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(71) Applicant: **EVOLUTIONARY GENOMICS, INC.**
[US/US]; 1376 Miners Drive, Suite 101, Lafayette, Colorado 80026 (US).

(72) Inventor: **MESSIER, Walter**; c/o EVOLUTIONARY GENOMICS, INC., 1376 Miners Drive, Suite 101, Lafayette, Colorado 80026 (US).

(74) Agents: **VEITENHEIMER, Erich E.** et al.; COOLEY LLP, 1299 Pennsylvania Avenue, N.W., Suite 700, Washington, District of Columbia 20004-2400 (US).

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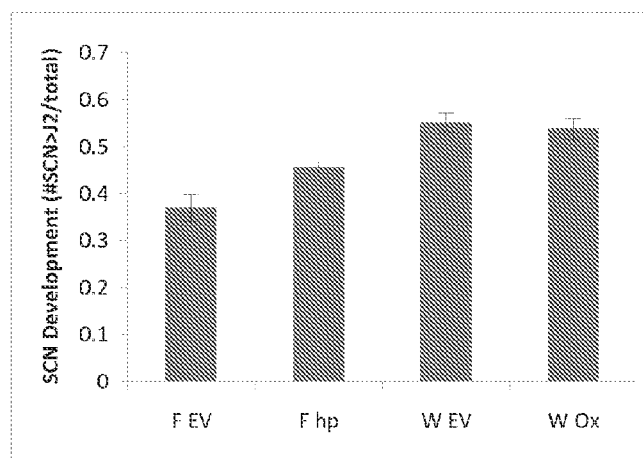


Figure 1.

(57) Abstract: The present invention provides the identification and use of EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations thereof for altering, e.g. increasing, pathogen tolerance and/or resistance in plants.



DIRIGENT GENE EG261 AND ITS ORTHOLOGS AND PARALOGS AND THEIR USES FOR PATHOGEN RESISTANCE IN PLANTS

5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. provisional application No. 61/789,463 filed on March 15, 2013 and U.S. provisional application No. 61/652,029 filed on May 25, 2012 each of which is hereby incorporated by reference in its entirety for all purposes.

10 DESCRIPTION OF THE TEXT FILE SUBMITTED ELECTRONICALLY

The contents of the text file submitted electronically herewith are incorporated herein by reference in their entirety: A computer readable format copy of the Sequence Listing (filename: EVOL_004_01WO_SeqList_ST25.txt, date recorded: May 22, 2013, file size 85 kilobytes).

15

TECHNICAL FIELD

The invention relates to the identification and use of nucleic acid sequences for pathogen resistance in plants.

20

BACKGROUND

“Dirigent” refers to genes or proteins which are members of a gene or protein family, respectively, members of which have been identified in many plants. Dirigents have been implicated in resistance to various types of pathogens in a range of different, and sometimes distantly related, plants.

Dirigent proteins confer a broad response to many pathogens in a number of plants species, including for conifers (Ralph et al., *Plant Molecular Biology* (2006) 60:21–40); cotton (L. Zhu, X. Zhang, L. Tu, F. Zeng, Y. Nie and X. Guo *Journal of Plant Pathology*. 2007. 89 (1), 41–45); barley (Ralph et al., *Plant Molecular Biology*, 2006, 60:21–40 _ DOI 10.1007/s11103-005-2226-y), barley, (Kristensen et al., *Plant Physiology*, June 1999, Vol. 120, pp. 501–512); orange trees (“Gene expression in *Citrus sinensis* (L.) Osbeck following infection with the bacterial pathogen *Candidatus Liberibacter asiaticus* causing Huanglongbing in Florida” Albrecht et al., *Plant Science*, Volume 175, Issue 3, September 2008, Pages 291–306); wheat (poster presentation: “Cloning and Transcriptional Profiling of a Dirigent-like Gene from Wheat Responding to Hessian Fly Infestation”, C. Williams, Poster PO910, Plant and Animal Genome 20); and pea (“Transgenic canola lines expressing pea defense gene DRR206 have resistance to aggressive blackleg isolates and to *Rhizoctonia solani* L.”, Wang and Fristensky, *Molecular Breeding* Volume 8, Number 3 (2001), 263–271, DOI: 10.1023/A:1013706400168). Thus, dirigents have been implicated in pathogen response in many plants, and this response has been shown for a number of different pathogens, including but not limited to fungi, bacteria, insects and nematodes.

The identification and use of dirigents is important to plant husbandry and crop production, particularly for commercial crop production in agronomy and horticulture.

SUMMARY OF THE INVENTION

The present invention provides the identification and use of EG261, homologs of EG261, orthologs of EG261, paralogues of EG261, and fragments and variations thereof for altering, e.g. conferring or increasing, pathogen tolerance and/or resistance in plants. Importantly, such pathogen altered, e.g., increased, tolerance and/or resistance can be obtained using conventional plant breeding methods, whereby such methods optionally also include using any of various biotechnological methods for verifying that the desired EG261, homologs of EG261, orthologs of EG261, paralogues of EG261, and fragments and variations thereof are present in the resulting crosses and offspring.

In embodiments, the present invention provides isolated nucleic acid sequences coding for EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations thereof. The present invention also provides chimeric genes, constructs, recombinant DNA, vectors, plant cells, plant tissues, plant parts, plant tissue
5 cultures and/or whole plants comprising such nucleic acid sequences.

In one embodiment, the present invention provides polynucleotides for altering and/or increasing pathogen tolerance and/or resistance comprising a nucleic acid sequence that shares at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at
10 least 96%, at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% identity to nucleic acids coding for EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations thereof.

The present invention further provides isolated amino acid sequences (e.g., a peptide, polypeptide and the like) comprising an amino acid sequence encoded by the nucleic acid
15 sequences for EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations thereof.

In some embodiments, the present invention provides isolated amino acid sequences which form a protein that shares an amino acid sequence having at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at
20 least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% identity to an amino acid sequence encoded by the nucleic acid sequences for EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations thereof.

In one embodiment, the present invention provides isolated amino acid sequences
25 which form a protein that shares an amino acid having at least 85%, at least 86%, at least 87%, at least 88%, at least 89%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% identity to an amino acid sequence encoded by the nucleic acid

sequences for EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations thereof.

The present invention also provides a chimeric gene comprising the isolated nucleic acid sequence of any one of the polynucleotides described above operably linked to suitable regulatory sequences.

The present invention also provides a recombinant construct comprising the chimeric genes as described above.

The present invention further comprises interfering RNA (RNAi) based on the expression of the nucleic acid sequences of the present invention, wherein such RNAi includes but is not limited to microRNA (miRNA) and small interfering RNA (siRNA) which can be used in gene silencing constructs.

The present invention also provides transformed host cells comprising the chimeric genes as described above. In one embodiment, said host cells are selected from the group consisting of bacteria, yeasts, filamentous fungi, algae, animals, and plants.

The present invention in another aspect provides plants comprising in its genome one or more genes as described herein, one or more genes with mutations as described herein, or the chimeric genes as described herein. In some embodiments, the plant is derived from a soybean variety sensitive to soybean cyst nematode (SCN), and wherein the gene comprises nucleic acid sequence encoding an EG261 ortholog derived from *G. pescadrensis*.

The present invention in another aspect provides plant seed obtained from the plants described herein, wherein the plants producing such seeds comprise in their genomes one or more genes as described herein, one or more genes with mutations as described herein, or the chimeric genes as described herein.

In some embodiments, the methods comprise introducing mutations in one or more nucleic acid sequences for EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations thereof.

In one aspect, the present invention provides methods of breeding plants to alter, e.g. confer or increase, pathogen tolerance and/or resistance. In one embodiment, such methods comprise making a cross between a first plant comprising one or more nucleic acid sequences

for EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations with a second plant of the same or different species to produce an F1 plant; backcrossing the F1 plant to the second plant; and repeating the backcrossing step to generate a near isogenic line, wherein the one or more nucleic acid sequences for EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations thereof are integrated into the genome of the second plant; wherein the near isogenic line derived from the second plant has altered, e.g. increased, pathogen tolerance and/or resistance. Optionally, such methods can be facilitated by using various biotechnological methods to verify that the nucleic acid sequences for EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations thereof are included in the second plant. In some embodiments, the first plant comprises a nucleic acid sequence encoding an EG261 ortholog derived from *G. pescadrensis*. In some embodiments, the second plant is a soybean variety sensitive to soybean cyst nematode (SCN).

5 The present invention provides isolated polynucleotides comprising a nucleic acid sequence selected from the group consisting of: (a) a nucleic acid sequence having at least 95% identical nucleotides to a nucleic acid sequence coding for EG261; (b) a nucleic acid sequence having at least 95% identical nucleotides to a nucleic acid sequence coding for a
10 homolog of EG261; (c) a nucleic acid sequence having at least 95% identical nucleotides to a nucleic acid sequence coding for an ortholog of EG261; (d) a nucleic acid sequence having at least 95% identical nucleotides to a nucleic acid sequence coding for a paralog of EG261; (e) complements of a nucleic acid sequence of (a), (b), (c) or (d); (f) reverse complements of a nucleic acid sequence of (a), (b), (c) or (d); (g) reverse sequences of a nucleic acid sequence
15 of (a), (b), (c) or (d); (h) mRNA sequences of a nucleic acid sequence of (a), (b), (c) or (d); and, (i) fragments and variations of a nucleic acid sequence of (a), (b), (c), (d), (e), (f), (g), and (h).

The present invention provides vectors comprising one or more of the polynucleotides described herein.

15 The present invention also provides genetic constructs comprising one or more of the isolated polynucleotides described herein.

The present invention also provides genetic constructs comprising, in the 5'-3' direction: (a) a promoter sequence, (b) one or more of the isolated polynucleotides described herein; and (c) a gene termination sequence.

5 In some embodiments of the present invention the genetic constructs comprise an open reading frame encoding a polypeptide capable of altering pathogen tolerance and/or resistance.

In some embodiments of the present invention the pathogen tolerance and/or resistance is conferred or enhanced.

10 In some embodiments of the present invention the pathogen is soybean cyst nematode.

The present invention further provides transgenic cells comprising the genetic constructs described herein.

The present invention also provides organisms comprising the transgenic cells described herein.

15 In some embodiments of the present invention the organism is a plant. In some specific embodiments of the present invention the plant is a soybean plant.

The present invention provides plants and progeny plants thereof having altered pathogen tolerance and/or resistance as a result of inheriting the polynucleotides described herein.

20 The present invention provides methods of producing hybrid seed which include crossing the plants and progeny plants described herein with a different plant of the same species, and harvesting the resultant seed.

The present invention also provides methods for modifying gene expression in a target organism comprising stably incorporating into the genome of the organism a genetic
25 construct described herein. In some such embodiments of the present invention the organism is a plant. In some specific embodiments the plant is a soybean plant, a garden bean, or a cowpea plant.

The present invention provides methods for producing a plant having altered pathogen tolerance and/or resistance comprising: (a) transforming a plant cell with a genetic construct

to provide a transgenic cell, wherein the genetic construct comprises: (i) a promoter sequence; (ii) an isolated polynucleotide sequence of the present invention as described herein; and (iii) a gene termination sequence; and (b) cultivating the transgenic cell under conditions conducive to regeneration and mature plant growth of a plant having altered pathogen tolerance and/or resistance. In some such embodiments the plant cell is a soybean plant cell, a garden bean plant cell, or a cowpea plant cell.

The present invention also provides methods for modifying a phenotype of a target organism, comprising stably incorporating into the genome of the target organism a genetic construct comprising: (a) a promoter sequence; (b) an isolated polynucleotide sequence of the present invention as described herein; and (c) a gene termination sequence. In some such embodiments the target organism is a plant. In some specific embodiments the plant is a soybean plant, a garden bean plant, or a cowpea plant.

The present invention provides processes of determining the presence or absence of a polynucleotide coding for EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations thereof in a plant, wherein the process comprises at least one of: (a) isolating nucleic acid molecules from said plant and amplifying sequences homologous to the polynucleotide; (b) isolating nucleic acid molecules from said plant and performing a Southern hybridization to detect the polynucleotide; (c) isolating proteins from said plant and performing a Western Blot using antibodies to a protein encoded by the polynucleotide; and/or (d) demonstrating the presence of mRNA sequences derived from a polynucleotide mRNA transcript and unique to the polynucleotide.

The present invention provides methods of breeding plants to produce altered pathogen tolerance and/or resistance comprising: i) making a cross between a first plant with an isolated polynucleotide sequence of claim 1 with a second plant to produce a F1 plant; ii) backcrossing the F1 plant to the second plant; and iii) repeating the backcrossing step to generate a near isogenic or isogenic line, wherein the isolated polynucleotide sequence of claim 1 is integrated into the genome of the second plant and the near isogenic or isogenic line derived from the second plant with the isolated polynucleotide sequence has conferred or enhanced pathogen tolerance and/or resistance compared to that of the second plant without

the isolated polynucleotide sequence. In some such embodiments of the present invention the plant is soybean. In some such embodiments of the present invention the pathogen is soybean cyst nematode. In some embodiments, the first plant comprises a nucleic acid sequence encoding an EG261 ortholog derived from *G. pescadrensis*. In some embodiments, the second plant is a soybean variety sensitive to soybean cyst nematode (SCN).

The present invention provides methods of producing a plant with conferred or enhanced pathogen tolerance and/or resistance, the process comprising: (a) crossing a first plant containing a polynucleotide coding for EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations thereof to a second plant, and harvesting the resultant seed; (b) determining the presence of the polynucleotide in the resultant seed or in cells or tissues of a plant grown from the resultant seed; wherein the determining comprises at least one of: (i) isolating nucleic acid molecules from the resultant seed or in cells or tissues of a plant grown from the resultant seed and amplifying sequences homologous to the polynucleotide; (ii) isolating nucleic acid molecules from the resultant seed or in cells or tissues of a plant grown from the resultant seed and performing a Southern hybridization to detect the polynucleotide; (iii) isolating proteins from the resultant seed or in cells or tissues of a plant grown from the resultant seed and performing a Western Blot using antibodies to a protein encoded by the polynucleotide; and/or (iv) demonstrating the presence in the resultant seed or in cells or tissues of a plant grown from the resultant seed of mRNA sequences derived from a polynucleotide mRNA transcript and unique to the polynucleotide. In some such embodiments the method further comprises confirming that the resultant seed or the cells or tissues of the plant grown from the resultant seed contain the polynucleotide. In some such embodiments the methods further comprise using the resultant plant containing the polynucleotide in a plant breeding scheme. In some embodiments the methods further comprise crossing the resultant plant containing the polynucleotide with another plant of the same species. In some embodiments, the first plant comprises a nucleic acid sequence encoding an EG261 ortholog derived from *G. pescadrensis*. In some embodiments, the second plant is a soybean variety sensitive to soybean cyst nematode (SCN).

The present invention also provides methods of introducing the EG261 orthologs conferring nematode resistance isolated according to the present invention into a nematode-susceptible plant of the same or different species in order to increase nematode resistance of the plant and the plants produced by such methods.

5 The present invention also provides methods of introducing the EG261 orthologs of the present invention into nematode-resistant plants of the same or different species in order to further increase nematode resistance of the plant and the plants produced by such methods. For example, according to the present invention, a gene encoding any one of the proteins of SEQ ID NOs: 9-15, 25, 26, 27, 28, 29, 30, 31, 32, 33, 37, 38, 39, 43, 44, 45, 51, 52, 53, 54,
10 55, 57, 59, 61, 63, 64, or 65 can be introduced into nematode resistant plants containing any nematode resistant traits such as *rhg1*, *rhg4*, or any other known QTLs such as those listed in Concibido et al., 2004 (A decade of QTL mapping for cyst nematode resistance in soybean, Crop Sci. 44: 1121-1131); Shannon et al., (2004) Breeding for resistance and tolerance, In: Biology and management of soybean cyst nematode, 2nd ed, P. Schmitt, J. A. Wrather, and R.
15 D. Riggs, (editors). Pg155-180; and Klink et al., (2013) Engineered Soybean Cyst Nematode Resistance, In: Soybean-Pest Resistance, Hany A. El-Shemy (editor), pg- 139-172.

The present invention also provides incorporating nematode resistance into a plant via non-transgenic (i.e., traditional) methods such as plant breeding and the plants produced by such methods. For example, according to the present invention, a cross can be made between
20 a first plant with a naturally-occurring or transgenic EG261 gene copy conferring nematode resistance, and a second plant to produce a F1 plant. This F1 plant can then be subjected to multiple backcrossings to generate a near isogenic or isogenic line, wherein the nematode resistant copy of EG261 is integrated into the genome of the second plant.

25 The EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and/or fragments and variations thereof of the present invention provide broad pathogen resistance in many, distantly related plant species. For example, orthologs of EG261 may be effective in a number of commercially important crop species, including, e.g., soybeans, garden beans, cowpeas, and cotton.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts results showing that silencing of EG261 reduces the SCN resistance of “Fayette”, a SCN-resistant variety of soybean.

Figure 2 depicts sequence alignments of dirigent proteins encoded by the dirigent
5 genes of SEQ ID NOs 9 to 15.

Figure 3 depicts sequence alignments of dirigent proteins encoded by the dirigent genes of *Solanaceae* family plant species.

Figure 4 depicts sequence alignments of dirigent proteins encoded by the dirigent genes of monocot plant species.

10

DETAILED DESCRIPTION

Definitions

The term “a” or “an” refers to one or more of that entity; for example, “a gene” refers
15 to one or more genes or at least one gene. As such, the terms “a” (or “an”), “one or more” and “at least one” are used interchangeably herein. In addition, reference to “an element” by the indefinite article “a” or “an” does not exclude the possibility that more than one of the elements is present, unless the context clearly requires that there is one and only one of the elements.

20 As used herein, the verb “comprise” as is used in this description and in the claims and its conjugations are used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded.

As used herein, the term “plant” refers to any living organism belonging to the kingdom Plantae (i.e., any genus/species in the Plant Kingdom), including but not limited to,
25 *Glycine spp.* (e.g., soybean), *Solanaceae* species (e.g., *Solanum lycopersicum*, *Solanum chmielewskii*, *Solanum habrochaites*, *Solanum corneliomulleri*, *Capsicum annuum*, *Solanum melongena*, *Solanum tuberosum*), *Phaseolus vulgaris*, *Vigna unguiculata*, *Coffea arabica*, *Zea mays*, *Sorghum spp.*, *Oryza sativa*, *Triticum spp.*, *Hordeum spp.*, *Gossypium hirsutum*, and *Heliotropium curassavicum*.

As used herein, the term “plant part” refers to any part of a plant including but not limited to the shoot, root, stem, seeds, fruits, stipules, leaves, petals, flowers, ovules, bracts, branches, petioles, internodes, bark, pubescence, tillers, rhizomes, fronds, blades, pollen, stamen, rootstock, scion and the like. The two main parts of plants grown in some sort of media, such as soil, are often referred to as the “above-ground” or “aerial” part, also often referred to as the “shoots”, and the “below-ground” part, also often referred to as the “roots”.

As used herein when discussing plants, the term “ovule” refers to the female gametophyte, whereas the term “pollen” means the male gametophyte.

As used herein, the term “plant tissue” refers to any part of a plant. Examples of plant organs include, but are not limited to the leaf, stem, root, tuber, seed, branch, pubescence, nodule, leaf axil, flower, pollen, stamen, pistil, petal, peduncle, stalk, stigma, style, bract, fruit, trunk, carpel, sepal, anther, ovule, pedicel, needle, cone, rhizome, stolon, shoot, pericarp, endosperm, placenta, berry, stamen, and leaf sheath.

As used herein, the term “phenotype” refers to the observable characters of an individual cell, cell culture, organism (e.g., a plant), or group of organisms which results from the interaction between that individual’s genetic makeup (i.e., genotype) and the environment.

As used herein, the term “nucleic acid” refers to a polymeric form of nucleotides of any length, either ribonucleotides or deoxyribonucleotides, or analogs thereof. This term refers to the primary structure of the molecule, and thus includes double- and single-stranded DNA, as well as double- and single-stranded RNA. It also includes modified nucleic acids such as methylated and/or capped nucleic acids, nucleic acids containing modified bases, backbone modifications, and the like. The terms “nucleic acid” and “nucleotide sequence” are used interchangeably.

As used herein, the term “nucleotide change” or “nucleotide modification” refers to, e.g., nucleotide substitution, deletion, and/or insertion, as is well understood in the art. For example, such nucleotide changes/modifications include mutations containing alterations that produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded protein or how the proteins are made. As another example, such nucleotide

changes/modifications include mutations containing alterations that produce replacement substitutions, additions, or deletions, that alter the properties or activities of the encoded protein or how the proteins are made.

As used herein, the terms "polypeptide," "peptide," and "protein" are used interchangeably herein to refer to polymers of amino acids of any length. These terms also include proteins that are post-translationally modified through reactions that include glycosylation, acetylation and phosphorylation.

As used herein, the term "protein modification" refers to, e.g., amino acid substitution, amino acid modification, deletion, and/or insertion, as is well understood in the art.

As used herein, the term "derived from" refers to the origin or source, and may include naturally occurring, recombinant, unpurified, or purified molecules. A nucleic acid or an amino acid derived from an origin or source may have all kinds of nucleotide changes or protein modification as defined elsewhere herein.

As used herein, the term "pathogen" refers to an agent that causes disease, especially a living microorganism such as an insect, a bacterium, virus, nematode or fungus.

As used herein, the term "nematode" refers to any of several worms of the phylum Nematoda, having unsegmented, cylindrical bodies, often narrowing at each end, and including parasitic forms such as the hookworm and pinworm. They are referred to as "roundworms" in some contexts. Nematode species are very difficult to distinguish, with over 28,000 having been described. Plant-parasitic nematodes include several groups causing severe crop losses. The most common genera are *Aphelenchoides* (foliar nematodes), *Ditylenchus*, *Globodera* (potato cyst nematodes), *Heterodera* (soybean cyst nematodes), *Longidorus*, *Meloidogyne* (root-knot nematodes), *Nacobbus*, *Pratylenchus* (lesion nematodes), *Trichodorus* and *Xiphinema* (dagger nematodes). Several phytoparasitic nematode species cause histological damages to roots, including the formation of visible galls (e.g. by root-knot nematodes), which are useful characters for their diagnostic in the field. Some nematode species transmit plant viruses through their feeding activity on roots. One of them is *Xiphinema index*, vector of grapevine fanleaf virus), an important disease of grapes.

Other nematodes attacks bark and forest trees. The most important representative of this group is *Bursaphelenchus xylophilus*, the pine wood nematode, present in Asia and America and recently discovered in Europe.

As used herein, the phrase “soybean cyst nematode” and the term “SCN” each refer to *Heterodera glycines*, a plant-parasitic nematode and a devastating pest of the soybean including *Glycine max*, worldwide. The nematode infects the roots of soybean, and the female nematode eventually becomes a cyst. Infection causes various symptoms that may include chlorosis of the leaves and stems, root necrosis, loss in seed yield and suppression of root and shoot growth. SCN has threatened the U.S. crop since the 1950s, reducing returns to soybean producers by \$500 million each year and reducing yields by as much as 75 percent. It is also a significant problem in the soybean growing areas of the world, including South America and Asia.

As used herein, the term “resistant”, or “resistance”, describes a plant, line or cultivar that shows fewer or reduced symptoms to a biotic pest or pathogen than a susceptible (or more susceptible) plant, line or variety to that biotic pest or pathogen. These terms are variously applied to describe plants that show no symptoms as well as plants showing some symptoms but that are still able to produce marketable product with an acceptable yield. Some lines that are referred to as resistant are only so in the sense that they may still produce a crop, even though the plants may appear visually stunted and the yield is reduced compared to uninfected plants. As defined by the International Seed Federation (ISF), a non-governmental, non-profit organization representing the seed industry (see “Definition of the Terms Describing the Reaction of Plants to Pests or Pathogens and to Abiotic Stresses for the Vegetable Seed Industry”, May 2005), the recognition of whether a plant is affected by or subject to a pest or pathogen can depend on the analytical method employed. Resistance is defined by the ISF as the ability of plant types to restrict the growth and development of a specified pest or pathogen and/or the damage they cause when compared to susceptible plant varieties under similar environmental conditions and pest or pathogen pressure. Resistant plant types may still exhibit some disease symptoms or damage. Two levels of resistance are defined. The term “high/standard resistance” is used for plant varieties that highly restrict the

growth and development of the specified pest or pathogen under normal pest or pathogen pressure when compared to susceptible varieties. "Moderate/intermediate resistance" is applied to plant types that restrict the growth and development of the specified pest or pathogen, but exhibit a greater range of symptoms or damage compared to plant types with high resistance. Plant types with intermediate resistance will show less severe symptoms than susceptible plant varieties, when grown under similar field conditions and pathogen pressure. Methods of evaluating resistance are well known to one skilled in the art. Such evaluation may be performed by visual observation of a plant or a plant part (e.g., leaves, roots, flowers, fruits et. al) in determining the severity of symptoms. For example, when each plant is given a resistance score on a scale of 1 to 5 based on the severity of the reaction or symptoms, with 1 being the resistance score applied to the most resistant plants (e.g., no symptoms, or with the least symptoms), and 5 the score applied to the plants with the most severe symptoms, then a line is rated as being resistant when at least 75% of the plants have a resistance score at a 1, 2, or 3 level, while susceptible lines are those having more than 25% of the plants scoring at a 4 or 5 level. If a more detailed visual evaluation is possible, then one can use a scale from 1 to 10 so as to broaden out the range of scores and thereby hopefully provide a greater scoring spread among the plants being evaluated.

Another scoring system is a root inoculation test based on the development of the necrosis after inoculation and its position towards the cotyledon (such as one derived from Bosland et al., 1991), wherein 0 stands for no symptom after infection; 1 stands for a small necrosis at the hypocotyl after infection; 2 stands a necrosis under the cotyledons after infection; 3 stands for necrosis above the cotyledons after infection; 4 stands for a necrosis above the cotyledons together with a wilt of the plant after infection, while eventually, 5 stands for a dead plant.

In addition to such visual evaluations, disease evaluations can be performed by determining the pathogen bio-density in a plant or plant part using electron microscopy and/or through molecular biological methods, such as protein hybridization (e.g., ELISA, measuring pathogen protein density) and/or nucleic acid hybridization (e.g., RT-PCR, measuring pathogen RNA density). Depending on the particular pathogen/plant combination,

a plant may be determined resistant to the pathogen, for example, if it has a pathogen RNA/DNA and/or protein density that is about 50%, or about 40%, or about 30%, or about 20%, or about 10%, or about 5%, or about 2%, or about 1%, or about 0.1%, or about 0.01%, or about 0.001%, or about 0.0001% of the RNA/DNA and/or protein density in a susceptible plant.

Methods used in breeding plants for disease resistance are similar to those used in breeding for other characters. It is necessary to know as much as possible about the nature of inheritance of the resistant characters in the host plant and the existence of physiological races or strains of the pathogen.

As used herein, the term “full resistance” is referred to as complete failure of the pathogen to develop after infection, and may either be the result of failure of the pathogen to enter the cell (no initial infection) or may be the result of failure of the pathogen to multiply in the cell and infect subsequent cells (no subliminal infection, no spread). The presence of full resistance may be determined by establishing the absence of pathogen protein or pathogen RNA in cells of the plant, as well as the absence of any disease symptoms in said plant, upon exposure of said plant to an infective dosage of pathogen (i.e. after ‘infection’). Among breeders, this phenotype is often referred to as “immune”. “Immunity” as used herein thus refers to a form of resistance characterized by absence of pathogen replication even when the pathogen is actively transferred into cells by e.g. electroporation.

As used herein, the term “partial resistance” is referred to as reduced multiplication of the pathogen in the cell, as reduced (systemic) movement of the pathogen, and/or as reduced symptom development after infection. The presence of partial resistance may be determined by establishing the systemic presence of low concentration of pathogen protein or pathogen RNA in the plant and the presence of decreased or delayed disease-symptoms in said plant upon exposure of said plant to an infective dosage of pathogen. Protein concentration may be determined by using a quantitative detection method (e.g. an ELISA method or a quantitative reverse transcriptase-polymerase chain reaction (RT-PCR)). Among breeders, this phenotype is often referred to as “intermediate resistant.”

As used herein, the term “tolerant” is used herein to indicate a phenotype of a plant wherein disease-symptoms remain absent upon exposure of said plant to an infective dosage of pathogen, whereby the presence of a systemic or local pathogen infection, pathogen multiplication, at least the presence of pathogen genomic sequences in cells of said plant and/or genomic integration thereof can be established. Tolerant plants are therefore resistant for symptom expression but symptomless carriers of the pathogen. Sometimes, pathogen sequences may be present or even multiply in plants without causing disease symptoms. This phenomenon is also known as “latent infection”. In latent infections, the pathogen may exist in a truly latent non-infectious occult form, possibly as an integrated genome or an episomal agent (so that pathogen protein cannot be found in the cytoplasm, while PCR protocols may indicate the present of pathogen nucleic acid sequences) or as an infectious and continuously replicating agent. A reactivated pathogen may spread and initiate an epidemic among susceptible contacts. The presence of a “latent infection” is indistinguishable from the presence of a “tolerant” phenotype in a plant.

As used herein, the term “susceptible” is used herein to refer to a plant having no or virtually no resistance to the pathogen resulting in entry of the pathogen into the plant and multiplication and systemic spread of the pathogen, resulting in disease symptoms. The term “susceptible” is therefore equivalent to “non-resistant”.

As used herein, the term “offspring” refers to any plant resulting as progeny from a vegetative or sexual reproduction from one or more parent plants or descendants thereof. For instance an offspring plant may be obtained by cloning or selfing of a parent plant or by crossing two parents plants and include selfings as well as the F1 or F2 or still further generations. An F1 is a first-generation offspring produced from parents at least one of which is used for the first time as donor of a trait, while offspring of second generation (F2) or subsequent generations (F3, F4, etc.) are specimens produced from selfings of F1's, F2's etc. An F1 may thus be (and usually is) a hybrid resulting from a cross between two true breeding parents (true-breeding is homozygous for a trait), while an F2 may be (and usually is) an offspring resulting from self-pollination of said F1 hybrids.

As used herein, the term “cross”, “crossing”, “cross pollination” or “cross-breeding” refer to the process by which the pollen of one flower on one plant is applied (artificially or naturally) to the ovule (stigma) of a flower on another plant.

As used herein, the term “cultivar” refers to a variety, strain or race of plant that has
5 been produced by horticultural or agronomic techniques and is not normally found in wild populations.

As used herein, the terms “dicotyledon,” “dicot” and “dicotyledonous” refer to a flowering plant having an embryo containing two seed halves or cotyledons. Examples include tobacco; tomato; the legumes, including peas, alfalfa, clover and soybeans; oaks;
10 maples; roses; mints; squashes; daisies; walnuts; cacti; violets and buttercups.

As used herein, the term “monocotyledon,” “monocot” or “monocotyledonous” refer to any of a subclass (Monocotyledoneae) of flowering plants having an embryo containing only one seed leaf and usually having parallel-veined leaves, flower parts in multiples of three, and no secondary growth in stems and roots. Examples include lilies; orchids; rice;
15 corn, grasses, such as tall fescue, goat grass, and Kentucky bluegrass; grains, such as wheat, oats and barley; irises; onions and palms.

As used herein, the term “gene” refers to any segment of DNA associated with a biological function. Thus, genes include, but are not limited to, coding sequences and/or the regulatory sequences required for their expression. Genes can also include nonexpressed
20 DNA segments that, for example, form recognition sequences for other proteins. Genes can be obtained from a variety of sources, including cloning from a source of interest or synthesizing from known or predicted sequence information, and may include sequences designed to have desired parameters.

