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(54) **PROTEIN KINASE STRESS-RELATED POLYPEPTIDES AND METHODS OF USE IN PLANTS**

STRESS-ASSOZIIERTE, PROTEIN KINASE-SPEZIFISCHE POLYPEPTIDE UND VERFAHREN ZUR VERWENDUNG IN PFLANZEN

POLYPEPTIDES DE PROTEINE KINASE ASSOCIES AU STRESS ET PROCEDES D'UTILISATION DANS LES PLANTES

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(56) References cited:
WO-A-01/77355 **WO-A-01/77356**
WO-A-98/26045 **WO-A2-01/45492**

- **DATABASE EMBL [Online] 7 March 2000 (2000-03-07), "ga71e12.y1 Moss EST library PPU Physcomitrella patens cDNA clone PEP_ SOURCE_ID:PPU121123 5' similar to TR:O65461 O65461 SERINE/THREONINE PROTEIN KINASE LIKE PROTEIN. ;, mRNA sequence." XP002320898 retrieved from EBI accession no. EM_PRO:AW510033 Database accession no. AW510033**
- **PIAO H L ET AL: "Constitutive over-expression of AtGSK1 induces NaCl stress responses in the absence of NaCl stress and results in enhanced NaCl tolerance in arabidopsis" PLANT JOURNAL, BLACKWELL SCIENTIFIC PUBLICATIONS, OXFORD, GB, vol. 27, no. 4, 2001, pages 305-314, XP002961273 ISSN: 0960-7412**
- **LAURIE S ET AL: "THE ROLE OF PROTEIN KINASES IN THE REGULATION OF PLANT GROWTH AND DEVELOPMENT" PLANT GROWTH REGULATION, DORDRECHT, NL, vol. 34, no. 3, July 2001 (2001-07), pages 253-265, XP009034127 ISSN: 0167-6903**

Printed by Jouve, 75001 PARIS (FR)

(Cont. next page)

- **EINZENBERGER ELISABETH ET AL: "Isolation and expression during pollen development of a tobacco cDNA clone encoding a protein kinase homologous to shaggy/glycogen synthase kinase-3" BIOCHIMICA ET BIOPHYSICA ACTA, vol. 1260, no. 3, 1995, pages 315-319, XP008049610 ISSN: 0006-3002 -& DATABASE UniProt [Online] 16 October 2001 (2001-10-16), "Shaggy-related protein kinase NtK-1 (EC 2.7.1.-)." XP002335171 retrieved from EBI accession no. UNIPROT:MSK1_TOBAC Database accession no. Q40518**
- **DATABASE TREMBL SEQUENCE LIBRARY EBI.HINXTON; 1 August 1998 (1998-08-01), BEVAN, M. ET AL.: "SerineThreonine protein kinase like protein, A. thaliana" XP002320899 accession no. WWW.EBI.AC.UK Database accession no. O65461**
- **DATABASE TREMBL SEQUENCE LIBRARY [Online] EBI.HINXTON; 1 October 2001 (2001-10-01), NAKAMURA, Y.: "Serine/Threonine protein kinase-likeprotein (Strubbelig receptor famiuly 7) - A. thaliana" XP002320900 accession no. WWW.EBI.AC.UK Database accession no. Q9LUL4**
- **JONAK CLAUDIA ET AL: "Stress signaling in plants: A mitogen-activated protein kinase pathway is activated by cold and drought" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF USA, NATIONAL ACADEMY OF SCIENCE. WASHINGTON, US, vol. 93, no. 20, 1996, pages 11274-11279, XP002176204 ISSN: 0027-8424**
- **MACHUKA J ET AL: "SEQUENCE ANALYSIS OF EXPRESSED SEQUENCE TAGS FROM AN ABA-TREATED CDNA LIBRARY IDENTIFIES STRESS RESPONSE GENES IN THE MOSS PHYSCOMITRELLA PATENS" PROCEEDINGS OF THE INTERNATIONAL CONGRESS ON TRANSPORTATION ELECTRONICS, IEEE, NEW YORK, NY, US, vol. 40, no. 4, 1999, pages 378-387, XP000946663**
- **DATABASE EMBL [Online] 23 January 2002 (2002-01-23), "Physcomitrella patens subsp. patens cDNA clone:pphn9a09, 5' end, single read." XP002320901 retrieved from EBI accession no. EM_PRO:BJ205855 Database accession no. BJ205855**
- **SAIJO ET AL.: 'A Ca2+-dependent protein kinase that endows rice plants with cold-and salt-stress tolerance functions in vascular bundles' PLANT CELL PHYSIOL. vol. 42, no. 11, 2001, pages 1228 - 1233, XP008034845**
- **LEE ET AL.: 'A highly conserved kinase is an essential component for stress tolerance in yeast and plant cells' PROC. NATL. ACAD. SCI. USA vol. 96, May 1999, pages 5873 - 5877, XP002127807**
- **URAO ET AL.: 'A transmembrane hybrid-type histidine kinase in arabidopsis functions as an osmosensor' PLANT CELL vol. 11, September 1999, pages 1743 - 1754, XP002212287**
- **SAIJO ET AL.: 'Over-expression of a single Ca2+-dependent protein kinase confers both cold and salt/drought tolerance on rice plants' PLANT J. vol. 23, no. 3, 2000, pages 319 - 327, XP002176206**

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Description

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] This invention relates generally to nucleic acid sequences encoding polypeptides that are associated with abiotic stress responses and abiotic stress tolerance in plants.

[0002] In particular, this invention relates to nucleic acid sequences encoding polypeptides that confer drought, cold, and/or salt tolerance to plants.

Background Art

[0003] Abiotic environmental stresses, such as drought stress, salinity stress, heat stress, and cold stress, are major limiting factors of plant growth and productivity. Crop losses and crop yield losses of major crops such as soybean, rice, maize (corn), cotton, and wheat caused by these stresses represent a significant economic and political factor and contribute to food shortages in many underdeveloped countries.

[0004] Plants are typically exposed during their life cycle to conditions of reduced environmental water content. Most plants have evolved strategies to protect themselves against these conditions of desiccation. However, if the severity and duration of the drought conditions are too great, the effects on development, growth, and yield of most crop plants are profound. Continuous exposure to drought conditions causes major alterations in the plant metabolism which ultimately lead to cell death and consequently yield losses.

[0005] Developing stress-tolerant plants is a strategy that has the potential to solve or mediate at least some of these problems. However, traditional plant breeding strategies to develop new lines of plants that exhibit resistance (tolerance) to these types of stresses are relatively slow and require specific resistant lines for crossing with the desired line. Limited germplasm resources for stress tolerance and incompatibility in crosses between distantly related plant species represent significant problems encountered in conventional breeding. Additionally, the cellular processes leading to drought, cold, and salt tolerance in model drought- and/or salt-tolerant plants are complex in nature and involve multiple mechanisms of cellular adaptation and numerous metabolic pathways. This multi-component nature of stress tolerance has not only made breeding for tolerance largely unsuccessful, but has also limited the ability to genetically engineer stress tolerance plants using biotechnological methods.

[0006] Drought and cold stresses, as well as salt stresses, have a common theme important for plant growth, and that is water availability. Plants are exposed during their entire life cycle to conditions of reduced environmental water content, and most plants have evolved strategies to protect themselves against these conditions of desiccation. However, if the severity and duration of the drought conditions are too great, the effects on plant development, growth and yield of most crop plants are profound. Furthermore, most of the crop plants are very susceptible to higher salt concentrations in the soil. Because high salt content in some soils results in less water being available for cell intake, high salt concentration has an effect on plants similar to the effect of drought on plants. Additionally, under freezing temperatures, plant cells lose water as a result of ice formation that starts in the apoplast and withdraws water from the symplast. A plant's molecular response mechanisms to each of these stress conditions are common, and protein kinases play an essential role in these molecular mechanisms.

[0007] Protein kinases represent a superfamily, and the members of this superfamily catalyze the reversible transfer of a phosphate group of ATP to serine, threonine, and tyrosine amino acid side chains on target polypeptides. Protein kinases are primary elements in signaling processes in plants and have been reported to play crucial roles in perception and transduction of signals that allow a cell (and the plant) to respond to environmental stimuli. In particular, receptor protein kinases (RPKs) represent one group of protein kinases that activate a complex array of intracellular signaling pathways in response to the extracellular environment (Van der Gear et al., 1994, Annu. Rev. Cell Biol. 10:251-337). RPKs are single-pass transmembrane polypeptides that contain an amino-terminal signal sequence, extracellular domains unique to each receptor, and a cytoplasmic kinase domain. Ligand binding induces homo- or hetero-dimerization of RPKs, and the resultant close proximity of the cytoplasmic domains results in kinase activation by transphosphorylation. Although plants have many polypeptides similar to RPKs, no ligand has been identified for these receptor-like kinases (RLKs). The majority of plant RLKs that have been identified belong to the family of Serine/Threonine (Ser/Thr) kinases, and most have extracellular Leucine-rich repeats (Becraft, PW., 1998, Trends Plant Sci. 3:384-388).

[0008] Another type of protein kinase is the Ca+-dependent protein kinase (CDPK).
This type of kinase has a calmodulin-like domain at the COOH terminus which allows response to Ca+ signals directly without calmodulin being present. Currently, CDPKs are the most prevalent Ser/Thr polypeptide kinases found in higher plants. Although their physiological roles remain unclear, they are induced by cold, drought, and abscisic acid (ABA) (Knight et al., 1991, Nature 352:524; Schroeder, J.I. and Thuleau, P., 1991, Plant Cell 3:555; Bush, D.S., 1995, Annu.

Rev. Plant Phys. Plant Mol. Biol. 46:95; Urao, T. et al., 1994, Mol. Gen. Genet. 244:331).

[0009] Another type of signaling mechanism involves members of the conserved SNF1 Serine/Threonine polypeptide kinase family. These kinases play essential roles in eukaryotic glucose and stress signaling. Plant SNF1-like kinases participate in the control of key metabolic enzymes, including HMGR, nitrate reductase, sucrose synthase, and sucrose phosphate synthase (SPS). Genetic and biochemical data indicate that sugar-dependent regulation of SNF1 kinases involves several other sensory and signaling components in yeast, plants, and animals.

