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(54) Title: COMPOSITIONS AND METHODS USING RNA INTERFERENCE OF CDPK-LIKE FOR CONTROL OF NEMATODES

(57) Abstract: The present invention concerns double stranded RNA compositions and transgenic plants capable of inhibiting expression of genes essential to establishing or maintaining nematode infestation in a plant, and methods associated therewith. Specifically, the invention relates to the use of RNA interference to inhibit expression of a target plant gene, which is a CDPK-like gene, and relates to the generation of plants that have increased resistance to parasitic nematodes.

Compositions and Methods Using RNA Interference of CDPK-Like For Control of Nematodes

CROSS REFERENCE TO RELATED APPLICATIONS

5 This application claims the priority benefit of U.S. Provisional Application Serial No.60/900,466 filed February 09, 2007.

FIELD OF THE INVENTION

[Para 1] The field of this invention is the control of nematodes, in particular the control of 10 soybean cyst nematodes. The invention also relates to the introduction of genetic material into plants that are susceptible to nematodes in order to increase resistance to nematodes.

BACKGROUND OF THE INVENTION

[Para 2] .Nematodes are microscopic roundworms that feed on the roots, leaves and stems 15 of more than 2,000 row crops, vegetables, fruits, and ornamental plants, causing an estimated $100 billion crop loss worldwide. A variety of parasitic nematode species infect crop plants, including root-knot nematodes (RKN), cyst- and lesion-forming nematodes. Root-knot nematodes, which are characterized by causing root gall formation at feeding sites, have a relatively broad host range and are therefore pathogenic on a large number of crop species. The cyst- and le-20 sion-forming nematode species have a more limited host range, but still cause considerable losses in susceptible crops.

[Para 3] Pathogenic nematodes are present throughout the United States, with the greatest concentrations occurring in the warm, humid regions of the South and West and in sandy soils. Soybean cyst nematode (Heterodera glycines), the most serious pest of soybean plants, was 25 first discovered in the United States in North Carolina in 1954. Some areas are so heavily infested by soybean cyst nematode (SCN) that soybean production is no longer economically possible without control measures. Although soybean is the major economic crop attacked by SCN, SCN parasitizes some fifty hosts in total, including field crops, vegetables, ornamentals, and weeds.

30 [Para 4] Signs of nematode damage include stunting and yellowing of leaves, and wilting of the plants during hot periods. However, nematode infestation can cause significant yield losses without any obvious above-ground disease symptoms. The primary causes of yield reduction are due to root damage underground. Roots infected by SCN are dwarfed or stunted. Nematode infestation also can decrease the number of nitrogen-fixing nodules on the roots, and may make 35 the roots more susceptible to attacks by other soil-borne plant pathogens.

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

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

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

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

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

[Para 10] Methods have been proposed for the genetic transformation of plants in order to confer increased resistance to plant parasitic nematodes. U.S. Patent Nos. 5,589,622 and 5,824,876 are directed to the identification of plant genes expressed specifically in or adjacent to the feeding site of the plant after attachment by the nematode. The promoters of these plant target genes can then be used to direct the specific expression of detrimental proteins or enzymes, or the expression of antisense RNA to the target gene or to general cellular genes. The plant promoters may also be used to confer nematode resistance specifically at the feeding site by transforming the plant with a construct comprising the promoter of the plant target gene linked to a gene whose product induces lethality in the nematode after ingestion.

[Para 11] Recently, RNA interference (RNAi), also referred to as gene silencing, has been proposed as a method for controlling nematodes. When double-stranded RNA (dsRNA) corresponding essentially to the sequence of a target gene or mRNA is introduced into a cell, expression from the target gene is inhibited (See e.g., U.S. Patent No. 6,506,559). U.S. Patent No. 6,506,559 demonstrates the effectiveness of RNAi against known genes in Caenorhabditis elegans, but does not demonstrate the usefulness of RNAi for controlling plant parasitic nematodes.

[Para 12] Use of RNAi to target essential nematode genes has been proposed, for example, in PCT Publication WO 01/96584, WO 01/17654, US 2004/0098761, US 2005/0091713, US 2005/0188438, US 2006/0037101, US 2006/0080749, US 2007/0199100, and US 2007/0250947.

[Para 13] A number of models have been proposed for the action of RNAi. In mammalian systems, dsRNAs larger than 30 nucleotides trigger induction of interferon synthesis and a global shut-down of protein syntheses, in a non-sequence-specific manner. However, U.S. Patent No. 6,506,559 discloses that in nematodes, the length of the dsRNA corresponding to the target gene sequence may be at least 25, 50, 100, 200, 300, or 400 bases, and that even larger dsRNAs were also effective at inducing RNAi in C. elegans. It is known that when hairpin RNA constructs comprising double stranded regions ranging from 98 to 854 nucleotides were trans-

formed into a number of plant species, the target plant genes were efficiently silenced. There is general agreement that in many organisms, including nematodes and plants, large pieces of dsRNA are cleaved into about 19-24 nucleotide fragments (siRNA) within cells, and that these siRNAs are the actual mediators of the RNAi phenomenon.

5 [Para 14] The various calcium-dependent protein kinases (CDPKs) in plants mediate a variety of responses to the environment. A specific CDPK in Medicago truncatula (CDPK1) was demonstrated to be necessary for the formation of symbiotic interactions between plants and Rhizobia and mycorrhizal fungi (see Ivashuta et al., (2005) Plant Cell 17: 2911-2921). Ivashuta et al. suggest that the CDPK1 is involved in the cell wall expansion and/or synthesis.

10 [Para 15] Although there have been numerous efforts to use RNAi to control plant parasitic nematodes, to date no transgenic nematode-resistant plant has been deregulated in any country. Accordingly, there continues to be a need to identify safe and effective compositions and methods for the controlling plant parasitic nematodes using RNAi, and for the production of plants having increased resistance to plant parasitic nematodes.

15

SUMMARY OF THE INVENTION

[Para 16] The present inventors have discovered that the down-regulation of calcium-dependent protein kinases (CDPKs or CDPL-like genes), exemplified by the G. max cDNA designated as 49806575, confers resistance to plant parasitic nematodes. This down-regulation

20 can be accomplished using RNAi that targets such CDPK-like genes.

[Para 17] In one embodiment, the invention provides a dsRNA molecule comprising (a) a first strand comprising a sequence substantially identical to a portion of a CDPK-like gene and (b) a second strand comprising a sequence substantially complementary to the first strand.

[Para 18] The invention is further embodied in a pool of dsRNA molecules comprising a multi-

25 plicity of RNA molecules each comprising a double stranded region having a length of about 19 to 24 nucleotides, wherein said RNA molecules are derived from a polynucleotide being substantially identical to a portion of a CDPK-like gene.

[Para 19] In another embodiment, the invention provides a transgenic nematode-resistant plant capable of expressing a dsRNA that is substantially identical to a portion of a CDPK-like gene.

30 [Para 20] In another embodiment, the invention provides a transgenic plant capable of expressing a pool of dsRNA molecules, wherein each dsRNA molecule comprises a double stranded region having a length of about 19-24 nucleotides and wherein the RNA molecules are derived from a polynucleotide substantially identical to a portion of a CDPK-like gene.

[Para 21] In another embodiment, the invention provides a method of making a transgenic

35 plant capable of expressing a pool of dsRNA molecules each of which is substantially identical

to a portion of a CDPK-like gene in a plant, said method comprising the steps of: a) preparing a nucleic acid having a region that is substantially identical to a portion of a CDPK-like gene, wherein the nucleic acid is able to form a double-stranded transcript of a portion of a CDPK-like gene once expressed in the plant; b) transforming a recipient plant with said nucleic acid; c)
5 producing one or more transgenic offspring of said recipient plant; and d) selecting the offspring for expression of said transcript.

[Para 22] The invention further provides a method of conferring nematode resistance to a plant, said method comprising the steps of: a) preparing a nucleic acid having a region that is substantially identical to a portion of a CDPK-like gene, wherein the nucleic acid is able to form
10 a double-stranded transcript of a portion of a CDPK-like gene once expressed in the plant; b) transforming a recipient plant with said nucleic acid; c) producing one or more transgenic offspring of said recipient plant; and d) selecting the offspring for nematode resistance.

The invention further provides an expression cassette and an expression vector comprising a sequence substantially identical to a portion of a CDPK-like gene.

15 In another embodiment, the invention provides a method for controlling the infection of a plant by a parasitic nematode, comprising the steps of transforming the plant with a dsRNA molecule operably linked to a root-preferred, nematode inducible or feeding site-preferred promoter, whereby the dsRNA comprising one strand that is substantially identical to a portion of a target nucleic acid essential to the formation, development or support of the feeding site, in particular
20 the formation, development or support of a syncytia or giant cell, thereby controlling the infection of the plant by the nematode by removing or functionally incapacitating the feeding site, syncytia or giant cell, wherein the target nucleic acid is a CDPK-like gene

BRIEF DESCRIPTION OF THE DRAWINGS

25 [Para 23] Figure 1 shows the table of SEQ ID NOs assigned to corresponding sequences. SEQ ID NO: 1 is the partial cDNA sequence from Glycine max Hygene clone 49806575, including the stop codon and 3' untranslated region. SEQ ID NO: 2 is the sense sequence of the fragment of 49806575 (SEQ ID NO: 1) used in the rooted explant assay of Example 2. SEQ ID NO; 3 is the amino acid sequence encoded by 49806575 (SEQ ID NO: 1). SEQ ID NO: 4 is the
30 cDNA sequence of Medicago Genbank accession AY821654, including non-coding and coding sequences. The first base of the coding region corresponds to nucleotide 147, and the last base of the stop codon corresponds to nucleotide 1829. SEQ ID NO: 5 is the synthesized sequence described in Example 1., and SEQ ID NO: 6 is the sequence of the TPP-like promoter (SEQ ID NO:6) described in co-pending USSN 60/874,375 and hereby incorporated by reference

[Para 24] Figures 2a-2c show amino acid alignment of CDPK-like proteins: the partial Glycine cDNA clone 49806575 (SEQ ID NO:3), the protein encoded by AY821654 from Medicago (SEQ ID NO: 7), the protein encoded by AY823957 from Medicago (SEQ ID NO: 9), the protein encoded by AF435451 from Nicotiana (SEQ ID NO: 11), the protein encoded by AY971376 from
5 Nicotiana (SEQ ID NO: 13), the protein encoded by AF030879 from Solanum (SEQ ID NO: 15), the protein encoded by At2g17890 from Arabidopsis (SEQ ID NO: 17), the protein encoded by At4g36070 from Arabidopsis (SEQ ID NO: 19), the protein encoded by At5g66210 from Arabidopsis (SEQ ID NO: 21), the protein encoded by NM_001052286 from Oryza (SEQ ID NO: 23), the protein encoded by NM_001065979 from Oryza (SEQ ID NO: 25) and the amplified product
10 from CDPK 5' RACE PCR, RKF195-3, from Glycine (SEQ ID NO:27). The alignment is performed in Vector NTI software suite (gap opening penalty = 10, gap extension penalty = 0.05, gap separation penalty = 8).

[Para 25] Figure 3 shows the global amino acid percent identity of exemplary CDPK-like genes: the protein encoded by the partial Glycine clone 49806575 (SEQ ID NO:3), the protein
15 encoded by RKF195-3, from Glycine (SEQ ID NO: 27), the protein encoded by AY821654 from Medicago (SEQ ID NO: 7), the protein encoded by AY823957 from Medicago (SEQ ID NO: 9), the protein encoded by AF435451 from Nicotiana (SEQ ID NO:11), the protein encoded by AY971376 from Nicotiana (SEQ ID NO:13), the protein encoded by AF030879 from Solanum (SEQ ID NO: 15), the protein encoded by At2g17890 from Arabidopsis (SEQ ID NO:17), the
20 protein encoded by At4g36070 from Arabidopsis (SEQ ID NO: 19), the protein encoded by At5g66210 from Arabidopsis (SEQ ID NO: 21), the protein encoded by NM_001052286 from Oryza (SEQ ID NO: 23) and the protein encoded by NM_001065979 from Oryza (SEQ ID NO: 25). Only the region overlapping with the partial cDNA clone 49806575 was included in the analysis. Pairwise alignments and percent identities were calculated using Needle of EMBOSS-
25 4.0.0 (Needleman, S. B. and Wunsch, C. D. (1970) J. Mol. Biol. 48, 443-453).

[Para 26] Figure 4 shows the global nucleotide percent identity of exemplary CDPK-like genes: the partial Glycine clone 49806575 (SEQ ID NO: 1), RKF195-3, from Glycine (SEQ ID NO:26), AY821654 from Medicago (SEQ ID NO:4), AY823957 from Medicago (SEQ ID NO: 8), AF435451 from Nicotiana (SEQ ID NO: 10), AY971376 from Nicotiana (SEQ ID NO: 12),
30 AF030879 from Solanum (SEQ ID NO: 14), .At2g17890 from Arabidopsis (SEQ ID NO: 16), At4g36070 from Arabidopsis (SEQ ID NO: 18), At5g66210 from Arabidopsis (SEQ ID NO: 20), NM_001052286 from Oryza (SEQ ID NO: 22), and NM_001065979 from Oryza (SEQ ID NO: 24). Only the region overlapping with the partial cDNA clone 49806575 was included in the analysis. Pairwise alignments and percent identities were calculated using Needle of EMBOSS-
35 4.0.0 (Needleman, S. B. and Wunsch, C. D. (1970) J. Mol. Biol. 48, 443-453).

[Para 27] Figures 5a-5g show various 21mers possible for exemplary CDPK-like genes of SEQ ID NO: 1, 2, 4, 5, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 26 or a polynucleotide sequence encoding a CDPK-like homolog by nucleotide position..

DETAILED DESCRIPTION OF THE INVENTION

5 [Para 28] The present invention may be understood more readily by reference to the following detailed description of the preferred embodiments of the invention and the examples included herein. Unless otherwise noted, the terms used herein are to be understood according to conventional usage by those of ordinary skill in the relevant art. In addition to the definitions of terms provided below, definitions of common terms in molecular biology may also be found in 10 Rieger et al., 1991 Glossary of genetics: classical and molecular, 5th Ed., Berlin: Springer-Verlag; and in Current Protocols in Molecular Biology, F.M. Ausubel et al., Eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., (1998 Supplement). It is to be understood that as used in the specification and in the claims, “a” or “an” can mean one or more, depending upon the context in which it is used. Thus, for exam- 15 ple, reference to “a cell” can mean that at least one cell can be utilized. It is to be understood that the terminology used herein is for the purpose of describing specific embodiments only and is not intended to be limiting.

