



US 20030166241A1

(19) **United States**

(12) **Patent Application Publication**  
**Famodu et al.**

(10) **Pub. No.: US 2003/0166241 A1**

(43) **Pub. Date: Sep. 4, 2003**

(54) **PLANT AMINO ACYL-TRNA SYNTHETASE**

(60) Provisional application No. 60/092,866, filed on Jul. 15, 1998.

(76) Inventors: **Omolayo O. Famodu**, Newark, DE (US); **Carl R. Simmons**, Des Moines, IA (US)

**Publication Classification**

Correspondence Address:

**CONNOLLY BOVE LODGE & HUTZ, LLP**  
**1220 N MARKET STREET**  
**P O BOX 2207**  
**WILMINGTON, DE 19899**

(51) **Int. Cl.<sup>7</sup>** ..... **C12N 9/64**; C07H 21/04; C12P 21/02; C12N 5/06  
(52) **U.S. Cl.** ..... **435/226**; 435/69.1; 435/325; 435/320.1; 536/23.2

(21) Appl. No.: **09/846,589**

(57) **ABSTRACT**

(22) Filed: **May 1, 2001**

This invention relates to an isolated nucleic acid fragment encoding an aminoacyl-tRNA synthetase. The invention also relates to the construction of a chimeric gene encoding all or a portion of the aminoacyl-tRNA synthetase, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the aminoacyl-tRNA synthetase in a transformed host cell.

**Related U.S. Application Data**

(62) Division of application No. 09/352,990, filed on Jul. 14, 1999, now Pat. No. 6,255,090.

## PLANT AMINO ACYL-TRNA SYNTHETASE

[0001] This application claims the benefit of U.S. Provisional Application No. 60/092,866, filed Jul. 15, 1998.

### FIELD OF THE INVENTION

[0002] This invention is in the field of plant molecular biology. More specifically, this invention pertains to nucleic acid fragments encoding aminoacyl-tRNA synthetase in plants and seeds.

### BACKGROUND OF THE INVENTION

[0003] All tRNAs have two functions: to chemically link to a specific amino acid and to recognize a codon in mRNA so that the linked amino acid can be added to a growing peptide chain during protein synthesis. In general there is at least one aminoacyl-tRNA synthetase for each of the twenty amino acids. A specific aminoacyl-tRNA synthetase links an amino acid to the 2' or 3' hydroxyl of the adenosine residue at the 3'-terminus of a tRNA molecule. Once its correct amino acid is attached, a tRNA then recognizes a codon in mRNA, thus delivering its amino acid to the growing polypeptide chain. These enzymatic functions are critical to gene expression (Neidhart et al. (1975) *Annu. Rev. Microbiol.* 29:215-250). Mutations in tRNA synthetases often result in alterations in protein synthesis and in some cases cell death.

[0004] Plants like other cellular organisms have aminoacyl-tRNA synthetases. However a complete description of the plant 'complement' of aminoacyl-tRNA synthetases has not been published. It is anticipated that plants will likely have at least forty aminoacyl-tRNA synthetases. Plants have three sites of protein synthesis: the cytoplasm, the mitochondria and the chloroplast. Accordingly, there could be as many as sixty aminoacyl-tRNA synthetases. Based on knowledge of other eukaryotes the cytoplasmic and mitochondrial aminoacyl-tRNA synthetases are expected to be encoded by the same gene. This gene should be nuclearly encoded and produce two alternate products, one with a mitochondrial specific transit peptide, and the other without this targeting signal. The chloroplast is the other site of protein synthesis in plants. Based on a few examples of known plant chloroplast specific aminoacyl-tRNA synthetase genes it appears that these genes are also nuclear-encoded. Chloroplast aminoacyl-tRNA synthetases are directed to the chloroplast by a transit peptide.

[0005] Because of the central role aminoacyl-tRNA synthetases play in protein synthesis any agent that inhibits or disrupts aminoacyl-tRNA synthetase activity is likely to be toxic. Indeed a number of aminoacyl-tRNA synthetase inhibitors (antibiotics and herbicides) are known (Zon et al. (1988) *Phytochemistry* 27(3):711-714 and Heacock et al. (1996) *Bioorganic Chemistry* 24(3):273-289). Thus it may be possible to develop new herbicides that target aminoacyl-tRNA synthetases and engineer aminoacyl-tRNA synthetases that are resistant to such herbicides. Accordingly, the availability of nucleic acid sequences encoding all or a portion of these enzymes would facilitate studies to better understand protein synthesis in plants, provide genetic tools for the manipulation of gene expression, and provide a possible target for herbicides.

### SUMMARY OF THE INVENTION

[0006] The instant invention relates to isolated nucleic acid fragments encoding aminoacyl-tRNA synthetase. Spe-

cifically, this invention concerns an isolated nucleic acid fragment encoding an aspartyl-tRNA synthetase, cysteinyl-tRNA synthetase, tryptophanyl-tRNA synthetase or tyrosyl-tRNA synthetase and an isolated nucleic acid fragment that is substantially similar to an isolated nucleic acid fragment encoding an aspartyl-tRNA synthetase, cysteinyl-tRNA synthetase, tryptophanyl-tRNA synthetase or tyrosyl-tRNA synthetase. In addition, this invention relates to a nucleic acid fragment that is complementary to the nucleic acid fragment encoding aspartyl-tRNA synthetase, cysteinyl-tRNA synthetase, tryptophanyl-tRNA synthetase or tyrosyl-tRNA synthetase.

[0007] An additional embodiment of the instant invention pertains to a polypeptide encoding all or a substantial portion of an aminoacyl-tRNA synthetase selected from the group consisting of aspartyl-tRNA synthetase, cysteinyl-tRNA synthetase, tryptophanyl-tRNA synthetase and tyrosyl-tRNA synthetase.

[0008] In another embodiment, the instant invention relates to a chimeric gene encoding an aspartyl-tRNA synthetase, cysteinyl-tRNA synthetase, tryptophanyl-tRNA synthetase or tyrosyl-tRNA synthetase, or to a chimeric gene that comprises a nucleic acid fragment that is complementary to a nucleic acid fragment encoding an aspartyl-tRNA synthetase, cysteinyl-tRNA synthetase, tryptophanyl-tRNA synthetase or tyrosyl-tRNA synthetase, operably linked to suitable regulatory sequences, wherein expression of the chimeric gene results in production of levels of the encoded protein in a transformed host cell that is altered (i.e., increased or decreased) from the level produced in an untransformed host cell.

[0009] In a further embodiment, the instant invention concerns a transformed host cell comprising in its genome a chimeric gene encoding an aspartyl-tRNA synthetase, cysteinyl-tRNA synthetase, tryptophanyl-tRNA synthetase or tyrosyl-tRNA synthetase, operably linked to suitable regulatory sequences. Expression of the chimeric gene results in production of altered levels of the encoded protein in the transformed host cell. The transformed host cell can be of eukaryotic or prokaryotic origin, and include cells derived from higher plants and microorganisms. The invention also includes transformed plants that arise from transformed host cells of higher plants, and seeds derived from such transformed plants.

[0010] An additional embodiment of the instant invention concerns a method of altering the level of expression of an aspartyl-tRNA synthetase, cysteinyl-tRNA synthetase, tryptophanyl-tRNA synthetase or tyrosyl-tRNA synthetase in a transformed host cell comprising: a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding an aspartyl-tRNA synthetase, cysteinyl-tRNA synthetase, tryptophanyl-tRNA synthetase or tyrosyl-tRNA synthetase; and b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of altered levels of aspartyl-tRNA synthetase, cysteinyl-tRNA synthetase, tryptophanyl-tRNA synthetase or tyrosyl-tRNA synthetase in the transformed host cell.

[0011] An additional embodiment of the instant invention concerns a method for obtaining a nucleic acid fragment encoding all or a substantial portion of an amino acid

sequence encoding an aspartyl-tRNA synthetase, cysteinyl-tRNA synthetase, tryptophanyl-tRNA synthetase or tyrosyl-tRNA synthetase.

[0012] A further embodiment of the instant invention is a method for evaluating at least one compound for its ability to inhibit the activity of an aspartyl-tRNA synthetase, cysteinyl-tRNA synthetase, tryptophanyl-tRNA synthetase or tyrosyl-tRNA synthetase, the method comprising the steps of: (a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding an aspartyl-tRNA synthetase, cysteinyl-tRNA synthetase, tryptophanyl-tRNA synthetase or tyrosyl-tRNA synthetase, operably linked to suitable regulatory sequences; (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of aspartyl-tRNA synthetase, cysteinyl-tRNA synthetase, tryptophanyl-tRNA synthetase or tyrosyl-tRNA synthetase in the transformed host cell; (c) optionally purifying the aspartyl-tRNA synthetase, cysteinyl-tRNA synthetase, tryptophanyl-tRNA synthetase or tyrosyl-tRNA synthetase expressed by the transformed host cell; (d) treating the aspartyl-tRNA synthetase, cysteinyl-tRNA synthetase, tryptophanyl-tRNA synthetase or tyrosyl-tRNA synthetase with a compound to be tested; and (e) comparing the activity of the aspartyl-tRNA synthetase, cysteinyl-tRNA synthetase, tryptophanyl-tRNA synthetase or tyrosyl-tRNA synthetase that has been treated with a test compound to the activity of an untreated aspartyl-tRNA synthetase, cysteinyl-tRNA synthetase, tryptophanyl-tRNA synthetase or tyrosyl-tRNA synthetase, thereby selecting compounds with potential for inhibitory activity.

#### BRIEF DESCRIPTION OF THE SEQUENCE DESCRIPTIONS

[0013] The invention can be more fully understood from the following detailed description and the accompanying Sequence Listing which form a part of this application.

[0014] 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. 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

| Aminoacyl-tRNA Synthetase |                   |              |              |
|---------------------------|-------------------|--------------|--------------|
| Protein                   | Clone Designation | SEQ ID NO:   |              |
|                           |                   | (Nucleotide) | (Amino Acid) |
| Aspartyl-tRNA Synthetase  | p0094.cssth73r    | 1            | 2            |
| Aspartyl-tRNA Synthetase  | rl0n.pk0015.g11   | 3            | 4            |
| Aspartyl-tRNA Synthetase  | sfl1.pk0046.e8    | 5            | 6            |
| Aspartyl-tRNA Synthetase  | wle1n.pk0021.e6   | 7            | 8            |
| Cysteinyl-tRNA Synthetase | p0119.cmtmt52r    | 9            | 10           |
| Cysteinyl-tRNA Synthetase | rsl1n.pk016.p18   | 11           | 12           |
| Cysteinyl-tRNA Synthetase | sfl1.pk0013.f9    | 13           | 14           |

TABLE 1-continued

| Aminoacyl-tRNA Synthetase    |                   |              |              |
|------------------------------|-------------------|--------------|--------------|
| Protein                      | Clone Designation | SEQ ID NO:   |              |
|                              |                   | (Nucleotide) | (Amino Acid) |
| Tryptophanyl-tRNA Synthetase | p0118.chsbl87r    | 15           | 16           |
| Tryptophanyl-tRNA Synthetase | sdp4c.pk033.n11   | 17           | 18           |
| Tryptophanyl-tRNA Synthetase | wlm4.pk0013.c12   | 19           | 20           |
| Tyrosyl-tRNA Synthetase      | cs1.pk0035.d2     | 21           | 22           |

[0015] 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 Research* 13:3021-3030 (1985) and in the *Biochemical Journal* 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

[0016] In the context of this disclosure, a number of terms shall be utilized. As used herein, a "nucleic acid fragment" is a polymer of RNA or DNA that is single- or double-stranded, optionally containing synthetic, non-natural or altered nucleotide bases. A nucleic acid fragment in the form of a polymer of DNA may be comprised of one or more segments of cDNA, genomic DNA or synthetic DNA.

[0017] 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.

[0018] 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 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.

[0019] 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 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 6× SSC, 0.5% SDS at room temperature for 15 min, then repeated with 2× SSC, 0.5% SDS at 45° C. for 30 min, and then repeated twice with 0.2× 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 0.2× SSC, 0.5% SDS was increased to 60° C. Another preferred set of highly stringent conditions uses two final washes in 0.1× SSC, 0.1% SDS at 65° C.

[0020] 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. Preferred are those nucleic acid fragments whose nucleotide sequences encode amino acid sequences that are 80% identical to the amino acid sequences reported herein. More preferred nucleic acid fragments encode amino acid sequences that are 90% identical to the amino acid sequences reported herein. Most preferred are nucleic acid fragments that encode amino acid sequences that are 95% identical to the amino acid sequences reported herein. Sequence alignments and percent identity calculations were performed using the Megalign program of the LASAR-GENE bioinformatics computing suite (DNASTAR Inc., Madison, Wis.). Multiple alignment of the sequences was performed using the Clustal 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 Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5.

[0021] 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. 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 [www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)). 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-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 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 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.

[0022] "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.

[0023] "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 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 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.

[0024] “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, or chimeric genes. A “transgene” is a gene that has been introduced into the genome by a transformation procedure.

[0025] “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 processing or stability, or translation of the associated coding sequence. Regulatory sequences may include promoters, translation leader sequences, introns, and polyadenylation recognition sequences.

[0026] “Promoter” refers to a nucleotide sequence capable of controlling the expression of a coding sequence or functional RNA. In general, a coding sequence 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 promoter. Promoters may be derived in their entirety from a native gene, or be composed of different elements derived from different promoters found in nature, or even comprise synthetic 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 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 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.

[0027] The “translation leader sequence” refers to a nucleotide sequence located between the promoter sequence of a

gene and the coding sequence. The 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) *Molecular Biotechnology* 3:225).

[0028] The “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.

[0029] “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 polypeptide by the cell. “cDNA” refers to a double-stranded DNA that is complementary to and derived from mRNA. “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. Pat. 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.

[0030] The term “operably linked” refers to the association of two or more nucleic acid fragments on a single nucleic acid fragment 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.

[0031] 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. “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. Pat. No. 5,231,020, incorporated herein by reference).

[0032] “Altered levels” 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.

[0033] “Mature” 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. “Precursor” 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.

[0034] 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 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 present should be removed and instead a nuclear localization signal included (Raikhel (1992) *Plant Phys.* 100:1627-1632).

[0035] “Transformation” refers to the transfer of a nucleic acid fragment into the genome of a host organism, resulting in genetically stable inheritance. Host organisms containing the transformed nucleic acid fragments are referred to as “transgenic” organisms. Examples of methods of plant transformation include *Agrobacterium*-mediated transformation (De Blaere et al. (1987) *Meth. Enzymol.* 143:277) and particle-accelerated or “gene gun” transformation technology (Klein et al. (1987) *Nature (London)* 327:70-73; U.S. Pat. No. 4,945,050, incorporated herein by reference).

[0036] 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”).

[0037] Nucleic acid fragments encoding at least a portion of several aminoacyl-tRNA synthetases have been isolated and identified by comparison of random plant cDNA sequences to public databases containing nucleotide and protein sequences 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 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).

[0038] For example, genes encoding other aspartyl-tRNA synthetase, cysteinyl-tRNA synthetase, tryptophanyl-tRNA synthetase or tyrosyl-tRNA synthetase enzymes, either as

cDNAs or genomic DNAs, could be isolated directly by using all or a portion of the instant 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, the entire sequences can be used directly to synthesize DNA probes by methods known to the skilled artisan such as random primer DNA labeling, nick translation, or 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.

[0039] 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) 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; Loh et al. (1989) *Science* 243:217). Products generated by the 3' and 5' RACE procedures can be combined to generate full-length cDNAs (Frohman and Martin (1989) *Techniques* 1:165).

[0040] 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; Maniatis).

[0041] The nucleic acid fragments of the instant invention may 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 aminoacyl-tRNA synthetase activity in those cells.

[0042] Overexpression of the proteins of the instant invention may be accomplished by first constructing a chimeric

gene in which the coding region is operably linked to a promoter capable of directing expression of a gene in the desired tissues at the desired stage of development. For reasons of convenience, the chimeric gene may comprise promoter sequences and translation leader sequences derived from the same genes. 3' Non-coding sequences encoding transcription termination signals may also be provided. The instant chimeric gene may also comprise one or more introns in order to facilitate gene expression.

[0043] Plasmid vectors comprising the instant chimeric gene can then 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 plasmid vector in order to successfully transform, select and propagate host cells containing the 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 analysis of DNA, Northern analysis of mRNA expression, Western analysis of protein expression, or phenotypic analysis.

