID NO:17  (250) FLGNHDNPRAVSHFGDDRPQWREASAKALATVTLTQRGTPFIFQGDELGM
SEQ ID NO:18  (251) FLSNHDNPRAVSHFGDDRPEWRTRSAKALATLALTQRATPFIYQGDELGM
SEQ ID NO:19  (248) FLGNHDNPRAVSHFGDDSPEWRAASAKALATMMLTQRATPFLYQGDELGM
SEQ ID NO:20  (248) FLSNHDNPRSVSHFGDDDPAWVERSAKVLATLILTQRGTPFLYQGEELGM

                    301                                                 350
SEQ ID NO:14  (301) TNYPFRQLNEFDDIEVKGFWQDYVQSGKVTATEFLDNVRLTSRDNSRTPF
SEQ ID NO:15  (301) TNYPFRQLNEFDDIEVKGFWQDYVQSGKVTATEFLDNVRLTSRDNSRTPF
 SEQ ID NO:5  (301) TNYPFKAIDEFDDIEVKGFWHDYVETGKVKADEFLQNVRLTSRDNSRTPF
 SEQ ID NO:2  (301) TNYPFKKIDDFDDVEVKGFWQDYVETGKVKAEEFLQNVRQTSRDNSRTPF
SEQ ID NO:16  (301) TNYPFKNIEEFDDIEVKGFWNDYVASGKVNAAEFLQEVRMTSRDNSRTPM
SEQ ID NO:17  (300) TNYPFKTLQDFDDIEVKGFFQDYVETGKATAEELLTNVALTSRDNARTPF
SEQ ID NO:18  (301) TNYPFTSLSEFDDIEVKGFWQDFVETGKVKPDVFLENVKQTSRDNSRTPF
SEQ ID NO:19  (298) TNYPFRGLEDYDDVEVKGQWRDFVESGKVSADEYLAHLRQTSRDNARTPM
SEQ ID NO:20  (298) TNYPFQTLDDFDDLEVAGRWRDVKHR--VSEEEYLANARAMGRDNSRTPM

                    351                                                 400
SEQ ID NO:14  (351) QWNDTLNAGFTRGKPWFHINPNYVEINAEREETREDSVLNYYKKMIQLRH
SEQ ID NO:15  (351) QWNDTLNAGFTRGKPWFHINPNYVEINAEREETREDSVLNYYKKMIQLRH
 SEQ ID NO:5  (351) QWDTSKNAGFTSGKPWFKVNPNYQEINAVSQVAQPDSVFNYYRQLIKIRH
 SEQ ID NO:2  (351) QWDASKNAGFTSGTPWLKINPNYKEINSADQINNPNSVFNYYRKLINIRH
SEQ ID NO:16  (351) QWNDSVNAGFTQGKPWFHLNPNYKQINAAREVNKPDSVFSYYRQLINLRH
SEQ ID NO:17  (350) QWDDSANAGFTTGKPWLKVNPNYTEINAAREIGDPKSVYSFYRNLISIRH
SEQ ID NO:18  (351) QWSNTAQAGFTTGTPWFRINPNYKNINAEEQTQNPDSIFHFYRQLIELRH
SEQ ID NO:19  (348) QWSDAPNGGFTTGKPWLAVNPNYPQVNAASQVDDPGSIYHHYRRLLEVRR
SEQ ID NO:20  (346) QWTGDPHGGFTTGKPWLAVNPNAATINAQDQAARPDSVLTHCRALIAWRR

