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(54) Title: PHYTOCHELATIN SYNTHASE

(57) Abstract: The present invention relates to an isolated nucleic acid fragment encoding a phytochelatin synthase. The invention also relates to the construction of a recombinant DNA construct encoding all or a portion of the phytochelatin synthase, in sense or antisense orientation, wherein expression of the recombinant DNA construct results in production of altered levels of the phytochelatin synthase in a transformed host cell.



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TITLE

PHYTOCHELATIN SYNTHASE

This application claims the benefit of U.S. Provisional Application
5 No. 60/310,521, filed August 7, 2001, the entire content of which is herein
incorporated by reference.

FIELD OF THE INVENTION

This invention is in the field of plant molecular biology. More specifically, this
invention includes nucleic acid fragments encoding phytochelatin synthase in plants
10 and seeds.

BACKGROUND OF THE INVENTION

Metal ions such as magnesium, copper, zinc, manganese, nickel, and iron are
essential for plant growth, in processes that range from respiration to
photosynthesis, but deleterious when present in excess amounts. Others such as
15 cadmium, aluminum, and lead have no nutritional value and are toxic. When
present in large amount in the soil, metals interfere with the uptake of essential ions,
biosynthesis of chlorophyll and nucleic acids, and lipid metabolism, thus profoundly
affecting plant growth and development (Ouariti et al. (1997) *Phytochemistry*
45:1343-1350; Dykema et al. (1999) *Plant Mol Biol* 41:139-150).

20 With the necessity to regulate metal ion uptake and achieve metal ion
homeostasis, plants have evolved a series of metal transporters and various metal-
binding polypeptides and proteins. Metallothioneins and phytochelatins are
intracellular sulfur-rich low molecular weight polypeptides that chelate metal ions
such as cadmium, zinc, copper, and mercury, and are thought to play a role in
25 detoxification. More recently, a group of metal transporters, the *ZIP* gene family,
was identified in plants (Guerinot (2000) *Biochim Biophys Acta* 1465:190-198).
IRT1, the first *ZIP* gene to be identified, encodes a protein that is able to transport
iron, zinc, manganese, and cadmium (Rogers et al. (2000) *Proc Natl Acad Sci USA*
97:12356-12360).

30 A novel class of polypeptides that are capable of being isoprenylated and
binding metal ions such as copper, nickel, and zinc has also been recently
discovered (Dykema et al. (1999) *Plant Mol Biol* 41:139-150). These proteins
appear to be soluble, unlike most isoprenylated proteins which are membrane-
associated. In terms of structure, they share the CXXC metal-binding motifs, and
35 contain repetitive regions rich in the amino acids Pro, Lys, Asp, Glu, and Gly,
predicted to form alpha-helices. Preceding the carboxyl-terminus is a flexible region
of 30-70 amino acids enriched in the amino acids Pro, Ala, Tyr, and Gly, predicted

to form turns (Dykema et al. (1999) *Plant Mol Biol* 41:139-150). The eight amino acids proximal to the carboxyl-terminal isoprenylation CaaX motif are highly conserved, with a consensus sequence of FSDENPNA (SEQ ID NO:21) followed by the CaaX motif (Dykema et al. (1999) *Plant Mol Biol* 41:139-150).

5 Phytochelatins are a class of posttranslationally synthesized peptides ((γ -Glu-Cys)_n-Xaa polymers, where n is 2-11) whose synthesis from glutathione is promoted by heavy metals. They bind heavy metals such as cadmium with high affinity and localize themselves bound with the heavy metal ions to the cell vacuoles, thus playing a role in detoxification. Their synthesis is mediated by the
10 enzyme phytochelatin synthase (γ -glutamylcysteine dipeptidyltranspeptidase, EC 2.3.2.15) using glutathione and related thiol tripeptides as substrate, via the net transfer of a γ -Glu-Cys unit from one thiol peptide to another or to a pre-existing phytochelatin molecule. Phytochelatin synthase is activated by heavy metals, a reflection more of the enzyme's requirement for glutathione-like peptides containing
15 blocked thiol groups for activity, rather than direct metal binding to the enzyme (Vatamaniuk et al. (2000) *J Biol Chem* 275:31451-31459). Nucleic acid fragments encoding phytochelatin synthase have been isolated from Arabidopsis, yeast, wheat, and worm (Ha et al. (1999) *Plant Cell* 11:1153-1163; Clemens et al. (1999) *EMBO J* 18:3325-3333; Vatamaniuk et al. (1999) *Proc Natl Acad Sci USA*
20 96:7110-7115; Vatamaniuk et al. (2000) *J Biol Chem* 275:31451-31459; Vatamaniuk et al. (2001) *J Biol Chem* 276:20817-20820).

It is apparent that by manipulating phytochelatin synthase expression, phytochelatin levels may be controlled, and consequently, heavy metal detoxification and tolerance by plants as well. Increasing phytochelatin synthase
25 expression may lead to increased heavy (transition) metal resistance which has a potential use as a selectable marker system for plant transformation studies. Also, plants may be engineered to grow in toxic metal rich soils or to remove pollutant metals from the environment through manipulating expression of phytochelatin synthase. Accordingly, the instant specification discloses nucleotide sequences
30 encoding phytochelatin synthase which may be used for the above mentioned applications.

SUMMARY OF THE INVENTION

The present invention concerns isolated polynucleotides comprising a nucleotide sequence encoding a polypeptide having phytochelatin synthase activity.
35 wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NOs:2, 12, 14, 16 or 18 have at least 70% sequence identity. It is preferred that the identity be at least 80%, it is more preferred that the identity is at

least 85%, it is even more preferred that the identity be at least 90%, it is even more preferred that the identity be at least 95%. The present invention also relates to isolated polynucleotides comprising the complement of the nucleotide sequence. More specifically, the present invention concerns isolated polynucleotides encoding the polypeptide sequence of SEQ ID NOs: 2, 12, 14, 16 or 18 or nucleotide sequences comprising the nucleotide sequence of SEQ ID NOs: 1, 11, 13, 15 or 17.

In a first embodiment, the present invention relates to an isolated polynucleotide comprising: (a) a first nucleotide sequence encoding a first polypeptide having phytochelatin synthase activity, wherein the amino acid sequence of the first polypeptide and the amino acid sequence of SEQ ID NO:12 or SEQ ID NO:18 have at least 70% sequence identity based on the ClustalV alignment method, (b) a second nucleotide sequence encoding a second polypeptide having phytochelatin synthase activity, wherein the amino acid sequence of the second polypeptide and the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:16 have at least 80% sequence identity based on the ClustalV alignment method, or (c) the complement of the nucleotide sequence of (a) or (b). The polypeptide preferably comprises the amino acid sequence of SEQ ID NO:12, 14, 16 or 18. The nucleotide sequence preferably comprises the nucleotide sequence of SEQ ID NOs:11, 13, 15 or 17. The polypeptide preferably has phytochelatin synthase activity.

In a second embodiment, the present invention concerns a recombinant DNA construct comprising any of the isolated polynucleotides of the present invention operably linked to at least one regulatory sequence, and a cell, a plant, and a seed comprising the recombinant DNA construct.

In a third embodiment, the present invention relates to a vector comprising any of the isolated polynucleotides of the present invention.

In a fourth embodiment, the present invention concerns a method for transforming a cell comprising transforming a cell with any of the isolated polynucleotides of the present invention, and the cell transformed by this method. Advantageously, the cell is eukaryotic, e.g., a yeast or plant cell, or prokaryotic, e.g., a bacterium.

In a fifth embodiment, the present invention relates to a method for producing a transgenic plant comprising transforming a plant cell with any of the isolated polynucleotides of the present invention and regenerating a plant from the transformed plant cell. The invention is also directed to the transgenic plant produced by this method, and seed obtained from this transgenic plant.

In a sixth embodiment, the present invention concerns a first nucleotide sequence which contains at least 30 nucleotides, and wherein the first nucleotide sequence is comprised by another polynucleotide, wherein the other polynucleotide includes: (a) a second nucleotide sequence, wherein the second nucleotide
5 sequence encodes a polypeptide having phytochelatin synthase activity, wherein the amino acid sequence of the polypeptide and the amino acid sequence of SEQ ID NOs:2, 12, 14, 16, or 18 having at least 80%, 85%, 90%, or 95% sequence identity, or (b) the complement of the second nucleotide sequence of (a).

In a seventh embodiment, the present invention relates to an isolated
10 polypeptide comprising an amino acid sequence having phytochelatin synthase activity, wherein the polypeptide comprises: (a) a first amino acid sequence, wherein the first amino acid sequence and the amino acid sequence of SEQ ID NO:12 or SEQ ID NO:18 have at least 70% sequence identity based on the ClustalV alignment method, or (b) a second amino acid sequence, wherein the second amino
15 acid sequence and the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:16 have at least 80% sequence identity based on the ClustalV alignment method. The first amino acid sequence preferably comprises the amino acid sequence of SEQ ID NOs:12 or 18, and the second amino acid sequence preferably comprises the amino acid sequence of SEQ ID NOs:14 or 16.

20 In an eighth embodiment, the invention concerns a method for isolating a polypeptide encoded by the polynucleotide of the present invention comprising isolating the polypeptide from a cell containing a recombinant DNA construct comprising the polynucleotide operably linked to at least one regulatory sequence.

In a ninth embodiment, this invention relates to a method for positive selection
25 of a transformed cell comprising: (a) transforming a host cell with the recombinant DNA construct of the present invention or an expression cassette of the present invention; and (b) growing the transformed host cell, preferably a plant cell, such as a monocot or a dicot, under conditions which allow expression of the phytochelatin synthase polynucleotide in an amount sufficient to complement a null mutant to
30 provide a positive selection means.

In a tenth embodiment, this invention concerns a method of altering the level of expression of a phytochelatin synthase protein in a host cell comprising:
(a) transforming a host cell with a recombinant DNA construct of the present invention; and (b) growing the transformed host cell under conditions that are
35 suitable for expression of the recombinant DNA construct wherein expression of the recombinant DNA construct results in production of altered levels of the phytochelatin synthase protein in the transformed host cell.

In an eleventh embodiment, this invention relates to a method for positive selection of a transformed cell comprising: (a) transforming a host cell, preferably a plant cell, such as a monocot or a dicot, with the recombinant DNA construct of the present invention or an expression cassette of the present invention; (b) exposing the transformed host cell to toxic or growth-inhibitory levels of metal ions, and (c) growing the transformed host cell under conditions which allow expression of the phytochelatin synthase polynucleotide in an amount sufficient to overcome the negative effects of metal ion exposure and provide a positive selection means.

BRIEF DESCRIPTION OF THE DRAWINGS AND SEQUENCE LISTING

The invention can be more fully understood from the following detailed description and the accompanying drawings and Sequence Listing which form a part of this application.

Figures 1A, 1B and 1C depict the amino acid sequence alignment of the following phytochelatin synthases: (a) SEQ ID NO:12, encoded by the nucleotide sequence derived from sunflower clone hss1c.pk019.p5 (SEQ ID NO:11), (b) SEQ ID NO:14, encoded by the nucleotide sequence corresponding to a contig (SEQ ID NO:13) of sequences derived from corn clones cpi1c.pk022.m11 (SEQ ID NO:1) and p0031.ccmbg14r (SEQ ID NO:3), (c) SEQ ID NO:16, encoded by the nucleotide sequence corresponding to a contig (SEQ ID NO:15) of sequences derived from rice clone rlsu0c.pk005.f18 (SEQ ID NO:7) and PCR fragment sequence, (d) SEQ ID NO:18, encoded by the nucleotide sequence corresponding to a contig (SEQ ID NO:17) of sequences derived from soybean clone sgs4c.pk002.g1 (SEQ ID NO:9), PCR fragment sequence and a published soybean EST sequence (NCBI General Identifier (GI) No. 17024112), (e) SEQ ID NO:19, from *Triticum aestivum* (NCBI GI No. 5757804) and (f) SEQ ID NO:20, from *Arabidopsis thaliana* (NCBI GI No. 5305736). Amino acids which are conserved among all and at least two sequences with an amino acid at that position are indicated with an asterisk (*). Dashes are used by the program to maximize alignment of the sequences. The consensus amino acid sequence is numbered below the alignment. The amino acid residues for each SEQ ID NO are given to the left of each line of sequence, and to the right of the last line of sequence.

Table 1 lists the polypeptides that are described herein, the designation of the cDNA clones that comprise the nucleic acid fragments encoding polypeptides representing all or a substantial portion of these polypeptides, and the corresponding identifier (SEQ ID NO:) as used in the attached Sequence Listing. Table 1 also identifies the cDNA clones as individual ESTs ("EST"), the sequences

of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), contigs assembled from two or more EST, FIS or PCR fragment sequences ("Contig"), or sequences encoding the entire protein or functionally active polypeptide derived from an FIS or a contig ("CGS"). The sequence descriptions and Sequence Listing attached hereto comply with the rules governing nucleotide and/or amino acid sequence disclosures in patent applications as set forth in 37 C.F.R. §1.821-1.825.

TABLE 1
Phytochelatin Synthase

Plant	Clone Designation	Status	SEQ ID NO:	
			(Nucleotide)	(Amino Acid)
Corn	cpi1c.pk022.m11 (FIS)	CGS	1	2
Corn	p0031.ccmbg14r	FIS	3	4
Rice	rca1n.pk015.j16	FIS	5	6
Rice	rlsu0c.pk005.f18	FIS	7	8
Soybean	sgs4c.pk002.g1	FIS	9	10
Sunflower	hss1c.pk019.p5 (FIS)	CGS	11	12
Corn	Contig of:	CGS	13	14
	cpi1c.pk022.m11 (FIS)			
	p0031.ccmbg14r (FIS)			
Rice	Contig of:	CGS	15	16
	rlsu0c.pk005.f18 (FIS)			
	PCR fragment sequence			
Soybean	Contig of:	CGS	17	18
	sgs4c.pk002.g1 (FIS)			
	PCR fragment sequence			
	GI No. 17024112			

SEQ ID NO:19 corresponds to the amino acid sequence of phytochelatin synthase from *Triticum aestivum* (NCBI GI No. 5757804).

SEQ ID NO:20 corresponds to the amino acid sequence of phytochelatin synthase from *Arabidopsis thaliana* (NCBI GI No. 5305736).

The Sequence Listing contains the one letter code for nucleotide sequence characters and the three letter codes for amino acids as defined in conformity with the IUPAC-IUBMB standards described in *Nucleic Acids Res.* 13:3021-3030 (1985) and in the *Biochemical J.* 219 (No. 2):345-373 (1984) which are herein incorporated by reference. The symbols and format used for nucleotide and amino acid sequence data comply with the rules set forth in 37 C.F.R. §1.822.

DETAILED DESCRIPTION OF THE INVENTION

The problem to be solved, therefore, was to identify polynucleotides that encode phytochelatin synthase proteins. These polynucleotides may be used in plant cells to alter metal ion accumulation in plants. More specifically, the polynucleotides of the instant invention may be used to create transgenic plants where the phytochelatin synthase levels are altered with respect to non-transgenic plants which would result in plants with increased heavy (transition) metal resistance which has a potential use as a selectable marker system for plant transformation studies. Also, plants may be engineered to grow in toxic metal rich soils or to remove pollutant metals from the environment through manipulating expression of phytochelatin synthase. The present invention provides polynucleotide and deduced polypeptide sequences corresponding to novel phytochelatin synthase genes from corn (*Zea mays*), rice (*Oryza sativa*), soybean (*Glycine max*) and sunflower (*Helianthus* sp.).

In the context of this disclosure, a number of terms shall be utilized. The terms "polynucleotide", "polynucleotide sequence", "nucleic acid sequence", and "nucleic acid fragment"/"isolated nucleic acid fragment" are used interchangeably herein. These terms encompass nucleotide sequences and the like. A polynucleotide may be a polymer of RNA or DNA that is single- or double-stranded, that optionally contains synthetic, non-natural or altered nucleotide bases. A polynucleotide in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA, synthetic DNA, or mixtures thereof. An isolated polynucleotide of the present invention may include at least 30 contiguous nucleotides, preferably at least 40 contiguous nucleotides, most preferably at least 60 contiguous nucleotides derived from SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15 or 17, or the complement of such sequences.