[0044] For some applications it may be useful to direct the instant polypeptides to different cellular compartments, or to facilitate its secretion from the cell. It is thus envisioned that the chimeric gene described above may be further supplemented by altering the coding sequence to encode the instant polypeptides with appropriate intracellular targeting sequences such as transit sequences (Keegstra (1989) *Cell* 56:247-253), signal sequences or sequences encoding endoplasmic reticulum localization (Chrispeels (1991) *Ann. Rev. Plant Phys. Plant Mol. Biol.* 42:21-53), or nuclear localization signals (Raikhel (1992) *Plant Phys.* 100:1627-1632) added and/or with targeting sequences that are already present removed. While the references cited give examples of each of these, the list is not exhaustive and more targeting signals of utility may be discovered in the future.

[0045] 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 chimeric gene designed for co-suppression of the instant polypeptide can be constructed by linking a gene or gene fragment encoding that polypeptide to plant promoter sequences. Alternatively, a chimeric gene 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 chimeric genes could be introduced into plants via transformation wherein expression of the corresponding endogenous genes are reduced or eliminated.

[0046] Molecular genetic solutions to the generation of plants with altered gene expression have a decided advantage over more traditional plant breeding approaches. Changes in plant phenotypes can be produced by specifically inhibiting expression of one or more genes by antisense inhibition or cosuppression (U.S. Pat. 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 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.

[0047] 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 chimeric genes 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, and is not an inherent part of the invention. 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.

[0048] 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 chimeric gene for production of the instant polypeptides. This chimeric gene could then be introduced into appropriate microorganisms via transformation to provide high level expression of the encoded aminoacyl-tRNA synthetase. An example of a vector for high level expression of the instant polypeptides in a bacterial host is provided (Example 9).

[0049] Additionally, the instant polypeptides can be used as targets to facilitate design and/or identification of inhibitors of those enzymes that may be useful as herbicides. This is desirable because the polypeptides described herein catalyze various steps in protein synthesis. Accordingly, inhibition of the activity of one or more of the enzymes described herein could lead to inhibition plant growth. Thus, the instant polypeptides could be appropriate for new herbicide discovery and design.

[0050] All or a substantial portion of the nucleic acid fragments of the instant invention may also be used as probes for genetically and physically mapping the genes that they are a part of, and 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 (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 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).

[0051] The production and use of plant gene-derived probes for use in genetic mapping is described in Bematzky and Tanksley (1986) *Plant Mol. Biol. Reporter* 4(1):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 isogenic lines, and other sets of individuals may be used for mapping. Such methodologies are well known to those skilled in the art.

[0052] 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 press 1996, pp. 319-346, and references cited therein).

[0053] In another embodiment, 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 to several hundred KB; see Laan et al. (1995) *Genome Research* 5:13-20), improvements in sensitivity may allow performance of FISH mapping using shorter probes.

[0054] 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.* 114(2):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) *Nature Genetics* 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.

[0055] 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; Koes et al. (1995) *Proc. Natl. Acad. Sci USA* 92:8149; Bensen et al. (1995) *Plant Cell* 7:75). 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 polypeptides. 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 polypeptides can be identified and obtained. This mutant plant can then be used to determine or confirm the natural function of the instant polypeptides disclosed herein.

## EXAMPLES

[0056] The present invention is further defined in the following Examples, in which all parts and percentages are by weight and degrees are Celsius, unless otherwise stated. It should be understood that these Examples, while indicating preferred 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.

### Example 1

#### Composition of cDNA Libraries; Isolation and Sequencing of cDNA Clones

[0057] cDNA libraries representing mRNAs from various corn, rice, soybean and wheat tissues were prepared. The characteristics of the libraries are described below.

TABLE 2

| cDNA Libraries from Corn, Rice, Soybean and Wheat |   |                 |
|---|---|-----------------|
| Library   | Tissue  | Clone           |
| cs1   | Corn leaf sheath from 5 week old plant  | cs1.pk0035.d2   |
| p0094   | Corn ear leaf sheath, 2-3 weeks after pollen shed*                                    | p0094.cssth73r  |
| p0118   | Corn pooled stem tissue from the 4-5 internodes subtending the tassel, V8-V12 stages* | p0118.chsbl87r  |
| p0119   | Corn ear shoot/w husk: V-12 stage*  | p0119.cmtmt52r  |
| r10n  | Rice 15 day old leaf*   | r10n.pk0015.g11 |
| rs11n   | Rice 15 day old seedling*   | rs11n.pk016.p18 |
| sdp4c   | Soybean developing embryo (9-11 mm)   | sdp4c.pk033.n11 |



TABLE 2-continued

| cDNA Libraries from Corn, Rice, Soybean and Wheat |  |                                |
|---|--|--------------------------------|
| Library   | Tissue   | Clone                          |
| sf1   | Soybean immature flower                                    | sf1.pk0013.f9<br>sf1.pk0046.e8 |
| wle1n   | Wheat leaf from 7 day old etiolated seedling*              | wle1n.pk0021.e6                |
| wlm4  | Wheat seedlings 4 hours after treatment with a fungicide** | wlm4.pk0013.c12                |

\*These libraries were normalized essentially as described in U.S. Pat. No. 5,482,845, incorporated herein by reference.

\*\*Fungicide: Application of 6-iodo-2-propoxy-3-propyl-4(3H)-quinazolinone synthesis and methods of using this compound are described in USSN 08/545, 827, incorporated herein by reference.

[0058] cDNA libraries may be prepared by any one of many methods available. For 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, Calif.). 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 pre-cut 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). The resulting ESTs are analyzed using a Perkin Elmer Model 377 fluorescent sequencer.

#### Example 2

##### Identification of cDNA Clones

[0059] cDNA clones encoding aminoacyl-tRNA synthetases were identified by conducting BLAST (Basic Local Alignment Search Tool; Altschul et al. (1993) *J. Mol. Biol.* 215:403-410; see also [www.ncbi.nlm.nih.gov/BLAST/](http://www.ncbi.nlm.nih.gov/BLAST/)) 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) *Nature Genetics* 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.

#### Example 3

##### Characterization of cDNA Clones Encoding Aspartyl-tRNA Synthetase

[0060] The BLASTX search using the EST sequences from clones listed in Table 3 revealed similarity of the polypeptides encoded by the cDNAs to aspartyl-tRNA synthetase from *Drosophila melanogaster* (NCBI Identifier No. gi 4512034), *Rattus norvegicus* (NCBI Identifier No. gi 135099) and *Homo sapiens* (NCBI Identifier No. gi 4557513). 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"), or contigs assembled from two or more ESTs ("Contig"):

TABLE 3

| BLAST Results for Sequences Encoding Polypeptides Homologous to <i>Drosophila melanogaster</i> , <i>Rattus norvegicus</i> and <i>Homo sapiens</i> Aspartyl-tRNA Synthetase |        |                     |
|--|--------|---------------------|
| Clone  | Status | BLAST pLog Score    |
| p0094.cssth73r   | FIS    | 134.00 (gi 4512034) |
| rl0n.pk0015.g11  | FIS    | 51.15 (gi 135099)   |
| sf1.pk0046.e8  | FIS    | 102.00 (gi 4557513) |
| wle1n.pk0021.e6  | FIS    | 21.40 (gi 4557513)  |

[0061] The data in Table 4 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOS: 2, 4, 6 and 8 and the *Drosophila melanogaster*, *Rattus norvegicus* and *Homo sapiens* aspartyl-tRNA synthetase sequences (SEQ ID NOS: 23, 24 and 25 respectively).

TABLE 4

| Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to <i>Drosophila melanogaster</i> , <i>Rattus norvegicus</i> and <i>Homo sapiens</i> Aspartyl-tRNA Synthetase |                     |
|--|---------------------|
| SEQ ID NO.   | Percent Identity to |
| 2  | 51% (gi 4512034)    |
| 4  | 65% (gi 135099)     |
| 6  | 51% (gi 4557513)    |
| 8  | 52% (gi 4557513)    |

[0062] Sequence alignments and percent identity calculations were performed using the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, Wis.). Multiple alignment of the sequences was performed using the Clustal 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 Clustal 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 a substantial portion of an aspartyl-tRNA synthetase. These sequences represent the first corn, rice, soybean and wheat sequences encoding aspartyl-tRNA synthetase.

#### Example 4

##### Characterization of cDNA Clones Encoding Cysteinyl-tRNA Synthetase

**[0063]** The BLASTX search using the EST sequences from clones listed in Table 5 revealed similarity of the polypeptides encoded by the cDNAs to cysteinyl-tRNA synthetase from *Haemophilus influenzae* (NCBI Identifier No. gi 1174501) and *Escherichia coli* (NCBI Identifier No. gi 41203). Shown in Table 5 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), or contigs assembled from two or more ESTs ("Contig"):

TABLE 5

| BLAST Results for Sequences Encoding Polypeptides Homologous to <i>Haemophilus influenzae</i> and <i>Escherichia coli</i> Cysteinyl-tRNA Synthetase |        |                     |
|---|--------|---------------------|
| Clone   | Status | BLAST pLog Score    |
| p0119.cmtmt52r  | FIS    | 104.00 (gi 1174501) |
| rsl1n.pk016.p18   | FIS    | 108.00 (gi 41203)   |
| sfl1.pk0013.f9  | FIS    | 117.00 (gi 1174501) |

**[0064]** The data in Table 6 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs: 10, 12 and 14 and the *Haemophilus influenzae* and *Escherichia coli* sequences (SEQ ID NOs: 26 and 27 respectively).

TABLE 6

| Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to <i>Haemophilus influenzae</i> and <i>Escherichia coli</i> Cysteinyl-tRNA Synthetase |                     |
|---|---------------------|
| SEQ ID NO.  | Percent Identity to |
| 10  | 43% (gi 1174501)    |
| 12  | 44% (gi 41203)      |
| 14  | 44% (gi 1174501)    |

**[0065]** Sequence alignments and percent identity calculations were performed using the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, Wis.). Multiple alignment of the sequences was performed using the Clustal 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 Clustal 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 a substantial portion of a cysteinyl-tRNA synthetase. These sequences represent the first corn, rice and soybean sequences encoding cysteinyl-tRNA synthetase.

#### Example 5

##### Characterization of cDNA Clones Encoding Tryptophanyl-tRNA Synthetase

**[0066]** The BLASTX search using the EST sequences from clones listed in Table 7 revealed similarity of the polypeptides encoded by the cDNAs to tryptophanyl-tRNA synthetase from *Synechocystis* sp. (NCBI Identifier No. gi 2501072). Shown in Table 7 are the BLAST results for individual ESTs ("EST"), the sequences of the entire cDNA inserts comprising the indicated cDNA clones ("FIS"), or contigs assembled from two or more ESTs ("Contig"):

TABLE 7

| BLAST Results for Sequences Encoding Polypeptides Homologous to <i>Synechocystis</i> sp. Tryptophanyl-tRNA Synthetase |        |                                  |
|---|--------|----------------------------------|
| Clone   | Status | BLAST pLog Score to (gi 2501072) |
| p0118.chsbl87r  | EST    | 104.00                           |
| sdp4c.pk033.n11   | FIS    | 103.00                           |
| wlm4.pk0013.c12   | FIS    | 43.22                            |

**[0067]** The data in Table 8 represents a calculation of the percent identity of the amino acid sequences set forth in SEQ ID NOs: 16, 18 and 24 and the *Synechocystis* sp. sequence (SEQ ID NO: 28).

TABLE 8

| Percent Identity of Amino Acid Sequences Deduced From the Nucleotide Sequences of cDNA Clones Encoding Polypeptides Homologous to <i>Synechocystis</i> sp. Tryptophanyl-tRNA Synthetase |                                  |
|---|----------------------------------|
| SEQ ID NO.  | Percent Identity to (gi 2501072) |
| 16  | 49%                              |
| 18  | 50%                              |
| 20  | 51%                              |

**[0068]** Sequence alignments and percent identity calculations were performed using the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, Wis.). Multiple alignment of the sequences was performed using the Clustal 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 Clustal 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 a substantial portion of a tryptophanyl-tRNA synthetase. These sequences represent the first corn, soybean and wheat sequences encoding tryptophanyl-tRNA synthetase.

#### Example 6

##### Characterization of cDNA Clones Encoding Tyrosyl-tRNA Synthetase

**[0069]** The BLASTX search using the EST sequence from the clone listed in Table 9 revealed similarity of the polypep-

tide encoded by the cDNA to tyrosyl-tRNA synthetase from *Bacillus caldotenax* (NCBI Identifier No. gi 135196). Shown in Table 9 are the BLAST results for the sequence of the entire cDNA insert comprising the indicated cDNA clone ("FIS"):

TABLE 9

| BLAST Results for Sequence Encoding Polypeptide Homologous to <i>Bacillus caldotenax</i> Tyrosyl-tRNA Synthetase |        |                                 |
|--|--------|---------------------------------|
| Clone  | Status | BLAST pLog Score to (gi 135196) |
| cs1.pk0035.d2  | FIS    | 62.52                           |

**[0070]** The data in Table 10 represents a calculation of the percent identity of the amino acid sequence set forth in SEQ ID NO:22 the *Bacillus caldotenax* sequence (SEQ ID NO: 29).

TABLE 10

| Percent Identity of Amino Acid Sequence Deduced From the Nucleotide Sequence of cDNA Clone Encoding Polypeptide Homologous to <i>Bacillus caldotenax</i> Tyrosyl-tRNA Synthetase |                                 |
|--|---------------------------------|
| SEQ ID NO.   | Percent Identity to (gi 135196) |
| 22   | 52%                             |

**[0071]** Sequence alignments and percent identity calculations were performed using the Megalign program of the LASARGENE bioinformatics computing suite (DNASTAR Inc., Madison, Wis.). Multiple alignment of the sequences was performed using the Clustal 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 Clustal 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 a substantial portion of a tyrosyl-tRNA synthetase. This sequence represent the first corn sequence encoding tyrosyl-tRNA synthetase.

#### Example 7

##### Expression of Chimeric Genes in Monocot Cells

**[0072]** A chimeric gene comprising a cDNA encoding the instant polypeptides 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 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 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 1.05 kb Sall-NcoI promoter fragment of the maize 27 kD zein gene and a 0.96 kb SmaI-Sall 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 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 chimeric gene encoding, in the 5' to 3' direction, the maize 27 kD zein promoter, a cDNA fragment encoding the instant polypeptides, and the 10 kD zein 3' region.

**[0073]** The chimeric gene 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 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.

**[0074]** 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 such as phosphinothricin. The pat gene in p35 S/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*.

**[0075]** The particle bombardment method (Klein et al. (1987) *Nature* 327:70-73) may be used to transfer genes to the callus culture cells. According to this method, gold particles (1 μm in diameter) are coated with DNA using the following technique. Ten μg of plasmid DNAs are added to 50 μL of a suspension of gold particles (60 mg per mL). Calcium chloride (50 μL of a 2.5 M solution) and spermidine free base (20 μL of a 1.0 M solution) are added to the particles. The suspension is 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 μL 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  $\mu\text{L}$  of ethanol. An aliquot (5  $\mu\text{L}$ ) of the DNA-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 Calif.), using a helium pressure of 1000 psi, a gap distance of 0.5 cm and a flying distance of 1.0 cm.

[0076] For bombardment, the embryogenic tissue is placed on filter paper over 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 membrane that bursts when the He pressure in the shock tube reaches 1000 psi.

[0077] Seven days after bombardment the tissue can be transferred to N6 medium that contains glufosinate (2 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 glufosinate. After 6 weeks, areas of about 1 cm in diameter of actively growing callus can be identified on some of the plates containing the glufosinate-supplemented medium. These calli may continue to grow when sub-cultured on the selective medium.

[0078] 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 8

##### Expression of Chimeric Genes in Dicot Cells

[0079] A seed-specific expression cassette composed of the promoter and transcription terminator from the gene encoding the  $\beta$  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 Nco I (which includes the ATG translation initiation codon), Sma I, Kpn I and Xba I. The entire cassette is flanked by Hind III sites.

[0080] The cDNA fragment of this gene may be generated by polymerase chain reaction (PCR) of the cDNA clone 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.

[0081] Soybean embryos may then be transformed with the expression vector comprising sequences encoding the instant polypeptides. 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.

[0082] 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.

[0083] Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Klein et al. (1987) *Nature* (London) 327:70, U.S. Pat. No. 4,945,050). A DuPont Biolistic™ PDS 1000/HE instrument (helium retrofit) can be used for these transformations.

[0084] 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 comprising the phaseolin 5' region, the fragment encoding the instant polypeptides 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.