[0010] Additionally, members of the Mitogen-Activated Protein Kinase (MAPK) family have been implicated in the actions of numerous environmental stresses in animals, yeasts and plants. It has been demonstrated that both MAPK-like kinase activity and mRNA levels of the components of MAPK cascades increase in response to environmental stress and plant hormone signal transduction. MAP kinases are components of sequential kinase cascades, which are activated by phosphorylation of threonine and tyrosine residues by intermediate upstream MAP kinase kinases (MAPKKs). The MAPKKs are themselves activated by phosphorylation of serine and threonine residues by upstream kinases (MAPKKKs). A number of MAP Kinase genes have been reported in higher plants.

[0011] Another major type of environmental stress is lodging, which refers to the bending of shoots or stems in response to wind, rain, pests or disease. Two types of lodging occur in cereals: root-lodging and stem breakage. The most common type of lodging is root lodging, which occurs early in the season. Stem-breakage, by comparison, occurs later in the season as the stalk becomes more brittle due to crop maturation. Stem breakage has greater adverse consequences on crop yield, since the plants cannot recover as well as from the earlier root-lodging.

[0012] Lodging in cereal crops is influenced by morphological (structural) plant traits as well as environmental conditions. Lodging in cereals is often a result of the combined effects of inadequate standing power of the crop and adverse weather conditions, such as rain, wind, and/or hail. Lodging is also variety (cultivar) dependent. For example, a tall, weak-stemmed wheat cultivar has a greater tendency to lodge than a semi-dwarf cultivar with stiffer straw. In addition, the tendency of a crop to lodge depends on the resistance especially of the lower internodes. This is because the lower internodes have to resist the greatest movement of force. The weight of the higher internodes of the stems plus leaves and heads in relation to the stem (culm) will affect the resistance of a crop to lodging. The heavier the higher parts of the stem are and the greater the distance from their center of gravity to the base of the stem, the greater is the movement of the forces acting upon the lower internodes and the roots. Supporting this argument, it was found that the breaking strength of the lowest internode and shoot per root ratio were the most suitable indices of lodging. Furthermore, plant morphological (structural) characteristics such as plant height, wall thickness, and cell wall lignification can affect the ability of the plant to resist a lateral force.

[0013] Severe lodging is very costly due to its effects on grain formation and associated harvesting problems and losses. It takes about twice the time to harvest a lodged crop than a standing one. Secondary growth in combination with a flattened crop makes harvesting difficult and can subsequently lead to poor grain quality. Yield loss comes from poor grain filling, head loss, and bird damage. Yield losses are most severe when a crop lodges during the ten days following head emergence. Yield losses at this stage will range between 15% and 40%. Lodging that occurs after the plant matures will not affect the yield but it may reduce the amount of harvestable grain. For instance, when lodging occurs after the plant matures, neck breakage and the loss of the whole head can result; these often lead to severe harvest losses. In theses cases, farmers who straight combine their grain will likely incur higher losses than those who swath them. Accordingly, it is desirable to identify genes expressed in lodging resistant plants that have the capacity to confer lodging resistance to the host plant and to other plant species.

[0014] Although some genes that are involved in stress responses in plants have been characterized, the characterization and cloning of plant genes that confer stress tolerance remains largely incomplete and fragmented. For example, certain studies have indicated that drought and salt stress in some plants may be due to additive gene effects, in contrast to other research that indicates specific genes are transcriptionally activated in vegetative tissue of plants under osmotic stress conditions. Although it is generally assumed that stress-induced proteins have a role in tolerance, direct evidence is still lacking, and the functions of many stress-responsive genes are unknown.

[0015] There is a need, therefore, to identify genes expressed in stress tolerant plants that have the capacity to confer stress tolerance to its host plant and to other plant species. Newly generated stress tolerant plants will have many advantages, such as an increased range in which the crop plants can be cultivated, by for example, decreasing the water requirements of a plant species. Other desirable advantages include increased resistance to lodging, the bending of shoots or stems in response to wind, rain, pests, or disease.

SUMMARY OF THE INVENTION

[0016] This invention fulfills in part the need to identify new, unique protein kinases capable of conferring stress tolerance to plants upon over-expression. The present invention describes a novel genus of Protein Kinase Stress-Related Polypeptides (PKSRPs) and PKSRP coding nucleic acids that are important for modulating a plant's response to an environmental stress. More particularly, over-expression of these PKSRP coding nucleic acids in a plant results

in the plant's increased tolerance to an environmental stress.

[0017] The present invention includes an isolated plant cell comprising a PKSRP coding nucleic acid, wherein expression of the nucleic acid sequence in the plant cell results in increased tolerance to environmental stress as compared to a wild type variety of the plant cell.Namely, described herein is PK-11 from *Physcomitrella patens.*

[0018] The invention provides in some embodiments that the PKSRP and coding nucleic acid are those that are found in members of the genus *Physcomitrella.*

In another preferred embodiment, the nucleic acid and polypeptide are from a *Physcomitrella patens* plant.

[0019] The invention provides that the environmental stress can be increased salinity, drought, temperature, or combinations thereof. In preferred embodiments, the environmental stress can be drought or cold temperature.

[0020] The invention further provides a seed produced by a transgenic plant transformed by a PKSRP coding nucleic acid, wherein the plant is true breeding for increased tolerance to environmental stress as compared to a wild type variety of the plant. The invention further provides a seed produced by a transgenic plant expressing a PKSRP, wherein the plant is true breeding for increased tolerance to environmental stress as compared to a wild type variety of the plant.

[0021] The invention further provides an agricultural product produced by any of the below-described transgenic plants, plant parts or seeds. The invention further provides an isolated PKSRP as described below. The invention further provides an isolated PKSRP coding nucleic acid, wherein the PKSRP coding nucleic acid codes for a PKSRP as described below.

[0022] The invention further provides an isolated recombinant expression vector comprising a PKSRP coding nucleic acid as described below, wherein expression of the vector in a host cell results in increased tolerance to environmental stress as compared to a wild type variety of the host cell. The invention further provides a host cell containing the vector and a plant containing the host cell.

[0023] The invention further provides a method of producing a transgenic plant with a PKSRP coding nucleic acid, wherein expression of the nucleic acid in the plant results in increased tolerance to environmental stress as compared to a wild type variety of the plant comprising: (a) transforming a plant cell with an expression vector comprising a PKSRP coding nucleic acid, and (b) generating from the plant cell a transgenic plant with an increased tolerance to environmental stress as compared to a wild type variety of the plant In preferred embodiments, the PKSRP and PKSRP coding nucleic acid are as described below.

[0024] Further described herein is a method of identifying a novel PKSRP, comprising (a) raising a specific antibody response to a PKSRP, or fragment thereof as described below; (b) screening putative PKSRP material with the antibody, wherein specific binding of the antibody to the material indicates the presence of a potentially novel PKSRP; and (c) identifying from the bound material a novel PKSRP in comparison to known PKSRP. Alternatively, hybridization with nucleic acid probes as described below can be used to identify novel PKSRP nucleic acids.

[0025] The present invention also provides methods of modifying stress tolerance of a plant comprising, modifying the expression of a PKSRP nucleic acid in the plant, wherein the PKSRP is as described below. The invention provides that this method can be performed such that the stress tolerance is either increased or decreased. Preferably, stress tolerance is increased in a plant via increasing expression of a PKSRP nucleic acid.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] Figure 1 shows a diagram of the plant expression vector pBPS-JH001 containing the super promoter driving the expression of the PKSRP coding nucleic acid ("Gene of Interest"). The components are: aacCI gentamycin resistance gene (Hajdukiewicz et al., 1994, Plant Molec. Biol. 25: 989-94), NOS promoter (Becker et al., 1992, Plant Molec. Biol. 20: 1195-97), g7T terminator (Becker et al., 1992), and NOSpA terminator (Jefferson et al., 1987, EMBO J. 6:3901-7).

[0027] Figure 2 shows a diagram of the plant expression vector pBPS-SC022 containing the super promoter driving the expression of the PKSRP coding nucleic acid (Gene of Interest"). The components are: NPTII kanamycin resistance gene (Hajdukiewicz et al., 1994, Plant Molec. Biol. 25: 989-98), AtAct2-1 promoter (An et al., 1996, Plant J. 10: 107-21), and OCS3 terminator (Weigel et al., 2000, Plant Physiol 122:1003-13).

DETAILED DESCRIPTION OF THE INVENTION

[0028] The present invention may be understood more readily by reference to the following detailed description of the preferred embodiments of the invention and the Examples included herein. However, before the present compounds, compositions, and methods are disclosed and described, it is to be understood that this invention is not limited to specific nucleic acids, specific polypeptides, specific cell types, specific host cells, specific conditions, or specific methods, etc., as such may, of course, vary, and the numerous modifications and variations therein will be apparent to those skilled in the art. It is also to be understood that the terminology used herein is for the purpose of describing specific embodiments only and is not intended to be limiting. In particular, the designation of the amino acid sequences as "Protein Kinase Stress-Related Polypeptides" (PKSRPs), in no way limits the functionality of those sequences.

[0029] The present invention describes a novel genus of Protein Kinase Stress-Related Polypeptides (PKSRPs) and PKSRP. coding nucleic acids that are important for modulating a plant's response to an environmental stress. More particularly, over-expression of these PKSRP coding nucleic acids in a plant results in the plant's increased tolerance to an environmental stress.

[0030] The present invention provides a transgenic plant cell transformed by a PKSRP coding nucleic acid, wherein expression of the nucleic acid sequence in the plant cell results in increased tolerance to environmental stress as compared to a wild type variety of the plant cell. The invention further provides transgenic plant parts and transgenic plants containing the plant cells described herein. In preferred embodiments, the transgenic plants and plant parts have increased tolerance to environmental stress as compared to a wild type variety of the plant. Plant parts include, but are not limited to, stems, roots, ovules, stamens, leaves, embryos, meristematic regions, callus tissue, gametophytes, sporophytes, pollen, microspores, and the like. In one embodiment, the transgenic plant is male sterile. Also provided is a plant seed produced by a transgenic plant transformed by a PKSRP coding nucleic acid, wherein the seed contains the PKSRP coding nucleic acid, and wherein the plant is true breeding for increased tolerance to environmental stress as compared to a wild type variety of the plant. The invention further provides a seed produced by a transgenic plant expressing a PKSRP, wherein the seed contains the PKSRP, and wherein the plant is true breeding for increased tolerance to environmental stress as compared to a wild type variety of the plant. The invention also provides an agricultural product produced by any of the below-described transgenic plants, plant parts, and plant seeds. Agricultural products include, but are not limited to, plant extracts, proteins, amino acids, carbohydrates, fats, oils, polymers, vitamins, and the like.