As used herein, the term “genotype” refers to the genetic makeup of an individual
25 cell, cell culture, tissue, organism (e.g., a plant), or group of organisms.

As used herein, the term “allele(s)” means any of one or more alternative forms of a gene, all of which alleles relate to at least one trait or characteristic. In a diploid cell, the two alleles of a given gene occupy corresponding loci on a pair of homologous chromosomes. Since the present invention relates to QTLs, i.e. genomic regions that may comprise one or

more genes or regulatory sequences, it is in some instances more accurate to refer to “haplotype” (i.e. an allele of a chromosomal segment) instead of “allele”, however, in those instances, the term “allele” should be understood to comprise the term “haplotype”. Alleles are considered identical when they express a similar phenotype. Differences in sequence are possible but not important as long as they do not influence phenotype.

As used herein, the term “locus” (plural: “loci”) refers to any site that has been defined genetically. A locus may be a gene, or part of a gene, or a DNA sequence that has some regulatory role, and may be occupied by different sequences.

As used herein, the term “molecular marker” or “genetic marker” refers to an indicator that is used in methods for visualizing differences in characteristics of nucleic acid sequences. Examples of such indicators are restriction fragment length polymorphism (RFLP) markers, amplified fragment length polymorphism (AFLP) markers, single nucleotide polymorphisms (SNPs), insertion mutations, microsatellite markers (SSRs), sequence- characterized amplified regions (SCARs), cleaved amplified polymorphic sequence (CAPS) markers or isozyme markers or combinations of the markers described herein which defines a specific genetic and chromosomal location. Mapping of molecular markers in the vicinity of an allele is a procedure which can be performed quite easily by the average person skilled in molecular-biological techniques which techniques are for instance described in Lefebvre and Chevre, 1995; Lorez and Wenzel, 2007, Srivastava and Narula, 2004, Meksem and Kahl, 2005, Phillips and Vasil, 2001. General information concerning AFLP technology can be found in Vos et al. (1995, AFLP: a new technique for DNA fingerprinting, *Nucleic Acids Res.* 1995 November 11; 23(21): 4407–4414).

As used herein, the term “hemizygous” refers to a cell, tissue or organism in which a gene is present only once in a genotype, as a gene in a haploid cell or organism, a sex-linked gene in the heterogametic sex, or a gene in a segment of chromosome in a diploid cell or organism where its partner segment has been deleted.

As used herein, the term “heterozygote” refers to a diploid or polyploid individual cell or plant having different alleles (forms of a given gene) present at least at one locus.

As used herein, the term “heterozygous” refers to the presence of different alleles (forms of a given gene) at a particular gene locus.

As used herein, the term “homozygote” refers to an individual cell or plant having the same alleles at one or more loci.

5 As used herein, the term “homozygous” refers to the presence of identical alleles at one or more loci in homologous chromosomal segments.

As used herein, the term “homologous” or “homolog” is known in the art and refers to related sequences that share a common ancestor or family member and are determined based on the degree of sequence identity. The terms “homology”, “homologous”, “substantially similar” and “corresponding substantially” are used interchangeably herein. Homologs usually control, mediate, or influence the same or similar biochemical pathways, yet particular homologs may give rise to differing phenotypes. It is therefore understood, as those skilled in the art will appreciate, that the invention encompasses more than the specific exemplary sequences. These terms describe the relationship between a gene found in one
10 species, subspecies, variety, cultivar or strain and the corresponding or equivalent gene in another species, subspecies, variety, cultivar or strain. For purposes of this invention homologous sequences are compared.

The term “homolog” is sometimes used to apply to the relationship between genes separated by the event of speciation (see “ortholog”) or to the relationship between genes
20 separated by the event of genetic duplication (see “paralog”).

The term “ortholog” refers to genes in different species that evolved from a common ancestral gene by speciation. Normally, orthologs retain the same function in the course of evolution. Identification of orthologs is critical for reliable prediction of gene function in newly sequenced genomes.

25 The term “paralog” refers to genes related by duplication within a genome. While orthologs generally retain the same function in the course of evolution, paralogs can evolve new functions, even if these are related to the original one.

“Homologous sequences” or “homologs” or “orthologs” are thought, believed, or known to be functionally related. A functional relationship may be indicated in any one of a

number of ways, including, but not limited to: (a) degree of sequence identity and/or (b) the same or similar biological function. Preferably, both (a) and (b) are indicated. The degree of sequence identity may vary, but in one embodiment, is at least 50% (when using standard sequence alignment programs known in the art), at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least about 91%, at least about 92%, at least about 93%, at least about 94%, at least about 95%, at least about 96%, at least about 97%, at least about 98%, or at least 98.5%, or at least about 99%, or at least 99.5%, or at least 99.8%, or at least 99.9%. Homology can be determined using software programs readily available in the art, such as those discussed in *Current Protocols in Molecular Biology* (F.M. Ausubel *et al.*, eds., 1987) Supplement 30, section 7.718, Table 7.71. Some alignment programs are MacVector (Oxford Molecular Ltd, Oxford, U.K.) and ALIGN Plus (Scientific and Educational Software, Pennsylvania). Other non-limiting alignment programs include Sequencher (Gene Codes, Ann Arbor, Michigan), AlignX, and Vector NTI (Invitrogen, Carlsbad, CA).

As used herein, the term “hybrid” refers to any individual cell, tissue or plant resulting from a cross between parents that differ in one or more genes.

As used herein, the term “inbred” or “inbred line” refers to a relatively true-breeding strain.

The term “single allele converted plant” as used herein refers to those plants which are developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered in addition to the single allele transferred into the inbred via the backcrossing technique.

As used herein, the term “line” is used broadly to include, but is not limited to, a group of plants vegetatively propagated from a single parent plant, via tissue culture techniques or a group of inbred plants which are genetically very similar due to descent from a common parent(s). A plant is said to “belong” to a particular line if it (a) is a primary transformant (T0) plant regenerated from material of that line; (b) has a pedigree comprised of a T0 plant of that line; or (c) is genetically very similar due to common ancestry (e.g., via inbreeding or selfing). In this context, the term “pedigree” denotes the lineage of a plant, e.g.

in terms of the sexual crosses affected such that a gene or a combination of genes, in heterozygous (hemizygous) or homozygous condition, imparts a desired trait to the plant.

As used herein, the terms “introgression”, “introgressed” and “introgressing” refer to the process whereby genes of one species, variety or cultivar are moved into the genome of another species, variety or cultivar, by crossing those species. The crossing may be natural or artificial. The process may optionally be completed by backcrossing to the recurrent parent, in which case introgression refers to infiltration of the genes of one species into the gene pool of another through repeated backcrossing of an interspecific hybrid with one of its parents. An introgression may also be described as a heterologous genetic material stably integrated in the genome of a recipient plant.

As used herein, the term “population” means a genetically homogeneous or heterogeneous collection of plants sharing a common genetic derivation.

As used herein, the term “variety” or “cultivar” means a group of similar plants that by structural features and performance can be identified from other varieties within the same species. The term “variety” as used herein has identical meaning to the corresponding definition in the International Convention for the Protection of New Varieties of Plants (UPOV treaty), of Dec. 2, 1961, as Revised at Geneva on Nov. 10, 1972, on Oct. 23, 1978, and on Mar. 19, 1991. Thus, “variety” means a plant grouping within a single botanical taxon of the lowest known rank, which grouping, irrespective of whether the conditions for the grant of a breeder's right are fully met, can be i) defined by the expression of the characteristics resulting from a given genotype or combination of genotypes, ii) distinguished from any other plant grouping by the expression of at least one of the said characteristics and iii) considered as a unit with regard to its suitability for being propagated unchanged.

As used herein, the term “mass selection” refers to a form of selection in which individual plants are selected and the next generation propagated from the aggregate of their seeds. More details of mass selection are described herein in the specification.

As used herein, the term “open pollination” refers to a plant population that is freely exposed to some gene flow, as opposed to a closed one in which there is an effective barrier to gene flow.

As used herein, the terms “open-pollinated population” or “open-pollinated variety” refer to plants normally capable of at least some cross-fertilization, selected to a standard, that may show variation but that also have one or more genotypic or phenotypic characteristics by which the population or the variety can be differentiated from others. A hybrid, which has no
5 barriers to cross-pollination, is an open-pollinated population or an open-pollinated variety.

As used herein, the term “self-crossing”, “self pollinated” or “self-pollination” means the pollen of one flower on one plant is applied (artificially or naturally) to the ovule (stigma) of the same or a different flower on the same plant.

As used herein, the term “cross”, “crossing”, “cross pollination” or “cross-breeding”
10 refer to the process by which the pollen of one flower on one plant is applied (artificially or naturally) to the ovule (stigma) of a flower on another plant.

As used herein, the term “derived from” refers to the origin or source, and may include naturally occurring, recombinant, unpurified, or purified molecules. A nucleic acid or an amino acid derived from an origin or source may have all kinds of nucleotide changes
15 or protein modification as defined elsewhere herein.

As used herein, the term “at least a portion” of a nucleic acid or polypeptide means a portion having the minimal size characteristics of such sequences, or any larger fragment of the full length molecule, up to and including the full length molecule. For example, a portion of a nucleic acid may be 12 nucleotides, 13 nucleotides, 14 nucleotides, 15 nucleotides, 16
20 nucleotides, 17 nucleotides, 18 nucleotides, 19 nucleotides, 20 nucleotides, 22 nucleotides, 24 nucleotides, 26 nucleotides, 28 nucleotides, 30 nucleotides, 32 nucleotides, 34 nucleotides, 36 nucleotides, 38 nucleotides, 40 nucleotides, 45 nucleotides, 50 nucleotides, 55 nucleotides, and so on, going up to the full length nucleic acid. Similarly, a portion of a polypeptide may be 4 amino acids, 5 amino acids, 6 amino acids, 7 amino acids, and so on, going up to the full
25 length polypeptide. The length of the portion to be used will depend on the particular application. A portion of a nucleic acid useful as hybridization probe may be as short as 12 nucleotides; in one embodiment, it is 20 nucleotides. A portion of a polypeptide useful as an epitope may be as short as 4 amino acids. A portion of a polypeptide that performs the function of the full-length polypeptide would generally be longer than 4 amino acids.

As used herein, "sequence identity" or "identity" in the context of two nucleic acid or polypeptide sequences includes reference to the residues in the two sequences which are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. Where sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences which differ by such conservative substitutions are said to have "sequence similarity" or "similarity." Means for making this adjustment are well-known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., according to the algorithm of Meyers and Miller, *Computer Applic. Biol. Sci.*, 4:11-17 (1988).

As used herein, the term "suppression" or "disruption" of regulation refers to reduced activity of regulatory proteins, and such reduced activity can be achieved by a variety of mechanisms including antisense, mutation knockout or RNAi. Antisense RNA will reduce the level of expressed protein resulting in reduced protein activity as compared to wild type activity levels. A mutation in the gene encoding a protein may reduce the level of expressed protein and/or interfere with the function of expressed protein to cause reduced protein activity.

As used herein, the terms "polynucleotide", "polynucleotide sequence", "nucleic acid sequence", "nucleic acid fragment", and "isolated nucleic acid fragment" are used interchangeably herein. These terms encompass nucleotide sequences and the like. A polynucleotide may be a polymer of RNA or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in

the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof. Nucleotides (usually found in their 5'-monophosphate form) are referred to by a single letter designation as follows: "A" for adenylate or deoxyadenylate (for RNA or DNA, respectively), "C" for cytidylate or deoxycytidylate, "G" for guanylate or deoxyguanylate, "U" for uridylate, "T" for deoxythymidylate, "R" for purines (A or G), "Y" for pyrimidines (C or T), "K" for G or T, "H" for A or C or T, "I" for inosine, "W" for A or T, "S" for G or C, and "N" for any nucleotide.

Messenger RNAs (mRNAs) are gene-coding polynucleotide sequences traditionally represented as strings of letters to marking the location of each of the ribonucleic acids: "A" for adenylate, "C" for cytidylate, "G" for guanylate, and "U" for uridylate. Sequences for mRNA genes are usually written in the 5' monophosphate to 3' hydroxyl orientation, and can also be represented by their complementary DNA (cDNA) sequence by replacing the "U" uridylates ribonucleotides with "T" deoxythymidylates. An example of this interchangeability between cDNA and mRNA sequences is illustrated in this application in the comparison of the cDNA sequence of EG261 from *Glycine pescadrensis* (SEQ ID No. 5), and its respective mRNA sequence (SEQ ID No: 66).

As used herein, "coding sequence" refers to a DNA sequence that codes for a specific amino acid sequence. "Regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA processing or stability, or translation of the associated coding sequence.

As used herein, "regulatory sequences" may include, but are not limited to, promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

As used herein, "promoter" refers to a DNA sequence capable of controlling the expression of a coding sequence or functional RNA. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an "enhancer" is a DNA sequence that can stimulate promoter activity, and may be an innate element of the promoter or a heterologous element inserted to

enhance the level or tissue-specificity of a promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic DNA segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to different environmental conditions. It is further recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, DNA fragments of some variation may have identical promoter activity. Promoters that cause a gene to be expressed in most cell types at most times are commonly referred to as “constitutive promoters”.

In some embodiments, the invention uses a Gmubi promoter to express EG261 genes in root tissue. In other embodiments, alternative promoters are utilized including, but not limited to the : Cauliflower mosaic virus (CaMV) 35S promoter and enhanced 35S (E35S) promoter, the Cassava vein mosaic virus (CsVMV) promoter, the Figwort mosaic virus (FMV) promoter, and the Strawberry vein banding virus (SVBV2) promoter (see also Hernandez-Garcia et al., 2010 (High level transgenic expression of soybean (Glycine max) GmERF and Gmubi gene promoters isolated by a novel promoter analysis pipeline. BMC Plant Biol. 2010 Nov 4;10:237), and Tucker et al., 2011 (Gene Expression Profiling and Shared Promoter Motif for Cell Wall-Modifying Proteins Expressed in Soybean Cyst Nematode-Infected Roots. Plant Phys. Vol 156 pp 319-329).

As used herein, the “3’ non-coding sequences” or “3’ UTR (untranslated region) sequence” refer to DNA sequences located downstream of a coding sequence and include polyadenylation recognition sequences and other sequences encoding regulatory signals capable of affecting mRNA processing or gene expression. The polyadenylation signal is usually characterized by affecting the addition of polyadenylic acid tracts to the 3’ end of the mRNA precursor. The use of different 3’ non-coding sequences is exemplified by Ingelbrecht, I. L., et al. (1989) Plant Cell 1:671-680.

As used herein, the term “operably linked” refers to the association of nucleic acid sequences on a single nucleic acid fragment so that the function of one is regulated by the other. For example, a promoter is operably linked with a coding sequence when it is capable of regulating the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in a sense or antisense orientation. In another example, the complementary RNA regions of the invention can be operably linked, either directly or indirectly, 5' to the target mRNA, or 3' to the target mRNA, or within the target mRNA, or a first complementary region is 5' and its complement is 3' to the target mRNA.

As used herein, the term “vector”, “plasmid”, or “construct” refers broadly to any plasmid or virus encoding an exogenous nucleic acid. The term should also be construed to include non-plasmid and non-viral compounds which facilitate transfer of nucleic acid into virions or cells, such as, for example, polylysine compounds and the like. The vector may be a viral vector that is suitable as a delivery vehicle for delivery of the nucleic acid, or mutant thereof, to a cell, or the vector may be a non-viral vector which is suitable for the same purpose. Examples of viral and non-viral vectors for delivery of DNA to cells and tissues are well known in the art and are described, for example, in Ma et al. (1997, Proc. Natl. Acad. Sci. U.S.A. 94:12744-12746). Examples of viral vectors include, but are not limited to, recombinant plant viruses. Non-limiting examples of plant viruses include, TMV-mediated (transient) transfection into tobacco (Tuipe, T-H et al (1993), J. Virology Meth, 42: 227-239), ssDNA genomes viruses (e.g., family *Geminiviridae*), reverse transcribing viruses (e.g., families *Caulimoviridae*, *Pseudoviridae*, and *Metaviridae*), dsNRA viruses (e.g., families *Reoviridae* and *Partitiviridae*), (-) ssRNA viruses (e.g., families *Rhabdoviridae* and *Bunyaviridae*), (+) ssRNA viruses (e.g., families *Bromoviridae*, *Closteroviridae*, *Comoviridae*, *Luteoviridae*, *Potyviridae*, *Sequiviridae* and *Tombusviridae*) and viroids (e.g., families *Pospiviroidae* and *Aysunviroidae*). Detailed classification information of plant viruses can be found in Fauquet et al (2008, "Geminivirus strain demarcation and nomenclature". *Archives of Virology* 153:783–821, incorporated herein by reference in its entirety), and Khan et al. (Plant viruses as molecular pathogens; Publisher Routledge, 2002,

ISBN 1560228954, 9781560228950). Examples of non-viral vectors include, but are not limited to, liposomes, polyamine derivatives of DNA, and the like.

The present invention provides an isolated nucleic acid sequence comprising a sequence selected from the group consisting of EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations thereof. In one embodiment, the present invention provides an isolated polynucleotide encoding the dirigent protein produced by the nucleic acid sequence for EG261, comprising a nucleic acid sequence that shares at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.1%, at least 99.2%, at least 99.3%, at least 99.4%, at least 99.5%, at least 99.6%, at least 99.7%, at least 99.8%, or at least 99.9% identity to EG261.

Methods of alignment of sequences for comparison are well known in the art. Various programs and alignment algorithms are described in: Smith and Waterman (*Adv. Appl. Math.*, 2:482, 1981); Needleman and Wunsch (*J. Mol. Biol.*, 48:443, 1970); Pearson and Lipman (*Proc. Natl. Acad. Sci.*, 85:2444, 1988); Higgins and Sharp (*Gene*, 73:237-44, 1988); Higgins and Sharp (*CABIOS*, 5:151-53, 1989); Corpet *et al.* (*Nuc. Acids Res.*, 16:10881-90, 1988); Huang *et al.* (*Comp. Appls Biosci.*, 8:155-65, 1992); and Pearson *et al.* (*Meth. Mol. Biol.*, 24:307-31, 1994). Altschul *et al.* (*Nature Genet.*, 6:119-29, 1994) presents a detailed consideration of sequence alignment methods and homology calculations.

The present invention also provides a chimeric gene comprising the isolated nucleic acid sequence of any one of the polynucleotides described above operably linked to suitable regulatory sequences.

The present invention also provides a recombinant construct comprising the chimeric gene as described above. In one embodiment, said recombinant construct is a gene silencing construct, such as used in RNAi gene silencing.

The expression vectors of the present invention will preferably include at least one selectable marker. Such markers include dihydrofolate reductase, G418 or neomycin resistance for eukaryotic cell culture and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria.

The present invention also provides a transformed host cell comprising the chimeric gene as described above. In one embodiment, said host cell is selected from the group consisting of bacteria, yeasts, filamentous fungi, algae, animals, and plants.

5 These sequences allow the design of gene-specific primers and probes for EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations thereof.

The term "primer" as used herein refers to an oligonucleotide which is capable of annealing to the amplification target allowing a DNA polymerase to attach, thereby serving as a point of initiation of DNA synthesis when placed under conditions in which synthesis of primer extension product is induced, i.e., in the presence of nucleotides and an agent for polymerization such as DNA polymerase and at a suitable temperature and pH. The (amplification) primer is preferably single stranded for maximum efficiency in amplification. Preferably, the primer is an oligodeoxyribonucleotide. The primer must be sufficiently long to prime the synthesis of extension products in the presence of the agent for polymerization. 10 The exact lengths of the primers will depend on many factors, including temperature and composition (A/T en G/C content) of primer. A pair of bi-directional primers consists of one forward and one reverse primer as commonly used in the art of DNA amplification such as in PCR amplification.

A probe comprises an identifiable, isolated nucleic acid that recognizes a target nucleic acid sequence. A probe includes a nucleic acid that is attached to an addressable location, a detectable label or other reporter molecule and that hybridizes to a target sequence. Typical labels include radioactive isotopes, enzyme substrates, co-factors, ligands, chemiluminescent or fluorescent agents, haptens, and enzymes. Methods for labelling and guidance in the choice of labels appropriate for various purposes are discussed, for example, 20 in Sambrook *et al.* (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989 and Ausubel *et al.* *Short Protocols in Molecular Biology*, 4th ed., John Wiley & Sons, Inc., 1999.

Methods for preparing and using nucleic acid probes and primers are described, for example, in Sambrook *et al.* (ed.), *Molecular Cloning: A Laboratory Manual*, 2nd ed., vol. 1-

3, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989; Ausubel *et al.* *Short Protocols in Molecular Biology*, 4th ed., John Wiley & Sons, Inc., 1999; and Innis *et al.* *PCR Protocols, A Guide to Methods and Applications*, Academic Press, Inc., San Diego, CA, 1990. Amplification primer pairs can be derived from a known sequence, for example, by
 5 using computer programs intended for that purpose such as PRIMER (Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge, MA). One of ordinary skill in the art will appreciate that the specificity of a particular probe or primer increases with its length. Thus, in order to obtain greater specificity, probes and primers can be selected that comprise at least 20, 25, 30, 35, 40, 45, 50 or more consecutive nucleotides of a target nucleotide
 10 sequences.

As used herein, the term “soybean” refers to a plant of the *Glycine* genus, including but not limited to the following species: *G. clandestine*, *G. facata*, *G. latifolia*, *G. latrobeana*, *G. canescens*, *G. tabacina*, *G. tomentella*, *G. soja*, *G. max*, *G. Arenaria*, *G. argyrea*, *G. canescens*, *G. clandestine*, *G. curvata*, *G. cyrtoloba*, *G. microphylla*, *G. pescadrensis*, *G.*
 15 *pinadanica*, *G. rubiginosa*, *G. soja*, and *G. Stenophita* (see for example U.S. patent 8,389,798, and Verma et al., (1996) Soybean: genetics, molecular biology and biotechnology, Verma, D. P. S.; Shoemaker, R. C. (editors)).

As used herein, the term “garden bean” or “bean” refers to a plant of the *Phaseolus* genus, including but not limited to the following species: *P. acutifolius*, *P. aegypticus*, *P.*
 20 *amblyosepalus*, *P. angustissimus*, *P. coccineus*, *P. filiformis*, *P. lunatus*, *P. Maculatus*, and *P. Vulgaris* (see for example Turley et al., 1999 (Phylogenetic Analysis of the Cultivated and Wild Species of *Phaseolus* (Fabaceae), Systematic Botany Vol. 24, No. 3 (Jul. –Sep., 199) pp 438-460)).

As used herein, the term “cowpea” refers to a plant of the *Vigna* genus, including but
 25 not limited to the following species: *V. aconitifolia*, *V. angularis*, *V. caracalla*, *V. lanceolata*, *V. o-wahuensis*, *V. umbellata*, *V. subterranea*, and *V. Unguiculata* (see for example Sylla Ba et al., 2004 (Genetic diversity in cowpea [*Vigna unguiculata* (L.) Walp.] as revealed by RAPD markers *Genetic Resources and Crop Evolution* 51: 539–550, 2004, and Pasquet et al.,

(2001) Cowpea. In: Tropical Plant Breeding. Charrier A., Jacquot M., Hamon S. and Nicolas D. (editors), Science publishers, Enfield, pp. 177–198.)).

EG261 Proteins

5 The present invention also provides polypeptides and amino acid sequences comprising at least a portion of the isolated proteins encoded by nucleotide sequences for EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations thereof.

10 The present invention also provides an isolated amino acid sequence encoded by the nucleic acid sequences of EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and/or fragments and variations thereof. In some embodiments, the present invention provides an isolated polypeptide comprising an amino acid sequence that shares at least about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%,
15 about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to an amino acid sequence encoded by the nucleic acid sequences of EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and/or fragments and variations thereof. In one embodiment, the present invention provides an isolated polypeptide comprising an amino acid sequence which encodes an amino acid sequence that shares at least about 85%, about 86%, about 87%, about
20 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, about 99.1%, about 99.2%, about 99.3%, about 99.4%, about 99.5%, about 99.6%, about 99.7%, about 99.8%, or about 99.9% identity to an amino acid sequence encoded by the nucleic acid sequences of EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and/or fragments and variations thereof.

25 The invention also encompasses variants and fragments of proteins of an amino acid sequence encoded by the nucleic acid sequences of EG261, homologs of EG261, orthologs of EG261 and/or paralogs of EG261. The variants may contain alterations in the amino acid sequences of the constituent proteins. The term “variant” with respect to a polypeptide refers to an amino acid sequence that is altered by one or more amino acids with respect to a

reference sequence. The variant can have “conservative” changes, or “nonconservative” changes, *e.g.*, analogous minor variations can also include amino acid deletions or insertions, or both.

Functional fragments and variants of a polypeptide include those fragments and variants that maintain one or more functions of the parent polypeptide. It is recognized that the gene or cDNA encoding a polypeptide can be considerably mutated without materially altering one or more of the polypeptide’s functions. First, the genetic code is well-known to be degenerate, and thus different codons encode the same amino acids. Second, even where an amino acid substitution is introduced, the mutation can be conservative and have no material impact on the essential function(s) of a protein. See, *e.g.*, *Stryer Biochemistry* 3rd Ed., 1988. Third, part of a polypeptide chain can be deleted without impairing or eliminating all of its functions. Fourth, insertions or additions can be made in the polypeptide chain for example, adding epitope tags, without impairing or eliminating its functions (Ausubel *et al. J. Immunol.* 159(5): 2502-12, 1997). Other modifications that can be made without materially impairing one or more functions of a polypeptide can include, for example, *in vivo* or *in vitro* chemical and biochemical modifications or the incorporation of unusual amino acids. Such modifications include, but are not limited to, for example, acetylation, carboxylation, phosphorylation, glycosylation, ubiquination, labelling, *e.g.*, with radionucleotides, and various enzymatic modifications, as will be readily appreciated by those well skilled in the art. A variety of methods for labelling polypeptides, and labels useful for such purposes, are well known in the art, and include radioactive isotopes such as ³²P, ligands which bind to or are bound by labelled specific binding partners (*e.g.*, antibodies), fluorophores, chemiluminescent agents, enzymes, and anti-ligands. Functional fragments and variants can be of varying length. For example, some fragments have at least 10, 25, 50, 75, 100, 200, or even more amino acid residues. These mutations can be natural or purposely changed. In some embodiments, mutations containing alterations that produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the proteins or how the proteins are made are an embodiment of the invention.

Conservative amino acid substitutions are those substitutions that, when made, least interfere with the properties of the original protein, that is, the structure and especially the function of the protein is conserved and not significantly changed by such substitutions. Conservative substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Further information about conservative substitutions can be found, for instance, in Ben Bassat *et al.* (*J. Bacteriol.*, 169:751-757, 1987), O'Regan *et al.* (*Gene*, 77:237-251, 1989), Sahin-Toth *et al.* (*Protein Sci.*, 3:240-247, 1994), Hochuli *et al.* (*Bio/Technology*, 6:1321-1325, 1988) and in widely used textbooks of genetics and molecular biology. The Blosum matrices are commonly used for determining the relatedness of polypeptide sequences. The Blosum matrices were created using a large database of trusted alignments (the BLOCKS database), in which pairwise sequence alignments related by less than some threshold percentage identity were counted (Henikoff *et al.*, *Proc. Natl. Acad. Sci. USA*, 89:10915-10919, 1992). A threshold of 90% identity was used for the highly conserved target frequencies of the BLOSUM90 matrix. A threshold of 65% identity was used for the BLOSUM65 matrix. Scores of zero and above in the Blosum matrices are considered "conservative substitutions" at the percentage identity selected. The following table shows exemplary conservative amino acid substitutions.

Original Residue	Very Highly - Conserved Substitutions	Highly Conserved Substitutions (from the Blosum90 Matrix)	Conserved Substitutions (from the Blosum65 Matrix)
Ala	Ser	Gly, Ser, Thr	Cys, Gly, Ser, Thr, Val
Arg	Lys	Gln, His, Lys	Asn, Gln, Glu, His, Lys
Asn	Gln; His	Asp, Gln, His, Lys, Ser, Thr	Arg, Asp, Gln, Glu, His, Lys, Ser, Thr
Asp	Glu	Asn, Glu	Asn, Gln, Glu, Ser
Cys	Ser	None	Ala
Gln	Asn	Arg, Asn, Glu, His, Lys, Met	Arg, Asn, Asp, Glu, His, Lys, Met, Ser
Glu	Asp	Asp, Gln, Lys	Arg, Asn, Asp, Gln, His, Lys, Ser
Gly	Pro	Ala	Ala, Ser
His	Asn; Gln	Arg, Asn, Gln, Tyr	Arg, Asn, Gln, Glu, Tyr
Ile	Leu; Val	Leu, Met, Val	Leu, Met, Phe, Val
Leu	Ile; Val	Ile, Met, Phe, Val	Ile, Met, Phe, Val

Lys	Arg; Gln; Glu	Arg, Asn, Gln, Glu	Arg, Asn, Gln, Glu, Ser,
Met	Leu; Ile	Gln, Ile, Leu, Val	Gln, Ile, Leu, Phe, Val
Phe	Met; Leu; Tyr	Leu, Trp, Tyr	Ile, Leu, Met, Trp, Tyr
Ser	Thr	Ala, Asn, Thr	Ala, Asn, Asp, Gln, Glu, Gly, Lys, Thr
Thr	Ser	Ala, Asn, Ser	Ala, Asn, Ser, Val
Trp	Tyr	Phe, Tyr	Phe, Tyr
Tyr	Trp; Phe	His, Phe, Trp	His, Phe, Trp
Val	Ile; Leu	Ile, Leu, Met	Ala, Ile, Leu, Met, Thr

In some examples, variants can have no more than 3, 5, 10, 15, 20, 25, 30, 40, 50, or 100 conservative amino acid changes (such as very highly conserved or highly conserved amino acid substitutions). In other examples, one or several hydrophobic residues (such as Leu, Ile, Val, Met, Phe, or Trp) in a variant sequence can be replaced with a different hydrophobic residue (such as Leu, Ile, Val, Met, Phe, or Trp) to create a variant functionally similar to the disclosed amino acid sequences encoded by the nucleic acid sequences of EG261, homologs of EG261, orthologs of EG261 and/or paralogs of EG261, and/or fragments and variations thereof.

In some embodiments, variants may differ from the disclosed sequences by alteration of the coding region to fit the codon usage bias of the particular organism into which the molecule is to be introduced. In other embodiments, the coding region may be altered by taking advantage of the degeneracy of the genetic code to alter the coding sequence such that, while the nucleotide sequence is substantially altered, it nevertheless encodes a protein having an amino acid sequence substantially similar to the disclosed amino acid sequences encoded by the nucleic acid sequences of EG261, homologs of EG261, orthologs of EG261 and/or paralogs of EG261, and/or fragments and variations thereof.

In some embodiments, functional fragments derived from the EG261 orthologs of the present invention are provided. The functional fragments can still confer resistance to pathogens when expressed in a plant. In some embodiments, the functional fragments contain at least the dirigent domain of a wild type EG261 orthologs, or functional variants thereof. In some embodiments, the functional fragments contain one or more conserved region shared by two or more EG261 orthologs, shared by two or more EG261 orthologs in

the same plant genus, shared by two or more dicot EG261 orthologs, and/or shared by two or more monocot EG261 orthologs. Non-limiting exemplary conserved regions are shown in Figures 2 to 4. The dirigent domain or conserved regions can be determined by any suitable computer program, such as NCBI protein BLAST program and NCBI Alignment program, or equivalent programs. In some embodiments, the functional fragments are 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more amino acids shorter compared to the EG261 orthologs of the present invention. In some embodiments, the functional fragments are made by deleting one or more amino acid of the EG261 orthologs of the present invention. In some embodiments, the functional fragments share at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more identity to the EG261 orthologs of the present invention.