[0085] To 50  $\mu\text{L}$  of a 60 mg/mL 1  $\mu\text{m}$  gold particle suspension is added (in order): 5  $\mu\text{L}$  DNA (1  $\mu\text{g}/\mu\text{L}$ ), 20  $\mu\text{L}$  spermidine (0.1 M), and 50  $\mu\text{L}$   $\text{CaCl}_2$  (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  $\mu\text{L}$  70% ethanol and resuspended in 40  $\mu\text{L}$  of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second each. Five  $\mu\text{L}$  of the DNA-coated gold particles are then loaded on each macro carrier disk.

[0086] 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 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.

[0087] 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 9

##### Expression of Chimeric Genes in Microbial Cells

**[0088]** 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 EcoR I and Hind III sites in pET-3a at their original positions. An oligonucleotide adaptor containing EcoR I and Hind III sites was inserted at the BamH I site of pET-3a. This created pET-3aM with additional unique cloning sites for insertion of genes into the expression vector. Then, the Nde I site at the position of translation initiation was converted to an Nco I site using oligonucleotide-directed mutagenesis. The DNA sequence of pET-3aM in this region, 5'-CATATGG, was converted to 5'-CCCATGG in pBT 430.

**[0089]** 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% NuSieve GTG™ low melting agarose gel (FMC). 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) 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, Beverly, Mass.). 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 polypeptides are then screened for the correct orientation with respect to the T7 promoter by restriction enzyme analysis.

**[0090]** 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 10

##### Evaluating Compounds for Their Ability to Inhibit the Activity of Aminoacyl-tRNA Synthetase

**[0091]** 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 9, 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<sub>6</sub>"). 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.

**[0092]** 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)<sub>6</sub> 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.

[0093] 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 aminoacyl-tRNA synthetases are presented by Zon et al. (1988) *Phytochemistry* 27(3):711-714 and Heacock et al. (1996) *Bioorganic Chemistry* 24(3):273-289.

---

 SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 29

<210> SEQ ID NO 1

<211> LENGTH: 1948

<212> TYPE: DNA

<213> ORGANISM: *Zea mays*

<400> SEQUENCE: 1

```

cgcacgatag ccgcccgcgt cgaccagagc actcccccggt cgtcgccacg atgtcgtctg      60
agcctccacc cgctctctct gcgcgcgcgc gagaggaact cgctgctgac ctttcgcgcg      120
ctacctctag caagaagcag cagaagaagg acgcgaggaa ggcggagaag gcagagcagc      180
gccacgctca gcagcagcag cagcagcagc cggcggacgc cgaggacccg ttcgcggcca      240
actacggcga ggtccccgctc gaggagatcc agtcaaaggc catctccggc cgctcgtggt      300
ccccatgtcgg cgacctcgac gactccgctg cgggccgctc cgtgcttata cgcggagccg      360
cgcaggccat ccgtccggtc agcaagaaga tggctttcgt cgtgctgcgc cagagtatg      420
gcaccgtgca gtgcgtgctc gtcgccagcg ccgacgccgg cgtcagcagc cagatggtc      480
gcttcgccac cgccctcagc aaggagtcca tcgtcgacgt tgaggcgcgc gctcctcccc      540
caaaggagcc cctcaaggcc accacacagc aggttgagat ccaagtgagg aagatcatt      600
gcatcaatag ggctattccg acccttccaa ttaacctga agatgcggct cggaggagg      660
cagattttga gaaggctgaa ttggctggag aaaagcttgt tcgcttggc caagtacct      720
gcttgaacta cagagctatt gatctacgaa caccctcga tcaagccata ttcggatcc      780
agtgtaaatg tgaaaacaaa tttagagatt ttttgttgc gaagaacttt gtgggatcc      840
acaccccaaa attgatttct ggatctagtg aagggggtgc ggctgtattc agcttctgt      900
acaatggtca acctgcttgt ttggcacaat cccctcagtt atacaagcaa tggctatct      960
ctggtgtggtt tgagcagata tttgaggtcg gccctgtggt tagagcagaa aattcaaaaa      1020
cacacagcca tctatgtgag ttcgttggtc ttgatgctga aatggagatt aaggagcatt      1080
atthttgaggt ctgtgacatt atagatggct tattcgtatc aatattttaa cacttgtctg      1140
aaaactgcaa gaaagaactc gaatcaataa acaggcagta tccattttaa cctctgaagt      1200
atctagacaa aacctttaag ctcaactatg aagaaggaat tcaaatgttg aaggaagccg      1260
gaacagaaat cgagcctatg ggtgacctca ataccgaagc tgagaaaaaa cttggtcggc      1320
ttgtcagggg aaagtatgac acagatthtt tcatcctgta tcggtatcct ttggtgttac      1380
gtccgttcta caccatgcct tggtatgaca acccagcgtc caccaattct tttgatgtct      1440
tcattcgagg cgaggagata atatctggag cacaaggat acacactcct gagctgctgg      1500
ccaagcgcgc gacagagtgt ggaatcgacg tgagcactat ctcggcctac attgaatcct      1560
tcagctatgg cgtgccgcca cacggcgggt tcgggggtggg tttggagagg gtggtgatgc      1620
tgttctgtgc cctgaacaac atcaggaaga cctcctggt cccgcgcgac ccgcagaggc      1680
tcgtgccgta agtttctgat tccaagcctg agtcttcgag tggctctacg agcagatccg      1740

```

-continued

---

```

atggtgttac catcagagtt gacttgcaat cttagctcct gaacctggcg gttaccgtgg 1800
atcagagttc ctgttgaatt tcacaaaagc ctacttgttc ctaatagatt gctgcaacca 1860
acaatattac gaccctttcg ggcttttctt cccgcctcac gtgttattct ggtctatact 1920
tgtttttaag tgcaagtatt gctcagtt 1948

```

```

<210> SEQ ID NO 2
<211> LENGTH: 546
<212> TYPE: PRT
<213> ORGANISM: Zea mays

```

```

<400> SEQUENCE: 2

```

```

Met Ser Ser Glu Pro Pro Pro Ala Ser Ser Ala Ala Ala Gly Glu Glu
 1           5           10
Leu Ala Ala Asp Leu Ser Ala Ala Thr Leu Ser Lys Lys Gln Gln Lys
 20           25           30
Lys Asp Ala Arg Lys Ala Glu Lys Ala Glu Gln Arg Gln Arg Gln Gln
 35           40           45
Gln Gln Gln Gln Gln Pro Ala Asp Ala Glu Asp Pro Phe Ala Ala Asn
 50           55           60
Tyr Gly Glu Val Pro Val Glu Glu Ile Gln Ser Lys Ala Ile Ser Gly
 65           70           75           80
Arg Ser Trp Ser His Val Gly Asp Leu Asp Asp Ser Ala Ala Gly Arg
 85           90           95
Ser Val Leu Ile Arg Gly Ala Ala Gln Ala Ile Arg Pro Val Ser Lys
 100          105          110
Lys Met Ala Phe Val Val Leu Arg Gln Ser Met Ser Thr Val Gln Cys
 115          120          125
Val Leu Val Ala Ser Ala Asp Ala Gly Val Ser Thr Gln Met Val Arg
 130          135          140
Phe Ala Thr Ala Leu Ser Lys Glu Ser Ile Val Asp Val Glu Gly Val
 145          150          155          160
Val Ser Leu Pro Lys Glu Pro Leu Lys Ala Thr Thr Gln Gln Val Glu
 165          170          175
Ile Gln Val Arg Lys Ile Tyr Cys Ile Asn Arg Ala Ile Pro Thr Leu
 180          185          190
Pro Ile Asn Leu Glu Asp Ala Ala Arg Ser Glu Ala Asp Phe Glu Lys
 195          200          205
Ala Glu Leu Ala Gly Glu Lys Leu Val Arg Val Gly Gln Asp Thr Arg
 210          215          220
Leu Asn Tyr Arg Ala Ile Asp Leu Arg Thr Pro Ser Asn Gln Ala Ile
 225          230          235          240
Phe Arg Ile Gln Cys Gln Val Glu Asn Lys Phe Arg Asp Phe Leu Leu
 245          250          255
Ser Lys Asn Phe Val Gly Ile His Thr Pro Lys Leu Ile Ser Gly Ser
 260          265          270
Ser Glu Gly Gly Ala Ala Val Phe Lys Leu Leu Tyr Asn Gly Gln Pro
 275          280          285
Ala Cys Leu Ala Gln Ser Pro Gln Leu Tyr Lys Gln Met Ala Ile Ser
 290          295          300
Gly Gly Phe Glu Arg Val Phe Glu Val Gly Pro Val Phe Arg Ala Glu
 305          310          315          320

```

-continued

Asn Ser Asn Thr His Arg His Leu Cys Glu Phe Val Gly Leu Asp Ala  
 325 330 335  
 Glu Met Glu Ile Lys Glu His Tyr Phe Glu Val Cys Asp Ile Ile Asp  
 340 345 350  
 Gly Leu Phe Val Ser Ile Phe Lys His Leu Ser Glu Asn Cys Lys Lys  
 355 360 365  
 Glu Leu Glu Ser Ile Asn Arg Gln Tyr Pro Phe Glu Pro Leu Lys Tyr  
 370 375 380  
 Leu Asp Lys Thr Phe Lys Leu Thr Tyr Glu Glu Gly Ile Gln Met Leu  
 385 390 395 400  
 Lys Glu Ala Gly Thr Glu Ile Glu Pro Met Gly Asp Leu Asn Thr Glu  
 405 410 415  
 Ala Glu Lys Lys Leu Gly Arg Leu Val Arg Glu Lys Tyr Asp Thr Asp  
 420 425 430  
 Phe Phe Ile Leu Tyr Arg Tyr Pro Leu Ala Val Arg Pro Phe Tyr Thr  
 435 440 445  
 Met Pro Cys Tyr Asp Asn Pro Ala Tyr Thr Asn Ser Phe Asp Val Phe  
 450 455 460  
 Ile Arg Gly Glu Glu Ile Ile Ser Gly Ala Gln Arg Ile His Thr Pro  
 465 470 475 480  
 Glu Leu Leu Ala Lys Arg Ala Thr Glu Cys Gly Ile Asp Val Ser Thr  
 485 490 495  
 Ile Ser Ala Tyr Ile Glu Ser Phe Ser Tyr Gly Val Pro Pro His Gly  
 500 505 510  
 Gly Phe Gly Val Gly Leu Glu Arg Val Val Met Leu Phe Cys Ala Leu  
 515 520 525  
 Asn Asn Ile Arg Lys Thr Ser Leu Phe Pro Arg Asp Pro Gln Arg Leu  
 530 535 540  
 Val Pro  
 545

<210> SEQ ID NO 3  
 <211> LENGTH: 730  
 <212> TYPE: DNA  
 <213> ORGANISM: Oryza sativa

<400> SEQUENCE: 3

gcacgagctt acacggcacg agcttacagg aattcaaatg ctgaaggaag ctggaacaga 60  
 aatcgaacc atgggtgacc tcaacactga agctgagaaa aaactaggcc ggcttgtaa 120  
 ggagaagtat ggaacagaat ttttcatcct ctatcgggat cctttggctg tgcgtccctt 180  
 ctacaccatg cctgtttatg acaaccacgc ttacagtaac tcttttgatg tctttattcg 240  
 aggagaggaa ataatatctg gagcacaag aatacattta ccagagctat tgacgaaacg 300  
 tgcaacagag tgtggaattg atgcgagtac ttttcatca tatatcgaat cgttcagcta 360  
 tgggtcacct cctcatgggt gttttgggtg cggcctggag aggggtgtaa tgctgttctg 420  
 cgccctaacc aacatcagga agacatcact tttcctcgc gatocacaaa ggctgggtgcc 480  
 ataatttgot ttttttccca agagcaaggt ttggactcag tacggactgg gcagttttcc 540  
 tcggctgggt tttttacctg gacattatgt togtatttat taatgtgctg tactgcaaaa 600  
 gctgctcctt tccacaacat ttggaatagt tgccgataca tttggaatag ggctcaacgt 660



-continued

---

```

tggcgttgtg atttcgttga tgatcccgct attcgtaca aaaaaaaaaa aaaaaaaaaa 720
aaaaaaaaaa 730

```

```

<210> SEQ ID NO 4
<211> LENGTH: 148
<212> TYPE: PRT
<213> ORGANISM: Oryza sativa

```

```

<400> SEQUENCE: 4

```

```

Met Leu Lys Glu Ala Gly Thr Glu Ile Glu Pro Met Gly Asp Leu Asn
 1             5             10             15
Thr Glu Ala Glu Lys Lys Leu Gly Arg Leu Val Lys Glu Lys Tyr Gly
          20             25             30
Thr Glu Phe Phe Ile Leu Tyr Arg Tyr Pro Leu Ala Val Arg Pro Phe
          35             40             45
Tyr Thr Met Pro Cys Tyr Asp Asn Pro Ala Tyr Ser Asn Ser Phe Asp
 50             55             60
Val Phe Ile Arg Gly Glu Glu Ile Ile Ser Gly Ala Gln Arg Ile His
 65             70             75             80
Leu Pro Glu Leu Leu Thr Lys Arg Ala Thr Glu Cys Gly Ile Asp Ala
          85             90             95
Ser Thr Ile Ser Ser Tyr Ile Glu Ser Phe Ser Tyr Gly Ala Pro Pro
          100            105            110
His Gly Gly Phe Gly Val Gly Leu Glu Arg Val Val Met Leu Phe Cys
          115            120            125
Ala Leu Asn Asn Ile Arg Lys Thr Ser Leu Phe Pro Arg Asp Pro Gln
          130            135            140
Arg Leu Val Pro
145

```

```

<210> SEQ ID NO 5
<211> LENGTH: 1109
<212> TYPE: DNA
<213> ORGANISM: Glycine max

```

```

<400> SEQUENCE: 5

```

```

gcacgaggtc atcagagaga atggcttcac cgttcaatgc ttggtgcagg cgcaggccga 60
tacggtgagc ccgagatgg tgaagttcgc cgctgcactc agccgcgagt ccatcgtcga 120
tgtcgaaggc gttgtttcga tcccctccgc tcccatcaaa ggcgccacac aacaggtgga 180
aattcaagtg aggaagttgt attgtgtcag tagggctgta cctactctgc ctattaatct 240
tgaggatgct gctcgaagtg aagttgaaat cgagacggct cttcaggctg gtgagcaact 300
tgttcgtggt aatcaggata cacgtctgaa ctttaggggt cttgatgtgc gaacgccagc 360
taatcaaggg attttccgca ttcagtctca agttggaat gcgtttagac aattcttatt 420
atctgaaggt ttttgtgaaa tccacactcc aaagttgata gctggatcta gtgagggagg 480
agctgctggt tttagactgg actacaaagg tcaacctgca tgccctggccc agtcacctca 540
gcttcacaag caaatgtcta tttgtggaga ttttggcgtg gtttttgaga ttggtcctgt 600
gttttagagca gaagattcct aactcacag goactctgtgt gagtttacag gtcttgatgt 660
tgaaatggag attaagaagc attactttga ggttatggat atagtcgata gattgtttgt 720
cgcaatgttt gacagtttga accagaattg taagaaggat ctggaagctg tcgggtctca 780

```

## -continued

---

```

gtatccattt gaaccttga agtatctgcg gacgacacta cggcttacat atgaagaagg      840
gattcagatg ctcaaggatg ttggagtaga aattgaacct tatggtgact tgaatactga      900
agcgaaaagg aaattgggct agctagtctc agagaaatat ggcacagagt tctatatact      960
tcaccggtag cctttggctg taaggccatt ctatacaatg ccttgctacg acaatcctgc     1020
atacagcaac tcgtttgatg tctttattcg aggtgaggag ataatttcag gagctcagcg     1080
tgttcatgtg ccagaatddd tggaacaag                                     1109

