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(54) Title: METHODS AND COMPOSITIONS FOR PLANT PEST CONTROL

(57) Abstract: The present invention is directed to controlling nematode infestation. The invention discloses methods and compositions for use in controlling nematode infestation by providing recombinant DNA molecules to the cells of a plant in order to achieve a reduction in nematode infestation. The invention is also directed to methods for making transgenic plants that express the recombinant DNA molecule for use in protecting plants from nematode infestation.



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METHODS AND COMPOSITIONS FOR PLANT PEST CONTROL

CROSS REFERENCE TO RELATED APPLICATIONS

- 5 This application claims the priority of U.S. Provisional Application Serial No. 61/027,473, filed February 10, 2008, the entire disclosure of which is incorporated herein by reference.

INCORPORATION-BY-REFERENCE OF SEQUENCE LISTING IN COMPUTER READABLE FORM

- 10 The Sequence Listing, which is a part of the present disclosure, includes a computer readable form 96 KB file entitled "MNDI005WOsequence" comprising nucleotide sequences of the present invention submitted via EFS-Web. The subject matter of the Sequence Listing is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

15 1. Field Of The Invention

The present invention relates to methods and compositions for pest or pathogen control in plants. More particularly, it discloses transgenic plant cells, plants and seeds comprising recombinant DNA and methods of making and using such plant cells, plants and seeds that are associated with pest resistance.

20 2. Description of Related Art

Plants and animals are targets for infection by many nematode pests. Improved methods for protecting plants from nematode infection are therefore desired since they would increase the amount and stability of food production.

- 25 There are numerous plant-parasitic nematode species, including various cyst nematodes (*e.g. Heterodera spp.*), root knot nematodes (*e.g. Meloidogyne spp.*), lesion nematodes (*e.g. Pratylenchus spp.*), dagger nematodes (*e.g. Xiphinema spp.*) and stem and bulb nematodes (*e.g. Ditylenchus spp.*), among others. Tylenchid nematodes (members of the order Tylenchida), including the families Heteroderidae, Meloidogynidae, and Pratylenchidae, are the largest and most economically important group of plant-parasitic nematodes. Other important plant-parasitic nematodes include Dorylaimid nematodes (*e.g. Xiphinema spp.*), among others. Nematode
- 30

species grow through a series of lifecycle stages and molts. Typically, there are five stages and four molts: egg stage; J1 (*i.e.* first juvenile stage); M1 (*i.e.* first molt); J2 (second juvenile stage; sometimes hatch from egg); M2; J3; M3; J4; M4; A (adult). Juvenile (“J”) stages are also sometimes referred to as larval (“L”) stages. Gene expression may be specific to one or more lifecycle stages. Nematodes have evolved as very successful parasites of both plants and animals and are responsible for significant economic losses in agriculture and livestock and for morbidity and mortality in humans. Nematode parasites of plants can inhabit all parts of plants, including roots, developing flower buds, leaves, and stems. Plant parasites are classified on the basis of their feeding habits into the broad categories migratory ectoparasites, migratory endoparasites, and sedentary endoparasites. Sedentary endoparasites, which include the root knot nematodes (*Meloidogyne* species, RKN), cyst nematodes (*Globodera* and *Heterodera* species) and reniform nematodes (*Rotylenchulus* species) induce feeding sites and establish long-term infections within roots that are often very damaging to crops. Nematode infection is a significant problem in the farming of many agriculturally significant crops. For example, soybean cyst nematode (*Heterodera glycines*, SCN) is believed to be responsible for yield losses in soybeans estimated to be in excess of \$1 billion per year in North America. Such damage is the result of the stunting of the soybean plant caused by the cyst nematode. The stunted plants have smaller root systems, show symptoms of mineral deficiencies in their leaves, and wilt easily. It is estimated that parasitic nematodes cost the horticulture and agriculture industries in excess of \$78 billion worldwide a year, based on an estimated average 12 percent annual loss spread across all major crops.

Traditional approaches for control of plant diseases have been the use of chemical treatment and the construction of interspecific hybrids between resistant crops and their wild-type relatives as sources of resistant germplasm. Chemical nematode control agents are not effective in eradicating nematode infestations. Because of the lack of selectivity, the chemical nematode control agents exert their effects on non-target fauna as well, often effectively sterilizing a field for a period of time following the application of nematode control agents. Nematicides such as Aldicarb and its environmental breakdown products are known to be highly toxic to mammals. As a result, government restrictions have been imposed on the use of these chemicals. The most widely used nematicide, methyl bromide, is scheduled to be

soon retired from use, and at present, there is no promising candidate to replace this treatment.

Methods employing plant biotechnology have provided effective means to control insect infestations, for instance through plant expression of an insect control agent. Biotechnologically-related nematode control agents have generally been reported to be nucleotides expressed by a plant that are selectively toxic to the target nematode when ingested by the nematode. However, there are few examples of effectively applied biotechnology methods to control nematode infection.

10 SUMMARY OF THE INVENTION

In one aspect, the invention provides agents effective as a plant nematode control agent. The effective compounds are, in one embodiment, methylketones not previously known to be toxic to plant parasitic nematodes. Additionally, the inventors have developed compositions and methods to express methylketones, such as 2-undecanone, 2-tridecanone and 2-pentadecanone, in the roots of plants that nematodes infect, to reduce or inhibit nematode growth, development, or the plant disease caused by nematode infection. In particular embodiments the method comprises production of transgenic plants containing one or more transgenes that provide for the production of 2-undecanone, 2-tridecanone and/or 2-pentadecanone in plant tissues susceptible to nematode infection.

In another aspect, the invention provides methods for construction and use of a transgene expression cassette comprising a methylketone synthase coding region and expression of the synthase in a plant cell, particularly the root cells of a plant. The invention provides for a transgenic plant comprising the transgene wherein the roots of the transgenic plant produce a methylketone. The methylketone synthase transgene, in certain embodiments, additionally comprises a sequence region comprising a heterologous plastid transit peptide molecule in operable linkage to the methylketone synthase coding region. By "heterologous" it is meant that a given sequence is not in its native context with respect to any other referenced sequence. Thus one sequence may be heterologous with respect to second, operably linked, sequence where both sequences can be isolated from the same species, but will be not be in their native orientation. A heterologous transit peptide operably linked to a given methylketone synthase coding region is therefore not a transit peptide normally

found in nature in an unmodified state in operable linkage to the methylketone synthase coding region.

In yet another aspect of the invention, modified DNA coding sequences comprising SEQ ID NO: 1 or 2 are provided that encode a methylketone synthase of
5 SEQ ID NO: 3; SEQ ID NO: 4 is provided encoding the methylketone synthase of SEQ ID NO: 5; and SEQ ID NO: 6 is provided encoding the methylketone synthase SEQ ID NO: 7. In certain embodiments, the DNA coding sequence encoding a polypeptide with methylketone synthase activity shares at least about 80%, 85%, 90%, 95%, 98%, or 99% percent sequence identity to any one or more of said SEQ ID
10 NOs.

In still yet another aspect of the invention, a heterologous fusion protein is provided that comprises a plastid transit peptide molecule (such as SEQ ID NO: 9 or 11) and a methylketone synthase molecule (such as SEQ ID NO: 13, 15, 17, 19, 21, 23, 25 or 27) or methylketone synthase molecule variant (such as SEQ ID NO: 29, 31,
15 33, 35, 37, or 39) with methylketone synthase activity, or a methylketone synthase molecule having at least about 80%, 85%, 90%, 95%, 98%, or 99% percent sequence identity to any one or more of said SEQ ID NOs.

In still yet another aspect of the invention, a transgene expression cassette is provided comprising a heterologous acyl carrier protein coding region that encodes
20 for an acyl carrier protein (such as SEQ ID NO: 41, 43, 45, 47, 49, 51, 53, 55, or 57) that is expressed in plant tissues with the transgene comprising the methylketone synthase coding region.

In still yet another aspect of the invention, a transgenic seed is provided comprising a heterologous plastid transit peptide molecule in operable linkage to the
25 methylketone synthase coding region. The transgenic seed may additionally comprise a transgene expression cassette comprising a heterologous acyl carrier protein coding region.

Other aspects of the invention are specifically directed to transgenic plant cells, and transgenic plants comprising a plurality of the plant cells, nuclei and
30 organelles, and progeny transgenic seed, embryo, ovule and transgenic pollen from such plants. A plant cell and parts thereof is selected from a population of transgenic plant cells transformed with a heterologous methylketone synthase coding region and may additionally comprise a heterologous acyl carrier protein coding region by selecting the transgenic plant cell from any population comprising the heterologous

coding region as compared to a cell that does not have the heterologous coding region.

This invention also provides methods for manufacturing non-natural, transgenic seed that can be used to produce a crop of transgenic plants with pest
5 resistance resulting from expression of a heterologous methylketone synthase coding region and in certain embodiments the co-expression of a heterologous acyl carrier protein coding region in the nucleus or organelle or cytoplasm of the plant cells making up the transgenic plants. The various aspects of this invention are especially useful for transgenic plants having nematode resistance activity that include, without
10 limitation, cereals including corn, wheat, barley, rye, and rice; vegetables; tomatoes; potatoes; clovers; legumes including beans, soybeans, peas and alfalfa; sugar cane; sugar beets; tobacco; cotton; rapeseed (canola); sunflower; safflower; and sorghum.

The present invention provides for a transgenic soybean plant comprising within its genome a heterologous methylketone synthase coding region and may
15 additionally comprise a heterologous acyl carrier protein coding region, wherein the plant is resistant to nematode infection or displays reduced disease symptoms caused by nematode infection.

The present invention further provides a method of increasing the yield of a nematode tolerant crop plant. The method comprises growing a crop plant
20 comprising a heterologous methylketone synthase coding region which may additionally comprise a heterologous acyl carrier protein coding region in the presence of nematodes.

Another aspect of the invention provides a method of producing a hybrid seed comprising acquiring hybrid seed from a nematode tolerant plant which also has a
25 stably-integrated heterologous nucleotide sequence encoding a methylketone synthase and may also have integrated a heterologous nucleotide sequence encoding an acyl carrier protein. The method further comprises producing a crop from plants grown from the hybrid seed, wherein a fraction of the plants produced from said hybrid seed are homozygous for the heterologous methylketone synthase coding sequence and if
30 present, the heterologous acyl carrier protein coding sequence, a fraction of the plants produced from said hybrid seed are hemizygous for the heterologous methylketone synthase coding sequence and if present, the heterologous acyl carrier protein coding sequence, and a fraction of the plants produced from the hybrid have no heterologous methylketone synthase coding sequence or heterologous acyl carrier protein coding

sequence; selecting plants which are homozygous and hemizygous; collecting seed from the selected plants, and planting the seed to produce further progeny plants; repeating the selecting and collecting steps at least once from these progeny plants to produce an inbred line; and crossing the inbred line with a second line to produce
5 hybrid seed. The plants of the invention are selected, without limitation, from the group of corn (maize), soybean, cotton, canola (rape), wheat, sunflower, sorghum, alfalfa, barley, millet, rice, tobacco, tomato, potato, fruit and vegetable crops, turfgrass, sugar cane, sugar beets, and safflower.