In some embodiments, functional chimeric or synthetic polypeptides derived from the EG261 orthologs of the present invention are provided. The functional chimeric or synthetic polypeptides can still confer resistance to pathogens when expressed in a plant. In some embodiments, the functional chimeric or synthetic polypeptides contain at least the dirigent domain of a wild type EG261 orthologs, or functional variants thereof. In some embodiments, the functional chimeric or synthetic polypeptides contain one or more conserved region shared by two or more EG261 orthologs, shared by two or more EG261 orthologs in the same plant genus, shared by two or more dicot EG261 orthologs, and/or shared by two or more monocot EG261 orthologs. Non-limiting exemplary conserved regions are shown in Figures 2 to 4. The dirigent domain or conserved regions can be determined by any suitable computer program, such as NCBI protein BLAST program and NCBI Alignment program, or equivalent programs. In some embodiments, the functional chimeric or synthetic polypeptides share at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more identity to the EG261 orthologs of the present invention.

Sequences of conserved regions can also be used to knock-down the level of one or more EG261 orthologs. In some embodiments, sequences of conserved regions can be used to make gene silencing molecules to target one or more EG261 orthologs. In some

embodiments, the gene silencing molecules are selected from the group consisting of double-stranded polynucleotides, single-stranded polynucleotides or Mixed Duplex Oligonucleotides. In some embodiments, the gene silencing molecules comprises a DNA/RNA fragment of about 10 bp, 15bp, 19 bp, 20 bp, 21 bp, 25 bp, 30 bp, 40bp, 50bp, 60bp, 70bp, 80bp, 90bp, 100bp, 150bp, 200pb, 250bp, 300bp, 350bp, 400bp, 500bp, 600bp, 700bp, 800bp, 900bp, 1000bp, or more polynucleotides, wherein the DNA/RNA fragment share at least 90%, 95%, 99%, or more identity to a conserved region of the EG261 orthologs sequences of the present invention, or complementary sequences thereof.

10 *Plant Transformation*

The present polynucleotides coding for EG261, homologs of EG261, orthologs of EG261 and/or paralogs of EG261, and/or fragments and variations thereof of the present invention can be transformed into soybean or other plant genera.

Methods of producing transgenic plants are well known to those of ordinary skill in the art. Transgenic plants can now be produced by a variety of different transformation methods including, but not limited to, electroporation; microinjection; microprojectile bombardment, also known as particle acceleration or biolistic bombardment; viral-mediated transformation; and Agrobacterium-mediated transformation. See, for example, U.S. Patent Nos. 5,405,765; 5,472,869; 5,538,877; 5,538,880; 5,550,318; 5,641,664; 5,736,369 and 5,736,369; International Patent Application Publication Nos. WO2002/038779 and WO/2009/117555; Lu et al., (Plant Cell Reports, 2008, 27:273-278); Watson et al., Recombinant DNA, Scientific American Books (1992); Hinchey et al., Bio/Tech. 6:915-922 (1988); McCabe et al., Bio/Tech. 6:923-926 (1988); Toriyama et al., Bio/Tech. 6:1072-1074 (1988); Fromm et al., Bio/Tech. 8:833-839 (1990); Mullins et al., Bio/Tech. 8:833-839 (1990); Hiei et al., Plant Molecular Biology 35:205-218 (1997); Ishida et al., Nature Biotechnology 14:745-750 (1996); Zhang et al., Molecular Biotechnology 8:223-231 (1997); Ku et al., Nature Biotechnology 17:76-80 (1999); and, Raineri et al., Bio/Tech. 8:33-38 (1990)), each of which is expressly incorporated herein by reference in their entirety.

Agrobacterium tumefaciens is a naturally occurring bacterium that is capable of inserting its DNA (genetic information) into plants, resulting in a type of injury to the plant known as crown gall. Most species of plants can now be transformed using this method, including cucurbitaceous species.

5 Microprojectile bombardment is also known as particle acceleration, biolistic bombardment, and the gene gun (Biolistic® Gene Gun). The gene gun is used to shoot pellets that are coated with genes (e.g., for desired traits) into plant seeds or plant tissues in order to get the plant cells to then express the new genes. The gene gun uses an actual explosive (.22 caliber blank) to propel the material. Compressed air or steam may also be
10 used as the propellant. The Biolistic® Gene Gun was invented in 1983-1984 at Cornell University by John Sanford, Edward Wolf, and Nelson Allen. It and its registered trademark are now owned by E. I. du Pont de Nemours and Company. Most species of plants have been transformed using this method.

The most common method for the introduction of new genetic material into a plant
15 genome involves the use of living cells of the bacterial pathogen *Agrobacterium tumefaciens* to literally inject a piece of DNA, called transfer or T-DNA, into individual plant cells (usually following wounding of the tissue) where it is targeted to the plant nucleus for chromosomal integration. There are numerous patents governing *Agrobacterium* mediated transformation and particular DNA delivery plasmids designed specifically for use with
20 *Agrobacterium*---for example, US4536475, EP0265556, EP0270822, WO8504899, WO8603516, US5591616, EP0604662, EP0672752, WO8603776, WO9209696, WO9419930, WO9967357, US4399216, WO8303259, US5731179, EP068730, WO9516031, US5693512, US6051757 and EP904362A1. *Agrobacterium*-mediated plant transformation involves as a first step the placement of DNA fragments cloned on plasmids
25 into living *Agrobacterium* cells, which are then subsequently used for transformation into individual plant cells. *Agrobacterium*-mediated plant transformation is thus an indirect plant transformation method. Methods of *Agrobacterium*-mediated plant transformation that involve using vectors with no T-DNA are also well known to those skilled in the art and can

have applicability in the present invention. See, for example, U.S. Patent No. 7,250,554, which utilizes P-DNA instead of T-DNA in the transformation vector.

A transgenic plant formed using *Agrobacterium* transformation methods typically contains a single gene on one chromosome, although multiple copies are possible. Such transgenic plants can be referred to as being hemizygous for the added gene. A more accurate name for such a plant is an independent segregant, because each transformed plant represents a unique T-DNA integration event (U.S. Patent No. 6,156,953). A transgene locus is generally characterized by the presence and/or absence of the transgene. A heterozygous genotype in which one allele corresponds to the absence of the transgene is also designated hemizygous (U.S. Patent No. 6,008,437).

Direct plant transformation methods using DNA have also been reported. The first of these to be reported historically is electroporation, which utilizes an electrical current applied to a solution containing plant cells (M. E. Fromm et al., *Nature*, 319, 791 (1986); H. Jones et al., *Plant Mol. Biol.*, 13, 501 (1989) and H. Yang et al., *Plant Cell Reports*, 7, 421 (1988). Another direct method, called "biolistic bombardment", uses ultrafine particles, usually tungsten or gold, that are coated with DNA and then sprayed onto the surface of a plant tissue with sufficient force to cause the particles to penetrate plant cells, including the thick cell wall, membrane and nuclear envelope, but without killing at least some of them (US 5,204,253, US 5,015,580). A third direct method uses fibrous forms of metal or ceramic consisting of sharp, porous or hollow needle-like projections that literally impale the cells, and also the nuclear envelope of cells. Both silicon carbide and aluminium borate whiskers have been used for plant transformation (Mizuno et al., 2004; Petolino et al., 2000; US5302523 US Application 20040197909) and also for bacterial and animal transformation (Kaepler et al., 1992; Raloff, 1990; Wang, 1995). There are other methods reported, and undoubtedly, additional methods will be developed. However, the efficiencies of each of these indirect or direct methods in introducing foreign DNA into plant cells are invariably extremely low, making it necessary to use some method for selection of only those cells that have been transformed, and further, allowing growth and regeneration into plants of only those cells that have been transformed.

For efficient plant transformation, a selection method must be employed such that whole plants are regenerated from a single transformed cell and every cell of the transformed plant carries the DNA of interest. These methods can employ positive selection, whereby a foreign gene is supplied to a plant cell that allows it to utilize a substrate present in the medium that it otherwise could not use, such as mannose or xylose (for example, refer US 5767378; US 5994629). More typically, however, negative selection is used because it is more efficient, utilizing selective agents such as herbicides or antibiotics that either kill or inhibit the growth of non-transformed plant cells and reducing the possibility of chimeras. Resistance genes that are effective against negative selective agents are provided on the introduced foreign DNA used for the plant transformation. For example, one of the most popular selective agents used is the antibiotic kanamycin, together with the resistance gene neomycin phosphotransferase (nptII), which confers resistance to kanamycin and related antibiotics (see, for example, Messing & Vierra, *Gene* 19: 259-268 (1982); Bevan et al., *Nature* 304:184-187 (1983)). However, many different antibiotics and antibiotic resistance genes can be used for transformation purposes (refer US 5034322, US 6174724 and US 6255560). In addition, several herbicides and herbicide resistance genes have been used for transformation purposes, including the bar gene, which confers resistance to the herbicide phosphinothricin (White et al., *Nucl Acids Res* 18: 1062 (1990), Spencer et al., *Theor Appl Genet* 79: 625-631(1990), US 4795855, US 5378824 and US 6107549). In addition, the dhfr gene, which confers resistance to the anticancer agent methotrexate, has been used for selection (Bourouis et al., *EMBO J.* 2(7): 1099-1104 (1983).

Non-limiting examples of binary vectors suitable for soybean species transformation and transformation methods are described by Yi et al. 2006 (Transformation of multiple soybean cultivars by infecting cotyledonary-node with *Agrobacterium tumefaciens*, *African Journal of Biotechnology* Vol. 5 (20), pp. 1989-1993, 16 October 2006), Paz et al., 2004 (Assessment of conditions affecting *Agrobacterium*-mediated soybean transformation using the cotyledonary node explant, *Euphytica* 136: 167-179, 2004), U.S. Patent Nos. 5,376,543, 5,416,011, 5,968,830, and 5,569,834, or by similar experimental procedures well known to

those skilled in the art. Soybean plants can be transformed by using any method described in the above references.

The expression control elements used to regulate the expression of a given protein can either be the expression control element that is normally found associated with the coding sequence (homologous expression element) or can be a heterologous expression control element. A variety of homologous and heterologous expression control elements are known in the art and can readily be used to make expression units for use in the present invention. Transcription initiation regions, for example, can include any of the various opine initiation regions, such as octopine, mannopine, nopaline and the like that are found in the Ti plasmids of *Agrobacterium tumefaciens*. Alternatively, plant viral promoters can also be used, such as the cauliflower mosaic virus 19S and 35S promoters (CaMV 19S and CaMV 35S promoters, respectively) to control gene expression in a plant (U.S. Patent Nos. 5,352,605; 5,530,196 and 5,858,742 for example). Enhancer sequences derived from the CaMV can also be utilized (U.S. Patent Nos. 5,164,316; 5,196,525; 5,322,938; 5,530,196; 5,352,605; 5,359,142; and 5,858,742 for example). Lastly, plant promoters such as prolifera promoter, fruit specific promoters, Ap3 promoter, heat shock promoters, seed specific promoters, etc. can also be used.

Either a gamete-specific promoter, a constitutive promoter (such as the CaMV or Nos promoter), an organ-specific promoter (such as the E8 promoter from tomato), or an inducible promoter is typically ligated to the protein or antisense encoding region using standard techniques known in the art. The expression unit may be further optimized by employing supplemental elements such as transcription terminators and/or enhancer elements.

Thus, for expression in plants, the expression units will typically contain, in addition to the protein sequence, a plant promoter region, a transcription initiation site and a transcription termination sequence. Unique restriction enzyme sites at the 5' and 3' ends of the expression unit are typically included to allow for easy insertion into a pre-existing vector.

In the construction of heterologous promoter/structural gene or antisense combinations, the promoter is preferably positioned about the same distance from the heterologous transcription start site as it is from the transcription start site in its natural setting. As is known in the art, however, some variation in this distance can be accommodated without loss of promoter function.

In addition to a promoter sequence, the expression cassette can also contain a transcription termination region downstream of the structural gene to provide for efficient termination. The termination region may be obtained from the same gene as the promoter sequence or may be obtained from different genes. If the mRNA encoded by the structural gene is to be efficiently processed, DNA sequences which direct polyadenylation of the RNA are also commonly added to the vector construct. Polyadenylation sequences include, but are not limited to the *Agrobacterium* octopine synthase signal (Gielen *et al.*, EMBO J 3:835-846 (1984)) or the nopaline synthase signal (Depicker *et al.*, Mol. and Appl. Genet. 1:561-573 (1982)). The resulting expression unit is ligated into or otherwise constructed to be included in a vector that is appropriate for higher plant transformation. One or more expression units may be included in the same vector. The vector will typically contain a selectable marker gene expression unit by which transformed plant cells can be identified in culture. Usually, the marker gene will encode resistance to an antibiotic, such as G418, hygromycin, bleomycin, kanamycin, or gentamicin or to an herbicide, such as glyphosate (Round-Up) or glufosinate (BASTA) or atrazine. Replication sequences, of bacterial or viral origin, are generally also included to allow the vector to be cloned in a bacterial or phage host; preferably a broad host range for prokaryotic origin of replication is included. A selectable marker for bacteria may also be included to allow selection of bacterial cells bearing the desired construct. Suitable prokaryotic selectable markers include resistance to antibiotics such as ampicillin, kanamycin or tetracycline. Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art. For instance, in the case of *Agrobacterium* transformations, T-DNA sequences will also be included for subsequent transfer to plant chromosomes.

To introduce a desired gene or set of genes by conventional methods requires a sexual cross between two lines, and then repeated back-crossing between hybrid offspring and one of the parents until a plant with the desired characteristics is obtained. This process, however, is restricted to plants that can sexually hybridize, and genes in addition to the
5 desired gene will be transferred.

Recombinant DNA techniques allow plant researchers to circumvent these limitations by enabling plant geneticists to identify and clone specific genes for desirable traits, such as improved fatty acid composition, and to introduce these genes into already useful varieties of plants. Once the foreign genes have been introduced into a plant, that plant can then be used
10 in conventional plant breeding schemes (e.g., pedigree breeding, single-seed-descent breeding schemes, reciprocal recurrent selection) to produce progeny which also contain the gene of interest.

Genes can be introduced in a site directed fashion using homologous recombination. Homologous recombination permits site-specific modifications in endogenous genes and thus
15 inherited or acquired mutations may be corrected, and/or novel alterations may be engineered into the genome. Homologous recombination and site-directed integration in plants are discussed in, for example, U.S. Patent Nos. 5,451,513; 5,501,967 and 5,527,695.

Breeding Methods

Open-Pollinated Populations. The improvement of open-pollinated populations of
20 such crops as rye, many maizes and sugar beets, herbage grasses, legumes such as alfalfa and clover, and tropical tree crops such as cacao, coconuts, oil palm and some rubber, depends essentially upon changing gene-frequencies towards fixation of favorable alleles while maintaining a high (but far from maximal) degree of heterozygosity. Uniformity in such
25 populations is impossible and trueness-to-type in an open-pollinated variety is a statistical feature of the population as a whole, not a characteristic of individual plants. Thus, the heterogeneity of open-pollinated populations contrasts with the homogeneity (or virtually so) of inbred lines, clones and hybrids.

Population improvement methods fall naturally into two groups, those based on purely phenotypic selection, normally called mass selection, and those based on selection with progeny testing. Interpopulation improvement utilizes the concept of open breeding populations; allowing genes for flow from one population to another. Plants in one population (cultivar, strain, ecotype, or any germplasm source) are crossed either naturally (e.g., by wind) or by hand or by bees (commonly *Apis mellifera* L. or *Megachile rotundata* F.) with plants from other populations. Selection is applied to improve one (or sometimes both) population(s) by isolating plants with desirable traits from both sources.

There are basically two primary methods of open-pollinated population improvement. First, there is the situation in which a population is changed en masse by a chosen selection procedure. The outcome is an improved population that is indefinitely propagable by random-mating within itself in isolation. Second, the synthetic variety attains the same end result as population improvement but is not itself propagable as such; it has to be reconstructed from parental lines or clones. These plant breeding procedures for improving open-pollinated populations are well known to those skilled in the art and comprehensive reviews of breeding procedures routinely used for improving cross-pollinated plants are provided in numerous texts and articles, including: Allard, *Principles of Plant Breeding*, John Wiley & Sons, Inc. (1960); Simmonds, *Principles of Crop Improvement*, Longman Group Limited (1979); Hallauer and Miranda, *Quantitative Genetics in Maize Breeding*, Iowa State University Press (1981); and, Jensen, *Plant Breeding Methodology*, John Wiley & Sons, Inc. (1988). For population improvement methods specific for soybean see, e.g., J.R. Wilcox, editor (1987) SOYBEANS: Improvement, Production, and Uses, Second Edition, American Society of Agronomy, Inc., Crop Science Society of America, Inc., and Soil Science Society of America, Inc., publishers, 888 pages.

Mass Selection. In mass selection, desirable individual plants are chosen, harvested, and the seed composited without progeny testing to produce the following generation. Since selection is based on the maternal parent only, and there is no control over pollination, mass selection amounts to a form of random mating with selection. As stated above, the purpose of mass selection is to increase the proportion of superior genotypes in the population.

Synthetics. A synthetic variety is produced by crossing inter se a number of genotypes selected for good combining ability in all possible hybrid combinations, with subsequent maintenance of the variety by open pollination. Whether parents are (more or less inbred) seed-propagated lines, as in some sugar beet and beans (*Vicia*) or clones, as in herbage grasses, clovers and alfalfa, makes no difference in principle. Parents are selected on general combining ability, sometimes by test crosses or topcrosses, more generally by polycrosses. Parental seed lines may be deliberately inbred (e.g. by selfing or sib crossing). However, even if the parents are not deliberately inbred, selection within lines during line maintenance will ensure that some inbreeding occurs. Clonal parents will, of course, remain unchanged and highly heterozygous.

Whether a synthetic can go straight from the parental seed production plot to the farmer or must first undergo one or two cycles of multiplication depends on seed production and the scale of demand for seed. In practice, grasses and clovers are generally multiplied once or twice and are thus considerably removed from the original synthetic.

While mass selection is sometimes used, progeny testing is generally preferred for polycrosses, because of their operational simplicity and obvious relevance to the objective, namely exploitation of general combining ability in a synthetic.

The number of parental lines or clones that enters a synthetic varies widely. In practice, numbers of parental lines range from 10 to several hundred, with 100-200 being the average. Broad based synthetics formed from 100 or more clones would be expected to be more stable during seed multiplication than narrow based synthetics.

Hybrids. A hybrid is an individual plant resulting from a cross between parents of differing genotypes. Commercial hybrids are now used extensively in many crops, including corn (maize), sorghum, sugar beet, sunflower and broccoli. Hybrids can be formed in a number of different ways, including by crossing two parents directly (single cross hybrids), by crossing a single cross hybrid with another parent (three-way or triple cross hybrids), or by crossing two different hybrids (four-way or double cross hybrids).

Strictly speaking, most individuals in an out breeding (i.e., open-pollinated) population are hybrids, but the term is usually reserved for cases in which the parents are

individuals whose genomes are sufficiently distinct for them to be recognized as different species or subspecies. Hybrids may be fertile or sterile depending on qualitative and/or quantitative differences in the genomes of the two parents. Heterosis, or hybrid vigor, is usually associated with increased heterozygosity that results in increased vigor of growth, survival, and fertility of hybrids as compared with the parental lines that were used to form the hybrid. Maximum heterosis is usually achieved by crossing two genetically different, highly inbred lines.

The production of hybrids is a well-developed industry, involving the isolated production of both the parental lines and the hybrids which result from crossing those lines. For a detailed discussion of the hybrid production process, see, e.g., Wright, *Commercial Hybrid Seed Production* 8:161-176, In *Hybridization of Crop Plants*.

Bulk Segregation Analysis (BSA). BSA, a.k.a. bulked segregation analysis, or bulk segregant analysis, is a method described by Michelmore et al. (Michelmore et al., 1991, Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proceedings of the National Academy of Sciences, USA*, 99:9828-9832) and Quarrie et al. (Quarrie et al., Bulk segregant analysis with molecular markers and its use for improving drought resistance in maize, 1999, *Journal of Experimental Botany*, 50(337):1299-1306).

For BSA of a trait of interest, parental lines with certain different phenotypes are chosen and crossed to generate F₂, doubled haploid or recombinant inbred populations with QTL analysis. The population is then phenotyped to identify individual plants or lines having high or low expression of the trait. Two DNA bulks are prepared, one from the individuals having one phenotype (e.g., resistant to pathogen), and the other from the individuals having reversed phenotype (e.g., susceptible to pathogen), and analyzed for allele frequency with molecular markers. Only a few individuals are required in each bulk (e.g., 10 plants each) if the markers are dominant (e.g., RAPDs). More individuals are needed when markers are co-dominant (e.g., RFLPs). Markers linked to the phenotype can be identified and used for breeding or QTL mapping.

Gene Pyramiding. The method to combine into a single genotype a series of target genes identified in different parents is usually referred as gene pyramiding. A non-limiting example of gene pyramiding scheme is shown in figure 3. The first part of a gene pyramiding breeding is called a pedigree and is aimed at cumulating one copy of all target genes in a single genotype (called root genotype). The second part is called the fixation steps and is aimed at fixing the target genes into a homozygous state, that is, to derive the ideal genotype (ideotype) from the root genotype. Gene pyramiding can be combined with marker assisted selection (MAS, see Hospital et al., 1992, 1997a, and 1997b, and Moreau et al, 1998) or marker based recurrent selection (MBRS, see Hospital et al., 2000).

RNA Interference (RNAi)

RNA interference (RNAi) is the process of sequence-specific, post-transcriptional gene silencing or transcriptional gene silencing in animals and plants, initiated by double-stranded RNA (dsRNA) that is homologous in sequence to the silenced gene. The preferred RNA effector molecules useful in this invention must be sufficiently distinct in sequence from any host polynucleotide sequences for which function is intended to be undisturbed after any of the methods of this invention are performed. Computer algorithms may be used to define the essential lack of homology between the RNA molecule polynucleotide sequence and host, essential, normal sequences.

The term “dsRNA” or “dsRNA molecule” or “double-strand RNA effector molecule” refers to an at least partially double-strand ribonucleic acid molecule containing a region of at least about 19 or more nucleotides that are in a double-strand conformation. The double-stranded RNA effector molecule may be a duplex double-stranded RNA formed from two separate RNA strands or it may be a single RNA strand with regions of self-complementarity capable of assuming an at least partially double-stranded hairpin conformation (i.e., a hairpin dsRNA or stem-loop dsRNA). In various embodiments, the dsRNA consists entirely of ribonucleotides or consists of a mixture of ribonucleotides and deoxynucleotides, such as RNA/DNA hybrids. The dsRNA may be a single molecule with regions of self-complementarity such that nucleotides in one segment of the molecule base pair with nucleotides in another segment of the molecule. In one aspect, the regions of self-

complementarity are linked by a region of at least about 3-4 nucleotides, or about 5, 6, 7, 9 to 15 nucleotides or more, which lacks complementarity to another part of the molecule and thus remains single-stranded (i.e., the "loop region"). Such a molecule will assume a partially double-stranded stem-loop structure, optionally, with short single stranded 5' and/or 3' ends. In one aspect the regions of self-complementarity of the hairpin dsRNA or the double-stranded region of a duplex dsRNA will comprise an Effector Sequence and an Effector Complement (e.g., linked by a single-stranded loop region in a hairpin dsRNA). The Effector Sequence or Effector Strand is that strand of the double-stranded region or duplex which is incorporated in or associates with RISC. In one aspect the double-stranded RNA effector molecule will comprise an at least 19 contiguous nucleotide effector sequence, preferably 19 to 29, 19 to 27, or 19 to 21 nucleotides, which is a reverse complement to the RNA of nucleic acid sequences coding for EG261, homologs of EG261, orthologs of EG261 and/or paralogs of EG261, and/or fragments and variations thereof, or an opposite strand replication intermediate. In one embodiment, said double-stranded RNA effector molecules are provided by providing to a soybean or other plant, plant tissue, or plant cell an expression construct comprising one or more double-stranded RNA effector molecules. In one embodiment, the expression construct comprises a double-strand RNA derived from any one of nucleic acid sequences coding for EG261, homologs of EG261, orthologs of EG261 and/or paralogs of EG261, and/or fragments and variations thereof. In other embodiments, the expression construct comprises a double-strand RNA derived from more than one sequences of nucleic acid sequences coding for EG261, homologs of EG261, orthologs of EG261 and/or paralogs of EG261, and/or fragments and variations thereof. In further embodiments, the expression construct comprises a double-strand RNA derived from more than one sequences of nucleic acid sequences coding for EG261, homologs of EG261, orthologs of EG261 and/or paralogs of EG261, and/or fragments and variations thereof, and one or more other genes involved in pathogen resistance. One skilled in the art will be able to design suitable double-strand RNA effector molecule based on the nucleotide sequences of nucleic acid sequences coding for EG261, homologs of EG261, orthologs of EG261 and/or paralogs of EG261, and/or fragments and variations thereof in the present invention.

In some embodiments, the dsRNA effector molecule of the invention is a “hairpin dsRNA”, a “dsRNA hairpin”, “short-hairpin RNA” or “shRNA”, i.e., an RNA molecule of less than approximately 400 to 500 nucleotides (nt), or less than 100 to 200 nt, in which at least one stretch of at least 15 to 100 nucleotides (e.g., 17 to 50 nt, 19 to 29 nt) is base paired with a complementary sequence located on the same RNA molecule (single RNA strand), and where said sequence and complementary sequence are separated by an unpaired region of at least about 4 to 7 nucleotides (or about 9 to about 15 nt, about 15 to about 100 nt, about 100 to about 1000 nt) which forms a single-stranded loop above the stem structure created by the two regions of base complementarity. The shRNA molecules comprise at least one stem-loop structure comprising a double-stranded stem region of about 17 to about 500 bp; about 17 to about 50 bp; about 40 to about 100 bp; about 18 to about 40 bp; or from about 19 to about 29 bp; homologous and complementary to a target sequence to be inhibited; and an unpaired loop region of at least about 4 to 7 nucleotides, or about 9 to about 15 nucleotides, about 15 to about 100 nt, about 250-500bp, about 100 to about 1000 nt, which forms a single-stranded loop above the stem structure created by the two regions of base complementarity. It will be recognized, however, that it is not strictly necessary to include a “loop region” or “loop sequence” because an RNA molecule comprising a sequence followed immediately by its reverse complement will tend to assume a stem-loop conformation even when not separated by an irrelevant “stuffer” sequence.

The expression construct of the present invention comprising DNA sequence which can be transcribed into one or more double-stranded RNA effector molecules can be transformed into a plant, wherein the transformed plant produces different fatty acid compositions than the untransformed plant. The target sequence to be inhibited by the dsRNA effector molecule include, but are not limited to, coding region, 5' UTR region, 3' UTR region of fatty acids synthesis genes. In one embodiment, the target sequence is from one or more nucleic acid sequences coding for EG261, homologs of EG261, orthologs of EG261 and/or paralogs of EG261, and/or fragments and variations thereof.

The effects of RNAi can be both systemic and heritable in plants. In plants, RNAi is thought to propagate by the transfer of siRNAs between cells through plasmodesmata. The

heritability comes from methylation of promoters targeted by RNAi; the new methylation pattern is copied in each new generation of the cell. A broad general distinction between plants and animals lies in the targeting of endogenously produced miRNAs; in plants, miRNAs are usually perfectly or nearly perfectly complementary to their target genes and induce direct mRNA cleavage by RISC, while animals' miRNAs tend to be more divergent in sequence and induce translational repression. Detailed methods for RNAi in plants are described in David Allis et al (Epigenetics, CSHL Press, 2007, ISBN 0879697245, 9780879697242), Sohail et al (Gene silencing by RNA interference: technology and application, CRC Press, 2005, ISBN 0849321417, 9780849321412), Engelke et al. (RNA Interference, Academic Press, 2005, ISBN 0121827976, 9780121827977), and Doran et al. (RNA Interference: Methods for Plants and Animals, CABI, 2009, ISBN 1845934105, 9781845934101), which are all herein incorporated by reference in their entireties for all purposes.

The present invention provides methods of producing soybeans or other plants containing altered and/or increased levels of pathogen tolerance and/or resistance. Such methods comprise utilizing the soybean or other plants comprising the chimeric genes as described above.

The present invention also provides methods of breeding soybean and other plants producing altered and/or increased levels of pathogen tolerance and/or resistance. In one embodiment, such methods comprise:

i) making a cross between the soybean or other plant species with nucleic acid sequences coding for EG261, homologs of EG261, orthologs of EG261 and/or paralogs of EG261, and/or fragments and variations thereof as described above to a second soybean or other plant species to make F1 plants;

ii) backcrossing said F1 plants to said second soybean or plant species, respectively;

iii) repeating backcrossing step until said nucleic acid sequences are integrated into the genome of said second soybean or other plant species, respectively. Optionally, such method can be facilitated by molecular markers.

The present invention provides methods of breeding species close to *Glycine max*, wherein said species produces altered and/or increased levels of pathogen tolerance and/or resistance. In one embodiment, such methods comprise

- 5 i) making a cross between the non-*Glycine max* species containing nucleic acid sequences coding for homologs of EG261, orthologs of EG261 and/or paralogs of EG261, and/or fragments and variations thereof as described above to *Glycine max* to make F1 plants;
- ii) backcrossing said F1 plants to *Glycine max*;
- 10 iii) repeating backcrossing step until said nucleic acid sequences are integrated into the genome of *Glycine max*. Special techniques (e.g., somatic hybridization) may be necessary in order to successfully transfer a gene from non-*Glycine max* species to *Glycine max*. Optionally, such method can be facilitated by molecular markers.

The present invention is further illustrated by the following examples that should not be construed as limiting. The contents of all references, patents, and published patent
15 applications cited throughout this application, as well as the Figures, are incorporated herein by reference in their entirety for all purposes.

EXAMPLES

Example 1. Identification and characterization of EG261 in domesticated soybean

20 (*Glycine max*)

This invention involves the discovery that the soybean gene referred to herein as ‘EG261’ codes for a dirigent protein that confers resistance to pathogens, including resistance to nematodes. There are 2 copies of EG261 in soybeans, but only one of the gene copies, which is referred to herein as “copy A,” is positively selected. The second copy, which is
25 referred to herein as “copy B,” is well-conserved and there is no evidence that it plays any role in nematode resistance (or in any other trait of agronomic interest). Unless otherwise specified herein, all references to EG261 are referring to copy A of the gene.

EG261 is very highly expressed in roots, as might be expected for a gene that confers resistance to SCN, as this is a primary route for SCN entrance to the plant.

It appears that EG261 maps into a predicted location (i.e., a QTL) on soybean chromosome 1 for SCN resistance. This QTL is supported by two different studies (Vergel C. Concibido, Brian W. Diers, and Prakash R. Arelli, *Crop Science*, VOL. 44, JULY–AUGUST 2004, 1121- 1131, and Pin Yue, David A. Sleper and Prakash R. Arelli, *Crop Science*, VOL. 41, SEPTEMBER–OCTOBER 2001, 1589-1595). However, this QTL is broad, and likely contains hundreds of genes, so the exact gene-of-interest has not previously been isolated. That positively-selected EG261 appears to fall within this QTL is consistent with our hypothesis that EG controls and confers SCN resistance. (It is noted, however, that the QTL location is still only tentatively mapped due to differences in size and structure of mapping populations, DNA marker platforms, and limited availability of DNA markers.)