The term "isolated" refers to materials, such as nucleic acid molecules and/or proteins, which are substantially free or otherwise removed from components that normally accompany or interact with the materials in a naturally occurring environment. Isolated polynucleotides may be purified from a host cell in which they naturally occur. Conventional nucleic acid purification methods known to skilled artisans may be used to obtain isolated polynucleotides. The term also embraces recombinant polynucleotides and chemically synthesized polynucleotides.

The term "recombinant" means, for example, that a nucleic acid sequence is made by an artificial combination of two otherwise separated segments of sequence, e.g., by chemical synthesis or by the manipulation of isolated nucleic

acids by genetic engineering techniques. A "recombinant DNA construct" comprises any of the isolated polynucleotides of the present invention operably linked to at least one regulatory sequence. The term "recombinant DNA construct" also embraces an isolated polynucleotide comprising a region encoding all or part of a functional RNA and at least one of the naturally occurring regulatory sequences directing expression in the source (e.g., organism) from which the polynucleotide was isolated, such as, but not limited to, an isolated polynucleotide comprising a nucleotide sequence encoding a phytochelatin synthase and the corresponding promoter and 3' end sequences directing expression in the source from which sequences were isolated.

As used herein, "contig" refers to a nucleotide sequence that is assembled from two or more constituent nucleotide sequences that share common or overlapping regions of sequence homology. For example, the nucleotide sequences of two or more nucleic acid fragments can be compared and aligned in order to identify common or overlapping sequences. Where common or overlapping sequences exist between two or more nucleic acid fragments, the sequences (and thus their corresponding nucleic acid fragments) can be assembled into a single contiguous nucleotide sequence.

As used herein, "substantially similar" refers to nucleic acid fragments wherein changes in one or more nucleotide bases results in substitution of one or more amino acids, but do not affect the functional properties of the polypeptide encoded by the nucleotide sequence. "Substantially similar" also refers to nucleic acid fragments wherein changes in one or more nucleotide bases does not affect the ability of the nucleic acid fragment to mediate alteration of gene expression by gene silencing through for example antisense or co-suppression technology.

"Substantially similar" also refers to modifications of the nucleic acid fragments of the instant invention such as deletion or insertion of one or more nucleotides that do not substantially affect the functional properties of the resulting transcript vis-à-vis the ability to mediate gene silencing or alteration of the functional properties of the resulting protein molecule. It is therefore understood that the invention encompasses more than the specific exemplary nucleotide or amino acid sequences and includes functional equivalents thereof. The terms "substantially similar" and "corresponding substantially" are used interchangeably herein.

Substantially similar nucleic acid fragments may be selected by screening nucleic acid fragments representing subfragments or modifications of the nucleic acid fragments of the instant invention, wherein one or more nucleotides are substituted, deleted and/or inserted, for their ability to affect the level of the

polypeptide encoded by the unmodified nucleic acid fragment in a plant or plant cell. For example, a substantially similar nucleic acid fragment representing at least 30 contiguous nucleotides, preferably at least 40 contiguous nucleotides, most preferably at least 60 contiguous nucleotides derived from the instant nucleic acid fragment can be constructed and introduced into a plant or plant cell. The level of the polypeptide encoded by the unmodified nucleic acid fragment present in a plant or plant cell exposed to the substantially similar nucleic acid fragment can then be compared to the level of the polypeptide in a plant or plant cell that is not exposed to the substantially similar nucleic acid fragment.

For example, it is well known in the art that antisense suppression and co-suppression of gene expression may be accomplished using nucleic acid fragments representing less than the entire coding region of a gene, and by using nucleic acid fragments that do not share 100% sequence identity with the gene to be suppressed. Moreover, alterations in a nucleic acid fragment which result in the production of a chemically equivalent amino acid at a given site, but do not effect the functional properties of the encoded polypeptide, are well known in the art. Thus, a codon for the amino acid alanine, a hydrophobic amino acid, may be substituted by a codon encoding another less hydrophobic residue, such as glycine, or a more hydrophobic residue, such as valine, leucine, or isoleucine. Similarly, changes which result in substitution of one negatively charged residue for another, such as aspartic acid for glutamic acid, or one positively charged residue for another, such as lysine for arginine, can also be expected to produce a functionally equivalent product. Nucleotide changes which result in alteration of the N-terminal and C-terminal portions of the polypeptide molecule would also not be expected to alter the activity of the polypeptide. Each of the proposed modifications is well within the routine skill in the art, as is determination of retention of biological activity of the encoded products. Consequently, an isolated polynucleotide comprising a nucleotide sequence of at least 30 (preferably at least 40, most preferably at least 60) contiguous nucleotides derived from a nucleotide sequence of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15 or 17, and the complement of such nucleotide sequences may be used to affect the expression and/or function of a phytochelatase in a host cell. A method of using an isolated polynucleotide to affect the level of expression of a polypeptide in a host cell (eukaryotic, such as plant or yeast, prokaryotic such as bacterial) may comprise the steps of: constructing an isolated polynucleotide of the present invention or an isolated recombinant DNA construct of the present invention; introducing the isolated polynucleotide or the isolated recombinant DNA construct into a host cell; measuring the level of a polypeptide or enzyme activity in

the host cell containing the isolated polynucleotide; and comparing the level of a polypeptide or enzyme activity in the host cell containing the isolated polynucleotide with the level of a polypeptide or enzyme activity in a host cell that does not contain the isolated polynucleotide.

5 Moreover, substantially similar nucleic acid fragments may also be characterized by their ability to hybridize. Estimates of such homology are provided by either DNA-DNA or DNA-RNA hybridization under conditions of stringency as is well understood by those skilled in the art (Hames and Higgins, Eds. (1985) Nucleic Acid Hybridisation, IRL Press, Oxford, U.K.). Stringency conditions can be adjusted
10 to screen for moderately similar fragments, such as homologous sequences from distantly related organisms, to highly similar fragments, such as genes that duplicate functional enzymes from closely related organisms. Post-hybridization washes determine stringency conditions. One set of preferred conditions uses a series of washes starting with 6X SSC, 0.5% SDS at room temperature for 15 min, then
15 repeated with 2X SSC, 0.5% SDS at 45°C for 30 min, and then repeated twice with 0.2X SSC, 0.5% SDS at 50°C for 30 min. A more preferred set of stringent conditions uses higher temperatures in which the washes are identical to those above except for the temperature of the final two 30 min washes in 0.2X SSC, 0.5% SDS was increased to 60°C. Another preferred set of highly stringent conditions
20 uses two final washes in 0.1X SSC, 0.1% SDS at 65°C.

Substantially similar nucleic acid fragments of the instant invention may also be characterized by the percent identity of the amino acid sequences that they encode to the amino acid sequences disclosed herein, as determined by algorithms commonly employed by those skilled in this art. Suitable nucleic acid fragments
25 (isolated polynucleotides of the present invention) encode polypeptides that are at least 70% identical, preferably at least 80% identical to the amino acid sequences reported herein. Preferred nucleic acid fragments encode amino acid sequences that are at least 85% identical to the amino acid sequences reported herein. More preferred nucleic acid fragments encode amino acid sequences that are at least
30 90% identical to the amino acid sequences reported herein. Most preferred are nucleic acid fragments that encode amino acid sequences that are at least 95% identical to the amino acid sequences reported herein. Suitable nucleic acid fragments not only have the above identities but typically encode a polypeptide having at least 50 amino acids, preferably at least 100 amino acids, more preferably
35 at least 150 amino acids, still more preferably at least 200 amino acids, and most preferably at least 250 amino acids.

It is well understood by one skilled in the art that many levels of sequence identity are useful in identifying related polypeptide sequences. Useful examples of percent identities are 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, or 95%, or any integer percentage from 55% to 100%. Sequence alignments and percent
5 identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the ClustalV method of alignment (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default
10 parameters for pairwise alignments using the ClustalV method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

A "substantial portion" of an amino acid or nucleotide sequence comprises an amino acid or a nucleotide sequence that is sufficient to afford putative identification of the protein or gene that the amino acid or nucleotide sequence comprises.
15 Amino acid and nucleotide sequences can be evaluated either manually by one skilled in the art, or by using computer-based sequence comparison and identification tools that employ algorithms such as BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see also the explanation of the BLAST algorithm on the world wide web site for the National
20 Center for Biotechnology Information at the National Library of Medicine of the National Institutes of Health). In general, a sequence of ten or more contiguous amino acids or thirty or more contiguous nucleotides is necessary in order to putatively identify a polypeptide or nucleic acid sequence as homologous to a known protein or gene. Moreover, with respect to nucleotide sequences, gene-
25 specific oligonucleotide probes comprising 30 or more contiguous nucleotides may be used in sequence-dependent methods of gene identification (e.g., Southern hybridization) and isolation (e.g., *in situ* hybridization of bacterial colonies or bacteriophage plaques). In addition, short oligonucleotides of 12 or more nucleotides may be used as amplification primers in PCR in order to obtain a
30 particular nucleic acid fragment comprising the primers. Accordingly, a "substantial portion" of a nucleotide sequence comprises a nucleotide sequence that will afford specific identification and/or isolation of a nucleic acid fragment comprising the sequence. The instant specification teaches amino acid and nucleotide sequences encoding polypeptides that comprise one or more particular plant proteins. The
35 skilled artisan, having the benefit of the sequences as reported herein, may now use all or a substantial portion of the disclosed sequences for purposes known to those skilled in this art. Accordingly, the instant invention comprises the complete

sequences as reported in the accompanying Sequence Listing, as well as substantial portions of those sequences as defined above.

“Codon degeneracy” refers to divergence in the genetic code permitting variation of the nucleotide sequence without effecting the amino acid sequence of an encoded polypeptide. Accordingly, the instant invention relates to any nucleic acid fragment comprising a nucleotide sequence that encodes all or a substantial portion of the amino acid sequences set forth herein. The skilled artisan is well aware of the “codon-bias” exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. Therefore, when synthesizing a nucleic acid fragment for improved expression in a host cell, it is desirable to design the nucleic acid fragment such that its frequency of codon usage approaches the frequency of preferred codon usage of the host cell.

“Synthetic nucleic acid fragments” can be assembled from oligonucleotide building blocks that are chemically synthesized using procedures known to those skilled in the art. These building blocks are ligated and annealed to form larger nucleic acid fragments which may then be enzymatically assembled to construct the entire desired nucleic acid fragment. “Chemically synthesized”, as related to a nucleic acid fragment, means that the component nucleotides were assembled *in vitro*. Manual chemical synthesis of nucleic acid fragments may be accomplished using well established procedures, or automated chemical synthesis can be performed using one of a number of commercially available machines. Accordingly, the nucleic acid fragments can be tailored for optimal gene expression based on optimization of the nucleotide sequence to reflect the codon bias of the host cell. The skilled artisan appreciates the likelihood of successful gene expression if codon usage is biased towards those codons favored by the host. Determination of preferred codons can be based on a survey of genes derived from the host cell where sequence information is available.

“Gene” refers to a nucleic acid fragment that expresses a specific protein, including regulatory sequences preceding (5' non-coding sequences) and following (3' non-coding sequences) the coding sequence. “Native gene” refers to a gene as found in nature with its own regulatory sequences. “Chimeric gene” refers any gene that is not a native gene, comprising regulatory and coding sequences that are not found together in nature. Accordingly, a chimeric gene may comprise regulatory sequences and coding sequences that are derived from different sources, or regulatory sequences and coding sequences derived from the same source, but arranged in a manner different than that found in nature. “Endogenous gene” refers to a native gene in its natural location in the genome of an organism. A “foreign-

gene" refers to a gene not normally found in the host organism, but that is introduced into the host organism by gene transfer. Foreign genes can comprise native genes inserted into a non-native organism, recombinant DNA constructs, or chimeric genes. A "transgene" is a recombinant DNA construct that has been
5 introduced into the genome by a transformation procedure.

"Coding sequence" refers to a nucleotide sequence that codes for a specific amino acid sequence. "Regulatory sequences" refer to nucleotide sequences located upstream (5' non-coding sequences), within, or downstream (3' non-coding sequences) of a coding sequence, and which influence the transcription, RNA
10 processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

"Promoter" refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence
15 is located 3' to a promoter sequence. The promoter sequence consists of proximal and more distal upstream elements, the latter elements often referred to as enhancers. Accordingly, an "enhancer" is a nucleotide sequence which can stimulate promoter activity and may be an innate element of the promoter or a heterologous element inserted to enhance the level or tissue-specificity of a
20 promoter. Promoters may be derived in their entirety from a native gene, or may be composed of different elements derived from different promoters found in nature, or may even comprise synthetic nucleotide segments. It is understood by those skilled in the art that different promoters may direct the expression of a gene in different tissues or cell types, or at different stages of development, or in response to
25 different environmental conditions. Promoters which cause a nucleic acid fragment to be expressed in most cell types at most times are commonly referred to as "constitutive promoters". New promoters of various types useful in plant cells are constantly being discovered; numerous examples may be found in the compilation by Okamuro and Goldberg (1989) *Biochemistry of Plants* 15:1-82. It is further
30 recognized that since in most cases the exact boundaries of regulatory sequences have not been completely defined, nucleic acid fragments of different lengths may have identical promoter activity.

"Translation leader sequence" refers to a nucleotide sequence located between the promoter sequence of a gene and the coding sequence. The
35 translation leader sequence is present in the fully processed mRNA upstream of the translation start sequence. The translation leader sequence may affect processing of the primary transcript to mRNA, mRNA stability or translation efficiency.

Examples of translation leader sequences have been described (Turner and Foster (1995) *Mol. Biotechnol.* 3:225-236).

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

“RNA transcript” refers to the product resulting from RNA polymerase-catalyzed transcription of a DNA sequence. When the RNA transcript is a perfect complementary copy of the DNA sequence, it is referred to as the primary transcript or it may be a RNA sequence derived from posttranscriptional processing of the primary transcript and is referred to as the mature RNA. “Messenger RNA (mRNA)” refers to the RNA that is without introns and that can be translated into polypeptides by the cell. “cDNA” refers to DNA that is complementary to and derived from an mRNA template. The cDNA can be single-stranded or converted to double stranded form using, for example, the Klenow fragment of DNA polymerase I. “Sense-RNA” refers to an RNA transcript that includes the mRNA and so can be translated into a polypeptide by the cell. “Antisense RNA” refers to an RNA transcript that is complementary to all or part of a target primary transcript or mRNA and that blocks the expression of a target gene (see U.S. Patent No. 5,107,065, incorporated herein by reference). The complementarity of an antisense RNA may be with any part of the specific nucleotide sequence, i.e., at the 5' non-coding sequence, 3' non-coding sequence, introns, or the coding sequence. “Functional RNA” refers to sense RNA, antisense RNA, ribozyme RNA, or other RNA that may not be translated but yet has an effect on cellular processes.

The term “operably linked” refers to the association of two or more nucleic acid fragments on a single polynucleotide so that the function of one is affected by the other. For example, a promoter is operably linked with a coding sequence when it is capable of affecting the expression of that coding sequence (i.e., that the coding sequence is under the transcriptional control of the promoter). Coding sequences can be operably linked to regulatory sequences in sense or antisense orientation.

The term “expression”, as used herein, refers to the transcription and stable accumulation of sense (mRNA) or antisense RNA derived from the nucleic acid fragment of the invention. Expression may also refer to translation of mRNA into a polypeptide. “Antisense inhibition” refers to the production of antisense RNA

transcripts capable of suppressing the expression of the target protein.

"Overexpression" refers to the production of a gene product in transgenic organisms that exceeds levels of production in normal or non-transformed organisms.

5 "Co-suppression" refers to the production of sense RNA transcripts capable of suppressing the expression of identical or substantially similar foreign or endogenous genes (U.S. Patent No. 5,231,020, incorporated herein by reference).