In a further aspect of the invention, control of agronomically important soil
10 inhabiting insects is contemplated, which include, but are not limited to *Diabrotica*, *Diaprepes*, *Pachnaeus*, *Asynonychus*, *Lycoriella*, *Sciara*, *Stenophylus*, and *Bradysia* among others. Broader acaricidal, insecticidal, and pest repellent properties are also contemplated.

DETAILED DESCRIPTION OF THE INVENTION

15 The invention relates to methods and compositions for pest control in plants, in particular nematode control. In one aspect, the invention relates to controlling, preventing or treating nematode infection in transgenic plants. The method comprises, in one embodiment, generation of transgenic plant containing a recombinant construct and expression of such construct to impart nematode resistance
20 to plants. The recombinant construct may comprise a nucleotide sequence encoding one or more proteins, wherein the sequence is operably linked to a heterologous promoter functional in a plant cell, and to cells transformed with the recombinant construct. Cells comprising (meaning including but not limited to) the recombinant construct may be prokaryotic or eukaryotic. In particular, they may be plant cells.
25 Plants and seeds derived from such transformed plant cells are also contemplated. The transgenic plants or parts thereof of the present invention, in one embodiment, produce one or more fatty acid compounds for which at least one is 2-tridecanone. 2-tridecanone is the major methylketone (76% of total volatile content) produced in *Lycopersicon hirsutum* (*Solanum habrochaites*) (compared to 21% 2-undecanone, and
30 3% 2-pentadecanone; Fridman, *et al.*, *Plant Cell* 17:1252-67, 2005). Higher plants synthesize fatty acids via a metabolic pathway involving an acyl carrier protein co-factor (ACP) and a fatty acid synthase (FAS) enzyme complex. The FAS complex

consists of about eight separate enzymes that catalyze thirty or more individual reaction steps, all of which, in plants, are located in the plastids.

The present invention provides heterologous molecules that are directed into the plastid of a plant to provide production of a methylketone, especially 2-tridecanone, from the FAS complex, including, but not limited to, nucleotides that encode polypeptides having methylketone synthase activity such as SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, or the amino acid sequence given in GenBank Accession AY701574. In certain embodiments, the polypeptide having methylketone synthase activity (*e.g.* allowing for production of methylketones such as 2-undecanone, 2-tridecanone, and 2-pentadecanone) may share at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity, to any one or more amino acid sequence(s) set forth in SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, or SEQ ID NO:39. The function of the encoded polypeptide may also be determined by measuring the efficacy of the presence of the transgene that encodes it in reducing nematode infection, growth, reproduction, or symptomatology. For instance, a reduction in root galls, cysts, or worm number of 20% or more, 25% or more, 50% or more, 80% or more, or 95% or more, in a transgenic plant comprising a heterologous nucleotide construct encoding methylketone synthase activity, relative to a control plant, for instance an otherwise isogenic plant not comprising the heterologous molecule, under similar conditions, indicates the presence of a functional molecule.

In certain embodiments, a heterologous molecule provided by the present invention that is directed into the plastid of a plant to provide production of a methylketone may share at least 80%, at least 85%, at least 90%, at least 95%, at least 98%, at least 99%, or 100% sequence identity at the nucleotide level with one or more sequence(s) as set forth in SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:34, SEQ ID NO:36, or SEQ ID NO:38; or any of SEQ ID NOs:58-61. Thus, in particular embodiments, the heterologous molecule may comprise a sequence encoding a heterologous chloroplast

transit peptide, for instance, without limitation, as shown in SEQ ID NO:9 or SEQ ID NO:11.

Likewise, in certain embodiments, a nucleotide of the present invention may further comprise a sequence that encodes an acyl carrier protein (*e.g.* ACP1), as set forth in any of SEQ ID NO:41, SEQ ID NO:43, SEQ ID NO:45, SEQ ID NO:47, SEQ ID NO:49, SEQ ID NO:51, SEQ ID NO:53, SEQ ID NO:55, or SEQ ID NO:57, or may comprise a sequence that encodes an acyl carrier protein with at least about 85%, 90%, 95%, 98%, or 99% sequence similarity to any of these sequences.

Yet another aspect of the invention provides methods for production and for use of one or more methylketone(s), such as 2-tridecanone, to control nematode infestation. Thus, methods for production of a methylketone, for instance in a plant cell, are provided. The methylketone may then be applied to soil prior to, during, or subsequent to planting of a crop, in order to control or reduce nematode infestation or symptomatology of crop plants grown in that soil.

Unless otherwise noted, terms are to be understood according to conventional usage by those of ordinary skill in the relevant art. Definitions of common terms in molecular biology may also be found in Rieger *et al.*, *Glossary of Genetics: Classical and Molecular*, 5th edition, Springer-Verlag: New York, 1991; and Lewin, *Genes V*, Oxford University Press: New York, 1994. The nomenclature for DNA bases as set forth at Title 37 of the United States Code of Federal Regulations, Part 1, section 1.822.

As used herein, a “transgenic plant” is any plant in which one or more, or all, of the cells of the plant include a transgene. A transgene may be integrated within a nuclear genome or organelle genome, or it may be extra-chromosomally replicating DNA. The term “transgene” means a nucleic acid that is partly or entirely heterologous, foreign, to a transgenic microbe, plant, animal, or cell into which it is introduced. Cells that make up various cell and tissue types of plants include but are not limited to seed, root, leaf, shoot, flower, pollen and ovule.

“Recombinant DNA” is a polynucleotide having a genetically engineered modification introduced through combination of endogenous and/or exogenous molecules in a transcription unit, manipulation via mutagenesis, restriction enzymes, and the like or simply by inserting multiple copies of a native transcription unit. Recombinant DNA may comprise DNA segments obtained from different sources, or DNA segments obtained from the same source, but which have been manipulated to

join DNA segments which do not naturally exist in the joined form. An isolated recombinant polynucleotide may exist, for example as a purified molecule, or integrated into a genome, such as a plant cell, or organelle genome or a microbe plasmid or genome. The polynucleotide comprises linked regulatory molecules that
5 cause transcription of an RNA in a plant cell.

As used herein, “percent identity” means the extent to which two optimally aligned DNA or protein segments are invariant throughout a window of alignment of components, for example nucleotide sequence or amino acid sequence. An “identity fraction” for aligned segments of a test sequence and a reference sequence is the
10 number of identical components that are shared by sequences of the two aligned segments divided by the total number of sequence components in the reference segment over a window of alignment which is the smaller of the full test sequence or the full reference sequence. “Percent identity” (“% identity”) is the identity fraction times 100.

15 “Expression” means transcription of DNA to produce RNA. The resulting RNA may be without limitation mRNA encoding a protein, antisense RNA, or a double-stranded RNA for use in RNAi technology. Expression also may refer to translation of RNA, *i.e.* the production of encoded protein from an mRNA.

As used herein, “promoter” means regulatory DNA molecules for initializing
20 transcription. A “plant promoter” is a promoter capable of initiating transcription in plant cells whether or not its origin is a plant cell. For example it is well known that certain *Agrobacterium* promoters are functional in plant cells. Thus, plant promoters include promoter DNA obtained from plants, plant viruses (in particular, double stranded DNA viruses) and bacteria such as *Agrobacterium* and *Bradyrhizobium*
25 bacteria. Constitutive promoters generally provide transcription in most or all of the cells of a plant. In particular, promoters such as the FMV promoter (FMV, US Patent 6,051,753), the enhanced 35S promoter (E35S, US Patent 5,359,142), rice actin promoter (US Patent 5,641,876), and various chimeric promoters (US Patent 6,660,911) are useful in the present invention. Examples of promoters under
30 developmental control include promoters that preferentially initiate transcription in certain tissues, such as leaves, roots, or seeds. Such promoters are referred to as “tissue-preferred”. Promoters that initiate transcription only in certain tissues are referred to as “tissue specific.”

A number of root-specific or root-enhanced promoters or fragments of such that provide enhanced expression in root tissues relative to other plant tissues have been identified and are known in the art (*e.g.* US Patents 5,110,732, 5,837,848, 5,837,876; 5,633,363; 5,459,252; 5,401,836; 7,196,247; 7,232,940; 7,119,254; and 5 7,078,589). Examples include root-enhanced or root-specific promoters such as the CaMV-derived as-1 promoter or the wheat POX1 promoter (U.S. Patent. 5,023,179), the acid chitinase gene promoter (Samac *et al.*, *Plant Mol. Biol.* 25:587-596 (1994); the root specific subdomains of the CaMV35S promoter (Lam *et al.*, *Proc. Natl. Acad. Sci. (U.S.A.)* 86:7890-7894 (1989); the root-enhanced ORF13 promoter from 10 *Agrobacterium rhizogenes* (Hansen *et al.*, *Mol. Gen. Genet.* 254:337-343 (1997); the promoter for the tobacco root-specific gene RB7 (U.S. Pat. No. 5,750,386); and the root cell-specific promoters reported by Conkling *et al.* (*Plant Physiol.* 93:1203-1211 (1990). Additional examples include RCc2 and RCc3, promoters that direct root-specific gene transcription in rice (Xu *et al.*, *Plant Mol. Biol.* 27:237, 1995); soybean 15 root-specific glutamine synthetase promoter (Hire *et al.*, *Plant Mol. Biol.* 20:207-218, 1992); root-specific control element in the GRP 1.8 gene of French bean (Keller and Baumgartner, *Plant Cell* 3:1051-1061, 1991.); a root-specific promoter of the mannopine synthase (MAS) gene of *Agrobacterium tumefaciens* (Sanger *et al.*, *Plant Mol. Biol.* 14:433-443, 1990); and full-length cDNA clone encoding cytosolic 20 glutamine synthetase (GS), which is expressed in roots and root nodules of soybean (Miao *et al.*, *Plant Cell* 3:11-22, 1991). See also Bogusz *et al.*, *Plant Cell* 2:633-641, 1990, where two root-specific promoters isolated from hemoglobin genes from the nitrogen-fixing non-legume *Parasponia andersonii* and the related non-nitrogen-fixing non-legume *Trema tomentosa* are described. Leach and Aoyagi (1991) 25 describe their analysis of the promoters of the highly expressed *rolC* and *rolD* root-inducing genes of *Agrobacterium rhizogenes* (see *Plant Science* (Limerick) 79:69-76). Additional root-preferred promoters include the VfENOD-GRP3 gene promoter (Kuster *et al.*, *Plant Mol. Biol.* 29(4):759-772, 1995); and *rolB* promoter (Capana *et al.*, *Plant Mol. Biol.* 25:681-691, 1994). Examples of nematode-induced promoters 30 include, for instance, the TobRB7 promoter (Opperman *et al.*, *Science* 263:221-223, 1994), and promoters described in U.S. Patents 6,262,344, and 7,193,136.