[0031] As used herein, the term "variety" refers to a group of plants within a species that share constant characters that separate them from the typical form and from other possible varieties within that species. While possessing at least one distinctive trait, a variety is also characterized by some variation between individuals within the variety, based primarily on the Mendelian segregation of traits among the progeny of succeeding generations. A variety is considered "true breeding" for a particular trait if it is genetically homozygous for that trait to the extent that, when the true-breeding variety is self-pollinated, a significant amount of independent segregation of the trait among the progeny is not observed. In the present invention, the trait arises from the transgenic expression of one or more DNA sequences introduced into a plant variety.

[0032] The present invention describes for the first time that the *Physcomitrella patens* PKSRP PK-11 is useful for increasing a plant's tolerance to environmental stress. As used herein, the term polypeptide refers to a chain of at least four amino acids joined by peptide bonds. The chain may be linear, branched, circular or combinations thereof. Accordingly, the present invention provides the isolated PKSRP PK-11, and homologs thereof. In a preferred embodiment, 1 the PKSRP is *Physcomitrella patens* Protein Kinase-11 (PK-11) polypeptide as defined in SEQ ID NO:12; and homologs and orthologs thereof. Homologs and orthologs of the amino acid sequences are defined below.

[0033] The PKSRPs of the present invention are preferably produced by recombinant DNA techniques. For example, a nucleic acid molecule encoding the polypeptide is cloned into an expression vector (as described below), the expression vector is introduced into a host cell (as described below) and the PKSRP is expressed in the host cell. The PKSRP can then be isolated from the cells by an appropriate purification scheme using standard polypeptide purification techniques. For the purposes of the invention, the term "recombinant polynucleotide" refers to a polynucleotide that has been altered, rearranged or modified by genetic engineering. Examples include any cloned polynucleotide, and polynucleotides that are linked or joined to heterologous sequences. The term "recombinant" does not refer to alterations to polynucleotides that result from naturally occurring events, such as spontaneous mutations. Alternative to recombinant expression, a PKSRP, or peptide can be synthesized chemically using standard peptide synthesis techniques. Moreover, native PKSRP can be isolated from cells (e.g., *Physcomitrella patens, Brassica napus, Glycine max, or Oryza sativa*), for example using an anti-PKSRP antibody, which can be produced by standard techniques utilizing a PKSRP or fragment thereof.

[0034] The invention further provides an isolated PKSRP coding nucleic acid. The present invention includes PKSRP coding nucleic acids that encode PKSRPs as described herein. In a preferred embodiment, the PKSRP coding nucleic acid is *Physcomitrella patens* Protein Kinase-11 (PK-11) nucleic acid as defined in SEQ ID NO:11; and homologs and orthologs thereof. Homologs and orthologs of the nucleotide sequences are defined below. In one preferred embodiment, the nucleic acid and polypeptide is isolated from the plant genus *Physcomitrella.* In another preferred embodiment, the nucleic acid and polypeptide is from a *Physcomitrella patens* (*P. patens*) plant.

[0035] As used herein, the term "environmental stress" refers to any sub-optimal growing condition and includes, but is not limited to, sub-optimal conditions associated with salinity, drought, temperature, metal, chemical, pathogenic, and oxidative stresses, or combinations thereof. In preferred embodiments, the environmental stress can be selected from one or more of the group consisting of salinity, drought, or temperature, or combinations thereof, and in particular, can be selected from one or more of the group consisting of high salinity, low water content, or low temperature. It is also to be understood that as used in the specification and in the claims, "a" or "an" can mean one or more, depending upon the context in which it is used. Thus, for example, reference to "a cell" can mean that at least one cell can be utilized.

[0036] As also used herein, the term "nucleic acid" and "polynucleotide" refer to RNA or DNA that is linear or branched,

single or double stranded, or a hybrid thereof. The term also encompasses RNA/DNA hybrids. These terms also encompass untranslated sequence located at both the 3' and 5' ends of the coding region of the gene: at least about 1000 nucleotides of sequence upstream from the 5' end of the coding region and at least about 200 nucleotides of sequence downstream from the 3' end of the coding region of the gene. Less common bases, such as inosine, 5-methylcytosine, 6-methyladenine, hypoxanthine and others can also be used for antisense, dsRNA and ribozyme pairing. For example, polynucleotides that contain C-5 propyne analogues of uridine and cytidine have been shown to bind RNA with high affinity and to be potent antisense inhibitors of gene expression. Other modifications, such as modification to the phosphodiester backbone, or the 2'-hydroxy in the ribose sugar group of the RNA can also be made. The antisense polynucleotides and ribozymes can consist entirely of ribonucleotides, or can contain mixed ribonucleotides and deoxyribonucleotides. The polynucleotides of the invention may be produced by any means, including genomic preparations, cDNA preparations, *in vitro* synthesis, RT-PCR, and *in vitro* or *in vivo* transcription.

[0037] An "isolated" nucleic acid molecule is one that is substantially separated from other nucleic acid molecules which are present in the natural source of the nucleic acid (i.e., sequences encoding other polypeptides). Preferably, an "isolated" nucleic acid is free of some of the sequences which naturally flank the nucleic acid (i.e., sequences located at the 5' and 3' ends of the nucleic acid) in its naturally occurring replicon. For example, a cloned nucleic acid is considered isolated. In various embodiments, the isolated PKSRP nucleic acid molecule can contain less than about 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb, or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in genomic DNA of the cell from which the nucleic acid is derived (e.g., a *Physcomitrella patens, a Brassica napus, a Glycine max,* or an *Oryza sativa* cell). A nucleic acid is also considered isolated if it has been altered by human intervention, or placed in a locus or location that is not its natural site, or if it is introduced into a cell by agroinfection. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, can be free from some of the other cellular material with which it is naturally associated, or culture medium when produced by recombinant techniques, or chemical precursors or other chemicals when chemically synthesized.

[0038] Specifically excluded from the definition of "isolated nucleic acids" are: naturally-occurring chromosomes (such as chromosome spreads), artificial chromosome libraries, genomic libraries, and cDNA libraries that exist either as an *in vitro* nucleic acid preparation or as a transfected/transformed host cell preparation, wherein the host cells are either an *in vitro* heterogeneous preparation or plated as a heterogeneous population of single colonies. Also specifically excluded are the above libraries wherein a specified nucleic acid makes up less than 5% of the number of nucleic acid inserts in the vector molecules. Further specifically excluded are whole cell genomic DNA or whole cell RNA preparations (including whole cell preparations that are mechanically sheared or enzymatically digested). Even further specifically excluded are the whole cell preparations found as either an *in vitro* preparation or as a heterogeneous mixture separated by electrophoresis wherein the nucleic acid of the invention has not further been separated from the heterologous nucleic acids in the electrophoresis medium (e.g., further separating by excising a single band from a heterogeneous band population in an agarose gel or nylon blot).

[0039] A nucleic acid molecule of the present invention, e.g., a nucleic acid molecule having the nucleotide sequence of SEQ ID NO:11, or a portion thereof, can be isolated using standard molecular biology techniques and the sequence information provided herein. Moreover, a nucleic acid molecule encompassing all or a portion of the sequence of SEQ ID NO:11, can be isolated by the polymerase chain reaction using oligonucleotide primers designed based upon this sequence. For example, mRNA can be isolated from plant cells (e.g., by the guanidinium-thiocyanate extraction procedure of Chirgwin et al., 1979, Biochemistry 18:5294-5299) and cDNA can be prepared using reverse transcriptase (e.g., Moloney MLV reverse transcriptase, available from Gibco/BRL, Bethesda, MD; or AMV reverse transcriptase, available from Seikagaku America, Inc., St. Petersburg, FL). Synthetic oligonucleotide primers for polymerase chain reaction amplification can be designed based upon the nucleotide sequence shown in SEQID NO:11. A nucleic acid molecule of the invention can be amplified using cDNA or, alternatively, genomic DNA, as a template and appropriate oligonucleotide primers according to standard PCR amplification techniques. The nucleic acid molecule so amplified can be cloned into an appropriate vector and characterized by DNA sequence analysis. Furthermore, oligonucleotides corresponding to a PKSRP nucleotide sequence can be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

[0040] In a preferred embodiment, an isolated nucleic acid molecule of the invention comprises the nucleotide sequence shown in SEQ ID NO:11. The cDNA may comprise a sequence encoding the PKSRPs, (i.e., the "coding region" as well as a 5' untranslated sequence and a 3' untranslated sequence.

The present invention also includes PKSRP coding nucleic acids that encode PKSRPs as described herein. Preferred is a PKSRP coding nucleic acid that encodes the PKSRP PK-11 as defined in SEQ ID NO:12.

[0041] Moreover, the nucleic acid molecule of the invention can comprise a portion of the coding region of the sequence in SEQ ID NO:11, for example, a fragment which can be used as a probe or primer or a fragment encoding a biologically active portion of a PKSRP. The nucleotide sequence determined from the cloning of the PKSRP gene from *Physcomitrella patens,* allows for the generation of probes and primers designed for use in identifying and/or cloning PKSRP homologs in other cell types and organisms, as well as PKSRP homologs from other related species. The portion of the coding

region can also encode a biologically active fragment of a PKSRP.

[0042] As used herein, the term "biologically active portion of" a PKSRP is intended to include a portion, e.g., a domain/motif, of a PKSRP that participates in modulation of stress tolerance in a plant, and more preferably, drought tolerance or salt tolerance. For the purposes of the present invention, modulation of stress tolerance refers to at least a 10% increase or decrease in the stress tolerance of a transgenic plant comprising a PKSRP expression cassette (or expression vector) as compared to the stress tolerance of a non-transgenic control plant. Methods for quantitating stress tolerance are provided at least in Example 7 below. In a preferred embodiment, the biologically active portion of a PKSRP increases a plant's tolerance to an environmental stress.

[0043] Biologically active portions of a PKSRP include peptides comprising amino acid sequences derived from the amino acid sequence of a PKSRP, e.g., an amino acid sequence of SEQ ID NO:12, or the amino acid sequence of a polypeptide identical to a PKSRP, which include fewer amino acids than a full length PKSRP or the full length polypeptide which is identical to a PKSRP, and exhibit at least one activity of a PKSRP. Typically, biologically active portions (e.g., peptides which are, for example, 5, 10, 15, 20, 30, 35, 36, 37, 38, 39, 40, 50, 100 or more amino acids in length) comprise a domain or motif with at least one activity of a PKSRP. Moreover, other biologically active portions in which other regions of the polypeptide are deleted, can be prepared by recombinant techniques and evaluated for one or more of the activities described herein. Preferably, the biologically active portions of a PKSRP include one or more selected domains/motifs or portions thereof having biological activity such as a kinase domain.