A "protein" or "polypeptide" is a chain of amino acids arranged in a specific order determined by the coding sequence in a polynucleotide encoding the polypeptide. Each protein or polypeptide has a unique function.

10 "Altered levels" or "altered expression" refers to the production of gene product(s) in transgenic organisms in amounts or proportions that differ from that of normal or non-transformed organisms.

"Mature protein" or the term "mature" when used in describing a protein refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed.

15 "Precursor protein" or the term "precursor" when used in describing a protein refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present. Pre- and propeptides may be but are not limited to intracellular localization signals.

20 A "chloroplast transit peptide" is an amino acid sequence which is translated in conjunction with a protein and directs the protein to the chloroplast or other plastid types present in the cell in which the protein is made. "Chloroplast transit sequence" refers to a nucleotide sequence that encodes a chloroplast transit peptide. A "signal peptide" is an amino acid sequence which is translated in
25 conjunction with a protein and directs the protein to the secretory system (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53). If the protein is to be directed to a vacuole, a vacuolar targeting signal (*supra*) can further be added, or if to the endoplasmic reticulum, an endoplasmic reticulum retention signal (*supra*) may be added. If the protein is to be directed to the nucleus, any signal peptide
30 present should be removed and instead a nuclear localization signal included (Raikhel (1992) *Plant Phys.* 100:1627-1632). A "mitochondrial signal peptide" is an amino acid sequence which directs a precursor protein into the mitochondria (Zhang and Glaser (2002) *Trends Plant Sci* 7:14-21).

35 "Transformation" refers to the transfer of a nucleic acid fragment into the genome of a host organism. Host organisms containing the transferred nucleic acid fragments are referred to as "transgenic" or "transformed" organisms. Examples of methods of plant transformation include *Agrobacterium*-mediated transformation (De

Blaere et al. (1987) *Meth. Enzymol.* 143:277; Ishida Y. et al. (1996) *Nature Biotech.* 14:745-750) and particle-accelerated or "gene gun" transformation technology (Klein et al. (1987) *Nature (London)* 327:70-73; U.S. Patent No. 4,945,050, incorporated herein by reference). Thus, isolated polynucleotides of the present invention can be
5 incorporated into recombinant constructs, typically DNA constructs, capable of introduction into and replication in a host cell. Such a construct can be a vector that includes a replication system and sequences that are capable of transcription and translation of a polypeptide-encoding sequence in a given host cell. A number of vectors suitable for stable transfection of plant cells or for the establishment of
10 transgenic plants have been described in, e.g., Pouwels et al., *Cloning Vectors: A Laboratory Manual*, 1985, supp. 1987; Weissbach and Weissbach, *Methods for Plant Molecular Biology*, Academic Press, 1989; and Flevin et al., *Plant Molecular Biology Manual*, Kluwer Academic Publishers, 1990. Typically, plant expression vectors include, for example, one or more cloned plant genes under the
15 transcriptional control of 5' and 3' regulatory sequences and a dominant selectable marker. Such plant expression vectors also can contain a promoter regulatory region (e.g., a regulatory region controlling inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific expression), a transcription initiation start site, a ribosome binding site, an RNA
20 processing signal, a transcription termination site, and/or a polyadenylation signal.

"Stable transformation" refers to the transfer of a nucleic acid fragment into a genome of a host organism, including both nuclear and organellar genomes, resulting in genetically stable inheritance. In contrast, "transient transformation" refers to the transfer of a nucleic acid fragment into the nucleus, or DNA-containing
25 organelle, of a host organism resulting in gene expression without integration or stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as "transgenic" or "transformed" organisms. The term "transformation" as used herein refers to both stable transformation and transient transformation.

30 The terms "recombinant construct", "expression construct" and "recombinant expression construct" are used interchangeably herein. These terms refer to a functional unit of genetic material that can be inserted into the genome of a cell using standard methodology well known to one skilled in the art. Such construct may be used by itself or may be used in conjunction with a vector. If a vector is
35 used, the choice of vector is dependent upon the method that will be used to transform host plants as is well known to those skilled in the art. .

Standard recombinant DNA and molecular cloning techniques used herein are well known in the art and are described more fully in Sambrook et al. *Molecular Cloning: A Laboratory Manual*; Cold Spring Harbor Laboratory Press: Cold Spring Harbor, 1989 (hereinafter "Maniatis").

5 "Motifs" or "subsequences" refer to short regions of conserved sequences of nucleic acids or amino acids that comprise part of a longer sequence. For example, it is expected that such conserved subsequences would be important for function, and could be used to identify new homologues in plants. It is expected that some or all of the elements may be found in a homologue. Also, it is expected that one or
10 two of the conserved amino acids in any given motif may differ in a true homologue.

"PCR" or "polymerase chain reaction" is well known by those skilled in the art as a technique used for the amplification of specific DNA segments (U.S. Patent Nos. 4,683,195 and 4,800,159).

The present invention concerns an isolated polynucleotide comprising a
15 nucleotide sequence encoding a phytochelatin synthase polypeptide having at least 70% or 80% sequence identity, based on the ClustalV method of alignment, when compared to a polypeptide of SEQ ID NOs:2, 4, 6, 8, 10, 12, 14, 16 or 18.

This invention also relates to the isolated complement of such polynucleotides, wherein the complement and the polynucleotide consist of the same number of
20 nucleotides, and the nucleotide sequences of the complement and the polynucleotide have 100% complementarity.

Nucleic acid fragments encoding at least a portion of several phytochelatin synthase protein have been isolated and identified by comparison of random plant cDNA sequences to public databases containing nucleotide and protein sequences
25 using the BLAST algorithms well known to those skilled in the art. The nucleic acid fragments of the instant invention may be used to isolate cDNAs and genes encoding homologous proteins from the same or other plant species. Isolation of homologous genes using sequence-dependent protocols is well known in the art. Examples of sequence-dependent protocols include, but are not limited to, methods
30 of nucleic acid hybridization, and methods of DNA and RNA amplification as exemplified by various uses of nucleic acid amplification technologies (e.g., polymerase chain reaction, ligase chain reaction).

For example, genes encoding other phytochelatin synthases, either as cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant
35 nucleic acid fragments as DNA hybridization probes to screen libraries from any desired plant employing methodology well known to those skilled in the art. Specific oligonucleotide probes based upon the instant nucleic acid sequences can be

designed and synthesized by methods known in the art (Maniatis). Moreover, an entire sequence can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling, nick translation, end-labeling techniques, or RNA probes using available *in vitro* transcription systems. In addition, specific primers can be designed and used to amplify a part or all of the instant sequences. The resulting amplification products can be labeled directly during amplification reactions or labeled after amplification reactions, and used as probes to isolate full length cDNA or genomic fragments under conditions of appropriate stringency.

In addition, two short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols to amplify longer nucleic acid fragments encoding homologous genes from DNA or RNA. The polymerase chain reaction may also be performed on a library of cloned nucleic acid fragments wherein the sequence of one primer is derived from the instant nucleic acid fragments, and the sequence of the other primer takes advantage of the presence of the polyadenylic acid tracts to the 3' end of the mRNA precursor encoding plant genes. Alternatively, the second primer sequence may be based upon sequences derived from the cloning vector. For example, the skilled artisan can follow the RACE protocol (Frohman et al. (1988) *Proc. Natl. Acad. Sci. USA* 85:8998-9002) to generate cDNAs by using PCR to amplify copies of the region between a single point in the transcript and the 3' or 5' end. Primers oriented in the 3' and 5' directions can be designed from the instant sequences. Using commercially available 3' RACE or 5' RACE systems (BRL), specific 3' or 5' cDNA fragments can be isolated (Ohara et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:5673-5677; Loh et al. (1989) *Science* 243:217-220). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques* 1:165). Consequently, a polynucleotide comprising a nucleotide sequence of at least 30 (preferably at least 40, most preferably at least 60) contiguous nucleotides derived from a nucleotide sequence of SEQ ID NOs:1, 3, 5, 7, 9, 11, 13, 15 or 17, and the complement of such nucleotide sequences, may be used in such methods to obtain a nucleic acid fragment encoding a substantial portion of an amino acid sequence of a polypeptide.

Availability of the instant nucleotide and deduced amino acid sequences facilitates immunological screening of cDNA expression libraries. Synthetic peptides representing portions of the instant amino acid sequences may be synthesized. These peptides can be used to immunize animals to produce polyclonal or monoclonal antibodies with specificity for peptides or proteins

comprising the amino acid sequences. These antibodies can be then be used to screen cDNA expression libraries to isolate full-length cDNA clones of interest (Lerner (1984) *Adv. Immunol.* 36:1-34; Maniatis).

In another embodiment, this invention concerns viruses and host cells
5 comprising either the recombinant DNA constructs of the invention as described herein or isolated polynucleotides of the invention as described herein. Examples of host cells which can be used to practice the invention include, but are not limited to, yeast, bacteria, and plants.

As was noted above, the nucleic acid fragments of the instant invention may
10 be used to create transgenic plants in which the disclosed polypeptides are present at higher or lower levels than normal or in cell types or developmental stages in which they are not normally found. This would have the effect of altering the level of phytochelatin synthase in those cells. It is apparent that by manipulating phytochelatin synthase expression, phytochelatin levels may be controlled, and
15 consequently, heavy metal detoxification and tolerance by plants as well. Increasing phytochelatin synthase expression may lead to increased heavy (transition) metal resistance which has a potential use as a selectable marker system for plant transformation studies. Also, plants may be engineered to grow in toxic metal rich soils or to remove pollutant metals from the environment through
20 increased expression of phytochelatin synthase. Accordingly, the instant specification discloses nucleotide sequences encoding phytochelatin synthase which may be used for the above mentioned applications.

Overexpression of the proteins of the instant invention may be accomplished by first constructing a recombinant DNA construct in which the coding region is
25 operably linked to a promoter capable of directing expression of a gene in the desired tissues at the desired stage of development. The recombinant DNA construct may comprise promoter sequences and translation leader sequences derived from the same genes. Non-coding 3' sequences comprising transcription termination signals may also be provided. The instant recombinant DNA construct
30 may also comprise one or more introns in order to facilitate gene expression.

Plasmid vectors comprising the instant isolated polynucleotide(s) (or recombinant DNA construct(s)) may be constructed. The choice of plasmid vector is dependent upon the method that will be used to transform host plants. The skilled artisan is well aware of the genetic elements that must be present on the
35 plasmid vector in order to successfully transform, select and propagate host cells containing the recombinant DNA construct or chimeric gene. The skilled artisan will also recognize that different independent transformation events will result in

different levels and patterns of expression (Jones et al. (1985) *EMBO J.* 4:2411-2418; De Almeida et al. (1989) *Mol. Gen. Genetics* 218:78-86), and thus that multiple events must be screened in order to obtain lines displaying the desired expression level and pattern. Such screening may be accomplished by Southern
5 analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, or phenotypic analysis.

For some applications it may be useful to direct the instant polypeptides to different cellular compartments, or to facilitate its secretion from the cell (Economou (1999) *Trends Microbiol.* 7:315-320; Fernandez et al. (2000) *Appl. Environ. Microbiol.* 66:5024-5029; Kjeldsen et al. (2002) *J. Biol. Chem.* 277:18245-18248; U.S. Patent No. 6,348,344). It is thus envisioned that the recombinant DNA construct(s) described above may be further supplemented by directing the coding sequence to encode the instant polypeptides with appropriate intracellular targeting sequences such as transit sequences (Keegstra (1989) *Cell* 56:247-253), signal
15 sequences or sequences encoding endoplasmic reticulum localization (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53), nuclear localization signals (Raikhel (1992) *Plant Phys.* 100:1627-1632) or mitochondrial signal sequences (Zhang and Glaser (2002) *Trends Plant Sci* 7:14-21) with or without removing targeting sequences that are already present. While the references cited give
20 examples of each of these, the list is not exhaustive and more targeting signals of use may be discovered in the future.

It may also be desirable to reduce or eliminate expression of genes encoding the instant polypeptides in plants for some applications. In order to accomplish this, a recombinant DNA construct designed for co-suppression of the instant
25 polypeptide can be constructed by linking a gene or gene fragment encoding that polypeptide to plant promoter sequences. Alternatively, a recombinant DNA construct designed to express antisense RNA for all or part of the instant nucleic acid fragment can be constructed by linking the gene or gene fragment in reverse orientation to plant promoter sequences. Either the co-suppression or antisense
30 recombinant DNA constructs could be introduced into plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

Molecular genetic solutions to the generation of plants with altered gene expression have a decided advantage over more traditional plant breeding
35 approaches. Changes in plant phenotypes can be produced by specifically inhibiting expression of one or more genes by antisense inhibition or cosuppression (U.S. Patent Nos. 5,190,931, 5,107,065 and 5,283,323). An antisense or

cosuppression construct would act as a dominant negative regulator of gene activity. While conventional mutations can yield negative regulation of gene activity these effects are most likely recessive. The dominant negative regulation available with a transgenic approach may be advantageous from a breeding perspective. In addition, the ability to restrict the expression of a specific phenotype to the reproductive tissues of the plant by the use of tissue specific promoters may confer agronomic advantages relative to conventional mutations which may have an effect in all tissues in which a mutant gene is ordinarily expressed.

The person skilled in the art will know that special considerations are associated with the use of antisense or cosuppression technologies in order to reduce expression of particular genes. For example, the proper level of expression of sense or antisense genes may require the use of different recombinant DNA constructs utilizing different regulatory elements known to the skilled artisan. Once transgenic plants are obtained by one of the methods described above, it will be necessary to screen individual transgenics for those that most effectively display the desired phenotype. Accordingly, the skilled artisan will develop methods for screening large numbers of transformants. The nature of these screens will generally be chosen on practical grounds. For example, one can screen by looking for changes in gene expression by using antibodies specific for the protein encoded by the gene being suppressed, or one could establish assays that specifically measure enzyme activity. A preferred method will be one which allows large numbers of samples to be processed rapidly, since it will be expected that a large number of transformants will be negative for the desired phenotype.

In another embodiment, the present invention concerns a phytochelatin synthase polypeptide having an amino acid sequence that is at least 70 or 80% identical, based on the ClustalV method of alignment, to a polypeptide of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16 or 18.

The instant polypeptides (or portions thereof) may be produced in heterologous host cells, particularly in the cells of microbial hosts, and can be used to prepare antibodies to these proteins by methods well known to those skilled in the art. The antibodies are useful for detecting the polypeptides of the instant invention *in situ* in cells or *in vitro* in cell extracts. Preferred heterologous host cells for production of the instant polypeptides are microbial hosts. Microbial expression systems and expression vectors containing regulatory sequences that direct high level expression of foreign proteins are well known to those skilled in the art. Any of these could be used to construct a recombinant DNA construct for production of the instant polypeptides. This recombinant DNA construct could then be introduced into

appropriate microorganisms via transformation to provide high level expression of the encoded phytochelatin synthase. An example of a vector for high level expression of the instant polypeptides in a bacterial host is provided (Example 6).

5 All or a substantial portion of the polynucleotides of the instant invention may also be used as probes for genetically and physically mapping the genes that they are a part of, and used as markers for traits linked to those genes. Such information may be useful in plant breeding in order to develop lines with desired phenotypes. For example, the instant nucleic acid fragments may be used as restriction fragment length polymorphism (RFLP) markers. Southern blots
10 (Maniatis) of restriction-digested plant genomic DNA may be probed with the nucleic acid fragments of the instant invention. The resulting banding patterns may then be subjected to genetic analyses using computer programs such as MapMaker (Lander et al. (1987) *Genomics* 1:174-181) in order to construct a genetic map. In addition, the nucleic acid fragments of the instant invention may be used to probe Southern
15 blots containing restriction endonuclease-treated genomic DNAs of a set of individuals representing parent and progeny of a defined genetic cross. Segregation of the DNA polymorphisms is noted and used to calculate the position of the instant nucleic acid sequence in the genetic map previously obtained using this population (Botstein et al. (1980) *Am. J. Hum. Genet.* 32:314-331).