[Para 29] Throughout this application, various patent and literature publications are referenced. The disclosures of all of these publications and those references cited within those publications 20 in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains.

[Para 30] A plant “CDPK-like gene” is defined herein as a gene having at least 70% sequence identity to the 49806575 polynucleotide having the sequence set forth in SEQ ID NO:1, which is the G. max CDPK-like gene. In accordance with the invention, CDPK-like genes include genes 25 having sequences such as those set forth in SEQ ID NOs:2, 4, 5, 8, 10, 12, 14, 16, 18, 20, 22, 24, and 26, which are homologs of the G. max CDPK-like gene. The CDPK-like genes defined herein encode polypeptides having at least 70% sequence identity to the G. max CDKP-like partial polypeptide having the sequence as set forth in SEQ ID NO:3. Such polypeptides include CDPK-like polypeptides having the sequences as set forth in SEQ ID NOs:7, 9, 11, 13, 15, 17, 30 19, 21, 23, 25 and 27.

[Para 31] Additional CDPK-like genes (CDPK-like gene homologs) may be isolated from plants other than soybean using the information provided herein and techniques known to those of skill in the art of biotechnology. For example, a nucleic acid molecule from a plant that hybridizes under stringent conditions to the nucleic acid of SEQ ID NO:1 can be isolated from plant 35 tissue cDNA libraries. Alternatively, mRNA can be isolated from plant cells (e.g., by the guanid-

inium-thiocyanate extraction procedure of Chirgwin et al., 1979, Biochemistry 18:5294-5299), and cDNA can be prepared using reverse transcriptase (e.g., Moloney MLV reverse transcriptase, available from Gibco/BRL, Bethesda, MD; or AMV reverse transcriptase, available from Seikagaku America, Inc., St. Petersburg, FL). Synthetic oligonucleotide primers for polymerase
5 chain reaction amplification can be designed based upon the nucleotide sequence shown in SEQ ID NO:1. Additional oligonucleotide primers may be designed that are based on the sequences of the CDPK-like genes having the sequences as set forth in SEQ ID NOs: 2, 4, 5, 8, 10, 12, 14, 16, 18, 20, 22, 24, and 26. Nucleic acid molecules corresponding to the CDPK-like target genes defined herein can be amplified using cDNA or, alternatively, genomic DNA, as a
10 template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid molecules so amplified can be cloned into appropriate vectors and characterized by DNA sequence analysis.

[Para 32] As used herein, “RNAi” or “RNA interference” refers to the process of sequence-specific post-transcriptional gene silencing in plants, mediated by double-stranded RNA
15 (dsRNA). As used herein, “dsRNA” refers to RNA that is partially or completely double stranded. Double stranded RNA is also referred to as small or short interfering RNA (siRNA), short interfering nucleic acid (siNA), short interfering RNA, micro-RNA (miRNA), and the like. In the RNAi process, dsRNA comprising a first strand that is substantially identical to a portion of a target gene e.g. a CDPK-like gene and a second strand that is complementary to the first strand is
20 introduced into a plant. After introduction into the plant, the target gene-specific dsRNA is processed into relatively small fragments (siRNAs) and can subsequently become distributed throughout the plant, leading to a loss-of-function mutation having a phenotype that, over the period of a generation, may come to closely resemble the phenotype arising from a complete or partial deletion of the target gene. Alternatively, the target gene-specific dsRNA is operably as-
25 sociated with a regulatory element or promoter that results in expression of the dsRNA in a tissue, temporal, spatial or inducible manner and may further be processed into relatively small fragments by a plant cell containing the RNAi processing machinery, and the loss-of-function phenotype is obtained. Also, the regulatory element or promoter may direct expression preferentially to the roots or syncytia or giant cell where the dsRNA may be expressed either constitu-
30 tively in those tissues or upon induction by the feeding of the nematode or juvenile nematode, such as J2 nematodes.

[Para 33] As used herein, taking into consideration the substitution of uracil for thymine when comparing RNA and DNA sequences, the term “substantially identical” as applied to dsRNA means that the nucleotide sequence of one strand of the dsRNA is at least about 80%-90%
35 identical to 20 or more contiguous nucleotides of the target gene, more preferably, at least

about 90-95% identical to 20 or more contiguous nucleotides of the target gene, and most preferably at least about 95%, 96%, 97%, 98% or 99% identical or absolutely identical to 20 or more contiguous nucleotides of the target gene. 20 or more nucleotides means a portion, being at least about 20, 21, 22, 23, 24, 25, 50, 100, 200, 300, 400, 500, 1000, 1500, consecutive bases or up to the full length of the target gene.

[Para 34] As used herein, “complementary” polynucleotides are those that are capable of base pairing according to the standard Watson-Crick complementarity rules. Specifically, purines will base pair with pyrimidines to form a combination of guanine paired with cytosine (G:C) and adenine paired with either thymine (A:T) in the case of DNA, or adenine paired with uracil (A:U) in the case of RNA. It is understood that two polynucleotides may hybridize to each other even if they are not completely complementary to each other, provided that each has at least one region that is substantially complementary to the other. As used herein, the term “substantially complementary” means that two nucleic acid sequences are complementary over at least at 80% of their nucleotides. Preferably, the two nucleic acid sequences are complementary over at least at 85%, 90%, 95%, 96%, 97%, 98%, 99% or more or all of their nucleotides. Alternatively, “substantially complementary” means that two nucleic acid sequences can hybridize under high stringency conditions. As used herein, the term “substantially identical” or “corresponding to” means that two nucleic acid sequences have at least 80% sequence identity. Preferably, the two nucleic acid sequences have at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% of sequence identity.

[Para 35] Also as used herein, the terms “nucleic acid” and “polynucleotide” refer to RNA or DNA that is linear or branched, single or double stranded, or a hybrid thereof. The term also encompasses RNA/DNA hybrids. When dsRNA is produced synthetically, less common bases, such as inosine, 5-methylcytosine, 6-methyladenine, hypoxanthine and others can also be used for antisense, dsRNA, and ribozyme pairing. For example, polynucleotides that contain C-5 propyne analogues of uridine and cytidine have been shown to bind RNA with high affinity and to be potent antisense inhibitors of gene expression. Other modifications, such as modification to the phosphodiester backbone, or the 2’-hydroxy in the ribose sugar group of the RNA can also be made.

[Para 36] As used herein, the term “control,” when used in the context of an infection, refers to the reduction or prevention of an infection. Reducing or preventing an infection by a nematode will cause a plant to have increased resistance to the nematode, however, such increased resistance does not imply that the plant necessarily has 100% resistance to infection. In preferred embodiments, the resistance to infection by a nematode in a resistant plant is greater than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% in comparison to a wild type plant that is

not resistant to nematodes. Preferably the wild type plant is a plant of a similar, more preferably identical genotype as the plant having increased resistance to the nematode, but does not comprise a dsRNA directed to the target gene. The plant's resistance to infection by the nematode may be due to the death, sterility, arrest in development, or impaired mobility of the nematode upon exposure to the plant comprising dsRNA specific to a gene essential for development or maintenance of a functional feeding site, syncytia, or giant cell. The term "resistant to nematode infection" or "a plant having nematode resistance" as used herein refers to the ability of a plant, as compared to a wild type plant, to avoid infection by nematodes, to kill nematodes or to hamper, reduce or stop the development, growth or multiplication of nematodes. This might be achieved by an active process, e.g. by producing a substance detrimental to the nematode, or by a passive process, like having a reduced nutritional value for the nematode or not developing structures induced by the nematode feeding site like syncytia or giant cells. The level of nematode resistance of a plant can be determined in various ways, e.g. by counting the nematodes being able to establish parasitism on that plant, or measuring development times of nematodes, proportion of male and female nematodes or, for cyst nematodes, counting the number of cysts or nematode eggs produced on roots of an infected plant or plant assay system.

[Para 37] The term "plant" is intended to encompass plants at any stage of maturity or development, as well as any tissues or organs (plant parts) taken or derived from any such plant unless otherwise clearly indicated by context. Plant parts include, but are not limited to, stems, roots, flowers, ovules, stamens, seeds, leaves, embryos, meristematic regions, callus tissue, anther cultures, gametophytes, sporophytes, pollen, microspores, protoplasts, hairy root cultures, rooted explant cultures and the like. The present invention also includes seeds produced by the plants of the present invention. In one embodiment, the seeds are true breeding for an increased resistance to nematode infection as compared to a wild-type variety of the plant seed. As used herein, a "plant cell" includes, but is not limited to, a protoplast, gamete producing cell, and a cell that regenerates into a whole plant. Tissue culture of various tissues of plants and regeneration of plants therefrom is well known in the art and is widely published.

[Para 38] As used herein, the term "transgenic" refers to any plant, plant cell, callus, plant tissue, or plant part that contains all or part of at least one recombinant polynucleotide. In many cases, all or part of the recombinant polynucleotide is stably integrated into a chromosome or stable extra-chromosomal element, so that it is passed on to successive generations. For the purposes of the invention, the term "recombinant polynucleotide" refers to a polynucleotide that has been altered, rearranged, or modified by genetic engineering. Examples include any cloned polynucleotide, or polynucleotides, that are linked or joined to heterologous sequences. The term "recombinant" does not refer to alterations of polynucleotides that result from naturally oc-

curring events, such as spontaneous mutations, or from non-spontaneous mutagenesis followed by selective breeding.

[Para 39] As used herein, the term “amount sufficient to inhibit expression” refers to a concentration or amount of the dsRNA that is sufficient to reduce levels or stability of mRNA or protein produced from a target gene in a plant. As used herein, “inhibiting expression” refers to the absence or observable decrease in the level of protein and/or mRNA product from a target gene. Inhibition of target gene expression may be lethal to the parasitic nematode either directly or indirectly through modification or eradication of the feeding site, syncytia, or giant cell, or such inhibition may delay or prevent entry into a particular developmental step (e.g., metamorphosis), if access to a fully functional feeding site, syncytia, or giant cell is associated with a particular stage of the parasitic nematode’s life cycle. The consequences of inhibition can be confirmed by examination of the plant root for reduction or elimination of cysts or other properties of the nematode or nematode infestation (as presented below in Example 2).

[Para 40] In accordance with the invention, a plant is transformed with a nucleic acid or a dsRNA, which specifically inhibits expression of a target gene (CDPK-like gene) in the plant that is essential for the development or maintenance of a feeding site, syncytia, or giant cell; ultimately affecting the survival, metamorphosis, or reproduction of the nematode. In one embodiment, the dsRNA is encoded by a vector that has been transformed into an ancestor of the infected plant. Preferably, the nucleic acid sequence expressing said dsRNA is under the transcriptional control of a root specific promoter or a parasitic nematode feeding cell-specific promoter or a nematode inducible promoter.

[Para 41] Accordingly, the dsRNA of the invention comprises a first strand is substantially identical to a portion of a CDPK-like gene such as the soybean 49806575 cDNA, and a second strand that is substantially complementary to the first strand. n preferred embodiments, the target gene is selected from the group consisting of: (a) a polynucleotide having the sequence set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 26; (b) a polynucleotide having at least 70% sequence identity to SEQ ID NO: 1, 2, 4, 5, 8, 10, 12, 14, 16, 18, 20, 22, 24 or 26; and (c) a polynucleotide from a plant that hybridizes under stringent conditions to a polynucleotide having the sequence set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10, 12, 14, 16, 18, 20, 22, 24 or 26. The length of the substantially identical double-stranded nucleotide sequences may be at least about 19, 20, 21, 22, 23, 24, 25, 50, 100, 200, 300, 400, 500, 1000, 1500, consecutive bases or up to the whole length of the CDPK-like gene. In a preferred embodiment, the length of the double-stranded nucleotide sequence is from about 19 to about 200-500 consecutive nucleotides in length. In another preferred embodiment, the dsRNA of the invention is substantially identical or is identical to bases 1 to 320 of SEQ ID NO: 2.

[Para 42] As discussed above, fragments of dsRNA larger than about 19-24 nucleotides in length are cleaved intracellularly by nematodes and plants to siRNAs of about 19-24 nucleotides in length, and these siRNAs are the actual mediators of the RNAi phenomenon. The table in Figures 51-5g sets forth exemplary 21-mers of the CDPK-like genes defined herein. This table can also be used to calculate the 19, 20, 22, 23 or 24-mers by adding or subtracting the appropriate number of nucleotides from each 21-mer. Thus the dsRNA of the present invention may range in length from about 19 nucleotides to about 320 nucleotides. Preferably, the dsRNA of the invention has a length from about 21 nucleotides to about 600 nucleotides. More preferably, the dsRNA of the invention has a length from about 21 nucleotides to about 500 nucleotides, or from about 21 nucleotides to about 400 nucleotides.

[Para 43] As disclosed herein, 100% sequence identity between the dsRNA and the target gene is not required to practice the present invention. While a dsRNA comprising a nucleotide sequence identical to a portion of the CDPK-like gene is preferred for inhibition, the invention can tolerate sequence variations that might be expected due to gene manipulation or synthesis, genetic mutation, strain polymorphism, or evolutionary divergence. Thus the dsRNAs of the invention also encompass dsRNAs comprising a mismatch with the target gene of at least 1, 2, or more nucleotides. For example, it is contemplated in the present invention that the 21mer dsRNA sequences exemplified in Figures 5a-5g may contain an addition, deletion or substitution of 1, 2, or more nucleotides, so long as the resulting sequence still interferes with the CDPK-like gene function.

[Para 44] Sequence identity between the dsRNAs of the invention and the CDPK-like target genes may be optimized by sequence comparison and alignment algorithms known in the art (see Gribskov and Devereux, Sequence Analysis Primer, Stockton Press, 1991, and references cited therein) and calculating the percent difference between the nucleotide sequences by, for example, the Smith-Waterman algorithm as implemented in the BESTFIT software program using default parameters (e.g., University of Wisconsin Genetic Computing Group). Greater than 80 % sequence identity, 90% sequence identity, or even 100% sequence identity, between the inhibitory RNA and the portion of the target gene is preferred. Alternatively, the duplex region of the RNA may be defined functionally as a nucleotide sequence that is capable of hybridizing with a portion of the target gene transcript under stringent conditions (e.g., 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 60°C hybridization for 12-16 hours; followed by washing at 65°C with 0.1%SDS and 0.1% SSC for about 15-60 minutes).