EG261 soybean sequences follow. Initiation codons shown in underlined lower case font. Termination codons shown in **bolded** lower case font. The UTR sequence is in lower case.

The cDNA sequence of EG261 (SEQ ID NO: 1), derived from cultivated soybean (*Glycine max*) follows:

5'-

agcaagagatgctcaaaccctaaattcacctacttcgatgcttccccaccctataaattcttgcatattaccactttcatcaccctaatcc
acttctcttaattgacacccacctaaccatgGCTTCCCACCTTCCTCAAATCCCTCCTTCTCCTCTCCA
CCTATGCCCTCACCATCTCAGCAGAATACACAGGCTTCGTGGGAACACTAGACCC
AAAGTCCATAGGCATACACCACAAGAAAACCCTAAGCCACTTCAGGTCTACTG
GCACGAAGTCTTCAGCGGAGAAAACCCACATCGGTTAGAATCATTCCTCACTC
CCCAAATACAACGCAACCACAACCTTCGGCTCCGTTGGAATCTTTGACACCCCTT
TAACCGTGGGACCTGAGGTGTACTCCAAGGTTGTTCGGAAAAGCCGAGGGGCTTGT
TTGCCTCCACGTCACAAACGCAGTTTGACCTGTTACTGATTTACAACCTTCGCGTTG
ACCCAAGGGAAGTACAACGGCAGCACCATCACGTTACGGGGAGGAGCCCCCTC
TCGGAGAAGGTGAGGGAGCTGCCCATTTGTTGGTGGTAGTGGGGTCTTCAAATTTG
CCACTGGGTATGTTGAGTCTAGGACGCTAAGTTTTGATCCCCAAACAAGGAATAA
CACGGTTCAGTTCGACGTGTATTTACTATtgatgattattgaatgtgtttttcatgttgatgcgttatcgct
ttggtctgtctcacctagtttcta

EG261 coding sequence (cultivated soybean; *Glycine max*, SEQ ID NO: 2):

atgGCTTCCCACCTTCCTCAAATCCCTCCTTCTCCTCTCCACCTATGCCCTCACCATCT
 5 CAGCAGAATACACAGGCTTCGTGGGAACACTAGACCCAAAGTCCATAGGCATAC
 ACCACAAGAAAACCCTAAGCCACTTCAGGTTCTACTGGCACGAAGTCTTCAGCG
 GAGAAAACCCACATCGGTTAGAATCATTCCTCACTCCCCAAATACAACGCAAC
 CACAACCTTCGGCTCCGTTGGAATCTTTGACACCCCTTTAACCGTGGGACCTGAG
 GTGTACTCCAAGGTTGTTCGGAAGCCGAGGGCTTGTTTGCCTCCACGTCACAAA
 10 CGCAGTTTGACCTGTTACTGATTTACAACCTTCGCGTTGACCCAAGGGAAGTACAA
 CGGCAGCACCATCACGTTACGGGGAGGAGCCCCCTCTCGGAGAAGGTGAGGGA
 GCTGCCCATTTGTTGGTGGTAGTGGGGTCTTCAAATTTGCCACTGGGTATGTTGAG
 TCTAGGACGCTAAGTTTTGATCCCCAAACAAGGAATAACACGGTTCAGTTCGACG
 TGTATATTTACTATtga

15

The EG261 cDNA sequence is more than 99% identical to Sequences 65260 and 33011 of U.S. Patent No. 7,569,389, each of which are identical to each other (SEQ ID NO: 3):

20 1 atcaccaat ccacttctc taattgacac ccacctaaac catggcttcc cacttctca
 61 aatectect tctectctc acctatgcc tcaccatctc agcagaatac acaggtctcg
 121 tgggaacact agacccaaag tccataggca tacaccacaa gaaaacccta agccacttca
 181 ggttctactg gcaegaagtc ttcagcggag aaaaceccac atcggttaga atcattccct
 241 cactccccaa atacaacgca accacaacct tcggctcgt tggaatctt gacacccctt
 25 301 taaccgtggg acctgaggtg tactccaagg ttgtcggaaa agccgagggc ttgttgcct
 361 ccacgtcaca aacgcagttt gacctgttac tgatttaca cttcgcgttg acccaaggga
 421 agtacaacgg cagcaccatc acgttcacgg ggaggagccc cctctcggag aaggtgaggg
 481 agctgcccat tgttggtggt agtggggtct tcaaatttgc cactgggtat gttgagtcta
 541 ggacgctaag tttgatecc caaacaagga ataacacggt tcagttcgac gtgtatattt

601 actattgatg attattgaaa gtgtttttt catgttgatg cgttatcgct ttggtctgtc
 661 tcacctagtt tctacataag ttctctctt tgaggggcatg tgggtcatg gaataaagtc
 721 atctttgggc aaaaaaaaaa aaaaaaaaaa

5 **Example 2. Identification of orthologs of EG261 in wild soybean species**

Using analysis techniques derived from molecular evolutionary biology, gene sequences from the cultivated (i.e., domesticated) soybean, *Glycine max*, were compared to orthologous genes from species of wild soybean that are relatives to *G. max* and that were suspected to be resistant to attack by the Soybean Cyst Nematode (SCN). This analysis was performed in high-throughput fashion, applying Evolutionary Genomics' proprietary software and patented approach. See, e.g., U.S. Patent No. 6,228,586; U.S. Patent No. 6,274,319; and U.S. Patent No. 6,280,953.

Most varieties of cultivated soybeans are susceptible to one or more races (now more commonly known as HG groups) of SCN, and even those soybean cultivars bred for resistance to SCN are now often losing that resistance as the cyst nematodes evolve the ability to evade the current resistance mechanisms. Present resistance mechanisms all depend upon only one or two genes, so are relatively easy targets for the evolution of resistance.

Wild soybean relatives were chosen for analysis; these species were those that the scientific literature suggested were tolerant to one or more races of SCN. These putatively SCN-resistant species were then tested in standard assays for SCN susceptibility/resistance. Multiple races (HG groups) were used to test each wild soybean accession. In multiple replicates, multiple plants of each wild soybean species were challenged by SCN; each challenge involved multiple SCN races/HG groups.

After demonstrating that several wild species were indeed resistant to SCN, these species were grown to maturity in order to collect RNA. Total RNA was prepared from individual plants of each of 5 SCN-resistant species. RNA was pooled from several tissues from each individual plant in order to maximize how comprehensively the transcriptome of that species was sampled. RNA was used to create cDNA libraries which were used to sequence the species' transcriptome. This was accomplished by Roche 454 high-throughput

next-gen sequencing, although any appropriate DNA sequencing method could have been used.

The resulting ESTs were compared on a gene-by-gene basis. The homologous sequences were analyzed to identify those that have nucleic acid sequence differences between the two species. Then molecular evolution analysis was conducted to evaluate quantitatively and qualitatively the evolutionary significance of the differences between orthologous genes derived from cultivated soybean (*G. max*) and each wild species (for example, *Glycine tabacina*) on a pairwise basis. Each wild species was also compared in pairwise fashion to every other wild soybean species. Automated bioinformatics analysis was then applied to each pairwise comparison and only those sequences that contain a nucleotide change (or changes) that yield evolutionarily significant change(s) were retained for further analysis. This enabled the identification of genes that have evolved to confer some evolutionary advantage as well as the identification of the specific evolved changes.

Any of several different molecular evolution analyses or Ka/Ks-type methods can be employed to evaluate quantitatively and qualitatively the evolutionary significance of the identified nucleotide changes between homologous gene sequences from related species. Kreitman and Akashi (1995) *Annu. Rev. Ecol. Syst.* 26:403-422; Li, *Molecular Evolution*, Sinauer Associates, Sunderland, Mass., 1997. For example, positive selection on proteins (i.e., molecular-level adaptive evolution) can be detected in protein-coding genes by pairwise comparisons of the ratios of nonsynonymous nucleotide substitutions per nonsynonymous site (Ka) to synonymous substitutions per synonymous site (Ks) (Li et al., 1985; Li, 1993). Any comparison of Ka and Ks may be used, although it is particularly convenient and most effective to compare these two variables as a ratio. Sequences are identified by exhibiting a statistically significant difference between Ka and Ks using standard statistical methods.

Preferably, the Ka/Ks analysis by Li *et al.* is used to carry out the present invention, although other analysis programs that can detect positively selected genes between species can also be used. Li et al. (1985) *Mol. Biol. Evol.* 2:150-174; Li (1993); see also J. Mol. Evol. 36:96-99; Messier and Stewart (1997) *Nature* 385:151-154; Nei (1987) *Molecular Evolutionary Genetics* (New York, Columbia University Press). The Ka/Ks method, which

comprises a comparison of the rate of non-synonymous substitutions per non-synonymous site with the rate of synonymous substitutions per synonymous site between homologous protein-coding regions of genes in terms of a ratio, is used to identify sequence substitutions that may be driven by adaptive selections as opposed to neutral selections during evolution.

5 A synonymous ("silent") substitution is one that, owing to the degeneracy of the genetic code, makes no change to the amino acid sequence encoded; a non-synonymous substitution results in an amino acid replacement. The extent of each type of change can be estimated as K_a and K_s , respectively, the numbers of synonymous substitutions per synonymous site and non-synonymous substitutions per non-synonymous site. Calculations of K_a/K_s may be
10 performed manually or by using software. An example of a suitable program is MEGA (Molecular Genetics Institute, Pennsylvania State University).

For the purpose of estimating K_a and K_s , either complete or partial protein-coding sequences are used to calculate total numbers of synonymous and non-synonymous substitutions, as well as non-synonymous and synonymous sites. The length of the
15 polynucleotide sequence analyzed can be any appropriate length. Preferably, the entire coding sequence is compared, in order to determine any and all significant changes. Publicly available computer programs, such as Li93 (Li (1993) J. Mol. Evol. 36:96-99) or INA, can be used to calculate the K_a and K_s values for all pairwise comparisons. This analysis can be further adapted to examine sequences in a "sliding window" fashion such that small numbers
20 of important changes are not masked by the whole sequence. "Sliding window" refers to examination of consecutive, overlapping subsections of the gene (the subsections can be of any length).

The comparison of non-synonymous and synonymous substitution rates is commonly represented by the K_a/K_s ratio. K_a/K_s has been shown to be a reflection of the degree to
25 which adaptive evolution has been at work in the sequence under study. Full length or partial segments of a coding sequence can be used for the K_a/K_s analysis. The higher the K_a/K_s ratio, the more likely that a sequence has undergone adaptive evolution and the non-synonymous substitutions are evolutionarily significant. See, for example, Messier and Stewart (1997).

Ka/Ks ratios significantly greater than unity strongly suggest that positive selection has fixed greater numbers of amino acid replacements than can be expected as a result of chance alone, and is in contrast to the commonly observed pattern in which the ratio is less than or equal to one. Nei (1987); Hughes and Nei (1988) *Nature* **335**:167 170; Messier and Stewart (1994) *Current Biol.* **4**:911 913; Kreitman and Akashi (1995) *Ann. Rev. Ecol. Syst.* **26**:403 422; Messier and Stewart (1997). Ratios less than one generally signify the role of negative, or purifying selection: there is strong pressure on the primary structure of functional, effective proteins to remain unchanged.

All methods for calculating Ka/Ks ratios are based on a pairwise comparison of the number of nonsynonymous substitutions per nonsynonymous site to the number of synonymous substitutions per synonymous site for the protein-coding regions of homologous genes from related species. Each method implements different corrections for estimating "multiple hits" (i.e., more than one nucleotide substitution at the same site). Each method also uses different models for how DNA sequences change over evolutionary time. Thus, preferably, a combination of results from different algorithms is used to increase the level of sensitivity for detection of positively-selected genes and confidence in the result.

It is understood that the methods described herein could lead to the identification of soybean polynucleotide sequences that are functionally related to soybean protein-coding sequences. Such sequences may include, but are not limited to, non-coding sequences or coding sequences that do not encode proteins. These related sequences can be, for example, physically adjacent to the soybean protein-coding sequences in the soybean genome, such as introns or 5'- and 3'-flanking sequences (including control elements such as promoters and enhancers). These related sequences may be obtained via searching a public genome database such as GenBank or, alternatively, by screening and sequencing an appropriate genomic library with a protein-coding sequence as probe. Methods and techniques for obtaining non-coding sequences using related coding sequence are well known to one skilled in the art.

After candidate genes were identified, the nucleotide sequences of the genes in each orthologous gene pair were carefully verified by standard DNA sequencing techniques and then Ka/Ks analysis was repeated for each carefully sequenced candidate gene pair.

More specifically to this Example, the software ran through all possible pairwise comparisons between putative orthologs of every gene from cultivated soybean, *G. max*, as compared to the likely orthologs from the wild species, looking for high Ka/Ks ratios. The software BLASTed (in automated fashion) every mRNA sequence from cultivated soybean against every sequence in the transcriptome that was sequenced from a wild relative, for example, *G. pescadrensis*. The software then performed Ka/Ks analysis for each gene pair (i.e., each set of 'orthologs'), flagging the gene pairs with high Ka/Ks scores. The software then compared every *G max* sequence against every sequence of another wild relative, for example, *G. tabacina*, again by doing a series of BLASTs, and then sifting through for high Ka/Ks scores. It thus does this for the transcriptome sequence of all the wild species in succession. This gives a set of candidates (see below) for subsequent analysis.

The software next compared every gene sequence in the transcriptome of *G. pescadrensis* against every sequence of *G. tabacina*, again doing BLASTs, etc. It thus ultimately compared all of the expressed genes represented in the utilized cDNA libraries of every soybean species against all the genes of every other soybean species, both wild and cultivated, the goal being to find every gene that shows evidence of positive selection. While software was used to accomplish this analysis it can be accomplished by one skilled in the art using a calculator, i.e., it can all be “done by hand” – the software simply speeds things up.

The flagged gene pairs that emerged were then individually and carefully re-sequenced in the lab to check the accuracy of the original high-throughput reads; thus false positives were eliminated.

Next, every remaining candidate gene pair with a high Ka/Ks score was examined to determine if the comparison was truly orthologous, or just an artifactual false positive caused by a paralogous comparison.

Following are provided the nucleotide sequences of the orthologous genes that were definitely, positively selected based on this analysis. Information about the geographic

locations from which each accession was originally collected, as well any other available data about each accession are also given below.

Glycine microphylla (SEQ ID NO: 4):

5 ttaattcatcactttcatcacccaatccact:cctctaattaacacccaccaacaccatgGCTTCCCACCTTCCTCAAATC
CCTCCTTCTCCTCTCCACCTATGCCCTCACCATCTCAGCAGAATACACAGGCTTCG
TGGGGACACTAGACCCAAAGTCCATAGGCATACACCACAAGAAAACCCTAAGCC
ACTTCAGGTTCTACTGGCACGAAGTCTTCAGCGGAGAAAACCCCAACACGGTTA
GAATCATTCCCTCACTCCCCAAATACAACACAACCACAACCTTCGGTTCCGTTGG
10 AATCTTTGACAACACTTTAACCGTGGGACCTGAGGTGTACTCCAAGGTTGCCGGA
AAAGCCGAGGGCTTGTTTGCCTCCACGTCACAAACGCAGTTTAACCTGTTACTGA
TTTACAGCTTCGCGTTGACCCAAGGGAAGTACAACGGCAGCACCATCACGTTTCAC
GGGGAGGAGCCCCCTCTCGGAGAAGGTGAGGGAGCTGCCCATTTGTTGGTGGCAG
TGGGGTCTTCAAATTTGCCACTGGGTATGTTGAGTCTAGGACGCTAAGTTTTGAT
15 CCCCCAACGAGGAATAACACGGTTCAGTTCGACGTGTATATTTACTATtgatgattattg
aatgtgtttttacatgttgatgtgtagagcttgggtgtgtgtcacttagtttcta

This sequence is from Accession PI505196 which was collected 30-Jul-1983.

Queensland, Australia. Locality: 5.4 km from road junction (Evelyn) towards Tumoulin.

20 Latitude: 17 deg 32 min S (-17.53333333), Longitude: 145 deg 26 min E (145.43333333)
Elevation: 900 meters.

Demonstrated to be resistant to SCN race 3 (Bauer, S., T. Hymowitz, and G. R. Noel.
2007. Soybean cyst nematode resistance derived from *Glycine tomentella* in amphiploid (*G.*
max x *G. tomentella*) hybrid lines. *Nematropica* 37:277-285).

25

Glycine pescadrensis (SEQ ID NO: 5):

ataaattcttgcaattaattcatcactttcatcacccaatccatttcctctaattaacacccacccaaaaccatgGCTTCCCACCTTCC
TCAAATCCCTCCTTCTCCTCTCCACCTATGCCCTCACCATCTCAGCAGAATACACA
GGCTTCGTGGGCACACTAGACCCAAAGTCCATAGGCATACACCACAAGAAAACC

CTAAACCACTTCAGGTTCTACTGGCACGAAGTCTTCAGCGGAGAAAACCCACAT
 CGGTTAGAATCATTCCCTCACTCCCCAAATACAACACAACCACAACCTTCGGTTC
 CGTTGGAATCTCTGACAACGCTTTAACCGTGGGACCTGAGGTGTACTCCAAGGTT
 GTCGGAAAAGCCGAGGGGCTTGTGTTGTCTCCACGTCACAAACGCAGTTTGACCTGT
 5 TACTGATTTACAACCTTCGCGTTGACCCAAGGGAAGTACAACGGCAGCACCATCAC
 GTTCACGGGGAGGAGCCCCCTCTCGGAGAAGGTGAGGGAGCTGCCCATTTGTAGG
 TGGCAGTGGGGTCTTCAAATTTTCCACTGGGTATGTTGAGTCTAGGACGCTAAGT
 TTTGATCCCCAAACGAGGAATAACACGGTTCAGTTCGACGTGTATATTTACTATtg
 atgattattgaatgtgtttttccatgttgatgtgttatagctttggatgtgtcacttagtttcta

10

This sequence is from Accession PI537287, which was collected 07-Apr-1988.
 Taiwan. Locality: Military area, Sea Stride (Penghu Bridge), Paisha Island, Pescadores
 Islands. Latitude: 23 deg 40 min 0 sec N (23.66666667), Longitude: 119 deg 34 min 48 sec E
 (119.58). Elevation: 5 meters.

15

Glycine tabacina (New South Wales, Australia, SEQ ID NO: 6):

catcacccaatccact:cctctaattaacacccaccaacaccatgGCTTCCCCTTCCTCAAATCCCTCCTTCT
 CCTCTCCACCTATGCCCTCACCATCTCAGCAGAATACACAGGCTTCGTGGGGACA
 CTAGACCCAAAGTCCATAGGCATACACCACAAGAAAACCCTAAGCCACTTCAGG
 20 TTCTACTGGCACGAAGTCTTCAGCGGAGAAAACCCACAAACGGTTAGAATCATT
 CCTCACTCCCCAAATACAACACAACCACAACCTTCGGTTCCGTTGGAATCTTTGA
 CAACACTTTAACCGTGGGACCTGAGGTGTACTCTAAGGTTGCCGGAAAAGCCGA
 GGGCTTGTGTTGCCTCCACGTCACAAACGCAGTTTAACCTGTTACTGATTTACAGCT
 TCGCGTTGACCCAAGGGAAGTACAACGGCAGCACCATCACGTTACGGGGAGGA
 25 GCCCCCTCTCGGAGAAGGTGAGGGAGCTGCCCATTTGTTGGTGGCAGTGGGGTCTT
 CAAATTTGCCACTGGGTATGTTGAGTCTAGGACGCTAAGTTTTGATCCCCAAACG
 AGGAATAACACGGTTCAGTTCGACGTGTATATTTACTATtgatgattattgaatgtgtttttacatgt
 tgatgtgttagagctttgggtgtgtcacttagtttcta

This sequence is from *G. tabacina* Accession PI446968, collected in: New South Wales, Australia, and some time prior to 1980. Locality: Toward west Wyalong, 35 km from Condobolin. Latitude: 33 deg 20 min S (-33.33333333), Longitude: 147 deg 8 min E (147.13333333) Elevation: 260 meters.

- 5 Demonstrated to be resistant to SCN race 3 (Bauer, S., T. Hymowitz, and G. R. Noel. 2007. Soybean cyst nematode resistance derived from *Glycine tomentella* in amphiploid (*G. max* x *G. tomentella*) hybrid lines. *Nematropica* 37:277-285).

Glycine tabacina (Taiwan, SEQ ID NO: 7):

- 10 cctacttcgatgcttccccaccttataaattcttgcaatcattcatcactttcatcaccatccatttcctctaattaacaccacccaaaaacc
atgGCTTCCCACCTTCCTCAAATCCCCTCCTTCTCCTCTCCACCTATGCCCTCACCATCT
CAGCAGAATACACAGGCTTCGTGGGCACACTAGACCCAAAGTCCATAGGCATAC
ACCACAAGAAAACCCTAAACCACTTCAGGTTCTACTGGCACGAAGTCTTCAGCG
GAGAAAACCCACATCGGTTAGAATCATTCCCTCACTCCCCAAATACAACACAAC
15 CACAACCTTCGGTTCCGTTGGAATCTCTGACAACGCTTTAACCGTGGGACCTGAG
GTGTACTCCAAGGTTGTTCGGAAAAGCCGAGGGGCTTGTTTGTCTCCACGTCACAAA
CGCAGTTTGACCTGTTACTGATTTACAACCTTCGCGTTGACCCAAGGGAAGTACAA
CGGCAGCACCATCACGTTACGGGGAGGAGCCCCCTCTCGGAGAAGGTGAGGGA
GCTGCCCATTTGTAGGTGGCAGTGGGGTCTTCAAATTTTCCACTGGGTATGTTGAG
20 TCTAGGACGCTAAGTTTTGATCCCCAAACGAGGAATAACACGGTTCAGTTCGACG
TGTATATTTACTATtgatgattattgaatgtgtttttccatgttgatgtgttatagctttggatgtgtcacttagttteta

- This sequence is from *G. tabacina* Accession PI559281, collected in Penghu, Taiwan, 08-Apr-1988. Locality: Airport (NE) on Chimei Island. Latitude: 23 deg 33 min 48 sec N (23.5633577), Longitude: 119 deg 37 min 41 sec E (119.628067).
- 25

Demonstrated to be resistant to SCN race 3 (Bauer, S., T. Hymowitz, and G. R. Noel. 2007. Soybean cyst nematode resistance derived from *Glycine tomentella* in amphiploid (*G. max* x *G. tomentella*) hybrid lines. *Nematropica* 37:277-285.)

Glycine tomentella (An alternate name for this species, used in some literature, is *G. latifolia*.) (Australia, SEQ ID NO: 8):

cacctactcgatgcttccccccaccttataaattcttgcattaattcatcactttcatcacccaatccatttctctaattaacacccaccaaa
 accatgGCTTCCCCTTCCCTCAAATCCCTCCTTCTCCTCTCCACCTATGCCCTCACCAT
 5 CTCAGCAGAATACACAGGCTTCGTGGGCACACTAGACCCAAAGTCCATAGGCAT
 ACACCACAAGAAAACCCTAAACCACTTCAGGTTCTACTGGCACGAAGTCTTCAGC
 GGAGAAAACCCACATCGGTTAGAATCATTCCCTCACTCCCCAAATACAACACA
 ACCACAACCTTCGGTTCGGTTGGAATCTCTGACAACGCTTTAACCGTGGGACCTG
 AGGTGTACTCCAAGGTTGTCTGGAAAAGCCGAGGGCTTGTTTGTCTCCACGTCACA
 10 AACGCAGTTTGACCTGTTACTGATTTACAACCTTCGCGTTGACCCAAGGGAAGTAC
 AACGGCAGCACCATCACGTTACGGGGAGGAGCCCCCTCTCGGAGAAGGTGAGG
 GAGCTGCCCATTTGTAGGTGGCAGTGGGGTCTTCAAATTTTCCACTGGGTATGTTG
 AGTCTAGGATGCTAAGTTTTGATCCCCAAACGAGGAATAACACGGTTCAGTTCGA
 CGTGTATATTTACTATtgatgattattgaatgtgttttccatgttgatgttatagcttggtatgtgtcacttagtttctatat
 15 aagtttccama

This sequence is from *G. tomentella* Accession PI 499946, collected in New South Wales, Australia. Locality: Delungra. Latitude: 29 deg 39 min 0 sec S (-29.65), Longitude: 150 deg 50 min E (150.83333333) Elevation: 600 meters.

20 Demonstrated to be resistant to SCN (Bauer, S., T. Hymowitz, and G. R. Noel. 2007. Soybean cyst nematode resistance derived from *Glycine tomentella* in amphiploid (*G. max* x *G. tomentella*) hybrid lines. *Nematropica* 37:277-285).

25 All of the above EG261 sequences from the wild relatives of *G. max* are all “copy A” (as given above for *G. max*). Copy A from each wild species has been strongly positively selected as compared to copy A of *G. max*. In addition, when compared to each other (i.e., not compared only to cultivated soybeans) EG261 copy A from each wild species examined has been strongly positively selected relative to each of the other wild species. This suggests that each wild species has likely been confronted by several different races of SCN, as well as

by different species and even categories of pathogens (such as bacteria, fungi, insects, and nematodes) so that each wild species of soybean has thus likely evolved/achieved resistance to multiple SCN races.

Our demonstration of the clear episodes of positive selection in the wild soybean species, relative to each of the other wild species, suggests strongly that the orthologs of EG261 from each of these wild species is of value for creating SCN resistance in cultivated soybeans.

Example 3. Characterization of orthologs of EG261 in wild soybean species

Plants from wild, related species containing the orthologs identified according to Example 2 were assayed for their actual ability to confer resistance. In this example, wild soybean species of the genus *Glycine* were tested for their innate ability to resist challenge by soybean cyst nematode (SCN). Germplasm accessions were obtained from the National Plant Germplasm System (NPGS).

Seeds were germinated and seedlings were tested for SCN resistance in conical containers. (Niblack, T., Tylka, G. L., Arelli, P., Bond, J., Diers, B., Donald, P., Faghihi, J., Ferris, V. R., Gallo, K., Heinz, R. D., Lopez-Nicora, H., Von Qualen, R., Welacky, T., and Wilcox, J. 2009. A standard greenhouse method for assessing soybean cyst nematode resistance in soybean: SCE08 (standardized cyst evaluation 2008). Online. *Plant Health Progress* doi:10.1094/PHP-2009-0513-01-RV.)

For those species that were confirmed to have innate ability to resist SCN, RNAs from several tissues (including root, stem, and leaf) were flash-frozen and both genomic DNA and RNA were prepared from the frozen tissues. RNAs from multiple tissues from each plant then were pooled for subsequent construction of cDNA libraries (one library per species). High-throughput 454 Titanium sequencing was performed for each cDNA library. It is noted, however, that any suitable high-throughput sequencing format would have been acceptable and using this particular method was not critical to the success of these experiments.

The resulting transcriptome sequence datasets for each species were analyzed in order to identify any positively selected transcripts using proprietary analysis software of Evolutionary Genomics, Inc.

Candidate genes were verified using protocols in transgenic soybean plants that test for effectiveness in protecting the transformed soybean plants from attack by the SCN organism as well as the *Phytophthora* fungus. We report here results from experiments where RNAi is used to knock out EG261 in an SCN-resistant line (Fayette). After knockout, the normally resistant Fayette line became SCN sensitive. The control for this experiment was RNAi silencing of EG261 in the Williams 82, which is SCN-sensitive: no difference in sensitivity was observed after silencing of EG261 in this line. These experiments are interpreted to mean that resistance to SCN is conferred by the EG261 gene; if one knocks out the EG261 allele in SCN-resistant lines, the result is that SCN resistance is then lost. (Interestingly, the allele of EG261 in the SCN-sensitive William 82 line is already either non-functional or at least not very effective in conferring SCN-resistance, so that silencing of the EG261 allele does not change the SCN-sensitive phenotype of Williams 82.) Importantly, the resistant line (Fayette) is one that was created by cross-breeding to soybean land-races that display SCN-resistance. (It is believed that the land-races became SCN-resistant because of introgression with wild SCN-resistant soybean species.)

Example 4. Silencing of EG261 eliminates the SCN-resistant phenotype in SCN-resistant soybeans

Construct pG2RNAi2 was produced carrying a hairpin to silence the target gene EG261 and the constructs were used to produce transgenic roots in the soybean variety 'Fayette.' Two replications of SCN demographics experiments were conducted for the 'Fayette' transgenic roots. For a detailed description of the test protocol used herein, see Melito et al. (2010) A nematode demographics assay in transgenic roots reveals no significant impacts of the Rhg1 locus LRR-Kinase on soybean cyst nematode resistance, BMC Plant Biology 10:104, 14 pages.

Approximately 250 hatched J2 nematodes were placed near the tip of each transgenic soybean root, with root identities blinded by assignment of randomized numbers. After two weeks, roots were fixed and stained with acid fuchsin and the number and growth stage of all nematodes within or attached to each root was assessed (average 76 total nematodes per root).

In the first of two replicate experiments, in the 'Fayette' transgenic roots, expression levels of EG261 are on average about 12% of Fayette wild-type. 11 of 14 transgenic roots had silencing >50% and were included; 3 roots were excluded due to poor silencing. In the second replicate experiment, tissue was saved but silencing was not checked. Therefore, all of the data was included (hence the phenotypic impact of silencing may be masked a bit due to inclusion of some non-silenced roots in the data that was obtained).

Data show significant shift toward susceptibility (Figure 1). Bars show the proportion of nematode population on each root that advanced past the J2 stage (mean $\pm$ std.error). F: Fayette (SCN-resistant); W: Williams 82 (SCN-susceptible); EV: transformed with empty vector; hp: transformed with EG261 hairpin gene silencing construct; Ox: transformed with construct expressing Glyma01g31770 under control of strong *G. max* ubiquitin promoter. ANOVA P value for similarity of means is 0.005 for F EV compared to F hp, and 0.97 for W EV compared to W Ox.

The control for this experiment was RNAi silencing of EG261 in the soybean cultivar Williams 82, which is SCN-sensitive; no difference in sensitivity was observed after silencing of EG261 in this line. These experiments were interpreted to mean that resistance to SCN is conferred by the EG261 gene; if one knocks out the EG261 allele in SCN-resistant lines, the result is that SCN resistance is then lost. However, the allele of EG261 in the sensitive William 82 line is already either non-functional or at least not very effective in conferring SCN-resistance, so that silencing of the EG261 allele does not change the SCN-sensitive phenotype of Williams 82. Importantly, the resistant line (Fayette) is one that was created by cross-breeding to soybean land-races that display SCN-resistance. (It is believed that the land-races became SCN-resistant because of introgression with wild SCN-resistant

soybean species.) This is strong evidence for the role of EG261 in influencing/controlling SCN resistance in soybeans.

Example 5. Transformation of tomato with EG261

‘Big Boy’ tomato plants can be transformed with a construct containing EG261 using standard transformation technology for tomato plants. See, e.g., Antonio Di Matteo, Maria Manuela Rigano, Adriana Sacco, Luigi Frusciante and Amalia Barone (2011). Genetic Transformation in Tomato: Novel Tools to Improve Fruit Quality and Pharmaceutical Production, Genetic Transformation, Prof. María Alvarez (Ed.), ISBN: 978-953-307-364-4, InTech.