20 The production and use of plant gene-derived probes for use in genetic mapping is described in Bernatzky and Tanksley (1986) *Plant Mol. Biol. Reporter* 4:37-41. Numerous publications describe genetic mapping of specific cDNA clones using the methodology outlined above or variations thereof. For example, F2 intercross populations, backcross populations, randomly mated populations, near
25 isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art.

Nucleic acid probes derived from the instant nucleic acid sequences may also be used for physical mapping (i.e., placement of sequences on physical maps; see Hoheisel et al. In: *Nonmammalian Genomic Analysis: A Practical Guide*, Academic
30 press 1996, pp. 319-346, and references cited therein).

Nucleic acid probes derived from the instant nucleic acid sequences may be used in direct fluorescence *in situ* hybridization (FISH) mapping (Trask (1991) *Trends Genet.* 7:149-154). Although current methods of FISH mapping favor use of large clones (several kb to several hundred kb; see Laan et al. (1995) *Genome Res.*
35 5:13-20), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

A variety of nucleic acid amplification-based methods of genetic and physical mapping may be carried out using the instant nucleic acid sequences. Examples include allele-specific amplification (Kazazian (1989) *J. Lab. Clin. Med.* 11:95-96), polymorphism of PCR-amplified fragments (CAPS; Sheffield et al. (1993) *Genomics* 16:325-332), allele-specific ligation (Landegren et al. (1988) *Science* 241:1077-1080), nucleotide extension reactions (Sokolov (1990) *Nucleic Acid Res.* 18:3671), Radiation Hybrid Mapping (Walter et al. (1997) *Nat. Genet.* 7:22-28) and Happy Mapping (Dear and Cook (1989) *Nucleic Acid Res.* 17:6795-6807). For these methods, the sequence of a nucleic acid fragment is used to design and produce primer pairs for use in the amplification reaction or in primer extension reactions. The design of such primers is well known to those skilled in the art. In methods employing PCR-based genetic mapping, it may be necessary to identify DNA sequence differences between the parents of the mapping cross in the region corresponding to the instant nucleic acid sequence. This, however, is generally not necessary for mapping methods.

Loss of function mutant phenotypes may be identified for the instant cDNA clones either by targeted gene disruption protocols or by identifying specific mutants for these genes contained in a maize population carrying mutations in all possible genes (Ballinger and Benzer (1989) *Proc. Natl. Acad. Sci USA* 86:9402-9406; Koes et al. (1995) *Proc. Natl. Acad. Sci USA* 92:8149-8153; Bensen et al. (1995) *Plant Cell* 7:75-84). The latter approach may be accomplished in two ways. First, short segments of the instant nucleic acid fragments may be used in polymerase chain reaction protocols in conjunction with a mutation tag sequence primer on DNAs prepared from a population of plants in which Mutator transposons or some other mutation-causing DNA element has been introduced (see Bensen, *supra*). The amplification of a specific DNA fragment with these primers indicates the insertion of the mutation tag element in or near the plant gene encoding the instant polypeptide. Alternatively, the instant nucleic acid fragment may be used as a hybridization probe against PCR amplification products generated from the mutation population using the mutation tag sequence primer in conjunction with an arbitrary genomic site primer, such as that for a restriction enzyme site-anchored synthetic adaptor. With either method, a plant containing a mutation in the endogenous gene encoding the instant polypeptide can be identified and obtained. This mutant plant can then be used to determine or confirm the natural function of the instant polypeptide disclosed herein.

EXAMPLES

The present invention is further illustrated in the following Examples, in which parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred
 5 embodiments of the invention, are given by way of illustration only. From the above discussion and these Examples, one skilled in the art can ascertain the essential characteristics of this invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, various modifications of the invention in
 10 addition to those shown and described herein will be apparent to those skilled in the art from the foregoing description. Such modifications are also intended to fall within the scope of the appended claims.

The disclosure of each reference set forth herein is incorporated herein by reference in its entirety.

EXAMPLE 1

Composition of cDNA Libraries; Isolation and Sequencing of cDNA Clones

cDNA libraries representing mRNAs from various corn (*Zea mays*), rice (*Oryza sativa*), soybean (*Glycine max*), and sunflower (*Helianthus* sp.) tissues were prepared. The characteristics of the libraries are described below.

TABLE 2

cDNA Libraries from Corn, Rice, Soybean, and Sunflower

Library	Tissue	Clone
cpi1c	Corn Treated with Chemicals Related to Biochemical Compound Synthesis*	cpi1c.pk022.m11
hss1c	Sclerotinia Infected Sunflower Plant	hss1c.pk019.p5
p0031	Corn Shoot Culture	p0031.ccmbg14r
rca1n	Rice Callus**	rca1n.pk015.j16
rlsu0c	Rice Leaf	rlsu0c.pk005.f18
sgs4c	Soybean Seed 2 Days After Germination	sgs4c.pk002.g1

*Chemicals used included sorbitol, egosterol, taxifolin, methotrexate, D-mannose, D-galactose, alpha-amino adipic acid, and ancymidol, all of which are commercially
 25 available from Calbiochem-Novabiochem Corp. (1-800-628-8470).

**This library was normalized essentially as described in U.S. Patent No. 5,482,845, incorporated herein by reference.

cDNA libraries may be prepared by any one of many methods available. For
 30 example, the cDNAs may be introduced into plasmid vectors by first preparing the cDNA libraries in Uni-ZAP™ XR vectors according to the manufacturer's protocol

(Stratagene Cloning Systems, La Jolla, CA). The Uni-ZAP™ XR libraries are converted into plasmid libraries according to the protocol provided by Stratagene. Upon conversion, cDNA inserts will be contained in the plasmid vector pBluescript. In addition, the cDNAs may be introduced directly into precut Bluescript II SK(+) vectors (Stratagene) using T4 DNA ligase (New England Biolabs), followed by transfection into DH10B cells according to the manufacturer's protocol (GIBCO BRL Products). Once the cDNA inserts are in plasmid vectors, plasmid DNAs are prepared from randomly picked bacterial colonies containing recombinant pBluescript plasmids, or the insert cDNA sequences are amplified via polymerase chain reaction using primers specific for vector sequences flanking the inserted cDNA sequences. Amplified insert DNAs or plasmid DNAs are sequenced in dye-primer sequencing reactions to generate partial cDNA sequences (expressed sequence tags or "ESTs"; see Adams et al., (1991) *Science* 252:1651-1656). The resulting ESTs are analyzed using a Perkin Elmer Model 377 fluorescent sequencer.

Full-insert sequence (FIS) data is generated utilizing a modified transposition protocol. Clones identified for FIS are recovered from archived glycerol stocks as single colonies, and plasmid DNAs are isolated via alkaline lysis. Isolated DNA templates are reacted with vector primed M13 forward and reverse oligonucleotides in a PCR-based sequencing reaction and loaded onto automated sequencers. Confirmation of clone identification is performed by sequence alignment to the original EST sequence from which the FIS request is made.

Confirmed templates are transposed via the Primer Island transposition kit (PE Applied Biosystems, Foster City, CA) which is based upon the *Saccharomyces cerevisiae* Ty1 transposable element (Devine and Boeke (1994) *Nucleic Acids Res.* 22:3765-3772). The *in vitro* transposition system places unique binding sites randomly throughout a population of large DNA molecules. The transposed DNA is then used to transform DH10B electro-competent cells (Gibco BRL/Life Technologies, Rockville, MD) via electroporation. The transposable element contains an additional selectable marker (named DHFR; Fling and Richards (1983) *Nucleic Acids Res.* 11:5147-5158), allowing for dual selection on agar plates of only those subclones containing the integrated transposon. Multiple subclones are randomly selected from each transposition reaction, plasmid DNAs are prepared via alkaline lysis, and templates are sequenced (ABI Prism dye-terminator ReadyReaction mix) outward from the transposition event site, utilizing unique primers specific to the binding sites within the transposon.

Sequence data is collected (ABI Prism Collections) and assembled using Phred/Phrap (P. Green, University of Washington, Seattle). Phred/Phrap is a public domain software program which re-reads the ABI sequence data, re-calls the bases, assigns quality values, and writes the base calls and quality values into editable output files. The Phrap sequence assembly program uses these quality values to increase the accuracy of the assembled sequence contigs. Assemblies are viewed by the Consed sequence editor (D. Gordon, University of Washington, Seattle).

In some of the clones the cDNA fragment corresponds to a portion of the 3'-terminus of the gene and does not cover the entire open reading frame. In order to obtain the upstream information one of two different protocols are used. The first of these methods results in the production of a fragment of DNA containing a portion of the desired gene sequence while the second method results in the production of a fragment containing the entire open reading frame. Both of these methods use two rounds of PCR amplification to obtain fragments from one or more libraries. The libraries some times are chosen based on previous knowledge that the specific gene should be found in a certain tissue and some times are randomly-chosen. Reactions to obtain the same gene may be performed on several libraries in parallel or on a pool of libraries. Library pools are normally prepared using from 3 to 5 different libraries and normalized to a uniform dilution. In the first round of amplification both methods use a vector-specific (forward) primer corresponding to a portion of the vector located at the 5'-terminus of the clone coupled with a gene-specific (reverse) primer. The first method uses a sequence that is complementary to a portion of the already known gene sequence while the second method uses a gene-specific primer complementary to a portion of the 3'-untranslated region (also referred to as UTR). In the second round of amplification a nested set of primers is used for both methods. The resulting DNA fragment is ligated into a pBluescript vector using a commercial kit and following the manufacturer's protocol. This kit is selected from many available from several vendors including Invitrogen (Carlsbad, CA), Promega Biotech (Madison, WI), and Gibco-BRL (Gaithersburg, MD). The plasmid DNA is isolated by alkaline lysis method and submitted for sequencing and assembly using Phred/Phrap, as above.

EXAMPLE 2

Identification of cDNA Clones

cDNA clones encoding phytochelatin synthases were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see also the explanation of the BLAST algorithm on the world wide web site for the National Center for Biotechnology Information at the National

Library of Medicine of the National Institutes of Health) searches for similarity to sequences contained in the BLAST "nr" database (comprising all non-redundant GenBank CDS translations, sequences derived from the 3-dimensional structure Brookhaven Protein Data Bank, the last major release of the SWISS-PROT protein sequence database, EMBL, and DDBJ databases). The cDNA sequences obtained in Example 1 were analyzed for similarity to all publicly available DNA sequences contained in the "nr" database using the BLASTN algorithm provided by the National Center for Biotechnology Information (NCBI). The DNA sequences were translated in all reading frames and compared for similarity to all publicly available protein sequences contained in the "nr" database using the BLASTX algorithm (Gish and States (1993) *Nat. Genet.* 3:266-272) provided by the NCBI. For convenience, the P-value (probability) of observing a match of a cDNA sequence to a sequence contained in the searched databases merely by chance as calculated by BLAST are reported herein as "pLog" values, which represent the negative of the logarithm of the reported P-value. Accordingly, the greater the pLog value, the greater the likelihood that the cDNA sequence and the BLAST "hit" represent homologous proteins.

ESTs submitted for analysis are compared to the genbank database as described above. ESTs that contain sequences more 5- or 3-prime can be found by using the BLASTn algorithm (Altschul et al (1997) *Nucleic Acids Res.* 25:3389-3402.) against the Du Pont proprietary database comparing nucleotide sequences that share common or overlapping regions of sequence homology. Where common or overlapping sequences exist between two or more nucleic acid fragments, the sequences can be assembled into a single contiguous nucleotide sequence, thus extending the original fragment in either the 5 or 3 prime direction. Once the most 5-prime EST is identified, its complete sequence can be determined by Full Insert Sequencing as described in Example 1. Homologous genes belonging to different species can be found by comparing the amino acid sequence of a known gene (from either a proprietary source or a public database) against an EST database using the tBLASTn algorithm. The tBLASTn algorithm searches an amino acid query against a nucleotide database that is translated in all 6 reading frames. This search allows for differences in nucleotide codon usage between different species, and for codon degeneracy.

EXAMPLE 3

Characterization of cDNA Clones Encoding Phytochelatin Synthase

The BLASTX search using the EST sequences from clones listed in Table 3 revealed similarity of the polypeptides encoded by the cDNAs to phytochelatin

synthases from *Triticum aestivum* (NCBI General Identifier (GI) No. 5757804; SEQ ID NO:19) and *Arabidopsis thaliana* (NCBI GI No. 5305736; SEQ ID NO:20).

Shown in Table 3 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), the sequences of contigs assembled from two or more EST, FIS or PCR sequences ("Contig"), or sequences encoding an entire protein, or functionally active polypeptide, derived from an EST, FIS or a contig ("CGS"):

TABLE 3

BLAST Results for Sequences Encoding Polypeptides Homologous to Phytochelatin Synthase

Clone	Status	BLAST Results	
		NCBI GI No.	pLog Score
cpi1c.pk022.m11 (FIS)	CGS	5757804	>180.00
p0031.ccmbg14r	FIS	5757804	93.10
rca1n.pk015.j16	FIS	5757804	27.70
rlsu0c.pk005.f18	FIS	5757804	159.00
sgs4c.pk002.g1	FIS	5757804	110.00
hss1c.pk019.p5 (FIS)	CGS	5305736	>180.00

The cDNA insert in the clone, hss1c.pk019.p5, was found to encode the entire sunflower phytochelatin synthase gene (SEQ ID NO:11). The corresponding amino acid sequence, SEQ ID NO:12, is the open-reading frame encoded by nucleotides 106-1563 of SEQ ID NO:11.

A sequence alignment of the corn phytochelatin synthase sequence, SEQ ID NO:2, with the wheat phytochelatin synthase sequence, SEQ ID NO:19, indicated that SEQ ID NO:2 was missing approximately 32 amino acids relative to the wheat protein, at a position around 360 amino acids into the open-reading frame. A contig of SEQ ID NO:1 and SEQ ID NO:3 was assembled and is shown as SEQ ID NO:13. This contig contains a 93 nucleotide insert relative to the nucleotide sequence of SEQ ID NO:1, occurring between the two G residues at positions 1285 and 1286 of SEQ ID NO:1. The corresponding open-reading frame for SEQ ID NO:13, from nucleotides 202-1722, is given as SEQ ID NO:14. SEQ ID NO:14 contains 31 amino acids more than SEQ ID NO:2, and gives a better alignment (Figures 1A-1C) with the functionally active phytochelatin synthase from wheat (Clemens et al. (1999) *EMBO J* 18:3325-3333). The 93 nucleotide insert in SEQ ID NO:13 begins with GT and ends with AG, the standard dinucleotides seen at the beginning and end of introns. SEQ ID NO:2 may represent an alternatively spliced (or aberrantly spliced) version of phytochelatin synthase from corn.

The sequence of the cDNA insert in rlsu0c.pk005.f18 was found not to encode the entire rice phytochelatin synthase. Consequently, PCR-based methods well known in the art and described in Example 1 were employed to obtain the entire coding sequence for a full-length rice phytochelatin synthase (SEQ ID NO:15). The corresponding open-reading frame for SEQ ID NO:15, from nucleotides 119-1624, is given as SEQ ID NO:16.

The sequence of the cDNA insert in sgs4c.pk002.g1 was found to encode a portion of the soybean phytochelatin synthase. The sequence of the insert in sgs4c.pk002.g1 is similar to amino acids 6-256 of the wheat phytochelatin synthase (SEQ ID NO:19). Consequently, PCR-based methods well known in the art and described in Example 1 were employed to obtain the missing 3' terminal sequence. The full-insert sequence of sgs4c.pk002.g1, PCR fragment sequence of the 3' end of the gene, and publically available soybean EST GI No. 17024112 were combined into a contig to obtain the complete gene sequence of the soybean phytochelatin synthase (SEQ ID NO:17). Soybean EST GI No. 17024112 contributed the first 67 nucleotides of SEQ ID NO:17. The corresponding 499 amino acid open-reading frame of SEQ ID NO:17, from nucleotides 53-1549, is given as SEQ ID NO:18.