The term “resistance,” or “tolerance” when used in the context of comparing the effectiveness of a transgene in a transgenic plant, refers to the ability of the transgenic plant to maintain a desirable phenotype when exposed to nematode

infestation pressures relative to the phenotype presented by a nematode sensitive non-transgenic plant under similar conditions. The level of resistance can be determined by comparing the physical characteristics of the transgenic plant to non-transgenic plants that either have or have not been exposed to nematode infection. Exemplary
5 physical characteristics to observe include plant height, an increase in population of plants that have ability to survive nematode challenge (that is, plants that come in contact with a parasitic nematode may have enhanced root growth, enhanced fruit or grain yield, and reproduction nematode infection or population increase rate). The product of expression of the recombinant DNA may be directly toxic to the nematode
10 (nematicidal) or may affect the mobility, host finding, feeding site establishment, fecundity or have other nematostatic effects.

“Transformed seed” is the seed which has been generated from the transformed plant. A transformed plant contains transformed cells. A transformed cell is a cell that has been altered by the introduction of an exogenous DNA molecule
15 or in the present invention comprises a heterologous methylketone synthase or a heterologous acyl carrier protein or a combination of both.

Nematodes include but are not limited to plant parasitic species, for example, *Heterodera* species, *Globodera* species, *Meloidogyne* species, *Rotylenchulus* species, *Hoplolaimus* species, *Belonolaimus* species, *Pratylenchus* species, *Longidorus*
20 species, *Paratrichodorus* species, *Ditylenchus* species, *Xiphinema* species, *Dolichodorus* species, *Helicotylenchus* species, *Radopholus* species, *Hirschmanniella* species, *Tylenchorhynchus* species, and *Trichodorus* species, and the like.

The present invention provides recombinant DNA constructs comprising a polynucleotide that, when incorporated in a plant cell, imparts to the plant resistance
25 to nematode infection or plant disease caused by the nematode infection. Such constructs also typically comprise a promoter operatively linked to said polynucleotide to provide for expression in the plant cells. Other construct components may include additional regulatory molecules, such as 5' leader regions or 3' untranslated regions (such as polyadenylation sites), intron regions, and transit or
30 signal peptides. Such recombinant DNA constructs can be assembled using methods known to those of ordinary skill in the art.

Recombinant constructs prepared in accordance with the present invention also generally include a 3' untranslated DNA region (UTR) that typically contains a polyadenylation sequence following the polynucleotide coding region. Examples of

useful 3' UTRs include but are not limited to those from the nopaline synthase gene of *Agrobacterium tumefaciens* (nos), a gene encoding the small subunit of a ribulose-1,5-bisphosphate carboxylase-oxygenase (rbcS), and the T7 transcript of *Agrobacterium tumefaciens*.

5 Constructs and vectors may also include a transit peptide for targeting of a protein product, particularly to a chloroplast, leucoplast or other plastid organelle, mitochondria, peroxisome, or vacuole or an extracellular location. For descriptions of the use of chloroplast transit peptides, see U.S. Patent 5,188,642 and U.S. Patent No. 5,728,925. Many chloroplast-localized proteins are expressed from nuclear genes
10 as precursors and are targeted to the chloroplast by a chloroplast transit peptide (CTP). Examples of other such isolated chloroplast proteins include, but are not limited to those associated with the small subunit (SSU) of ribulose-1,5,-bisphosphate carboxylase, ferredoxin, ferredoxin oxidoreductase, the light-harvesting complex protein I and protein II, thioredoxin F, enolpyruvyl shikimate phosphate synthase
15 (EPSPS) and transit peptides described in US Patent 7,193,133. It has been demonstrated in vivo and in vitro that non-chloroplast proteins may be targeted to the chloroplast by use of protein fusions with a heterologous CTP and that the CTP is sufficient to target a protein to the chloroplast. Incorporation of a suitable chloroplast transit peptide, such as, the *Arabidopsis thaliana* EPSPS CTP (CTP2, Klee *et al.*, *Mol.*
20 *Gen. Genet.* 210:437-442, 1987), and the *Petunia hybrida* EPSPS CTP (CTP4, della-Cioppa *et al.*, *Proc. Natl. Acad. Sci. USA* 83:6873-6877, 1986) has been show to target heterologous EPSPS protein sequences to chloroplasts in transgenic plants. The production of glyphosate tolerant plants by expression of a fusion protein comprising an amino-terminal CTP with a glyphosate resistant EPSPS enzyme is well known by
25 those skilled in the art, (U.S. Patent No. 5,627,061, U.S. Patent No. 5,633,435, U.S. Patent No. 5,312,910, EP 0218571, EP 189707, EP 508909, and EP 924299). Those skilled in the art will recognize that various chimeric constructs can be made that utilize the functionality of a CTP to import various methylketone synthases or acyl carrier proteins into the plant cell plastid.

30 Stable methods for plant transformation include virtually any method by which DNA can be introduced into a cell, such as by direct delivery of DNA (for example, by PEG-mediated transformation of protoplasts, by electroporation, by agitation with silicon carbide fibers, and by acceleration of DNA coated particles), by *Agrobacterium*-mediated transformation, by viral or other vectors. One preferred

method of plant transformation is microprojectile bombardment, for example, as illustrated in U.S. Patents 5,015,580 (soy), 5,550,318 (maize), 5,538,880 (maize), 6,153,812 (wheat), 6,160,208 (maize), 6,288,312 (rice) and 6,399,861 (maize), and 6,403,865 (maize).

5 Detailed procedures for *Agrobacterium*-mediated transformation of plants, especially crop plants, include, for example, procedures disclosed in U. S. Patents 5,004,863, 5,159,135, 5,518,908, 5,846,797, and 6,624,344 (cotton); 5,416,011, 5,569,834, 5,824,877, 5,914,451, 6,384,301, and 7,002,058 (soy); 5,591,616, 5,981,840, and 7,060,876 (maize); 5,463,174 and 5,750,871 (Brassica species, including rapeseed and canola), and in U. S. Patent Application Publications 10 2004/0244075 (maize), 2004/0087030 (cotton) and 2005/0005321 (soybean). Additional procedures for *Agrobacterium*-mediated transformation are disclosed in WO9506722 (maize). Similar methods have been reported for many plant species, both dicots and monocots, including, among others, peanut (Cheng *et al.*, *Plant Cell* 15 *Rep.*, 15:653, 1996); asparagus (Bytebier *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 84:5345, 1987); barley (Wan and Lemaux, *Plant Physiol.*, 104:37, 1994); rice (Toriyama *et al.*, *Bio/Technology*, 6:10, 1988; Zhang *et al.*, *Plant Cell Rep.*, 7:379, 1988; wheat (Vasil *et al.*, *Bio/Technology*, 10:667, 1992; Becker *et al.*, *Plant J.*, 5:299, 1994), alfalfa (Masoud *et al.*, *Transgen. Res.*, 5:313, 1996); Brassica species 20 (Radke *et al.*, *Plant Cell Rep.*, 11:499-505, 1992); and tomato (Sun *et al.*, *Plant Cell Physiol.*, 47:426-431, 2006). Transgenic plant cells and transgenic plants can also be obtained by transformation with other vectors, such as but not limited to viral vectors (for example, tobacco etch virus (TEV), barley stripe mosaic virus (BSMV), and the viruses referenced in Edwardson and Christie, "The Potyvirus Group: Monograph 25 No. 16", 1991, Agric. Exp. Station, Univ. of Florida), plasmids, cosmids, YACs (yeast artificial chromosomes), BACs (bacterial artificial chromosomes) or any other suitable cloning vector, when used with an appropriate transformation protocol such as but not limited to bacterial infection (for example, with *Agrobacterium* as described above), binary bacterial artificial chromosome constructs, direct delivery of 30 DNA (for example, via PEG-mediated transformation, desiccation/inhibition-mediated DNA uptake, electroporation, agitation with silicon carbide fibers, and microprojectile bombardment). It would be clear to one of ordinary skill in the art that various transformation methodologies can be used and modified for production of stable transgenic plants from any number of plant species of interest. For example the

construction of stably inherited recombinant DNA constructs and mini-chromosomes can be used as vectors for the construction of transgenic plants (U.S. Patent 7,235,716).

Plants of the present invention include, but are not limited to, Acacia, alfalfa, aneth, apple, apricot, artichoke, arugula, asparagus, avocado, banana, barley, beans, beet, blackberry, blueberry, broccoli, brussels sprouts, cabbage, canola, cantaloupe, carrot, cassava, cauliflower, celery, cherry, cilantro, citrus, clementine, coffee, corn, cotton, cucumber, Douglas fir, eggplant, endive, escarole, eucalyptus, fennel, figs, forest trees, gourd, grape, grapefruit, honey dew, jicama, kiwifruit, lettuce, leeks, lemon, lime, loblolly pine, mango, melon, mushroom, nut, oat, okra, onion, orange, an ornamental plant, papaya, parsley, pea, peach, peanut, pear, pepper, persimmon, pine, pineapple, plantain, plum, pomegranate, poplar, potato, pumpkin, quince, radiata pine, radicchio, radish, rapeseed, raspberry, rice, rye, sorghum, Southern pine, soybean, spinach, squash, strawberry, sugarbeet, sugarcane, sunflower, sweet potato, sweetgum, tangerine, tea, tobacco, tomato, turf, a vine, watermelon, wheat, yams, and zucchini. Crop plants are defined as plants which are cultivated to produce one or more commercial products. Examples of such crops or crop plants include but are not limited to soybean, canola, rape, cotton (cottonseeds), peanut, sunflower, pigeon pea, chickpea, and the like, and grains such as corn, wheat, rice, oat, millet, and rye, and the like. Rape, rapeseed and canola are used synonymously in the present disclosure.