[0044] The invention also provides PKSRP chimeric or fusion polypeptides. As used herein, a PKSRP "chimeric polypeptide" or "fusion polypeptide" comprises a PKSRP operatively linked to a non-PKSRP. A PKSRP refers to a polypeptide having an amino acid sequence corresponding to a PKSRP, whereas a non-PKSRP refers to a polypeptide having an amino acid sequence corresponding to a polypeptide which is not substantially identical to the PKSRP, e.g., a polypeptide that is different from the PKSRP and is derived from the same or a different organism. As used herein with respect to the fusion polypeptide, the term "operatively linked" is intended to indicate that the PKSRP and the non-PKSRP are fused to each other so that both sequences fulfill the proposed function attributed to the sequence used. The non-PKSRP can be fused to the N-terminus or C-terminus of the PKSRP. For example, in one embodiment, the fusion polypeptide is a GST-PKSRP fusion polypeptide in which the PKSRP sequences are fused to the C-terminus of the GST sequences. Such fusion polypeptides can facilitate the purification of recombinant PKSRPs. In another embodiment, the fusion polypeptide is a PKSRP containing a heterologous signal sequence at its N-terminus. In certain host cells (e.g., mammalian host cells), expression and/or secretion of a PKSRP can be increased through use of a heterologous signal sequence.

[0045] Preferably, a PKSRP chimeric or fusion polypeptide of the invention is produced by standard recombinant DNA techniques. For example, DNA fragments coding for the different polypeptide sequences are ligated together in-frame in accordance with conventional techniques, for example by employing blunt-ended or stagger-ended termini for ligation, restriction enzyme digestion to provide for appropriate termini, filling-in of cohesive ends as appropriate, alkaline phosphatase treatment to avoid undesirable joining and enzymatic ligation. In another embodiment, the fusion gene can be synthesized by conventional techniques including automated DNA synthesizers. Alternatively, PCR amplification of gene fragments can be carried out using anchor primers which give rise to complementary overhangs between two consecutive gene fragments which can subsequently be annealed and re-amplified to generate a chimeric gene sequence (See, e.g., Current Protocols in Molecular Biology, eds. Ausubel et al. John Wiley & Sons: 1992). Moreover, many expression vectors are commercially available that already encode a fusion moiety (e.g., a GST polypeptide). A PKSRP encoding nucleic acid can be cloned into such an expression vector such that the fusion moiety is linked in-frame to the PKSRP.

[0046] In addition to fragments and fusion polypeptides of the PKSRPs described herein, the present invention includes homologs and analogs of naturally occurring PKSRPs and PKSRP encoding nucleic acids in a plant. "Homologs" are defined herein as two nucleic acids or polypeptides that have similar, or substantially identical, nucleotide or amino acid sequences, respectively. Homologs include allelic variants, orthologs, paralogs, agonists and antagonists of PKSRPs as defined hereafter. The term "homolog" further encompasses nucleic acid molecules that differ from the nucleotide sequence shown in SEQ ID NO:11, (and portions thereof) due to degeneracy of the genetic code and thus encode the same PKSRP as that encoded by the nucleotide sequence shown in SEQ ID NO:11.

As used herein a "naturally occurring" PKSRP refers to a PKSRP amino acid sequence that occurs in nature. Preferably, a naturally occurring PKSRP comprises the amino acid sequence SEQ ID NO:12.

[0047] An agonist of the PKSRP can retain substantially the same, or a subset, of the biological activities of the PKSRP. An antagonist of the PKSRP can inhibit one or more of the activities of the naturally occurring form of the PKSRP. For example, the PKSRP antagonist can competitively bind to a downstream or upstream member of the cell membrane component metabolic cascade that includes the PKSRP, or bind to a PKSRP that mediates transport of compounds across such membranes, thereby preventing translocation from taking place.

[0048] Nucleic acid molecules corresponding to natural allelic variants and analogs, orthologs and paralogs of a PKSRP cDNA can be isolated based on their identity to the *Physcomitrella patens,* PKSRP nucleic acids described herein using PKSRP cDNAs, or a portion thereof, as a hybridization probe according to standard hybridization techniques

under stringent hybridization conditions. In an alternative embodiment, homologs of the PKSRP can be identified by screening combinatorial libraries of mutants, e.g., truncation mutants, of the PKSRP for PKSRP agonist or antagonist activity. In one embodiment, a variegated library of PKSRP variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. A variegated library of PKSRP variants can be produced by, for example, enzymatically ligating a mixture of synthetic oligonucleotides into gene sequences such that a degenerate set of potential PKSRP sequences is expressible as individual polypeptides, or alternatively, as a set of larger fusion polypeptides (e.g., for phage display) containing the set of PKSRP sequences therein. There are a variety of methods that can be used to produce libraries of potential PKSRP homologs from a degenerate oligonucleotide sequence. Chemical synthesis of a degenerate gene sequence can be performed in an automatic DNA synthesizer, and the synthetic gene is then ligated into an appropriate expression vector. Use of a degenerate set of genes allows for the provision, in one mixture, of all of the sequences encoding the desired set of potential PKSRP sequences. Methods for synthesizing degenerate oligonucleotides are known in the art. See, e.g., Narang, S.A., 1983, Tetrahedron 39:3; Itakura et al., 1984, Annu. Rev. Biochem. 53:323; Itakura et al., 1984, Science 198:1056; Ike et al., 1983, Nucleic Acid Res. 11:477.

[0049] In addition, libraries of fragments of the PKSRP coding regions can be used to generate a variegated population of PKSRP fragments for screening and subsequent selection of homologs of a PKSRP. In one embodiment, a library of coding sequence fragments can be generated by treating a double stranded PCR fragment of a PKSRP coding sequence with a nuclease under conditions wherein nicking occurs only about once per molecule, denaturing the double stranded DNA, renaturing the DNA to form double stranded DNA, which can include sense/antisense pairs from different nicked products, removing single stranded portions from reformed duplexes by treatment with S1 nuclease, and ligating the resulting fragment library into an expression vector. By this method, an expression library can be derived which encodes N-terminal, C-terminal, and internal fragments of various sizes of the PKSRP.

[0050] Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation,and for screening cDNA libraries for gene products having a selected property. Such techniques are adaptable for rapid screening of the gene libraries generated by the combinatorial mutagenesis of PKSRP homologs. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. Recursive ensemble mutagenesis (REM), a new technique that enhances the frequency of functional mutants in the libraries, can be used in combination with the screening assays to identify PKSRP homologs (Arkin and Yourvan, 1992, PNAS 89:7811-7815; Delgrave et al., 1993, Polypeptide Engineering 6(3):327-331). Further, cell based assays can be exploited to analyze a variegated PKSRP library, using methods well known in the art. A method of identifying a novel PKSRP, comprising (a) raising a specific antibody response to a PKSRP, or a fragment thereof, as described herein; (b) screening putative PKSRP material with the antibody, wherein specific binding of the antibody to the material indicates the presence of a potentially novel PKSRP; and (c) analyzing the bound material in comparison to known PKSRP, to determine its novelty is further described herein.

[0051] As stated above, the present invention includes PKSRPs and homologs thereof. To determine the percent sequence identity of two amino acid sequences (e.g., one of the sequences of SEQ ID NO: 12, and a mutant form thereof), the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of one polypeptide for optimal alignment with the other polypeptide or nucleic acid). The amino acid residues at corresponding amino acid positions are then compared. When a position in one sequence (e.g., the sequence of SEQ ID NO:12, is occupied by the same amino acid residue as the corresponding position in the other sequence (e.g., a mutant form of the sequence selected from the polypeptide of SEQ ID NO:12 then the molecules are identical at that position. The same type of comparison can be made between two nucleic acid sequences.

[0052] The percent sequence identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., percent sequence identity = numbers of identical positions/total numbers of positions x 100). Preferably, the isolated amino acid homologs included in the present invention are at least 70%, and more preferably at least 70-75%, 75-80%, 80-85%, 85-90% or 90-95%, and most preferably at least 96%, 97%, 98%, 99% or more identical to an entire amino acid sequence shown in SEQ ID NO:12. In yet another embodiment, the isolated amino acid homologs included in the present invention are at least 70%, and more preferably at least 70-75%, 75-80%, 80-85%, 85-90% or 90-95%, and most preferably at least 96%, 97%, 98%, 99% or more identical to an entire amino acid sequence encoded by a nucleic acid sequence shown in SEQ ID NO: 11.

In other embodiments, the PKSRP amino acid homologs have sequence identity over at least 15 contiguous amino acid residues, more preferably at least 25 contiguous amino acid residues, and most preferably at least 35 contiguous amino acid residues of SEQ ID NO: 12.

[0053] In another preferred embodiment, an isolated nucleic acid homolog of the invention comprises a nucleotide sequence which encodes a polypeptide having at least 70% sequence identity with a polypeptide as defined in SEQ ID NO: 12 and which is at least 70%, more preferably at least 70-75%, 75-80%, 80-85%, 85-90% or 90-95%, and even

more preferably at least 95%, 96%, 97%, 98%, 99% or more identical to a nucleotide sequence shown in (SEQ ID NO:11, The preferable length of sequence comparison for nucleic acids is at least 75 nucleotides, more preferably at least 100 nucleotides and most preferably the entire length of the coding region.

[0054] It is further preferred that the isolated nucleic acid homolog of the invention encodes a PKSRP, or portion thereof, that is at least 85% identical to an amino acid sequence of SEQ ID NO: 12, and that functions as a modulator of an environmental stress response in a plant In a more preferred embodiment, overexpression of the nucleic acid homolog in a plant increases the tolerance of the plant to an environmental stress. In a further preferred embodiment, the nucleic acid homolog encodes a PKSRP that functions as a protein kinase.

[0055] For the purposes of the invention, the percent sequence identity between two nucleic acid or polypeptide sequences may be determined using the Vector NTI 6.0 (PC) software package (InforMax, 7600 Wisconsin Ave., Bethesda, MD 20814). A gap opening penalty of 15 and a gap extension penalty of 6.66 are used for determining the percent identity of two nucleic acids. A gap opening penalty of 10 and a gap extension penalty of 0.1 are used for determining the percent identity of two polypeptides. All other parameters are set at the default settings. For purposes of a multiple alignment (Clustal W algorithm), the gap opening penalty is 10, and the gap extension penalty is 0.05 with blosum62 matrix. It is to be understood that for the purposes of determining sequence identity when comparing a DNA sequence to an RNA sequence, a thymidine nucleotide is equivalent to a uracil nucleotide.