Transformation of tomato plants with a construct containing EG261 can be accomplished with either the cultivated/the wild soybean orthologs included in this patent, or any of the other EG261 orthologs described in this application, including, the tomato ortholog (SEQ ID NO 16).

The transformed tomato plants can be tested for disease resistance as compared to the untransformed, control ‘Big Boy’ tomato plants.

Diseases which can be tested for conferred or enhanced pathogen tolerance and/or resistance as a result of such transformation include root rot caused by *Rhizoctonia solani*, *Fusarium solani* and *Sclerotium rolfsii*. For procedures used to test for tolerance/resistance to these root diseases see, e.g., Abd-El-Kareem, F., Nehal S. El-Mougy, Nadia G. El-Gamal and Y.O. Fotouh (2006) Use of Chitin and Chitosan Against Tomato Root Rot Disease under Greenhouse Conditions Research, Journal of Agriculture and Biological Sciences, 2(4): 147-152.

The transformed tomato plants showing conferred and/or enhanced resistance to one or more of these root diseases can be tested to confirm the presence of EG261 by using one or more of the following procedures: (a) isolating nucleic acid molecules from said plant and amplifying sequences homologous to the EG261 polynucleotide; (b) isolating nucleic acid molecules from said plant and performing a Southern hybridization to detect the EG261 polynucleotide; (c) isolating proteins from said plant and performing a Western Blot using

antibodies to a protein encoded by the EG261 polynucleotide; and/or (d) demonstrating the presence of mRNA sequences derived from a EG261 polynucleotide mRNA transcript and unique to the EG261 polynucleotide.

5 **Example 6. Tomato breeding with tomato plants expressing EG261**

A 'Big Boy' tomato plant with a conferred copy of the coding sequence for EG261 as obtained in Example 6 can be crossed to a plant of the 'Early Girl' variety and the resultant progeny can be tested for tolerance/resistance to the three root diseases.

10 The presence of the EG261 polynucleotide can be confirmed in the resultant 'Early Girl' progeny according to the procedures set forth in Example 5.

In a further procedure, the transformed 'Early Girl' tomato plant can be backcrossed one or more times to 'Early Girl' to produce a near isogenic or isogenic 'Early Girl' tomato with the coding sequence for EG261.

15

Example 7. Transformation of maize with EG261

'B73' and/or 'Mol7' inbred maize plants can be transformed with a construct containing EG261 using standard transformation technology for maize. See, e.g., Sidorov and Duncan, 2008 (Agrobacterium-Mediated Maize Transformation: Immature Embryos Versus
20 Callus, Methods in Molecular Biology, 526:47-58); Frame et al., 2002 (Agrobacterium tumefaciens-Mediated Transformation of Maize Embryos Using a Standard Binary Vector System, Plant Physiology, May 2002, Vol. 129, pp. 13-22); and, Ahmadabadi et al., 2007 (A leaf-based regeneration and transformation system for maize (Zea mays L.), TransgenicRes. 16, 437-448).

25 Transformation of maize plants with a construct containing EG261 can be accomplished with either the cultivated/the wild soybean orthologs included in this patent, or with the maize ortholog of these EG261 genes.

The transformed maize plants can be tested for nematode/pest resistance as compared to the untransformed, control 'B73' or 'Mol7' maize plants. For procedures used to test for

tolerance/resistance to nematodes see, *e.g.*, Sasser, et al. (1984) Standardization of Host Suitability Studies and Reporting of Resistance to Root-Knot Nematodes, Crop Nematode Research & Control Project (CNRCP), a cooperative publication of The Department of Plant Pathology, North Carolina State University and the United States Agency for International Development, North Carolina State University Graphics, Raleigh, NC, 10 pages; and, Cook, R. and K. Evans (1987) Resistance and Tolerance, In: Principles and practice of nematode control in plants, R.H. Brown and B.R. Kerry (editors), pg. 179-231.

The transformed maize plants showing conferred and/or enhanced resistance to one or more races of nematodes can be tested to confirm the presence of EG261 by using one or more of the following procedures: (a) isolating nucleic acid molecules from said plant and amplifying sequences homologous to the EG261 polynucleotide; (b) isolating nucleic acid molecules from said plant and performing a Southern hybridization to detect the EG261 polynucleotide; (c) isolating proteins from said plant and performing a Western Blot using antibodies to a protein encoded by the EG261 polynucleotide; and/or (d) demonstrating the presence of mRNA sequences derived from a EG261 polynucleotide mRNA transcript and unique to the EG261 polynucleotide.

The transformed maize plants with confirmed tolerance and/or resistance to one or more nematode races can be maintained by self-pollinating the plants, harvesting the resultant seed and growing the resistant plants from such harvested seed.

Example 8. Maize breeding with maize plants expressing EG261

A 'B73' or 'Mo17' corn inbred plant with a conferred copy of the coding sequence for EG261 as obtained in Example 7 can be crossed to a transformed or non-transformed inbred 'Mo17' 'B73' plant, respectively, and the resultant hybrid F1 progeny can be tested for tolerance/resistance to nematodes.

The presence of the EG261 polynucleotide can be confirmed in the resultant hybrid progeny according to the procedures set forth in Example 7.

In a further procedure, the transformed hybrid maize plant can be backcrossed one or more times to its recurrent parent (i.e., either 'B73' or 'Mo17' as applicable) to produce a near isogenic or isogenic maize inbred with the coding sequence for EG261.

5 **Example 9. EG261 overexpression**

Overexpression of EG261 was tested using two replications of the soybean variety 'Williams 82.' For a detailed description of the test protocol used herein, *see* Melito et al. (2010) A nematode demographics assay in transgenic roots reveals no significant impacts of the Rhg1 locus LRR-Kinase on soybean cyst nematode resistance, BMC Plant Biology
10 10:104, 14 pages.

Expression level in "overexpression roots" was found to be 4.3-fold greater, however native EG261 gene is expressed very highly, only 5-fold less than ubiquitin, so these expression levels appear to be consistent with expectations.

Data show no significance difference from normal 'Williams 82' (i.e., without
15 overexpression of EG261), but these inconclusive results are probably because expression levels were not as rigorously controlled as was desirable.

Example 10. Identification of orthologs of EG261 in other plant species

Using analysis techniques derived from molecular evolutionary biology, gene
20 sequences from the cultivated (i.e., domesticated) soybean, *Glycine max*, were used to identify orthologous genes from other plant species. The following table provides the SEQ ID Nos. of the nucleotide sequences of genes that are orthologous to the positively selected soybean EG261 copy A gene. Predicted amino acid sequences, and information about the geographic locations from which each accession was originally collected, as well any other
25 available information about each accession are also given below.

Plant species	Nucleotide sequence SEQ ID NO.	Amino acid sequence SEQ ID NO.	Geographic locations and accession information
<i>Solanum lycopersicum</i> (or <i>Solanum esculentum</i>)	16	25	cultivated tomato
<i>Solanum peruvianum</i>	17	26	Peruvian tomato, a.k.a.,

Plant species	Nucleotide sequence SEQ ID NO.	Amino acid sequence SEQ ID NO.	Geographic locations and accession information
			Peruvian nightshade
<i>Solanum chmielewskii</i>	18	27	
<i>Solanum habrochaetes</i>	19	28	
<i>Solanum corneliomulleri</i>	20	29	
<i>Capsicum annuum</i>	21	30	chili pepper
<i>Capsicum annuum</i>	22	31	hot pepper
<i>Solanum melongena</i>	23	32	Eggplant
<i>Solanum tuberosum</i>	24	33	Potato
<i>Phaseolus vulgaris</i>	34	37	Common bean varieties “Blue Lake” (a bush type snap bean that was developed from the Blue Lake Pole bean), Henderson Bush Lima Bean (bean variety dates to 1885), Dixie Speckled Butterpea Lima Bean, Hidatsa Red Indian, and Kabouli Black Garbanzo Asian Bean (collected in Kabul, Afghanistan)
<i>Phaseolus vulgaris</i>	35	38	Common bean variety Scarlet Emperor (dates at least to 1750, was originally from Central America)
<i>Phaseolus vulgaris</i>	36	39	Jacob’s Cattle Bean
<i>Coffea arabica</i>	40	43	coffee plant, Allele A
<i>Coffea arabica</i>	41	44	coffee plant, Allele B
<i>Capsicum annuum</i>	42	45	cultivated pepper (“ornamental” pepper)
<i>Zea mays</i>	46	51	
<i>Sorghum sp.</i>	47	52	
<i>Oryza sativa,</i>	48	53	
<i>Triticum sp.</i>	49	54	
<i>Hordeum sp.</i>	50	55	
<i>Gossypium hirsutum</i>	56	57	Variety FJ600364
<i>Gossypium hirsutum</i>	58 64 65	59	Variety FJ600365
<i>Vigna unguiculata (Cowpea)</i>	60	61	Monkey tail (collected in Africa)
<i>Vigna unguiculata (Cowpea)</i>	62	63	Pea, varieties Risina Del

Plant species	Nucleotide sequence SEQ ID NO.	Amino acid sequence SEQ ID NO.	Geographic locations and accession information
			Trasiorfino (collected in Perugia, Italy) and Shanty Pea (collected in South Carolina, USA)

Example 11. Characterization of orthologs of EG261 isolated from additional plant species

5 The gene orthologs from wild, related species can be assayed for their ability to confer resistance. (In this example, corresponding wild plant species can also be tested for their innate ability to resist challenge by one or more parasitic nematodes. Seeds are germinated and seedlings are tested for resistance to one or more parasitic nematodes by any suitable standard testing methods.)

10 For each species examined, the species-specific ortholog of EG261 can be analyzed for evidence of positive selection, using proprietary analysis software of Evolutionary Genomics, Inc. Orthologs that appear to have been positively selected can then be assayed for the ability to confer resistance as described for soybean (*G. max*) in Examples 3 and 4.

15 **Example 12. Silencing of EG261 eliminates the nematode resistant phenotype in nematode-resistant plants**

20 Constructs are produced carrying an RNAi silencing DNA fragment (e.g., a hairpin, a double strand, an antisense, an inverted repeat, etc.) targeting each one of the EG261 orthologs isolated in Example 10. These constructs are used to produce transgenic plants for each plant species.

 Hatched J2 nematodes are placed near the tip of each transgenic plant. After a time sufficient for nematode infection, roots are fixed and stained with acid fuchsin and the number and growth stage of all nematodes within or attached to each root is assessed.

Data shows significant shift toward susceptibility in one or more transgenic plant species compared to their corresponding wild-type nematode-resistant control plant species.

Example 13. Introducing EG261 orthologs into heterologous plant species

5 In some embodiments, the EG261 orthologs conferring nematode resistance isolated according to the present invention from one plant species can be introduced into a nematode-susceptible plant of the same species in order to create nematode resistance into the plant.

However, in some other embodiments, the EG261 orthologs of the present invention isolated from one plant species can be introduced into a nematode-susceptible plant of
10 different species of the same genus, or a plant species of different genus in order to introduce nematode resistance into the plant.

For example, a gene encoding any one of the proteins of SEQ ID NOs: 9-15 which were isolated from soybean can be also introduced into other plant species, such as *Solanaceae* species (e.g., *Solanum lycopersicum*, *Solanum chemielewskii*, *Solanum*
15 *habrochaites*, *Solanum corneliomulleri*, *Capsicum annuum*, *Solanum melongena*, *Solanum tuberosum*), *Phaseolus vulgaris*, *Vigna unguiculata*, *Coffea arabica*, *Zea mays*, *Sorghum* spp., *Oryza sativa*, *Triticum* spp., *Hordeum* spp., *Gossypium hirsutum*, and *Heliotropium curassavicum*. Similarly, a gene encoding anyone of the proteins of SEQ ID NOs: 25, 26, 27, 28, 29, 30, 31, 32, 33, 37, 38, 39, 43, 44, 45, 51, 52, 53, 54, 55, 57, 59, 61, 63, 64, and 65 can
20 be introduced into other plant species.

Following the methods described in Example 11, plant species comprising a heterologous EG261 gene or an ortholog thereof are created and tested. The results will confirm that one or more EG261 genes or orthologs thereof can introduce nematode resistance when heterologously expressed in a plant species different from the plant species
25 from which the heterologous EG261 genes or orthologs thereof were isolated.

Example 14. Introducing nematode resistance into nematode-susceptible plants

This example shows that a gain-of-SCN-resistance phenotype in a previously SCN-sensitive soybean cultivar was observed when the EG261 ortholog from a wild SCN-resistant

soybean species (*Glycine pescadrensis*) was transformed into the SCN-sensitive 'Williams 82' soybean cultivar. That is, by using transformation (knock-in) a cultivated SCN-sensitive soybean cultivar becomes SCN resistant. In fact, the level of resistance achieved is roughly equivalent to that of the only known commercially-available SCN-resistant soybean cultivar.

5 The results indicate that, by using an EG261 ortholog isolated from a nematode resistant plant to transform a plant that one can achieve SCN suppression, to near zero in some cases, in that plant.

More specifically, EG261 orthologs from 2 species, (*G. pescadrensis* and *G. microphylla*) were tested.

10 The *G. pescadrensis* EG261 ORF with 5' and 3' UTR was PCR amplified from cDNA and ligated into pCR8. A NOS terminator was cloned into pSM101-Gmubi to create an over-expression construct with a strong promoter and terminator. Alternative promoters for root expression as discussed elsewhere herein or are well known to those skilled in the art could be used instead. The *G. pescadrensis* EG261 ORF was excised from pCR8 (5' PstI, 3'
15 BamHI) and ligated into pSM101-Gmubi-NOS. Vector pSM101 – Gmubi – *G. pescadrensis* EG261 - NOS was verified by sequencing.

Similarly, the *G. microphylla* EG261 ORF with UTR was PCR-amplified from cDNA and ligated into pCR8. A NOS terminator was cloned into pSM101-Gmubi to create an over-expression construct with a strong promoter and terminator. The *G. microphylla* EG261 ORF
20 was excised from pCR8 (5' PstI, 3' BamHI) and ligated into pSM101-Gmubi-NOS. Vector pSM101 – Gmubi – *G. microphylla* EG261 - NOS was verified by sequencing.

These constructs were transformed into *Agrobacterium rhizogenes*. Cotyledons of SCN-susceptible genotype ('Williams 82') and SCN-resistant genotype ('Fayette') were germinated and then transformed with *Agrobacterium* containing these EG261 over-
25 expression constructs. Hairy roots emerged from these cotyledons and GFP positive roots (those expressing the genes of interest as well as an empty control vector) were harvested and

subcultured (approximately 10 roots per genotype with each construct). SCN cysts were crushed and eggs hatched to produce infective juveniles (J2). Approximately 220 sterile J2 were placed onto these transgenic hairy roots.

5 The second-stage juvenile, or J2, is the only life stage that soybean cyst nematode can penetrate roots. The first-stage juvenile occurs in the egg, and third- and fourth-stages occur in the roots. The J2 enters the root moving through the plant cells to the vascular tissue where it feeds. The J2 induces cell division in the root to form specialized feeding sites. As the nematode feeds, it swells. The female swells so much that her posterior end bursts out of the root and she becomes visible to the naked eye. In contrast, the adult male regains a wormlike shape, and he leaves the root in order to find and fertilize the large females. The female continues to feed as she lays 200 to 400 eggs in a yellow gelatinous matrix, forming an egg sac which remains inside her. She then dies and her cuticle hardens forming a cyst. The eggs may hatch when conditions in the soil are favorable, the larvae developing inside the cyst and the biological cycle repeating itself. There are usually three generations in the year. In the autumn or in unfavorable conditions, the cysts containing dormant larvae may remain intact in the soil for several years. The juvenile molts to the J3 and begins enlarging as the reproductive system develops. Nematodes which become females are no longer able to leave the root. They continue to enlarge as they go through the J3 and J4 stages. During this time, cells around the head of the nematode enlarge to form nurse cells or giant cells. For root-knot nematode, galls will typically develop on the root. Upon becoming adults, root-knot nematodes will begin to lay eggs (up to several hundred) which are contained in a gelatinous matrix at the posterior end of the body. The egg mass may be within the root or partly or wholly exposed on the root surface while the swollen body of the female remains within the root. Therefore, we count the J2 and J3 numbers to assess the extent of the nematode infestation.

As mentioned above, variety 'Williams' was used as a SCN susceptible cultivar and variety 'Fayette' was used as a SCN-resistant control. Experimental results from three independent experiments are provided in the table below. The testing methods used herein

are described in Melito et al. (BMC Plant Biology, 2010, 10:104, incorporated herein by reference in its entirety). In the following table, “EV” refers to ‘empty vector’, i.e., a control plant that has been transformed with a vector construct that lacks the experimental gene but that otherwise consists of the complete vector. “OX” refers to ‘over-expression’, i.e., those experimental plants in which the vector contains the experimental EG261 ortholog, and the experimental knock-in gene is actively expressed at high levels. “W” refers to the SCN-sensitive Williams 82 soybean cultivar, while “F” refers to the normally SCN-resistant soybean cultivar. “Microph” refers to the EG261 ortholog derived from *G. microphylla*, while “Pesc” refers to the EG261 ortholog derived from *G. pescadrensis*.

10

Independent Experiment #1					
Sample	Sample Description	J2	J3 (and >+ Males)	Notes	(J3 & Above / Total)
1	W-pescOX	32	13		0.289
2	W-pescOX	38	7		0.156
3	W-microphOX	8	9		0.529
4	W-EV	24	19	3 males	0.442
5	W-pescOX	11	3		0.214
6	W-EV	14	19		0.576
7	W-microphOX	19	12	Confusing	0.387
8	F-EV	20	3	2 males	0.130
9	W-EV	16	25		0.610
10	W-EV	22	27	2 males	0.551
11	W-EV	8	6		0.429
12	W-microphOX	10	9		0.474
13	W-pescOX	30	10	6 males	0.250
14	W-EV	38	4		0.095
15	W-pescOX	22	5		0.185
16	W-pescOX	50	41		0.451
17	W-microphOX	37	21		0.362
18	W-pescOX	32	13		0.289
19	W-microphOX	4	9		0.692
20	W-microphOX	18	20		0.526
21	W-EV	4	16		0.800
22	W-microphOX	5	20		0.800
23	W-microphOX				
24	F-EV	6	5	Faint stain	0.455

Independent Experiment #1					
Sample	Sample Description	J2	J3 (and >+ Males)	Notes	(J3 & Above / Total)
25	F-EV	32	16		0.333
26	W-microphOX	8	16		0.667
27	F-EV	8	9	Faint stain	0.529
28	W-EV	9	5		0.357
29	F-EV	8	11		0.579
30	W-microphOX	14	5		0.263
31	W-microphOX	10	23	8 males	0.697
32	F-EV	40	9		0.184
33	W-pescOX	3	10		0.769
34	W-EV	8	13		0.619
35	W-EV	11	5	Faint - Not confident at all	0.313
36	W-EV	3	7		0.700
37	W-EV	2	9		0.818
38	F-EV	5	7		0.583
39	W-microphOX	3	10		0.769
40	W-microphOX	7	7		0.500
41	W-EV	4	11		0.733
42	W-microphOX	8	6	Faint, hard to count	0.429
43	F-EV	27	9		0.250
44	W-EV	6	11		0.647
45	W-EV	15	9		0.375
46	F-EV	67	20		0.230

Independent Experiment #2					
Sample	Sample Description	J2	J3 (and >+ Males)	Notes	(J3 & Above / Total)
1	W-pescOX	25	11		0.306
2	F-EV	48	27		0.360
3	W-pescOX	44	63		0.589
4	W-pescOX	57	53		0.482
5	W-pescOX	35	51	Very susceptible	0.593
6	W-microphOX	31	25		0.446
7	F-EV	54	53		0.495
8	W-EV	37	58	Looks kind of susceptible? Recount?	0.611
9	F-EV	49	15		0.234

Independent Experiment #2					
Sample	Sample Description	J2	J3 (and >+ Males)	Notes	(J3 & Above / Total)
10	W-microphOX	64	80		0.556
11	F-EV	60	16		0.211
12	W-pescOX	20	37		0.649
13	F-EV	53	72		0.576
15	W-microphOX			Contaminated	
16	W-EV	27	55		0.671
17	W-pescOX	28	37		0.569
18	W-microphOX	30	48		0.615
19	W-EV	54	31		0.365
20	W-pescOX	46	49		0.516
21	W-EV	21	42	Hard to count	0.667
22	F-EV	94	23		0.197
23	W-pescOX	66	25		0.275
24	W-pescOX	46	47		0.505
25	W-microphOX	14	14		0.500
26	W-EV	14	40		0.741
27	W-microphOX	95	37		0.280
28	W-pescOX	27	24		0.471
29	W-EV	15	39		0.722
30	F-EV	30	69		0.697
31	F-EV	86	26	Hard to count	0.232
32	W-EV	53	57		0.518
33	W-EV	58	30		0.341
34	F-EV	96	41		0.299
35	W-EV	82	24		0.226
36	W-microphOX	86	53	Hard to count	0.381
37	W-pescOX	68	49		0.419
38	W-EV	53	65		0.551
39	W-microphOX	89	86		0.491
40	F-EV	76	34		0.309
41	W-EV	44	38		0.463
42	W-EV	82	53		0.393
43	F-EV			Contaminated	
44	F-EV	59	43		0.422
45	W-pescOX	80	39		0.328
46	W-microphOX	45	85		0.654
47	W-pescOX	85	44		0.341

Independent Experiment #3					
Sample	Sample Description	J2	J3 (and >+ Males)	Notes	(J3 & Above / Total)

Independent Experiment #3					
Sample	Sample Description	J2	J3 (and >+ Males)	Notes	(J3 & Above / Total)
1	W-EV	24	11		0.314285714
2	F-EV			Contaminated	
3	F-EV	39	15		0.277777778
4	W-pescOX	24	13		0.351351351
5	W-pescOX	26	4		0.133333333
6	F-EV	34	15		0.306122449
7	W-pescOX	32	20		0.384615385
8	W-microphOX	17	14		0.451612903
9	W-EV	7	11		0.611111111
10	W-pescOX	19	11		0.366666667
11	W-EV	21	12		0.363636364
12	W-pescOX	31	20		0.392156863
13	W-pescOX	11	9		0.45
14	W-EV	17	11		0.392857143
15	W-EV	10	7		0.411764706
16	W-EV	17	11		0.392857143
17	F-EV	37	8		0.177777778
18	W-EV	27	13		0.325
19	F-EV	38	13		0.254901961
20	F-EV	40	23		0.365079365
21	W-microphOX	15	21		0.583333333
22	W-microphOX	18	11		0.379310345
23	W-microphOX	21	30		0.588235294
24	W-EV	4	9		0.692307692
25	W-pescOX	16	14		0.466666667
26	W-microphOX	12	12		0.5
27	W-pescOX	19	23		0.547619048
28	W-microphOX	32	30		0.483870968
29	F-EV	40	20		0.333333333
30	W-EV	19	22		0.536585366
31	F-EV	8	12		0.6
32	W-pescOX	11	14		0.56
33	W-EV	17	15		0.46875
34	F-EV	28	15		0.348837209
35	W-EV			Less than 5 Nematodes	
36	W-microphOX	15	19		0.558823529
37	F-EV	10	7		0.411764706
38	W-pescOX	14	6		0.3
39	F-EV	19	11		0.366666667
40	W-microphOX	10	17		0.62962963

Independent Experiment #3					
Sample	Sample Description	J2	J3 (and >+ Males)	Notes	(J3 & Above / Total)
41	W-microphOX	15	17		0.53125
42	W-EV	14	11		0.44
43	W-microphOX	19	23		0.547619048
44	W-microphOX	18	30		0.625
45	W-microphOX	22	11		0.333333333
46	W-pescOX	12	8		0.4
47	F-EV	24	18		0.428571429
				J3 & Above/Total normalized to W-EV within each experiment	SE
F-EV				0.722940836	0.05183849
W-EV				0.999999999	0.051787021
W-microOX				1.04598414	0.046093455
W-pescOX				0.824925891	0.052329931

Examination of the results make clear that transformation with the EG261 ortholog from *G. pescadrensis* causes the normally SCN-sensitive Williams 82 cultivar to acquire a level of SCN-resistance that approximates that of the SCN-resistant cultivar Fayette. Although the *G. microphylla* ortholog didn't appear to show obvious SCN suppression in the initial assessment, this may not rule out the effectiveness of the *G. microphylla* EG261 ortholog, given the number of variables that can affect transformation experiments. (We are repeating this first-pass experiment for the *G. microphylla* ortholog.) In contrast, the EG261 ortholog from *G. pescadrensis* is clearly capable of conferring resistance to SCN when introduced into SCN-sensitive soybean plants. We are currently investigating the effectiveness of EG261 orthologs from additional wild soybean relatives that are SCN tolerant.

Example 15. Transformation of beans and cowpeas with EG261

Other agriculturally relevant species such as beans (*Phaseolus vulgaris*) and cowpeas (*Vigna unguiculata*) can also be transformed with constructs containing EG261 using standard transformation technology for beans and cowpeas respectively. See, e.g., Brasileiro

et al., 1996 (Susceptibility of common and tepary beans to *Agrobacterium* spp. Strains and improvement of *Agrobacterium*-mediated transformation using microprojectile bombardment, J. Am. Soc. Hortic. Sci. 121, 810–815); Estrada-Navarrete et al., 2007 (Fast, efficient and reproducible genetic transformation of *Phaseolus* spp. by *Agrobacterium* rhizogenes, Nat. Protoc 2007;2(7):1819-24); Ivo et al., 2008 (Biolistic-mediated genetic transformation of cowpea (*Vigna unguiculata*) and stable Mendelian inheritance of transgenes, Plant Cell Rep 27:1475-83); Popelka et al., 2006 (Genetic transformation of cowpea (*Vigna unguiculata* L.) and stable transmission of the transgenes to progeny, Plant Cell Rep 2006; 25:304-12).

Transformation of beans or cowpea plants with a construct containing EG261 can be accomplished with either the cultivated/the wild soybean orthologs included in this patent, or with their respective bean (*Phaseolus vulgaris*), or cowpea (*Vigna unguiculata*) orthologs of these EG261 genes.

The transformed bean plants can be tested for nematode/pest resistance as compared to the untransformed, control ‘Condor,’ ‘Matterhorn,’ ‘Sedona,’ ‘Olathe,’ or ‘Montcalm’ bean plants. The transformed cowpea plants can also be tested for nematode/pest resistance as compared to the untransformed, control ‘IT 82E-16,’ ‘TVu 1390,’ or ‘B301’ cowpea plants. For procedures used to test for tolerance/resistance to nematodes see, e.g., Sasser, et al. (1984) Standardization of Host Suitability Studies and Reporting of Resistance to Root-Knot Nematodes, Crop Nematode Research & Control Project (CNRCP), a cooperative publication of The Department of Plant Pathology, North Carolina State University and the United States Agency for International Development, North Carolina State University Graphics, Raleigh, NC, 10 pages; and, Cook, R. and K. Evans (1987) Resistance and Tolerance, In: Principles and practice of nematode control in plants, R.H. Brown and B.R. Kerry (editors), pg. 179-231.

The transformed bean or cowpea plants showing conferred and/or enhanced resistance to one or more races of nematodes can be tested to confirm the presence of EG261 by using one or more of the following procedures: (a) isolating nucleic acid molecules from said plant and amplifying sequences homologous to the EG261 polynucleotide; (b) isolating nucleic

acid molecules from said plant and performing a Southern hybridization to detect the EG261 polynucleotide; (c) isolating proteins from said plant and performing a Western Blot using antibodies to a protein encoded by the EG261 polynucleotide; and/or (d) demonstrating the presence of mRNA sequences derived from a EG261 polynucleotide mRNA transcript and
5 unique to the EG261 polynucleotide.

The transformed bean or cowpea plants with confirmed tolerance and/or resistance to one or more nematode races can be maintained by self-pollinating the plants, harvesting the resultant seed and growing the resistant plants from such harvested seed.

10 **Example 16. Improving nematode resistance by introducing EG261 genes into nematode resistant plant varieties.**

In some embodiments, the EG261 orthologs conferring nematode resistance isolated according to the present invention can be introduced into a nematode-susceptible plant of the same or different species in order to increase nematode resistance of the plant.

15 However, in some other embodiments, the EG261 orthologs of the present invention can be introduced into nematode-resistant plants of the same or different species in order to further increase nematode resistance of the plant.

For example, a gene encoding any one of the proteins of SEQ ID NOs: 9-15, 25, 26, 27, 28, 29, 30, 31, 32, 33, 37, 38, 39, 43, 44, 45, 51, 52, 53, 54, 55, 57, 59, 61, 63, 64, or 65
20 can be introduced into nematode resistant plants containing any nematode resistant traits such as *rhg1*, *rhg4*, or any other known QTLs such as those listed in Concibido et al., 2004 (A decade of QTL mapping for cyst nematode resistance in soybean, Crop Sci. 44: 1121-1131); Shannon et al., (2004) Breeding for resistance and tolerance, In: Biology and management
25 of soybean cyst nematode, 2nd ed, P. Schmitt, J. A. Wrather, and R. D. Riggs, (editors). pg155-180; and Klink et al., (2013) Engineered Soybean Cyst Nematode Resistance, In: Soybean-Pest Resistance, Hany A. El-Shemy (editor), pg- 139-172.

Following the methods described in Example 11, nematode resistant plants comprising a heterologous EG261 gene or an ortholog thereof are created and tested. The

results will confirm that one or more EG261 genes or orthologs thereof can improve nematode resistance when expressed in a plant species with previously existing resistance to nematodes.

5 **Example 17. Conferring nematode resistance into nematode-susceptible plants via non-transgenic methods.**

As set-forth in the Examples above, nematode resistance is can be introduced into a nematode-susceptible plant via the genetic transformation of an EG261 gene or ortholog into the genome of said plant.

10 In other embodiments, nematode resistance can be incorporated into a plant via non-transgenic (i.e., traditional) methods such as plant breeding. For example a cross can be made between a first plant with a naturally-occurring or transgenic EG261 gene copy conferring nematode resistance, and a second plant to produce a F1 plant. This F1 plant can then be subjected to multiple backcrossings to generate a near isogenic or isogenic line,
15 wherein the nematode resistant copy of EG261 is integrated into the genome of the second plant.

For example, *G. microphylla*, or *G. Pescadrensis* plants with naturally-occurring gene copies of EG261 can be crossed with a second plant, to produce a F1 plant. This F1 plant can then be subjected to multiple backcrossings to generate a near isogenic or isogenic line,
20 wherein the nematode resistance copy of EG261 is integrated into the genome of the second plant. Similarly, transgenic plants containing copies of *G. microphylla*, or *G. Pescadrensis* EG261 genes can be crossed with a second plant, to produce a F1 plant. This F1 plant can then be subjected to multiple backcrossings to generate a near isogenic or isogenic line, wherein the nematode resistance copy of EG261 is integrated into the genome of the second
25 plant.

Unless defined otherwise, all technical and scientific terms herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials, similar or equivalent to those described herein, can be used in the practice or testing of the present invention, the preferred methods

and materials are described herein. All publications, patents, patent publications, and nucleic acid and amino acid sequences cited are incorporated by reference herein in their entirety for all purposes.

5 The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention.

10 While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features hereinbefore set forth and as follows in the scope of the appended claims.

SEQUENCE LISTINGS

SEQ ID NO: 1

cDNA sequence of EG261 from *Glycine max*.