[Para 45] When dsRNA of the invention has a length longer than about 21 nucleotides, for example from about 50 nucleotides to about 1000 nucleotides, it will be cleaved randomly to dsRNAs of about 21 nucleotides within the plant or parasitic nematode cell, the siRNAs. The

cleavage of a longer dsRNA of the invention will yield a pool of about 21mer dsRNAs (ranging from about 19mers to about 24mers), derived from the longer dsRNA. This pool of about 21mer dsRNAs is also encompassed within the scope of the present invention, whether generated intracellularly within the plant or nematode or synthetically using known methods of oligonucleotide synthesis.

[Para 46] The siRNAs of the invention have sequences corresponding to fragments of about 19-24 contiguous nucleotides across the entire sequence of the CDPK-like target gene. For example, a pool of siRNA of the invention derived from the CDPK-like gene as set forth in SEQ ID NO:1, 2, 4, 5, 8, 10, 12, 14, 16, 18, 20, 22, 24 or 26 may comprise a multiplicity of RNA molecules which are selected from the group consisting of oligonucleotides substantially identical to the 21mer nucleotides of SEQ ID NO:1, 2, 4, 5, 8, 10, 12, 14, 16, 18, 20, 22, 24 or 26 found in Figures 5a-5g. A pool of siRNA of the invention derived from the CDPK-like target gene of SEQ ID NO:1 may also comprise any combination of the specific RNA molecules having any of the 21 contiguous nucleotide sequences derived from SEQ ID NO:1 set forth in Figures 5a-5g. Further, as noted above, multiple specialized Dicers in plants generate siRNAs typically ranging in size from 19nt to 24nt (See Henderson et al., 2006. Nature Genetics 38:721-725.). The siRNAs of the present invention can range from about 19 contiguous nucleotide sequences to about 24 contiguous nucleotide sequences. Similarly, a pool of siRNA of the invention may comprise a multiplicity of RNA molecules having any of about 19, 20, 21, 22, 23, or 24 contiguous nucleotide sequences derived from SEQ ID NO:1, 2, 4, 5, 8, 10, 12, 14, 16, 18, 20, 22, 24 or 26. Alternatively, the pool of siRNA of the invention may comprise a multiplicity of RNA molecules having a combination of any of about 19, 20, 21, 22, 23, and/or 24 contiguous nucleotide sequences derived from SEQ ID NO: 1.

[Para 47] The dsRNA of the invention may optionally comprise a single stranded overhang at either or both ends. The double-stranded structure may be formed by a single self-complementary RNA strand (i.e. forming a hairpin loop) or two complementary RNA strands. RNA duplex formation may be initiated either inside or outside the cell. When the dsRNA of the invention forms a hairpin loop, it may optionally comprise an intron, as set forth in US 2003/0180945A1 or a nucleotide spacer, which is a stretch of sequence between the complementary RNA strands to stabilize the hairpin transgene in cells. Methods for making various dsRNA molecules are set forth, for example, in WO 99/53050 and in U.S. Pat. No. 6,506,559. The RNA may be introduced in an amount that allows delivery of at least one copy per cell. Higher doses of double-stranded material may yield more effective inhibition.

[Para 48] In another embodiment, the invention provides an isolated recombinant expression vector comprising a nucleic acid encoding a dsRNA molecule as described above, wherein ex-

pression of the vector in a host plant cell results in increased resistance to a parasitic nematode as compared to a wild-type variety of the host plant cell. As used herein, the term “vector” refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a “plasmid,” which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host plant cell into which they are introduced. Other vectors are integrated into the genome of a host plant cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as “expression vectors.” In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, “plasmid” and “vector” can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., potato virus X, tobacco rattle virus, and Geminivirus), which serve equivalent functions.

[Para 49] The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host plant cell, which means that the recombinant expression vector includes one or more regulatory sequences, e.g. promoters, selected on the basis of the host plant cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. With respect to a recombinant expression vector, the terms “operatively linked” and “in operative association” are interchangeable and are intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in a host plant cell when the vector is introduced into the host plant cell). The term “regulatory sequence” is intended to include promoters, enhancers, and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990) and Gruber and Crosby, in: Methods in Plant Molecular Biology and Biotechnology, Eds. Glick and Thompson, Chapter 7, 89-108, CRC Press: Boca Raton, Florida, including the references therein. Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cells and those that direct expression of the nucleotide sequence only in certain host cells or under certain conditions. It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of dsRNA desired, etc.

The expression vectors of the invention can be introduced into plant host cells to thereby produce dsRNA molecules of the invention encoded by nucleic acids as described herein.

[Para 50] In accordance with the invention, the recombinant expression vector comprises a regulatory sequence operatively linked to a nucleotide sequence that is a template for one or
5 both strands of the dsRNA molecules of the invention. In one embodiment, the nucleic acid molecule further comprises a promoter flanking either end of the nucleic acid molecule, wherein the promoters drive expression of each individual DNA strand, thereby generating two complementary RNAs that hybridize and form the dsRNA. In another embodiment, the nucleic acid molecule comprises a nucleotide sequence that is transcribed into both strands of the dsRNA
10 on one transcription unit, wherein the sense strand is transcribed from the 5' end of the transcription unit and the antisense strand is transcribed from the 3' end, wherein the two strands are separated by about 3 to about 500 base pairs or more, and wherein after transcription, the RNA transcript folds on itself to form a hairpin. In accordance with the invention, the spacer region in the hairpin transcript may be any DNA fragment.

15 [Para 51] According to the present invention, the introduced polynucleotide may be maintained in the plant cell stably if it is incorporated into a non-chromosomal autonomous replicon or integrated into the plant chromosomes. Alternatively, the introduced polynucleotide may be present on an extra-chromosomal non-replicating vector and be transiently expressed or transiently active. Whether present in an extra-chromosomal non-replicating vector or a vector that is inte-
20 grated into a chromosome, the polynucleotide preferably resides in a plant expression cassette. A plant expression cassette preferably contains regulatory sequences capable of driving gene expression in plant cells that are operatively linked so that each sequence can fulfill its function, for example, termination of transcription by polyadenylation signals. Preferred polyadenylation signals are those originating from Agrobacterium tumefaciens t-DNA such as the gene 3 known
25 as octopine synthase of the Ti-plasmid pTiACH5 (Gielen et al., 1984, EMBO J. 3:835) or functional equivalents thereof, but also all other terminators functionally active in plants are suitable. As plant gene expression is very often not limited on transcriptional levels, a plant expression cassette preferably contains other operatively linked sequences like translational enhancers such as the overdrive-sequence containing the 5'-untranslated leader sequence from tobacco
30 mosaic virus enhancing the polypeptide per RNA ratio (Gallie et al., 1987, Nucl. Acids Research 15:8693-8711). Examples of plant expression vectors include those detailed in: Becker, D. et al., 1992, New plant binary vectors with selectable markers located proximal to the left border, Plant Mol. Biol. 20:1195-1197; Bevan, M.W., 1984, Binary Agrobacterium vectors for plant transformation, Nucl. Acid. Res. 12:8711-8721; and Vectors for Gene Transfer in Higher Plants;

in: Transgenic Plants, Vol. 1, Engineering and Utilization, eds.: Kung and R. Wu, Academic Press, 1993, S. 15-38.

[Para 52] Plant gene expression should be operatively linked to an appropriate promoter conferring gene expression in a temporal-preferred, spatial-preferred, cell type-preferred, and/or tissue-preferred manner. Promoters useful in the expression cassettes of the invention include any promoter that is capable of initiating transcription in a plant cell present in the plant's roots. Such promoters include, but are not limited to those that can be obtained from plants, plant viruses and bacteria that contain genes that are expressed in plants, such as Agrobacterium and Rhizobium. Preferably, the expression cassette of the invention comprises a root-specific promoter, a pathogen inducible promoter, or a nematode inducible promoter. More preferably the nematode inducible promoter is a parasitic nematode feeding cell-specific promoter. A parasitic nematode feeding site-specific promoter may be specific for syncytial cells or giant cells or specific for both kinds of cells. A promoter is inducible, if its activity, measured on the amount of RNA produced under control of the promoter, is at least 30%, 40%, 50% preferably at least 60%, 70%, 80%, 90% more preferred at least 100%, 200%, 300% higher in its induced state, than in its un-induced state. A promoter is cell-, tissue- or organ-specific, if its activity , measured on the amount of RNA produced under control of the promoter, is at least 30%, 40%, 50% preferably at least 60%, 70%, 80%, 90% more preferred at least 100%, 200%, 300% higher in a particular cell-type, tissue or organ, then in other cell-types or tissues of the same plant, preferably the other cell-types or tissues are cell types or tissues of the same plant organ, e.g. a root. In the case of organ specific promoters, the promoter activity has to be compared to the promoter activity in other plant organs, e.g. leafs, stems, flowers or seeds.

[Para 53] The promoter may be constitutive, inducible, developmental stage-preferred, cell type-preferred, tissue-preferred or organ-preferred. Constitutive promoters are active under most conditions. Non-limiting examples of constitutive promoters include the CaMV 19S and 35S promoters (Odell et al., 1985, Nature 313:810-812), the sX CaMV 35S promoter (Kay et al., 1987, Science 236:1299-1302), the Sep1 promoter, the rice actin promoter (McElroy et al., 1990, Plant Cell 2:163-171), the Arabidopsis actin promoter, the ubiquitin promoter (Christensen et al., 1989, Plant Molec. Biol. 18:675-689); pEmu (Last et al., 1991, Theor. Appl. Genet. 81:581-588), the figwort mosaic virus 35S promoter, the Smas promoter (Velten et al., 1984, EMBO J. 3:2723-2730), the GRP1-8 promoter, the cinnamyl alcohol dehydrogenase promoter (U.S. Patent No. 5,683,439), promoters from the T-DNA of Agrobacterium, such as mannopine synthase, nopaline synthase, and octopine synthase, the small subunit of ribulose biphosphate carboxylase (ssuRUBISCO) promoter, and the like. Promoters that express the dsRNA in a cell that is contacted by parasitic nematodes are preferred. Alternatively, the promoter may drive

expression of the dsRNA in a plant tissue remote from the site of contact with the nematode, and the dsRNA may then be transported by the plant to a cell that is contacted by the parasitic nematode, in particular cells of, or close by nematode feeding sites, e.g. syncytial cells or giant cells.

5 [Para 54] Inducible promoters are active under certain environmental conditions, such as the presence or absence of a nutrient or metabolite, heat or cold, light, pathogen attack, anaerobic conditions, and the like. For example, the promoters TobRB7, AtRPE, AtPyk10, Gemini19, and AtHMG1 have been shown to be induced by nematodes (for a review of nematode-inducible promoters, see Ann. Rev. Phytopathol. (2002) 40:191-219; see also U.S. Pat. No. 6,593,513).
10 Method for isolating additional promoters, which are inducible by nematodes are set forth in U.S. Pat. Nos. 5,589,622 and 5,824,876. Other inducible promoters include the hsp80 promoter from Brassica, being inducible by heat shock; the PPDK promoter is induced by light; the PR-1 promoter from tobacco, Arabidopsis, and maize are inducible by infection with a pathogen; and the Adh1 promoter is induced by hypoxia and cold stress. Plant gene expression can also be
15 facilitated via an inducible promoter (For review, see Gatz, 1997, Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:89-108). Chemically inducible promoters are especially suitable if time-specific gene expression is desired. Non-limiting examples of such promoters are a salicylic acid inducible promoter (PCT Application No. WO 95/19443), a tetracycline inducible promoter (Gatz et al., 1992, Plant J. 2:397-404) and an ethanol inducible promoter (PCT Application No.
20 WO 93/21334).

[Para 55] Developmental stage-preferred promoters are preferentially expressed at certain stages of development. Tissue and organ preferred promoters include, but are not limited to, those that are preferentially expressed in certain tissues or organs, such as leaves, roots, seeds, or xylem. Examples of tissue preferred and organ preferred promoters include, but are
25 not limited to fruit-preferred, ovule-preferred, male tissue-preferred, seed-preferred, integument-preferred, tuber-preferred, stalk-preferred, pericarp-preferred, and leaf-preferred, stigma-preferred, pollen-preferred, anther-preferred, a petal-preferred, sepal-preferred, pedicel-preferred, silique-preferred, stem-preferred, root-preferred promoters and the like. Seed preferred promoters are preferentially expressed during seed development and/or germination. For
30 example, seed preferred promoters can be embryo-preferred, endosperm preferred and seed coat-preferred. See Thompson et al., 1989, BioEssays 10:108. Examples of seed preferred promoters include, but are not limited to cellulose synthase (celA), Cim1, gamma-zein, globulin-1, maize 19 kD zein (cZ19B1) and the like.

[Para 56] Other suitable tissue-preferred or organ-preferred promoters include the napin-gene
35 promoter from rapeseed (U.S. Patent No. 5,608,152), the USP-promoter from Vicia faba (Bae-

umlein et al., 1991, Mol Gen Genet. 225(3):459-67), the oleosin-promoter from Arabidopsis (PCT Application No. WO 98/45461), the phaseolin-promoter from Phaseolus vulgaris (U.S. Patent No. 5,504,200), the Bce4-promoter from Brassica (PCT Application No. WO 91/13980), or the legumin B4 promoter (LeB4; Baeumlein et al., 1992, Plant Journal, 2(2):233-9), as well as promoters conferring seed specific expression in monocot plants like maize, barley, wheat, rye, rice, etc. Suitable promoters to note are the lpt2 or lpt1-gene promoter from barley (PCT Application No. WO 95/15389 and PCT Application No. WO 95/23230) or those described in PCT Application No. WO 99/16890 (promoters from the barley hordein-gene, rice glutelin gene, rice oryzin gene, rice prolamin gene, wheat gliadin gene, wheat glutelin gene, oat glutelin gene, Sorghum kasirin-gene, and rye secalin gene).

[Para 57] Other promoters useful in the expression cassettes of the invention include, but are not limited to, the major chlorophyll a/b binding protein promoter, histone promoters, the Ap3 promoter, the β-conglycin promoter, the napin promoter, the soybean lectin promoter, the maize 15kD zein promoter, the 22kD zein promoter, the 27kD zein promoter, the g-zein promoter, the waxy, shrunken 1, shrunken 2, and bronze promoters, the Zm13 promoter (U.S. Patent No. 5,086,169), the maize polygalacturonase promoters (PG) (U.S. Patent Nos. 5,412,085 and 5,545,546), and the SGB6 promoter (U.S. Patent No. 5,470,359), as well as synthetic or other natural promoters.