[0056] In another aspect, the invention provides an isolated nucleic acid comprising a polynucleotide that encodes a polypeptide having at least 70% sequence identity with a polypeptide as defined in SEQ ID NO: 12 and that hybridizes to the polynucleotide of SEQ ID NO:11, under stringent conditions. More particularly, an isolated nucleic acid molecule of the invention is at least 15 nucleotides in length and hybridizes under stringent conditions to the nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO:11

In other embodiments, the nucleic acid is at least 30, 50, 100, 250 or more nucleotides in length. Preferably an isolated nucleic acid homolog of the invention comprises a nucleotide sequence which hybridizes under highly stringent conditions to the nucleotide sequence shown in SEQ ID NO:11, and functions as a modulator of stress tolerance in a plant In a further preferred embodiment, overexpression of the isolated nucleic acid homolog in a plant increases a plant's tolerance to an environmental stress. In an even further preferred embodiment, the isolated nucleic acid homolog encodes a PKSRP that functions as a protein kinase.

[0057] As used herein with regard to hybridization for DNA to DNA blot, the term "stringent conditions" refers to hybridization overnight at 60˚C in 10X Denharts solution, 6X SSC, 0.5% SDS and 100 μg/ml denatured salmon sperm DNA. Blots are washed sequentially at 62˚C for 30 minutes each time in 3X SSC/0.1% SDS, followed by 1X SSC/0.1% SDS and finally 0.1X SSC/0.1% SDS. As also used herein, "highly stringent conditions" refers to hybridization overnight at 65˚C in 10X Denharts solution, 6X SSC, 0.5% SDS and 100 μg/ml denatured salmon sperm DNA. Blots are washed sequentially at 65 ˚C for 30 minutes each time in 3X SSC/0.1% SDS, followed by 1X SSC/0.1% SDS and finally 0.1X SSC/0.1% SDS. Methods for nucleic acid hybridizations are described in Meinkoth and Wahl, 1984, Anal. Biochem. 138:267-284; Ausubel et al. eds, 1995, Current Protocols in Molecular Biology, Chapter 2, Greene Publishing and Wiley-Interscience, New York; and Tijssen, 1993, Laboratory Techniques in Biochemistry and Molecular Biology: Hybridization with Nucleic Acid Probes, Part I, Chapter 2, Elsevier, New York. Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent or highly stringent conditions to a sequence of SEQ ID NO:11 corresponds to a naturally occurring nucleic acid molecule. As used herein, a "naturally occurring" nucleic acid molecule refers to an RNA or DNA molecule having a nucleotide sequence that occurs in nature (e.g., encodes a natural polypeptide). In one embodiment, the nucleic acid encodes a naturally occurring *Physcomitrella patens* PKSRP.

[0058] Using the above-described methods, and others known to those of skill in the art, one of ordinary skill in the art can isolate homologs of the PKSRPs comprising amino acid sequences shown in SEQ ID NO:12.

One subset of these homologs are allelic variants. As used herein, the term "allelic variant" refers to a nucleotide sequence containing polymorphisms that lead to changes in the amino acid sequences of a PKSRP and that exist within a natural population (e.g., a plant species or variety). Such natural allelic variations can typically result in 1-5% variance in a PKSRP nucleic acid. Allelic variants can be identified by sequencing the nucleic acid sequence of interest in a number of different plants, which can be readily carried out by using hybridization probes to identify the same PKSRP genetic locus in those plants. Any and all such nucleic acid variations and resulting amino acid polymorphisms or variations in a PKSRP that are the result of natural allelic variation and that do not alter the functional activity of a PKSRP, are intended to be within the scope of the invention.

[0059] Moreover, nucleic acid molecules encoding PKSRPs from the same or other species such as PKSRP analogs, orthologs, and paralogs, are intended to be within the scope of the present invention. As used herein, the term "analogs" refers to two nucleic acids that have the same or similar function, but that have evolved separately in unrelated organisms. As used herein, the term "orthologs" refers to two nucleic acids from different species, but that have evolved from a common ancestral gene by speciation. Normally, orthologs encode polypeptides having the same or similar functions. As also used herein, the term "paralogs" refers to two nucleic acids that are related by duplication within a genome. Paralogs usually have different functions, but these functions may be related (Tatusov, R.L. et al., 1997, Science 278

(5338):631-637). Analogs, orthologs and paralogs of a naturally occurring PKSRP can differ from the naturally occurring PKSRP by post-translational modifications, by amino acid sequence differences, or by both. Post-translational modifications include *in vivo* and *in vitro* chemical derivatization of polypeptides, e.g., acetylation, carboxylation, phosphorylation, or glycosylation, and such modifications may occur during polypeptide synthesis or processing or following treatment with isolated modifying enzymes. In particular, orthologs of the invention will generally exhibit at least 80-85%, more preferably, 85-90% or 90-95%, and most preferably 95%, 96%, 97%, 98% or even 99% identity or sequence identity with all or part of a naturally occurring PKSRP amino acid sequence and will exhibit a function similar to a PKSRP. Preferably, a PKSRP ortholog of the present invention functions as a modulator of an environmental stress response in a plant and/or functions as a protein kinase. More preferably, a PKSRP ortholog increases the stress tolerance of a plant. In one embodiment, the PKSRP orthologs maintain the ability to participate in the metabolism of compounds necessary for the construction of cellular membranes in a plant, or in the transport of molecules across these membranes.

[0060] In addition to naturally-occurring variants of a PKSRP sequence that may exist in the population, the skilled artisan will further appreciate that changes can be introduced by mutation into a nucleotide sequence of SEQ ID NO: 11, thereby leading to changes in the amino acid sequence of the encoded PKSRP, without altering the functional activity of the PKSRP. For example, nucleotide substitutions leading to amino acid substitutions at "non-essential" amino acid residues can be made in a sequence of SEQ ID NO:11.

A "non-essential" amino acid residue is a residue that can be altered from the wild-type sequence of one of the PKSRPs without altering the activity of said PKSRP, whereas an "essential" amino acid residue is required for PKSRP activity. Other amino acid residues, however, (e.g., those that are not conserved or only semi-conserved in the domain having PKSRP activity) may not be essential for activity and thus are likely to be amenable to alteration without altering PKSRP activity.

[0061] Accordingly, another aspect of the invention pertains to nucleic acid molecules encoding PKSRPs that contain changes in amino acid residues that are not essential for PKSRP activity. Such PKSRPs differ in amino acid sequence from a sequence contained in SEQ ID NO:12, yet retain at least one of the PKSRP activities described herein. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence encoding a polypeptide, wherein the polypeptide comprises an amino acid sequence at least about 70% identical to an amino acid sequence of SEQ ID NO:12 Preferably, the polypeptide encoded by the nucleic acid molecule is at least about, 70-75%, 75-80%, 80-85%, 85-90%, 90-95% identical to the sequence of SEQ ID NO:12 and more preferably at least about 96%, 97%, 98%, or 99% identical to the sequence of SEQ ID NO:12. The preferred PKSRP homologs of the present invention participate in the a stress tolerance response in a plant, or more particularly, participate in the transcription of a polypeptide involved in a stress tolerance response in a plant, and/or function as a protein kinase.

[0062] An isolated nucleic acid molecule encoding a PKSRP having sequence identity with a polypeptide sequence of ID NO:12, can be created by introducing one or more nucleotide substitutions, additions or deletions into a nucleotide sequence of SEQ ID NO:11, respectively, such that one or more amino acid substitutions, additions, or deletions are introduced into the encoded polypeptide. Mutations can be introduced into the sequence of SEQ ID NO:11, by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more predicted non-essential amino acid residues. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain.

[0063] Families of amino acid residues having similar side chains have been defined in the art. These families include amino acids with basic side chains (e.g., lysine, arginine, histidine), acidic side chains (e.g., aspartic acid, glutamic acid), uncharged polar side chains (e.g., glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (e.g., threonine, valine, isoleucine) and aromatic side chains (e.g., tyrosine, phenylalanine, tryptophan, histidine). Thus, as predicted nonessential amino acid residue in a PKSRP is preferably replaced with another amino acid residue from the same side chain family. Alternatively, in another embodiment, mutations can be introduced randomly along all or part of a PKSRP coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for a PKSRP activity described herein to identify mutants that retain PKSRP activity. Following mutagenesis of the sequence of SEQ ID NO: 11, the encoded polypeptide can be expressed recombinantly and the activity of the polypeptide can be determined by analyzing the stress tolerance of a plant expressing the polypeptide as described in Example 7.

[0064] Additionally, optimized PKSRP nucleic acids can be created. Preferably, an optimized PKSRP nucleic acid encodes a PKSRP that functions as a protein kinase and/or modulates a plant's tolerance to an environmental stress, and more preferably increases a plant's tolerance to an environmental stress upon its overexpression in the plant. As used herein, "optimized" refers to a nucleic acid that is genetically engineered to increase its expression in a given plant or animal. To provide plant optimized PKSRP nucleic acids, the DNA sequence of the gene can be modified to 1) comprise codons preferred by highly expressed plant genes; 2) comprise an A+T content in nucleotide base composition to that substantially found in plants; 3) form a plant initiation sequence; or 4) eliminate sequences that cause destabilization, inappropriate polyadenylation, degradation, and termination of RNA, or that form secondary structure hairpins or RNA splice sites. Increased expression of PKSRP nucleic acids in plants can be achieved by utilizing the distribution

frequency of codon usage in plants in general or a particular plant. Methods for optimizing nucleic acid expression in plants can be found in EPA 0359472; EPA 0385962; PCT Application No. WO 91/16432; U.S. Patent No. 5,380,831; U.S. Patent No. 5,436,391; Perlack et al., 1991, Proc. Natl. Acad. Sci. USA 88:3324-3328; and Murray et al., 1989, Nucleic Acids Res. 17:477-498.