Shown in Table 4 are the BLASTX results for the complete gene sequences of phytochelatin synthase from corn, rice and soybean. Table 4 shows the similarity of the polypeptides encoded by the corn, rice and soybean contigs, to the phytochelatin synthase from *Triticum aestivum* (NCBI GI No. 5757804; SEQ ID NO:19).

TABLE 4

BLAST Results for Sequences Encoding Polypeptides Homologous to Wheat Phytochelatin Synthase (NCBI GI No. 5757804)

Sequence	Plant	Status	pLog Score
Contig of: cpi1c.pk022.m11 (FIS) p0031.ccmbg14r (FIS)	corn	CGS	>180
Contig of: rlsu0c.pk005.f18 (FIS) PCR fragment sequence	rice	CGS	>180
Contig of: sgs4c.pk002.g1 (FIS) PCR fragment sequence GI No. 17024112	soybean	CGS	158

Figures 1A, 1B and 1C present an alignment of the amino acid sequences set forth in SEQ ID NOs:12, 14, 16, 18, 19 (wheat; GI No. 5757804), and 20 (*Arabidopsis*; GI No. 5305736). The data in Table 5 represents a calculation of the percent sequence identities of the amino acid sequences set forth in SEQ ID NOs:2, 12, 14, 16 and 18, when compared to the phytochelatin synthases from *Triticum aestivum* (NCBI GI No. 5757804; SEQ ID NO:19) and *Arabidopsis thaliana* (NCBI GI No. 5305736; SEQ ID NO:20).

TABLE 5

Percent Identity of Amino Acid Sequences Deduced from the Nucleotide Sequences Encoding Polypeptides Homologous to Phytochelatin Synthase

Sequence	SEQ ID NO.	% Identity to GI No. 5757804	% Identity to GI No. 5305736
cpi1c.pk022.m11 (FIS)	2	73.7	52.9
hss1c.pk019.p5 (FIS)	12	57.6	62.7
Contig of:	14	75.6	56.1
cpi1c.pk022.m11 (FIS)			
p0031.ccmbg14r (FIS)			
Contig of:	16	76.6	56.3
rlsu0c.pk005.f18 (FIS)			
PCR fragment sequence			
Contig of:	18	53.3	52.6
sgs4c.pk002.g1 (FIS)			
PCR fragment sequence			
GI No. 17024112			

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the ClustalV method of alignment (Higgins and Sharp (1989) *CABIOS*. 5:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the ClustalV method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode the complete phytochelatin synthase from corn, rice, soybean and sunflower. A sequence for a different phytochelatin synthase gene from soybean has recently been described (GI No. 18699092; Oven et al. (2002) *J. Biol. Chem.* 277:4747-

4754). The soybean phytochelatase synthase of SEQ ID NO:18 has 56.4% sequence identity with the amino acid sequence of GI No. 18699092.

EXAMPLE 4

Expression of Recombinant DNA Constructs in Monocot Cells

5 A recombinant DNA construct comprising a cDNA encoding the instant polypeptide in sense orientation with respect to the maize 27 kD zein promoter that is located 5' to the cDNA fragment, and the 10 kD zein 3' end that is located 3' to the cDNA fragment, can be constructed. The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone, plant cDNA or
10 plant cDNA libraries, using appropriate oligonucleotide primers. Cloning sites (NcoI or SmaI) can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the digested vector pML103 as described below. Amplification is then performed in a standard PCR. The amplified DNA is then digested with restriction enzymes NcoI and SmaI and fractionated on an
15 agarose gel. The appropriate band can be isolated from the gel and combined with a 4.9 kb NcoI-SmaI fragment of the plasmid pML103. Plasmid pML103 has been deposited under the terms of the Budapest Treaty at ATCC (American Type Culture Collection, 10801 University Blvd., Manassas, VA 20110-2209), and bears accession number ATCC 97366. The DNA segment from pML103 contains a
20 1.05 kb SmaI-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-SmaI fragment from the 3' end of the maize 10 kD zein gene in the vector pGem9Zf(+) (Promega). Vector and insert DNA can be ligated at 15°C overnight, essentially as described (Maniatis). The ligated DNA may then be used to transform *E. coli* XL1-Blue (Epicurian Coli XL-1 Blue™; Stratagene). Bacterial
25 transformants can be screened by restriction enzyme digestion of plasmid DNA and limited nucleotide sequence analysis using the dideoxy chain termination method (Sequenase™ DNA Sequencing Kit; U.S. Biochemical). The resulting plasmid construct would comprise a recombinant DNA construct encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding the instant
30 polypeptide, and the 10 kD zein 3' region.

The recombinant DNA construct described above can then be introduced into corn cells by the following procedure. Immature corn embryos can be dissected from developing caryopses derived from crosses of the inbred corn lines H99 and LH132. The embryos are isolated 10 to 11 days after pollination when they are 1.0
35 to 1.5 mm long. The embryos are then placed with the axis-side facing down and in contact with agarose-solidified N6 medium (Chu et al. (1975) *Sci. Sin. Peking* 18:659-668). The embryos are kept in the dark at 27°C. Friable embryogenic

callus consisting of undifferentiated masses of cells with somatic proembryoids and embryoids borne on suspensor structures proliferates from the scutellum of these immature embryos. The embryogenic callus isolated from the primary explant can be cultured on N6 medium and sub-cultured on this medium every 2 to 3 weeks.

5 The plasmid, p35S/Ac (obtained from Dr. Peter Eckes, Hoechst Ag, Frankfurt, Germany) may be used in transformation experiments in order to provide for a selectable marker. This plasmid contains the *Pat* gene (see European Patent Publication 0 242 236) which encodes phosphinothricin acetyl transferase (PAT). The enzyme PAT confers resistance to herbicidal glutamine synthetase inhibitors
10 such as phosphinothricin. The *pat* gene in p35S/Ac is under the control of the 35S promoter from cauliflower mosaic virus (Odell et al. (1985) *Nature* 313:810-812) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*.

The particle bombardment method (Klein et al. (1987) *Nature* 327:70-73) may
15 be used to transfer genes to the callus culture cells. According to this method, gold particles (1 μ m in diameter) are coated with DNA using the following technique. Ten μ g of plasmid DNAs are added to 50 μ L of a suspension of gold particles (60 mg per mL). Calcium chloride (50 μ L of a 2.5 M solution) and spermidine free base (20 μ L of a 1.0 M solution) are added to the particles. The suspension is
20 vortexed during the addition of these solutions. After 10 minutes, the tubes are briefly centrifuged (5 sec at 15,000 rpm) and the supernatant removed. The particles are resuspended in 200 μ L of absolute ethanol, centrifuged again and the supernatant removed. The ethanol rinse is performed again and the particles resuspended in a final volume of 30 μ L of ethanol. An aliquot (5 μ L) of the DNA-
25 coated gold particles can be placed in the center of a Kapton™ flying disc (Bio-Rad Labs). The particles are then accelerated into the corn tissue with a Biolistic™ PDS-1000/He (Bio-Rad Instruments, Hercules CA), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

For bombardment, the embryogenic tissue is placed on filter paper over
30 agarose-solidified N6 medium. The tissue is arranged as a thin lawn and covered a circular area of about 5 cm in diameter. The petri dish containing the tissue can be placed in the chamber of the PDS-1000/He approximately 8 cm from the stopping screen. The air in the chamber is then evacuated to a vacuum of 28 inches of Hg. The macrocarrier is accelerated with a helium shock wave using a rupture
35 membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

Seven days after bombardment the tissue can be transferred to N6 medium that contains bialaphos (5 mg per liter) and lacks casein or proline. The tissue

continues to grow slowly on this medium. After an additional 2 weeks the tissue can be transferred to fresh N6 medium containing bialophos. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the bialophos-supplemented medium. These calli may continue to grow when sub-cultured on the selective medium.

Plants can be regenerated from the transgenic callus by first transferring clusters of tissue to N6 medium supplemented with 0.2 mg per liter of 2,4-D. After two weeks the tissue can be transferred to regeneration medium (Fromm et al. (1990) *Bio/Technology* 8:833-839).

EXAMPLE 5

Expression of Recombinant DNA Constructs in Dicot Cells

A seed-specific expression cassette composed of the promoter and transcription terminator from the gene encoding the β subunit of the seed storage protein phaseolin from the bean *Phaseolus vulgaris* (Doyle et al. (1986) *J. Biol. Chem.* 261:9228-9238) can be used for expression of the instant polypeptides in transformed soybean. The phaseolin cassette includes about 500 nucleotides upstream (5') from the translation initiation codon and about 1650 nucleotides downstream (3') from the translation stop codon of phaseolin. Between the 5' and 3' regions are the unique restriction endonuclease sites NcoI (which includes the ATG translation initiation codon), SmaI, KpnI and XbaI. The entire cassette is flanked by HindIII sites.

The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone, plant cDNA or plant cDNA libraries, using appropriate oligonucleotide primers. Cloning sites can be incorporated into the oligonucleotides to provide proper orientation of the DNA fragment when inserted into the expression vector. Amplification is then performed as described above, and the isolated fragment is inserted into a pUC18 vector carrying the seed expression cassette.

Soybean embryos may then be transformed with the expression vector comprising sequences encoding the instant polypeptide. To induce somatic embryos, cotyledons, 3-5 mm in length dissected from surface sterilized, immature seeds of the soybean cultivar A2872, can be cultured in the light or dark at 26°C on an appropriate agar medium for 6-10 weeks. Somatic embryos which produce secondary embryos are then excised and placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos which multiplied as early, globular staged embryos, the suspensions are maintained as described below.

Soybean embryogenic suspension cultures can be maintained in 35 mL liquid media on a rotary shaker, 150 rpm, at 26°C with florescent lights on a 16:8 hour day/night schedule. Cultures are subcultured every two weeks by inoculating approximately 35 mg of tissue into 35 mL of liquid medium.

5 Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Klein et al. (1987) *Nature* (London) 327:70-73, U.S. Patent No. 4,945,050). A DuPont Biolistic™ PDS1000/HE instrument (helium retrofit) can be used for these transformations.

10 A selectable marker gene which can be used to facilitate soybean transformation is a chimeric gene composed of the 35S promoter from cauliflower mosaic virus (Odell et al. (1985) *Nature* 313:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from *E. coli*; Gritz et al. (1983) *Gene* 25:179-188) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The seed expression cassette
15 comprising the phaseolin 5' region, the fragment encoding the instant polypeptide and the phaseolin 3' region can be isolated as a restriction fragment. This fragment can then be inserted into a unique restriction site of the vector carrying the marker gene.

To 50 µL of a 60 mg/mL 1 µm gold particle suspension is added (in order):
20 5 µL DNA (1 µg/µL), 20 µL spermidine (0.1 M), and 50 µL CaCl₂ (2.5 M). The particle preparation is then agitated for three minutes, spun in a microfuge for 10 seconds and the supernatant removed. The DNA-coated particles are then washed once in 400 µL 70% ethanol and resuspended in 40 µL of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second
25 each. Five µL of the DNA-coated gold particles are then loaded on each macro carrier disk.

Approximately 300-400 mg of a two-week-old suspension culture is placed in an empty 60x15 mm petri dish and the residual liquid removed from the tissue with a pipette. For each transformation experiment, approximately 5-10 plates of tissue
30 are normally bombarded. Membrane rupture pressure is set at 1100 psi and the chamber is evacuated to a vacuum of 28 inches mercury. The tissue is placed approximately 3.5 inches away from the retaining screen and bombarded three times. Following bombardment, the tissue can be divided in half and placed back into liquid and cultured as described above.

35 Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post bombardment with fresh media containing 50 mg/mL hygromycin. This selective media can be refreshed weekly.

Seven to eight weeks post bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into individual flasks to generate new, clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or regenerated into whole plants by maturation and germination of individual somatic embryos.

EXAMPLE 6

Expression of Recombinant DNA Constructs in Microbial Cells

The cDNA fragment of the gene may be generated by polymerase chain reaction (PCR) of the cDNA clone, plant cDNA or plant cDNA libraries, using appropriate oligonucleotide primers. The cDNAs encoding the instant polypeptides can be inserted into the T7 *E. coli* expression vector pBT430. This vector is a derivative of pET-3a (Rosenberg et al. (1987) *Gene* 56:125-135) which employs the bacteriophage T7 RNA polymerase/T7 promoter system. Plasmid pBT430 was constructed by first destroying the EcoRI and HindIII sites in pET-3a at their original positions. An oligonucleotide adaptor containing EcoRI and Hind III sites was inserted at the BamHI site of pET-3a. This created pET-3aM with additional unique cloning sites for insertion of genes into the expression vector. Then, the NdeI site at the position of translation initiation was converted to an NcoI site using oligonucleotide-directed mutagenesis. The DNA sequence of pET-3aM in this region, 5'-CATATGG, was converted to 5'-CCCATGG in pBT430.

Plasmid DNA containing a cDNA may be appropriately digested to release a nucleic acid fragment encoding the protein. This fragment may then be purified on a 1% low melting agarose gel. Buffer and agarose contain 10 µg/ml ethidium bromide for visualization of the DNA fragment. The fragment can then be purified from the agarose gel by digestion with GELase™ (Epicentre Technologies, Madison, WI) according to the manufacturer's instructions, ethanol precipitated, dried and resuspended in 20 µL of water. Appropriate oligonucleotide adapters may be ligated to the fragment using T4 DNA ligase (New England Biolabs (NEB), Beverly, MA). The fragment containing the ligated adapters can be purified from the excess adapters using low melting agarose as described above. The vector pBT430 is digested, dephosphorylated with alkaline phosphatase (NEB) and deproteinized with phenol/chloroform as described above. The prepared vector pBT430 and fragment can then be ligated at 16°C for 15 hours followed by transformation into DH5 electrocompetent cells (GIBCO BRL). Transformants can be selected on agar

plates containing LB media and 100 µg/mL ampicillin. Transformants containing the gene encoding the instant polypeptide are then screened for the correct orientation with respect to the T7 promoter by restriction enzyme analysis.

For high level expression, a plasmid clone with the cDNA insert in the correct orientation relative to the T7 promoter can be transformed into *E. coli* strain BL21(DE3) (Studier et al. (1986) *J. Mol. Biol.* 189:113-130). Cultures are grown in LB medium containing ampicillin (100 mg/L) at 25°C. At an optical density at 600 nm of approximately 1, IPTG (isopropylthio-β-galactoside, the inducer) can be added to a final concentration of 0.4 mM and incubation can be continued for 3 h at 25°. Cells are then harvested by centrifugation and re-suspended in 50 µL of 50 mM Tris-HCl at pH 8.0 containing 0.1 mM DTT and 0.2 mM phenyl methylsulfonyl fluoride. A small amount of 1 mm glass beads can be added and the mixture sonicated 3 times for about 5 seconds each time with a microprobe sonicator. The mixture is centrifuged and the protein concentration of the supernatant determined. One µg of protein from the soluble fraction of the culture can be separated by SDS-polyacrylamide gel electrophoresis. Gels can be observed for protein bands migrating at the expected molecular weight.

EXAMPLE 7

Assaying for Phytochelatin Synthase Activity

The polypeptides described herein may be produced using any number of methods known to those skilled in the art. Such methods include, but are not limited to, expression in bacteria as described in Example 6, or expression in eukaryotic cell culture, *in planta*, and using viral expression systems in suitably infected organisms or cell lines. The instant polypeptides may be expressed either as mature forms of the proteins as observed *in vivo* or as fusion proteins by covalent attachment to a variety of enzymes, proteins or affinity tags. Common fusion protein partners include glutathione S-transferase ("GST"), thioredoxin ("Trx"), maltose binding protein, and C- and/or N-terminal hexahistidine polypeptide ("His)₆"). The fusion proteins may be engineered with a protease recognition site at the fusion point so that fusion partners can be separated by protease digestion to yield intact mature enzyme. Examples of such proteases include thrombin, enterokinase and factor Xa. However, any protease can be used which specifically cleaves the peptide connecting the fusion protein and the enzyme.