```

&lt;210&gt; SEQ ID NO 6

&lt;211&gt; LENGTH: 369

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Glycine max

&lt;400&gt; SEQUENCE: 6

```

His Glu Val Ile Arg Glu Asn Gly Phe Thr Val Gln Cys Leu Val Gln
  1           5           10           15
Ala Gln Ala Asp Thr Val Ser Pro Gln Met Val Lys Phe Ala Ala Ala
          20           25           30
Leu Ser Arg Glu Ser Ile Val Asp Val Glu Gly Val Val Ser Ile Pro
          35           40           45
Ser Ala Pro Ile Lys Gly Ala Thr Gln Gln Val Glu Ile Gln Val Arg
          50           55           60
Lys Leu Tyr Cys Val Ser Arg Ala Val Pro Thr Leu Pro Ile Asn Leu
          65           70           75           80
Glu Asp Ala Ala Arg Ser Glu Val Glu Ile Glu Thr Ala Leu Gln Ala
          85           90           95
Gly Glu Gln Leu Val Arg Val Asn Gln Asp Thr Arg Leu Asn Phe Arg
          100          105          110
Val Leu Asp Val Arg Thr Pro Ala Asn Gln Gly Ile Phe Arg Ile Gln
          115          120          125
Ser Gln Val Gly Asn Ala Phe Arg Gln Phe Leu Leu Ser Glu Gly Phe
          130          135          140
Cys Glu Ile His Thr Pro Lys Leu Ile Ala Gly Ser Ser Glu Gly Gly
          145          150          155          160
Ala Ala Val Phe Arg Leu Asp Tyr Lys Gly Gln Pro Ala Cys Leu Ala
          165          170          175
Gln Ser Pro Gln Leu His Lys Gln Met Ser Ile Cys Gly Asp Phe Gly
          180          185          190
Arg Val Phe Glu Ile Gly Pro Val Phe Arg Ala Glu Asp Ser Tyr Thr
          195          200          205
His Arg His Leu Cys Glu Phe Thr Gly Leu Asp Val Glu Met Glu Ile
          210          215          220
Lys Lys His Tyr Phe Glu Val Met Asp Ile Val Asp Arg Leu Phe Val
          225          230          235          240
Ala Met Phe Asp Ser Leu Asn Gln Asn Cys Lys Lys Asp Leu Glu Ala
          245          250          255
Val Gly Ser Gln Tyr Pro Phe Glu Pro Leu Lys Tyr Leu Arg Thr Thr
          260          265          270
Leu Arg Leu Thr Tyr Glu Glu Gly Ile Gln Met Leu Lys Asp Val Gly
          275          280          285

```

-continued

Val Glu Ile Glu Pro Tyr Gly Asp Leu Asn Thr Glu Ala Glu Arg Lys  
 290 295 300  
 Leu Gly Gln Leu Val Ser Glu Lys Tyr Gly Thr Glu Phe Tyr Ile Leu  
 305 310 315 320  
 His Arg Tyr Pro Leu Ala Val Arg Pro Phe Tyr Thr Met Pro Cys Tyr  
 325 330 335  
 Asp Asn Pro Ala Tyr Ser Asn Ser Phe Asp Val Phe Ile Arg Gly Glu  
 340 345 350  
 Glu Ile Ile Ser Gly Ala Gln Arg Val His Val Pro Glu Phe Leu Glu  
 355 360 365

Gln

<210> SEQ ID NO 7  
 <211> LENGTH: 836  
 <212> TYPE: DNA  
 <213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 7

tacacatgca gactttcagt gagtttttgt tctcggactt gggatccaca gtccaaagtt 60  
 gattggtgga tcaagtgaac ttggtgcatc tccattcaag ctggcgtaca attaccaacc 120  
 tgcttattta gcgagctctc tacaatcata caagcaaatg agcatctgtg gtggctttgg 180  
 gcgctgtgtt gaggctggtc cggtatntag atcagaaaaa tcaaacactc acaggcatct 240  
 atgtgagttt attgggttgg atgcagaaat ggagattaag gagcactact ttgaggtttg 300  
 tgatatcata gattgctaata ttagcaata ttcaaacacc caaatgaaaa ttgtcagaag 360  
 gaactcgaga caataaatag gcagtatcca tttgaacctc tgaagtacct agagaaaacg 420  
 ttgaagctaa cgtacgagga agggatataa atgctcaagg tttcattctg gaatcctcta 480  
 ggcagggtgc ttgcaatccc ctacatctcg gotgcaacaa aaaagacca acgaggctgt 540  
 tgtttcaagc tcagaccctc ttcattgcac gcggtgctag aaggagaact gggttgtggt 600  
 gctgttgctg gtcgttttcc tttttacttt tgcactttgg ccgtcataaa cgatacatgc 660  
 ttgtccctcg gatggatctc tttctctccc tggatctttt aaacaggtgt tgtgattaa 720  
 attgtgataa atcagtgttc atcactaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaaaa 780  
 aatctcgagg gggggcccgg tactgttcac cgcgtggcgc cgggctagag actagt 836

<210> SEQ ID NO 8  
 <211> LENGTH: 98  
 <212> TYPE: PRT  
 <213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 8

Val Phe Val Leu Gly Leu Gly Ile His Ser Pro Lys Leu Ile Gly Gly  
 1 5 10 15  
 Ser Ser Glu Leu Gly Ala Ser Pro Phe Lys Leu Ala Tyr Asn Tyr Gln  
 20 25 30  
 Pro Ala Tyr Leu Ala Gln Ser Leu Gln Ser Tyr Lys Gln Met Ser Ile  
 35 40 45  
 Cys Gly Gly Phe Gly Arg Val Phe Glu Ala Gly Pro Val Phe Arg Ser  
 50 55 60  
 Glu Lys Ser Asn Thr His Arg His Leu Cys Glu Phe Ile Gly Leu Asp  
 65 70 75 80

-continued

Ala Glu Met Glu Ile Lys Glu His Tyr Phe Glu Val Cys Asp Ile Ile  
 85 90 95

Asp Cys

<210> SEQ ID NO 9  
 <211> LENGTH: 2085  
 <212> TYPE: DNA  
 <213> ORGANISM: Zea mays

&lt;400&gt; SEQUENCE: 9

ggaaaccgtg tttcgacggg ccgcagtgagg cagtggttg gcccatcgaa cccacttgcc 60  
 actcacttcc acctgaacct tgccctgctt tctctcgacg actcccctgt ccccgccgcc 120  
 gccgcccggc caaatccctt tccgctctgt tctggcctct ggggcttcta ggttagcgcg 180  
 tgcgaccacc atggccgagg aggtccaggc tccactttcc gccaccatgg cgaaggaggc 240  
 ccagtcgccc ccgtccgcaa ccatagcggg ggcgacggcg ccgcccgcgc tcttattatt 300  
 taactccttt acgaagaggg aggagccatt ccagccccgg gtagagggga aggtagggat 360  
 gtacgtctgt ggcgtcactc cctacgactt tagccacatc ggccacgcgc gtgcctacgt 420  
 cgccttcgac gtcctctaca ggtaccttaa attcttgggg tatgaagttg aatagtcccg 480  
 taatttcaag gatattgatg acaagattat taagcgtgcc aatgaacgcg gtgaacacgt 540  
 aacaagcctg agtagccagt ttatcaatga atttcttctt gacatgactg agctccagtg 600  
 cttgcctcct acctgcgagc cacgggtaac agaacacatt gagcatatta taaagttgat 660  
 aacacagata atggagaatg gcaaagccta tgctattgaa ggagatgttt acttttcagt 720  
 tgaaagtttt cctgaatata tcagtttata tggaagaaaa tttgatcaaa atcaggcagg 780  
 tgcaacgggt gcttttgata caagaaagcg taatcctgca gacttcgcac tctggaaaagc 840  
 tgcaaaggag ggtgaacctt ttgggatag cccttggggc cgtggaagac caggttgcca 900  
 tattgaatgc agcgaatga gtgctcacta tttaggacat gtattcgata ttcatggtgg 960  
 ggggaaagat ttgatttttc ctcatcatga gaatgagctt gcacaaagcc gcgcagctta 1020  
 tctctgatagc gaggtcaaat gctggatgca caatggcttt gttaacaagg atgataaaaa 1080  
 aatggcaaaa tcagataata actttttcac gattagagat atcattgctc tttaccatcc 1140  
 aatggcttta agatttttct tgatgcccac acattataga tcagatgtta accattctga 1200  
 tcaagcgcct gagattgcat ctgatcgtgt ctactacatt tatcagactc tatatgactg 1260  
 tgaggaagtg ttagctacat atcgtgaaga gggctacctt ctcccagtcg cgtctgagga 1320  
 gcaaaatctg attggtaagc accattcaga attcttgaaa catatgtcga atgatcttaa 1380  
 aaccacagat gttctggacc gttgcttcat ggagctgctg aaggccataa acagcagtct 1440  
 gaatgatttg aagaaatgc agcaaaaaat agaacagcaa aagaagaaac agcaacagca 1500  
 gaagaagcag caacagcaga agcagcagca acagaagcaa cagcaattgc aaaaacagcc 1560  
 agaagattat attcaagctc tgattgcact ggaacagaa cttaaaaaca aattgtctat 1620  
 acttggtctg atgccatctt catctttggc agaggtactg aagcaattga aggacaaatc 1680  
 attaaagcga gcagggctga ctgaagaaca attgcaagag cagattgagc agagaaatgt 1740  
 cgcaaggaag aataagcagt ttgagatata tgatggaatc aggaaaaacc ttgctaccaa 1800  
 aggcacgcgc ctgatggagc aaccttctgg tacagtatgg agaccatgcg aaccagagcg 1860

-continued

---

```

gtctgaagag tcatgattag ctcaactgact caacaagtga tggcgggtgta aatgagatt 1920
tttgctgag ggcagttatc gcattttgaa gactaacaaa aatcgccatc tctggatgtg 1980
gtattctaca gggtagggtt tccaggttga ctcaccagtt aaaacatgca tttctggttg 2040
tataacaagc aatgaacccc atatataatac ttgacagttg actcc 2085

```

```

<210> SEQ ID NO 10
<211> LENGTH: 599
<212> TYPE: PRT
<213> ORGANISM: Zea mays

```

```

<400> SEQUENCE: 10

```

```

Thr Leu Pro Cys Leu Leu Ser Thr Thr Pro Leu Ser Pro Pro Pro Pro
 1          5          10          15
Pro Pro Gln Ile Pro Phe Arg Val Cys Leu Ala Ser Gly Ala Ser Arg
 20          25          30
Leu Ala Arg Ala Thr Thr Met Ala Glu Glu Val Gln Ala Pro Leu Ser
 35          40          45
Ala Thr Met Ala Lys Glu Ala Gln Ser Pro Pro Ser Ala Thr Ile Ala
 50          55          60
Glu Ala Thr Ala Pro Pro Gln Leu Leu Leu Phe Asn Ser Phe Thr Lys
 65          70          75          80
Arg Glu Glu Pro Phe Gln Pro Arg Val Glu Gly Lys Val Gly Met Tyr
 85          90          95
Val Cys Gly Val Thr Pro Tyr Asp Phe Ser His Ile Gly His Ala Arg
100          105          110
Ala Tyr Val Ala Phe Asp Val Leu Tyr Arg Tyr Leu Lys Phe Leu Gly
115          120          125
Tyr Glu Val Glu Tyr Val Arg Asn Phe Thr Asp Ile Asp Asp Lys Ile
130          135          140
Ile Lys Arg Ala Asn Glu Arg Gly Glu Thr Val Thr Ser Leu Ser Ser
145          150          155          160
Gln Phe Ile Asn Glu Phe Leu Leu Asp Met Thr Glu Leu Gln Cys Leu
165          170          175
Pro Pro Thr Cys Glu Pro Arg Val Thr Glu His Ile Glu His Ile Ile
180          185          190
Lys Leu Ile Thr Gln Ile Met Glu Asn Gly Lys Ala Tyr Ala Ile Glu
195          200          205
Gly Asp Val Tyr Phe Ser Val Glu Ser Phe Pro Glu Tyr Leu Ser Leu
210          215          220
Ser Gly Arg Lys Phe Asp Gln Asn Gln Ala Gly Ala Arg Val Ala Phe
225          230          235          240
Asp Thr Arg Lys Arg Asn Pro Ala Asp Phe Ala Leu Trp Lys Ala Ala
245          250          255
Lys Glu Gly Glu Pro Phe Trp Asp Ser Pro Trp Gly Arg Gly Arg Pro
260          265          270
Gly Trp His Ile Glu Cys Ser Ala Met Ser Ala His Tyr Leu Gly His
275          280          285
Val Phe Asp Ile His Gly Gly Gly Lys Asp Leu Ile Phe Pro His His
290          295          300
Glu Asn Glu Leu Ala Gln Ser Arg Ala Ala Tyr Pro Asp Ser Glu Val
305          310          315          320

```

-continued

---

Lys Cys Trp Met His Asn Gly Phe Val Asn Lys Asp Asp Lys Lys Met  
 325 330 335

Ala Lys Ser Asp Asn Asn Phe Phe Thr Ile Arg Asp Ile Ile Ala Leu  
 340 345 350

Tyr His Pro Met Ala Leu Arg Phe Phe Leu Met Arg Thr His Tyr Arg  
 355 360 365

Ser Asp Val Asn His Ser Asp Gln Ala Leu Glu Ile Ala Ser Asp Arg  
 370 375 380

Val Tyr Tyr Ile Tyr Gln Thr Leu Tyr Asp Cys Glu Glu Val Leu Ala  
 385 390 395 400

Thr Tyr Arg Glu Glu Gly Thr Ser Leu Pro Val Pro Ser Glu Glu Gln  
 405 410 415

Asn Leu Ile Gly Lys His His Ser Glu Phe Leu Lys His Met Ser Asn  
 420 425 430

Asp Leu Lys Thr Thr Asp Val Leu Asp Arg Cys Phe Met Glu Leu Leu  
 435 440 445

Lys Ala Ile Asn Ser Ser Leu Asn Asp Leu Lys Lys Leu Gln Gln Lys  
 450 455 460

Ile Glu Gln Gln Lys Lys Lys Gln Gln Gln Gln Lys Lys Gln Gln Gln  
 465 470 475 480

Gln Lys Gln Gln Gln Gln Lys Gln Gln Gln Leu Gln Lys Gln Pro Glu  
 485 490 495

Asp Tyr Ile Gln Ala Leu Ile Ala Leu Glu Thr Glu Leu Lys Asn Lys  
 500 505 510

Leu Ser Ile Leu Gly Leu Met Pro Ser Ser Ser Leu Ala Glu Val Leu  
 515 520 525

Lys Gln Leu Lys Asp Lys Ser Leu Lys Arg Ala Gly Leu Thr Glu Glu  
 530 535 540

Gln Leu Gln Glu Gln Ile Glu Gln Arg Asn Val Ala Arg Lys Asn Lys  
 545 550 555 560

Gln Phe Glu Ile Ser Asp Gly Ile Arg Lys Asn Leu Ala Thr Lys Gly  
 565 570 575

Ile Ala Leu Met Asp Glu Pro Ser Gly Thr Val Trp Arg Pro Cys Glu  
 580 585 590

Pro Glu Arg Ser Glu Glu Ser  
 595

<210> SEQ ID NO 11  
 <211> LENGTH: 1957  
 <212> TYPE: DNA  
 <213> ORGANISM: Oryza sativa

<400> SEQUENCE: 11

```

cgccagttct agggttagct cgtcggcgtc cagccctctc actctccccc tccgctctca    60
cgatggcgga gagcggaag ccgacgccgc agctggagct cttcaactcg atgacgaaga    120
agaaggagct cttcgagccg cttgtggagg ggaaggccg catgtatgtg tgccggctca    180
cgccctacga cttcagccac atcggccacg cccgcgccta cgtgccttc gacgtctct    240
acaggtatct taaattcttg gggtagcagg togaatatgt gcgcaacttc actgatattg    300
atgacaagat tatcaaacga gcaaatgaag ctggtgaaac tgtaactagc ttgagcagcc    360
ggtttattaa tgaattcctt ctogatatgg ctgagctcca gtgcttacc ccaacttgtg    420
    
```