                    401                                                 450
SEQ ID NO:14  (401) HIPALVYGAYQDLNPQDNTVYAYTRTLGNERYLVVVNFKEYPVRYTLPAN
SEQ ID NO:15  (401) HIPALVYGAYQDLNPQDNTVYAYTRTLGNERYLVVVNFKEYPVRYTLPAN
 SEQ ID NO:5  (401) NIPALTYGTYTDLDPANDSVYAYTRSLGAEKYLVVVNFQEQVMRYKLPDN
 SEQ ID NO:2  (401) DIPALTYGSYIDLDPDNNSVYAYTRTLGAEKYLVVINFKEEVMHYTLPGD
SEQ ID NO:16  (401) QIPALTSGEYRDLDPQNNQVYAYTRILDNEKYLVVVNFKPEQLHYALPDN
SEQ ID NO:17  (400) ETPALSTGSYRDIDPSNADVYAYTRSQDGETYLVVVNFKAEPRSFTLPDG
SEQ ID NO:18  (401) ATPAFTYGTYQDLDPNNNEVLAYTRELNQQRYLVVVNFKEKPVHYVLPKT
SEQ ID NO:19  (398) QTPALIHGQFRDLDPANPKVFAYTRTLDDKRYLVLINFTRETVAYDLPEG
SEQ ID NO:20  (396) GSVDLREGDYRDIDPDHPQVFAYRR---GEGLLVLLNFGRETVRYALPEG

                    451                                 489
SEQ ID NO:14  (451) DAIEEVVIDT--QQQAAAPHSTSLSLSPWQAGVYKLR--
SEQ ID NO:15  (451) DAIEEVVIDT--QQQATAPHSTSLSLSPWQAGVYKLR--
 SEQ ID NO:5  (451) LSIEKVIIESN-SKNVVKKNDSLLELKPWQSGVYKLNQ-
 SEQ ID NO:2  (451) LSINKVITENN-SHTIVNKNDRQLRLEPWQSGIYKLNP-
SEQ ID NO:16  (451) LTIASSLLENV-HQPSLQENASTLTLAPWQAGIYKLN--
SEQ ID NO:17  (450) MHIAETLIESS-SPAAPAAGAASLELQPWQSGIYKVK--
SEQ ID NO:18  (451) LSIKQSLLESG-QKDKVEPNATTLELQPWQSGIYQLN--
SEQ ID NO:19  (448) LKIAATLLDNGAAQESMQPGAASVTLQPWQATIYRL---
SEQ ID NO:20  (443) LAIESAAFG------AVEIAGRVVALTGWSFVILTVRDR
```

Figure 4: Matrix table of truncated sucrose isomerase homologs

	SEQ ID NO:14	SEQ ID NO:15	SEQ ID NO:16	SEQ ID NO:17	SEQ ID NO:18	SEQ ID NO:19	SEQ ID NO:2	SEQ ID NO:20	SEQ ID NO:5
SEQ ID NO:14	100	99.2	70.6	68.3	67.5	55.9	73.1	49.5	74.3
SEQ ID NO:15	99.2	100	70.2	67.9	67.3	55.6	72.7	49.3	73.9
SEQ ID NO:16	70.6	70.2	100	66	67.1	60.2	73.1	51.6	73.3
SEQ ID NO:17	68.3	67.9	66	100	66.5	57.8	68	51.5	68.2
SEQ ID NO:18	67.5	67.3	67.1	66.5	100	59.3	68.2	51.2	68.6
SEQ ID NO:19	55.9	55.6	60.2	57.8	59.3	100	57	61.5	55.7
SEQ ID NO:2	73.1	72.7	73.1	68	68.2	57	100	51.6	83.4
SEQ ID NO:20	49.5	49.3	51.6	51.5	51.2	61.5	51.6	100	49.6
SEQ ID NO:5	74.3	73.9	73.3	68.2	68.6	55.7	83.4	49.6	100

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2008/051382

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/82 C12N9/90

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 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 practical, search terms used)
EPO-Internal, WPI Data, MEDLINE, BIOSIS, EMBASE, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 786 140 A (MATTES RALF [DE] ET AL) 28 July 1998 (1998-07-28) the whole document	1,2
A	WO 2004/005504 A (SUNGENE GMBH & CO KGAA [DE]; SONNEWALD UWE [DE]; BOERNKE FREDERIK [DE]) 15 January 2004 (2004-01-15) cited in the application the whole document	1-20

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

☒ See patent family annex.

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

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Date of the actual completion of the international search	Date of mailing of the international search report
28 May 2008	09/06/2008

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2008/051382

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