Purification of the instant polypeptides, if desired, may utilize any number of separation technologies familiar to those skilled in the art of protein purification. Examples of such methods include, but are not limited to, homogenization, filtration, centrifugation, heat denaturation, ammonium sulfate precipitation, desalting, pH

precipitation, ion exchange chromatography, hydrophobic interaction chromatography and affinity chromatography, wherein the affinity ligand represents a substrate, substrate analog or inhibitor. When the instant polypeptides are expressed as fusion proteins, the purification protocol may include the use of an affinity resin which is specific for the fusion protein tag attached to the expressed enzyme or an affinity resin containing ligands which are specific for the enzyme. For example, the instant polypeptides may be expressed as a fusion protein coupled to the C-terminus of thioredoxin. In addition, a (His)₆ peptide may be engineered into the N-terminus of the fused thioredoxin moiety to afford additional opportunities for affinity purification. Other suitable affinity resins could be synthesized by linking the appropriate ligands to any suitable resin such as Sepharose-4B. In an alternate embodiment, a thioredoxin fusion protein may be eluted using dithiothreitol; however, elution may be accomplished using other reagents which interact to displace the thioredoxin from the resin. These reagents include β -mercaptoethanol or other reduced thiol. The eluted fusion protein may be subjected to further purification by traditional means as stated above, if desired. Proteolytic cleavage of the thioredoxin fusion protein and the enzyme may be accomplished after the fusion protein is purified or while the protein is still bound to the ThioBond[®] affinity resin or other resin.

Crude, partially purified or purified enzyme, either alone or as a fusion protein, may be utilized in assays for the evaluation of compounds for their ability to inhibit enzymatic activation of the instant polypeptides disclosed herein. Assays may be conducted under well known experimental conditions which permit optimal enzymatic activity. For example, assays for phytochelatin synthase are presented by Ha et al. (1999) *Plant Cell* 11:1153-1163 and Vatamaniuk et al. (1999) *Proc Natl Acad Sci USA* 96:7110-7115.

EXAMPLE 8

Expression of Recombinant DNA Constructs in Yeast Cells

The polypeptides encoded by the polynucleotides of the instant invention may be expressed in a yeast (*Saccharomyces cerevisiae*) strain YPH. Plasmid DNA, plant cDNA or plant cDNA libraries may be used as template to amplify the portion encoding the phytochelatin synthase. Amplification may be performed using the GC melt kit (Clontech) with a 1 M final concentration of GC melt reagent and using a Perkin Elmer 9700 thermocycler. The amplified insert may then be incubated with a modified pRS315 plasmid (NCBI General Identifier No. 984798; Sikorski, R. S. and Hieter, P. (1989) *Genetics* 122:19-27) that has been digested with Not I and Spe I. Plasmid pRS315 has been previously modified by the insertion of a bidirectional

gal1/10 promoter between the Xho I and Hind III sites. The plasmid may then be transformed into the YPH yeast strain using standard procedures where the insert recombines through gap repair to form the desired transformed yeast strain (Hua, S. B. et al. (1997) *Plasmid* 38:91-96).

5 Yeast cells may be prepared according to a modification of the methods of Pompon et al. (Pompon, D. et al. (1996) *Meth. Enz.* 272:51-64). Briefly, a yeast colony will be grown overnight (to saturation) in SG (-Leucine) medium at 30°C with good aeration. A 1:50 dilution of this culture will be made into 500 mL of YPGE medium with adenine supplementation and allowed to grow at 30°C with good
10 aeration to an OD₆₀₀ of 1.6 (24-30 h). Fifty mL of 20% galactose will be added, and the culture allowed to grow overnight at 30°C. The cells will be recovered by centrifugation at 5,500 rpm for five minutes in a Sorvall GS-3 rotor. The cell pellet resuspended in 500 mL of 0.1 M potassium phosphate buffer (pH 7.0) and then allowed to grow at 30°C for another 24 hours.

15 The cells may be recovered by centrifugation as described above and the presence of the polypeptide of the instant invention determined by HPLC/mass spectrometry or any other suitable method.

EXAMPLE 9

Expression of Recombinant DNA Constructs in Insect Cells

20 The cDNA fragment of the gene may be generated by polymerase chain reaction (PCR) of the cDNA clone, plant cDNA or plant cDNA libraries, using appropriate oligonucleotide primers. The cDNAs encoding the instant polypeptides may be introduced into the baculovirus genome itself. For this purpose the cDNAs may be placed under the control of the polyhedron promoter, the IE1 promoter, or
25 any other one of the baculovirus promoters. The cDNA, together with appropriate leader sequences is then inserted into a baculovirus transfer vector using standard molecular cloning techniques. Following transformation of *E. coli* DH5 α , isolated colonies are chosen and plasmid DNA is prepared and is analyzed by restriction enzyme analysis. Colonies containing the appropriate fragment are isolated,
30 propagated, and plasmid DNA is prepared for cotransfection.

Spodoptera frugiperda cells (Sf-9) are propagated in ExCell® 401 media (JRH Biosciences, Lenexa, KS) supplemented with 3.0% fetal bovine serum. Lipofectin® (50 μ L at 0.1 mg/mL, Gibco/BRL) is added to a 50 μ L aliquot of the transfer vector containing the toxin gene (500 ng) and linearized polyhedrin-negative
35 AcNPV (2.5 μ g, Baculogold® viral DNA, Pharmigen, San Diego, CA). Sf-9 cells (approximate 50% monolayer) are co-transfected with the viral DNA/transfer vector solution. The supernatant fluid from the co-transfection experiment is collected at 5

days post-transfection and recombinant viruses are isolated employing standard plaque purification protocols, wherein only polyhedrin-positive plaques are selected (O'Reilly et al. (1992), *Baculovirus Expression Vectors: A Laboratory Manual*, W. H. Freeman and Company, New York.). Sf-9 cells in 35 mM petri
5 dishes (50% monolayer) are inoculated with 100 μ L of a serial dilution of the viral suspension, and supernatant fluids are collected at 5 days post infection. In order to prepare larger quantities of virus for characterization, these supernatant fluids are used to inoculate larger tissue cultures for large-scale propagation of recombinant viruses. Expression of the instant polypeptides encoded by the recombinant
10 baculovirus is confirmed by any of the methods mentioned in Example 7.

CLAIMS

What is claimed is:

1. An isolated polynucleotide comprising:

(a) a first nucleotide sequence encoding a first polypeptide having

5 phytochelatin synthase activity, wherein the amino acid sequence of the first polypeptide and the amino acid sequence of SEQ ID NO:12 or SEQ ID NO:18 have at least 70% sequence identity based on the ClustalV alignment method,

10 (b) a second nucleotide sequence encoding a second polypeptide having phytochelatin synthase activity, wherein the amino acid sequence of the second polypeptide and the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:16 have at least 80% sequence identity based on the ClustalV alignment method, or

(c) the complement of the nucleotide sequence of (a) or (b).

15 2. The polynucleotide of Claim 1, wherein the amino acid sequence of the first polypeptide and the amino acid sequence of SEQ ID NO:12 or SEQ ID NO:18 have at least 80% sequence identity based on the ClustalV alignment method.

20 3. The polynucleotide of Claim 1, wherein the amino acid sequence of the first polypeptide and the amino acid sequence of SEQ ID NO:12 or SEQ ID NO:18 have at least 85% sequence identity based on the ClustalV alignment method, or wherein the amino acid sequence of the second polypeptide and the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:16 have at least 85% sequence identity based on the ClustalV alignment method.

25 4. The polynucleotide of Claim 1, wherein the amino acid sequence of the first polypeptide and the amino acid sequence of SEQ ID NO:12 or SEQ ID NO:18 have at least 90% sequence identity based on the ClustalV alignment method, or wherein the amino acid sequence of the second polypeptide and the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:16 have at least 90% sequence identity based on the ClustalV alignment method.

30 5. The polynucleotide of Claim 1, wherein the amino acid sequence of the first polypeptide and the amino acid sequence of SEQ ID NO:12 or SEQ ID NO:18 have at least 95% sequence identity based on the ClustalV alignment method, or wherein the amino acid sequence of the second polypeptide and the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:16 have at least 95% sequence identity
35 based on the ClustalV alignment method.

6. The polynucleotide of Claim 1, wherein the amino acid sequence of the first polypeptide comprises the amino acid sequence of SEQ ID NO:12 or SEQ ID

NO:18, or wherein the amino acid sequence of the second polypeptide comprises the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:16.

7. The polynucleotide of Claim 1, wherein the nucleotide sequence of the first polynucleotide comprises the nucleotide sequence of SEQ ID NO:11 or SEQ ID NO:17, or wherein the nucleotide sequence of the second polynucleotide comprises the nucleotide sequence of SEQ ID NO:13 or SEQ ID NO:15.

8. A vector comprising the polynucleotide of Claim 1.

9. A recombinant DNA construct comprising the polynucleotide of Claim 1 operably linked to at least one regulatory sequence.

10. A method for transforming a cell, comprising transforming a cell with the polynucleotide of Claim 1.

11. A cell comprising the recombinant DNA construct of Claim 9.

12. A method for production of a polypeptide having phytochelatin synthase activity comprising the steps of cultivating the cell of Claim 11 under conditions that allow for the synthesis of the polypeptide and isolating the polypeptide from the cultivated cells, from the culture medium, or from both the cultivated cells and the culture medium.

13. A method for producing a plant comprising transforming a plant cell with the polynucleotide of Claim 1 and regenerating a plant from the transformed plant cell.

14. A plant comprising the recombinant DNA construct of Claim 9.

15. A seed comprising the recombinant DNA construct of Claim 9.

16. An isolated polypeptide having phytochelatin synthase activity, wherein the polypeptide comprises:

(a) a first amino acid sequence, wherein the first amino acid sequence and the amino acid sequence of SEQ ID NO:12 or SEQ ID NO:18 have at least 70% sequence identity based on the ClustalV alignment method, or

(b) a second amino acid sequence, wherein the second amino acid sequence and the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:16 have at least 80% sequence identity based on the ClustalV alignment method.

17. The polypeptide of Claim 16, wherein the amino acid sequence of the first polypeptide and the amino acid sequence of SEQ ID NO:12 or SEQ ID NO:18 have at least 80% sequence identity based on the ClustalV alignment method.

18. The polypeptide of Claim 16, wherein the amino acid sequence of the first polypeptide and the amino acid sequence of SEQ ID NO:12 or SEQ ID NO:18 have at least 85% sequence identity based on the ClustalV alignment method, or wherein

the amino acid sequence of the second polypeptide and the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:16 have at least 85% sequence identity based on the ClustalV alignment method.

19. The polypeptide of Claim 16, wherein the amino acid sequence of the first polypeptide and the amino acid sequence of SEQ ID NO:12 or SEQ ID NO:18 have at least 90% sequence identity based on the ClustalV alignment method, or wherein the amino acid sequence of the second polypeptide and the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:16 have at least 90% sequence identity based on the ClustalV alignment method.

20. The polypeptide of Claim 16, wherein the amino acid sequence of the first polypeptide and the amino acid sequence of SEQ ID NO:12 or SEQ ID NO:18 have at least 95% sequence identity based on the ClustalV alignment method, or wherein the amino acid sequence of the second polypeptide and the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:16 have at least 95% sequence identity based on the ClustalV alignment method.

21. The polypeptide of Claim 16, wherein the amino acid sequence of the first polypeptide comprises the amino acid sequence of SEQ ID NO:12 or SEQ ID NO:18, or wherein the amino acid sequence of the second polypeptide comprises the amino acid sequence of SEQ ID NO:14 or SEQ ID NO:16.

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SEQ ID NO:14	1	MAAVASLYRRVLSPSPPAIDFASPEGKRLFAEALAAAGTMEGFFPLVSVFQTQSEPAFCGL	*****
SEQ ID NO:16	1	MAA-MASLYRRVLSPSPPAVEFASPEGKRLFSEALESGTLQGFNLSYFQTQSEPAFCGL	***
SEQ ID NO:18	1	MAS--PGLYRRVLSPSP-IEFASPEGKRLFGEALERGTQGFNLSYFQTQSEPAYCGL	***
SEQ ID NO:19	1	ME--VASLYRRVLSPSPPAVEFASAEKRLFAEALQGGTMEGFFNLSYFQTQSEPAFCGL	***
SEQ ID NO:20	1	M--AMASLYRRSLSPSPPAIDFSSAEGKLIFNEALQKGTMEGFFRLISYFQTQSEPAYCGL	*****
	1		60
SEQ ID NO:12	61	ATLAMVLNALSIDPGRKWKGPPWRWFDESMLDCEPLETVKAGISFGKVVCLAHACAGAKV	***
SEQ ID NO:14	61	ASLAVVLNALAIDPGRRWKGPWRWFDESMLDCEPLDKVKAEGITFGKVACLAHCSGADV	***
SEQ ID NO:16	60	ASLSVVLNALAIDPGRQWKGPWRWFDESMLDCEPLDKVKAEGITFAKLAHLAHACAGANV	***
SEQ ID NO:18	58	ATLSVVLNALAIDPGRKWKGPPWRWFDESMLDCEPLAKVKLEGITFGKVACLAHACAGANV	***
SEQ ID NO:19	59	ASLSVVLNALAIDPGRPWKGPWRWFDESMLDCEPLHKKVKAEGITFGKVACLAHACAGARV	***
SEQ ID NO:20	59	ASLSVVLNALSIDPGRKWKGPPWRWFDESMLDCEPLEVVKKEGISFGKVVCLAHACAGAKV	***
	61		120
SEQ ID NO:12	121	EAFTNQTSIDFRKHVVACSSDDCHVIA SYNRA TFKQTGAGHFSPIGGYHAGRDMVLI	***
SEQ ID NO:14	121	QSFRTNRVTIHLRRLHLCVSSQDCHLIAS YHRRPFGQTGTGHFSPIGGYHAGQDMVLI	***
SEQ ID NO:16	120	RSEADQSTIHDFFHHLVRSASSQDCHLIAS YHRKPKFKQTGTGHFSPIGGYHAGQDMALI	***
SEQ ID NO:18	118	EAERSDQSSVDFFNRVISCSSDCHVIVSYHRTPLNQTGIGHFSVPVGGYHAERDMVIV	***
SEQ ID NO:19	119	QSEADQTTIHDFAHLTRCASSQDCHLISSYHRSFPKQTGTGHFSPIGGYHAEKDMALI	***
SEQ ID NO:20	119	EAERTSQSTIDDFRKFVVKCTSSENCHMISTYHRGVFKQTGTGHFSPIGGYNAERDMALI	***
	121		180