Transformation methods to provide transgenic plant cells and transgenic plants containing stably integrated recombinant DNA are preferably practiced in tissue culture on media and in a controlled environment. Recipient cell targets include but are not limited to meristem cells, callus, immature embryos or parts of embryos, gametic cells such as microspores, pollen, sperm, and egg cells. Any cell from which a fertile plant can be regenerated is contemplated as a useful recipient cell for practice of the invention. Callus can be initiated from various tissue sources, including, but not limited to, immature embryos or parts of embryos, seedling apical meristems, microspores, and the like. Those cells which are capable of proliferating as callus can serve as recipient cells for genetic transformation. Practical transformation methods and materials for making transgenic plants of this invention (for example, various media and recipient target cells, transformation of immature embryos, and subsequent regeneration of fertile transgenic plants) are disclosed, for example, in U. S. Patents 6,194,636 and 6,232,526 and U. S. Patent Application Publication 2004/0216189.

In general transformation practice, DNA is introduced into only a small percentage of target cells in any one transformation experiment. Marker genes are generally used to provide an efficient system for identification of those cells that are transformed by a transgenic DNA construct. Preferred marker genes provide selective markers which confer resistance to a selective agent, such as an antibiotic or herbicide. Any of the antibiotics or herbicides to which a plant cell may be resistant can be a useful agent for selection. Potentially transformed cells are exposed to the selective agent. In the population of surviving cells will be those cells where, generally, the resistance-conferring gene is expressed at sufficient levels to permit cell survival in the presence of the selective agent. Cells can be tested further to confirm integration of the recombinant DNA. Commonly used selective marker genes include those conferring resistance to antibiotics such as kanamycin or paromomycin (*nptII*), hygromycin B (*aph IV*), gentamycin (*aac3* and *aacC4*) and glufosinate (*bar* or *pat*), glyphosate (EPSPS), and dicamba (dicamba monooxygenase). Examples of useful selective marker genes and selection agents are illustrated in U. S. Patents 5,550,318, 5,633,435, 5,780,708, and 6,118,047. Screenable markers or reporters, such as markers that provide an ability to visually identify transformants can also be employed. Non-limiting examples of useful screenable markers include, for example, a gene expressing a protein that produces a detectable color by acting on a chromogenic substrate (for example, *beta*-glucuronidase, GUS, *uidA*, or luciferase, *luc*) or that itself is detectable, such as green fluorescent protein (GFP, *gfp*) or an immunogenic molecule. Those of skill in the art will recognize that many other useful markers or reporters are available for use.

Trait Stacking and Breeding:

The recombinant DNA constructs of the invention can be stacked with other recombinant DNA for imparting additional agronomic traits (such as in the case of transformed plants, traits including but not limited to herbicide resistance, insect resistance, cold germination tolerance, water deficit tolerance, enhanced yield, enhanced quality, fungal, viral, and bacterial disease resistance) for example, by expressing other transgenes. The recombinant DNA constructs of the present invention can also be transformed into plant varieties that carry natural pest or pathogen resistance genes to enhance the efficacy of the resistance phenotype. Constructs for coordinated decrease and/or increase of gene expression are disclosed

in U.S. Patent Application Publication 2004/0126845 A1. Seeds of transgenic, fertile plants can be harvested and used to grow progeny generations, including hybrid generations, of transgenic plants of this invention that include the recombinant DNA construct in their genome. Thus, in addition to direct transformation of a plant with a recombinant DNA construct of this invention, transgenic plants of the invention can be prepared by crossing a first plant having the recombinant DNA with a second plant lacking the construct. For example, the recombinant DNA can be introduced into a plant line that is amenable to transformation to produce a transgenic plant, which can be crossed with a second plant line to introgress the recombinant DNA into the resulting progeny. A transgenic plant of the invention can be crossed with a plant line having other recombinant DNA or naturally occurring genetic regions that confers one or more additional trait(s) (such as, but not limited to, herbicide resistance, pest or disease resistance, environmental stress resistance, modified nutrient content, and yield improvement) to produce progeny plants having recombinant DNA that confers both the desired target sequence expression behavior and the additional trait(s). Typically, in such breeding for combining traits the transgenic plant donating the additional trait is a male line and the transgenic plant carrying the base traits is the female line. The progeny of this cross segregate such that some of the plant will carry the DNA for both parental traits and some will carry DNA for one parental trait; such plants can be identified by markers associated with parental recombinant DNA. Progeny plants carrying DNA for both parental traits can be crossed back into the female parent line multiple times, for example, usually 6 to 8 generations, to produce a progeny plant with substantially the same genotype as one original transgenic parental line but for the recombinant DNA of the other transgenic parental line.

The transgenic plant, plant part, seed or progeny plants of the present invention can be processed into products useful in commerce. These products include but are not limited to meal, flour, oil, hay, starch, juice, protein extract, and fiber.

EXAMPLES

The following examples are included to illustrate embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent techniques discovered by the inventor to function well in the practice of the invention. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in

the specific embodiments which are disclosed and still obtain a like or similar result without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

Example 1

The example illustrates the surprising nematicidal efficacy of various methylketones. Methylketones of various chain lengths were tested *in vitro* against *C. elegans* L1 and L4 larvae and *M. incognita* pre-parasitic J2 larvae, the dispersal larval stage found in the soil. As illustrated in Table 1, nematicidal activity was observed for the medium-length methylketones (10-14 carbon chain lengths). The table shows the *in vitro* IC30 values (in parts per million) of various methylketones effective against *C. elegans* L1 and L4 larvae and *M. incognita* J2 larvae. IC30 is defined as the concentration of the methylketone at which 30 percent of the nematodes are killed after an exposure of 4 hours for *C. elegans* and 24 hours for *M. incognita*.

Table 1. *In vitro* efficacy of various methylketones on nematodes.

Compound	<i>C. elegans</i>		<i>M. incognita</i>
	vs.L1	vs.L4	vs.J2
2-heptanone	>400	>400	400
2-nonanone	12.5	>400	200
2-decanone	6.3	>400	25
2-undecanone	6.3	25	50
2-dodecanone	3.2	25	50
2-tridecanone	3.2	12.5	50
2-tetradecanone	3.2	>100	25
2-pentadecanone	12.5	>100	400

20

Whole plant assays were used to determine the efficacy of the methylketones on the infection of soybean plants and tomato plants by nematodes, *H. glycines* and *M. incognita*, respectively. The seeds were planted in 100 percent sand in two-inch square plastic pots and grown to a sufficient size for treatment. Methylketone chemical treatment was applied when the soybean plants showed the first trifoliate beginning to emerge and when the tomato plants reached the 2-3 leaf stage.

25

Following methylketone treatment, nematodes were inoculated into each pot and for soybeans are then incubated for 28 days before harvest and for tomatoes incubated 21 days before harvest.

To each of four pots, five milliliters of the appropriate chemical solution is applied to the surface making sure to avoid contact with the base of the plant. Immediately following the chemical application, the pot surface is wetted sufficiently to water in the chemical. One milligram of chemical per four pots is approximately equivalent to one kilogram per hectare of chemical. A standard test uses four replications. For rates above 2 kg/ha, the desired amount of chemical is weighed into a 30 ml vial (example: 8 kg/ha rate = 8 mg chemical in 30 ml vial). The chemical is dissolved in 2 ml of an appropriate solvent, generally acetone. For rates below 2 kg/ha, 2 milligrams of chemistry is weighed into the vial and dissolved in 2 ml of the solvent. The appropriate amount of chemical concentrate is then applied into a separate 30 ml vial and solvent is added to bring the volume to 2 ml (example 0.5 kg/ha = 0.5 ml of concentrate + 1.5 ml solvent). Each dissolved concentrate is then brought to a total of 20 milliliters using 0.05% Triton X-100 surfactant solution.

Nematode eggs, either SCN or RKN, are added to distilled water to create a concentration of 1000 vermiform eggs per liter of water. At least four hours after chemical treatment the eggs are applied to the treated pots plus non-treated check plants. A small hole about 1 cm deep is punched into the pot surface. One milliliter of the nematode egg slurry is applied into the hole. Immediately afterwards the hole is gently covered. Watering of the test plants is then restricted to a minimum volume needed to prevent wilting for a period of 24 hours. After the 24 hour restricted watering, normal sub-irrigation watering is done for the duration of the test.

The 2-undecanone, 2-tridecanone, and 2-pentadecanone are tested in greenhouse studies against *M. incognita* infection of tomato roots in sand. The tomato plants are commercial varieties sensitive to nematode infection (e.g., Mountain Spring) and do not accumulate the methylketones that are found in the leaf trichomes of some wild tomato species. Shown in Table 2 is a high level of nematicidal activity of various methylketones observed against root knot nematode (*M. incognita*) inoculated into treated pots containing tomato plants. 2-tridecanone is highly effective at controlling nematode-induced galling at both 40 kilograms per hectare (kg/ha) (100% control) and 8 kg/ha (97% control), while 2-undecanone and 2-pentadecanone also demonstrated nematode control. The listed kilograms/hectare

(kg/ha) rating is based upon the surface area of the test pots; 1 kg/ha equates to about 1.65 mg compound per kilogram of soil in these assays.

Table 2. Activity of various methylketones on root knot nematode disease.

Compound	Rate (kg/ha)	% Galled Roots	% Control
2-undecanone	40	21	65%
2-undecanone	8	39	35%
2-tridecanone	40	0	100%
2-tridecanone	8	2	97%
2-pentadecanone	40	38	37%
2-pentadecanone	8	45	25%
No Compound added	-	60	NA

- 5 2-tridecanone was also assayed in the greenhouse for control of soybean cyst nematode (*H. glycines*) in soybeans (Table 3). Nematode control (#cysts/plant) is observed at 40 kg/ha (96% control relative to the non-treated) and 8 kg/ha (80% control relative to the non-treated) when 2-tridecanone was applied as a soil drench prior to nematode inoculation.

10 **Table 3. Efficacy of methylketone on cyst nematode infection of soybean**

Compound	Rate (kg/ha)	#cysts/plant	% Control
2-tridecanone	40	2	96%
2-tridecanone	8	10	80%
No Compound added	-	49	NA

Example 2

- This example provides descriptions of compositions in use or contemplated for use in controlling plant parasitic nematodes singularly or in any combination. Table 4 provides a list of the compositions. A crop transformation base vector comprising selection expression cassettes and elements necessary for the maintenance of the plasmid in a bacterial cell is used to assemble DNA segments (promoters, leaders, introns, 3'UTR) that provide regulatory activity when operably linked to DNA segments that provide functionality in the present invention. The assembly of these DNA segments can be accomplished using methods known in the art of recombinant DNA technology. DNA coding sequences of the present invention such as any one or more of the DNA molecules identified as SEQ ID NO: 1, 2, 4, 6, 8, 10, 12, 14, 16, 18,

20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 59, 60, and 61 are cloned and inserted into an expression cassette or inserted into operable linkage with another coding sequence or genetic element of an expression cassette. Other genetic elements can be selected and tested by those skilled in the art that provide functional expression of a methylketone in plant tissues.