5 agcaagagatgctcaaaacccaaattcacctacttcgatgcttccccccaccttataaaattcttgcattaatttacc
acttttcatcacccaatccacttccctctaattgacacccacctaaccATGGCTTCCCACCTTCCTCAAATCCCTCC
TTCTCCTCTCCACCTATGCCCTCACCATCTCAGCAGAATACACAGGCTTCGTGGGAACACTAGACCCAAAGTCCA
TAGGCATACACCACAAGAAAACCTAAGCCACTTCAGGTTCTACTGGCACGAAGTCTTCAGCGGAGAAAAACCCCA
CATCGGTTAGAAATCATTCCCTCACTCCCCAAATACAACGCAACCACAACCTTCGGCTCCGTTGGAATCTTTGACA
10 CCCCTTTAACCGTGGGACCTGAGGTGTACTCCAAGGTTGTTCGGAAGAGCCGAGGGCTTGTTTGCCTCCACGTCAC
AAACGCAGTTTGACCTGTTACTGATTTACAACCTTCGCGTTGACCCAAGGGAAGTACAACGGCAGCACCATCACGT
TCACGGGGAGGAGCCCCCTCTCGGAGAAGGTGAGGGAGCTGCCCATTTGTTGGTGGTAGTGGGGTCTTCAAATTTG
CCACTGGGTATGTTGAGTCTAGGACGCTAAGTTTTGATCCCCAAACAAGGAATAACACGGTTCAGTTCGACGTGT
ATATTTACTATTGATgattattgaatgtgttttttcatgttgatgcgttatcgcttgggtctgtctcacctagt
15 ttcta

SEQ ID NO: 2

coding sequence of EG261 from cultivated soybean *Glycine max*.

ATGGCTTCCCACCTTCCTCAAATCCCTCCTTCTCCTCTCCACCTATGCCCTCACCATCTCAGCAGAATACACAGGC
20 TTCGTGGGAACACTAGACCCAAAGTCCATAGGCATACACCACAAGAAAACCTAAGCCACTTCAGGTTCTACTGG
CACGAAGTCTTCAGCGGAGAAAAACCCACATCGGTTAGAAATCATTCCCTCACTCCCCAAATACAACGCAACCACA
ACCTTCGGCTCCGTTGGAATCTTTGACACCCCTTTAACCGTGGGACCTGAGGTGTACTCCAAGGTTGTTCGGAAGAA
GCCGAGGGCTTGTTTGCCTCCACGTCACAAACGCAGTTTGACCTGTTACTGATTTACAACCTTCGCGTTGACCCAA
GGGAAGTACAACGGCAGCACCATCACGTTACGCGGGAGGAGCCCCCTCTCGGAGAAGGTGAGGGAGCTGCCCATTT
25 GTTGGTGGTAGTGGGGTCTTCAAATTTGCCACTGGGTATGTTGAGTCTAGGACGCTAAGTTTTGATCCCCAAACA
AGGAATAACACGGTTCAGTTCGACGTGTATATTTACTATTGA

SEQ ID NO: 3

30 EG261 sequence from *Glycine max* as found in SEQ ID 65260 of U.S. Patent No.
7,569,389.

atcacccaatccacttccctctaattgacacccacctaaccATGGCTTCCCACCTTCCTCAAATCCCTCCTTCTCC
TCTCCACCTATGCCCTCACCATCTCAGCAGAATACACAGGCTTCGTGGGAACACTAGACCCAAAGTCCATAGGCA
35 TACACCACAAGAAAACCTAAGCCACTTCAGGTTCTACTGGCACGAAGTCTTCAGCGGAGAAAAACCCACATCGG
TTAGAATCATTCCCTCACTCCCCAAATACAACGCAACCACAACCTTCGGCTCCGTTGGAATCTTTGACACCCCTT
TAACCGTGGGACCTGAGGTGTACTCCAAGGTTGTTCGGAAGAGCCGAGGGCTTGTTTGCCTCCACGTCACAAACGC
AGTTTGACCTGTTACTGATTTACAACCTTCGCGTTGACCCAAGGGAAGTACAACGGCAGCACCATCACGTTACGCG
GGAGGAGCCCCCTCTCGGAGAAGGTGAGGGAGCTGCCCATTTGTTGGTGGTAGTGGGGTCTTCAAATTTGCCACTG
40 GGTATGTTGAGTCTAGGACGCTAAGTTTTGATCCCCAAACAAGGAATAACACGGTTCAGTTCGACGTGTATATTT
ACTATTGATgattattgaaagtgttttttcatgttgatgcgttatcgcttgggtctgtctcacctagttttctac
ataagtttccctcttttgagggcatgtggtgtcatggaataaagtcatcttgggcaaaaaaaaaaaaaaaaaaaaa

SEQ ID NO: 4

45 cDNA of EG261 from *Glycine microphylla*.

ttaattcatcacttttcatcacccaatccactccctctaattaacacccaccaacaccATGGCTTCCCACCTTCCTCA
AATCCCTCCTTCTCCTCTCCACCTATGCCCTCACCATCTCAGCAGAATACACAGGCTTCGTGGGGACACTAGACC
CAAAGTCCATAGGCATACACCACAAGAAAACCTAAGCCACTTCAGGTTCTACTGGCACGAAGTCTTCAGCGGAG
AAAACCCCAACACGGTTAGAATCATTCCCTCACTCCCCAAATACAACACAACCACAACCTTCGGTTCCGTTGGAA
50 TCTTTGACAACACTTTAACCGTGGGACCTGAGGTGTACTCCAAGGTTGCCGGAAGAGCCGAGGGCTTGTTTGCCT
CCACGTCACAAACGCAGTTTAACCTGTTACTGATTTACAGCTTCGCGTTGACCCAAGGGAAGTACAACGGCAGCA
CCATCACGTTACGCGGGAGGAGCCCCCTCTCGGAGAAGGTGAGGGAGCTGCCCATTTGTTGGTGGCAGTGGGGTCT

TCAAATTTGCCACTGGGTATGTTGAGTCTAGGACGCTAAGTTTTGATCCCCAAACGAGGAATAACACGGTTCAGT
TCGACGTGTATATTTACTATTGAtgattattgaatgtgttttttacatgttgatgtgttagagctttggtgtgtg
tcacttagtttcta

5 SEQ ID NO: 5

cDNA EG261 from *Glycine pescadrensis*.

ataaattcttgcattaattcatcactttcatcacccaatccatttcctctaattaacacccaccaaaaaccATGGC
TTCCCACTTCCTCAAATCCCTCCTTCTCCTCTCCACCTATGCCCTCACCATCTCAGCAGAATACACAGGCTTCGT
GGGCACACTAGACCCAAAGTCCATAGGCATACACCACAAGAAAACCCCTAAACCACTTCAGGTTCTACTGGCAGCA
10 AGTCTTCAGCGGAGAAAACCCACATCGGTTAGAATCATTCCTCACTCCCCAAATACAACACAACCACAACCTT
CGGTTCCGTTTGAATCTCTGACAACGCTTTAACCGTGGGACCTGAGGTGTACTCCAAGGTTGTTCGGAAAAGCCGA
GGGCTTGTTTGTCTCCACGTCACAAACGCGAGTTTGACCTGTTACTGATTTACAACCTTCGCGTTGACCCAAGGGAA
GTACAACGGCAGCACCATCACGTTACGCGGGAGGAGCCCCCTCTCGGAGAAGGTGAGGGAGCTGCCCATTTGTAGG
TGGCAGTGGGGTCTTCAAATTTTCCACTGGGTATGTTGAGTCTAGGACGCTAAGTTTTGATCCCCAAACGAGGAA
15 TAACACGGTTCAGTTCGACGTGTATATTTACTATTGAtgattattgaatgtgtttttccatgttgatgtgttat
agctttggtatgtgtcacttagtttcta

SEQ ID NO: 6

cDNA of EG261 from *Glycine tabacina* (New South Wales, Australia).

20 catcacccaatccactcctctaattaacacccaccaaacaccATGGCTTCCCACTTCCTCAAATCCCTCCTTCTCC
TCTCCACCTATGCCCTCACCATCTCAGCAGAATACACAGGCTTCGTGGGGACACTAGACCCAAAGTCCATAGGCA
TACACCACAAGAAAACCCCTAAGCCACTTCAGGTTCTACTGGCAGCAAGTCTTCAGCGGAGAAAACCCACAACGG
TTAGAATCATTCCTCACTCCCCAAATACAACACAACCACAACCTTCGCGTTCCGTTGGAATCTTTGACAACACTT
TAACCGTGGGACCTGAGGTGTACTCTAAGGTTGCCGGAAGCCGAGGGCTTGTTTGCTCCACGTCACAAACGC
25 AGTTTAACTGTTACTGATTTACAGCTTCGCGTTGACCCAAGGGAAGTACAACGGCAGCACCATCACGTTACGG
GGAGGAGCCCCCTCTCGGAGAAGGTGAGGGAGCTGCCCATTTGTTGGTGGCAGTGGGGTCTTCAAATTTGCCACTG
GGTATGTTGAGTCTAGGACGCTAAGTTTTGATCCCCAAACGAGGAATAACACGGTTCAGTTCGACGTGTATATTT
ACTATTGAtgattattgaatgtgttttttacatgttgatgtgttagagctttggtgtgtgtcacttagtttcta

30

SEQ ID NO: 7

cDNA of EG261 from *Glycine tabacina* (Taiwan).

cctaacttcgatgtttccccacacttataaattcttgcattaattcatcactttcatcacccaatccatttcctct
aattaacacccaccaaaaaccATGGCTTCCCACTTCCTCAAATCCCTCCTTCTCCTCTCCACCTATGCCCTCACC
35 TCTCAGCAGAATACACAGGCTTCGTGGGCACACTAGACCCAAAGTCCATAGGCATACACCACAAGAAAACCCCTAA
ACCACTTCAGGTTCTACTGGCAGCAAGTCTTCAGCGGAGAAAACCCACATCGGTTAGAATCATTCCTCACTCC
CCAAATACAACACAACCACAACCTTCGCGTTCCGTTGGAATCTCTGACAACGCTTTAACCGTGGGACCTGAGGTGT
ACTCCAAGGTTGTTCGGAAAAGCCGAGGGCTTGTTTGTCTCCACGTCACAAACGCGAGTTTGACCTGTTACTGATTT
ACAACCTTCGCGTTGACCCAAGGGAAGTACAACGGCAGCACCATCACGTTTCAGGGGAGGAGCCCCCTCTCGGAGA
40 AGGTGAGGGAGCTGCCCATTTGTAGGTGGCAGTGGGGTCTTCAAATTTTCCACTGGGTATGTTGAGTCTAGGACGC
TAAGTTTTGATCCCCAAACGAGGAATAACACGGTTCAGTTCGACGTGTATATTTACTATTGAtgattattgaatg
tgttttttccatgttgatgtgttatagctttggtatgtgtcacttagtttcta

SEQ ID NO: 8

45 cDNA of EG261 from *Glycine tomentella* (Australia).

cactacttcgatgtttccccacacttataaattcttgcattaattcatcactttcatcacccaatccatttc
tctaattaacacccaccaaaaaccATGGCTTCCCACTTCCTCAAATCCCTCCTTCTCCTCTCCACCTATGCCCTCA
CCATCTCAGCAGAATACACAGGCTTCGTGGGCACACTAGACCCAAAGTCCATAGGCATACACCACAAGAAAACCC
TAAACCACTTCAGGTTCTACTGGCAGCAAGTCTTCAGCGGAGAAAACCCACATCGGTTAGAATCATTCCTCAC
50 TCCCCAAATACAACACAACCACAACCTTCGCGTTCCGTTGGAATCTCTGACAACGCTTTAACCGTGGGACCTGAGG
TGTACTCCAAGGTTGTTCGGAAAAGCCGAGGGCTTGTTTGTCTCCACGTCACAAACGCGAGTTTGACCTGTTACTGA
TTTACAACCTTCGCGTTGACCCAAGGGAAGTACAACGGCAGCACCATCACGTTTCAGGGGAGGAGCCCCCTCTCGG

AGAAGGTGAGGGAGCTGCCCCATTGTAGGTGGCAGTGGGGTCTTCAAATTTTCCACTGGGTATGTTGAGTCTAGGA
TGCTAAGTTTTTGATCCCCAAACGAGGAATAACACGGTTCAGTTCGACGTGTATATTACTATTGatgattattga
atgtgttttttccatgttgatgtgttatagctttggtatgtgtcacttagtttctatataagtttccama

5 SEQ ID NO: 9
EG261 polypeptide encoded by SEQ ID NO: 1, *Glycine max*.
MASHFLKSLLLLSTYALTISA EYTG FVGTLDPKSIGIHHKKTLSHFRFYWHEVFSGENPTSVRIIPSLPKYNATT
TFGSVGIFDPTPLTVGPEVYSKVVGKAEGLFASSTQTFDLLLLIYNFALTQGKYNGSTITFTGRSPLSEKVRELPI
VGGSGVFKFATGYVESRSLSFDPQTRNNTVQFDVYIYY

10 SEQ ID NO: 10
EG261 polypeptide encoded by SEQ ID NO:3, *Glycine max*.
MASHFLKSLLLLSTYALTISA EYTG FVGTLDPKSIGIHHKKTLSHFRFYWHEVFSGENPTSVRIIPSLPKYNATT
TFGSVGIFDPTPLTVGPEVYSKVVGKAEGLFASSTQTFDLLLLIYNFALTQGKYNGSTITFTGRSPLSEKVRELPI
15 VGGSGVFKFATGYVESRSLSFDPQTRNNTVQFDVYIYY

SEQ ID NO: 11
EG261 polypeptide encoded by SEQ ID NO:4, *Glycine microphylla*.
20 MASHFLKSLLLLSTYALTISA EYTG FVGTLDPKSIGIHHKKTLSHFRFYWHEVFSGENPTTVRIIPSLPKYNNTT
TFGSVGIFDNTLTVGPEVYSKVAGKAEGLFASSTQTFDLLLLIYSFALTQGKYNGSTITFTGRSPLSEKVRELPI
VGGSGVFKFATGYVESRSLSFDPQTRNNTVQFDVYIYY

25 SEQ ID NO: 12
EG261 polypeptide encoded by SEQ ID NO:5, *Glycine pescadrensis*.
MASHFLKSLLLLSTYALTISA EYTG FVGTLDPKSIGIHHKKTLSHFRFYWHEVFSGENPTSVRIIPSLPKYNNTT
TFGSVGISDNALTVGPEVYSKVVGKAEGLFVSTSTQTFDLLLLIYNFALTQGKYNGSTITFTGRSPLSEKVRELPI
VGGSGVFKFSTGYVESRSLSFDPQTRNNTVQFDVYIYY

30 SEQ ID NO: 13
EG261 polypeptide encoded by SEQ ID NO:6, *Glycine tabacina* (New South
Wales, Australia)
35 MASHFLKSLLLLSTYALTISA EYTG FVGTLDPKSIGIHHKKTLSHFRFYWHEVFSGENPTTVRIIPSLPKYNNTT
TFGSVGIFDNTLTVGPEVYSKVAGKAEGLFASSTQTFDLLLLIYSFALTQGKYNGSTITFTGRSPLSEKVRELPI
VGGSGVFKFATGYVESRSLSFDPQTRNNTVQFDVYIYY

40 SEQ ID NO: 14
EG261 polypeptide encoded by SEQ ID NO:7, *Glycine tabacina* (Taiwan).
MASHFLKSLLLLSTYALTISA EYTG FVGTLDPKSIGIHHKKTLSHFRFYWHEVFSGENPTSVRIIPSLPKYNNTT
TFGSVGISDNALTVGPEVYSKVVGKAEGLFVSTSTQTFDLLLLIYNFALTQGKYNGSTITFTGRSPLSEKVRELPI
VGGSGVFKFSTGYVESRSLSFDPQTRNNTVQFDVYIYY

45 SEQ ID NO: 15
EG261 polypeptide encoded by SEQ ID NO:8, *Glycine tomentella* (Australia).
MASHFLKSLLLLSTYALTISA EYTG FVGTLDPKSIGIHHKKTLSHFRFYWHEVFSGENPTSVRIIPSLPKYNNTT
TFGSVGISDNALTVGPEVYSKVVGKAEGLFVSTSTQTFDLLLLIYNFALTQGKYNGSTITFTGRSPLSEKVRELPI
50 VGGSGVFKFSTGYVESRMLSFDPQTRNNTVQFDVYIYY

SEQ ID NO: 16

partial cDNA of EG261 ortholog from cultivated tomato (*Solanum lycopersicum* or *Solanum esculentum*).

cattccATGGCCAACTAATACTCCAAATCTTCACCATTTCCTCTTCCTTTCTCTGGTGGCCTTTTCGCGCCACC
 5 GGAGAAGAAGATAATTATATTTTTGGAAAAATCCATAAAACAAAAAACCACCAAGGTTAAGAAAGGAAAAAATCAGT
 CATTTTTCGATTTTTTTGGCATGATATTTTAAGTGGTTCAAAACCAACATCAATGATGATTATTCACCACCTAAA
 AACACAACAACAGGTTTTGGACAAATGAATATGATAGATAATGCCTTAACCTTAGGACCAAACTAAGTTCCAAG
 ATTGTTGGTAGGGCACAAGGGTTTTATGGTGCTGCTTCACTTAATGATGTTGGATTAAATGATGGTTATGAATTTT
 GCTTTTATTGAAGGGAAATATAATGGAAGTACTTTTACTATACTTGGTCGGAATCCGGTGTTCGAGAAGGTGAGA
 10 GAGATGGCGGTGATCGGAGGGAGTGGGCTTTTCCGATTTGCTAGAGGATATGTTCAAGCTAGTACTCATTCATGG
 GATTTCAAACTGGAGATGCTACTGTTCAAGTATGATGCTTATGTCTTTGCATTATTGAGGTTTACTAATTTTATA
 TATTTTCATCGTGTCAATTTATG

SEQ ID NO: 17

cDNA of EG261 ortholog in Peruvian tomato (also known as Peruvian
 15 nightshade, *Solanum peruvianum*).

tcaaagaattttactttccattccATGGCCAACTAATACTCCAAATCTTCACCATTTCCTCTTCCTTTCTCTGGT
 GGCCTTTTCGCGCCACCAGGAGAAGAAGATAATTATGTTTTTGGAAAAATCCATAAAACAAAAAACCACCAAGGTTAAG
 AAAGGAAAAATTCAGTCATTTTTCGATTTTATTGGCATGATATTTTAAGTGGTTCAAAACCAACATCAATGATGAT
 20 TATTCACCACCTAAAAACACAACAACAGGTTTTGGACAAATGAATATGATAGATAATGCCTTAACCTTAGGACC
 AAAACTAAGTTCCAAGATTGTTGGTAGGGCACAAGGGTTTTATGGTGCTGCTTCACTTAATGATGTTGGATTAAAT
 GATGGTTATGAATTTTGTCTTTTATTGAAGGGAAATATAATGGAAGTACTTTTACTATACTTGGTCGGAATCCGGT
 GTTCGAGAAGGTGAGAGAGATGGCGGTGATCGGAGGGAGTGGGCTTTTCCGATTTGCTAGAGGATATGTTGAAGC
 TAGTACTCATTCATGGGATTTCAAACTGGAGATGCTACTGTTCAAGTATGATGCTTATGTCTTGCATTATTGAgg
 25 tttactaattttatatatattttcatcgtgtcaatttatgcgacctagtt

SEQ ID NO: 18

partial cDNA of EG261 ortholog in *Solanum chmielewskii*.

attccATGGCCAACTAATACTCCAAATCTTCACCATTTCCTCTTCCTTTCTCTGGTGGCCTTTTCGCGCCACACG
 30 GAGAAGAAGATAATTATATTTTTGGAAAAATCCATAAAACAAAAAACCACCAAGGTTAAGAAAGGAAAAAATCAGTC
 ATTTTCGATTTTATTGGCATGATATTTTAAGTGGTTCAAAACCAACATCAATGATGATTATTCACCACCTAAAA
 ACACAACAACAGGTTTTGGACAAATGAATATGATAGATAATGCCTTAACCTTAGGACCAAACTAAGTTCCAAGA
 TTGTTGGTAGGGCACAAGGGTTTTATGGTGCTGCTTCACTTAATGATGTTGGATTAAATGATGGTTATGAATTTTG
 CTTTTATTGAAGGGAAATATAATGGAAGTACTTTTACTATACTTGGTCGGAATCCGGTGTTCGAGAAGGTGAGAG
 35 AGATGGCGGTGATAGGAGGGAGTGGGCTTTTCCGATTTGCTAGAGGATATGTTCAAGCTAGTACTCATTCATGGG
 ATTTCAAACTGGAGATGCTACTGTTC

SEQ ID NO: 19

cDNA of EG261 ortholog in *Solanum habrochaites*.

tactttccattccATGGCCAACTAATACTCCAAATCTTCACCATTTCCTCTTCCTTTCTCTGGTGGCCTTTTCGS
 40 GCCACCGGAGAAGAAGATAATYATATTTTTGAAAAATCCATAAAACAAAAAACCACCAAGGTTAAGAAAGSAAAA
 TTCAGTCATTTTCGATTTTATTGGCATGATATTTAAGTGGTTCAAAACCAACATCAATGATGATTATTCACCA
 CCTAAAAACACAACAACAGGTTTTGGACAAATGAATATGATAGATAATGCCTTAACCTTAGGACCAAACTAAGT
 TCCAAGATTGTTGGTAGGGCACAAGGGTTTTATGGTGCTGCTTCACTTAATGATGTTGGATTAAATGATGGTTATG
 45 AATTTTGCTTTTATTGAAGGGAAATAYAAATGGAAGTACTTTTACTATACTTGGTCGGAATCCGGTGTTCGAGAAG
 GTGAGAGAGATGGCGGTGATCGGAGGGAGTGGGCTTTTCCGATTTGCTAGAGGATATGTTCAAGCTAGTACTCAT
 TCATGGGATTTCAAACTGGAGATGCTACTGTTCAAGTATGATGCTTATGTCTTGCATTATTGAggtttactaatt
 ttatatat

SEQ ID NO: 20

cDNA of EG261 ortholog in *Solanum corneliomulleri*.

cttcaccaactgactcaaagaattttactttccattccATGGCCAACTAATACTCCTAATCTTCACCATTTCCTGTC
 50 TTCCTTTCTCTGGTGGCCTTTTCGTGCCACCGGAGAAGAAGATAATTATATTTTTGGAAAAATCCATAAAACAAAAA

CCCACAAGGTTAAGAAAGGAAAAATTCAGTCATTTTCGATTTTATTGGCATGATATTTTAAGTGGTTCAAAACCA
 ACATCAATGATGATTATTCACCACCTAAAAACACAACAACAGGTTTTGGACAAATGAATATGATAGATAATGCC
 TTAACCTTAGGACCAAACTAAGTTCCAAGATTGTTGGAAGGGCACAAGGGTTTTATGGTGTCTGCTTCACCTAAT
 GATGTTGGATTAATGATGGTTATGAATTTTGCTTTTATTGAAGGGAAATATAATGGAAGTACTTTTACTATACTT
 5 GGTCCGAATCCGGTGTTCGAGAAGGTGAGAGAGATGGCGGTGATCGGAGGGAGTGGGCTTTTCCGATTTGCTAGA
 GGATATGTTCAAGCTAGTACTCATTTCATGGGATTTCAAAACTGGAGATGCTACTGTTTCAGTATGATGCTTATGTC
 TTGCATTATTGAAGgtttactaattttatatatattttcatc

SEQ ID NO: 21

cDNA of EG261 ortholog in chili pepper (*Capsicum annuum*).

gcacgaggctcaaagaatctacttccATGGCCAACTAATACTCCAAATCTTCTCCATTTCCGTTCTCTATTAC
 TGGTAGCCTTTCCAGCCACGGGAGAAGAAGATCATATTTTGGAAAAATCCATAAATGAAAAGTCCATGAGGCTAA
 AAAGGGAAAAACTCAGCCATTTTCAGATTTTATTGGCACGACGTCCTCAGTGGCTCCAAACCAACATCTATGATAA
 TTATCCACCTCCCAAAAATACTACCACAGGCTTTGGCCAAATGAATATGATAGATAATGCCCTAACCTTAGGAC
 15 CAGAGCTGAGTTCCAGGATAGTTGGAAGGGCACAAGGGTTTTACGCTGCTGCTTCACTAAATGATGTTGGCTTAA
 TGATGGTCATGAACCTTTGCTTTTATTGAAGGAAAAATATAATGGGAGCACCTTCACATATACTTGGACGAAATCCGG
 TATTTGAGAAGGTGAGAGAGATGGCCGTGATCGGCGGCAGTGGGCTTTTCCGATTTGCTAGAGGATATGTTTCAGG
 CTAGTACTCATTTCATTGGATTTCAAAACTGGCGATGCTACTGTTTCAGTATGATGCCATATGTTTTGCATTATTTGAa
 gtttactaatattatatatcact

SEQ ID NO: 22

cDNA of EG261 ortholog in hot pepper (*Capsicum annuum*).

gcacgaggtaacttccATGGCCAACTAATACTCCAAATCTTCTCCATTTCCGTTCTCTATTCACTGGTAGCCTTT
 CCAGCCACGGGAGAAGAAGATCATATTTTGGAAAAATCCATAAATGAAAAGTCCATGAGGCTAAAAAGGGAAAAA
 25 CTCAGCCATTTTCAGATTTTATTGGCACGACGTCCTCAGTGGCTCCAAACCAACATCTATGATAATTATCCACCT
 CCCAAAAATACTACCACAGGCTTTGGCCAAATGAATATGATAGATAATGCCCTAACCTTAGGACCAGAGCTGAGT
 TCCAGGATAGTTGGAAGGGCACAAGGGTTTTACGCTGCTGCTTCACTAAATGATGTTGGCTTAATGATGGTCATG
 AACTTTGCTTTTATTGAAGGAAAAATATAATGGGAGCACCTTCACATATACTTGGACGAAATCCGGTATTTGAGAAG
 GTGAGAGAGATGGCCGTGATCGGCGGCAGTGGGCTTTTCCGATTTGCTAGAGGATATGTTTCAGGCTAGTACTCAT
 30 TCATTGGATTTCAAAACTGGCGATGCTACTGTTTCAGTATGATGCCATATGTTTTGCATTATTTGAagtttactaata
 ttatatatcactcctaactcggctagctggccttttaattaataattatgtcg

SEQ ID NO: 23

cDNA of EG261 ortholog in eggplant (*Solanum melongena*).

ATGGCCAACTATCACTCCAAATCTTCAACATCTCCATTCTCCTTTTTCTGGTTGCCTTTCCGGCAACCGGAGAA
 GAAGATAATTATACTTTTGGAAAAATCCATAAATAAAAGTCTATGAGGTTAAGAAAGGAGAAACTCAGCCATTTTC
 AGATTTTATTGGCATGATGTCCTAAGTGGCTCAAAACCAACATCAATGATGATTATTCACCACCTAAAAACACC
 ACAACAGGTTTTGGACAAATGAATATGATAGATAATGCCCTTAACCTTAGGAGCAGAGTTGAGTTCCAAGATTGTT
 GGAAGGGCACAAGGGTTTTACGCTGCTGCTTCACTTAATGATGTTGGATTAATGATGGTAATGAATTTTGCTTTT
 40 ATTGAAGGGAAATATAATGGAAGCACTTTCACTATACTTGGACGGAAATCCGGTGTGTTGAGAAGGTGAGGGAGATG
 GCGGTGATCGGAGGAAGTGGACTTTTCCGATTTGCTAGAGGATATGTTTCAGGCCAGTACTCATTTCATGGGATTAC
 AAAACTGGAGATGCTACTGTGAAGTATGATGCTTA

SEQ ID NO: 24

cDNA of EG261 ortholog in Potato (*Solanum tuberosum*).

gaaaattctccaccaagtgactcaaagaatttacttccattccATGGCCAACTAATACTCCAAATCTTCAACAT
 TTCCATCTTCTTTCTCTGGTGGCCTTTCCGGCCACCGGAGAAGAAGATACTTATATTTTTTGGAAAAATCTATAAAA
 CAAAAAACCACAAAGGTTAAAAAAGGAAAAATTCAGTCATTTTCGATTTTATTGGCATGATATTCTAAGTGGTTC
 50 AAAACCAACATCAATGATGATTATTCACCATCTAAAAACACAACAACAGGTTTTGGGCAAATGAATATGATAGA
 TAATGCCTTAACCTTAGGACCAGAATTAAGTTCCAAGATTGTTGGAAGGGCGCAAGGGTTTTATGGTGTCTGCTTC
 ACTTAATGATGTTGGTTTAAATGATGGTTATGAATTTTGCTTTTATTGAAGGGAAATATAATGGAAGTACTTTTAC

TATACTTGGTCGGAATCCGGTGTTCGAGAAGGTGAGAGAGATGGCGGTGATCGGAGGGAGTGGGCTTTTCCGATT
 TGCTAGAGGATATGTTGAAGCTAGTACTCATTTCATGGGATTTTAAACTGGAGATGCTACGGTTCAGTATGATGC
 TTATGTCTTGCATTATTAAGgttttactaattttgtatgttttcgttgtgtcaatttataagatttagtttaac
 tggatactaaatttatgaaatatcccatTTTgttcaaaaaaaaaaaaaaaaaaaaaa

5

SEQ ID NO: 25

EG261 polypeptide encoded by SEQ ID NO: 16, EG261 ortholog in Cultivated tomato (*Solanum lycopersicum* or *Solanum esculentum*).

10

MAKLILQIFTISLFLSLVAFRATGEEDNYIFGKSINKKPTRLRKEKISHFRFFWHDILSGSKPTSMMIIPPPKNT
 TTGFGQMN MIDNALT LGPKLSSKIVGRAQGFYGAASLNDVGLMMVMNFAFIEGKYNGSTFTILGRNPVFEKVREM
 AVIGGSGLFRFARGYVQASTHSWDFKTGDATVQYDAYV FALLRFTNFIYFHRVNLX

SEQ ID NO: 26

15 EG261 polypeptide encoded by SEQ ID NO: 17, EG261 ortholog in Peruvian tomato (also known as Peruvian nightshade, *Solanum peruvianum*).

MAKLILQIFTISLFLSLVAFRATGEEDNYVFGKSINKKPTRLRKEKFSHFRFYWHDILSGSKPTSMMIIPPPKNT
 TTGFGQMN MIDNALT LGPKLSSKIVGRAQGFYGAASLNDVGLMMVMNFAFIEGKYNGSTFTILGRNPVFEKVREM
 AVIGGTGLFRFARGYVEASTHSWDFKTGDATVQYDAYVLHY

20

SEQ ID NO: 27

EG261 polypeptide encoded by SEQ ID NO: 18, EG261 ortholog in *Solanum chmielewskii*.

25

MAKLILQIFTISLFLSLVAFRATGEEDNYIFGKSINKKPTRLRKEKISHFRFYWHDILSGSKPTSMMIIPPPKNT
 TTGFGQMN MIDNALT LGPKLSSKIVGRAQGFYGAASLNDVGLMMVMNFAFIEGKYNGSTFTILGRNPVFEKVREM
 AVIGGSGLFRFARGYVQASTHSWDFKTGDATVX

SEQ ID NO: 28

30 EG261 polypeptide encoded by SEQ ID NO: 19, ortholog in *Solanum habrochaites*.

MAKLILQIFTISLFLSLVAFRATGEEDNXIFEKSINKKPTRLRKKXKFSHFRFYWHDILSGSKPTSMMIIPPPKNT
 TTGFGQMN MIDNALT LGPKLSSKIVGRAQGFYGAASLNDVGLMMVMNFAFIEGKYNGSTFTILGRNPVFEKVREM
 AVIGGSGLFRFARGYVQASTHSWDFKTGDATVQYDAYVLHY

35

SEQ ID NO: 29

EG261 polypeptide encoded by SEQ ID NO: 20, ortholog in *Solanum corneliomulleri*.