FIG. 1A

```
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SEQ ID NO:14 181 LDVAREFKYPPHWPVPLQLLWEAMNTTDDSTGLRGFMILSRKVAAPSLLYTVSCRDENWKR *** **
SEQ ID NO:16 180 LDVAREFKYPPHWPVPLPLLWEAMNTTDDATGLRGFMILSRHTAAPSLLYTVSCRDESWKS *** **
SEQ ID NO:18 178 LDVAREFKYPPHWPVPLTLLWEGMSTIDQATRLRRGYMIISRLNRAPSLITYTVSCRHEGWSS *** **
SEQ ID NO:19 179 LDVAREFKYPPHWPVPLTLLWDAMNTTDEATGLRGFMIVSRSSAPSLITYTVSCGHGSWS *** **
SEQ ID NO:20 179 LDVAREFKYPPHWPVPLKLLWEAMDSIDQSTGKRRGFMILSRPHREPGLLYTLSCKDESWE 240
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* ** * *
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241
** * * *
301 QERLSIKGEILKQVQETELYKYVAD--FLDSPC-----SGQEASLTEIAASVCCQAGF ** ***
301 KERFLKENVLKQVRDTRLFAIVHDLQYANKPCYNC-SSPEDDLSLTRIAAVVCCQGAAM ***
300 EERLILKEMILQVRDTELFRLVRELQFTKQCCSC-SYSSDDDSFTTRIAASVCCQGAAL ***
298 KGRLAIKADILEQIRTTGLEFKHVTRWLDSESSCNTLANLGDKDMLPALAASVCCQAADL ***
299 KERFLKEKVLQIIRDTDLFRVVHELQYPKGLCGSC-SSSDEDSIAEIAATVCCQGAAF ***
299 KSRLKLKQLVLKEVHETELFKHINK--FLST-----VGYEDSLTYAAAKACCQGAEL 360
301
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FIG. 1B

SEQ ID NO:12	* * * * *	353 LK--GNSESSDGFCCGETQVHCICKNNGAMPHVTIVSGVTNGIGEQHVDMLVPSLTN---	
SEQ ID NO:14		360 LS--GNLVPDAFCCRETSEFCVQANGD-GLKTVISGSVCEGSEQGVDMLLPMSSPGAS	
SEQ ID NO:16		359 LT--GNLSSKDGFCRETCEKCVQVDGD-GPKTVVTGTAVSGVNEQSVDMLLPISTLETS	
SEQ ID NO:18		358 LTVCGRIGLSGGKCCSQIDVKHLNADSENPTVLSGIVTTGGSEQGVDDVLVPLCQREPS	
SEQ ID NO:19		358 LS--GNLVS RDGFCCRETCKICIEANGD-GLKTVISGTVVSKGNEQAVDLLLLPTSSSKTS	
SEQ ID NO:20		349 LS--GS--PSKEFCCRETCKVCKIKGPD--SEGTVVTGVVVRDNEQKVVDLLVPSTQTE--	420
		361	
SEQ ID NO:12	* * * * *	408 -----RIGLHPASSDVLIALIALPPQIWSGIKDDTLLREIDTLVSMNLPPLLQE	
SEQ ID NO:14		417 -SCSNLKSNAVYPSSVDLTVLLALHPNTWLGIKDEKLKAEFQTLISTDSLPDDLKR	
SEQ ID NO:16		416 -VCNSNSSNEVVYPSRTDILTVLLALHPSTWVGIKDERLKAEFQSLISTDILHDDLKR	
SEQ ID NO:18		418 RLCLSNEGHCIGMHPSTADVLTVLLALPLHTWSGIKEEKLVEALSLLATEDLPPLLQE	
SEQ ID NO:19		415 -LCNSNLKSKIVKYPSSDVLTVLLVLQPNWLGIKDENVKAEFQSLVSTDNLPDLKQ	
SEQ ID NO:20		402 --CEC---GPEATYPAGNDVFTALLALPPQTWSGIKDQALMHMKQLISMASLPPLLQE	480
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SEQ ID NO:12	* * * * *	459 EVMHLRSQLYILKRCKDNELEKDL SAP-L---	486
SEQ ID NO:14		476 EILHLRRQLYLYLKACKEECEDAEQSPKQOC	507
SEQ ID NO:16		475 EILHLRRQLHYVRSCKEEYGDVPQSH----	502
SEQ ID NO:18		478 EVLELRDQLHFLMT-----DISAPS--PS	499
SEQ ID NO:19		474 EILHLRRQLHYLAGCKGQACQEPSP-----	500
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FIG. 1C

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Val Val Leu Asn Ala Leu Ala Ile Asp Pro Gly Arg Arg Trp Lys Gly
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Pro Trp Arg Trp Phe Asp Glu Ser Met Leu Asp Cys Cys Glu Pro Leu
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 Arg Leu Lys Ala Glu Phe Gln Ser Leu Ile Ser Thr Asp Ile Leu His
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 <213> Oryza sativa

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 aagcagccat gttgtagttg ctcatattca agtgaatgat attcctttac ccggattgca 660

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<212> PRT

<213> Oryza sativa

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Thr Gly His Phe Ser Pro Ile Gly Gly Tyr His Ala Gly Gln Asp Met
      35              40              45

Ala Leu Ile Leu Asp Val Ala Arg Phe Lys Tyr Pro Pro His Trp Val
      50              55              60

Pro Leu Pro Leu Leu Trp Glu Ala Met Asn Thr Thr Asp Asp Ala Thr
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Gly Leu Leu Arg Gly Phe Met Leu Ile Ser Arg His Thr Ala Ala Pro
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Ser Leu Leu Tyr Thr Val Ser Cys Arg Asp Glu Ser Trp Lys Ser Met
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Ala Lys Tyr Cys Met Glu Asp Val Pro Asp Leu Leu Lys Asp Glu Ser
      115              120              125

Val Asp Asn Val Pro Ala Leu Leu Ser Arg Leu Val Lys Ser Leu Pro
      130              135              140

Ala Asn Ala Gly Asn Leu Ile Lys Trp Val Ile Glu Val Arg Arg Gln
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Glu Glu Gly Gly Ser Gly Leu Ser Lys Glu Glu Glu Glu Arg Leu Ile
      165              170              175

Leu Lys Glu Met Ile Leu Gln Gln Val Arg Asp Thr Glu Leu Phe Arg
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Leu Val Arg Glu Leu Gln Phe Thr Lys Gln Pro Cys Cys Ser Cys Ser
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Tyr Ser Ser Asp Asp Asp Ser Phe Thr Arg Ile Ala Ala Ser Val Cys
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 Phe Cys Cys Arg Glu Thr Cys Phe Lys Cys Val Gln Val Asp Gly Asp
 245 250 255
 Gly Pro Lys Thr Val Val Thr Gly Thr Ala Val Ser Gly Val Asn Glu
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 Gln Ser Val Asp Met Leu Leu Pro Ile Ser Thr Leu Glu Thr Ser Val
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 Cys Asn Ser Asn Ser Ser Asn Glu Val Val Lys Tyr Pro Ser Arg Thr
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 Asp Ile Leu Thr Val Leu Leu Leu Ala Leu His Pro Ser Thr Trp Val
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 Gly Ile Lys Asp Glu Arg Leu Lys Ala Glu Phe Gln Ser Leu Ile Ser
 325 330 335
 Thr Asp Ile Leu His Asp Asp Leu Lys Arg Glu Ile Leu His Leu Arg
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 Arg Gln Leu His Tyr Val Arg Ser Cys Lys Glu Glu Glu Tyr Gly Asp
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 Pro Val Pro Gln Ser His
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 <211> 967
 <212> DNA
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Arg	Gly	Thr	Met	Gln	Gly	Phe	Phe	Lys	Leu	Ile	Ser	Tyr	Tyr	Gln	Thr
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Gln	Ser	Glu	Pro	Ala	Tyr	Cys	Gly	Leu	Ala	Thr	Leu	Ser	Val	Val	Leu
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Lys	Leu	Glu	Gly	Ile	Thr	Phe	Gly	Lys	Val	Ala	Cys	Leu	Ala	Arg	Cys
		115					120					125			
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Val	Ile	Val	Ser	Tyr	His	Arg	Thr	Pro	Leu	Asn	Gln	Thr	Gly	Ile	Gly
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Arg	Arg	Gly	Tyr	Met	Ile	Ile	Ser	Arg	Leu	Asn	Arg	Ala	Pro	Ser	Ile
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Leu	Tyr	Thr	Val	Ser	Cys	Arg	His	Glu	Gly	Trp	Ser	Ser	Val	Ala	Lys
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 <212> DNA
 <213> *Helianthus* sp.

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Phe Gln Thr Gln Ser Glu Pro Ala Tyr Cys Gly Leu Ala Thr Leu Ala
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 Met Val Leu Asn Ala Leu Ser Ile Asp Pro Gly Arg Lys Trp Lys Gly
 65 70 75 80
 Pro Trp Arg Trp Phe Asp Glu Ser Met Leu Asp Cys Cys Glu Pro Leu
 85 90 95
 Glu Thr Val Lys Ala Lys Gly Ile Ser Phe Gly Lys Val Val Cys Leu
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 Ala His Cys Ala Gly Ala Lys Val Glu Ala Phe Arg Thr Asn Gln Thr
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 Ser Ile Asp Glu Phe Arg Lys His Val Val Ala Cys Ser Ser Ser Asp
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 Asp Cys His Val Ile Ala Ser Tyr Asn Arg Ala Thr Phe Lys Gln Thr
 145 150 155 160
 Gly Ala Gly His Phe Ser Pro Ile Gly Gly Tyr His Ala Gly Arg Asp
 165 170 175
 Met Val Leu Ile Leu Asp Val Ala Arg Phe Lys Tyr Pro Pro His Trp
 180 185 190
 Val Pro Leu Lys Leu Leu Trp Glu Ala Met Asp Thr Val Asp Gln Ala
 195 200 205
 Ser Gly Tyr His Arg Gly Phe Met Leu Val Ser Arg Leu Gln Arg Pro
 210 215 220
 Pro Ala Leu Leu Tyr Thr Leu Ser Cys Lys His Glu Ser Trp Val Asn
 225 230 235 240
 Ile Ala Lys Tyr Leu Thr Glu Asp Val Pro Glu Leu Leu Ser Ser Lys
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 Asn Val Lys Asp Val Lys Asp Val Leu Ser Ile Val Phe Ser Ser Leu
 260 265 270
 Pro Ser Lys Phe Leu Glu Phe Ile Thr Trp Val Pro Glu Val Arg Arg
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 Thr Glu Glu Gly Asp Gln Ser Leu Thr Pro Glu Glu Gln Glu Arg Leu
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 Ser Ile Lys Gly Glu Ile Leu Lys Gln Val Gln Glu Thr Glu Leu Tyr
 305 310 315 320
 Lys Tyr Val Ala Asp Phe Leu Asp Ser Pro Cys Ser Gly Gln Glu Ala
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 Ser Leu Thr Glu Ile Ala Ala Ser Val Cys Cys Gln Gly Ala Gly Phe
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 355 360 365

Gln Val His Cys Ile Lys Asn Asn Gly Ala Met Pro His Val Thr Ile
 370 375 380
 Val Ser Gly Thr Val Thr Asn Gly Ile Gly Glu Gln His Val Asp Met
 385 390 395 400
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 405 410 415
 Asp Val Leu Ile Ala Leu Leu Leu Ala Leu Pro Pro Gln Ile Trp Ser
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 Gly Ile Lys Asp Asp Thr Leu Leu Arg Glu Ile Asp Thr Leu Val Ser
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 Met Asp Asn Leu Pro Thr Leu Leu Gln Glu Glu Val Met His Leu Arg
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 <212> DNA
 <213> Zea mays

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 <211> 507
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      20             25             30

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      35             40             45

Phe Gln Thr Gln Ser Glu Pro Ala Phe Cys Gly Leu Ala Ser Leu Ala
      50             55             60

Val Val Leu Asn Ala Leu Ala Ile Asp Pro Gly Arg Arg Trp Lys Gly
      65             70             75             80

Pro Trp Arg Trp Phe Asp Glu Ser Met Leu Asp Cys Cys Glu Pro Leu
      85             90             95

Asp Lys Val Lys Ala Glu Gly Ile Thr Phe Gly Lys Val Ala Cys Leu
      100            105            110

Ala His Cys Ser Gly Ala Asp Val Gln Ser Phe Arg Thr Asn Arg Val
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Thr Ile His Asp Leu Arg Arg His Leu Ile Arg Cys Val Ser Ser Gln
      130            135            140

Asp Cys His Leu Ile Ala Ser Tyr His Arg Arg Pro Phe Gly Gln Thr
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Gly Thr Gly His Phe Ser Pro Ile Gly Gly Tyr His Ala Gly Gln Asp
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Met Val Leu Ile Leu Asp Val Ala Arg Phe Lys Tyr Pro Pro His Trp
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Val Pro Leu Gln Leu Leu Trp Glu Ala Met Asn Thr Thr Asp Asp Ser
      195            200            205

Thr Gly Leu Leu Arg Gly Phe Met Leu Ile Ser Arg Lys Val Ala Ala
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Pro Ser Leu Leu Tyr Thr Val Ser Cys Arg Asp Glu Asn Trp Lys Arg
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Met Ser Lys Tyr Cys Val Glu Asp Leu Pro Ser Leu Leu Lys Ala Gly
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 Ala Ile Val His Asp Leu Gln Tyr Ala Asn Lys Pro Cys Tyr Asn Cys
 325 330 335
 Ser Ser Pro Ser Glu Asp Asp Ser Leu Thr Arg Ile Ala Ala Val Val
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 Cys Cys Gln Gly Ala Ala Met Leu Ser Gly Asn Leu Val Pro Arg Asp
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 Ala Phe Cys Cys Arg Glu Thr Ser Phe Glu Cys Val Gln Ala Asn Gly
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 Asp Gly Leu Lys Thr Val Ile Ser Gly Ser Val Val Cys Glu Gly Ser
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 Glu Gln Gly Val Asp Met Leu Leu Pro Met Ser Ser Pro Gly Ala Ser
 405 410 415
 Ser Cys Asn Ser Asn Leu Lys Ser Asn Ala Val Lys Tyr Pro Ser Ser
 420 425 430
 Val Asp Val Leu Thr Val Leu Leu Leu Ala Leu His Pro Asn Thr Trp
 435 440 445
 Leu Gly Ile Lys Asp Glu Lys Leu Lys Ala Glu Phe Gln Thr Leu Ile
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 Ser Thr Asp Ser Leu Pro Asp Asp Leu Lys Arg Glu Ile Leu His Leu
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<211> 1951

<212> DNA

<213> Oryza sativa

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gaagtattgc atggaagatg taccgatctt tcttaaggat gagagtgtag acaatgttcc 900
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ctcttggttag tgacaactga caactagcgg ggaggcacac gctaattgtg cggctggtgt 1860
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<210> 16

<211> 502

<212> PRT

<213> *Oryza sativa*

<400> 16

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Met Ala Ala Met Ala Ser Leu Tyr Arg Arg Val Leu Pro Ser Pro Pro
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Ala Val Glu Phe Ala Ser Glu Glu Gly Lys Arg Leu Phe Ser Glu Ala
          20                      25                      30

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Leu Glu Ser Gly Thr Leu Gln Gly Phe Phe Asn Leu Ile Ser Val Phe
          35                      40                      45

```

```

Gln Thr Gln Ser Glu Pro Ala Phe Cys Gly Leu Ala Ser Leu Ser Val
          50                      55                      60

```

```

Val Leu Asn Ala Leu Ala Ile Asp Pro Gly Arg Gln Trp Lys Gly Pro
          65                      70                      75                      80