[Para 58] In accordance with the present invention, the expression cassette comprises an expression control sequence operatively linked to a nucleotide sequence that is a template for one or both strands of the dsRNA. The dsRNA template comprises (a) a first stand having a sequence substantially identical to from about 19 to about 500, or up to the full length, consecutive nucleotides of SEQ ID NO: 1, 2, 4, 5, 8, 10, 12, 14, 16, 18, 20, 22, 24 or 26; and (b) a second strand having a sequence substantially complementary to the first strand. In further embodiments, a promoter flanks either end of the template nucleotide sequence, wherein the promoters drive expression of each individual DNA strand, thereby generating two complementary RNAs that hybridize and form the dsRNA. In alternative embodiments, the nucleotide sequence is transcribed into both strands of the dsRNA on one transcription unit, wherein the sense strand is transcribed from the 5' end of the transcription unit and the anti-sense strand is transcribed from the 3' end, wherein the two strands are separated by about 3 to about 500 base pairs, and wherein after transcription, the RNA transcript folds on itself to form a hairpin.

[Para 59] In another embodiment, the vector contains a bidirectional promoter, driving expression of two nucleic acid molecules, whereby one nucleic acid molecule codes for the sequence substantially identical to a portion of a CDPK-like gene and the other nucleic acid molecule codes for a second sequence being substantially complementary to the first strand and

capable of forming a dsRNA, when both sequences are transcribed.. A bidirectional promoter is a promoter capable of mediating expression in two directions.

[Para 60] In another embodiment, the vector contains two promoters one mediating transcription of the sequence substantially identical to a portion of a CDPK-like gene and another promoter mediating transcription of a second sequence being substantially complementary to the first strand and capable of forming a dsRNA, when both sequences are transcribed. The second promoter might be a different promoter. A different promoter means a promoter having a different activity in regard to cell or tissue specificity, or showing expression on different inducers for example, pathogens, abiotic stress or chemicals. For example, one promoter might by constitutive or tissue specific and another might be tissue specific or inducible by pathogens. In one embodiment one promoter mediates the transcription of one nucleic acid molecule suitable for over-expression of a CDPK-like gene, while another promoter mediates tissue- or cell-specific transcription or pathogen inducible expression of the complementary nucleic acid

[Para 61] The invention is also embodied in a transgenic plant capable of expressing the dsRNA of the invention and thereby inhibiting the CDPK-like genes in the roots, feeding site, syncytia and/or giant cell. The plant or transgenic plant may be any plant, such like, but not limited to trees, cut flowers, ornamentals, vegetables or crop plants. The plant may be from a genus selected from the group consisting of Medicago, Lycopersicon, Brassica, Cucumis, Solanum, Juglans, Gossypium, Malus, Vitis, Antirrhinum, Populus, Fragaria, Arabidopsis, Picea, Capsicum, Chenopodium, Dendranthema, Pharbitis, Pinus, Pisum, Oryza, Zea, Triticum, Triticale, Secale, Lolium, Hordeum, Glycine, Pseudotsuga, Kalanchoe, Beta, Helianthus, Nicotiana, Cucurbita, Rosa, Fragaria, Lotus, Medicago, Onobrychis, trifolium, Trigonella, Vigna, Citrus, Linum, Geranium, Manihot, Daucus, Raphanus, Sinapis, Atropa, Datura, Hyoscyamus, Nicotiana, Petunia, Digitalis, Majorana, Ciahorium, Lactuca, Bromus, Asparagus, Antirrhinum, Heterocallis, Nemesis, Pelargonium, Panieum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Browaalia, Phaseolus, Avena, and Allium, or the plant may be selected from a genus selected from the group consisting of Arabidopsis, Medicago, Lycopersicon, Brassica, Cucumis, Solanum, Juglans, Gossypium, Malus, Vitis, Antirrhinum, Brachipodium, Populus, Fragaria, Arabidopsis, Picea, Capsicum, Chenopodium, Dendranthema, Pharbitis, Pinus, Pisum, Oryza, Zea, Triticum, Triticale, Secale, Lolium, Hordeum, Glycine, Pseudotsuga, Kalanchoe, Beta, Helianthus, Nicotiana, Cucurbita, Rosa, Fragaria, Lotus, Medicago, Onobrychis, trifolium, Trigonella, Vigna, Citrus, Linum, Geranium, Manihot, Daucus, Raphanus, Sinapis, Atropa, Datura, Hyoscyamus, Nicotiana, Petunia, Digitalis, Majorana, Ciahorium, Lactuca, Bromus, Asparagus, Antirrhinum, Heterocallis, Nemesis, Pelargonium, Panicum, Pennisetum, Ranunculus, Senecio,

Salpiglossis, Browaalia, Phaseolus, Avena, and Allium. In one embodiment the plant is a monocotyledonous plant or a dicotyledonous plant.

[Para 62] Preferably the plant is a crop plant. Crop plants are all plants, used in agriculture. Accordingly in one embodiment the plant is a monocotyledonous plant, preferably a plant of the family Poaceae, Musaceae, Liliaceae or Bromeliaceae, preferably of the family Poaceae. Accordingly, in yet another embodiment the plant is a Poaceae plant of the genus Zea, Triticum, Oryza, Hordeum, Secale, Avena, Saccharum, Sorghum, Pennisetum, Setaria, Panicum, Eleusine, Miscanthus, Brachypodium, Festuca or Lolium. When the plant is of the genus Zea, the preferred species is Z. mays. When the plant is of the genus Triticum, the preferred species is T. aestivum, T. speltae or T. durum. When the plant is of the genus Oryza, the preferred species is O. sativa. When the plant is of the genus Hordeum, the preferred species is H. vulgare. When the plant is of the genus Secale, the preferred species S. cereale. When the plant is of the genus Avena, the preferred species is A. sativa. When the plant is of the genus Saccarum, the preferred species is S. officinarum. When the plant is of the genus Sorghum, the preferred species is S. vulgare, S. bicolor or S. sudanense. When the plant is of the genus Pennisetum, the preferred species is P. glaucum. When the plant is of the genus Setaria, the preferred species is S. italica. When the plant is of the genus Panicum, the preferred species is P. miliaceum or P. virgatum. When the plant is of the genus Eleusine, the preferred species is E. coracana. When the plant is of the genus Miscanthus, the preferred species is M. sinensis. When the plant is a plant of the genus Festuca, the preferred species is F. arundinaria, F. rubra or F. pratensis. When the plant is of the genus Lolium, the preferred species is L. perenne or L. multiflorum. Alternatively, the plant may be Triticosecale.

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

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

[Para 63] In one embodiment the plant is a Fabaceae plant and the target gene is substan-
25 tially similar to SEQ ID NO: 1, 2, 4, 5, 8 or 26. In a further embodiment the plant is a Brassicaceae plant and the target gene is substantially identical to SEQ ID NO: 16,18 or 20. In an alternative embodiment the plant is a Solanaceae plant and the target gene is substantially identical to SEQ ID NO: 10, 12 or 14. In a further embodiment the plant is a Poaceae plant and the target gene is substantially identical to SEQ ID NO: 22 or 24.

30 [Para 64] Suitable methods for transforming or transfecting host cells including plant cells are well known in the art of plant biotechnology. Any method may be used to transform the recombinant expression vector into plant cells to yield the transgenic plants of the invention. General methods for transforming dicotyledenous plants are disclosed, for example, in U.S. Pat. Nos. 4,940,838; 5,464,763, and the like. Methods for transforming specific dicotyledenous
35 plants, for example, cotton, are set forth in U.S. Pat. Nos. 5,004,863; 5,159,135; and 5,846,797.

Soybean transformation methods are set forth in U.S. Pat. Nos. 4,992,375; 5,416,011; 5,569,834; 5,824,877; 6,384,301 and in EP 0301749B1 may be used. Transformation methods may include direct and indirect methods of transformation. Suitable direct methods include polyethylene glycol induced DNA uptake, liposome-mediated transformation (US 4,536,475),
5 biolistic methods using the gene gun (Fromm ME et al., Bio/Technology. 8(9):833-9, 1990; Gordon-Kamm et al. Plant Cell 2:603, 1990), electroporation, incubation of dry embryos in DNA-comprising solution, and microinjection. In the case of these direct transformation methods, the plasmids used need not meet any particular requirements. Simple plasmids, such as those of the pUC series, pBR322, M13mp series, pACYC184 and the like can be used. If intact plants
10 are to be regenerated from the transformed cells, an additional selectable marker gene is preferably located on the plasmid. The direct transformation techniques are equally suitable for dicotyledonous and monocotyledonous plants.

[Para 65] Transformation can also be carried out by bacterial infection by means of Agrobacterium (for example EP 0 116 718), viral infection by means of viral vectors (EP 0 067 553;
15 US 4,407,956; WO 95/34668; WO 93/03161) or by means of pollen (EP 0 270 356; WO 85/01856; US 4,684,611). Agrobacterium based transformation techniques (especially for dicotyledonous plants) are well known in the art. The Agrobacterium strain (e.g., Agrobacterium tumefaciens or Agrobacterium rhizogenes) comprises a plasmid (Ti or Ri plasmid) and a T-DNA element which is transferred to the plant following infection with Agrobacterium. The T-DNA
20 (transferred DNA) is integrated into the genome of the plant cell. The T-DNA may be localized on the Ri- or Ti-plasmid or is separately comprised in a so-called binary vector. Methods for the Agrobacterium-mediated transformation are described, for example, in Horsch RB et al. (1985) Science 225:1229. The Agrobacterium-mediated transformation is best suited to dicotyledonous plants but has also been adapted to monocotyledonous plants. The transformation of plants by
25 Agrobacteria is described in, for example, White FF, Vectors for Gene Transfer in Higher Plants, Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press, 1993, pp. 15 - 38; Jenes B et al. Techniques for Gene Transfer, Transgenic Plants, Vol. 1, Engineering and Utilization, edited by S.D. Kung and R. Wu, Academic Press, 1993, pp. 128-143; Potrykus (1991) Annu Rev Plant Physiol Plant Molec Biol 42:205- 225.
30 Transformation may result in transient or stable transformation and expression. Although a nucleotide sequence of the present invention can be inserted into any plant and plant cell falling within these broad classes, it is particularly useful in crop plant cells.

[Para 66] The transgenic plants of the invention may be crossed with similar transgenic plants or with transgenic plants lacking the nucleic acids of the invention or with non-transgenic
35 plants, using known methods of plant breeding, to prepare seeds. Further, the transgenic plant

of the present invention may comprise, and/or be crossed to another transgenic plant that comprises one or more nucleic acids, thus creating a "stack" of transgenes in the plant and/or its progeny. The seed is then planted to obtain a crossed fertile transgenic plant comprising the nucleic acid of the invention. The crossed fertile transgenic plant may have the particular expression cassette inherited through a female parent or through a male parent. The second plant may be an inbred plant. The crossed fertile transgenic may be a hybrid. Also included within the present invention are seeds of any of these crossed fertile transgenic plants. The seeds of this invention can be harvested from fertile transgenic plants and be used to grow progeny generations of transformed plants of this invention including hybrid plant lines comprising the DNA construct.

[Para 67] "Gene stacking" can also be accomplished by transferring two or more genes into the cell nucleus by plant transformation. Multiple genes may be introduced into the cell nucleus during transformation either sequentially or in unison. Multiple genes in plants or target pathogen species can be down-regulated by gene silencing mechanisms, specifically RNAi, by using a single transgene targeting multiple linked partial sequences of interest. Stacked, multiple genes under the control of individual promoters can also be over-expressed to attain a desired single or multiple phenotype. Constructs containing gene stacks of both over-expressed genes and silenced targets can also be introduced into plants yielding single or multiple agronomically important phenotypes. In certain embodiments the nucleic acid sequences of the present invention can be stacked with any combination of polynucleotide sequences of interest to create desired phenotypes. The combinations can produce plants with a variety of trait combinations including but not limited to disease resistance, herbicide tolerance, yield enhancement, cold and drought tolerance. These stacked combinations can be created by any method including but not limited to cross breeding plants by conventional methods or by genetic transformation. If the traits are stacked by genetic transformation, the polynucleotide sequences of interest can be combined sequentially or simultaneously in any order. For example if two genes are to be introduced, the two sequences can be contained in separate transformation cassettes or on the same transformation cassette. The expression of the sequences can be driven by the same or different promoters.

[Para 68] In accordance with this embodiment, the transgenic plant of the invention is produced by a method comprising the steps of providing a CDPK-like target gene, preparing an expression cassette having a first region that is substantially identical to a portion of the selected CDPK-like gene and a second region which is complementary to the first region, transforming the expression cassette into a plant, and selecting progeny of the transformed plant which express the dsRNA construct of the invention.

[Para 69] Increased resistance to nematode infection is a general trait wished to be inherited into a wide variety of plants. Increased resistance to nematode infection is a general trait wished to be inherited into a wide variety of plants. The present invention may be used to reduce crop destruction by any plant parasitic nematode. Preferably, the parasitic nematodes belong to nematode families inducing giant or syncytial cells. Nematodes inducing giant or syncytial cells are found in the families Longidoridae, Trichodoridae, Heterodidae, Meloidogynidae, Pratylenchidae or Tylenchulidae. In particular in the families Heterodidae and Meloidogynidae.

[Para 70] Accordingly, parasitic nematodes targeted by the present invention belong to one or more genus selected from the group of Naccobus, Cactodera, Dolichodera, Globodera, Heterodera, Punctodera, Longidorus or Meloidogyne. In a preferred embodiment the parasitic nematodes belong to one or more genus selected from the group of Naccobus, Cactodera, Dolichodera, Globodera, Heterodera, Punctodera or Meloidogyne. In a more preferred embodiment the parasitic nematodes belong to one or more genus selected from the group of Globodera, Heterodera, or Meloidogyne. In an even more preferred embodiment the parasitic nematodes belong to one or both genus selected from the group of Globodera or Heterodera. In another embodiment the parasitic nematodes belong to the genus Meloidogyne.