-continued

```

agccacgtgt gacggatcac attgaacata ttatagagtt gataaccaag ataatggaga 480
atgggaaagc ctatgctatg gaaggagatg tttacttttc agttgatact ttcctgagtt 540
atctcagttt atctggaagc aagtttagatc ataactcttc tggttcgagg gttgctgtcg 600
atacaagaaa gcggaacctc gcagactttg cgctgtggaa ggctgctaag gaaggcgaac 660
ctttctggga tagcccatgg gccctgggta gaccaggatg gcatattgaa tgcagtgcaa 720
tgagtgtcoa ttatttagga catgtgtttg atatccatgg tggagggaaa gatctgatat 780
ttcctcatca tgagaatgag cttgctcaga gccgggcagc ttatccagaa agtgagggtca 840
aatgttggat gcacaatggg tttgttaaca aggatgatca gaaaatgtca aagtcagata 900
aaaattttct cacaaatcca gatattattg atctgtacca tcccatggct ttgagggttt 960
tctctgatgog cacacattac agaggagatg tgaatcactc tgacaaagca cttgagatag 1020
catctgatog tgtctactac atatacaga ctttatatga ctgtgaggaa gtgttgcctc 1080
aatatcgtgg agagaatata tctgtcccgg tccctgttga ggaacaagat atggtaaca 1140
agcaccattc agaattcttg gaatctatgg cggatgatct tagaacaaca gatgttctgg 1200
atggcctttc tgacttgctg aaggcaatta acagcaatct gaatgatttt aagaagttgc 1260
aacagaagct agagcagcaa aagaagaaac aacaacagca gaagcagcag aagcaaaagc 1320
agcagcagcg acagaaacaa ccagaagaat atattcaagc tatgtttgca cttgagacag 1380
aaattaaaaa taaaatatct atccttggtc tgatgccacc ttcttccttg gcagaggcac 1440
tgaagcaact taaggataaa gctttgaaga gagcaggggt gactgaagaa ctgttgacag 1500
agcaaatgta gcagagaact gctgcaagga aaaacaagca gtttgatgtg tctgacaaa 1560
tcaggaaaca gctaggcagc aaagcagatg ccctcatgga tgaacctact ggtacagtat 1620
ggagaccatg cgagccagag tctgaatagt cacatgattg atttgtgctt tggtaaacag 1680
gtgatggtag aaactggaaa atttaaccaa gcacatctgc tgaattggtg taaattgatg 1740
cagatcaaca tttttttttg taattttgta ggggtttaag ttcactggcc aactgaaact 1800
tgcgtttctc gtgtgtgtaag aagcaaaacc ccatatactg atatactcga ggactccctt 1860
gttgatgatt atgctttgga ttggaatatt gaagtcaaat cataattaca tttgcatgat 1920
caaaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaa 1957

```

&lt;210&gt; SEQ ID NO 12

&lt;211&gt; LENGTH: 548

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Oryza sativa

&lt;400&gt; SEQUENCE: 12

```

Pro Val Leu Gly Leu Ala Arg Arg Arg Pro Ala Leu Ser Leu Ser Pro
 1           5           10          15
Ser Ala Leu Thr Met Ala Glu Ser Ala Lys Pro Thr Pro Gln Leu Glu
          20           25           30
Leu Phe Asn Ser Met Thr Lys Lys Lys Glu Leu Phe Glu Pro Leu Val
          35           40           45
Glu Gly Lys Val Arg Met Tyr Val Cys Gly Val Thr Pro Tyr Asp Phe
          50           55           60
Ser His Ile Gly His Ala Arg Ala Tyr Val Ala Phe Asp Val Leu Tyr
          65           70           75           80

```

-continued

---

Arg Tyr Leu Lys Phe Leu Gly Tyr Glu Val Glu Tyr Val Arg Asn Phe  
                   85                                  90                                  95  
 Thr Asp Ile Asp Asp Lys Ile Ile Lys Arg Ala Asn Glu Ala Gly Glu  
                   100                                  105                                  110  
 Thr Val Thr Ser Leu Ser Ser Arg Phe Ile Asn Glu Phe Leu Leu Asp  
                   115                                  120                                  125  
 Met Ala Gln Leu Gln Cys Leu Pro Pro Thr Cys Glu Pro Arg Val Thr  
                   130                                  135                                  140  
 Asp His Ile Glu His Ile Ile Glu Leu Ile Thr Lys Ile Met Glu Asn  
                   145                                  150                                  155                                  160  
 Gly Lys Ala Tyr Ala Met Glu Gly Asp Val Tyr Phe Ser Val Asp Thr  
                   165                                  170                                  175  
 Phe Pro Glu Tyr Leu Ser Leu Ser Gly Arg Lys Leu Asp His Asn Leu  
                   180                                  185                                  190  
 Ala Gly Ser Arg Val Ala Val Asp Thr Arg Lys Arg Asn Pro Ala Asp  
                   195                                  200                                  205  
 Phe Ala Leu Trp Lys Ala Ala Lys Glu Gly Glu Pro Phe Trp Asp Ser  
                   210                                  215                                  220  
 Pro Trp Gly Arg Gly Arg Pro Gly Trp His Ile Glu Cys Ser Ala Met  
                   225                                  230                                  235                                  240  
 Ser Ala His Tyr Leu Gly His Val Phe Asp Ile His Gly Gly Gly Lys  
                   245                                  250                                  255  
 Asp Leu Ile Phe Pro His His Glu Asn Glu Leu Ala Gln Ser Arg Ala  
                   260                                  265                                  270  
 Ala Tyr Pro Glu Ser Glu Val Lys Cys Trp Met His Asn Gly Phe Val  
                   275                                  280                                  285  
 Asn Lys Asp Asp Gln Lys Met Ser Lys Ser Asp Lys Asn Phe Phe Thr  
                   290                                  295                                  300  
 Ile Arg Asp Ile Ile Asp Leu Tyr His Pro Met Ala Leu Arg Phe Phe  
                   305                                  310                                  315                                  320  
 Leu Met Arg Thr His Tyr Arg Gly Asp Val Asn His Ser Asp Lys Ala  
                   325                                  330                                  335  
 Leu Glu Ile Ala Ser Asp Arg Val Tyr Tyr Ile Tyr Gln Thr Leu Tyr  
                   340                                  345                                  350  
 Asp Cys Glu Glu Val Leu Ser Gln Tyr Arg Gly Glu Asn Ile Ser Val  
                   355                                  360                                  365  
 Pro Val Pro Val Glu Glu Gln Asp Met Val Asn Lys His His Ser Glu  
                   370                                  375                                  380  
 Phe Leu Glu Ser Met Ala Asp Asp Leu Arg Thr Thr Asp Val Leu Asp  
                   385                                  390                                  395                                  400  
 Gly Phe Thr Asp Leu Leu Lys Ala Ile Asn Ser Asn Leu Asn Asp Phe  
                   405                                  410                                  415  
 Lys Lys Leu Gln Gln Lys Leu Glu Gln Gln Lys Lys Lys Gln Gln Gln  
                   420                                  425                                  430  
 Gln Lys Gln Gln Lys Gln Lys Gln Gln Gln Ala Gln Lys Gln Pro Glu  
                   435                                  440                                  445  
 Glu Tyr Ile Gln Ala Met Phe Ala Leu Glu Thr Glu Ile Lys Asn Lys  
                   450                                  455                                  460  
 Ile Ser Ile Leu Gly Leu Met Pro Pro Ser Ser Leu Ala Glu Ala Leu  
                   465                                  470                                  475                                  480





-continued

---

```

ctaaatcgga tgcaatcagg aaggatttgg ctgtacttgg tattactctt atggacagtc 1680
caaatggcac aacttggagg cctgccattc ctcttccact tcaagagctg ctctaagtca 1740
agagttgttc aacatctcca aagcaaaacc aagaaatgta agttactaggt tctcgtgata 1800
tggaatcaa ttataagga tgccacgggt gtatctcgtc atcaacttct cagaatgata 1860
aaggcgacc cttcttaact ctgtatgccg taaaaacatg gattacaatt tacgttttga 1920
tagagatgtg cttagtgtag ttgtcttggg gaccaatatt gaattttttt tttttcttca 1980
tataccgggc ttttaacccc tagagtattc atagtttcaa cgaatttgag tttcagatta 2040
atattaaaaa aaatagtgcg actatcacta gagtagtggt atgtttctac tttctagagt 2100
agcttcgggt taatattgag aaagacattt tttttgtggt gataatgaat tttctgtgtg 2160
tttttaaaaa aaaaaaaaaa aaa 2183

```

&lt;210&gt; SEQ ID NO 14

&lt;211&gt; LENGTH: 574

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Glycine max

&lt;400&gt; SEQUENCE: 14

```

Thr Ile Thr Leu Phe Gly Cys Glu Phe Gly Met Ser Trp Ser Gly Ala
 1           5           10          15
Lys Met Gly Thr Val Ser Leu Leu Lys Cys Tyr Arg Pro Phe Phe Ser
           20           25           30
Met Leu Phe Pro His Ser Ala Pro Pro Arg Leu His Ala Ala Ile Phe
           35           40           45
Arg Ser Lys Asn Phe Ser Phe Cys Ala Thr Ser Ser Pro Pro Leu Thr
           50           55           60
Ala Glu Lys Gly Cys Gly Lys Ser Asp Ala Glu Cys Pro Thr Leu Pro
           65           70           75           80
Glu Val Trp Leu His Asn Thr Met Ser Arg Thr Lys Glu Leu Phe Lys
           85           90           95
Pro Lys Val Glu Ser Lys Val Gly Met Tyr Val Cys Gly Val Thr Ala
           100          105          110
Tyr Asp Leu Ser His Ile Gly His Ala Arg Val Tyr Val Asn Phe Asp
           115          120          125
Leu Leu Tyr Arg Tyr Phe Lys His Leu Gly Phe Glu Val Cys Tyr Val
           130          135          140
Arg Asn Phe Thr Asp Val Asp Asp Lys Ile Ile Ala Arg Ala Lys Glu
           145          150          155          160
Leu Gly Glu Asp Pro Ile Ser Leu Ser Trp Arg Tyr Cys Glu Glu Phe
           165          170          175
Cys Gln Asp Met Val Thr Leu Asn Cys Leu Ser Pro Ser Val Glu Pro
           180          185          190
Lys Val Ser Glu His Met Pro Gln Ile Ile Asp Met Ile Glu Lys Ile
           195          200          205
Leu Asn Asn Gly Tyr Ala Tyr Ile Val Asp Gly Asp Val Tyr Phe Asn
           210          215          220
Val Glu Lys Phe Pro Glu Tyr Gly Lys Leu Ser Ser Arg Asp Leu Glu
           225          230          235          240
Asp Asn Arg Ala Gly Glu Arg Val Ala Val Asp Ser Arg Lys Lys Asn
           245          250          255

```

-continued

Pro Ala Asp Phe Ala Leu Trp Lys Ser Ala Lys Pro Gly Glu Pro Phe  
 260 265 270  
 Trp Glu Ser Pro Trp Gly Pro Gly Arg Pro Gly Trp His Ile Glu Cys  
 275 280 285  
 Ser Ala Met Ser Ala Ala Tyr Leu Gly Tyr Ser Phe Asp Ile His Gly  
 290 295 300  
 Gly Gly Ile Asp Leu Val Phe Pro His His Glu Asn Glu Ile Ala Gln  
 305 310 315 320  
 Ser Cys Ala Ala Cys Lys Lys Ser Asp Ile Ser Ile Trp Met His Asn  
 325 330 335  
 Gly Phe Val Thr Ile Asp Ser Val Lys Met Ser Lys Ser Leu Gly Asn  
 340 345 350  
 Phe Phe Thr Ile Arg Gln Val Ile Asp Val Tyr His Pro Leu Ala Leu  
 355 360 365  
 Arg Tyr Phe Leu Met Ser Ala His Tyr Arg Ser Pro Ile Asn Tyr Ser  
 370 375 380  
 Asn Ile Gln Leu Glu Ser Ala Ser Asp Arg Val Phe Tyr Ile Tyr Glu  
 385 390 395 400  
 Thr Leu His Glu Cys Glu Ser Phe Leu Asn Gln His Asp Gln Arg Lys  
 405 410 415  
 Asp Ser Thr Pro Pro Asp Thr Leu Asp Ile Ile Asp Lys Phe His Asp  
 420 425 430  
 Val Phe Leu Thr Ser Met Ser Asp Asp Leu His Thr Pro Val Val Leu  
 435 440 445  
 Ala Gly Met Ser Asp Pro Leu Lys Ser Ile Asn Asp Leu Leu His Ala  
 450 455 460  
 Arg Lys Gly Lys Lys Gln Gln Phe Arg Ile Glu Ser Leu Ser Ala Leu  
 465 470 475 480  
 Glu Lys Ser Val Arg Asp Val Leu Thr Val Leu Gly Leu Met Pro Ala  
 485 490 495  
 Ser Tyr Ser Glu Val Leu Gln Gln Leu Lys Val Lys Ala Leu Lys Arg  
 500 505 510  
 Ala Asn Phe Thr Glu Glu Glu Val Leu Gln Lys Ile Glu Glu Arg Ala  
 515 520 525  
 Thr Ala Arg Met Gln Lys Glu Tyr Ala Lys Ser Asp Ala Ile Arg Lys  
 530 535 540  
 Asp Leu Ala Val Leu Gly Ile Thr Leu Met Asp Ser Pro Asn Gly Thr  
 545 550 555 560  
 Thr Trp Arg Pro Ala Ile Pro Leu Pro Leu Gln Glu Leu Leu  
 565 570

<210> SEQ ID NO 15  
 <211> LENGTH: 633  
 <212> TYPE: DNA  
 <213> ORGANISM: Zea mays

<400> SEQUENCE: 15

gcacacacgt cggtcctcaaac acgcgcgcgc cgctcgcggc ttctccaacc aaagccgtgc 60  
 agccaaatcc gaagggtagc gtagcacggg gacgacgcca tgagccgcgc gtcctctcc 120  
 cagctcctcc accgtccgcc gcacttcgcg tacacctgct taaggagtgg cgttggtgcc 180  
 cgaggaggag tgctcgttc tggtcatccac ccaactccgc gtctcaattg cagcgcggtt 240

-continued

---

```

gaagccgttc ccgccccac cgaggaggcg cctgctcctc aggcaaggaa gaaaagagta    300
gtttctggtg tacagccaac aggatcggtt caccttgaa attatctagg ggcaattaag    360
aattgggttg cacttcagga ttcatatgag acattctttt tcatcgtgga tcttcatgca    420
attactttac catatgaggc gccactgctt tctaaagcaa caagaagcac tgctgcaata    480
tatcttgcat gtggcgtcga cagctccaag gcttctatct ttgtacagtc tcatgtccgt    540
gctcatgttg agttgatgtg gctattgagt tcttctactc ctattggctg gctgaataga    600
atgatccagt tcaaagagaa gtctcgcaag gcg                                633

```

```

<210> SEQ ID NO 16
<211> LENGTH: 410
<212> TYPE: PRT
<213> ORGANISM: Zea mays

```

```

<400> SEQUENCE: 16

```

```

His Gly Asp Asp Ala Met Ser Arg Ala Leu Leu Ser His Val Leu His
 1           5           10          15
Arg Pro Pro His Phe Ala Tyr Thr Cys Leu Arg Ser Gly Val Gly Ala
          20           25          30
Arg Gly Gly Val Leu Ala Ser Gly Ile His Pro Leu Arg Arg Leu Asn
      35           40          45
Cys Ser Ala Val Glu Ala Val Pro Gly Pro Thr Glu Glu Ala Pro Ala
      50           55          60
Pro Gln Ala Arg Lys Lys Arg Val Val Ser Gly Val Gln Pro Thr Gly
      65           70          75          80
Ser Val His Leu Gly Asn Tyr Leu Gly Ala Ile Lys Asn Trp Val Ala
          85           90          95
Leu Gln Asp Ser Tyr Glu Thr Phe Phe Phe Ile Val Asp Leu His Ala
          100          105          110
Ile Thr Leu Pro Tyr Glu Ala Pro Leu Leu Ser Lys Ala Thr Arg Ser
          115          120          125
Thr Ala Ala Ile Tyr Leu Ala Cys Gly Val Asp Ser Ser Lys Ala Ser
          130          135          140
Ile Phe Val Gln Ser His Val Arg Ala His Val Glu Leu Met Trp Leu
          145          150          155          160
Leu Ser Ser Ser Thr Pro Ile Gly Trp Leu Asn Arg Met Ile Gln Phe
          165          170          175
Lys Glu Lys Ser Arg Lys Ala Gly Asp Glu Asn Val Gly Val Ala Leu
          180          185          190
Leu Thr Tyr Pro Val Leu Met Ala Ser Asp Ile Leu Leu Tyr Gln Ser
          195          200          205
Asp Leu Val Pro Val Gly Glu Asp Gln Thr Gln His Leu Glu Leu Thr
          210          215          220
Arg Glu Ile Ala Glu Arg Val Asn Asn Leu Tyr Gly Gly Arg Lys Trp
          225          230          235          240
Lys Lys Leu Gly Gly Arg Gly Gly Leu Leu Phe Lys Val Pro Glu Ala
          245          250          255
Leu Ile Pro Pro Ala Gly Ala Arg Val Met Ser Leu Thr Asp Gly Leu
          260          265          270
Ser Lys Met Ser Lys Ser Ala Pro Ser Asp Gln Ser Arg Ile Asn Leu
          275          280          285