Table 4. Descriptions of genetic elements.

SEQ ID NO:	Name	Description
SEQ ID NO:1	MKS1a	A codon-optimized polynucleotide sequence variant for <i>L. hirsutum</i> methylketone synthase
SEQ ID NO:2	MKS1b	A codon-optimized polynucleotide sequence variant for <i>L. hirsutum</i> methylketone synthase
SEQ ID NO:3	MKS1	Amino acid sequence of the methylketone synthase protein from <i>L. hirsutum</i>
SEQ ID NO:4	LhMKS1	Polynucleotide sequence for a codon-optimized <i>L. hirsutum</i> methylketone synthase
SEQ ID NO:5	LhMKS1 protein variant	Amino acid sequence variant of the methylketone synthase protein from <i>L. hirsutum</i>
SEQ ID NO:6	LsMKS1	Polynucleotide sequence for a codon-optimized <i>Lycopersicon esculentum</i> (<i>Solanum lycopersicum</i>) methylketone synthase
SEQ ID NO:7	LsMKS1 protein	Amino acid sequence of the methylketone synthase protein from <i>L. esculentum</i>
SEQ ID NO:8	AtCTP2	A polynucleotide sequence encoding a chloroplast transit peptide from <i>A. thaliana</i> EPSPS protein
SEQ ID NO:9	AtCTP2 protein	Amino acid sequence of the chloroplast transit peptide from <i>A. thaliana</i> EPSPS protein
SEQ ID NO:10	PhCTP4	A polynucleotide sequence encoding a chloroplast transit peptide from <i>Petunia hybrida</i> EPSPS protein
SEQ ID NO:11	PhCTP4 protein	Amino acid sequence of the chloroplast transit peptide from <i>Petunia hybrida</i> EPSPS protein
SEQ ID NO:12	CTP2-MKS1a	Polynucleotide sequence of the AtCTP2 chloroplast transit peptide fused to the MKS1a codon optimized sequence encoding methylketone synthase

SEQ ID NO:13	CTP2-MKS1a protein	Amino acid sequence of the heterologous AtCTP2-MKS1 fusion protein
SEQ ID NO:14	CTP4-MKS1a	Polynucleotide sequence of the PhCTP4 chloroplast transit peptide fused to the MKS1a codon optimized sequence encoding methylketone synthase
SEQ ID NO:15	CTP4-MKS1a protein	Amino acid sequence of the heterologous PhCTP4-MKS1a fusion protein
SEQ ID NO:16	CTP2-MKS1b	Polynucleotide sequence of the CTP2 chloroplast transit peptide fused to the MKS1b codon optimized sequence encoding methylketone synthase
SEQ ID NO:17	CTP2-MKS1b protein	Amino acid sequence of the heterologous AtCTP2-MKS1b fusion protein
SEQ ID NO:18	CTP4-MKS1b	Polynucleotide sequence of the PhCTP4 chloroplast transit peptide fused to the MKS1b codon optimized sequence encoding methylketone synthase
SEQ ID NO:19	CTP4-MKS1b protein	Amino acid sequence of the heterologous PhCTP4-MKS1b fusion protein
SEQ ID NO:20	CTP2-LhMKS1	Polynucleotide sequence of the AtCTP2 chloroplast transit peptide fused to the LhMKS1 sequence encoding methylketone synthase
SEQ ID NO:21	CTP2-LhMKS1 protein	Amino acid sequence of the heterologous AtCTP2-LhMKS1 fusion protein
SEQ ID NO:22	CTP4-LhMKS1	Polynucleotide sequence of the PhCTP4 chloroplast transit peptide fused to the LhMKS1 sequence encoding methylketone synthase
SEQ ID NO:23	CTP4-LhMKS1 protein	Amino acid sequence of the heterologous PhCTP4-LhMKS1 fusion protein
SEQ ID NO:24	CTP2-LsMKS1	Polynucleotide sequence of the AtCTP2 chloroplast transit peptide fused to the LsMKS1 sequence encoding methylketone synthase
SEQ ID NO:25	CTP2-LsMKS1 protein	Amino acid sequence of the heterologous AtCTP2-LsMKS1 fusion protein

SEQ ID NO:26	CTP4-LsMKS1	Polynucleotide sequence of the PhCTP4 chloroplast transit peptide fused to the LsMKS1 sequence encoding methylketone synthase
SEQ ID NO:27	CTP4-LsMKS1 protein	Amino acid sequence of the heterologous PhCTP4-LsMKS1 fusion protein
SEQ ID NO:28	CTP2-MKS1a_sN	Polynucleotide sequence of the AtCTP2 chloroplast transit peptide fused to the MKS1s sequence encoding an Alanine to Serine active site variant of methylketone synthase
SEQ ID NO:29	CTP2-MKS1a_sN protein	Amino acid sequence of the AtCTP2 chloroplast transit peptide fused to the MKS1s sequence having an Alanine to Serine active site variant of methylketone synthase
SEQ ID NO:30	CTP2-MKS1a_Ad	Polynucleotide sequence of the AtCTP2 chloroplast transit peptide fused to the MKS1s sequence encoding an Asparagine to Aspartic acid active site variant of methylketone synthase
SEQ ID NO:31	CTP2-MKS1a_Ad protein	Amino acid sequence of the AtCTP2 chloroplast transit peptide fused to the MKS1s sequence having an Asparagine to Aspartic acid active site variant of methylketone synthase
SEQ ID NO:32	CTP2-MKS1a_sd	Polynucleotide sequence of the AtCTP2 chloroplast transit peptide fused to the MKS1s sequence encoding a double variant Alanine to Serine and Asparagine to Aspartic acid active site variant of methylketone synthase
SEQ ID NO:33	CTP2-MKS1a_sd protein	Amino acid sequence of the AtCTP2 chloroplast transit peptide fused to the MKS1s sequence having a double variant Alanine to Serine and Asparagine to Aspartic acid active site variant of methylketone synthase
SEQ ID NO:34	CTP2-LsMKS1_sN	Polynucleotide sequence of the AtCTP2 chloroplast transit peptide fused to the LsMKS1s sequence encoding an Alanine to Serine active site variant of methylketone synthase
SEQ ID NO:35	CTP2-LsMKS1_sN protein	Amino acid sequence of the AtCTP2 chloroplast transit peptide fused to the LsMKS1s sequence encoding an Alanine to Serine active site variant of methylketone synthase

SEQ ID NO:36	CTP2-LsMKS1_Ad	Polynucleotide sequence of the AtCTP2 chloroplast transit peptide fused to the LsMKS1s sequence encoding an Asparagine to Aspartic acid active site variant of methylketone synthase
SEQ ID NO:37	CTP2-LsMKS1_Ad protein	Amino acid sequence of the AtCTP2 chloroplast transit peptide fused to the LsMKS1s sequence encoding an Asparagine to Aspartic acid active site variant of methylketone synthase
SEQ ID NO:38	CTP2-LsMKS1_sd	Polynucleotide sequence of the AtCTP2 chloroplast transit peptide fused to the LsMKS1s sequence encoding a double variant Alanine to Serine and Asparagine to Aspartic acid active site variant of methylketone synthase
SEQ ID NO:39	CTP2-LsMKS1_sd protein	Amino acid sequence of the AtCTP2 chloroplast transit peptide fused to the LsMKS1s sequence having a double variant Alanine to Serine and Asparagine to Aspartic acid active site variant of methylketone synthase
SEQ ID NO:40	LhACP1-PI126449	Polynucleotide sequence of an Acyl carrier protein ACP1 coding sequence from PI126449
SEQ ID NO:41	LhACP1-PI126449 protein	Amino acid sequence of an Acyl carrier protein ACP1 from PI126449
SEQ ID NO:42	LhACP2-PI126449	Polynucleotide sequence of an Acyl carrier protein ACP2 coding sequence from PI126449
SEQ ID NO:43	LhACP2-PI126449 protein	Amino acid sequence of an Acyl carrier protein ACP2 from PI126449
SEQ ID NO:44	LhACP1-LA1777	Polynucleotide sequence of an Acyl carrier protein ACP1 coding sequence from LA1777
SEQ ID NO:45	LhACP1-LA1777 protein	Amino acid sequence of an Acyl carrier protein ACP1 from LA1777
SEQ ID NO:46	LeACP2	Polynucleotide sequence of an Acyl carrier protein ACP2 from <i>L. esculentum</i>
SEQ ID NO:47	LeACP2 protein	Amino acid sequence of an Acyl carrier protein ACP2 from <i>L. esculentum</i>
SEQ ID NO:48	StACP2	Polynucleotide sequence of an Acyl carrier protein ACP2 from <i>Solanum tuberosum</i>
SEQ ID NO:49	StACP2 protein	Amino acid sequence of an Acyl carrier protein ACP2 from <i>Solanum tuberosum</i>

SEQ ID NO:50	ScACP2	Polynucleotide sequence of an Acyl carrier protein ACP2 from <i>Solanum chacoense</i>
SEQ ID NO:51	ScACP2 protein	Amino acid sequence of an Acyl carrier protein ACP2 from <i>Solanum chacoense</i>
SEQ ID NO:52	NtACP2	Polynucleotide sequence of an Acyl carrier protein ACP2 from <i>Nicotiana tabacum</i>
SEQ ID NO:53	NtACP2 protein	Amino acid sequence of an Acyl carrier protein ACP2 from <i>Nicotiana tabacum</i>
SEQ ID NO:54	PhACP2	Polynucleotide sequence of an Acyl carrier protein ACP2 from <i>Petunia hybrida</i>
SEQ ID NO:55	PhACP2 protein	Amino acid sequence of an Acyl carrier protein ACP2 from <i>Petunia hybrida</i>
SEQ ID NO:56	CaACP2	Polynucleotide sequence of an Acyl carrier protein ACP2 from <i>Capsicum annum</i>
SEQ ID NO:57	CaACP2 protein	Amino acid sequence of an Acyl carrier protein ACP2 from <i>Capsicum annum</i>
SEQ ID NO:58	LeMKS1 homolog	Polynucleotide sequence of an MKS1 homolog from <i>L. esculentum</i>
SEQ ID NO:59	StMKS1 homolog	Polynucleotide sequence of an MKS1 homolog from <i>S. tuberosum</i>
SEQ ID NO:60	NtMKS1 homolog	Polynucleotide sequence of an MKS1 homolog from <i>N. tabacum</i>
SEQ ID NO:61	CaMKS1 homolog	Polynucleotide sequence of an MKS1 homolog from <i>C. annum</i>
SEQ ID NO: 62	Act7 intron	558 nucleotide actin 7 intron sequence from <i>A. thaliana</i>

Example 3

This example describes generation of tomato or soybean transgenic hairy roots expressing MKS and the nematode infection assay. Hairy root cultures allow the rapid growth of root tissue on a large scale which can be used for testing the effectiveness of the gene of interest as set forth herein, for controlling plant parasitic nematode infestation of a crop plant. Hairy roots are characterized by fast growth, frequent branching, plagiotropism, and the ability to synthesize the same compounds as the roots of the intact plant (David *et al.*, *Biotechnology* 2:73–76, 1984). Transfer and integration of the genes located on the root-inducing plasmid Ri of *Agrobacterium rhizogenes* into the plant genome and their expression therein (White and Nester, *J Bacteriol.*, 141:1134-1141, 1980). These types of roots continue to

grow in vitro on hormone-free medium and also exhibit a high degree of genetic stability (Aird *et al.*, *Plant Cell Tiss. Org. Cult.* 15: 47–57, 1988). The natural ability of the soil bacterium *A. rhizogenes* to transform genes into a host plant genome results in roots being formed at the site of infection. Infection of the plant with *A.*
5 *rhizogenes*, leads to the integration and expression of T-DNA in the plant genome, which causes development of a hairy root. Hairy root cultures grow rapidly, show plagiotropic root growth and are highly branched on hormone-free medium.