```

```

Trp Arg Trp Phe Asp Glu Ser Met Leu Asp Cys Cys Glu Pro Leu Asp
          85                      90                      95

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Lys Val Lys Ala Glu Gly Ile Thr Phe Ala Lys Leu Ala Cys Leu Ala
          100                     105                     110

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```

His Cys Ala Gly Ala Asn Val Arg Ser Phe Arg Ala Asp Gln Ser Thr
          115                     120                     125

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Ile His Asp Phe Arg His His Leu Val Arg Ser Ala Ser Ser Gln Asp
 130                      135                      140

Cys His Leu Ile Ala Ser Tyr His Arg Lys Pro Phe Lys Gln Thr Gly
145                      150                      155                      160

Thr Gly His Phe Ser Pro Ile Gly Gly Tyr His Ala Gly Gln Asp Met
                      165                      170                      175

Ala Leu Ile Leu Asp Val Ala Arg Phe Lys Tyr Pro Pro His Trp Val
                      180                      185                      190

Pro Leu Pro Leu Leu Trp Glu Ala Met Asn Thr Thr Asp Asp Ala Thr
                      195                      200                      205

Gly Leu Leu Arg Gly Phe Met Leu Ile Ser Arg His Thr Ala Ala Pro
210                      215                      220

Ser Leu Leu Tyr Thr Val Ser Cys Arg Asp Glu Ser Trp Lys Ser Met
225                      230                      235                      240

Ala Lys Tyr Cys Met Glu Asp Val Pro Asp Leu Leu Lys Asp Glu Ser
                      245                      250                      255

Val Asp Asn Val Pro Ala Leu Leu Ser Arg Leu Val Lys Ser Leu Pro
                      260                      265                      270

Ala Asn Ala Gly Asn Leu Ile Lys Trp Val Ile Glu Val Arg Arg Gln
                      275                      280                      285

Glu Glu Gly Gly Ser Gly Leu Ser Lys Glu Glu Glu Glu Arg Leu Ile
290                      295                      300

Leu Lys Glu Met Ile Leu Gln Gln Val Arg Asp Thr Glu Leu Phe Arg
305                      310                      315                      320

Leu Val Arg Glu Leu Gln Phe Thr Lys Gln Pro Cys Cys Ser Cys Ser
                      325                      330                      335

Tyr Ser Ser Asp Asp Asp Ser Phe Thr Arg Ile Ala Ala Ser Val Cys
                      340                      345                      350

Cys Gln Gly Ala Ala Leu Leu Thr Gly Asn Leu Ser Ser Lys Asp Gly
                      355                      360                      365

Phe Cys Cys Arg Glu Thr Cys Phe Lys Cys Val Gln Val Asp Gly Asp
370                      375                      380

Gly Pro Lys Thr Val Val Thr Gly Thr Ala Val Ser Gly Val Asn Glu
385                      390                      395                      400

Gln Ser Val Asp Met Leu Leu Pro Ile Ser Thr Leu Glu Thr Ser Val
                      405                      410                      415

Cys Asn Ser Asn Ser Ser Asn Glu Val Val Lys Tyr Pro Ser Arg Thr
                      420                      425                      430

Asp Ile Leu Thr Val Leu Leu Leu Ala Leu His Pro Ser Thr Trp Val
435                      440                      445

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Gly Ile Lys Asp Glu Arg Leu Lys Ala Glu Phe Gln Ser Leu Ile Ser
 450 455 460
 Thr Asp Ile Leu His Asp Asp Leu Lys Arg Glu Ile Leu His Leu Arg
 465 470 475 480
 Arg Gln Leu His Tyr Val Arg Ser Cys Lys Glu Glu Glu Tyr Gly Asp
 485 490 495
 Pro Val Pro Gln Ser His
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 <211> 1920
 <212> DNA
 <213> Glycine max

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 gaagaagctg ttcggtgaag cgcttgagcg aggaaccatg caaggcttct tcaagctaatt 180
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 cctcaatgcc cttgccattg accctggaag gaaatggaaa ggtccttgga gatggtttga 300
 cgagtccatg ttggattgct gtgagccttt ggccaagggt aaattggaag gcattacgtt 360
 cggtaaaagt gcatgcttgg ctcgatgtaa tggagctaag gttgaagcct ttcgatcgga 420
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 tcaagcaacc agacttcgta gggggtacat gattatttct aggcttaaca gagcaccatc 720
 tatactttat actgtgagtt gtagacatga aggttgagag agtggttgcca aatttctaac 780
 cgaagatgtc cctcaacttc taaagtcaga ggatctaaaa gacattcagg aagtactctc 840
 tcttgctttt aaatctctc ccagtgaatt gagaggggta ataacatgga ttgctgaagt 900
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 caaggctgac atactggaac agattcgaac aactggactc ttcaaacacg tgacaagggtg 1020
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 accagcactt gctgccagtg tttgttgcca agcggcagat cttttgactg tttgtggtag 1140
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 agaccaactc catttttctca tgactgatat cagtgtcctt tctccctcat gatactattg 1560
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 catcaggtgt gtccttgaac ttggaatcaa tacagcatta gcattgctgt ggtgctatgt 1680
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 <211> 499
 <212> PRT
 <213> Glycine max

<400> 18

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          20           25           30

Arg Gly Thr Met Gln Gly Phe Phe Lys Leu Ile Ser Tyr Tyr Gln Thr
          35           40           45

Gln Ser Glu Pro Ala Tyr Cys Gly Leu Ala Thr Leu Ser Val Val Leu
 50           55           60

Asn Ala Leu Ala Ile Asp Pro Gly Arg Lys Trp Lys Gly Pro Trp Arg
 65           70           75           80

Trp Phe Asp Glu Ser Met Leu Asp Cys Cys Glu Pro Leu Ala Lys Val
          85           90           95

Lys Leu Glu Gly Ile Thr Phe Gly Lys Val Ala Cys Leu Ala Arg Cys
          100          105          110

Asn Gly Ala Lys Val Glu Ala Phe Arg Ser Asp Gln Ser Ser Val Asp
          115          120          125

Asp Phe Arg Asn Arg Val Ile Ser Cys Ser Ser Ser Glu Asp Cys His
          130          135          140

Val Ile Val Ser Tyr His Arg Thr Pro Leu Asn Gln Thr Gly Ile Gly
          145          150          155          160

His Phe Ser Pro Val Gly Gly Tyr His Ala Glu Arg Asp Met Val Leu
          165          170          175

Val Leu Asp Val Ala Arg Phe Lys Tyr Pro Pro His Trp Val Pro Leu
          180          185          190

Thr Leu Leu Trp Glu Gly Met Ser Thr Ile Asp Gln Ala Thr Arg Leu
          195          200          205

Arg Arg Gly Tyr Met Ile Ile Ser Arg Leu Asn Arg Ala Pro Ser Ile
          210          215          220

Leu Tyr Thr Val Ser Cys Arg His Glu Gly Trp Ser Ser Val Ala Lys
          225          230          235          240

Phe Leu Thr Glu Asp Val Pro Gln Leu Leu Lys Ser Glu Asp Leu Lys
          245          250          255

Asp Ile Gln Glu Val Leu Ser Leu Ala Phe Lys Ser Pro Pro Ser Glu
          260          265          270

Leu Arg Gly Leu Ile Thr Trp Ile Ala Glu Val Arg Arg Gln Glu Asp
          275          280          285

Gly Asn Leu Thr Leu Ser Glu Glu Glu Lys Gly Arg Leu Ala Ile Lys
          290          295          300

Ala Asp Ile Leu Glu Gln Ile Arg Thr Thr Gly Leu Phe Lys His Val
          305          310          315          320

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Thr Arg Trp Leu Asp Ser Glu Ser Ser Cys Cys Asn Thr Leu Ala Asn
 325 330 335
 Leu Gly Asp Lys Asp Met Leu Pro Ala Leu Ala Ala Ser Val Cys Cys
 340 345 350
 Gln Ala Ala Asp Leu Leu Thr Val Cys Gly Arg Leu Gly Leu Ser Gly
 355 360 365
 Gly Lys Cys Cys Ser Gln Ile Asp Val Lys His Leu Asn Ala Asp Ser
 370 375 380
 Glu Asn Pro Val Thr Leu Val Ser Gly Ile Val Thr Thr Gly Gly Gly
 385 390 395 400
 Ser Glu Gln Gly Val Asp Val Leu Val Pro Leu Cys Gln Arg Glu Pro
 405 410 415
 Ser Arg Leu Cys Leu Ser Asn Glu Gly His Cys Ile Gly Met His Pro
 420 425 430
 Ser Thr Ala Asp Val Leu Thr Val Leu Leu Leu Ala Leu Pro Leu His
 435 440 445
 Thr Trp Ser Gly Ile Lys Glu Glu Lys Leu Arg Val Glu Ala Leu Ser
 450 455 460
 Leu Leu Ala Thr Glu Asp Leu Pro Pro Leu Leu Gln Glu Glu Val Leu
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 Phe Leu Arg Asp Gln Leu His Phe Leu Met Thr Asp Ile Ser Ala Pro
 485 490 495
 Ser Pro Ser

<210> 19
 <211> 500
 <212> PRT
 <213> Triticum aestivum

<400> 19
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 Gln Gly Gly Thr Met Glu Gly Phe Phe Asn Leu Ile Ser Tyr Phe Gln
 35 40 45
 Thr Gln Ser Glu Pro Ala Phe Cys Gly Leu Ala Ser Leu Ser Val Val
 50 55 60
 Leu Asn Ala Leu Ala Ile Asp Pro Gly Arg Pro Trp Lys Gly Pro Trp
 65 70 75 80
 Arg Trp Phe Asp Glu Ser Met Leu Asp Cys Cys Glu Pro Leu His Lys
 85 90 95

Val Lys Ala Glu Gly Ile Thr Phe Gly Lys Val Val Cys Leu Ala His
 100 105 110
 Cys Ala Gly Ala Arg Val Gln Ser Phe Arg Ala Asp Gln Thr Thr Ile
 115 120 125
 His Asp Phe Arg Ala His Leu Thr Arg Cys Ala Ser Ser Gln Asp Cys
 130 135 140
 His Leu Ile Ser Ser Tyr His Arg Ser Pro Phe Lys Gln Thr Gly Thr
 145 150 155 160
 Gly His Phe Ser Pro Ile Gly Gly Tyr His Ala Glu Lys Asp Met Ala
 165 170 175
 Leu Ile Leu Asp Val Ala Arg Phe Lys Tyr Pro Pro His Trp Val Pro
 180 185 190
 Leu Thr Leu Leu Trp Asp Ala Met Asn Thr Thr Asp Glu Ala Thr Gly
 195 200 205
 Leu Leu Arg Gly Phe Met Leu Val Ser Arg Arg Ser Ser Ala Pro Ser
 210 215 220
 Leu Leu Tyr Thr Val Ser Cys Gly His Gly Ser Trp Lys Ser Met Ala
 225 230 235 240
 Lys Tyr Cys Val Glu Asp Val Pro Asn Leu Leu Lys Asp Glu Ser Leu
 245 250 255
 Asp Asn Val Thr Thr Leu Leu Ser Arg Leu Val Glu Ser Leu Pro Ala
 260 265 270
 Asn Ala Gly Asp Leu Ile Lys Cys Val Ile Glu Val Arg Arg Lys Glu
 275 280 285
 Glu Gly Glu Ser Ser Leu Ser Lys Glu Glu Lys Glu Arg Leu Phe Leu
 290 295 300
 Lys Glu Lys Val Leu Gln Gln Ile Arg Asp Thr Asp Leu Phe Arg Val
 305 310 315 320
 Val His Glu Leu Gln Tyr Pro Lys Gly Leu Cys Gly Ser Cys Ser Ser
 325 330 335
 Ser Ser Asp Glu Asp Ser Leu Ala Glu Ile Ala Ala Thr Val Cys Cys
 340 345 350
 Gln Gly Ala Ala Phe Leu Ser Gly Asn Leu Val Ser Arg Asp Gly Phe
 355 360 365
 Cys Cys Arg Glu Thr Cys Ile Lys Cys Ile Glu Ala Asn Gly Asp Gly
 370 375 380
 Leu Lys Thr Val Ile Ser Gly Thr Val Val Ser Lys Gly Asn Glu Gln
 385 390 395 400
 Ala Val Asp Leu Leu Leu Pro Thr Ser Ser Ser Lys Thr Ser Leu Cys
 405 410 415

Asn Ser Asn Leu Lys Ser Lys Ile Val Lys Tyr Pro Ser Ser Thr Asp
 420 425 430
 Val Leu Thr Val Leu Leu Leu Val Leu Gln Pro Asn Thr Trp Leu Gly
 435 440 445
 Ile Lys Asp Glu Asn Val Lys Ala Glu Phe Gln Ser Leu Val Ser Thr
 450 455 460
 Asp Asn Leu Pro Asp Leu Leu Lys Gln Glu Ile Leu His Leu Arg Arg
 465 470 475 480
 Gln Leu His Tyr Leu Ala Gly Cys Lys Gly Gln Glu Ala Cys Gln Glu
 485 490 495
 Pro Pro Ser Pro
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 <211> 485
 <212> PRT
 <213> Arabidopsis thaliana

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 Gln Lys Gly Thr Met Glu Gly Phe Phe Arg Leu Ile Ser Tyr Phe Gln
 35 40 45
 Thr Gln Ser Glu Pro Ala Tyr Cys Gly Leu Ala Ser Leu Ser Val Val
 50 55 60
 Leu Asn Ala Leu Ser Ile Asp Pro Gly Arg Lys Trp Lys Gly Pro Trp
 65 70 75 80
 Arg Trp Phe Asp Glu Ser Met Leu Asp Cys Cys Glu Pro Leu Glu Val
 85 90 95
 Val Lys Glu Lys Gly Ile Ser Phe Gly Lys Val Val Cys Leu Ala His
 100 105 110
 Cys Ser Gly Ala Lys Val Glu Ala Phe Arg Thr Ser Gln Ser Thr Ile
 115 120 125
 Asp Asp Phe Arg Lys Phe Val Val Lys Cys Thr Ser Ser Glu Asn Cys
 130 135 140
 His Met Ile Ser Thr Tyr His Arg Ser Val Phe Lys Gln Thr Gly Asn
 145 150 155 160
 Gly His Phe Ser Pro Ile Gly Gly Tyr Asn Ala Glu Arg Asp Met Ala
 165 170 175
 Leu Ile Leu Asp Val Ala Arg Phe Lys Tyr Pro Pro His Trp Val Pro

180					185					190					
Leu	Lys	Leu	Leu	Trp	Glu	Ala	Met	Asp	Ser	Ile	Asp	Gln	Ser	Thr	Gly
	195						200					205			
Lys	Arg	Arg	Gly	Phe	Met	Leu	Ile	Ser	Arg	Pro	His	Arg	Glu	Pro	Gly
	210					215					220				
Leu	Leu	Tyr	Thr	Leu	Ser	Cys	Lys	Asp	Glu	Ser	Trp	Ile	Glu	Ile	Ala
225						230					235				240
Lys	Tyr	Leu	Lys	Glu	Asp	Val	Pro	Arg	Leu	Val	Ser	Ser	Gln	His	Val
				245					250					255	
Asp	Ser	Val	Glu	Lys	Ile	Ile	Ser	Val	Val	Phe	Lys	Ser	Leu	Pro	Ser
			260					265					270		
Asn	Phe	Asn	Gln	Phe	Ile	Arg	Trp	Val	Ala	Glu	Ile	Arg	Ile	Thr	Glu
		275					280					285			
Asp	Ser	Asn	Gln	Asn	Leu	Ser	Ala	Glu	Glu	Lys	Ser	Arg	Leu	Lys	Leu
	290					295					300				
Lys	Gln	Leu	Val	Leu	Lys	Glu	Val	His	Glu	Thr	Glu	Leu	Phe	Lys	His
305						310					315				320
Ile	Asn	Lys	Phe	Leu	Ser	Thr	Val	Gly	Tyr	Glu	Asp	Ser	Leu	Thr	Tyr
				325					330					335	
Ala	Ala	Ala	Lys	Ala	Cys	Cys	Gln	Gly	Ala	Glu	Ile	Leu	Ser	Gly	Ser
			340					345					350		
Pro	Ser	Lys	Glu	Phe	Cys	Cys	Arg	Glu	Thr	Cys	Val	Lys	Cys	Ile	Lys
		355					360					365			
Gly	Pro	Asp	Asp	Ser	Glu	Gly	Thr	Val	Val	Thr	Gly	Val	Val	Val	Arg
	370					375					380				
Asp	Gly	Asn	Glu	Gln	Lys	Val	Asp	Leu	Leu	Val	Pro	Ser	Thr	Gln	Thr
385						390					395				400
Glu	Cys	Glu	Cys	Gly	Pro	Glu	Ala	Thr	Tyr	Pro	Ala	Gly	Asn	Asp	Val
				405					410					415	
Phe	Thr	Ala	Leu	Leu	Leu	Ala	Leu	Pro	Pro	Gln	Thr	Trp	Ser	Gly	Ile
			420				425						430		
Lys	Asp	Gln	Ala	Leu	Met	His	Glu	Met	Lys	Gln	Leu	Ile	Ser	Met	Ala
		435					440					445			
Ser	Leu	Pro	Thr	Leu	Leu	Gln	Glu	Glu	Val	Leu	His	Leu	Arg	Arg	Gln
	450					455					460				
Leu	Gln	Leu	Leu	Lys	Arg	Cys	Gln	Glu	Asn	Lys	Glu	Glu	Asp	Asp	Leu
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Ala	Ala	Pro	Ala	Tyr											
				485											

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<211> 8

<212> PRT

<213> Artificial Sequence

<220>

<223> Consensus motif in isoprenylated metal-binding proteins

<400> 21

Phe Ser Glu Asp Asn Pro Asn Ala

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