```

-continued

---

Leu Asp Pro Lys Asp Val Ile Ala Asn Lys Ile Lys Arg Cys Lys Thr  
 290 295 300

Asp Ser Phe Pro Gly Met Glu Phe Asp Asn Pro Glu Arg Pro Glu Cys  
 305 310 315 320

Arg Asn Leu Leu Ser Ile Tyr Gln Ile Ile Thr Glu Lys Thr Lys Glu  
 325 330 335

Glu Val Val Ser Glu Cys Gln His Met Asn Trp Gly Thr Phe Lys Thr  
 340 345 350

Thr Leu Thr Glu Ala Leu Ile Asp His Leu Gln Pro Ile Gln Val Arg  
 355 360 365

Tyr Glu Glu Ile Met Ser Asp Pro Ala Tyr Leu Asp Asn Val Leu Leu  
 370 375 380

Glu Gly Ala Val Lys Ala Ala Glu Ile Ala Asp Ile Thr Leu Asn Asn  
 385 390 395 400

Val Tyr Gln Ala Met Gly Phe Leu Arg Arg  
 405 410

<210> SEQ ID NO 17  
 <211> LENGTH: 1536  
 <212> TYPE: DNA  
 <213> ORGANISM: Glycine max

<400> SEQUENCE: 17

```

gcacgagggg agatgagcgt ttcacatttc gcggttctat cgtcgtggtg ttgtccacgc      60
ttggcccctt ctctgtcgcg tgcttcaacc cttcgttctc gcatccgggtg ttgtactact      120
ctcactgcta cttcttcaga gactcccact ccaaccttcg tgaagaaacg agtagtgctg      180
ggggttcagc ccacgggctc aattcacctc ggaaactatt ttggcgccat caagaattgg      240
gttgcccttc agaagtgtga tgatacactt ttcttcattg tggacctgca cgcgattaca      300
ttaccatatg acaccaaca attatctaag gctacaaggt caactgctgc tatttaccta      360
gcatgtggag tggatccttc aaaggcttca gtatttgtac agtctcatgt tcgggcacat      420
gtagaattga tgtggctgct aagttccaca acaccaattg gttggctgaa caaaatgata      480
caatttaaa agaaatctcg caaggcggga gatgaagaag ttgggggtgc ccttttgact      540
tatcctgttc tgatggcttc tgatatactt ctatatcagt ctgattttgt cctgtttggt      600
gaagatcaaa agcagcactt ggagttgact cgtgacttgg ctgaacgggt taataattta      660
tatggaggaa gaaagtggaa gaaattagcc ggttatgaca gccgaggtgg tactatattt      720
aaggttccag agccccttat acctccagcc ggagcccgga taatgtccct aactgatggc      780
ctgtccaaga tgtcaaagtc tgcaccttct gatcaatcca gaatcaatat tcttgatcct      840
aaagatctca tagcaaaca gatcaaactg tgcaaaactg attcatttcc tggcttgtaa      900
tttgacaact ctgagaggcc tgaatgtaac aatcttgttt ccatatacca gcttatttca      960
ggaaagacga aagaggaagt tgtgcaggaa tgccaaaaca tgaactgggg cacattcaaa     1020
cctcttttaa cagatgcctt gattgatcat ttgcatccca ttcaggttcg ctatgaggaa     1080
atcatgtccg attcaggtta ttagatgga gttttagcac aagggtgctag aaatgcagca     1140
gatatagcag attctacact taataatatt taccaagcaa tgggattttt taagagacag     1200
tgataattga tgccaaataa attaaagatt ggcgagacgt caacttaaaa gctaacttct     1260
ggatgattca tgatgggcct caaaattttg gagtaactct atggacatat acttgactac     1320
    
```

-continued

---

```

tggaatgga aagattattg atgcaaagcc taaaggtccc attagttcct gatgcaatgg 1380
gctttgtatc tccttcattt ttctccgagt atggtcgttg ccttcatttt atattttatt 1440
gtttcaatct ctttcattat ttacttgtat tttataatga attcagcata ttgataaatt 1500
gttccgccat tgtattttaa aaaaaaaaa aaaaaa 1536

```

```

<210> SEQ ID NO 18
<211> LENGTH: 400
<212> TYPE: PRT
<213> ORGANISM: Glycine max

<400> SEQUENCE: 18

```

```

Ala Arg Gly Lys Met Ser Val Ser His Phe Ala Val Leu Ser Ser Cys
 1          5          10          15
Cys Cys Pro Arg Leu Ala Pro Ser Leu Ser Arg Ala Ser Thr Leu Arg
          20          25          30
Ser Arg Ile Arg Cys Cys Thr Thr Leu Thr Ala Thr Ser Ser Glu Thr
          35          40          45
Pro Thr Pro Thr Phe Val Lys Lys Arg Val Val Ser Gly Val Gln Pro
          50          55          60
Thr Gly Ser Ile His Leu Gly Asn Tyr Phe Gly Ala Ile Lys Asn Trp
          65          70          75          80
Val Ala Leu Gln Asn Val Tyr Asp Thr Leu Phe Phe Ile Val Asp Leu
          85          90          95
His Ala Ile Thr Leu Pro Tyr Asp Thr Gln Gln Leu Ser Lys Ala Thr
          100          105          110
Arg Ser Thr Ala Ala Ile Tyr Leu Ala Cys Gly Val Asp Pro Ser Lys
          115          120          125
Ala Ser Val Phe Val Gln Ser His Val Arg Ala His Val Glu Leu Met
          130          135          140
Trp Leu Leu Ser Ser Thr Thr Pro Ile Gly Trp Leu Asn Lys Met Ile
          145          150          155          160
Gln Phe Lys Glu Lys Ser Arg Lys Ala Gly Asp Glu Glu Val Gly Val
          165          170          175
Ala Leu Leu Thr Tyr Pro Val Leu Met Ala Ser Asp Ile Leu Leu Tyr
          180          185          190
Gln Ser Asp Phe Val Pro Val Gly Glu Asp Gln Lys Gln His Leu Glu
          195          200          205
Leu Thr Arg Asp Leu Ala Glu Arg Val Asn Asn Leu Tyr Gly Gly Arg
          210          215          220
Lys Trp Lys Lys Leu Gly Gly Tyr Asp Ser Arg Gly Gly Thr Ile Phe
          225          230          235          240
Lys Val Pro Glu Pro Leu Ile Pro Pro Ala Gly Ala Arg Ile Met Ser
          245          250          255
Leu Thr Asp Gly Leu Ser Lys Met Ser Lys Ser Ala Pro Ser Asp Gln
          260          265          270
Ser Arg Ile Asn Ile Leu Asp Pro Lys Asp Leu Ile Ala Asn Lys Ile
          275          280          285
Lys Arg Cys Lys Thr Asp Ser Phe Pro Gly Leu Glu Phe Asp Asn Ser
          290          295          300
Glu Arg Pro Glu Cys Asn Asn Leu Val Ser Ile Tyr Gln Leu Ile Ser
          305          310          315          320

```

-continued

Gly Lys Thr Lys Glu Glu Val Val Gln Glu Cys Gln Asn Met Asn Trp  
 325 330 335  
 Gly Thr Phe Lys Pro Leu Leu Thr Asp Ala Leu Ile Asp His Leu His  
 340 345 350  
 Pro Ile Gln Val Arg Tyr Glu Glu Ile Met Ser Asp Ser Gly Tyr Leu  
 355 360 365  
 Asp Gly Val Leu Ala Gln Gly Ala Arg Asn Ala Ala Asp Ile Ala Asp  
 370 375 380  
 Ser Thr Leu Asn Asn Ile Tyr Gln Ala Met Gly Phe Phe Lys Arg Gln  
 385 390 395 400

<210> SEQ ID NO 19  
 <211> LENGTH: 725  
 <212> TYPE: DNA  
 <213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 19

ctcgtgccga attcggcacg aggcggttca ttatttaagg ttcctgaagc cttatccct 60  
 ccagcagggg cccgtgtgat gtccttaact gatggcctct ccaagatgtc gaagtctgct 120  
 ccttcagatt tgtctcgcat taaccttctt gaccctaatg atgtgattgt gaacaaaatc 180  
 aaacgctgca aaactgactc gtcctctggc ttggaattcg acaaccgaga gaggccggaa 240  
 tgcaaaaatc ttctctcagt ctaccagatc atcactggaa aaacgaaaga ggaagtgttt 300  
 agtgaatgcc aagatatgaa ctgggggacg ttcaaggtta cccttacgga tgccttaatt 360  
 gatcatctgc aacctattca ggctcgatac gaggagatca tgtctgatcc aggttatttg 420  
 gacaatgttc tgctaaatgg ggcagggaaa gctctgaga tagcagacgc caccctcaac 480  
 aacgtctaoc aagccatggg tttcttgccg agatagcata tgtagaacat tttttataac 540  
 tgcacaatgc tagttttgca ctgtttggcc tttctgctag tggactgat aagcgttttg 600  
 tttgatatgc ttggattagc cttttgttcc tggttattat ggacactggt aataggattt 660  
 aaaaggatta tttactgaaa aaaaaaaaaa aaaaaaaaaa attaaaaggg ggcgcgctga 720  
 ccata 725

<210> SEQ ID NO 20  
 <211> LENGTH: 171  
 <212> TYPE: PRT  
 <213> ORGANISM: Triticum aestivum

<400> SEQUENCE: 20

Leu Val Pro Asn Ser Ala Arg Gly Gly Ser Leu Phe Lys Val Pro Glu  
 1 5 10 15  
 Ala Leu Ile Pro Pro Ala Gly Ala Arg Val Met Ser Leu Thr Asp Gly  
 20 25 30  
 Leu Ser Lys Met Ser Lys Ser Ala Pro Ser Asp Leu Ser Arg Ile Asn  
 35 40 45  
 Leu Leu Asp Pro Asn Asp Val Ile Val Asn Lys Ile Lys Arg Cys Lys  
 50 55 60  
 Thr Asp Ser Leu Pro Gly Leu Glu Phe Asp Asn Pro Glu Arg Pro Glu  
 65 70 75 80  
 Cys Lys Asn Leu Leu Ser Val Tyr Gln Ile Ile Thr Gly Lys Thr Lys  
 85 90 95

-continued

---

Glu Glu Val Val Ser Glu Cys Gln Asp Met Asn Trp Gly Thr Phe Lys  
                   100                  105                  110

Val Thr Leu Thr Asp Ala Leu Ile Asp His Leu Gln Pro Ile Gln Val  
           115                  120                  125

Arg Tyr Glu Glu Ile Met Ser Asp Pro Gly Tyr Leu Asp Asn Val Leu  
           130                  135                  140

Leu Asn Gly Ala Gly Lys Ala Ser Glu Ile Ala Asp Ala Thr Leu Asn  
   145                  150                  155                  160

Asn Val Tyr Gln Ala Met Gly Phe Leu Arg Arg  
                   165                  170

<210> SEQ ID NO 21  
 <211> LENGTH: 1062  
 <212> TYPE: DNA  
 <213> ORGANISM: Zea mays

<400> SEQUENCE: 21

```

gcacgagggg catcacgctg ctggatttcc tgagagaggt gggccgtttt gcacgcgtgg      60
gtacaatgat cgccaaggag agcgtcaaga agcgtcttgc gtcggaagac gggatgagct      120
acaccgagtt tacctaccag ctgctgcagg gctacgactt cctttacatg ttcaagaata      180
tggtgtgcaaa tggtcagatc gggggcagcg atcagtgggg gaacatcaca gcgggaactg      240
agttgatcag aaaaatcttg caggttgaag gggcgcatgg actcacattc caacttctgc      300
tgaagagcga cgggtacaaa ttggaaaaga cggaggatgg ggcaatctgg ctctcttcga      360
agatgctttc tccttacaag ttctatcagt acttcttgc ggtgccagac atcgatgtca      420
tcaggtttat gaagatcctg acgttccctg gcttggatga gattctggag ctagaagact      480
cgatgaagaa gcctggctat gtgccaacaa ctgttcagaa gaggcttgca gaagaggtga      540
cgcgatttgt tcatggcgag gagggattgg aggaggcatt gaaggcaacc gaggccttga      600
gacctgggtc tcagacacaa ttggatgcac aaacaattga ggggatagca gatgatgtgc      660
cttcagcttc tttagcttat gatcaagtgt tcaagtctcc acttattgat ttggctgttt      720
ccacaggttt gctcactagt aagtcagcag ttaagcggct tattaagcaa ggtggtctgt      780
acttgaataa cgtgaggatt gatagtgagg ataagctggt tgaggaaggt gatatagttg      840
atgggaaggt gctctgtgtg tctgctggaa agaagaacaa gatggtgtgt aggatatctt      900
gactactctt atttgttctt tataacttat tttagccatt gaggagaaaa gtaacggtgt      960
tgtgtcttca aaactcaaat gagctgtcta tgagcataca gattgttata ttggagaggt     1020
tgaacacacc ttttttttgc ctctaaaaaa aaaaaaaaaa aa                               1062
  
```

<210> SEQ ID NO 22  
 <211> LENGTH: 299  
 <212> TYPE: PRT  
 <213> ORGANISM: Zea mays

<400> SEQUENCE: 22

Thr Arg Asp Ile Thr Leu Leu Asp Phe Leu Arg Glu Val Gly Arg Phe  
 1                  5                  10                  15

Ala Arg Val Gly Thr Met Ile Ala Lys Glu Ser Val Lys Lys Arg Leu  
           20                  25                  30

Ala Ser Glu Asp Gly Met Ser Tyr Thr Glu Phe Thr Tyr Gln Leu Leu  
   35                  40                  45



-continued

Gln Gly Tyr Asp Phe Leu Tyr Met Phe Lys Asn Met Gly Val Asn Val  
 50 55 60  
 Gln Ile Gly Gly Ser Asp Gln Trp Gly Asn Ile Thr Ala Gly Thr Glu  
 65 70 75 80  
 Leu Ile Arg Lys Ile Leu Gln Val Glu Gly Ala His Gly Leu Thr Phe  
 85 90 95  
 Pro Leu Leu Leu Lys Ser Asp Gly Thr Lys Phe Gly Lys Thr Glu Asp  
 100 105 110  
 Gly Ala Ile Trp Leu Ser Ser Lys Met Leu Ser Pro Tyr Lys Phe Tyr  
 115 120 125  
 Gln Tyr Phe Phe Ala Val Pro Asp Ile Asp Val Ile Arg Phe Met Lys  
 130 135 140  
 Ile Leu Thr Phe Leu Ser Leu Asp Glu Ile Leu Glu Leu Glu Asp Ser  
 145 150 155 160  
 Met Lys Lys Pro Gly Tyr Val Pro Asn Thr Val Gln Lys Arg Leu Ala  
 165 170 175  
 Glu Glu Val Thr Arg Phe Val His Gly Glu Glu Gly Leu Glu Glu Ala  
 180 185 190  
 Leu Lys Ala Thr Glu Ala Leu Arg Pro Gly Ala Gln Thr Gln Leu Asp  
 195 200 205  
 Ala Gln Thr Ile Glu Gly Ile Ala Asp Asp Val Pro Ser Cys Ser Leu  
 210 215 220  
 Ala Tyr Asp Gln Val Phe Lys Ser Pro Leu Ile Asp Leu Ala Val Ser  
 225 230 235 240  
 Thr Gly Leu Leu Thr Ser Lys Ser Ala Val Lys Arg Leu Ile Lys Gln  
 245 250 255  
 Gly Gly Leu Tyr Leu Asn Asn Val Arg Ile Asp Ser Glu Asp Lys Leu  
 260 265 270  
 Val Glu Glu Gly Asp Ile Val Asp Gly Lys Val Leu Leu Leu Ser Ala  
 275 280 285  
 Gly Lys Lys Asn Lys Met Val Val Arg Ile Ser  
 290 295

&lt;210&gt; SEQ ID NO 23

&lt;211&gt; LENGTH: 346

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Drosophila melanogaster

&lt;400&gt; SEQUENCE: 23

Met Val Asp Lys Val Ala Asn Gly Val Ser Lys Lys Gly Ala Lys Lys  
 1 5 10 15  
 Ala Lys Ala Ala Lys Lys Ala Lys Ala Asn Ala Ser Thr Ala Ala Ala  
 20 25 30  
 Asn Asn Ser Gly Gly Asp Ser Ala Asp His Ala Ala Gly Arg Tyr Gly  
 35 40 45  
 Ser Met Ser Lys Asp Lys Arg Ser Arg Asn Val Val Ser Ser Gly Val  
 50 55 60  
 Gly Lys Gly Val Trp Val Arg Gly Arg Val His Thr Ser Arg Ala Lys  
 65 70 75 80  
 Gly Lys Cys Arg Ser Ser Thr Val Cys Ala Val Gly Asp Val Ser Lys  
 85 90 95