For soybean hairy roots, *A. rhizogenes* strain K599 is grown and maintained on LB, minimal A, or yeast extract and peptone (YEP) media. Methods for
10 generation of transgenic tomato hairy root cultures for evaluating lesion or root knot nematodes are not significantly different other than the use of *A. rhizogenes* D1 strain. Soybean seeds are surface-sterilized by setting in chlorine gas under controlled conditions for 12-16 hours, and then aerating in a clean air hood for at least 30 minutes. Seeds are germinated in Petri dishes containing ¼ MS.

15 The hypocotyl or cotyledons of 6-days-old seedlings are wounded using a scalpel. The wounded cotyledons are then immersed in freshly grown *A. rhizogenes* containing the construct and subsequently vacuum infiltrated. Cotyledons are cultured under the same conditions used for seed germination with the exception that the antibiotic cefotaxime is added to the ¼ MS agar plates to prevent the *A.*
20 *rhizogenes* from subsequent growth. Adventitious roots are excised from hypocotyls or cotyledons inoculated with *A. rhizogenes*. The putative transformed roots are cultured on Gamborg's B-5 agar containing 3% sucrose plus 3 g/l Gelrite, BASTA, and cefotaxime). Roots passing selection are transferred to fresh media and maintained. Cultured roots are maintained in an incubator, without light, set at 24-30°
25 C. Roots are maintained on Gamborg's B-5 agar. A piece of root tip is excised and transferred to fresh medium every 2-4 weeks.

Following hairy root line selection, roots for the plant nematode bioassay are transferred to fresh plates containing Gamborg's B-5 medium and allowed to grow for approximately two weeks to provide sufficient tissue for nematode infection before
30 inoculation with a mixed population of root lesion nematodes or second-stage juveniles of soybean cyst nematode (SCN) or root knot nematode (RKN). Individual hairy root tips are placed on infection plates. 20 plates are used for testing transformed roots for reaction to lesion, SCN or RKN. Each plate contains a transformed root from a separate integration. An additional 20 plates containing a

transformed lesion susceptible, SCN-susceptible or RKN-susceptible control and an additional 20 plates containing a transformed SCN-resistant or RKN-resistant control are also tested. Transformed controls are empty vectors. Plates are then inoculated with approximately 400 axenic lesion worms or 1000 sterile *H. glycines* J2s or 450
5 sterile *M. incognita* J2s and incubated at 26-28 °C (SCN or RKN) or 25 °C or 30 °C (lesion nematode).

Approximately six weeks after inoculation with *M. incognita* or five weeks after inoculation with *H. glycines*, infected tomato or soybean hairy roots are removed from the agar plates and the number of galls or cysts counted. For SCN hairy root
10 plates cysts are counted directly, whereas for RKN gall numbers may be estimated. Gall scores are weighted estimates based on size. A scale is created at the beginning of scoring process. The smallest galls are given a score of 1 and as the galled areas become larger the gall score increases. The scale is then used to rate each gall on each plate in the experiment. Egg numbers are also scored at 42 days for RKN
15 infections in tomato hairy roots. At 42 days post-infection, plates are microwaved and sieved to collect the roots. The roots are weighed, then blended in a 10% bleach solution and poured over a series of sieves to remove the root debris and collect the eggs. Eggs are removed from each plate and are counted. For lesion nematodes, plates are harvested after approximately 56 days by placing roots in glass bowls filled
20 with sterilized water containing 50 mg/L carbenicillin and 50 mg/L kanamycin. After 9-10 days to allow the worms to exit the roots, the worms are counted under a microscope. To determine weights, root bowls are then microwaved to melt the agar and roots are collected with a sieve. The extra water is absorbed with a paper towel and the root weights recorded.

25 Axenic lesion, SCN and RKN larvae are prepared for use with the hairy root culture system. Axenic SCN J2s are produced as follows. Clean soybean cyst nematode eggs (*i.e.*, eggs with soil and other debris removed) are collected and placed in a 50 ml centrifuge vial containing 30 ml of a 10% bleach solution. The bleach solution is mildly agitated and then left to settle for 2-3 minutes. The vial is mildly
30 agitated again to re-suspend the eggs and then centrifuged for 1 minute at 1000 rpm. Under a sterile hood, the bleach solution is removed into a receptacle and 25 ml of sterile water is added into the vial of eggs. The vial is recapped under the sterile hood, mildly agitated to re-suspend the eggs and centrifuged for 1 minute at 1000 rpm. Under the sterile hood, this liquid is poured off and 25 ml of sterile water is

again placed in the vial. The vial is recapped under the sterile hood and the process of agitation and centrifugation repeated. This process of washing the eggs with sterile water is repeated approximately 4 times to thoroughly rinse the bleach from the eggs. Following the last rinse under the sterile hood the liquid is removed leaving about 1-2 ml of egg concentrate. Axenic eggs are hatched by incubating them on the surface of moist filter paper resting in a solution of 5 mM zinc sulfate just deep enough to cover the surface of the filter paper. After 2-3 days J2 larvae are collected in the solution underneath the filter paper. J2s are centrifuged and further cleaned using chlorhexidine (Atkinson *et al.*, *J. Nematol.* 28:209-215, 1996).

10 Axenic RKN larvae are prepared by collecting eggs by placing chopped RKN infected roots into a blender with a sufficient quantity of 10% bleach solution. The blender is pulsed on/off for 5 second intervals. This process is repeated 5-6 times. The root slurry is then passed through a series of sieves where the eggs and small debris are collected in a 500 micron sieve. Any remaining bleach solution is thoroughly rinsed from this egg/debris. Twenty milliliters of the egg/debris is added to a 50 ml conical tube and 20 ml of a 40% sucrose solution is added into the bottom of the tube, bringing the total volume to 40 milliliters. This solution is then centrifuged at 3750 rpm for 5 minutes to separate the eggs from the debris. After centrifugation, the eggs are removed and thoroughly rinsed to remove any remaining sucrose solution. Eggs are then placed into a hatch bowl containing filter paper moistened with just enough aerated tap water to cover the eggs. After 1-2 days J2 larvae are collected in the solution underneath the filter paper. J2 larvae are centrifuged and further cleaned using chlorhexidine (Atkinson *et al.* (1996, see above).

25 Axenic lesion larvae are prepared from lesion nematodes grown on corn explant plates. The nematodes are harvested by placing roots with medium onto filter paper supported by a wire sieve in a sterilized glass bowl which has been filled with sterilized water containing 50 mg/L carbenicillin and 50 mg/L kanamycin. The amount of the water is sufficient to submerge the agar, and the bowls are stored at room temperature (25°C) for two days. The sieve is removed and the solution poured into a 50 ml conical tube, which was then centrifuged for 5 minutes at 3500 x g at room temperature. After the worms settle to the bottom of the tube (further 15 minute incubation), the supernatant is decanted. Sterilized water is then added to the worm

pellet containing 12 mg/L of the antifungal compound Imazilil and 50 mg/L kanamycin.

The following are results found after the transgenic expression of various combinations of promoters, transit peptides and methylketone synthase coding sequences for control of plant parasitic nematode infections in hairy roots. SCN cysts in the transgenic soybean hairy root inoculated plates are counted and the average number of cysts per replication (Rep 1 and Rep 2) tabulated. The results shown in Table 5 demonstrate that transgenic soybean roots containing the chimeric CTP-methylketone synthase coding region provides resistance to SCN infection, where all treatments having a heterologous CTP fused to a methylketone synthase show a reduction in the average cyst counts compared to the transgenic empty vector control, 4211. The constructs that lack a heterologous CTP (FMV-LsMKS1 and FMV-LhMKS1) do not show a reduction in cyst counts.

Table 5. Transgenic soybean roots expressing MKS reduce SCN infection (cysts).

	control	Test constructs	Test constructs
	D4211	E35S-ctp2/mks1	E35S-ctp4/mks1
Rep 1	31.4	12.6	21.9
Rep 2	18.3	9.1	-
	4211	E35Sp-ctp2/mks1	E35Sp-ctp4/mks1
Rep 1	19.4	17.6	13
Rep 2	18.4	11.2	13.7
	4211	FMV-ctp2/LsMKS1	E35Sp-ctp2/LsMKS1
Rep 1	33	16.5	19.3
Rep 2	25.4	15	22.9
	4211	FMV-LsMKS1(no CTP)	FMV-LhMKS1 (no CTP)
Rep 1	21	27	23
Rep 2	18	17	18

As can be seen in table 6 below, the expression of MKS constructs containing CTP leaders either with or without certain targeted active site mutations leads to reduction in the ability of root knot nematode to infect plants roots. In addition to the elements listed in the table above, the constructs shown contain a ~540 nucleotide actin 7 intron incorporated into the 5' untranslated region (UTR) of the fused methylketone synthase transcript and a visual fluorescent DsRED marker (driven by the FMV promoter) co-expressed in the T-DNA, downstream of the MKS open reading frame.