40

MAKLILLIFTISVFLSLVAFRATGEEDNYIFGKSINKKPTRLRKEKFSHFRFYWHDILSGSKPTSMMIIPPPKNT
 TTGFGQMN MIDNALT LGPKLSSKIVGRAQGFYGAASLNDVGLMMVMNFAFIEGKYNGSTFTILGRNPVFEKVREM
 AVIGGSGLFRFARGYVQASTHSWDFKTGDATVQYDAYVLHY

SEQ ID NO: 30

45 EG261 polypeptide encoded by SEQ ID NO: 21, ortholog in chili pepper (*Capsicum annuum*).

MAKLILQIFSISVLYSLVAFRATGEEDHIFGKSINEKSMRLKREKLSHFRFY
 WHDVLSGSKPTSMMIIPPPKNTTTGFGQMN MIDNALT LGPELSSRIVGRAQGFYAAASLN
 DVGLMMVMNFAFIEGKYNGSTFTILGRNPVFEKVREMAVIGGSGLFRFARGYVQASTHSL
 DFKTGDATVQYDAYVLHY

50

SEQ ID NO: 31

EG261 polypeptide encoded by SEQ ID NO: 22, ortholog in hot pepper (*Capsicum annuum*).

MAKLILQIFSIISVLYSLVAFPATGEEDHIFGKSINEKSMRLKREKLSHFRFYWHD
VLSGSKPTSMIIIPPPKNTTTFGFGQNMIDNALTLGPELSSSRIVGRAQGFYAAASLNDVG
5 LMMVMNFAFIEGKYNGSTFTILGRNPVFEKVREMAVIGGSGLFRFARGYVQASTHSLDFK
TGDATVQYDAYVLHY

SEQ ID NO: 32

EG261 polypeptide encoded by SEQ ID NO: 23, ortholog in eggplant (*Solanum melongena*)

MAKLSLQIFTISILLFLVAFPATGEEDNYTFGKSINKKSMRLRKEKLSHFRFYWHDVLSG
10 SKPTSMIIIPPPKNTTTFGFGQNMIDNALTLGAELSSKIVGRAQGFYAAASLNDVGLMMV
MNFAFIEGKYNGSTFTILGRNPVFEKVREMAVIGGSGLFRFARGYVQASTHSWDYKTGDA
TVKYDAX

SEQ ID NO: 33

EG261 polypeptide encoded by SEQ ID NO: 24, ortholog in **Potato** (*Solanum tuberosum*):

MAKIILQIFTISIFLSLVAFFPATGEEDTYIFGKSINKKPTRLKKEK
20 FSHFRFYWHDILSGSKPTSMIIIPPSKNTTTFGFGQNMIDNALTLGPELSSKIVGRAQGF
YGAASLNDVGLMMVMNFAFIEGKYNGSTFTILGRNPVFEKVREMAVIGGSGLFRFARGYV
EASTHSWDFKTGDATVQYDAYVLHY

SEQ ID NO: 34

Partial cDNA of EG261 ortholog in common bean (*Phaseolus vulgaris*), varieties "Blue Lake" (this common bean variety is a bush type snap bean that was developed from the Blue Lake Pole bean), Henderson Bush Lima Bean (this bean variety dates to 1885), Dixie Speckled Butterpea Lima Bean, Hidatsa Red Indian, and Kabouli Black Garbanzo Asian Bean (collected in
30 Kabul, Afghanistan).

tcctctaaccacacaaaaccATGGCTACCAAATTCCTCTTATCGTTCCTTCTCATCTCCTGCTATGTCTCTCCAT
CTCAGGAGAGAAAAGAAACAGGTTTCGTGGGCTCAGTAGACCCTAAGTCCTTAAGCTACAAGAAGAAGCACACTCT
TAGCCACTTCAGGTTCTATTGGCAAGAAATCTTCAGTGGGAAGCAACCCCTCCTCGGTTAGAATCATTCACCACA
35 ACCAAAGTACAGCACAAACCACACCTTCGGTTCGGTGGGAGTATTGACAAACGTGTTGACCCCTAGGACCCGAGTT
GTACTCAAAGGTTGTGGGAAGTGCTGAAGGGTTGTACTCCTCCACATCACAAAAGGAGTTTGCCCTTTTGGTGAT
TACGAACCTTTGTGTTGACCGAAGGGAAGTACAATGGTAGCACCATCACGTTTCGTGGGGAGAAGTCCCATTGCTCA
GAAGGTGAGGGAGATGCCTGTGGTTGGTGGCAGTGGTGTTCAGATTTGCCAGGGGCTTTGTTGAGTCCAGGAC
TCTGTCTTTTGATCCCCAAACGAGGAACAACAATGAGTACAACGTCTACGTTTACCACATAAttgttgtgttt
40 tataggatgtaaggagtttgatcattgttgttcttcaaaaaggctgta

SEQ ID NO: 35

cDNA of EG261 ortholog in common bean (*Phaseolus vulgaris*), variety Scarlet Emperor (this variety dates at least to 1750, was originally from Central America)

acttcctctaaccacacaaaaccATGGCTACCAAATTCCTCTTATCGTTCCTTCTCATCTCCTGCTATGTCTCTC
CATCTCAGGAGAGAAAAGAAACAGGTTTCGTGGGCTCAGTAGACCCTAAGTCCTTAAGCTACAAGAAGAAGCACAC
45 TCTTAGCCACTTCAGGTTCTATTGGCAGCAAAATCTTCAGTGGGAAGCAACCCCTCCTCGGTTAGAATCATTCACC
ACAACCAAAGTACAGCACAAACCACACCTTCGGTTCGGTGGGAGTATTGACAAACGTGTTGACCCCTAGGACCCGA
GTTGTACTCAAAGGTTGTGGGAAGTGCTGAAGGGTTGTACTCCTCCACATCACAAAAGGAGTTTGCCCTTTTGGT
50 GATTACGAACCTTTGTGTTGACCGAAGGGAAGTACAATGGTAGCACCATCACGTTTCGTGGGGAGAAGTCCCATTGC
TCAGAAGGTGAGGGAGATGCCTGTCTGGTGGCAGTGGTGTTCAGATTTGCCAGGGGCTTTGTTGAGTCCAG

GACTCTGGCTTTTGATCCCCAAACGAGGAACAACACAATTGAGTACAACGCTCTACGTTTACCACTAAttgttgtg
cttttaaggatgttaaggagtttgatcattgttgttgttc

SEQ ID NO: 36

- 5 Partial cDNA of EG261 ortholog in Jacob's Cattle Bean
GAAACAGGTTTCGTGGGCTCAGTAGACCCCTAAGTCCTTAAGCTACAAGAAGAAGCACACYCTTAGCCACTTCAGG
TTCTATTGGCACGAAATCTTCAGTGGGAAGCAACCCCTCCTCGGTTAGAATCATTCACCACAACCAAAGTACAGC
ACAACCACCACCTTCGGTTTCGGTGGGAGTATTCGACAACGTGTTGACCCTAGGACCCGAGTTGTACTCAAAGGTT
GTGGGAAGTGCTGAAGGGTGTACTCCTCCACATCACAAAAGGAGTTTGCCCTTTTGGTGATTACGAACCTTTGTG
10 TTGACCGAAGGGAAGTACAATGGTAGCACCATCACGTTTCGTGGGGAGAAGTCCCATTGCTCAGAAGGTGAGGGAG
ATGCCTGTGGTTGGYGGCAGTGGTGTTCAGATTTCGCAGGGGCTTTGTTGAGTCCAGGACTCTGTCTTTTGAT
CCCCAAACGAGGAACAACACAATTGAGTACAACGCTCTACGTTTACCACTAAttgttgtgtttataggatgttaa
ggagtttgatcattgttgttcttcaaaaaggctgta

- 15 SEQ ID NO: 37

EG261 polypeptide encoded by SEQ ID NO: 34, EG261 ortholog in common bean
(*Phaseolus vulgaris*), varieties "Blue Lake", Henderson Bush Lima Bean,
Dixie Speckled Butterpea Lima Bean, Hidatsa Red Indian, and Kabouli Black
Garbanzo Asian Bean

- 20 MATKFLLSFLLISCYVLSISGEKETGFVGSVDPKSLSYKKKHTLSHFRFYWHEI
FSGSNPSSVRIIPPQPKYSTTTTTFGSGVGFNDVLTGLPELYSKVVGSAEGLYSSTSQKEF
ALLVITNFEVLTEGKYNGSTITFVGRSPIAQKVREMPVVGSGVFRFARGFVESRTLSDP
QTRNNTIEYNVYVYH

- 25 SEQ ID NO: 38

EG261 polypeptide encoded by SEQ ID NO: 35, ortholog in common bean
(*Phaseolus vulgaris*), variety Scarlet Emperor

- MATKFLLSFLLISCYVLSISGEKETGFVGSVDPKSLSYKKKHTLSHFRFYWHE
IFSGSNPSSVRIIPPQPKYSTTTTTFGSGVGFNDVLTGLPELYSKVVGSAEGLYSSTSQKE
30 FALLVITNFEVLTEGKYNGSTITFVGRSPIAQKVREMPVVGSGVFRFARGFVESRTLAFD
PQTRNNTIEYNVYVYH

SEQ ID NO: 39

EG261 polypeptide encoded by SEQ ID NO: 36, ortholog in Jacob's Cattle Bean

- 35 ETGFVGSVDPKSLSYKKKHTLSHFRFYWHEIFSGSNPSSVRIIPPQPKYSTTTTTFGSGV
FDNVLTGLPELYSKVVGSAEGLYSSTSQKEFALLVITNFEVLTEGKYNGSTITFVGRSPIA
QKVREMPVVGSGVFRFARGFVESRTLSDPQTRNNTIEYNVYVYH

SEQ ID NO: 40

- 40 Partial cDNA of EG261 ortholog in coffee plant (*Coffea arabica*), Allele A
ATGGCTAGAATTTACAGCATTTCTCTCCTCACCATCTTCATCTTCATTTCTGTCATCACCCTTCAGGTCACCTTAT
GGTGATGAAGAATACGAGTTTGTCAAAGCAATTGATCCAAAAGTAGCACTAAAGATGAAGAAAGAAAAGCTAAGC
CTTTTCAGGTTCTACTGGCACGACATCCTCAGTGGCAAAGCACCTACTTCAGTCATGGTGGTGGCCACCCTCCGAAA
ACCAATTCATTCAGTCTTTTGGCCTGGTGAACATGATCGATAATCCTTTAACTGTCGGCCCCGAGCTCAGCTCA
45 AAATTGGTCCGGAGGGCTCAAGGGTTTATGCATCAGCTTCACAAGAGGAAATTGGCTTCTTGATGACCATGAAC
TTTGCTTTCAGTGAAGGTAAAGTATAATGGAAGCACCCCTACCGTGTTAGGGAGGAATCCGGTGCTCAAAAAGGTG
AGGGAGATGCCGGTGGTCCGGCGGAAGTGGGCTTTTCCGATTTGCTAATGGTTATGCTCAGGCATCAACCCACAAC
TTTGACCCCAAGACTGGTGTATGCTGTTGTTGAGTACAACATCTATGTTATGCATTTATTGAtgatcgtgggattgt
tttcccttagagatcggactttttcccttggcttttgttttcttttgtctcttggattttctgctagaaattttg
50 tggggcataatatatcca

SEQ ID NO: 41

Partial cDNA of EG261 ortholog in coffee plant (*Coffea arabica*), Allele B
 ATGGCTAGAATTTTCAGCATTTCTCTCTCCTCACCATCTTCATCTTCATTTCTTGCATCACCCTTCAGGTCACCTTAT
 GGTGATGAAGAATACGAGTTTGTCAAAGCAATTGATCCAAAAGTAGCACTAAAGATGAAGAAAGAAAAGCTAAGC
 CTTTTTCAGGTTCTACTGGCAGCAGCATCTCAGTGGCAAAGCACCTACTTCAGTCATGGTGGTGCCACCTCCGAAA
 5 ACCAATTCATTCAGTCTTTTGGCCTGGTGAACATGATCGATAATCCGTTAACTGTTGGCCCCGGACCTCAGCTCA
 AAATTGGTCGGGAGGGGCTCAAGGGTTTTATGCATCAGCTTCACAGGAGGAAAATTGGCTTCTTGTGATGACCATGAAC
 TTTGCTTTCACTGAAGGTAAGTATAATGGAAGCACCTTCACCGTGTAGGGAGGAATCCGGTGTCTCAAAAAGGTG
 AGGGAGATGCCGGTAGTCGGCGGAAGTGGGCTTTTCCGATTTGCTAATGGTTATGCTCAGGCATCAACCCACAAC
 TTTGACCCCAAGACTGGTGTGCTGTTGTTGAGTACAACATCTATGTTATGCATTATTGATGATcgtgggattgt
 10 tttcccttagagatcggactttttccctttgcttttgcttttcttttctctcttggtatttctgctagaaaattttg
 tggggcataatatatcca

SEQ ID NO: 42

Partial cDNA of EG261 ortholog in cultivated pepper ("ornamental" pepper)
 15 aatctactttccATGGCCAAACTAATACTCCAAATCTTCTCCATTTCCGTTCTCTATTCACTGGTAGCCTTTCCAG
 CCACGGGAGAAGAAGATCATATTTTTGGAAAAATCCATAAATGAAAAGTCCATGAGGCTAAAAAGGGAAAAACTCA
 GCCATTTTCAGATTTTATTGGCAGCAGCTCCTCAGTGGCTCCAAACCAACATCTATGATAATTATCCACCTCCCA
 AAAATACTACCACAGGCTTTGGCCAAATGAATATGATAGATAATGCCCTAACCTTAGGACCAGAGCTGAGTTCCA
 GGATAGTTGGAAGGGGCACAAGGGTTTTACGCTGCTGCTTCACTAAATGATGTTGGCTTAATGATGGTCATGAAC
 20 TTGCTTTTATTGAAGGAAAATATAATGGGAGCACCTTCACTATACTTGGACGAAATCCGGTATTTGAGAAGGTGA
 GAGAGATGGCCGTGATCGGCGGCAGTGGGCTTTTCCGATTTGCTAGAGGATATGTTTCAGGCTAGTACTCATTAT
 TGGATTTCAAACTGGCGATGCTACTGTTTCAGTATGATGCCTATGTTTTGCATTATTGAagtttactaatattat
 atatcactctaatacggctagctggcttttaattaataattatgtcg

SEQ ID NO: 43

EG261 polypeptide encoded by SEQ ID NO: 40, ortholog in coffee plant
 (*Coffea arabica*), Allele A

MARISAFPLLTIFIFISCITVQVTYGDDEEYEFVKAIDPKVALKMKKEKLSLFRFYWHDIL
 SGKAPTSVMVPPPKTNSFTAFGLVNMIDNPLTVGPDLSSKLVGRAQGFYASASQEEIGF
 30 LMTMNFATTEGKYNGSTLTVLGRNPVLKKVREMPVVGSGSLFRFANGYAQASTHNFDPKT
 GDAVVEYNIYVMHY

SEQ ID NO: 44

EG261 polypeptide encoded by SEQ ID NO: 41, ortholog in coffee plant
 (*Coffea arabica*), Allele B

MARISAFPLLTIFIFISCITVQVTYGDDEEYEFVKAIDPKVALKMKKEKLSLFRFYWHDIL
 SGKAPTSVMVPPPKTNSFTAFGLVNMIDNPLTVGPDLSSKLVGRAQGFYASASQEEIGF
 35 LMTMNFATTEGKYNGSTLTVLGRNPVLKKVREMPVVGSGSLFRFANGYAQASTHNFDPKT
 GDAVVEYNIYVMHY

SEQ ID NO: 45

EG261 polypeptide encoded by SEQ ID NO: 42, ortholog in cultivated pepper
 ("ornamental" pepper)

MAKLILQIFISISVLYSLVAFPATGEEDHIFGKSINEKSMRLKREKLSHFRFYWHDVL
 45 SGSKPTSMIIIPPKNTTGFQGMNIDNALTLPDLSSRIVGRAQGFYAAASLNDVGLM
 MVMNFATIEGKYNGSTLTILGRNPVFEKVREMAVIGSGSLFRFARGYVQASTHSDFKTG
 DATVQYDAYVLHY

SEQ ID NO: 46

Partial cDNA of EG261 ortholog in maize, GenBank NM_001156097

accacacgtacgcccacaccagataatacaactacagcacagcacccgacacacacctctcaagtaacccagcaggca
 ccATGGCCGCCCGCTGCCCTCCTCCTCCTGCTACTGCCAACGACCTGATGGCCCGCTCGGCGGCGTCCG

GCGGCGAGAAGAGCACGCACATCAAGCTGTACTGGCACGACGTGGTGAGCGGGCCGAGCCCCGACGGCGGTGCCGG
 TGGCGCAGGGCGGGCGGTGACCAACACCTCCAAGACCGCTTCGGCATGGTGGTGGTGATCGACGACCCGCTGACCG
 AGGGCCCCGACCTCAAGTCTCCAAGCCGCTCGGCCGCGCGCAGGGCACCTACGTGCGGCGGGGCAAGGACGAGC
 TCTCCCTGATGATGAACATGAATTTTCGTGTTCCAGGCCGGCGAGTACAACGGCAGCACCGTCGCCATCATGGGGC
 5 GGAACGCCGTGTTTCGACGCCGTCCGCGAGATGGCCGTCGTGGGCGGCACCGGCGCGTTTACGGATGGCGCGCGGGT
 ACGCGCAGGCCCCGACGCACACCTTCGACCTCAACACCGGCGACGCCACCGTCGAGTACAACCTCTACATCAAGC
 ACTAGctagctagcccatcggtttgtgtttctgattgttggtgctcatatatgaacacgatcgaactccataat
 tgtttgtgagctcaatttgtgccactggcttttgcagttttggtgaaaaagaagaggtaattaaaggacgata
 gcttcgggtcggttgtaagctgacttcgatttaacttgcacaatgattgaggacttaaagaataaagatagtaataag
 10 actttgttttatctgatcaaaaaaaaaaaaaaaaaa

SEQ ID NO: 47

Partial cDNA of EG261 ortholog in sorghum, GenBank CD430191

aaaaaaaaaccaccaccaccatcgccATGGCCACCACCACGCTCTTCTCTCTCTGCGCCGCCGCGGCACCTCG
 15 CATCAGCGGCAGCGCCGCGGACGACACCGGGTTACGACCTTCAAGCTCTACTTCCACGACATCGTGGCGGGGA
 CGTCGTCCCCAACCGCGGTGCGGATCGCGCAGGCGGCGTCGTCCAACACCTCCTCCACCTCCTTCGGCGCCGTGG
 TGGCCATCGACGACCTCTCACACCGGGGCCACGCGGTTCGTCCGGCACCGAGATCGGGCGCGCCAGGGCACCT
 ACACGTTTCGCGGACAGACACGTTTCGGCTCCTCATGGTGTGATGAACCTTCGTGTTTCACCGCCGCGGACACACAACG
 GCAGCACGCTCTCCATCCTCGGCCGGAACGAGGTGCTCACCGACGTCCGCGAGATGAGCATCGTCGGCGGCAGCG
 20 GAAAGTTCCGCATGGCCAAGGGCTATGTCCAGGCGCACACCATTTGATTCCGGCGCCACCACCGGGGAGACCGTCG
 TCCAGTACACCGTCAACGTCAAGACGCCCTAGctagcttagttggtttcttgctccggccggcgccgggggctgct
 ggtgctgccatggcttggtgtattgtgtgctctccggcagctttgtacacacctgcttttgccgttcttcgggtg
 ttagtacatgacatgacatgttgtagattgagatttcagatcgactcgatcttcactactattgagcgggattta
 25 tttggatttgc

SEQ ID NO: 48

Partial cDNA of EG261 ortholog in rice, GenBank CT835590

GAGACGACGGCGACGACGACGCACATCAAGGTGTACTGGCACGACGTGGTGAGCGGGCCGAGCCCCGACGGCGGTG
 30 CAGGTGGCGAGGGCGGGCGACGACCAACTCGTCGGCGAGCTTCTTCGGCGCCGTGGTGGTGATCGACGACCCGCTG
 ACGTCGGGCCCCGACCTGAACGCCTCGTCGCCGGTGGGCCCCGCGCCAGGGCACCTACGTGAGCGCCGGCAAGGAC
 ACGGTGGCGCTGCTCATGAACATGAACCTTCGTCTTCCAGTCCGGCAGGTACAACGGCAGCACCGTCGCCATCATG
 GGCCGCAACGAGGTCTTCGCCGCCGTCCGCGAGATGGCCGTCGTCCGGCGGCACCGGCGTCTTCGGGTGGGCCCCG
 GGCTACGCCAGGCCCCGACCCACACCTTCGACATGAAGACCGGCGACGCCACCGTTGAGTACAACCTCTACATC
 35 AACCCTGAactagtcacatctttctcatgagtttttttttaccttttcagaaattatggatttagttaatagtta
 atttttacttagcaccaataattgatgattaatcttgcttggt

SEQ ID NO: 49

cDNA of EG261 ortholog in wheat, GenBank BJ260151

aagggaaatcATGGCCTCTGCCGTGCTCTTCGTCTCTCGCCCTGGCCACCATGCAACCGCAGACCGCGTCGTC
 40 CCAGAAGGAGACGCACCTCAAGGTGTACTGGCACGACGTGGTGAGCGGACCGGACCCGACGTGAGTCCGGTGGC
 GCGCGCGACACGACCAACACCTCCAAGACAGCCTTCGGCGTCGTCATGGTCATGGACAACCTCACTCACCGAGGG
 GCCGAGCCTCAACTCATCCAGGCTCATGGGCCGCGCCAGGGCACCTACATCGCCGCCGGCAAGGACCGAGCTGGC
 GCTGCTCATGCTCATGAACCTTCCTTTTCACCGCCGGCAAGTACAACGGCAGCAGCGTCGCCATTATGGGTTCGCAA
 45 CGCCGTGTTACCGAGGTCCGCGAGATGGCTGTGTCGGCGGTACCGGCGTTCAGGTGGGCTCCAGGGTACGC
 GCAGGCCAGGACGCACACCTTGGACCTCAAGACCGGCGACGCCACCGTTGAGTACAACGTATTCATCATGCACTA
 Gtc

SEQ ID NO: 50

Partial cDNA of EG261 ortholog in barley, GenBank AK373627

ggactactcgacagaaaaatccATGGCCTCTGCAGCGCTCTTCTTTGTCTCTCTCGCCCTGGCCACAATGCTGCCG
 50 CAGACCGCGTCGTCCGAGAAGGAGACGCACCTCAAGGTGTACTGGCACGACGTGGTGAGCGGGCCGACCCGACG

TCGGTGCCGGTGGCGCGTGGCGCCACGACCAACACCTCCAAGACAGCCTTCGGCGTCGTCATGGTCATCGACAAC
 CCACTCACCGAGGGGGGAGCCTCAACTCATCCAGGCTCATGGGCGCGCCAGGGCACCTACATCGCCGCCGGC
 AAGGACCAGCTGGCGCTGCTCATGCTCATGAACCTTCGTCTTCACTGCGCGCGAGTACAACGGCAGCAGCGTCGCC
 ATTATGGGTCGCAACGCCGTGTTCCACCGAGGTCCGCGAGATGGCTGTCGTCGGCGGTACCGGCCTTTTCAGGTGG
 5 GCTCGTGGGTACGCGCAGGCCAGGACGCACACCTTGGACCTCAAGACCGCGGACGCCACCGTTGAGTACAAAGTA
 TTCGTCATGCACTAGtcgtctcggccgtgggtcacattttaccaggacttttgtatacaactcaagttacaagaata
 atttatttttttttgcggatgaagaataaattttatttatatgcgttacttacggaaaacttttctcacaattcgt
 gtgtgtgtcattcacaattgatggtgctgcccgtttaatattgatattgtattattgt

10 SEQ ID NO: 51

EG261 polypeptide encoded by SEQ ID NO:46, ortholog in maize, predicted
 MAAVPLLLLLLLPPTLMAASAASGGEKSTHIKLYWHDVVS GPSP TAVPVAQA AVTNTSK
 TAFGMVVVIDDPLTEGPD LKSSKPLGRAQGT YV GAGKDEL SLMNMNFV FQAGEYNGSTV
 15 AIMGRNAVFD AVREMAVVG GTGA FRMARGYA QARTHTFDLNTGDATVEYNLYIKH

SEQ ID NO: 52

EG261 polypeptide encoded by SEQ ID NO: 47, ortholog in sorghum, predicted
 MATTTLFLLLCAAAALASAAAAADDTGFTT FKLYFHDIVAGTSSPTAVRIAQA ASSNTSS
 20 TSFGAVVAID DPLTTGPTRRSSGTEIGRAQGT YTFADQTT FGLLMVMNFV FTAGDHNGSTL
 SILGRNEVLTDVREMSIVGGSGKFRMAKGYVQAHTIDSGATTGETVVQYTVNVKTP

SEQ ID NO: 53

EG261 polypeptide encoded by SEQ ID NO: 48, ortholog in rice, predicted
 25 ETTATTTTHIKVYWHDVVS GPSP TAVQVARAATTNSSASFFGAVVVIDDPLTSGPDLNASSP
 VGRAQGT YVSAGKDTVALLMNMNFV FQSGRYNGSTVAIMGRNEVF AAVREMAVVG GTGVFR
 WARGYAQARTHTFD MKTGDATVEYNLYINH

SEQ ID NO: 54

EG261 polypeptide encoded by SEQ ID NO: 49, ortholog in wheat, predicted
 MASAVLFVLLALATMQPQTASSQKETHLKVYWHDVVS GPDPTSV PVARATTTNTSKTAFG
 30 VVMVMDNSLTEGPSLNSSRLMGRAQGT YIAAGKDQLALLMLMNFV FTAGKYNGSSVAIMG
 RNAVFTEVREMAVVG GTGVFRWAPGYA QARTHTLDLKTGDATVEYNVFIMH

SEQ ID NO: 55

EG261 polypeptide encoded by SEQ ID NO: 50, ortholog in barley, predicted
 40 MASAAFFVLLALATMLPQTASSEKETHLKVYWHDVVS GPNPTSV PVARAATTNTSKTAF
 GVVMVIDNPLTEGGSINSSRLMGRAQGT YIAAGKDQLALLMLMNFV FTAGEYNGSSVAIM
 GRNAVFTVREMAVVG GTGVFRWARGYA QARTHTLDLKTGDATVEYKVFVMH

SEQ ID NO: 56

Partial cDNA of EG261 ortholog in cotton (*Gossypium hirsutum*), variety
 FJ600364
 ATGGCTAAACTTT CAGAAAACACCTAGCTGCCCACCTTCATCTTCACCATTTTCATCGTTTCAGCTTTAGCTGAA
 AACGGCAGTAGCTTCGCGAGAACCATGGACAAGAAGGTGTTGGGGATGAAGAAGGAAAAGCTCAGCCACTTTAGG
 CTATACTGGCACGACATTGTTGGCGGGAAAAACGCGACGGCGGTTCCGGTGGTTTTCCCATCGAGGAATTCAACG
 ACGGCGTTCCGTATGATCAGTGTAATAGACGATCCTTTAACGATGAGACCTGAATTAAGTTCGAAAATGGTGGGA
 AGAGCACAAGGGTTTTACTCGGCGGCGTCACAACAAGAAGTAGGATTGTTGATGGCGATGAATTTTGCTTTTATG
 GAAGGGAAATATAATGGGAGTACGATAACGATATTGGGGAGGAACACGGTGTTCCTCAAAGGCGAGGGAAATGCCG

GTGATCGGCGGTAGTGGACTATTTAGGTTTGCTAGAGGGTATGTTGAAGCTAGAACGCATCTTTTTGATCCTGCT
ACTGGTGATGCTGTGGTTCAGTATGATTGTTATGTTATGCATTATTGA

SEQ ID NO: 57

EG261 polypeptide encoded by SEQ ID NO: 56 ortholog in cotton (*Gossypium hirsutum*), variety FJ600364

MAKLSENTLAAHFIFTIFIVSALAENGSSSFARTMDKKVLGMKKEKLSHFRLYWHDIVGGK
NATAVPVVFPSRNSTTAFGMISVIDDPLTMRPELSSKMVGRAQGFYSAASQQEVGLLMAM
NFAFMEGKYNGSTITILGRNTVFSKAREMPVIGGSGLFRFARGYVEARTHLPATGDAV
VQYDCYVMHY

SEQ ID NO: 58

Partial cDNA EST of EG261 ortholog in cotton (*Gossypium hirsutum*), variety FJ600365

ATGGCTAGGATTCTCTCTCTTAGCTTCCAAATTCATCTTCTTATCCATTTTATCCTCCTCAGGCGTCATCCGA
TGCACCCGAGGCGGAAAACAATGACGATCATGGCTTCATCCAGAGCCTTGACCGAGAGTCGATGGGCTTAAAAAAA
GAAAAGCTAAGTCACCTTTCGCATCTACTGGCACGACATTGTTAGTGGCCGCAATGCTACGTCGATACGAGTGGTT
CGACCTTCTAACGCATCGGTAACAGGGTTCCGAATAATCAACATGATCGACAATCCATTAACCTTAGGGCCGAAC
CTAAGCTCGAACTAGTGGGAAGAGCACAAGGGTTCTACGCACTCTCGTCACAAGAAGAAGTGGGATTGTTGATG
TCGATGAACTTTGCTTTTCACGGAAGGGGAAATACAATGGTAGCACGATCACAGTGTGGGGAGAAACCCAGTTTTTC
AACAAAGTGAGGGGAAATGCGGGTGATCGGAGGCAGCGGACTTTTCCGATTTCGCCCGAGGCATGTTCAAGCAAGA
ACTAATACATTAAACTTGACAACCGGAGATGCCATTGTTGAATACACTTGTATGTGATGCATTATTGA

SEQ ID NO: 59

EG261 polypeptide encoded by SEQ ID NO: 58, ortholog in cotton (*Gossypium hirsutum*), variety FJ600364

MARIPLLLASKFIFLSILSSSGVIRCTRGEMNDDHGFIQSLDRESMGLKKEKLSHFRIYW
HDIVSGRNATSIRVVRPSNASVTGFGIINMIDNPLTLGPNLSSKLVGRAQGFYALSSQEE
VGLLMSMNFAFTEGKYNGSTITVLGRNPVFNKVREMRVIGGSGLFRFARGYVQARTNTLN
LTTGDAIVEYTCYVMHY

SEQ ID NO: 60

Partial cDNA of EG261 ortholog in Monkey Tail (*Heliotropium curassavicum*), collected in Africa

gctctaaccaccataatcATGGCTACCAAATTCCTCTTATCGTTCCTTTTCATCTCCTGCTATGTCCTCTCCATC
TCAGCAGACAAAGAAACAGGTTTCGTGGGCCCCAGTTGATCCTAAGTCCGTAAGCTACAAGAAGAAGCACACATTT
AGCCACTTCAGGTTCTATTGGCACGAAATCTTCAGTGGAAGCAACCCCTCCTCTGTTAGAAATCGTTCCACCACAA
CCAAAGTACAGCACAAACCACCACCTTCGGTTCTGTGGGAGTATTCGACAAACGTGTTGACCCTTAGGACCCGAGTTG
TACTCAAAGGTTGTGGGAAGTGCCGAAGGGTTGTACTCCTCTGCTTCACAAAAGGAGTTTGCCCTTTTGGTCATT
ATGAACTTCGCGTTGACCGAAGGGAAGTACAATGGTAGCACCATCACGTTTCGTGGGGAGAAGCCCCATCGCTCAA
AAGGTGAGAGAGATGCCTGTAATTGGTGGCACCGGTGTTTTAGATTGTCAGGGGCTATGTTGAGTCCTCGACC
ATCACCTTTGATCCTCAAACGAGGAACAACACAATTGAGTACAACGTCTATGTTTACCACATAATTgttgtgtgtt
gaaggatgttgaggagtttgatcattgttgttcttggtgttcttgaaaaaggcttagtgtgcc