-continued

---

Met Val Lys Ala Gly Asn Lys Ser Asp Ala Lys Val Ala Val Ser Ser  
100 105 110

Lys Ser Cys Thr Ser Ser Val Val Ser Ala Lys Ala Asp Ala Ser Arg  
115 120 125

Asn Ala Asp Asp Ala Gly Asn Arg Val Asn Asp Thr Arg Asp Asn Arg  
130 135 140

Val Asp Arg Thr Ala Asn Ala Arg Ala Gly Val Cys Arg Arg Asp Thr  
145 150 155 160

Gly Thr His Thr Lys Ser Ala Ala Ser Gly Gly Ala Asn Val Thr Val  
165 170 175

Ser Tyr Lys Asp Ser Ala Tyr Ala Ser Tyr Lys Met Ala Ala Ala Asp  
180 185 190

Asp Lys Val Tyr Thr Val Gly Ala Val Arg Ala Asp Ser Asn Thr His  
195 200 205

Arg His Thr Val Gly Asp Met Ala Lys Tyr His Tyr His Val His Thr  
210 215 220

Gly Asn Thr Thr Ser Lys Gly Arg Asp Lys Tyr Ala Lys Ser Val Gly  
225 230 235 240

Tyr Lys Val Asp Ala Lys Ala Asp Gly Val Ala Met Arg Ala Gly Val  
245 250 255

Thr Gly Asp Asp Ser Thr Asn Lys Gly Arg Val Lys Ala Lys Tyr Asp  
260 265 270

Thr Asp Tyr Asp Lys Ala Arg Tyr Thr Met Asp Asn Asn Val Tyr Ser  
275 280 285

Asn Ser Tyr Asp Met Met Arg Gly Ser Gly Ala Arg His Asp Tyr Arg  
290 295 300

Ala Lys His His Gly Asp Thr Ser Lys Ala Ala Tyr Ser Arg Tyr Gly  
305 310 315 320

Cys His Ala Gly Gly Gly Met Arg Val Val Met Tyr Gly Asp Asn  
325 330 335

Arg Lys Thr Ser Met Arg Asp Lys Arg Thr  
340 345

&lt;210&gt; SEQ ID NO 24

&lt;211&gt; LENGTH: 501

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Rattus norvegicus

&lt;400&gt; SEQUENCE: 24

Met Pro Ser Ala Asn Ala Ser Arg Lys Gly Gln Glu Lys Pro Arg Glu  
1 5 10 15

Ile Val Asp Ala Ala Glu Asp Tyr Ala Lys Glu Arg Tyr Gly Val Ser  
20 25 30

Ser Met Ile Gln Ser Gln Glu Lys Pro Asp Arg Val Leu Val Arg Val  
35 40 45

Lys Asp Leu Thr Val Gln Lys Ala Asp Glu Val Val Trp Val Arg Ala  
50 55 60

Arg Val His Thr Ser Arg Ala Lys Gly Lys Gln Cys Phe Leu Val Leu  
65 70 75 80

Arg Gln Gln Gln Phe Asn Val Gln Ala Leu Val Ala Val Gly Asp His  
85 90 95

-continued

---

Ala Ser Lys Gln Met Val Lys Phe Ala Ala Asn Ile Asn Lys Glu Ser  
100 105 110

Ile Ile Asp Val Glu Gly Ile Val Arg Lys Val Asn Gln Lys Ile Gly  
115 120 125

Ser Cys Thr Gln Gln Asp Val Glu Leu His Val Gln Lys Ile Tyr Val  
130 135 140

Ile Ser Leu Ala Glu Pro Arg Leu Pro Leu Gln Leu Asp Asp Ala Ile  
145 150 155 160

Arg Pro Glu Val Glu Gly Glu Glu Asp Gly Arg Ala Thr Val Asn Gln  
165 170 175

Asp Thr Arg Leu Asp Asn Arg Ile Ile Asp Leu Arg Thr Ser Thr Ser  
180 185 190

Gln Ala Ile Phe His Leu Gln Ser Gly Ile Cys His Leu Phe Arg Glu  
195 200 205

Thr Leu Ile Asn Lys Gly Phe Val Glu Ile Gln Thr Pro Lys Ile Ile  
210 215 220

Ser Ala Ala Ser Glu Gly Glu Ala Asn Val Phe Thr Val Ser Tyr Phe  
225 230 235 240

Lys Ser Asn Ala Tyr Leu Ala Gln Ser Pro Gln Leu Tyr Lys Gln Met  
245 250 255

Cys Ile Cys Ala Asp Phe Glu Lys Val Phe Cys Ile Gly Pro Val Phe  
260 265 270

Arg Ala Glu Asp Ser Asn Thr His Arg His Leu Thr Glu Phe Val Gly  
275 280 285

Leu Asp Ile Glu Met Ala Phe Asn Tyr His Tyr His Glu Val Val Glu  
290 295 300

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

Phe Gln Thr Glu Ile Gln Thr Val Asn Lys Gln Phe Pro Cys Glu Pro  
325 330 335

Phe Lys Phe Leu Glu Pro Thr Leu Arg Leu Glu Tyr Cys Glu Ala Leu  
340 345 350

Ala Met Leu Arg Glu Ala Gly Val Glu Met Asp Asp Glu Glu Asp Leu  
355 360 365

Ser Thr Pro Asn Glu Lys Leu Leu Gly Arg Leu Val Lys Glu Lys Tyr  
370 375 380

Asp Thr Asp Phe Tyr Val Leu Asp Lys Tyr Pro Leu Ala Val Arg Pro  
385 390 395 400

Phe Tyr Thr Met Pro Asp Pro Arg Asn Pro Lys Gln Ser Asn Ser Tyr  
405 410 415

Asp Met Phe Met Arg Gly Glu Glu Ile Leu Ser Gly Ala Gln Arg Ile  
420 425 430

His Asp Pro Gln Leu Leu Thr Glu Arg Ala Leu His His Gly Ile Asp  
435 440 445

Leu Glu Lys Ile Lys Ala Tyr Ile Asp Ser Phe Arg Phe Gly Ala Pro  
450 455 460

Pro His Ala Gly Gly Gly Ile Gly Leu Glu Arg Val Thr Met Leu Phe  
465 470 475 480

Leu Gly Leu His Asn Val Arg Gln Thr Ser Met Phe Pro Arg Asp Pro  
485 490 495

-continued

---

Lys Arg Leu Thr Pro  
500

<210> SEQ ID NO 25  
<211> LENGTH: 500  
<212> TYPE: PRT  
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 25

Met Pro Ser Ala Thr Gln Arg Lys Ser Gln Glu Lys Pro Arg Glu Ile  
1 5 10 15

Met Asp Ala Ala Glu Asp Tyr Ala Lys Glu Arg Tyr Gly Ile Ser Ser  
20 25 30

Met Ile Gln Ser Gln Glu Lys Pro Asp Arg Val Leu Val Arg Val Arg  
35 40 45

Asp Leu Thr Ile Gln Lys Ala Asp Glu Val Val Trp Val Arg Ala Arg  
50 55 60

Val His Thr Ser Arg Ala Lys Gly Lys Gln Cys Phe Leu Val Leu Arg  
65 70 75 80

Gln Gln Gln Phe Asn Val Gln Ala Leu Val Ala Val Gly Asp His Ala  
85 90 95

Ser Lys Gln Met Val Lys Phe Ala Ala Asn Ile Asn Lys Glu Ser Ile  
100 105 110

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

Cys Thr Gln Gln Asp Val Glu Leu His Val Gln Lys Ile Tyr Val Ile  
130 135 140

Ser Leu Ala Glu Pro Arg Leu Pro Leu Gln Leu Asp Asp Ala Val Arg  
145 150 155 160

Pro Glu Gln Glu Gly Glu Glu Glu Gly Arg Ala Thr Val Asn Gln Asp  
165 170 175

Thr Arg Leu Asp Asn Arg Val Ile Asp Leu Arg Thr Ser Thr Ser Gln  
180 185 190

Ala Val Phe Arg Leu Gln Ser Gly Ile Cys His Leu Phe Arg Glu Thr  
195 200 205

Leu Ile Asn Lys Gly Phe Val Glu Ile Gln Thr Pro Lys Ile Ile Ser  
210 215 220

Ala Ala Ser Glu Gly Gly Ala Asn Val Phe Thr Val Ser Tyr Phe Lys  
225 230 235 240

Asn Asn Ala Tyr Leu Ala Gln Ser Pro Gln Leu Tyr Lys Gln Met Cys  
245 250 255

Ile Cys Ala Asp Phe Glu Lys Val Phe Ser Ile Gly Pro Val Phe Arg  
260 265 270

Ala Glu Asp Ser Asn Thr His Arg His Leu Thr Glu Phe Val Gly Leu  
275 280 285

Asp Ile Glu Met Ala Phe Asn Tyr His Tyr His Glu Val Met Glu Glu  
290 295 300

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

Gln Thr Glu Ile Gln Thr Val Asn Lys Gln Phe Pro Cys Glu Pro Phe  
325 330 335

Lys Phe Leu Glu Pro Thr Leu Arg Leu Glu Tyr Cys Glu Ala Leu Ala  
340 345 350

-continued

Met Leu Arg Glu Ala Gly Val Glu Met Gly Asp Glu Asp Asp Leu Ser  
 355 360 365  
 Thr Pro Asn Glu Lys Leu Leu Gly His Leu Val Lys Glu Lys Tyr Asp  
 370 375 380  
 Thr Asp Phe Tyr Ile Leu Asp Lys Tyr Pro Leu Ala Val Arg Pro Phe  
 385 390 395 400  
 Tyr Thr Met Pro Asp Pro Arg Asn Pro Lys Gln Ser Lys Ser Tyr Asp  
 405 410 415  
 Met Phe Met Arg Gly Glu Glu Ile Leu Ser Gly Ala Gln Arg Ile His  
 420 425 430  
 Asp Pro Gln Leu Leu Thr Glu Arg Ala Leu His His Gly Asn Asp Leu  
 435 440 445  
 Glu Lys Ile Lys Ala Tyr Ile Asp Ser Phe Arg Phe Gly Ala Pro Pro  
 450 455 460  
 His Ala Gly Gly Gly Ile Gly Leu Glu Arg Val Thr Met Leu Phe Leu  
 465 470 475 480  
 Gly Leu His Asn Val Arg Gln Thr Ser Met Phe Pro Arg Asp Pro Lys  
 485 490 495  
 Arg Leu Thr Pro  
 500

<210> SEQ ID NO 26  
 <211> LENGTH: 459  
 <212> TYPE: PRT  
 <213> ORGANISM: Haemophilus influenzae Rd

<400> SEQUENCE: 26

Met Leu Lys Ile Phe Asn Thr Leu Thr Arg Glu Lys Glu Ile Phe Lys  
 1 5 10 15  
 Pro Ile His Glu Asn Lys Val Gly Met Tyr Val Cys Gly Val Thr Val  
 20 25 30  
 Tyr Asp Leu Cys His Ile Gly His Gly Arg Thr Phe Val Cys Phe Asp  
 35 40 45  
 Val Ile Ala Arg Tyr Leu Arg Ser Leu Gly Tyr Asp Leu Thr Tyr Val  
 50 55 60  
 Arg Asn Ile Thr Asp Val Asp Asp Lys Ile Ile Lys Arg Ala Leu Glu  
 65 70 75 80  
 Asn Lys Glu Thr Cys Asp Gln Leu Val Asp Arg Met Val Gln Glu Met  
 85 90 95  
 Tyr Lys Asp Phe Asp Ala Leu Asn Val Leu Arg Pro Asp Phe Glu Pro  
 100 105 110  
 Arg Ala Thr His His Ile Pro Glu Ile Ile Glu Ile Val Glu Lys Leu  
 115 120 125  
 Ile Lys Arg Gly His Ala Tyr Val Ala Asp Asn Gly Asp Val Met Phe  
 130 135 140  
 Asp Val Glu Ser Phe Lys Glu Tyr Gly Lys Leu Ser Arg Gln Asp Leu  
 145 150 155 160  
 Glu Gln Leu Gln Ala Gly Ala Arg Ile Glu Ile Asn Glu Ile Lys Lys  
 165 170 175  
 Asn Pro Met Asp Phe Val Leu Trp Lys Met Ser Lys Glu Asn Glu Pro  
 180 185 190

-continued

Ser Trp Ala Ser Pro Trp Gly Ala Gly Arg Pro Gly Trp His Ile Glu  
 195 200 205

Cys Ser Ala Met Asn Cys Lys Gln Leu Gly Glu Tyr Phe Asp Ile His  
 210 215 220

Gly Gly Gly Ser Asp Leu Met Phe Pro His His Glu Asn Glu Ile Ala  
 225 230 235 240

Gln Ser Cys Cys Ala His Gly Gly Gln Tyr Val Asn Tyr Trp Ile His  
 245 250 255

Ser Gly Met Ile Met Val Asp Lys Glu Lys Met Ser Lys Ser Leu Gly  
 260 265 270

Asn Phe Phe Thr Ile Arg Asp Val Leu Asn His Tyr Asn Ala Glu Ala  
 275 280 285

Val Arg Tyr Phe Leu Leu Thr Ala His Tyr Arg Ser Gln Leu Asn Tyr  
 290 295 300

Ser Glu Glu Asn Leu Asn Leu Ala Gln Gly Ala Leu Glu Arg Leu Tyr  
 305 310 315 320

Thr Ala Leu Arg Gly Thr Asp Gln Ser Ala Val Ala Phe Gly Gly Glu  
 325 330 335

Asn Phe Val Ala Thr Phe Arg Glu Ala Met Asp Asp Asp Phe Asn Thr  
 340 345 350

Pro Asn Ala Leu Ser Val Leu Phe Glu Met Ala Arg Glu Ile Asn Lys  
 355 360 365

Leu Lys Thr Glu Asp Val Glu Lys Ala Asn Gly Leu Ala Ala Arg Leu  
 370 375 380

Arg Glu Leu Gly Ala Ile Leu Gly Leu Leu Gln Gln Glu Pro Glu Lys  
 385 390 395 400

Phe Leu Gln Ala Gly Ser Asn Asp Asp Glu Val Ala Lys Ile Glu Ala  
 405 410 415

Leu Ile Lys Gln Arg Asn Glu Ala Arg Thr Ala Lys Asp Trp Ser Ala  
 420 425 430

Ala Asp Ser Ala Arg Asn Glu Leu Thr Ala Met Gly Ile Val Leu Glu  
 435 440 445

Asp Gly Pro Asn Gly Thr Thr Trp Arg Lys Gln  
 450 455

<210> SEQ ID NO 27  
 <211> LENGTH: 461  
 <212> TYPE: PRT  
 <213> ORGANISM: Escherichia coli  
 <400> SEQUENCE: 27