[0065] As used herein, "frequency of preferred codon usage" refers to the preference exhibited by a specific host cell in usage of nucleotide codons to specify a given amino acid. To determine the frequency of usage of a particular codon in a gene, the number of occurrences of that codon in the gene is divided by the total number of occurrences of all codons specifying the same amino acid in the gene. Similarly, the frequency of preferred codon usage exhibited by a host cell can be calculated by averaging frequency of preferred codon usage in a large number of genes expressed by the host cell. It is preferable that this analysis be limited to genes that are highly expressed by the host cell. The percent deviation of the frequency of preferred codon usage for a synthetic gene from that employed by a host cell is calculated first by determining the percent deviation of the frequency of usage of a single codon from that of the host cell followed by obtaining the average deviation over all codons. As defined herein, this calculation includes unique codons (i.e., ATG and TGG). In general terms, the overall average deviation of the codon usage of an optimized gene from that of a host cell is calculated using the equation $1A = n = 1\ Z\ X_n - Y_n\ X_n$ times 100 Z where X_n = frequency of usage for codon n in the host cell; Y_n = frequency of usage for codon n in the synthetic gene; n represents an individual codon that specifies an amino acid; and the total number of codons is Z. The overall deviation of the frequency of codon usage, A, for all amino acids should preferably be less than about 25%, and more preferably less than about 10%.

[0066] Hence, a PKSRP nucleic acid can be optimized such that its distribution frequency of codon usage deviates, preferably, no more than 25% from that of highly expressed plant genes and, more preferably, no more than about 10%. In addition, consideration is given to the percentage G+C content of the degenerate third base (monocotyledons appear to favor G+C in this position, whereas dicotyledons do not). It is also recognized that the XCG (where X is A, T, C, or G) nucleotide is the least preferred codon in dicots whereas the XTA codon is avoided in both monocots and dicots. Optimized PKSRP nucleic acids of this invention also preferably have CG and TA doublet avoidance indices closely approximating those of the chosen host plant (i.e., *Physcomitrella patens*). More preferably these indices deviate from that of the host by no more than about 10-15%.

[0067] In addition to the nucleic acid molecules encoding the PKSRPs described above, another aspect of the invention pertains to isolated nucleic acid molecules that are antisense thereto. Antisense polynucleotides are thought to inhibit gene expression of a target polynucleotide by specifically binding the target polynucleotide and interfering with transcription, splicing, transport, translation, and/or stability of the target polynucleotide. Methods are described in the prior art for targeting the antisense polynucleotide to the chromosomal DNA, to a primary RNA transcript, or to a processed mRNA. Preferably, the target regions include splice sites, translation initiation codons, translation termination codons, and other sequences within the open reading frame.

[0068] The term "antisense," for the purposes of the invention, refers to a nucleic acid comprising a polynucleotide that is sufficiently complementary to all or a portion of a gene, primary transcript, or processed mRNA, so as to interfere with expression of the endogenous gene. "Complementary" polynucleotides are those that are capable of base pairing according to the standard Watson-Crick complementarity rules. Specifically, purines will base pair with pyrimidines to form a combination of guanine paired with cytosine (G:C) and adenine paired with either thymine (A:T) in the case of DNA, or adenine paired with uracil (A:U) in the case of RNA. It is understood that two polynucleotides may hybridize to each other even if they are not completely complementary to each other, provided that each has at least one region that is substantially complementary to the other. The term "antisense nucleic acid" includes single stranded RNA as well as double-stranded DNA expression cassettes that can be transcribed to produce an antisense RNA. "Active" antisense nucleic acids are antisense RNA molecules that are capable of selectively hybridizing with a primary transcript or mRNA encoding a polypeptide having at least 80% sequence identity with the polypeptide of SEQ ID NO:12,

[0069] The antisense nucleic acid can be complementary to an entire PKSRP coding strand, or to only a portion thereof. In one embodiment, an antisense nucleic acid molecule is antisense to a "coding region" of the coding strand of a nucleotide sequence encoding a PKSRP. The term "coding region" refers to the region of the nucleotide sequence comprising codons that are translated into amino acid residues

In another embodiment, the antisense nucleic acid molecule is antisense to a "noncoding region" of the coding strand of a nucleotide sequence encoding a PKSRP. The term "noncoding region" refers to 5' and 3' sequences that flank the coding region that are not translated into amino acids (i.e., also referred to as 5' and 3' untranslated regions). The antisense nucleic acid molecule can be complementary to the entire coding region of PKSRP mRNA, but more preferably is an oligonucleotide which is antisense to only a portion of the coding or noncoding region of PKSRP mRNA. For example, the antisense oligonucleotide can be complementary to the region surrounding the translation start site of PKSRP mRNA. An antisense oligonucleotide can be, for example, about 5, 10, 15, 20, 25, 30, 35, 40, 45 or 50 nucleotides in length. Typically, the antisense molecules of the present invention comprise an RNA having 60-100% sequence identity with at least 14 consecutive nucleotides of SEQ ID NO:11 or a polynucleotide encoding SEQ ID NO:12, Preferably, the sequence identity will be at least 70%, more preferably at least 75%, 80%, 85%, 90%, 95%, 98% and most preferably

99%.

[0070] An antisense nucleic acid of the invention can be constructed using chemical synthesis and enzymatic ligation reactions using procedures known in the art. For example, an antisense nucleic acid (e.g., an antisense oligonucleotide) can be chemically synthesized using naturally occurring nucleotides or variously modified nucleotides designed to increase the biological stability of the molecules or to increase the physical stability of the duplex formed between the antisense and sense nucleic acids, e.g., phosphorothioate derivatives and acridine substituted nucleotides can be used. Examples of modified nucleotides which can be used to generate the antisense nucleic acid include 5-fluorouracil, 5-bromouracil, 5-chlorouracil, 5-iodouracil, hypoxanthine, xanthine, 4-acetylcytosine, 5-(carboxyhydroxylmethyl) uracil, 5-carboxymethylaminomethyl-2-thiouridine, 5-carboxymethylaminomethyluracil, dihydrouracil, beta-D-galactosylqueosine, inosine, N6-isopentenyladenine, 1-methylguanine, 1-methylinosine, 2,2-dimethylguanine, 2-methyladenine, 2-methylguanine, 3-methylcytosine, 5-methylcytosine, N6-adenine, 7-methylguanine, 5-methylaminomethyluracil, 5-methoxyaminomethyl-2-thiouracil, beta-D-mannosylqueosine, 5'-methoxycarboxymethyluracil, 5-methoxyuracil, 2-methylthio-N6-isopentenyladenine, uracil-5-oxyacetic acid (v), wybutoxosine, pseudouracil, queosine, 2-thiocytosine, 5-methyl-2-thiouracil, 2-thiouracil, 4-thiouracil, 5-methyluracil, uracil-5-oxyacetic acid methylester, uracil-5-oxyacetic acid (v), 5-methyl-2-thiouracil, 3-(3-amino-3-N-2-carboxypropyl) uracil, (acp3)w, and 2,6-diaminopurine. Alternatively, the antisense nucleic acid can be produced biologically using an expression vector into which a nucleic acid has been subcloned in an antisense orientation (i.e., RNA transcribed from the inserted nucleic acid will be of an antisense orientation to a target nucleic acid of interest, described further in the following subsection).

[0071] In yet another embodiment, the antisense nucleic acid molecule of the invention is an α-anomeric nucleic acid molecule. An α-anomeric nucleic acid molecule forms specific double-stranded hybrids with complementary RNA in which, contrary to the usual β-units, the strands run parallel to each other (Gaultier et al., 1987, Nucleic Acids. Res. 15: 6625-6641). The antisense nucleic acid molecule can also comprise a 2'-o-methylribonucleotide (Inoue et al., 1987, Nucleic Acids Res. 15:6131-6148) or a chimeric RNA-DNA analogue (Inoue et al., 1987, FEBS Lett. 215:327-330).

[0072] The antisense nucleic acid molecules of the invention are typically administered to a cell or generated *in situ* such that they hybridize with or bind to cellular mRNA and/or genomic DNA encoding a PKSRP to thereby inhibit expression of the polypeptide, e.g., by inhibiting transcription and/or translation. The hybridization can be by conventional nucleotide complementarity to form a stable duplex, or, for example, in the case of an antisense nucleic acid molecule which binds to DNA duplexes, through specific interactions in the major groove of the double helix. The antisense molecule can be modified such that it specifically binds to a receptor or an antigen expressed on a selected cell surface, e.g., by linking the antisense nucleic acid molecule to a peptide or an antibody which binds to a cell surface receptor or antigen. The antisense nucleic acid molecule can also be delivered to cells using the vectors described herein. To achieve sufficient intracellular concentrations of the antisense molecules, vector constructs in which the antisense nucleic acid molecule is placed under the control of a strong prokaryotic, viral, or eukaryotic (including plant) promoter are preferred.

[0073] As an alternative to antisense polynucleotides, ribozymes, sense polynucleotides, or double stranded RNA (dsRNA) can be used to reduce expression of a PKSRP polypeptide. By "ribozyme" is meant a catalytic RNA-based enzyme with ribonuclease activity which is capable of cleaving a single-stranded nucleic acid, such as an mRNA, to which it has a complementary region. Ribozymes (e.g., hammerhead ribozymes described in Haselhoff and Gerlach, 1988, Nature 334:585-591) can be used to catalytically cleave PKSRP mRNA transcripts to thereby inhibit translation of PKSRP mRNA. A ribozyme having specificity for a PKSRP-encoding nucleic acid can be designed based upon the nucleotide sequence of a PKSRP cDNA, as disclosed herein (i.e. SEQ ID NO:11, or on the basis of a heterologous sequence to be isolated according to methods taught in this invention. For example, a derivative of a Tetrahymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in a PKSRP-encoding mRNA. See, e.g., U.S. Patent Nos. 4,987,071 and 5,116,742 to Cech et al. Alternatively, PKSRP mRNA can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules. See, e.g., Bartel, D. and Szostak, J.W., 1993, Science 261:1411-1418. In preferred embodiments, the ribozyme will contain a portion having at least 7, 8, 9, 10, 12, 14, 16, 18 or 20 nucleotides, and more preferably 7 or 8 nucleotides, that have 100% complementarity to a portion of the target RNA. Methods for making ribozymes are known to those skilled in the art. See, e.g., U.S. Patent Nos. 6,025,167; 5,773,260; and 5,496,698.

[0074] The term "dsRNA," as used herein, refers to RNA hybrids comprising two strands of RNA. The dsRNAs can be linear or circular in structure. In a preferred embodiment, dsRNA is specific for a polynucleotide encoding either the polypeptide of SEQ ID NO:12, or a polypeptide having at least 70% sequence identity with SEQ ID NO:12

The hybridizing RNAs may be substantially or completely complementary. By "substantially complementary," is meant that when the two hybridizing RNAs are optimally aligned using the BLAST program as described above, the hybridizing portions are at least 95% complementary. Preferably, the dsRNA will be at least 100 base pairs in length. Typically, the hybridizing RNAs will be of identical length with no over hanging 5' or 3' ends and no gaps. However, dsRNAs having 5' or 3' overhangs of up to 100 nucleotides may be used in the methods of the invention.