SEQ ID NO: 61

EG261 polypeptide encoded by SEQ ID NO: 60, ortholog in Monkey Tail

MATKFLLSFLFISCYVLSISADKETGFVGPVDPKSVSYKKKHTFSHFYWHEIFSGSNP
SSVRIVPPQPKYSTTTTFGSVGVFDNVLTLPPELYSKVVGSAEGLYSSASQKEFALLVIM
NFALTEGKYNGSTITFVGRSPLAQKVREMPVIGGTGVFRFARGYVESSTITFDPQTRNNT
IEYNVYVYH

gctctaaccaccataatcAIGGCTACCAAAATTCCTCTTATCGTTCCCTTTTCATCTCCTGCTATGTCCTCTCCATC
TCAGCAGACAAAAGAAACAGGTTTCGTGGGCCAGTTGATCCTAAGTCCGTAAGCTACAAGAAGAAGCACACATTT
AGCCACTTCAGGTTCTATTGGCACGAAATCTTCAGTGGGAAGCAACCCCTTCCTCTGTTAGAAFCGTTCCACCACAA
CCAAAGTACAGCACAAACCACCACCTTCGGTTCCTGTGGGAGTATTCGATAACGTGTTGACCCTAGGACCCGAGTTG
TACTCAAAGGTTGTGGGAAGTGCCGAAGGGTTGTA CTCTCTGCTTCACAAAAGGAGTTTGCCCTTTTGGTCATT
ATGAACTTCGCGTTGACCGAAGGGGAAGTACAATGGTAGCACCATCACGTTTCGTGGGGAGAAGCCCCATCGCTCAA
AAGGTAAGAGAGATGCGTGTGATTGGTGGCACCGGTGTTTTTCAGATTTGCCAGGGGCTATGTTTGAGTCCCTCGACC
ATCACCTTCGATTCCTCAAACGAGGAACAACACAAATTCAGTACAACGTCTATGTTTACCACtaattgttggtgttt
gaaggatgttgaggagtttgatcattgttggttcttgaaaaaggccttagtgtgcc

MATKFLLSFLFISCYVLSISADKETGEVGPVDPKSVSYKKKHFTFSHFRFYWHEIFSGSNP
SSVRIVPPQPKYSTTTTTFGSGVGFDDNVLTLGPELYSKVVGSAEGLYSSASQKEFALLVIM
NFALTEGKYNGSTITFVGRSPIAQKVREMPVIGGTGVFRFARGYVESSTITFDPPQTRNNT
IEYNVYVYH

cggtgcagaagATGGCTAGGATTCCTCTTCTCTTAGCTTCCAAATTCATCTTCTTATCCATTTTATCCTCCTCAGG
CGTCAATCCGATGCACCCGAGGCGAAAAACAATGACGATCATGGCTTTCATCCAGAGCCTTGACCGAGAGTTCGATGGG
CTTAAAAAAAAGAAAAGCTAAGTCACTTTTCGCATCTACTGGCACGACATTTGTTAGTGGCCGCAATGCTACGTGAT
ACGAGTGGTTTCGACCTTCTAACGCATCGGTAACAGGGTTTCGGAAATAATCAACATGATCGACAAATCCATTAAACCTT
AGGGCCGAACCTAAGCTCGAAACTAGTGGGAAGAGCACAAAGGGTTTCTACGCACTCTCGTCACAAGAAGAAGTGGG
ATTTGTTGATGTCGATGAACCTTTGCTTTTCACGGAAGGGGAAATACAATGGTAGCACGATCACAGTGTGGGGAGAAA
CCAGTTTTTCAACAAAAGTGGAGGAAAATGCGGGTGTATCGGAGGCAGCGGACTTTTCCGAATTCGCCCGAGGCTATGT
TCAAGCAAGAAGTAATAACATTAAACTTTGACAAACCGGAGATGCCATTGTTGAATACACTTGTATGTGATGCATTA
TTGAatggtgtgacccatttgccttgcattattataaaagttaaatttgcctttaatcat

ATFGGCTAGGATTCCCTCTTCTCTTAGCTTCCAAATTCATCTTCTTATCCATTTTATCCCTCTCAGGCGTCATCCGA
TGCACCCGAGGCGAAAAACAATGACGATCATGGCTTCATCCAGAGCCTTGACCGAGAGTCGATGGGCTTAAAAAAA
GAAAAGCTAAGTCACCTTTTCGCATCTACTGGCACGACATTGTTAGTGGCCGCAATGCTACGTCGATACGAGTGGTTF
CGACCTTCTAACGCATCGGTAACAGGGTTTCGGAAATAATCAACATGATCGACAATCCATTTAACCTTAGGGCCGGAAC
CTAAGCTCGAAAAGTAGTGGGAAGAGCACAAAGGGTTTCTACGCACTCTCTGTCACAAGAAGAGTGGGATTGTTGATG
TCGATGAACCTTTGCTTTTACCGGAAGGGGAAATACAAATGGTAGCACGATCACAGTGTGTTGGGGAGAAAACCCAGTTTTTT
AACAAAAGTGAGGGGAAATGCGGGTGATCGGAGGCAGCGGACTTTTTCCGATTTCGCCCGAGGCTATGTTTCAAGCAAGA
ACTAATACATTAAACTTTGACAAACCGGAGATGCCATTGTTGAATACACTTGTATGTGATGCATTATTGA

1 auaaaauucuuugcauuuaauucaucaucauuuicaucaucccaauuccaauuuccucuaauuuuacacccaccacaaaaccAUGGC
 UUCCCCACUUCCUCUAAAAUCCCUCCUUCUCCUCUUCACCCCUAUGCCCUACCCAUUCUCAGCAGAAUACACAGGCUUUCGU

GGGCACACUAGACCCAAAGUCCAUAGGCAUACACCACAAGAAAACCCUAAACCACUUCAGGUUCUACUGGCACGA
AGUCUUCAGCGGAGAAAACCCACAUCCGUUAGAAUCAUCCCUCACUCCCCAAAUACAACACAACCACAACCUU
CGGUUCCGUUGGAAUCUCUGACAACGCUUUAACCGUGGGACCUGAGGUGUACUCCAAGGUUGUCGGAAAAGCCGA
GGGCUUGUUUGUCUCCACGUCACAAACGCAGUUUGACCUGUUACUGAUUUACAACUUCGCGUUGACCCAAGGGAA
GUACAACGGCAGCACCAUCACGUUCACGGGGAGGAGCCCCUCUCGGAGAAGGUGAGGGAGCUGCCCAUUGUAGG
UGGCAGUGGGGUCUCAAUUUUCCACUGGGUAUGUUGAGUCUAGGACGCUAAGUUUUUGAUCCCCAAACGAGGAA
UAACACGGUUCAGUUCGACGUGUAUUUUACUAUugaugauuuuuugaauguguuuuuuuccauguugauguguuau
agcuuugguaugugucacuuaguucua

CLAIMS:

1. An isolated, recombinant, or synthetic polynucleotide comprising a nucleic acid sequence selected from the group consisting of:
 - (a) a nucleic acid sequence having at least 95% identical nucleotides to a nucleic acid sequence coding for EG261 selected from the group consisting of SEQ ID NOs: 1, 2, 16, 17, 18, 19, 20, 21, 22, 23, 24, 34, 35, 36, 40, 41, 42, 46, 47, 48, 49, 50, 56, 58, 60, and 62;
 - (b) a nucleic acid sequence having at least 95% identical nucleotides to a nucleic acid sequence coding for a homolog of EG261 selected from the group consisting of SEQ ID NOs: 1, 2, 16, 17, 18, 19, 20, 21, 22, 23, 24, 34, 35, 36, 40, 41, 42, 46, 47, 48, 49, 50, 56, 58, 60, and 62;
 - (c) a nucleic acid sequence having at least 95% identical nucleotides to a nucleic acid sequence coding for an ortholog of EG261 selected from the group consisting of SEQ ID NOs: 1, 2, 16, 17, 18, 19, 20, 21, 22, 23, 24, 34, 35, 36, 40, 41, 42, 46, 47, 48, 49, 50, 56, 58, 60, and 62;
 - (d) a nucleic acid sequence having at least 95% identical nucleotides to a nucleic acid sequence coding for a paralog of EG261 selected from the group consisting of SEQ ID NOs: 1, 2, 16, 17, 18, 19, 20, 21, 22, 23, 24, 34, 35, 36, 40, 41, 42, 46, 47, 48, 49, 50, 56, 58, 60, and 62 ;
 - (e) complements of a nucleic acid sequence of (a), (b), (c) or (d);
 - (f) reverse complements of a nucleic acid sequence of (a), (b), (c) or (d);
 - (g) reverse sequences of a nucleic acid sequence of (a), (b), (c) or (d);
 - (h) mRNA sequence of nucleic acid sequence of (a), (b), (c) or (d); and,
 - (i) fragments and variations of a nucleic acid sequence of (a), (b), (c), (d), (e), (f), (g) and (h).
2. A vector comprising the polynucleotide of claim 1.

3. A genetic construct comprising an isolated, recombinant, or synthetic polynucleotide of claim 1.
4. A genetic construct comprising, in the 5'-3' direction: (a) a promoter sequence, (b) an isolated, recombinant, or synthetic polynucleotide of claim 1; and (c) a gene termination sequence.
5. The genetic construct of claim 3 or claim 4, wherein the isolated, recombinant, or synthetic polynucleotide comprises an open reading frame encoding a polypeptide capable of altering pathogen tolerance and/or resistance.
6. The genetic construct of claim 5, wherein the pathogen tolerance and/or resistance is conferred or enhanced.
7. The genetic construct of claim 5 or claim 6, wherein the pathogen is soybean cyst nematode.
8. A transgenic cell comprising a genetic construct of claim 3, claim 4, claim 5, claim 6 or claim 7.
9. An organism comprising a transgenic cell of claim 8.
10. A plant comprising a transgenic cell of claim 3, claim 4, claim 5, claim 6 or claim 7, or a part or propagule or progeny thereof.
11. Progeny plants of the plant of claim 10, wherein the progeny plants have an altered pathogen tolerance and/or resistance as a result of inheriting the polynucleotide.

12. A method of producing hybrid seed comprising crossing the plant of claim 10 or a progeny plant of claim 11 with a different plant of the same species, and harvesting the resultant seed.
13. A method for modifying gene expression in a target organism comprising stably incorporating into the genome of the organism a genetic construct according to claim 3, claim 4, claim 5, claim 6 or claim 7.
14. The method of claim 13, wherein the organism is a plant.
15. The method of claim 14, wherein the plant is a soybean plant (*Glycine max*).
16. A method for producing a plant having altered pathogen tolerance and/or resistance comprising: (a) transforming a plant cell with a genetic construct to provide a transgenic cell, wherein the genetic construct comprises: (i) a promoter sequence; (ii) an isolated, recombinant, or synthetic polynucleotide sequence of claim 1; and (c) a gene termination sequence; and (b) cultivating the transgenic cell under conditions conducive to regeneration and mature plant growth of a plant having altered pathogen tolerance and/or resistance.
17. A method for modifying a phenotype of a target organism, comprising stably incorporating into the genome of the target organism a genetic construct comprising: (a) a promoter sequence; (b) an isolated, recombinant, or synthetic polynucleotide sequence of claim 1; and (c) a gene termination sequence.
18. The method of claim 16 or claim 17, wherein the target organism is a plant.
19. A process of determining the presence or absence of a polynucleotide coding for EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations thereof in a plant, wherein the process comprises at least one of:

- (a) isolating nucleic acid molecules from said plant and amplifying sequences homologous to the polynucleotide;
- (b) isolating nucleic acid molecules from said plant and performing a Southern hybridization to detect the polynucleotide;
- (c) isolating proteins from said plant and performing a Western Blot using antibodies to a protein encoded by the polynucleotide; and/or
- (d) demonstrating the presence of mRNA sequences derived from a polynucleotide mRNA transcript and unique to the polynucleotide.

20. A method of breeding plants to produce altered pathogen tolerance and/or resistance comprising:

- i) making a cross between a first plant with an isolated, recombinant, or synthetic polynucleotide sequence of claim 1 with a second plant to produce a F1 plant;
- ii) backcrossing the F1 plant to the second plant; and
- iii) repeating the backcrossing step to generate a near isogenic or isogenic line, wherein the isolated, recombinant, or synthetic polynucleotide sequence of claim 1 is integrated into the genome of the second plant and the near isogenic or isogenic line derived from the second plant with the isolated, recombinant, or synthetic polynucleotide sequence has conferred or enhanced pathogen tolerance and/or resistance compared to that of the second plant without the isolated, recombinant, or synthetic polynucleotide sequence.

21. The method of claim 20, wherein the plant is soybean (*Glycine max*).

22. The method of claim 20 or claim 21, wherein the pathogen is soybean cyst nematode.

23. A method of producing a plant with conferred or enhanced pathogen tolerance and/or resistance, the process comprising:

- (b) crossing a first plant containing a polynucleotide coding for EG261, homologs of EG261, orthologs of EG261, paralogs of EG261, and fragments and variations thereof to a second plant, and harvesting the resultant seed;

(b) determining the presence of the polynucleotide in the resultant seed or in cells or tissues of a plant grown from the resultant seed; wherein the determining comprises at least one of:

- (i) isolating nucleic acid molecules from the resultant seed or in cells or tissues of a plant grown from the resultant seed and amplifying sequences homologous to the polynucleotide;
- (ii) isolating nucleic acid molecules from the resultant seed or in cells or tissues of a plant grown from the resultant seed and performing a Southern hybridization to detect the polynucleotide;
- (iii) isolating proteins from the resultant seed or in cells or tissues of a plant grown from the resultant seed and performing a Western Blot using antibodies to a protein encoded by the polynucleotide; and/or
- (iv) demonstrating the presence in the resultant seed or in cells or tissues of a plant grown from the resultant seed of mRNA sequences derived from a polynucleotide mRNA transcript and unique to the polynucleotide.

24. The method of claim 23, further comprising confirming that the resultant seed or the cells or tissues of the plant grown from the resultant seed contain the polynucleotide.

25. The method of claim 24, further comprising using the resultant plant containing the polynucleotide in a plant breeding scheme.

26. The method of claim 24 or claim 25, further comprising crossing the resultant plant containing the polynucleotide with another plant of the same species.

27. An isolated, recombinant, or synthetic polypeptide, wherein the polypeptide comprises an amino acid sequence having at least 90% identity to a polypeptide encoded by an EG261 gene selected from the group consisting of SEQ ID NOs: 1, 2, 16, 17, 18, 19, 20, 21, 22, 23, 24, 34, 35, 36, 40, 41, 42, 46, 47, 48, 49, 50, 56, 58, 60, and 62, wherein the isolated, recombinant, or synthetic polypeptide when expressed in a plant species induces resistance to a plant pathogen.

28. The isolated, recombinant, or synthetic polypeptide of claim 27, wherein the plant pathogen is nematode.
29. The isolated, recombinant, or synthetic polypeptide of claim 27, wherein the polypeptide comprises an amino acid sequence comprising anyone of SEQ ID NOs: 9-15, 25, 26, 27, 28, 29, 30, 31, 32, 33, 37, 38, 39, 43, 44, 45, 51, 52, 53, 54, 55, 57, 59, 61, 63, 64 and 65.
30. Use of an isolated, recombinant, or synthetic polynucleotide of claim 1.
31. Use of an isolated, recombinant, or synthetic polypeptide of claim 27.
32. The genetic construct of claim 4, wherein the promoter sequence is heterologous to the isolated, recombinant, or synthetic polynucleotide of claim 1.
33. The transgenic cell of claim 8, wherein the isolated, recombinant, or synthetic polynucleotide is heterologous to the transgenic cell.
34. The organism of claim 9, wherein the isolated, recombinant, or synthetic polynucleotide is heterologous to the organism.
35. The plant of claim 10, wherein the isolated, recombinant, or synthetic polynucleotide is heterologous to the plant.
36. The method of claim 16, wherein the plant is a soybean variety sensitive to soybean cyst nematode (SCN), and wherein the nucleic acid sequence encoding an EG261 ortholog is derived from *G. pescadrensis*.

37. The method of claim 20, wherein the first plant comprises a nucleic acid sequence encoding an EG261 ortholog derived from *G. pescadrensis*, and wherein the second plant is a soybean variety sensitive to soybean cyst nematode (SCN).

38. The method of claim 23, wherein the first plant comprises a nucleic acid sequence encoding an EG261 ortholog derived from *G. pescadrensis*, and wherein the second plant is a soybean variety sensitive to soybean cyst nematode (SCN).

Figure 1.

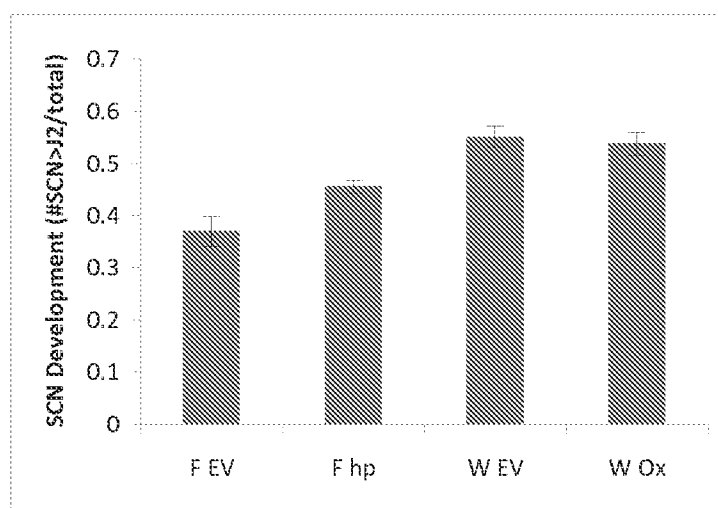


Figure 2.

SEQ_ID_NO_9	MASHFLKSLLLSTYALPISAEYTGFGVGLDPKSGIGHKKTLSHFRFYWHEVFSGENPT
SEQ_ID_NO_10	MASHFLKSLLLSTYALPISAEYTGFGVGLDPKSGIGHKKTLSHFRFYWHEVFSGENPT
SEQ_ID_NO_12	MASHFLKSLLLSTYALPISAEYTGFGVGLDPKSGIGHKKTLNHFRFYWHEVFSGENPT
SEQ_ID_NO_14	MASHFLKSLLLSTYALPISAEYTGFGVGLDPKSGIGHKKTLNHFRFYWHEVFSGENPT
SEQ_ID_NO_15	MASHFLKSLLLSTYALPISAEYTGFGVGLDPKSGIGHKKTLNHFRFYWHEVFSGENPT
SEQ_ID_NO_11	MASHFLKSLLLSTYALPISAEYTGFGVGLDPKSGIGHKKTLSHFRFYWHEVFSGENPT
SEQ_ID_NO_13	MASHFLKSLLLSTYALPISAEYTGFGVGLDPKSGIGHKKTLSHFRFYWHEVFSGENPT

SEQ_ID_NO_9	SVRIIPSLPKYNATTTFGSVGIFDPELTVGEPEVYSKVVGKAEGLFASTSQTFDILLIYN
SEQ_ID_NO_10	SVRIIPSLPKYNATTTFGSVGIFDPELTVGEPEVYSKVVGKAEGLFASTSQTFDILLIYN
SEQ_ID_NO_12	SVRIIPSLPKYNATTTFGSVGISDNALTVEPEVYSKVVGKAEGLFVSTSQTFDILLIYN
SEQ_ID_NO_14	SVRIIPSLPKYNATTTFGSVGISDNALTVEPEVYSKVVGKAEGLFVSTSQTFDILLIYN
SEQ_ID_NO_15	SVRIIPSLPKYNATTTFGSVGISDNALTVEPEVYSKVVGKAEGLFVSTSQTFDILLIYN
SEQ_ID_NO_11	TVRIIPSLPKYNATTTFGSVGIFDNTLTVGEPEVYSKVAGKAEGLFASTSQTFNILLIYS
SEQ_ID_NO_13	TVRIIPSLPKYNATTTFGSVGIFDNTLTVGEPEVYSKVAGKAEGLFASTSQTFNILLIYS
	:***** *..*****:*****:*****:*****
SEQ_ID_NO_9	FALTQGYNGSTTTFTGRSPELSEKVRPELPIVGGSGVFKFATGYVESRTLSFDEQTRNNTV
SEQ_ID_NO_10	FALTQGYNGSTTTFTGRSPELSEKVRPELPIVGGSGVFKFATGYVESRTLSFDEQTRNNTV
SEQ_ID_NO_12	FALTQGYNGSTTTFTGRSPELSEKVRPELPIVGGSGVFKFSTGYVESRTLSFDEQTRNNTV
SEQ_ID_NO_14	FALTQGYNGSTTTFTGRSPELSEKVRPELPIVGGSGVFKFSTGYVESRTLSFDEQTRNNTV
SEQ_ID_NO_15	FALTQGYNGSTTTFTGRSPELSEKVRPELPIVGGSGVFKFSTGYVESRMLSFDPQTRNNTV
SEQ_ID_NO_11	FALTQGYNGSTTTFTGRSPELSEKVRPELPIVGGSGVFKFATGYVESRTLSFDEQTRNNTV
SEQ_ID_NO_13	FALTQGYNGSTTTFTGRSPELSEKVRPELPIVGGSGVFKFATGYVESRTLSFDEQTRNNTV
	*****:*****:*****:*****:***** *****
SEQ_ID_NO_9	QFVYIYY
SEQ_ID_NO_10	QFVYIYY
SEQ_ID_NO_12	QFVYIYY
SEQ_ID_NO_14	QFVYIYY
SEQ_ID_NO_15	QFVYIYY
SEQ_ID_NO_11	QFVYIYY
SEQ_ID_NO_13	QFVYIYY

Figure 3.

SEQ_ID_NO_25	Cultivated_tomato	-----MAKLILQIFTISLFLSLVAFRAATGEEDNYIFGKSN
SEQ_ID_NO_27	Solanum_chmielewskii	-----MAKLILQIFTISLFLSLVAFRAATGEEDNYIFGKSN
SEQ_ID_NO_28	Solanum_habrochaites	-----MAKLILQIFTISLFLSLVAFRAATGEEDNYIFGKSN
SEQ_ID_NO_26	Peruvian_tomato	-----MAKLILQIFTISLFLSLVAFRAATGEEDNYIFGKSN
SEQ_ID_NO_29	Solanum_corneliomulleri	-----MAKLILQIFTISLFLSLVAFRAATGEEDNYIFGKSN
SEQ_ID_NO_33	Potato	-----MAKLILQIFTISLFLSLVAFRAATGEEDNYIFGKSN
SEQ_ID_NO_32	Eggplant	-----MAKLILQIFTISLFLSLVAFRAATGEEDNYIFGKSN
SEQ_ID_NO_30	Chili_pepper	-----MAKLILQIFSVLYSLVAFRAATGEED-HIFGKSN
SEQ_ID_NO_31	Hot_pepper	-----MAKLILQIFSVLYSLVAFRAATGEED-HIFGKSN
		: * **:: ***** *
SEQ_ID_NO_25	Cultivated_tomato	KKPTRLRKEKISHERFYWHDLILSGSKPTSMIIPPPKNTTTTGFQGMNID
SEQ_ID_NO_27	Solanum_chmielewskii	KKPTRLRKEKISHERFYWHDLILSGSKPTSMIIPPPKNTTTTGFQGMNID
SEQ_ID_NO_28	Solanum_habrochaites	KKPTRLRKXKESHHERFYWHDLILSGSKPTSMIIPPPKNTTTTGFQGMNID
SEQ_ID_NO_26	Peruvian_tomato	KKPTRLRKEKESHHERFYWHDLILSGSKPTSMIIPPPKNTTTTGFQGMNID
SEQ_ID_NO_29	Solanum_corneliomulleri	KKPTRLRKEKESHHERFYWHDLILSGSKPTSMIIPPPKNTTTTGFQGMNID
SEQ_ID_NO_33	Potato	KKPTRLRKEKESHHERFYWHDLILSGSKPTSMIIPPPKNTTTTGFQGMNID
SEQ_ID_NO_32	Eggplant	KKSMRLRKEKLSHERFYWHDLVLSGSKPTSMIIPPPKNTTTTGFQGMNID
SEQ_ID_NO_30	Chili_pepper	EKSMRLRKEKLSHERFYWHDLVLSGSKPTSMIIPPPKNTTTTGFQGMNID
SEQ_ID_NO_31	Hot_pepper	EKSMRLRKEKLSHERFYWHDLVLSGSKPTSMIIPPPKNTTTTGFQGMNID
		:* **:: *:*****:*****:*****:*****:*****:*****
SEQ_ID_NO_25	Cultivated_tomato	NALTLPKLSSKIVGRAQGFYGAASLNDVGLMMVMNFAFIEGKYNSTFT
SEQ_ID_NO_27	Solanum_chmielewskii	NALTLPKLSSKIVGRAQGFYGAASLNDVGLMMVMNFAFIEGKYNSTFT
SEQ_ID_NO_28	Solanum_habrochaites	NALTLPKLSSKIVGRAQGFYGAASLNDVGLMMVMNFAFIEGKYNSTFT
SEQ_ID_NO_26	Peruvian_tomato	NALTLPKLSSKIVGRAQGFYGAASLNDVGLMMVMNFAFIEGKYNSTFT
SEQ_ID_NO_29	Solanum_corneliomulleri	NALTLPKLSSKIVGRAQGFYGAASLNDVGLMMVMNFAFIEGKYNSTFT
SEQ_ID_NO_33	Potato	NALTLPKLSSKIVGRAQGFYGAASLNDVGLMMVMNFAFIEGKYNSTFT
SEQ_ID_NO_32	Eggplant	NALTLPKLSSKIVGRAQGFYGAASLNDVGLMMVMNFAFIEGKYNSTFT
SEQ_ID_NO_30	Chili_pepper	NALTLPKLSSKIVGRAQGFYGAASLNDVGLMMVMNFAFIEGKYNSTFT
SEQ_ID_NO_31	Hot_pepper	NALTLPKLSSKIVGRAQGFYGAASLNDVGLMMVMNFAFIEGKYNSTFT
		*****:*****:*****:*****:*****:*****:*****

Figure 3. Continued

SEQ_ID_NO_25	Cultivated_tomato	ILGRNPVEKVR	EMAVIGGSG	LFRFARGY	VQASTHSW	DFKTGDA	TVQYDA
SEQ_ID_NO_27	Solanum_chmielewskii	ILGRNPVEKVR	EMAVIGGSG	LFRFARGY	VQASTHSW	DFKTGDA	TVX---
SEQ_ID_NO_28	Solanum_habrochaites	ILGRNPVXEKVR	EMAVIGGSG	LFRFARGY	VQASTHSW	DFKTGDA	TVQYDA
SEQ_ID_NO_26	Peruvian_tomato	ILGRNPVEKVR	EMAVIGGTG	LFRFARGY	VEASTHSW	DFKTGDA	TVQYDA
SEQ_ID_NO_29	Solanum_corneliomulleri	ILGRNPVEKVR	EMAVIGGSG	LFRFARGY	VQASTHSW	DFKTGDA	TVQYDA
SEQ_ID_NO_33	Potato	ILGRNPVEKVR	EMAVIGGSG	LFRFARGY	VEASTHSW	DFKTGDA	TVQYDA
SEQ_ID_NO_32	Eggplant	ILGRNPVEKVR	EMAVIGGSG	LFRFARGY	VQASTHSW	DYKTGDA	TVKYDA
SEQ_ID_NO_30	Chili_pepper	ILGRNPVEKVR	EMAVIGGSG	LFRFARGY	VQASTHS	LD	DFKTGDA
SEQ_ID_NO_31	Hot_pepper	ILGRNPVEKVR	EMAVIGGSG	LFRFARGY	VQASTHS	LD	DFKTGDA
		*****	*****;	*****	*****	*****	*****
		YVFALLEFT	NEFYFHRV	NLX-----			
		YVLHY-----					
		YVLHY-----					
		YVLHY-----					
		YVLHYG-F	TNEVCFRC	VNEIRFSL	TGY		
		X-----					
		YVLHY-----					
		YVLHY-----					

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SEQ ID NO: 51	maize	MAAAVPELLLLLLPTTLMASASAASGGEKSTHIKLYHWDVVSPE-PTAVFVAQAAVNTS	59
SEQ ID NO: 53	rice	-----TTAT-----TTHIKYVHWDVVSPE-PTAVQVAPAAATNSS	36
SEQ ID NO: 54	wheat	-MAS-AVLEFVLLALATMQPQTASS--QRETHLKVYHWDVVSPE-PTSVPVARATNTS	55
SEQ ID NO: 55	barley	-MASAALEFVLLALATMLPQTASS----ERETHLKVYHWDVVSPE-PTSVPVARATNTS	56
SEQ ID NO: 52	sorghum	-MATTELELLCAAALASAAAAADDTGETTEKLYFHDIWAGTSSPTAVRIQAASNTS	59
		::: * :*:*:*:*:*: * :*:*:*:*: * :*: * :*: * :*: * :*: *	
SEQ ID NO: 51	maize	KTAFQMVVIDDPLETEGDLKSKPLGRAGQTYGAGKDELIMMNNNEVFQAGRYNGST	119
SEQ ID NO: 53	rice	ASFGAVVVIDDPLETSGPDINASSPVGRAGQTYVSAGKDTVALIMNNNEVFQSGRYNGST	96
SEQ ID NO: 54	wheat	KTAGVVMVMENSLTEGFSLSRLMGPAQGTIAAGKDQALIMLMELFTAGRYNGSS	115
SEQ ID NO: 55	barley	KTAGGVNVVIDNPLETGGSLNSRLMGRAQGTIIAAGKDQALIMLMNEVFTAGRYNGSS	116
SEQ ID NO: 52	sorghum	STSEGAVVAIDDDPLETTCFTKSSGTEIGRAQCTYTEADQTHGLMVMNEVFTAGDHNGST	119
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SEQ ID NO: 51	maize	VAINGRNAVFDAVREMAVVGGTGAFRMARGYAQARTHTFDLTGDTATVEYNLYINH-	175
SEQ ID NO: 53	rice	VAIMGRNEVFRAVREMAVVGGTGVRFWARGYAQARTHTTFMKTGDTATVEYNLYINH-	152
SEQ ID NO: 54	wheat	VAIMGRNAVFTVREMAVVGGTGTVFRWARGYAQARTHTLDLKTGDTATVEYNFMH-	171
SEQ ID NO: 55	barley	VAINGRNAVFTVREMAVVGGTGTVFRWARGYAQARTHTLDLKTGDTATVEYKVFVMH-	172
SEQ ID NO: 52	sorghum	LSILGRNEVLTDVREMSIVGGSGKFRMARGVQANTIDSGATGETVWQYTVNVKTP	176
		: : : * * * * * : : * * * * * : : * * * * * : : * * * * * : : * * * * * : :	