Table 6. Transgenic tomato roots expressing MKS reduce root knot nematode infection (eggs).

control	Test construct	Test construct	Test construct
8221	E35sp-ctp2/LsMKS1	E35sp-ctp2/LsMKS1_sN	E35sp-ctp2/LsMKS1_sd
1884.5	968.5	-	1333.4
3059.8	1927.4	-	1558
716.7	-	283.3	298.1

As can be seen in table 7 below, the expression of MKS constructs containing CTP leaders either with or without certain targeted active site mutations leads to reduction in the ability of root lesion nematodes to infect plants roots. In addition to the elements listed in the table above, the constructs shown contain a ~540 nucleotide actin 7 intron incorporated into the 5' untranslated region (UTR) of the fused methylketone synthase transcript and a visual fluorescent DsRED marker (driven by the FMV promoter) co-expressed in the T-DNA, downstream of the MKS open reading frame.

Table 7. Transgenic tomato roots expressing MKS reduce lesion nematode infection (larvae).

control	Test construct	Test construct	Test construct
8221	E35sp-ctp2/LsMKS1	E35sp-ctp2/LsMKS1_sN	E35sp-ctp2/LsMKS1_sd
5857.8	4842.4	3926.2	4897.2

Example 4

This example describes a plant transformation method useful in producing transgenic soybean plants and transgenic seed. Other methods are known in the art of plant cell transformation that can be applied using the DNA constructs of the present invention.

For *Agrobacterium* mediated transformation, soybean seeds are germinated overnight and the meristem explants excised (see US Patent No. 7,002,058). The meristems and the explants are placed in a wounding vessel. Soybean explants and induced *Agrobacterium* cells from a strain containing plasmid DNA with the expression cassettes of the present invention and a plant selectable marker cassette are

mixed within about 14 hours from the time of initiation of seed germination and wounded using sonication. Following wounding, explants are placed in co-culture for 2-5 days at which point they are transferred to selection media for 6-8 weeks to allow selection and growth of transgenic shoots. Trait positive shoots are harvested after
5 approximately 6-8 weeks and placed into selective rooting media for 2-3 weeks. Shoots producing roots are transferred to the greenhouse and potted in soil. Shoots that remain healthy on selection but that do not produce roots are transferred to non-selective rooting media for an additional two weeks. Roots from any shoots that produce roots off selection are tested for expression of the plant selectable marker
10 before they are transferred to the greenhouse and potted in soil. Additionally, a DNA construct can be transferred into the genome of a soybean cell by particle bombardment and the cell regenerated into a fertile soybean plant as described in U.S. Patent 5,015,580.

Transgenic soybean plant cells are transformed with recombinant DNA of this
15 invention. Progeny transgenic plants and seed of the transformed plant cells are selected that provide pest resistance, especially nematode resistance.

Example 5

A soybean cyst nematode pot assay is used to evaluate the resistance of
20 transgenic soybean plants comprising the methylketone synthase coding sequence to infection by and reproduction of the soybean cyst nematode (*Heterodera glycines*) on roots. Three or four inch diameter square pots are filled with clean sand and watered thoroughly. Transgenic and control soybean seeds, or alternatively any rooted plant parts, are planted one per pot in the center of the pot and watered well to remove air
25 pockets. The pots are incubated in the greenhouse or growth chamber at 20° C to 30° C until the plants reached a suitable age for inoculation. Soybeans started from seed are typically inoculated 2-3 weeks after planting, while transplants are inoculated 1-3 days after planting. The test inoculum consists of eggs from ripe *H. glycines* cysts collected from the soil and roots of infested soybean plants. A 250 micron mesh sieve
30 is used to collect the cysts, which are then crushed in a Tenbroeck glass tissue homogenizer to release the eggs. The eggs are further purified by sieving and centrifugation over 40 percent sucrose solution at 4000 RPM for 5 minutes. Inoculum for an experiment consisted of water containing 500 vermiform eggs per mL. Five mL of the egg suspension is applied over the surface of the sand containing the test

plants and the eggs are lightly watered in. The test plants are then returned to the greenhouse or growth chamber and incubated for 3-4 weeks to allow for root infection and cyst formation. The roots are then harvested by gently removing the pot and sand and rinsing in water. The severity of nematode infection is measured by counting the
5 number of nematode cysts adhering to the root system. Alternatively, the sand and roots could be diluted in water and passed over a 250 micron sieve to collect and concentrate the cysts for storage or counting.

Example 6

This example describes the detection and measurement of the recombinant
10 DNA construct in the transgenic plant cell. Detecting or measuring transcription of the recombinant DNA construct in the transgenic plant cell of the invention can be achieved by any suitable method, including protein detection methods (for example, western blots, ELISAs, and other immunochemical methods), measurements of enzymatic activity, or nucleic acid detection methods (for example, Southern blots,
15 northern blots, PCR, RT-PCR, fluorescent *in situ* hybridization). Such methods are well known to those of ordinary skill in the art as evidenced by the numerous handbooks available; see, for example, Joseph Sambrook and David W. Russell, "Molecular Cloning: A Laboratory Manual" (third edition), Cold Spring Harbor Laboratory Press, NY, 2001; Frederick M. Ausubel *et al.* (editors) "Short Protocols in
20 Molecular Biology" (fifth edition), John Wiley and Sons, 2002; John M. Walker (editor) "Protein Protocols Handbook" (second edition), Humana Press, 2002; and Leandro Peña (editor) "Transgenic Plants: Methods and Protocols", Humana Press, 2004.

DNA sequence information provided by the invention allows for the
25 preparation of relatively short DNA (or RNA) sequences having the ability to specifically hybridize to DNA sequences of the selected polynucleotides disclosed herein. The polynucleotides disclosed in the present invention include SEQ ID NO: 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 59, 60, and 61. In these aspects, nucleic acid probes of an
30 appropriate length are prepared. The ability of the nucleic acid probes to specifically hybridize to one or more of these gene coding sequences lends them particular utility in a variety of embodiments. Most importantly, the probes may be used in a variety of assays for detecting the presence of complementary sequences in a given sample.

In certain embodiments, it is advantageous to use oligonucleotide primers. The sequence of such primers is designed using a portion of a polynucleotide sequence of the present invention to be homologous or complementary to the sequence for use in detecting, amplifying a defined polynucleotide segment using PCRTM technology (A Guide to Methods and Applications, Academic Press: San Diego, 1990). PCR primer pairs can be derived from a known sequence, for example, by using computer programs intended for that purpose such as Primer (Version 0.5, © (1991, Whitehead Institute for Biomedical Research, Cambridge, Mass.). Primers and probes based on the sequences disclosed herein can be used to confirm and, if necessary, to modify the disclosed sequences by conventional methods, for example, by re-cloning and re-sequencing. Exemplary PCR reaction conditions may include: Component Amount/Volume required sub-library aliquot 1 µl Gene-specific primer 1, 1 µl (100 pmol, GenomeWalkerTM) Adaptor primer 1 (AP1), 1 µl dNTP mix (10 mM of each dNTP), 1 µl DMSO 2.5 µl (or 2-5% final concentration) 10X PCR buffer, 5 µl (final concentration of 1X) Amplitaq GoldTM, 0.5 µl distilled water for final reaction volume of 50 µl reaction conditions for primary PCR:

- A. 9 minutes at 95° C;
- B. 94° C for 2 seconds, 70° C for 3 minutes; repeat 94° C/70° C cycling for total of 7 times;
- 20 C. 94° C for 2 seconds, 65° C for 3 minutes; repeat 94° C/65° C cycling for total of 36 times;
- D. 65° C. for 4 minutes as a final extension;
- E. 10° C. for an extended incubation

NESTED PCR (secondary PCR reaction) Component Amount/Volume Required 1:50 dilution of the primary PCR reaction; 1 µl Gene-specific primer 2; 1 µl (100 pmol, GenomeWalkerTM Adaptor primer 2; 1 µl or 3 (AP2 or AP3), dNTP mix (10 mM of each dNTP); 1 µl DMSO; 2.5 µl 10X PCR buffer containing MgCl₂; 5 µl (final concentration of 1X) Amplitaq GoldTM; 0.5 µl distilled water to final reaction volume of 50 µl reaction. Conditions for Nested PCR:

- 30 A. 9 minutes at 95° C;
- B. 94° C for 2 seconds, 70° C for 3 minutes; repeat 94° C/70° C cycling for total of 5 times;
- C. 94° C for 2 seconds, 65° C for 3 minutes; repeat 94° C/65° C cycling for total of 24 times;

D. 65° C for 4 minutes as a final extension;

E. 10° C for an extended incubation.

PCR conditions can be modified from the described conditions by those skilled in the method to produce an amplicon.

- 5 Detection of foreign gene expression in transgenic plant is monitored by an immunological method for example ELISA (enzyme-linked immunosorbent assays) for a quantitative determination of the level of corresponding protein obtained. Quantitative determination of the encoded protein in the leaves of transgenic plants is performed using ELISA, for example as disclosed in Clark *et al.*; ELISA Techniques.
- 10 In: Weissbach A, Weissbach H (eds) *Methods in Enzymology* 118:742-766, Academic Press, Florida (1986).

 All publications and patents referenced herein are intended to be herein incorporated by reference in their entirety.

CLAIMS

- Claim 1. A polynucleotide comprising a sequence encoding plant methylketone synthase operably linked to a heterologous transit peptide.
- Claim 2. The polynucleotide of claim 1, wherein the plant methylketone synthase comprises an amino acid sequence with at least about 85% identity to a polypeptide selected from the group consisting of SEQ ID NO: 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, and 39, wherein the polypeptide has methylketone synthase activity.
- Claim 3. The polynucleotide of claim 1, wherein the transit peptide is a chloroplast transit peptide.
- Claim 4. The polynucleotide of claim 3, wherein the chloroplast transit peptide is selected from the group consisting of an EPSPS chloroplast transit peptide, a small subunit ribulose-1,5,-bisphosphate carboxylase chloroplast transit peptide, a ferredoxin chloroplast transit peptide, a ferredoxin oxidoreductase chloroplast transit peptide, a light-harvesting complex protein I and protein II chloroplast transit peptide, and a thioredoxin F chloroplast transit peptide.
- Claim 5. The polynucleotide of claim 1, wherein the sequence encoding plant methylketone synthase exhibits at least about 80% percent sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO: 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, and 38.
- Claim 6. A construct comprising the polynucleotide of claim 1 operably linked to a promoter functional in plants.
- Claim 7. A plant cell comprising the polynucleotide of claim 1.
- Claim 8. The plant cell of claim 7, wherein the transit peptide is a chloroplast transit peptide.
- Claim 9. The plant cell of claim 7, wherein said plant cell is from a seed, root, leaf, shoot, flower, pollen, or ovule.
- Claim 10. The plant cell of claim 7, wherein said cell produces a methylketone.