[Para 71] When the parasitic nematodes are of the genus Globodera, the species are preferably from the group consisting of G. achilleae, G. artemisiae, G. hypolysi, G. mexicana, G. millefolii, G. mali, G. pallida, G. rostochiensis, G. tabacum, and G. virginiae. In another preferred embodiment the parasitic Globodera nematodes includes at least one of the species G. pallida, G. tabacum, or G. rostochiensis. When the parasitic nematodes are of the genus Heterodera, the species may be preferably from the group consisting of H. avenae, H. carotae, H. ciceri, H. cruciferae, H. delvii, H. elachista, H. filipjevi, H. gambiensis, H. glycines, H. goettingiana, H. graduni, H. humuli, H. hordecalis, H. latipons, H. major, H. medicaginis, H. oryzicola, H. pakistanensis, H. rosii, H. sacchari, H. schachtii, H. sorghi, H. trifolii, H. urticae, H. vigni and H. zeae. In another preferred embodiment the parasitic Heterodera nematodes include at least one of the species H. glycines, H. avenae, H. cajani, H. gottingiana, H. trifolii, H. zeae or H. schachtii. In a more preferred embodiment the parasitic nematodes includes at least one of the species H. glycines or H. schachtii. In a most preferred embodiment the parasitic nematode is the species H. glycines.

[Para 72] When the parasitic nematodes are of the genus Meloidogyne, the parasitic nematode may be selected from the group consisting of M. acronea, M. arabica, M. arenaria, M. artiellia, M. brevicauda, M. camelliae, M. chitwoodi, M. cofeicola, M. esigua, M. graminicola, M. hapla, M. incognita, M. indica, M. inornata, M. javanica, M. lini, M. mali, M. microcephala, M. microtyla, M. naasi, M. salasi and M. thamesi. In a preferred embodiment the parasitic nema-

todes includes at least one of the species M. javanica, M. incognita, M. hapla, M. arenaria or M. chitwoodi.

[Para 73] The following examples are not intended to limit the scope of the claims to the invention, but are rather intended to be exemplary of certain embodiments. Any variations in the exemplified methods that occur to the skilled artisan are intended to fall within the scope of the present invention.

EXAMPLE 1: Binary Vector Construction for Soybean Transformation

[Para 74] This exemplified method employs a binary vector containing the target gene corresponding to soybean cDNA clone 49806575. Clone 49806575 was identified by searching a proprietary database of cDNA sequences using the public Medicago truncatula sequence AY821654. The expression vector consists of the synthesized fragment (SEQ ID NO :5), which in turn is comprised of a 320 bp antisense portion of 49806575 gene, a spacer, a sense fragment of the target gene (SEQ ID NO:2) corresponding to nucleotides 50-372 of SEQ ID NO:1, and a vector backbone. In this vector, RCB562, dsRNA for the 49806575 target gene was expressed under a syncytia or root preferred promoter, TPP-like promoter (SEQ ID NO: 6, see co-pending application U.S. patent application 60/874,375, hereby incorporated by reference). The TPP-like promoter drives transgene expression preferentially in roots and/or syncytia or giant cells. The selection marker for transformation in this vector was a mutated AHAS gene from Arabidopsis thaliana that conferred resistance to the herbicide Arsenal (Imazapyr, BASF Corporation, Mount Olive, NJ). The expression of mutated AHAS was driven by the parsley ubiquitin promoter (See Plesch, G. and Ebneth, M., “Method for the stable expression of nucleic acids in transgenic plants, controlled by a parsley ubiquitin promoter”, WO 03/102198, hereby incorporated by reference.).

EXAMPLE 2: Use of Soybean Plant Assay System to Detect Resistance to SCN Infection

[Para 75] The rooted explant assay was employed to demonstrate dsRNA expression and resulting nematode resistance. This assay can be found in co-pending application USSN 12/001,234, the contents of which are hereby incorporated by reference.

[Para 76] Clean soybean seeds from soybean cultivar were surface sterilized and germinated. Three days before inoculation, an overnight liquid culture of the disarmed Agrobacterium culture, for example, the disarmed A. rhizogenes strain K599 containing the binary vector RCB562, was initiated. The next day the culture was spread onto an LB agar plate containing kanamycin as a selection agent. The plates were incubated at 28°C for two days. One plate was prepared for every 50 explants to be inoculated. Cotyledons containing the proximal end from its connection with the seedlings were used as the explant for transformation. After removing the cotyledons the surface was scraped with a scalpel around the cut site. The cut and scraped cotyledon was the target for Agrobacterium inoculation. The prepared explants were dipped onto the disarmed thick A. rhizogenes colonies prepared above so that the colonies were visible on the cut and scraped surface. The explants were then placed onto 1% agar in Petri dishes for co-cultivation under light for 6-8 days.

[Para 77] After the transformation and co-cultivation soybean explants were transferred to rooting induction medium with a selection agent, for example S-B5-708 for the mutated acetohydroxy acid synthase (AHAS) gene (Sathasivan et al., Plant Phys. 97:1044-50, 1991). Cultures were maintained in the same condition as in the co-cultivation step. The S-B5-708 medium comprises: 0.5X B5 salts, 3mM MES, 2% sucrose, 1X B5 vitamins, 400µg/ml Timentin, 0.8% Noble agar, and 1 µM Imazapyr (selection agent for AHAS gene) (BASF Corporation, Florham Park, NJ) at pH5.8.

Two to three weeks after the selection and root induction, transformed roots were formed on the cut ends of the explants. Explants were transferred to the same selection medium (S-B5-708 medium) for further selection. Transgenic roots proliferated well within one week in the medium and were ready to be subcultured.

[Para 78] Strong and white soybean roots were excised from the rooted explants and cultured in root growth medium supplemented with 200 mg/l Timentin (S-MS-606 medium) in six-well plates. Cultures were maintained at room temperature under the dark condition. The S-MS-606 medium comprises: 0.2X MS salts and B5 vitamins, 2% sucrose, and 200mg/l Timentin at pH5.8.

One to five days after sub-culturing, the roots were inoculated with surface sterilized nematode juveniles in multi-well plates for either gene of interest or promoter construct assay. As a control, soybean cultivar Williams 82 control vector and Jack control vector roots were used. The root cultures of each line that occupied at least half of the well were inoculated with surface-decontaminated race 3 of soybean cyst nematode (SCN) second stage juveniles (J2) at the level of 500 J2/well. The plates were then sealed and put back into the incubator at 25ºC in darkness. Several independent root lines were generated from each binary vector transformation and the lines were used for bioassay. Four weeks after nematode inoculation, the cysts in each well were counted.

[Para 79] For each transformed line, the average number of cysts per line, the percent female index and the standard error values were determined across several replicated wells (Female index = average number of SCN cysts developing on the transgenic roots expressed as percentage of the average number of cysts developing on the W82 wild type susceptible control roots). Multiple independent, biologically replicated experiments were run to compare cyst numbers between RCB562 transformants and susceptible Williams82 lines. The results show that RCB562 transformed roots had statistically significant reductions (p-value ≤ 0.05) in cyst count over multiple transgenic lines and a general trend of reduced cyst count in the majority of transgenic lines assayed.

Example 3 RACE To Determine Full Transcribed Sequence

[Para 80] A full length transcript sequence with high homology to the partial cDNA clone 49806575 (SEQ ID NO: 1) was isolated using the GeneRacer Kit (L1502-01) from Invitrogen by following the manufacturers instructions. Total RNA from soybean roots harvested 6 days after
5 infection with SCN was prepared according to the Invitrogen GeneRacer Kit protocol to generate dephosphorylated and decapped RNA ligated to the GeneRacer RNA Oligo described by SEQ ID NO:28. The prepared RNA was reverse transcribed according to the GeneRacer Kit protocol and used as the RACE library template for PCR to isolate 5' cDNA ends using primary and secondary (nested) PCR reactions according to the GeneRacer Kit protocol. The primers
10 used for the primary PCR reaction are described by SEQ ID NOs 29 and 31. The secondary nested PCR reaction primers are described by SEQ ID NOs 30 and 32.

[Para 81] Products from secondary PCR reaction were separated by gel electrophoresis. Specific products were purified from agarose gel and cloned into pCR4-TOPO vectors (Invitrogen) following manufacturers instructions, Resulting colonies were miniprepped and sequenced. One
15 of the full length fragments described as SEQ ID NO:26 (RKF195-3_2) had high percent identity with SEQ ID NO:1 (49806575 cDNA sequence). The alignment between proteins encoded by the partial Glycine max 49806575 sequence, the full length Glycine max RKF195-3_2 and CDPK-like genes from other plant species is shown in Figures 2a-2d.

[Para 82] Those skilled in the art will recognize, or will be able to ascertain using no more than
20 routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

1. A dsRNA molecule comprising i) a first strand comprising a sequence substantially identical to a portion of a CDPK-like gene, and ii) a second strand comprising a sequence substantially complementary to the first strand, wherein the portion of the CDPK-like gene is from a polynucleotide selected from the group consisting of:
   a) a polynucleotide comprising a sequence as set forth in SEQ ID NO:1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26;
   b) a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO:3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27;
   c) a polynucleotide having 70% sequence identity to a polynucleotide having a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26;
   d) a polynucleotide encoding a polypeptide having 70% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27;
   e) a polynucleotide comprising a fragment of at least 19 consecutive nucleotides, or at least 50 consecutive nucleotides, or at least 100 consecutive nucleotides, or at least 200 consecutive nucleotides of a polynucleotide having a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26;
   f) a polynucleotide comprising a fragment encoding a biologically active portion of a polypeptide having a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27;
   g) a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising at least 19 consecutive nucleotides, or at least 50 consecutive nucleotides, or at least 100 consecutive nucleotides, or at least 200 consecutive nucleotides of a polynucleotide having a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26; and
   h) a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising at least 19 consecutive nucleotides, or at least 50 consecutive nucleotides, or at least 100 consecutive nucleotides, or at least 200 consecutive nucleotides of a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27.

2. The dsRNA of claim 1, wherein the polynucleotide comprises a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26.

3. The dsRNA of claim 1, wherein the polynucleotide has 70% sequence identity to a polynu-
5 cleotide having a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26.

4. The dsRNA of claim 1, wherein the polynucleotide encodes a polypeptide having a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27.

10

5. The dsRNA of claim 1, wherein the polynucleotide encodes a polypeptide having 70% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27.

15 6. The dsRNA of claim 1, wherein the a polynucleotide hybridizes under stringent conditions to a polynucleotide comprising a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26.

7. The dsRNA of claim 1, wherein the polynucleotide comprises a fragment encoding a biologi-
20 cally active portion of a polypeptide having a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27.

8. A pool of dsRNA molecules comprising a multiplicity of RNA molecules each comprising a double stranded region having a length of about 19 to 24 nucleotides, wherein said dsRNA
25 molecules are derived from a polynucleotide selected from the group consisting of:
   a) a polynucleotide comprising a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27;

   b) a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27;

30 c) a polynucleotide having 90% sequence identity to a polynucleotide having a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26; and

   d) a polynucleotide encoding a polypeptide having 90% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27.

9. The pool of dsRNA of claim 8, wherein the polynucleotide comprises a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26.

10. The pool of dsRNA of claim 8, wherein the polynucleotide has 90% sequence identity to a polynucleotide having a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26.

11. The pool of dsRNA of claim 8, wherein the polynucleotide encodes a polypeptide having a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27.

12. The pool of dsRNA of claim 8, wherein the polynucleotide encodes a polypeptide having 90% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO:2, 8, 10, 12, 14, 16, 18, or 20.

13. A transgenic plant capable of expressing a dsRNA that is substantially identical to a portion of a CDPK-like gene, wherein the portion of the CDPK-like gene is from a polynucleotide selected from the group consisting of:
   a) a polynucleotide comprising a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26;
   b) a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27;
   c) a polynucleotide having 70% sequence identity to a polynucleotide having a sequence as set forth in SEQ ID NO:1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26;
   d) a polynucleotide encoding a polypeptide having 70% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27;
   e) a polynucleotide comprising a fragment of at least 19 consecutive nucleotides, or at least 50 consecutive nucleotides, or at least 100 consecutive nucleotides, or at least 200 consecutive nucleotides of a polynucleotide having a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26;
   f) a polynucleotide comprising a fragment encoding a biologically active portion of a polypeptide having a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27;

g) a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising at least 19 consecutive nucleotides, or at least 50 consecutive nucleotides, or at least 100 consecutive nucleotides, or at least 200 consecutive nucleotides of a polynucleotide having a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24,
5 or 26; and

h) a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising at least 19 consecutive nucleotides, or at least 50 consecutive nucleotides, or at least 100 consecutive nucleotides, or at least 200 consecutive nucleotides of a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15,
10 17, 19, 21, 23, 25, or 27.

14. The transgenic plant of claim 13, wherein the dsRNA comprises a multiplicity of RNA molecules each comprising a double stranded region having a length of about 19 to 24 nucleotides, wherein said RNA molecules are derived from a portion of a polynucleotide selected
15 from the group consisting of:
a) a polynucleotide comprising a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26;

b) a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27;

20 c) a polynucleotide having 90% sequence identity to a polynucleotide having a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26; and

d) a polynucleotide encoding a polypeptide having 90% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27.

25 15. The transgenic plant of claim 13, wherein the plant is selected from the group consisting of soybean, potato, tomato, peanuts, cotton, cassava, coffee, coconut, pineapple, citrus trees, banana, corn, rape, beet, sunflower, sorghum, wheat, oats, rye, barley, rice, green bean, lima bean, pea, and tobacco.

30 16. The transgenic plant of claim 13, wherein the plant is soybean.

17. The transgenic plant of claim 13, wherein the polynucleotide comprises a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26.

18. The transgenic plant of claim 13, wherein the polynucleotide has 70% sequence identity to a polynucleotide having a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26.

5

19. The transgenic plant of claim 13, wherein the polynucleotide encodes a polypeptide having a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27.

20. The transgenic plant of claim 13, wherein the polynucleotide encodes a polypeptide having
10 70% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27.

21. A method of making a transgenic plant capable of expressing a dsRNA that inhibits expression of an CDPK-like gene in the plant, said method comprises the steps of i) preparing a
15 nucleic acid having a region that is substantially identical to a portion of the CDPK-like gene, wherein the nucleic acid is able to form a double-stranded transcript once expressed in the plant; ii) transforming a recipient plant with said nucleic acid; iii) producing one or more transgenic offspring of said recipient plant; and iv) selecting the offspring for expression of said transcript,
20 wherein the portion of the CDPK-like gene is from a polynucleotide selected from the group consisting of:
    a) a polynucleotide comprising a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26;

    b) a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO: 3,
25 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27;

    c) a polynucleotide having 70% sequence identity to a polynucleotide having a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26;

    d) a polynucleotide encoding a polypeptide having 70% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27;

30 e) a polynucleotide comprising a fragment of at least 19 consecutive nucleotides, or at least 50 consecutive nucleotides, or at least 100 consecutive nucleotides, or at least 200 consecutive nucleotides of a polynucleotide having a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26;

f) a polynucleotide comprising a fragment encoding a biologically active portion of a polypeptide having a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27;

g) a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising at least 19 consecutive nucleotides, or at least 50 consecutive nucleotides, or at least 100 consecutive nucleotides, or at least 200 consecutive nucleotides of a polynucleotide having a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26; and

h) a polynucleotide hybridizing under stringent conditions to a polynucleotide comprising at least 19 consecutive nucleotides, or at least 50 consecutive nucleotides, or at least 100 consecutive nucleotides, or at least 200 consecutive nucleotides of a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27.