Met Leu Lys Ile Phe Asn Thr Leu Thr Arg Gln Lys Glu Glu Phe Lys  
 1 5 10 15

Pro Ile His Ala Gly Glu Val Gly Met Tyr Val Cys Gly Ile Thr Val  
 20 25 30

Tyr Asp Leu Cys His Ile Gly His Gly Arg Thr Phe Val Ala Phe Asp  
 35 40 45

Val Val Ala Arg Tyr Leu Arg Phe Leu Gly Tyr Lys Leu Lys Tyr Val  
 50 55 60

Arg Asn Ile Thr Asp Ile Asp Asp Lys Ile Ile Lys Arg Ala Asn Glu  
 65 70 75 80

-continued

Asn Gly Glu Ser Phe Val Ala Met Val Asp Arg Met Ile Ala Glu Met  
 85 90 95  
 His Lys Asp Phe Asp Ala Leu Asn Ile Leu Arg Pro Asp Met Glu Pro  
 100 105 110  
 Arg Ala Thr His His Ile Ala Glu Ile Ile Glu Leu Thr Glu Gln Leu  
 115 120 125  
 Ile Ala Lys Gly His Ala Tyr Val Ala Asp Asn Gly Asp Val Met Phe  
 130 135 140  
 Asp Val Pro Thr Asp Pro Thr Tyr Gly Val Leu Ser Arg Gln Asp Leu  
 145 150 155 160  
 Asp Gln Leu Gln Ala Gly Ala Arg Val Asp Val Val Asp Asp Lys Arg  
 165 170 175  
 Asn Pro Met Asp Phe Val Leu Trp Lys Met Ser Lys Glu Gly Glu Pro  
 180 185 190  
 Ser Trp Pro Ser Pro Trp Gly Ala Gly Arg Pro Gly Trp His Ile Glu  
 195 200 205  
 Cys Ser Ala Met Asn Cys Lys Gln Leu Gly Asn His Phe Asp Ile His  
 210 215 220  
 Gly Gly Gly Ser Asp Leu Met Phe Pro His His Glu Asn Glu Ile Ala  
 225 230 235 240  
 Gln Ser Thr Cys Ala His Asp Gly Gln Tyr Val Asn Tyr Trp Met His  
 245 250 255  
 Ser Gly Met Val Met Val Asp Arg Glu Lys Met Ser Lys Ser Leu Gly  
 260 265 270  
 Asn Phe Phe Thr Val Arg Asp Val Leu Lys Tyr Tyr Asp Ala Glu Thr  
 275 280 285  
 Val Arg Tyr Phe Leu Met Ser Gly His Tyr Arg Ser Gln Leu Asn Tyr  
 290 295 300  
 Ser Glu Glu Asn Leu Lys Gln Ala Arg Ala Val Glu Arg Leu Tyr  
 305 310 315 320  
 Thr Ala Leu Arg Gly Thr Asp Lys Thr Val Ala Pro Ala Gly Gly Glu  
 325 330 335  
 Ala Phe Glu Ala Arg Phe Ile Glu Ala Met Asp Asp Asp Phe Asn Thr  
 340 345 350  
 Pro Glu Ala Tyr Ser Val Leu Phe Asp Met Ala Arg Glu Val Asn Arg  
 355 360 365  
 Leu Lys Ala Glu Asp Met Ala Ala Ala Asn Ala Met Ala Ser His Leu  
 370 375 380  
 Arg Lys Leu Ser Ala Val Leu Gly Leu Leu Glu Gln Glu Pro Glu Ala  
 385 390 395 400  
 Phe Leu Gln Ser Gly Ala Gln Ala Asp Asp Ser Glu Val Ala Glu Ile  
 405 410 415  
 Glu Ala Leu Ile Gln Gln Arg Leu Asp Ala Arg Lys Ala Lys Asp Trp  
 420 425 430  
 Ala Ala Ala Asp Ala Ala Arg Asp Arg Leu Asn Glu Met Gly Ile Val  
 435 440 445  
 Leu Glu Asp Gly Pro Gln Gly Thr Thr Trp Arg Arg Lys  
 450 455 460

&lt;210&gt; SEQ ID NO 28

&lt;211&gt; LENGTH: 377

-continued

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Synechocystis sp.

&lt;400&gt; SEQUENCE: 28

Met Lys Asn Cys Glu Asn Asp His Arg Phe Thr Thr Val Ser Ser Gly  
1 5 10 15  
Lys Ala Trp Gly Gln Leu His Arg Phe Pro Ser Leu Ile Lys Phe Asn  
20 25 30  
Phe Ala His Arg Ser Thr Thr Ala Met Asp Lys Pro Arg Ile Leu Ser  
35 40 45  
Gly Val Gln Pro Thr Gly Asn Leu His Leu Gly Asn Tyr Leu Gly Ala  
50 55 60  
Ile Arg Ser Trp Val Glu Gln Gln Gln His Tyr Asp Asn Phe Phe Cys  
65 70 75 80  
Val Val Asp Leu His Ala Ile Thr Val Pro His Asn Pro Gln Thr Leu  
85 90 95  
Ala Gln Asp Thr Leu Thr Ile Ala Ala Leu Tyr Leu Ala Cys Gly Ile  
100 105 110  
Asp Leu Gln Tyr Ser Thr Ile Phe Val Gln Ser His Val Ala Ala His  
115 120 125  
Ser Glu Leu Ala Trp Leu Leu Asn Cys Val Thr Pro Leu Asn Trp Leu  
130 135 140  
Glu Arg Met Ile Gln Phe Lys Glu Lys Ala Val Lys Gln Gly Glu Asn  
145 150 155 160  
Val Ser Val Gly Leu Leu Asp Tyr Pro Val Leu Met Ala Ala Asp Ile  
165 170 175  
Leu Leu Tyr Asp Ala Asp Lys Val Pro Val Gly Glu Asp Gln Lys Gln  
180 185 190  
His Leu Glu Leu Thr Arg Asp Ile Val Ile Arg Ile Asn Asp Lys Phe  
195 200 205  
Gly Arg Glu Asp Ala Pro Val Leu Lys Leu Pro Glu Pro Leu Ile Arg  
210 215 220  
Lys Glu Gly Ala Arg Val Met Ser Leu Ala Asp Gly Thr Lys Lys Met  
225 230 235 240  
Ser Lys Ser Asp Glu Ser Glu Leu Ser Arg Ile Asn Leu Leu Asp Pro  
245 250 255  
Pro Glu Met Ile Lys Lys Lys Val Lys Lys Cys Lys Thr Asp Pro Gln  
260 265 270  
Arg Gly Leu Trp Phe Asp Asp Pro Glu Arg Pro Glu Cys His Asn Leu  
275 280 285  
Leu Thr Leu Tyr Thr Leu Leu Ser Asn Gln Thr Lys Glu Ala Val Ala  
290 295 300  
Gln Glu Cys Ala Glu Met Gly Trp Gly Gln Phe Lys Pro Leu Leu Thr  
305 310 315 320  
Glu Thr Ala Ile Ala Ala Leu Glu Pro Ile Gln Ala Lys Tyr Ala Glu  
325 330 335  
Ile Leu Ala Asp Arg Gly Glu Leu Asp Arg Ile Ile Gln Ala Gly Asn  
340 345 350  
Ala Lys Ala Ser Gln Thr Ala Gln Gln Thr Leu Ala Arg Val Arg Asp  
355 360 365  
Ala Leu Gly Phe Leu Ala Pro Pro Tyr  
370 375



-continued

---

<210> SEQ ID NO 29

<211> LENGTH: 419

<212> TYPE: PRT

<213> ORGANISM: Bacillus caldotenax

<400> SEQUENCE: 29

```

Met Asp Leu Leu Ala Glu Leu Gln Trp Arg Gly Leu Val Asn Gln Thr
 1           5           10          15
Thr Asp Glu Asp Gly Leu Arg Lys Leu Leu Asn Glu Glu Arg Val Thr
           20           25           30
Leu Tyr Cys Gly Phe Asp Pro Thr Ala Asp Ser Leu His Ile Gly Asn
           35           40           45
Leu Ala Ala Ile Leu Thr Leu Arg Arg Phe Gln Gln Ala Gly His Arg
           50           55           60
Pro Ile Ala Leu Val Gly Gly Ala Thr Gly Leu Ile Gly Asp Pro Ser
           65           70           75           80
Gly Lys Lys Ser Glu Arg Thr Leu Asn Ala Lys Glu Thr Val Glu Ala
           85           90           95
Trp Ser Ala Arg Ile Lys Glu Gln Leu Gly Arg Phe Leu Asp Phe Glu
           100          105          110
Ala Asp Gly Asn Pro Ala Lys Ile Lys Asn Asn Tyr Asp Trp Ile Gly
           115          120          125
Pro Leu Asp Val Ile Thr Phe Leu Arg Asp Val Gly Lys His Phe Ser
           130          135          140
Val Asn Tyr Met Met Ala Lys Glu Ser Val Gln Ser Arg Ile Glu Thr
           145          150          155          160
Gly Ile Ser Phe Thr Glu Phe Ser Tyr Met Met Leu Gln Ala Tyr Asp
           165          170          175
Phe Leu Arg Leu Tyr Glu Thr Glu Gly Cys Arg Leu Gln Ile Gly Gly
           180          185          190
Ser Asp Gln Trp Gly Asn Ile Thr Ala Gly Leu Glu Leu Ile Arg Lys
           195          200          205
Thr Lys Gly Glu Ala Arg Ala Phe Gly Leu Thr Ile Pro Leu Val Thr
           210          215          220
Lys Ala Asp Gly Thr Lys Phe Gly Lys Thr Glu Ser Gly Thr Ile Trp
           225          230          235          240
Leu Asp Lys Glu Lys Thr Ser Pro Tyr Glu Phe Tyr Gln Phe Trp Ile
           245          250          255
Asn Thr Asp Asp Arg Asp Val Ile Arg Tyr Leu Lys Tyr Phe Thr Phe
           260          265          270
Leu Ser Lys Glu Glu Ile Glu Ala Leu Glu Gln Glu Leu Arg Glu Ala
           275          280          285
Pro Glu Lys Arg Ala Ala Gln Lys Ala Leu Ala Glu Glu Val Thr Lys
           290          295          300
Leu Val His Gly Glu Glu Ala Leu Arg Gln Ala Ile Arg Ile Ser Glu
           305          310          315          320
Ala Leu Phe Ser Gly Asp Ile Ala Asn Leu Thr Ala Ala Glu Ile Glu
           325          330          335
Gln Gly Phe Lys Asp Val Pro Ser Phe Val His Glu Gly Gly Asp Val
           340          345          350

```

-continued

---

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Leu | Val | Glu | Leu | Leu | Val | Ser | Ala | Gly | Ile | Ser | Pro | Ser | Lys | Arg |
|     | 355 |     |     |     |     |     | 360 |     |     |     |     | 365 |     |     |     |
| Gln | Ala | Arg | Glu | Asp | Ile | Gln | Asn | Gly | Ala | Ile | Tyr | Val | Asn | Gly | Glu |
|     | 370 |     |     |     |     | 375 |     |     |     |     | 380 |     |     |     |     |
| Arg | Leu | Gln | Asp | Val | Gly | Ala | Ile | Leu | Thr | Ala | Glu | His | Arg | Leu | Glu |
|     | 385 |     |     |     | 390 |     |     |     |     | 395 |     |     |     |     | 400 |
| Gly | Arg | Phe | Thr | Val | Ile | Arg | Arg | Gly | Lys | Lys | Lys | Tyr | Tyr | Leu | Ile |
|     |     |     |     | 405 |     |     |     |     | 410 |     |     |     |     |     | 415 |

---

Arg Tyr Ala

---

What is claimed is:

1. An isolated nucleic acid fragment encoding an aspartyl-tRNA synthetase comprising a member selected from the group consisting of:

- (a) an isolated nucleic acid fragment encoding an amino acid sequence that is at least 80% identical to the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO: 2, 4, 6 and 8;
- (b) an isolated nucleic acid fragment that is complementary to (a).

2. The isolated nucleic acid fragment of claim 1 wherein nucleic acid fragment is a functional RNA.

3. The isolated nucleic acid fragment of claim 1 wherein the nucleotide sequence of the fragment comprises the sequence set forth in a member selected from the group consisting of SEQ ID NO: 1, 3, 5 and 7.

4. A chimeric gene comprising the nucleic acid fragment of claim 1 operably linked to suitable regulatory sequences.

5. A transformed host cell comprising the chimeric gene of claim 4.

6. An aspartyl-tRNA synthetase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO: 2, 4, 6 and 8

7. An isolated nucleic acid fragment encoding a cysteinyl-tRNA synthetase comprising a member selected from the group consisting of:

- (a) an isolated nucleic acid fragment encoding an amino acid sequence that is at least 80% identical to the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO: 10, 12 and 14;
- (b) an isolated nucleic acid fragment that is complementary to (a).

8. The isolated nucleic acid fragment of claim 7 wherein nucleic acid fragment is a functional RNA.

9. The isolated nucleic acid fragment of claim 7 wherein the nucleotide sequence of the fragment comprises the sequence set forth in a member selected from the group consisting of SEQ ID NO: 9, 11 and 13.

10. A chimeric gene comprising the nucleic acid fragment of claim 7 operably linked to suitable regulatory sequences.

11. A transformed host cell comprising the chimeric gene of claim 10.

12. A cysteinyl-tRNA synthetase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO: 10, 12 and 14.

13. An isolated nucleic acid fragment encoding a tryptophanyl-tRNA synthetase comprising a member selected from the group consisting of:

- (a) an isolated nucleic acid fragment encoding an amino acid sequence that is at least 80% identical to the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO: 16, 18 and 20;
- (b) an isolated nucleic acid fragment that is complementary to (a).

14. The isolated nucleic acid fragment of claim 13 wherein nucleic acid fragment is a functional RNA.

15. The isolated nucleic acid fragment of claim 13 wherein the nucleotide sequence of the fragment comprises the sequence set forth in a member selected from the group consisting of SEQ ID NO: 15, 17 and 19.

16. A chimeric gene comprising the nucleic acid fragment of claim 13 operably linked to suitable regulatory sequences.

17. A transformed host cell comprising the chimeric gene of claim 16.

18. A tryptophanyl-tRNA synthetase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in a member selected from the group consisting of SEQ ID NO: 16, 18 and 20.

19. An isolated nucleic acid fragment encoding a tyrosyl-tRNA synthetase comprising a member selected from the group consisting of:

- (a) an isolated nucleic acid fragment encoding an amino acid sequence that is at least 80% identical to the amino acid sequence set forth in SEQ ID NO: 22;
- (b) an isolated nucleic acid fragment that is complementary to (a).

20. The isolated nucleic acid fragment of claim 19 wherein nucleic acid fragment is a functional RNA.

21. The isolated nucleic acid fragment of claim 19 wherein the nucleotide sequence of the fragment comprises the sequence set forth in SEQ ID NO: 21.

22. A chimeric gene comprising the nucleic acid fragment of claim 19 operably linked to suitable regulatory sequences.

23. A transformed host cell comprising the chimeric gene of claim 22.

24. A tyrosyl-tRNA synthetase polypeptide comprising all or a substantial portion of the amino acid sequence set forth in SEQ ID NO: 22.

25. A method of altering the level of expression of an aminoacyl-tRNA synthetase in a host cell comprising:

- (a) transforming a host cell with the chimeric gene of any of claims **4, 10, 16** and **22**; and
- (b) growing the transformed host cell produced in step (a) under conditions that are suitable for expression of the chimeric gene

wherein expression of the chimeric gene results in production of altered levels of an aminoacyl-tRNA synthetase in the transformed host cell.

**26.** A method of obtaining a nucleic acid fragment encoding all or a substantial portion of the amino acid sequence encoding an aminoacyl-tRNA synthetase comprising:

- (a) probing a cDNA or genomic library with the nucleic acid fragment of any of claims **1, 7, 13** and **19**;
- (b) identifying a DNA clone that hybridizes with the nucleic acid fragment of any of claims **1, 7, 13** and **19**;
- (c) isolating the DNA clone identified in step (b); and
- (d) sequencing the cDNA or genomic fragment that comprises the clone isolated in step (c)

wherein the sequenced nucleic acid fragment encodes all or a substantial portion of the amino acid sequence encoding an aminoacyl-tRNA synthetase.

**27.** A method of obtaining a nucleic acid fragment encoding a substantial portion of an amino acid sequence encoding an aminoacyl-tRNA synthetase comprising:

- (a) synthesizing an oligonucleotide primer corresponding to a portion of the sequence set forth in any of SEQ ID NOs: **1, 3, 5, 7, 9, 11, 13, 15, 17, 19** and **21**; and
- (b) amplifying a cDNA insert present in a cloning vector using the oligonucleotide primer of step (a) and a primer representing sequences of the cloning vector

wherein the amplified nucleic acid fragment encodes a substantial portion of an amino acid sequence encoding an aminoacyl-tRNA synthetase.

**28.** The product of the method of claim 26.

**29.** The product of the method of claim 27.

**30.** A method for evaluating at least one compound for its ability to inhibit the activity of an aminoacyl-tRNA synthetase, the method comprising the steps of:

- (a) transforming a host cell with a chimeric gene comprising a nucleic acid fragment encoding an aminoacyl-tRNA synthetase, operably linked to suitable regulatory sequences;
- (b) growing the transformed host cell under conditions that are suitable for expression of the chimeric gene wherein expression of the chimeric gene results in production of the aminoacyl-tRNA synthetase encoded by the operably linked nucleic acid fragment in the transformed host cell;
- (c) optionally purifying the aminoacyl-tRNA synthetase expressed by the transformed host cell;
- (d) treating the aminoacyl-tRNA synthetase with a compound to be tested; and
- (e) comparing the activity of the aminoacyl-tRNA synthetase that has been treated with a test compound to the activity of an untreated aminoacyl-tRNA synthetase,

thereby selecting compounds with potential for inhibitory activity.

\* \* \* \* \*