[0075] The dsRNA may comprise ribonucleotides or ribonucleotide analogs, such as 2'-O-methyl ribosyl residues, or

combinations thereof. See, e.g., U.S. Patent Nos. 4,130,641 and 4,024,222. A dsRNA polyriboinosinic acid:polyribocytidylic acid is described in U.S. patent 4,283,393. Methods for making and using dsRNA are known in the art. One method comprises the simultaneous transcription of two complementary DNA stands, either in vivo, or in a single *in vitro* reaction mixture. See, e.g., U.S. Patent No. 5,795,715. In one embodiment, dsRNA can be introduced into a plant or plant cell directly by standard transformation procedures. Alternatively, dsRNA can be expressed in a plant cell by transcribing two complementary RNAs.

[0076] Other methods for the inhibition of endogenous gene expression, such as triple helix formation (Moser et al., 1987, Science 238:645-650 and Cooney et al., 1988, Science 241:456-459) and cosuppression (Napoli et al., 1990, The Plant Cell 2:279-289) are known in the art. Partial and full-length cDNAs have been used for the cosuppression of endogenous plant genes. See, e.g., U.S. Patent Nos. 4,801,340, 5,034,323, 5,231,020, and 5,283,184; Van der Kroll et al., 1990, The Plant Cell 2:291-299; Smith et al., 1990, Mol. Gen. Genetics 224:477-481 and Napoli et al., 1990, The Plant Cell 2:279-289.

[0077] For sense suppression, it is believed that introduction of a sense polynucleotide blocks transcription of the corresponding target gene. The sense polynucleotide will have at least 65% sequence identity with the target plant gene or RNA. Preferably, the percent identity is at least 80%, 90%, 95% or more. The introduced sense polynucleotide need not be full length relative to the target gene or transcript Preferably, the sense polynucleotide will have at least 65% sequence identity with at least 100 consecutive nucleotides of SEQ ID NO: 11

The regions of identity can comprise introns and and/or exons and untranslated regions. The introduced sense polynucleotide may be present in the plant cell transiently, or may be stably integrated into a plant chromosome or extrachromosomal replicon.

[0078] Alternatively, PKSRP gene expression can be inhibited by targeting nucleotide sequences complementary to the regulatory region of a PKSRP nucleotide sequence (e.g., a PKSRP promoter and/or enhancer) to form triple helical structures that prevent transcription of a PKSRP gene in target cells. See generally, Helene, C., 1991, Anticancer Drug Des. 6(6):569-84; Helene, C. et al., 1992, Ann. N.Y. Acad. Sci. 660:27-36; and Maher, L.J., 1992, Bioassays 14(12): 807-15.

[0079] In addition to the PKSRP nucleic acids and polypeptides described above, the present invention encompasses these nucleic acids and polypeptides attached to a moiety. These moieties include, but are not limited to, detection moieties, hybridization moieties, purification moieties, delivery moieties, reaction moieties; binding moieties, and the like. A typical group of nucleic acids having moieties attached are probes and primers. Probes and primers typically comprise a substantially isolated oligonucleotide. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, preferably about 25, more preferably about 40, 50 or 75 consecutive nucleotides of a sense strand of the sequence set forth in SEQ ID NO:11, an anti-sense sequence of the sequence set forth in SEQ ID NO:11, or naturally occurring mutants thereof. Primers based on a nucleotide sequence of SEQ ID NO:11, can be used in PCR reactions to clone PKSRP homologs. Probes based on the PKSRP nucleotide sequences can be used to detect transcripts or genomic sequences encoding the same or substantially identical polypeptides. In preferred embodiments, the probe further comprises a label group attached thereto, e.g. the label group can be a radioisotope, a fluorescent compound, an enzyme, or an enzyme co-factor. Such probes can be used as a part of a genomic marker test kit for identifying cells which express a PKSRP, such as by measuring a level of a PKSRP-encoding nucleic acid, in a sample of cells, e.g., detecting PKSRP mRNA levels or determining whether a genomic PKSRP gene has been mutated or deleted.

[0080] In particular, a useful method to ascertain the level of transcription of the gene (an indicator of the amount of mRNA available for translation to the gene product) is to perform a Northern blot. For reference, see, for example, Ausubel et al., 1988, Current Protocols in Molecular Biology, Wiley: New York. The information from a Northern blot at least partially demonstrates the degree of transcription of the transformed gene. Total cellular RNA can be prepared from cells, tissues or organs by several methods, all well-known in the art, such as that described in Bormann, E.R. et al., 1992, Mol. Microbiol. 6:317-326. To assess the presence or relative quantity of polypeptide translated from this mRNA, standard techniques, such as a Western blot, may be employed. These techniques are well known to one of ordinary skill in the art. See, for example, Ausubel et al., 1988, Current Protocols in Molecular Biology, Wiley: New York.

[0081] The invention further provides an isolated recombinant expression vector comprising a PKSRP nucleic acid as described above, wherein expression of the vector in a host cell results in increased tolerance to environmental stress as compared to a wild type variety of the host cell. As used herein, the term "vector" refers to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid," which refers to a circular double stranded DNA loop into which additional DNA segments can be ligated. Another type of vector is a viral vector, wherein additional DNA segments can be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) are integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such

vectors are referred to herein as "expression vectors." In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses, and adeno-associated viruses), which serve equivalent functions.

[0082] The recombinant expression vectors of the invention comprise a nucleic acid of the invention in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression, which is operatively linked to the nucleic acid sequence to be expressed. As used herein with respect to a recombinant expression vector, "operatively linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner which allows for expression of the nucleotide sequence (e.g., in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers, and other expression control elements (e.g., polyadenylation signals). Such regulatory sequences are described, for example, in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990) and Gruber and Crosby, in: Methods in Plant Molecular Biology and Biotechnology, eds. Glick and Thompson, Chapter 7, 89-108, CRC Press: Boca Raton, Florida, including the references therein. Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cells and those that direct expression of the nucleotide sequence only in certain host cells or under certain conditions. It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to be transformed, the level of expression of polypeptide desired, etc. The expression vectors of the invention can be introduced into host cells to thereby produce polypeptides or peptides, including fusion polypeptides or peptides, encoded by nucleic acids as described herein (e.g., PKSRPs, mutant forms of PKSRPs, fusion polypeptides, etc.).

[0083] The recombinant expression vectors of the invention can be designed for expression of PKSRPs in prokaryotic or eukaryotic cells. For example, PKSRP genes can be expressed in bacterial cells such as *C. glutamicum,* insect cells (using baculovirus expression vectors), yeast and other fungal cells (See Romanos, M.A. et al., 1992, Foreign gene expression in yeast: a review, Yeast 8:423-488; van den Hondel, C.A.M.J.J. et al., 1991, Heterologous gene expression in filamentous fungi, in: More Gene Manipulations in Fungi, J.W. Bennet & L.L. Lasure, eds., p. 396-428: Academic Press: San Diego; and van den Hondel, C.A.M.J.J. & Punt, P.J., 1991, Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of Fungi, Peberdy, J.F. et al., eds., p. 1-28, Cambridge University Press: Cambridge), algae (Falciatore et al., 1999, Marine Biotechnology 1(3):239-251), ciliates of the types: Holotrichia, Peritrichia, Spirotrichia, Suctoria, Tetrahymena, Paramecium, Colpidium, Glaucoma, Platyophrya, Potomacus, Pseudocohnilembus, Euplotes, Engelmaniella, and Stylonychia, especially of the genus Stylonychia lemnae with vectors following a transformation method as described in PCT Application No. WO 98/01572, and multicellular plant cells (See Schmidt, R. and Willmitzer, L., 1988, High efficiency Agrobacterium tumefaciens-mediated transformation of Arabidopsis thaliana leaf and cotyledon explants, Plant Cell Rep. 583-586; Plant Molecular Biology and Biotechnology, C Press, Boca Raton, Florida, chapter 6/7, S.71-119 (1993); F.F. White, B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, eds. Kung und R. Wu, 128-43, Academic Press: 1993; Potrykus, 1991, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42:205-225 and references cited therein) or mammalian cells. Suitable host cells are discussed further in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press: San Diego, CA (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro,* for example using T7 promoter regulatory sequences and T7 polymerase.

[0084] Expression of polypeptides in prokaryotes is most often carried out with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion polypeptides. Fusion vectors add a number of amino acids to a polypeptide encoded therein, usually to the amino terminus of the recombinant polypeptide but also to the C-terminus or fused within suitable regions in the polypeptides. Such fusion vectors typically serve three purposes: 1) to increase expression of a recombinant polypeptide; 2) to increase the solubility of a recombinant polypeptide; and 3) to aid in the purification of a recombinant polypeptide by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction of the fusion moiety and the recombinant polypeptide to enable separation of the recombinant polypeptide from the fusion moiety subsequent to purification of the fusion polypeptide. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin, and enterokinase.

[0085] Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith, D.B. and Johnson, K.S., 1988, Gene 67:31-40), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding polypeptide, or polypeptide A, respectively, to the target recombinant polypeptide. In one embodiment, the coding sequence of the PKSRP is cloned into a pGEX expression vector to create a vector encoding a fusion polypeptide comprising, from the N-terminus to the C-terminus, GST-thrombin cleavage site-X polypeptide. The fusion polypeptide can be purified by affinity chromatography using glutathione-agarose resin. Recombinant PKSRP unfused to GST can be recovered by cleavage of the fusion polypeptide with thrombin.

[0086] Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amann et al., 1988, Gene 69:301-315) and pET 11d (Studier et al., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 60-89). Target gene expression from the pTrc vector relies on host RNA polymerase transcription from a hybrid trp-lac fusion promoter. Target gene expression from the pET 11d vector relies on transcription from a T7 gn10-lac fusion promoter mediated by a co-expressed viral RNA polymerase (T7 gnl). This viral polymerase is supplied by host strains BL21(DE3) or HMS174(DE3) from a resident λ prophage harboring a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

[0087] One strategy to maximize recombinant polypeptide expression is to express the polypeptide in a host bacteria with an impaired capacity to proteolytically cleave the recombinant polypeptide (Gottesman, S., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 119-128). Another strategy is to alter the sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in the bacterium chosen for expression, such as *C. glutamicum* (Wada et al., 1992, Nucleic Acids Res. 20:2111-2118). Such alteration of nucleic acid sequences of the invention can be carried out by standard DNA synthesis techniques.