- Claim 11. The plant cell of claim 10, wherein said methylketone is 2-undecanone or 2-tridecanone or 2-pentadecanone.
- Claim 12. The plant cell of claim 7, wherein said cell is a crop plant cell.
- Claim 13. The plant cell of claim 7, wherein said cell is from a plant selected from the group selected from cotton, soybean, canola, corn, wheat, rice, sunflower, sorghum, sugarcane, potato, tomato, and a tree.
- Claim 14. A plant or a part thereof comprising the polynucleotide of claim 1.
- Claim 15. The plant or part thereof of claim 14, wherein the part thereof is selected from the group consisting of a seed, pollen, a root, a leaf, a shoot, a flower and an ovule.
- Claim 16. A processed product comprising a plant tissue comprising the polynucleotide of claim 1.
- Claim 17. The processed product of claim 16, selected from the group consisting of meal, flour, oil, hay, starch, juice, protein extract, and fiber.
- Claim 18. A method for controlling a pathogen or pest in a plant comprising expressing in the plant the construct of claim 6.
- Claim 19. The method for controlling a pathogen or pest in a plant of claim 18, wherein the polynucleotide sequence comprises a sequence that encodes a second heterologous transit peptide operably linked to a sequence that encodes an acyl carrier protein.
- Claim 20. The method of claim 19, wherein the pathogen or pest is a nematode.
- Claim 21. The method of claim 20, wherein the nematode is selected from the group consisting of *Heterodera* species, *Globodera* species, *Meloidogyne* species, *Rotylenchulus* species, *Hoplolaimus* species, *Belonolaimus* species, *Pratylenchus* species, *Longidorus* species, *Paratrichodorus* species, *Ditylenchus* species, *Xiphinema* species, *Dolichodorus* species, *Helicotylenchus* species, *Radopholus* species, *Hirschmanniella* species, *Tylenchorhynchus* species, and *Trichodorus* species.

- Claim 22. The method of claim 19, wherein the pathogen or pest is an insect pest.
- Claim 23. The method of claim 22, wherein the insect pest is selected from the group consisting of *Diabrotica*, *Diaprepes*, *Pachnaeus*, *Asynonychus*, *Lycoriella*, *Sciara*, *Stenophlus*, and *Bradysia*.
- Claim 24. A method of producing seed, comprising crossing a plant of claim 14 with itself or a second plant.
- Claim 25. The method of producing seed of claim 24, wherein the polynucleotide sequence comprises a sequence that encodes a second heterologous transit peptide operably linked to a sequence that encodes an acyl carrier protein.
- Claim 26. The polynucleotide of claim 1, further comprising a sequence that encodes a second heterologous transit peptide operably linked to a sequence that encodes an acyl carrier protein.
- Claim 27. The polynucleotide of claim 26, wherein the acyl carrier protein comprises an amino acid sequence exhibiting at least about 85% identity to a polypeptide selected from the group consisting of SEQ ID NO: 41, 43, 45, 47, 49, 51, 53, 55, and 57.
- Claim 28. The polynucleotide of claim 26, wherein said second transit peptide is a chloroplast transit peptide.
- Claim 29. The polynucleotide of claim 26, wherein said second transit peptide is selected from the group consisting of an EPSPS chloroplast transit peptide, a small subunit ribulose-1,5,-bisphosphate carboxylase chloroplast transit peptide, a ferredoxin chloroplast transit peptide, a ferredoxin oxidoreductase chloroplast transit peptide, a light-harvesting complex protein I and protein II chloroplast transit peptide, and a thioredoxin F chloroplast transit peptide.
- Claim 30. The polynucleotide of claim 26, wherein said sequence that encodes an acyl carrier protein exhibits at least about 80% sequence identity to a polynucleotide sequence selected from the group consisting of SEQ ID NO: 40, 42, 44, 46, 48, 50, 52, 54, and 56.

- Claim 31. The plant cell of claim 7, wherein the polynucleotide sequence comprises a sequence that encodes a second heterologous transit peptide operably linked to a sequence that encodes an acyl carrier protein.
- Claim 32. The plant or part thereof of claim 14, wherein the polynucleotide sequence comprises a sequence that encodes a second heterologous transit peptide operably linked to a sequence that encodes an acyl carrier protein.
- Claim 33. A processed product of a plant, plant part, seed or progeny, wherein the product comprises the plant cell of claim 7.
- Claim 34. The processed product of claim 33, wherein the processed product is selected from the group consisting of meal, flour, oil, hay, starch, juice, protein extract, and fiber.

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2009/033560

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N15/82 C12N15/52 C12N9/00 A01H5/00

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N 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, BIOSIS, EMBASE, COMPENDEX, Sequence Search, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages*	Relevant to claim No.
X	FRIDMAN EYAL ET AL: "Metabolic, genomic, and biochemical analyses of glandular trichomes from the wild tomato species <i>Lycopersicon hirsutum</i> identify a key enzyme in the biosynthesis of methylketones" PLANT CELL, vol. 17, no. 4, April 2005 (2005-04), pages 1252-1267, XP002527594 ISSN: 1040-4651 the whole document -/--	1-34



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

14 May 2009

Date of mailing of the international search report

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Name and mailing address of the ISA/

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Bucka, Alexander

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2009/033560

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	-& DATABASE EMBL [Online] 9 April 2005 (2005-04-09), "Lycopersicon hirsutum f. glabratum MKS1 (MKS1) mRNA, complete cds." XP002527597 retrieved from EBI accession no. EMBL:AY701574 Database accession no. AY701574 -----	
A	GU ET AL: "Evaluation and identification of potential organic nematocidal volatiles from soil bacteria" SOIL BIOLOGY AND BIOCHEMISTRY, PERGAMON, OXFORD, GB, vol. 39, no. 10, 20 July 2007 (2007-07-20), pages 2567-2575, XP022162265 ISSN: 0038-0717 table 3 -----	20,21
A	FRIDMAN E ET AL: "Tomato glandular trichomes as a model system for exploring evolution of specialized metabolism in a single cell" RECENT ADVANCES IN PHYTOCHEMISTRY, PLENUM, NEW YORK, NY, US, vol. 40, 1 January 2006 (2006-01-01), pages 115-130, XP008105949 ISSN: 0079-9920 the whole document -----	1-34
A	WO 03/016551 A (THOMPSON BOYCE PLANT RES [US]; KLESSIG DANIEL F [US]; KUMAR DHIRENDRA) 27 February 2003 (2003-02-27) figure 4 -----	1-34
A	WO 2005/118823 A (CERES INC [US]; CHRISTENSEN CORY R [US]; APUYA NESTOR [US]; FELDMANN K) 15 December 2005 (2005-12-15) sequence 29 -----	1-34
A	ERMING WANG ET AL: "Transgenic Nicotiana Tabacum L. with enhanced trichome exudate cembratrieneols has reduced aphid infestation in the field" MOLECULAR BREEDING, KLUWER ACADEMIC PUBLISHERS, DO, vol. 13, no. 1, 1 January 2004 (2004-01-01), pages 49-57, XP019258628 ISSN: 1572-9788 the whole document -----	1-34
	-/--	

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2009/033560

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HOPPMANN V ET AL: "The potato granule bound starch synthase chloroplast transit peptide directs recombinant proteins to plastids" JOURNAL OF PLANT PHYSIOLOGY, FISCHER, STUTTGART, DE, vol. 159, no. 10, 1 January 2002 (2002-01-01), pages 1061-1067, XP004955303 ISSN: 0176-1617 page 1061, right-hand column - page 1062, left-hand column -----	1-34
A	WEI ET AL: "Expression and subcellular compartmentation of Aspergillus niger beta-glucosidase in transgenic tobacco result in an increased insecticidal activity on whiteflies (Bemisia tabaci)" PLANT SCIENCE, ELSEVIER IRELAND LTD, IE, vol. 172, no. 6, 5 May 2007 (2007-05-05), pages 1175-1181, XP022059849 ISSN: 0168-9452 the whole document -----	1-34
A	ANTONIOUS GEORGE F: "Production and quantification of methyl ketones in wild tomato accessions" JOURNAL OF ENVIRONMENTAL SCIENCE AND HEALTH PART B PESTICIDES FOOD CONTAMINANTS AND AGRICULTURAL WASTES, vol. B36, no. 6, November 2001 (2001-11), pages 835-848, XP008105955 ISSN: 0360-1234 the whole document -----	1-34
A	WU S ET AL: "Redirection of cytosolic or plastidic isoprenoid precursors elevates terpene production in plants" NATURE BIOTECHNOLOGY NOVEMBER 2006 NATURE PUBLISHING GROUP US, vol. 24, no. 11, November 2006 (2006-11), pages 1441-1447, XP002527595 the whole document ----- -/--	1-34

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2009/033560

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	FERY R L ET AL: "GENETIC ANALYSIS OF 2-TRIDECANONE CONCENTRATION, LEAF TRICHOME CHARACTERISTICS, AND TOBACCO HORNWORM RESISTANCE IN TOMATO" JOURNAL OF THE AMERICAN SOCIETY FOR HORTICULTURAL SCIENCE, AMERICAN SOCIETY FOR HORTICULTURAL SCIENCE, ALEXANDRIA, VA, US, vol. 112, no. 5, 1 January 1987 (1987-01-01), pages 886-891, XP008105981 ISSN: 0003-1062 the whole document	1-34
A	NIENHUIS J ET AL: "RESTRICTION FRAGMENT LENGTH POLYMORPHISM ANALYSIS OF LOCI ASSOCIATED WITH INSECT RESISTANCE IN TOMATO" CROP SCIENCE, CROP SCIENCE SOCIETY OF AMERICA, MADISON, WI, US, [Online] vol. 27, no. 4, 1 July 1987 (1987-07-01), pages 797-803, XP008105982 ISSN: 0011-183X Retrieved from the Internet: URL: http://crop.scijournals.org/cgi/reprin t/27/4/797> the whole document	1-34
A	MALIEPAARD CHRIS ET AL: "Mapping of QTLs for glandular trichome densities and Trialeurodes vaporariorum (greenhouse whitefly) resistance in an F-2 from Lycopersicon esculentum x Lycopersicon hirsutum f. glabratum" HEREDITY, vol. 75, no. 4, 1995, pages 425-433, XP002527596 ISSN: 0018-067X the whole document	1-34

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2009/033560

Patent document cited in search report		Publication date		Patent family member(s)		Publication date
WO 03016551	A	27-02-2003	AU	2002323249 A1		03-03-2003
WO 2005118823	A	15-12-2005	AU	2005250447 A1		15-12-2005
			BR	PI0510481 A		06-11-2007
			CA	2568367 A1		15-12-2005
			EP	1753865 A2		21-02-2007