22. The method of claim 21, wherein the dsRNA comprises a multiplicity of RNA molecules each comprising a double stranded region having a length of about 19 to 24 nucleotides, wherein said RNA molecules are derived from a polynucleotide selected from the group consisting of:

a) a polynucleotide comprising a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26;

b) a polynucleotide encoding a polypeptide having a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27;

c) a polynucleotide having 90% sequence identity to a polynucleotide having a sequence as set forth in SEQ ID NO: 1, 2, 4, 5, 8, 10 12, 14, 16, 18, 20, 22, 24, or 26; and

d) a polynucleotide encoding a polypeptide having 90% sequence identity to a polypeptide having a sequence as set forth in SEQ ID NO: 3, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, or 27.

23. The method of claim 21, wherein the plant is selected from the group consisting of soybean, potato, tomato, peanuts, cotton, cassava, coffee, coconut, pineapple, citrus trees, banana, corn, rape, beet, sunflower, sorghum, wheat, oats, rye, barley, rice, green bean, lima bean, pea, and tobacco.

24. The method of claim 21, wherein the plant is soybean.

25. The method of claim 21, wherein the dsRNA is expressed in plant roots or syncytia.

Figure 1

Gene Name	Species	SEQ ID NO:
49806575 partial cDNA sequence	Glycine max	1
Sense sequence of the 49806575 fragment	Glycine max	2
translation of the partial 49806575 EST	Glycine max	3
AY821654 DNA	Medicago truncatula	4
Synthesized sequence described in Example 1	Glycine max	5
TPP promoter	Arabidopsis thaliana	6
AY821654 protein	Medicago truncatula	7
AY823957 DNA	Medicago truncatula	8
AY823957 protein	Medicago truncatula	9
AF435451 DNA	Nicotiana tabacum	10
AF435451 protein	Nicotiana tabacum	11
AY971376 DNA	Nicotiana tabacum	12
AY971376 protein	Nicotiana tabacum	13
AF030879 DNA	Solanum tuberosum	14
AF030879 protein	Solanum tuberosum	15
At2g17890 DNA	Arabidopsis thaliana	16
At2g17890 protein	Arabidopsis thaliana	17
At4g36070 DNA	Arabidopsis thaliana	18
At4g36070 protein	Arabidopsis thaliana	19
At5g66210 DNA	Arabidopsis thaliana	20
At5g66210 protein	Arabidopsis thaliana	21
NM_001052286 DNA	Oryza sativa	22
NM_001052286 protein	Oryza sativa	23
NM_001065979 DNA	Oryza sativa	24
NM_001065979 protein	Oryza sativa	25
RKF195-3 DNA	Glycine max	26
RKF195-3 protein	Glycine max	27
GeneRacer RNA Oligo	unknown	28
GeneRacer 5′ Primer	unknown	29
GeneRacer 5′ Nested Primer	unknown	30
49806575 5′ RACE Primer	unknown	31
49806575_5′ RACE _Nested primer	unknown	32

[

FIGURE 2a

```
Hygene_49806575     (1) --------------------------------------------------
     Gm_RKF195-3    (1) -MGLCFSSTKVSGSSSNNNNN--------NNASSNRNR-------KCSAA
     St_AF030879    (1) -MGSCFSSSKVSGSNSNTPSTNNTATNTNTTVNVHPNRRETSKAPSTTVV
     Nt_AF435451    (1) MGNNCFSSSKVSGSNSNTPST----TATATTVNVRRNK---ANPPSTSTI
     Mt_AY821654    (1) -MGLCFSSTKVVSGSNSNTTNNDNRKRNQSTTTDTTVT------------
     Mt_AY823957    (1) -MGLCFSSTKVVSGS-NSNTT--------NNDNRKRNQ-------STTTD
     Nt_AY971376    (1) -MGSCFSSSKVSGSNSNTPST------TTTNVNVHHNR---PSTTTTTTV
       At4g36070    (1) -MGLCFSSPK-ATRRGTGSRNPNPDSPTQGKASEKVSN------------
       At2g17890    (1) -MGLCFSSAAKSSGHNRSSRNPHPHPPLTVVKSRPPRSP-------CS-F
       At5g66210    (1) -MGVCFSAIRVTGASSSRRSS-----------------------------
Os_NM_001065979     (1) -MGLCSSSSARRDAGTPGGG------------------------------
Os_NM_001052286     (1) -MGACFSSHTATAAADGGSGK-----------------------------

Hygene_49806575     (1) --------------------------------------------------
     Gm_RKF195-3   (35) PAAAAPPEPVTPQKKQPSQAQRRRVPEESRKNPRAKDKAGARRQ-GTRVP
     St_AF030879   (50) NSRNQEGSNYNRGKGNINQKNQQK--QPRNSQQ--NVKPSSRRQ-GGVIP
     Nt_AF435451   (44) TSTKQEGSHCNKQKVKDNHKSQHQKQQPRNSQQNVKKHNNGRRQKSGVIA
     Mt_AY821654   (38) -----VTTATTAAQKQTAQRRKGGSNETAQKKNHHQHHRLKEKTGSKHVP
     Mt_AY823957   (34) TTVTVTTATTAAQKQTAQRRKGGSNETAQKKNHHQHHRLKEKTG-SKHVP
     Nt_AY971376   (41) TSRKQEGSNYNRDKGNINTKNSHQKQQPRSSQQNVVVKPSSRRQSGGVIP
       At4g36070   (37) --------------------------------KNKKNTKKIQLRHQGGIP
       At2g17890   (42) MAVTIQKDHRTQPRRNATAKKTPTRHTPPHGKVREKVISNNGRRHGETIP
       At5g66210   (21) -----------------------Q--TKSKAAPTPIDTKASTKRRTGSIP
Os_NM_001065979    (20) -------------------------------NGAGNKDNAGRKG---IVA
Os_NM_001052286    (21) -----------------------R--QQRKGDHKGKLPDGGGGE---KEK

Hygene_49806575     (1) --------------------------------------------------
     Gm_RKF195-3   (84) CGKRTDFGYEKDFENRFSLGKLLGHGQFGYTYVGIDKKNGDRVAVKRLEK
     St_AF030879   (95) CGKRTDFGYDKDFEKRYTIGKLLGHGQFGYTYVATDKSSGDRVAVKRIEK
     Nt_AF435451   (94) CGKRTDFGYDKDFDKRFTIGKLLGHGQFGYTYVATHKSNGDRVAVKRIEK
     Mt_AY821654   (83) CGKRTDFGYEKDFDKRFSLGKLLGHGQFGYTYVGVDKSNGDRVAVKRLEK
     Mt_AY823957   (83) CGKRTDFGYEKDFDKRFSLGKLLGHGQFGYTYVGVDKSNGDRVAVKRLEK
     Nt_AY971376   (91) CGKRTDFGYDKDFDKRYTIGKLLGHGQFGYTYVATDRSSGDRVAVKKIEK
       At4g36070   (55) YGKRIDFGYAKDFDNRYTIGKLLGHGQFGFTYVATDNNNGNRVAVKRIDK
       At2g17890   (92) YGKRVDFGYAKDFDHRYTIGKLLGHGQFGYTYVATDKKTGDRVAVKKIDK
       At5g66210   (46) CGKRTDFGYSKDFHDHYTIGKLLGHGQFGYTYVAIHRPNGDRVAVKRLDK
Os_NM_001065979    (36) CGKRTDFGYDKDFEARYALGKLLGHGQFGYTFAAVDRRSSERVAVKRIDK
Os_NM_001052286    (43) EAARVEFGYERDFEGRYQVGRLLGHGQFGYTFAATDRASGDRVAVKRIDK

Hygene_49806575     (1) --------------------------------------------------
     Gm_RKF195-3  (134) SKMVLPIAVEDVKREVKILKELTGHENVVQFFNAFEDDSYVYIVMELCEG
     St_AF030879  (145) NKMVLPIAVEDVKREVKILKALGRHENVVQFYNSFEDHNYVYIVMELCEG
     Nt_AF435451  (144) NKMVLPIAVEDVKREVKILKALSGHENVVQFNNAFEDDNYVYIVMELCEG
     Mt_AY821654  (133) AKMVLPIAVEDVKREVKILKELTGHENVVQFYNAFDDDSYVYIVMELCEG
     Mt_AY823957  (133) AKMVLPIAVEDVKREVKILKELTGHENVVQFYNAFDDDSYVYIVMELCEG
     Nt_AY971376  (141) NKMVLPIAVEDVKREVKILKALAGHENVVQFYNSFEDDNYVYIVMELCEG
       At4g36070  (105) AKMTQPIEVEDVKREVKILQALGGHENVVGFHNAFEDKTYIYIVMELCDG
       At2g17890  (142) AKMTIPIAVEDVKREVKILQALTGHENVVRFYNAFEDKNSVYIVMELCEG
       At5g66210   (96) SKMVLPIAVEDVKREVQILIALSGHENVVQFHNAFEDDDYVYIVMELCEG
Os_NM_001065979    (86) NKMVLPVAVEDVKREVKILKALQGHENVVHFYNAFEDDNYVYIVMELCEG
Os_NM_001052286    (93) AKMVRPVAVEDVKREVKILKELKGHENIVHFYNAFEDDSYVYIVMELCEG
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FIGURE 2b

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Hygene_49806575    (1) --------------------------------------------------
    Gm_RKF195-3  (184) GELLDRILAKKDSRYTEKDAAVVVRQMLKVAAECHLHGLVHRDMKPENFL
    St_AF030879  (195) GELLDDCQKTVG---IREDAAIVVPQMLKVAAECHLHGLVHRDMKPENFL
    Nt_AF435451  (194) GELLDRILAKKDSRYAEKDAAIVVRQMLKEAAQCHLHGLVHRDMKPENFL
    Mt_AY821654  (183) GELLDRILNKKDSRYTEKDAAVVVRQMLKVAAQCHLHGLVHRDMKPENFL
    Mt_AY823957  (183) GELLDRILNKKDSRYTEKDAAVVVRQMLKVAAQCHLHGLVHRDMKPENFL
    Nt_AY971376  (191) GELLDRILSKKDSRYTEKDAAIVVRQMLKVAAECHLHGLVHRDMKPENFL
      At4g36070  (155) GELLDRILAKKDSRYTEKDAAVVVRQMLKVAAECHLRGLVHRDMKPENFL
      At2g17890  (192) GELLDRILARKDSRYSERDAAVVVRQMLKVAAECHLRGLVHRDMKPENFL
      At5g66210  (146) GELLDRILSKKGNRYSEKDAAVVVRQMLKVAGECHLHGLVHRDMKPENFL
Os_NM_001065979  (136) GELLDRILAKKDSRYSEKDAAVVVRQMLKVAAECHLHGLVHRDMKPENFL
Os_NM_001052286  (143) GELLDRILAKKNSRYSEKDAAVVVRQMLKVAAECHLHGLVHRDMKPENFL

Hygene_49806575    (1) --------------------------------------------------
    Gm_RKF195-3  (234) FKSTKEDSPLKATDFGLSDFIKPGKRFQDIVGSAYYVAPEVLKRKSGPES
    St_AF030879  (242) FKSTKEDSPLKATDFGSSDFIRPGK-VQDIVGSAYYVAPEVLKRRSGPES
    Nt_AF435451  (244) FKSSKEDSPLKATDFGLSDFIRPGKKFQDIVGSAYYVAPEVLKRRSGPES
    Mt_AY821654  (233) FKSNKEDSALKATDFGLSDFIKPGKRFQDIVGSAYYVAPEVLKRKSGPES
    Mt_AY823957  (233) FKSNKEDSALKATDFGLSDFIKPGKRFQDIVGSAYYVAPEVLKRKSGPES
    Nt_AY971376  (241) FKLFKGGFAIKSTDFGLSDFIRPGKKFQDIVGSAYYVAPEVLKRRSGPES
      At4g36070  (205) FKSTEEGSSLKATDFGLSDFIKPGVKFQDIVGSAYYVAPEVLKRRSGPES
      At2g17890  (242) FKSTEEDSPLKATDFGLSDFIKPGKKFHDIVGSAYYVAPEVLKRRSGPES
      At5g66210  (196) FKSAQLDSPLKATDFGLSDFIKPGKRFHDIVGSAYYVAPEVLKRRSGPES
Os_NM_001065979  (186) FKSTKEDSSLKATDFGLSDFIRPGKHFRDIVGSAYYVAPEVLKRKSGPES
Os_NM_001052286  (193) FKSTKEDSPLKATDFGLSDFIKPGKKFHDIVGSAYYVAPEVLKRRSGPES

Hygene_49806575    (1) --------------------------------------------------
    Gm_RKF195-3  (284) DVWSIGVITYILLCGRRPFWDKTEDGIFKEVLRNKPDFRRKPWPTISNAA
    St_AF030879  (291) DVWSIGVITYILLCGRRPFWDKTEDGIFKEVLRNKPDFRRKPWSNISNSA
    Nt_AF435451  (294) DEWSIGVITYILLCGRRRFWDKTEDGIFKEVLRNKPDFRRKPWPTISNSA
    Mt_AY821654  (283) DVWSIGVITYILLCGRRPFWDKTEDGIFKEVLRNKPDFRRKPWPTISNAA
    Mt_AY823957  (283) DVWSIGVITYILLCGRRPFWDKTEDGIFKEVLRNKPDFRRKPWPTISNAA
    Nt_AY971376  (291) DVWSIGVITYILLCGRRRFWDKTEDGIFKEVLRNKPDFRRKPWPNISNSA
      At4g36070  (255) DVWSIGVITYILLCGRRPFWDKTQDGIFNEVMRKKPDFREVPWPTISNGA
      At2g17890  (292) DVWSIGVISYILLCGRRPFWDKTEDGIFKEVLKNKPDFRRKPWPTISNSA
      At5g66210  (246) DVWSIGVITYILLCGRRPFWDRTEDGIFKEVLRNKPDFSRKPWATISDSA
Os_NM_001065979  (236) DVWSIGVITYILLCGRRPFWDKTEDGIFKEVLKNKPDFRRKPWPNITPCA
Os_NM_001052286  (243) DVWSIGVITYILLCGRRPFWNKTEDGIFREVLRNKPDFRKKPWPGISSGA

Hygene_49806575    (1) ----------DPRARYTAAQALSHPWVREGGEALEIPIDISVLNNMRQFV
    Gm_RKF195-3  (334) KDFMKKLLVKDPRARYAAAQALSHPWVREGGEALEIPIDISVLNNMRQFV
    St_AF030879  (341) KDFVKKLLVKDPRARLTAAQALSHPWVREGGDASEIPLDISVLSNMRQFV
    Nt_AF435451  (344) KDFVKKLLVKDPRARLTAAQALSHPWVREGGDASEIPLDISVLSNMRQFV
    Mt_AY821654  (333) KDFVKKLLVKDPRARLTAAQALSHPWVREGGEASEIPIDISVLNNMRQFV
    Mt_AY823957  (333) KDFVKKLLVKDPRARLTAAQALSHPWVREGGEASEIPIDISVLNNMRQFV
    Nt_AY971376  (341) KDFVKKLLVKDPRARLTAAQALSHPWVREGGIASEIPLDISVLSNMRQFV
      At4g36070  (305) KDFVKKLLVKEPRARLTAAQALSHSWVKEGGEASEVPIDISVLNNMRQFV
      At2g17890  (342) KDFVKKLLVKDPRARLTAAQALSHPWVREGGDASEIPIDISVLNNMRQFV
      At5g66210  (296) KDFVKKLLVKDPRARLTAAQALSHAWVREGGNATDIPVDISVLNNLRQFV
Os_NM_001065979  (286) KDFVQKLLVKDPRARLTAAQALSHEWVREGGQASDIPLDISVLHNMRQFV
Os_NM_001052286  (293) KDFVKKLLVKNPRARLTAAQALSHPWVREGGEASEIPVDISVLSNMRQFV
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FIGURE 2c

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Hygene_49806575   (41) KYSRLKQFALRALASTLNEGELSDLKDQFDAIDVDKNGSISLEEMRQALA
     Gm_RKF195-3  (384) KYSRLKQFALRALASTLNEGELSDLKDQFDAIDVDKNGSISLEEMRQALA
     St_AF030879  (391) RYSHLKQFALRALRSTLDEEEIADLRDQFSAIDVDKNGVISLEEMRQALA
     Nt_AF435451  (394) KYSRLKQFALRALASTVDEEELADVRDQFSAIDVDKNGVISLEEMRQALA
     Mt_AY821654  (383) KYSRLKQFALRALASTLNEGELSDLKDQFDAIDVDKNGAISLEEMRQALA
     Mt_AY823957  (383) KYSRLKQFALRALASTLNEGELSDLKDQFDAIDVDKNGAISLEEMRQALA
     Nt_AY971376  (391) RYSRLKQFALRALASTLDEEELSDLKDQFSAIDVDKNGVISLEEMRQALA
       At4g36070  (355) KFSRLKQIALRALAKTINEDELDDLRDQFDAIDIDKNGSISLEEMRQALA
       At2g17890  (392) KFSRLKQFALRALATTLDEEELADLRDQFDAIDVDKNGVISLEEMRQALA
       At5g66210  (346) RYSRLKQFALRALASTLDEAEISDLRDQFDAIDVDKNGVISLEEMRQALA
Os_NM_001065979  (336) KYSRFKQFALRALASTLNAEELSDLRDQFNAIDVDKNGTISLEELKQALA
Os_NM_001052286  (343) KYSRFKQFALRALASTLKEEELADLKDQFDAIDVDKSGSISIEEMRHALA

Hygene_49806575   (91) KDQPWKLKESRVLEILQAIDSNTDGLVDFTEFVAATLHVHQLEEHDSDKW
     Gm_RKF195-3  (434) KDQPWKLKESRVLEILQAIDSNTDGLVDFTEFVAATLHVHQLEEHDSDKW
     St_AF030879  (441) KDLPWKMKESRVLEILQAIDSNTDGLVDFPEFVAATLHVHQLEEHNSAKW
     Nt_AF435451  (444) KDLPWKMKESRVLEILQAIDSNSDGLLDFPEFVAATLHVHQLEEHNSIKW
     Mt_AY821654  (433) KDLPWKLKESRVLEILQAIDSNTDGLVDFTEFVAATLHVHQLEEHDSDKW
     Mt_AY823957  (433) KDLPWKLKESRVLEILQAIDSNTDGLVDFTEFVAATLHVHQLEEHDSDKW
     Nt_AY971376  (441) KDLPWKMKESRVLEILQAIDSNTDGLVDFPEFVAATLHVHQLEEHNSTKW
       At4g36070  (405) KDVPWKLKDARVAEILQANDSNTDGLVDFTEFVVAALHVNQLEEHDSEKW
       At2g17890  (442) KDHPWKLKDARVAEILQAIDSNTDGFVDFGEFVAAALHVNQLEEHDSEKW
       At5g66210  (396) KDLPWKLKDSRVAEILEAIDSNTDGLVDFTEFVAAALHVHQLEEHDSEKW
Os_NM_001065979  (386) KDVPWRLKGPRVLEIVEAIDSNTDGLVDFEEFVAATLHVHQLVEHDTEKW
Os_NM_001052286  (393) KDLPWRLKGPRVLEIIQAIDSNTDGLVDFEEFVAATLHIHQMAELDSERW

Hygene_49806575  (141) QQRSQAAFEKFDLDKDGYITPDELRMH--TGLRGSIDPLLEEADIDKDGK
     Gm_RKF195-3  (484) QQRSQAAFEKFDLDKDGYITPDELRMH--TGLRGSIDPLLEEADTDKDGK
     St_AF030879  (491) QQRSQAAFEKFDVDRDGFITPEELKMH--TGLRGSIDPLLEEADIDKDGK
     Nt_AF435451  (494) QERSQAAFEEFDVDRDGFITPEELRMH--TGLKGSIDPLLEEADIDKDGK
     Mt_AY821654  (483) QQRSQAAFEKFDIDKDGYITPEELRMH--TGMRGSIDPLLEEADIDKDGK
     Mt_AY823957  (483) QQRSQAAFEKFDIDKDGYITPEELRMH--TGMRGSIDPLLEEADIDKDGK
     Nt_AY971376  (491) QQRSQAAFEKFDVDRDGFITPEELKMH--TGLKGSIDPLLEEADIDKDGK
       At4g36070  (455) QQRSRAAFDKFDIDGDGFITPEELRLQ--TGLKGSIEPLLEEADVDEDGR
       At2g17890  (492) QQRSRAAFEKFDIDGDGFITAEELRMH--TGLKGSIEPLLEEADIDNDGK
       At5g66210  (446) QLRSRAAFEKFDLDKDGYITPEELRMH--TGLRGSIDPLLDEADIDRDGK
Os_NM_001065979  (436) KSLSQAAFDKFDVDGDGYITSDELRMQ--TGLKGSIDPLLEEADIDRDGK
Os_NM_001052286  (443) GLRCQAAFSKFDLDGDGYITPDELRMVQHTGLKGSIEPLLEEADIDKDGR

Hygene_49806575  (189) ISLPEFRRLLRTASMGSRTVMSPSHRHHRKI---
     Gm_RKF195-3  (532) ISLPEFRRLLRTASMGSRTVMSPSHRHHRKI---
     St_AF030879  (539) ISISEFRRLLRTASMTS-----PTVRDSRGM---
     Nt_AF435451  (542) ISLSEFRRLLRTASISSRMVTSPTVRGSRKS---
     Mt_AY821654  (531) ISLPEFRRLLRTASIGSRNVTSPTLRHRRI----
     Mt_AY823957  (531) ISLPEFRRLLRTASIGSRNVTSPTLRHRRI----
     Nt_AY971376  (539) ISLSEFRRLLRTASMSS-----PTVRDSRRNVAL
       At4g36070  (503) ISINEFRRLLRSASLKSKNVKSPPGYQLSQKM--
       At2g17890  (540) ISLQEFRRLLRTASIKSRNVRSPPGYLISRKV--
       At5g66210  (494) ISLHEFRRLLRTASISSQRAPSPAGHRNLR----
Os_NM_001065979  (484) ISLDEFRRLLKTASMSSRNVQTPRSVHRS-----
Os_NM_001052286  (493) ISLSEFRKLLRTASMS--NLPSPRGPPNPQPL--
```

FIGURE 3

Percent identity (amino acid)

	49806575	Gm_ RKF195-3	Mt_ AY821654	Mt_ AY823957	Nt_ AF435451	Nt_ AY971376	St_ AF030879	At2g17890	At4g36070	At5g66210	Os_NM_ 001052286	Os_NM_ 001065979
49806575	100	99	94	94	83	86	84	81	76	82	78	79
Gm_RKF195-3			93	93	82	86	83	80	76	82	77	78
Mt_AY821654				100	86	88	86	84	78	83	78	79
Mt_AY823957					86	88	86	84	78	83	78	79
Nt_AF435451						92	91	82	73	79	76	76
Nt_AY971376							94	83	74	83	78	79
St_AF030879								81	73	82	76	76
At2g17890									84	82	75	76
At4g36070										75	72	72
At5g66210											75	76
Os_NM_001052286												78
Os_NM_001065979												

FIGURE 4

Percent identity (nucleotide acid)

	49806575	Gm_ RKF195-3	Mt_ AY823957	Mt_ AY821654	Nt_ AF435451	Nt_ AY971376	St_ AF030879	At2g17890	At4g36070	At5g66210	Os_NM_ 001052286	Os_NM_ 001065979
49806575	100	100	90	90	76	75	78	76	74	76	72	73
Gm_RKF195-3		100	90	90	76	74	77	76	74	76	72	73
Mt_AY823957			100	100	75	74	76	75	74	76	72	74
Mt_AY821654				100	75	74	76	75	74	76	72	74
Nt_AF435451					100	85	86	72	70	73	71	71
Nt_AY971376						100	92	72	68	74	69	71
St_AF030879							100	74	70	75	70	72
At2g17890								100	81	77	69	72
At4g36070									100	73	68	70
At5g66210										100	69	70
Os_NM_001052286											100	73
Os_NM_001065979												100

Figure 5a

nucleotide positions of 21mers of CDPK-like genes									
e.g. SEQ ID NO: 1, 2, 4, 5, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 26									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
1	21	37	57	73	93	109	129	145	165
2	22	38	58	74	94	110	130	146	166
3	23	39	59	75	95	111	131	147	167
4	24	40	60	76	96	112	132	148	168
5	25	41	61	77	97	113	133	149	169
6	26	42	62	78	98	114	134	150	170
7	27	43	63	79	99	115	135	151	171
8	28	44	64	80	100	116	136	152	172
9	29	45	65	81	101	117	137	153	173
10	30	46	66	82	102	118	138	154	174
11	31	47	67	83	103	119	139	155	175
12	32	48	68	84	104	120	140	156	176
13	33	49	69	85	105	121	141	157	177
14	34	50	70	86	106	122	142	158	178
15	35	51	71	87	107	123	143	159	179
16	36	52	72	88	108	124	144	160	180
17	37	53	73	89	109	125	145	161	181
18	38	54	74	90	110	126	146	162	182
19	39	55	75	91	111	127	147	163	183
20	40	56	76	92	112	128	148	164	184
21	41	57	77	93	113	129	149	165	185
22	42	58	78	94	114	130	150	166	186
23	43	59	79	95	115	131	151	167	187
24	44	60	80	96	116	132	152	168	188
25	45	61	81	97	117	133	153	169	189
26	46	62	82	98	118	134	154	170	190
27	47	63	83	99	119	135	155	171	191
28	48	64	84	100	120	136	156	172	192
29	49	65	85	101	121	137	157	173	193
30	50	66	86	102	122	138	158	174	194
31	51	67	87	103	123	139	159	175	195
32	52	68	88	104	124	140	160	176	196
33	53	69	89	105	125	141	161	177	197
34	54	70	90	106	126	142	162	178	198
35	55	71	91	107	127	143	163	179	199
36	56	72	92	108	128	144	164	180	200

Figure 5b

nucleotide positions of 21mers of CDPK-like genes									
e.g. SEQ ID NO: 1, 2, 4, 5, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 26									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
181	201	217	237	253	273	289	309	325	345
182	202	218	238	254	274	290	310	326	346
183	203	219	239	255	275	291	311	327	347
184	204	220	240	256	276	292	312	328	348
185	205	221	241	257	277	293	313	329	349
186	206	222	242	258	278	294	314	330	350
187	207	223	243	259	279	295	315	331	351
188	208	224	244	260	280	296	316	332	352
189	209	225	245	261	281	297	317	333	353
190	210	226	246	262	282	298	318	334	354
191	211	227	247	263	283	299	319	335	355
192	212	228	248	264	284	300	320	336	356
193	213	229	249	265	285	301	321	337	357
194	214	230	250	266	286	302	322	338	358
195	215	231	251	267	287	303	323	339	359
196	216	232	252	268	288	304	324	340	360
197	217	233	253	269	289	305	325	341	361
198	218	234	254	270	290	306	326	342	362
199	219	235	255	271	291	307	327	343	363
200	220	236	256	272	292	308	328	344	364
201	221	237	257	273	293	309	329	345	365
202	222	238	258	274	294	310	330	346	366
203	223	239	259	275	295	311	331	347	367
204	224	240	260	276	296	312	332	348	368
205	225	241	261	277	297	313	333	349	369
206	226	242	262	278	298	314	334	350	370
207	227	243	263	279	299	315	335	351	371
208	228	244	264	280	300	316	336	352	372
209	229	245	265	281	301	317	337	353	373
210	230	246	266	282	302	318	338	354	374
211	231	247	267	283	303	319	339	355	375
212	232	248	268	284	304	320	340	356	376
213	233	249	269	285	305	321	341	357	377
214	234	250	270	286	306	322	342	358	378
215	235	251	271	287	307	323	343	359	379
216	236	252	272	288	308	324	344	360	380

Figure 5c

nucleotide positions of 21mers of CDPK-like genes									
e.g. SEQ ID NO: 1, 2, 4, 5, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 26									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
361	381	397	417	433	453	469	489	505	525
362	382	398	418	434	454	470	490	506	526
363	383	399	419	435	455	471	491	507	527
364	384	400	420	436	456	472	492	508	528
365	385	401	421	437	457	473	493	509	529
366	386	402	422	438	458	474	494	510	530
367	387	403	423	439	459	475	495	511	531
368	388	404	424	440	460	476	496	512	532
369	389	405	425	441	461	477	497	513	533
370	390	406	426	442	462	478	498	514	534
371	391	407	427	443	463	479	499	515	535
372	392	408	428	444	464	480	500	516	536
373	393	409	429	445	465	481	501	517	537
374	394	410	430	446	466	482	502	518	538
375	395	411	431	447	467	483	503	519	539
376	396	412	432	448	468	484	504	520	540
377	397	413	433	449	469	485	505	521	541
378	398	414	434	450	470	486	506	522	542
379	399	415	435	451	471	487	507	523	543
380	400	416	436	452	472	488	508	524	544
381	401	417	437	453	473	489	509	525	545
382	402	418	438	454	474	490	510	526	546
383	403	419	439	455	475	491	511	527	547
384	404	420	440	456	476	492	512	528	548
385	405	421	441	457	477	493	513	529	549
386	406	422	442	458	478	494	514	530	550
387	407	423	443	459	479	495	515	531	551
388	408	424	444	460	480	496	516	532	552
389	409	425	445	461	481	497	517	533	553
390	410	426	446	462	482	498	518	534	554
391	411	427	447	463	483	499	519	535	555
392	412	428	448	464	484	500	520	536	556
393	413	429	449	465	485	501	521	537	557
394	414	430	450	466	486	502	522	538	558
395	415	431	451	467	487	503	523	539	559
396	416	432	452	468	488	504	524	540	560

Figure 5d

nucleotide positions of 21mers of CDPK-like genes									
e.g. SEQ ID NO: 1, 2, 4, 5, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 26									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
541	561	577	597	613	633	649	669	685	705
542	562	578	598	614	634	650	670	686	706
543	563	579	599	615	635	651	671	687	707
544	564	580	600	616	636	652	672	688	708
545	565	581	601	617	637	653	673	689	709
546	566	582	602	618	638	654	674	690	710
547	567	583	603	619	639	655	675	691	711
548	568	584	604	620	640	656	676	692	712
549	569	585	605	621	641	657	677	693	713
550	570	586	606	622	642	658	678	694	714
551	571	587	607	623	643	659	679	695	715
552	572	588	608	624	644	660	680	696	716
553	573	589	609	625	645	661	681	697	717
554	574	590	610	626	646	662	682	698	718
555	575	591	611	627	647	663	683	699	719
556	576	592	612	628	648	664	684	700	720
557	577	593	613	629	649	665	685	701	721
558	578	594	614	630	650	666	686	702	722
559	579	595	615	631	651	667	687	703	723
560	580	596	616	632	652	668	688	704	724
561	581	597	617	633	653	669	689	705	725
562	582	598	618	634	654	670	690	706	726
563	583	599	619	635	655	671	691	707	727
564	584	600	620	636	656	672	692	708	728
565	585	601	621	637	657	673	693	709	729
566	586	602	622	638	658	674	694	710	730
567	587	603	623	639	659	675	695	711	731
568	588	604	624	640	660	676	696	712	732
569	589	605	625	641	661	677	697	713	733
570	590	606	626	642	662	678	698	714	734
571	591	607	627	643	663	679	699	715	735
572	592	608	628	644	664	680	700	716	736
573	593	609	629	645	665	681	701	717	737
574	594	610	630	646	666	682	702	718	738
575	595	611	631	647	667	683	703	719	739
576	596	612	632	648	668	684	704	720	740

Figure 5e

nucleotide positions of 21mers of CDPK-like genes									
e.g. SEQ ID NO: 1, 2, 4, 5, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 26									
nucleotide to nu-cleotide		nucleotide to nu-cleotide		nucleotide to nu-cleotide		nucleotide to nu-cleotide		nucleotide to nu-cleotide	
721	741	757	777	793	813	829	849	865	885
722	742	758	778	794	814	830	850	866	886
723	743	759	779	795	815	831	851	867	887
724	744	760	780	796	816	832	852	868	888
725	745	761	781	797	817	833	853	869	889
726	746	762	782	798	818	834	854	870	890
727	747	763	783	799	819	835	855	871	891
728	748	764	784	800	820	836	856	872	892
729	749	765	785	801	821	837	857	873	893
730	750	766	786	802	822	838	858	874	894
731	751	767	787	803	823	839	859	875	895
732	752	768	788	804	824	840	860	876	896
733	753	769	789	805	825	841	861	877	897
734	754	770	790	806	826	842	862	878	898
735	755	771	791	807	827	843	863	879	899
736	756	772	792	808	828	844	864	880	900
737	757	773	793	809	829	845	865	881	901
738	758	774	794	810	830	846	866	882	902
739	759	775	795	811	831	847	867	883	903
740	760	776	796	812	832	848	868	884	904
741	761	777	797	813	833	849	869	885	905
742	762	778	798	814	834	850	870	886	906
743	763	779	799	815	835	851	871	887	907
744	764	780	800	816	836	852	872	888	908
745	765	781	801	817	837	853	873	889	909
746	766	782	802	818	838	854	874	890	910
747	767	783	803	819	839	855	875	891	911
748	768	784	804	820	840	856	876	892	912
749	769	785	805	821	841	857	877	893	913
750	770	786	806	822	842	858	878	894	914
751	771	787	807	823	843	859	879	895	915
752	772	788	808	824	844	860	880	896	916
753	773	789	809	825	845	861	881	897	917
754	774	790	810	826	846	862	882	898	918
755	775	791	811	827	847	863	883	899	919
756	776	792	812	828	848	864	884	900	920

Figure 5f

nucleotide positions of 21mers of CDPK-like genes									
e.g. SEQ ID NO: 1, 2, 4, 5, 8, 10, 12, 14, 16, 18, 20, 22, 24, or 26									
nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide		nucleotide to nucleotide	
901	921	937	957	973	993	109	129		
902	922	938	958	974	994	110	130		
903	923	939	959	975	995	111	131		
904	924	940	960	976	996	112	132		
905	925	941	961	977	997	113	133		
906	926	942	962	978	998	114	134		
907	927	943	963	979	999	115	135		
908	928	944	964	980	1000	116	136		
909	929	945	965	981	1001	117	137		
910	930	946	966	982	1002	118	138		
911	931	947	967	983	1003	119	139		
912	932	948	968	984	1004	120	140		
913	933	949	969	985	1005	121	141		
914	934	950	970	986	1006	122	142		
915	935	951	971	987	1007	123	143		
916	936	952	972	988	1008	124	144		
917	937	953	973	989	1009	125	145		
918	938	954	974	990	1010				
919	939	955	975	991	1011				
920	940	956	976	992	1012				
921	941	957	977	993	1013	…..	…..		
922	942	958	978	994	1014	…..	…..		
923	943	959	979	995	1015	n-5	n+15		
924	944	960	980	996	1016	n-4	n+16		
925	945	961	981	997	1017	n-3	n+17		
926	946	962	982	998	1018	n-2	n+18		
927	947	963	983	999	1019	n-1	n+19		
928	948	964	984	1000	1020	n	n+20		
929	949	965	985	1001	1021				
930	950	966	986	1002	1022				
931	951	967	987	1003	1023				
932	952	968	988	1004	1024				
933	953	969	989	1005	1025				
934	954	970	990	1006	1026				
935	955	971	991	1007	1027				
936	956	972	992	1008	1028				

Figure 5g

n = total number of nucleotides of the entire length of an CDPK-like encoding polynucleotide – 20.

For example:

n = 1025 (=1045-20) for SEQ ID NO:1;

n =300 (=320-20) for SEQ ID NO:2;

n = 1991 (=2211-20) for SEQ ID NO:4;

n =720 (=740-20) for SEQ ID NOs:5;

n = 1663 (=1683-20) for SEQ ID NO:8;

n =1699 (=1719-20) for SEQ ID NO:10;

n = 1684 (=1704-20) for SEQ ID NO:12;

n =1675(=1695-20) for SEQ ID NO:14;

n = 1696 (=1716-20) for SEQ ID NO:16;

n = 1585 (=1605-20) for SEQ ID NO:18;

n = 1552 (=1572-20) for SEQ ID NO:20;

n = 1549 (=1569-20) for SEQ ID NO:22;

n = 1519 (=1539-20) for SEQ ID NO:24;

n = 1669 (=1689-20) for SEQ ID NO:26;

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2008/051482

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/82

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

B. FIELDS SEARCHED

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

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, Sequence Search, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	IVASHUTA S. ET AL.: "RNA interference identifies a calcium-dependent protein kinase involved in medicago truncatula root development" THE PLANT CELL, vol. 17, November 2005 (2005-11), pages 2911-2921, XP002478172 abstract page 2912, column 1, paragraph 2 figure 6 figure 7 figure 8 page 2917, column 2, paragraph 4 - page 2918, column 1, paragraph 2 "METHODS" ----- -/--	1-25

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 April 2008	11/06/2008

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Keller, Yves

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GARGANTINI P. ET AL.: "A CDPK isoform participates in the regulation of nodule number in Medicago truncatula" THE PLANT JOURNAL, vol. 48, 2006, pages 843-856, XP002478173 abstract page 844, column 1, paragraph 3 page 844, column 2, paragraph 4 - page 845, column 1, paragraph 1 figure 3 figure 4 page 848, column 2, paragraph 2 page 850, column 1, paragraph 1 - page 851, column 1, paragraph 1 page 853, column 2, paragraph 2	1-25
Y	LUDWIG A A ET AL: "CDPK-mediated signalling pathways: Specificity and cross-talk" JOURNAL OF EXPERIMENTAL BOTANY, OXFORD UNIVERSITY PRESS, GB, vol. 55, no. 395, January 2004 (2004-01), pages 181-188, XP002343024 ISSN: 0022-0957 abstract page 181, column 2, paragraph 1 - paragraph 2 page 182, column 2, paragraph 2 page 186, column 1, paragraph 4 - column 2, paragraph 2	1-25
Y	GHEYSEN ET AL: "RNAi from plants to nematodes" TRENDS IN BIOTECHNOLOGY, ELSEVIER PUBLICATIONS, CAMBRIDGE, GB, vol. 25, no. 3, 24 January 2007 (2007-01-24), pages 89-92, XP005896599 ISSN: 0167-7799 the whole document	1-25
Y	US 2004/031072 A1 (LA ROSA ET AL.) 12 February 2004 (2004-02-12) sequence 213143 claim 2 paragraph [0021] paragraphs [0009] - [0015] paragraph [0042] paragraph [0047] paragraph [0086] paragraph [0089]	1-25
A	WO 2006/020821 A (BASF PLANT SCIENCE GMBH [DE]; REN PEIFENG [US]; HUANG XIANG [US]; CHAU) 23 February 2006 (2006-02-23)	

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2008/051482

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2004/005485 A (UNIV KANSAS STATE [US]; TRICK HAROLD N [US]; ROE JUDITH L [US]; TODD T) 15 January 2004 (2004-01-15) -----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2008/051482

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

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

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

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

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

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

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

see additional sheet

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

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

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

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

1-25 (partially)

Remark on Protest

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

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

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-25 (partially)

   A ds RNA having a sequence substantially identical to a portion/fragment of a CDPK like gene/protein of SEQ ID No 1, 2 or 3, transgenic plants containing the same and related subject matter.

---

2. claims: 1-25 (partially)

   A ds RNA having a sequence substantially identical to a portion/fragment of a CDPK like gene/protein respectively of SEQ ID No 4 and 7,transgenic plants containing the same, and related subject matter.

---

3. claims: 1-25 (partially)

   A ds RNA having a sequence substantially identical to a portion/fragment of a CDPK like gene/protein respectively of SEQ ID No 8 and 9,transgenic plants containing the same, and related subject matter.

---

4. claims: 1-25 (partially)

   A ds RNA having a sequence substantially identical to a portion/fragment of a CDPK like gene/protein respectively of SEQ ID No 10 and 11,transgenic plants containing the same, and related subject matter.

---

5. claims: 1-25 (partially)

   A ds RNA having a sequence substantially identical to a portion/fragment of a CDPK like gene/protein respectively of SEQ ID No 12 and 13, transgenic plants containing the same, and related subject matter.

---

6. claims: 1-25 (partially)

   A ds RNA having a sequence substantially identical to a portion/fragment of a CDPK like gene/protein respectively of SEQ ID No 14 and 15, transgenic plants containing the same, and related subject matter.

---

7. claims: 1-25 (partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A ds RNA having a sequence substantially identical to a portion/fragment of a CDPK like gene/protein respectively of SEQ ID No 16 and 17, transgenic plants containing the same, and related subject matter.

---

8. claims: 1-25 (partially)

A ds RNA having a sequence substantially identical to a portion/fragment of a CDPK like gene/protein respectively of SEQ ID No 18 and 19, transgenic plants containing the same, and related subject matter.

---

9. claims: 1-25 (partially)

A ds RNA having a sequence substantially identical to a portion/fragment of a CDPK like gene/protein respectively of SEQ ID No 20 and 21, transgenic plants containing the same, and related subject matter.

---

10. claims: 1-25 (partially)

A ds RNA having a sequence substantially identical to a portion/fragment of a CDPK like gene/protein respectively of SEQ ID No 22 and 23, transgenic plants containing the same, and related subject matter.

---

11. claims: 1-25 (partially)

A ds RNA having a sequence substantially identical to a portion/fragment of a CDPK like gene/protein respectively of SEQ ID No 24 and 25, transgenic plants containing the same, and related subject matter.

---

12. claims: 1-25 (partially)

A ds RNA having a sequence substantially identical to a portion/fragment of a CDPK like gene/protein respectively of SEQ ID No 26 and 27, transgenic plants containing the same, and related subject matter.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2008/051482

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2004031072 A1	12-02-2004	US 2004034888 A1	19-02-2004
		US 2006236419 A1	19-10-2006
WO 2006020821 A	23-02-2006	AR 050211 A1	04-10-2006
		EP 1778848 A2	02-05-2007
		US 2006037101 A1	16-02-2006
WO 2004005485 A	15-01-2004	AU 2003247951 A1	23-01-2004
		BR PI0312580 A	10-10-2006
		US 2004098761 A1	20-05-2004