[0088] In another embodiment, the PKSRP expression vector is a yeast expression vector. Examples of vectors for expression in yeast *S. cerevisiae* include pYepSec1 (Baldari, et al., 1987, EMBO J. 6:229-234), pMFa (Kurjan and Herskowitz, 1982, Cell 30:933-943), pJRY88 (Schultz et al., 1987, Gene 54:113-123), and pYES2 (Invitrogen Corporation, San Diego, CA). Vectors and methods for the construction of vectors appropriate for use in other fungi, such as the filamentous fungi, include those detailed in: van den Hondel, C.A.M.J.J. & Punt, P.J., 1991, "Gene transfer systems and vector development for filamentous fungi," in: Applied Molecular Genetics of Fungi, J.F. Peberdy, et al., eds., p. 1-28, Cambridge University Press: Cambridge.

[0089] Alternatively, the PKSRPs of the invention can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of polypeptides in cultured insect cells (e.g., Sf 9 cells) include the pAc series (Smith et al., 1983, Mol. Cell Biol. 3:2156-2165) and the pVL series (Lucklow and Summers, 1989, Virology 170:31-39).

[0090] In yet another embodiment, a PKSRP nucleic acid of the invention is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include pCDM8 (Seed, B., 1987, Nature 329:840) and pMT2PC (Kaufman et al., 1987, EMBO J. 6:187-195). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, Adenovirus 2, cytomegalovirus, and Simian Virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells, see chapters 16 and 17 of Sambrook, J., Fritsh, E. F., and Maniatis, T. Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

[0091] In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid preferentially in a particular cell type (e.g., tissue-specific regulatory elements are used to express the nucleic acid). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert et al., 1987, Genes Dev. 1:268-277), lymphoid-specific promoters (Calame and Eaton, 1988, Adv. Immunol. 43:235-275), in particular promoters of T cell receptors (Winoto and Baltimore, 1989, EMBO J. 8:729-733) and immunoglobulins (Banerji et al., 1983, Cell 33:729-740; Queen and Baltimore, 1983, Cell 33:741-748), neuron-specific promoters (e.g., the neurofilament promoter; Byrne and Ruddle, 1989, PNAS 86:5473-5477), pancreas-specific promoters (Edlund et al., 1985, Science 230:912-916), and mammary gland-specific promoters (e.g., milk whey promoter; U.S. Patent No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, for example, the murine hox promoters (Kessel and Gruss, 1990, Science 249:374-379) and the fetopolypeptide promoter (Campes and Tilghman, 1989, Genes Dev. 3:537-546).

[0092] For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these integrants, a gene that encodes a selectable marker (e.g., resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Preferred selectable markers include those which confer resistance to drugs, such as G418, hygromycin, and methotrexate, or in plants that confer resistance towards a herbicide such as glyphosate or glufosinate. Nucleic acid molecules encoding a selectable marker can be introduced into a host cell on the same vector as that encoding a PKSRP or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid molecule can be identified by, for example, drug selection (e.g., cells that have incorporated the selectable marker gene will survive, while the other cells die).

[0093] In a preferred embodiment of the present invention, the PKSRPs are expressed in plants and plants cells such as unicellular plant cells (e.g. algae) (See Falciatore et al., 1999, Marine Biotechnology 1(3):239-251 and references therein) and plant cells from higher plants (e.g., the spermatophytes, such as crop plants). A PKSRP may be "introduced" into a plant cell by any means, including transfection, transformation or transduction, electroporation, particle bombard-

ment, agroinfection, and the like. One transformation method known to those of skill in the art is the dipping of a flowering plant into an *Agrobacteria* solution, wherein the *Agrobacteria* contains the PKSRP nucleic acid, followed by breeding of the transformed gametes.

[0094] Other suitable methods for transforming or transfecting host cells including plant cells can be found in Sambrook, et al., Molecular Cloning: A Laboratory Manual. 2nd, ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, and other laboratory manuals such as Methods in Molecular Biology, 1995, Vol. 44, Agrobacterium protocols, ed: Gartland and Davey, Humana Press, Totowa, New Jersey. As biotic and abiotic stress tolerance is a general trait wished to be inherited into a wide variety of plants like maize, wheat, rye, oat, triticale, rice, barley, soybean, peanut, cotton, rapeseed and canola, manihot, pepper, sunflower and tagetes, solanaceous plants like potato, tobacco, eggplant, and tomato, Vicia species, pea, alfalfa, bushy plants (coffee, cacao, tea), Salix species, trees (oil palm, coconut), perennial grasses, and forage crops, these crop plants are also preferred target plants for a genetic engineering as one further embodiment of the present invention. Forage crops include, but are not limited to, Wheatgrass, Canarygrass, Bromegrass, Wildrye Grass, Bluegrass, Orchardgrass, Alfalfa, Salfoin, Birdsfoot Trefoil, Alsike Clover, Red Clover, and Sweet Clover.

[0095] In one embodiment of the present invention, transfection of a PKSRP into a plant is achieved by *Agrobacterium* mediated gene transfer. *Agrobacterium* mediated plant transformation can be performed using for example the GV3101 (pMP90) (Koncz and Schell, 1986, Mol. Gen. Genet. 204:383-396) or LBA4404 (Clontech) *Agrobacterium tumefaciens* strain. Transformation can be performed by standard transformation and regeneration techniques (Deblaere et al., 1994, Nucl. Acids Res. 13:4777-4788; Gelvin, Stanton B. and Schilperoort, Robert A, Plant Molecular Biology Manual, 2nd Ed. - Dordrecht : Kluwer Academic Publ., 1995. - in Sect., Ringbuc Zentrale Signatur: BT11-P ISBN 0-7923-2731-4; Glick, Bernard R.; Thompson, John E., Methods in Plant Molecular Biology and Biotechnology, Boca Raton : CRC Press, 1993 360 S., ISBN 0-8493-5164-2). For example, rapeseed can be transformed via cotyledon or hypocotyl transformation (Moloney et al., 1989, Plant cell Report 8:238-242; De Block et al., 1989, Plant Physiol. 91:694-701). Use of antibiotics for *Agrobacterium* and plant selection depends on the binary vector and the *Agrobacterium* strain used for transformation. Rapeseed selection is normally performed using kanamycin as selectable plant marker. *,Agrobacterium* mediated gene transfer to flax can be performed using, for example, a technique described by Mlynarova et al., 1994, Plant Cell Report 13:282-285. Additionally, transformation of soybean can be performed using for example a technique described in European Patent No. 0424 047, U.S. Patent No. 5,322,783, European Patent No. 0397 687, U.S. Patent No. 5,376,543, or U.S. Patent No. 5,169,770. Transformation of maize can be achieved by particle bombardment, polyethylene glycol mediated DNA uptake or via the silicon carbide fiber technique. (See, for example, Freeling and Walbot "The maize handbook" Springer Verlag: New York (1993) ISBN 3-540-97826-7). A specific example of maize transformation is found in U.S. Patent No. 5,990,387, and a specific example of wheat transformation can be found in PCT Application No. WO 93/07256.

[0096] According to the present invention, the introduced PKSRP may be maintained in the plant cell stably if it is incorporated into a non-chromosomal autonomous replicon or integrated into the plant chromosomes. Alternatively, the introduced PKSRP may be present on an extra-chromosomal non-replicating vector and be transiently expressed or transiently active.

[0097] In one embodiment, a homologous recombinant microorganism can be created wherein the PKSRP is integrated into a chromosome, a vector is prepared which contains at least a portion of a PKSRP gene into which a deletion, addition, or substitution has been introduced to thereby alter, e.g., functionally disrupt, the PKSRP gene. Preferably, the PKSRP gene is a *Physcomitrella patens* PKSRP gene, but it can be a homolog from a related plant or even from a mammalian, yeast, or insect source. In one embodiment, the vector is designed such that, upon homologous recombination, the endogenous PKSRP gene is functionally disrupted (i.e., no longer encodes a functional polypeptide; also referred to as a knock-out vector). Alternatively, the vector can be designed such that, upon homologous recombination, the endogenous PKSRP gene is mutated or otherwise altered but still encodes a functional polypeptide (e.g., the upstream regulatory region can be altered to thereby alter the expression of the endogenous PKSRP). To create a point mutation via homologous recombination, DNA-RNA hybrids can be used in a technique known as chimeraplasty (Cole-Strauss et al., 1999, Nucleic Acids Research 27(5):1323-1330 and Kmiec, 1999 Gene therapy American Scientist. 87 (3):240-247). Homologous recombination procedures in *Physcomitrella patens* are also well known in the art and are contemplated for use herein.

[0098] Whereas in the homologous recombination vector, the altered portion of the PKSRP gene is flanked at its 5' and 3' ends by an additional nucleic acid molecule of the PKSRP gene to allow for homologous recombination to occur between the exogenous PKSRP gene carried by the vector and an endogenous PKSRP gene, in a microorganism or plant. The additional flanking PKSRP nucleic acid molecule is of sufficient length for successful homologous recombination with the endogenous gene. Typically, several hundreds of base pairs up to kilobases of flanking DNA (both at the 5' and 3' ends) are included in the vector. See, e.g., Thomas, K.R., and Capecchi, M.R., 1987, Cell 51:503 for a description of homologous recombination vectors or Strepp et al., 1998, PNAS, 95 (8):4368-4373 for cDNA based recombination in *Physcomitrella patens).* The vector is introduced into a microorganism or plant cell (e.g., via polyethylene

glycol mediated DNA), and cells in which the introduced PKSRP gene has homologously recombined with the endogenous PKSRP gene are selected using art-known techniques.

[0099] In another embodiment, recombinant microorganisms can be produced that contain selected systems which allow for regulated expression of the introduced gene. For example, inclusion of a PKSRP gene on a vector placing it under control of the lac operon permits expression of the PKSRP gene only in the presence of IPTG. Such regulatory systems are well known in the art.

[0100] Whether present in an extra-chromosomal non-replicating vector or a vector that is integrated into a chromosome, the PKSRP polynucleotide preferably resides in a plant expression cassette. A plant expression cassette preferably contains regulatory sequences capable of driving gene expression in plant cells that are operatively linked so that each sequence can fulfill its function, for example, termination of transcription by polyadenylation signals. Preferred polyadenylation signals are those originating from *Agrobacterium tumefaciens* t-DNA such as the gene 3 known as octopine synthase of the Ti-plasmid pTiACH5 (Gielen et al., 1984, EMBO J. 3:835) or functional equivalents thereof but also all other terminators functionally active in plants are suitable. As plant gene expression is very often not limited on transcriptional levels, a plant expression cassette preferably contains other operatively linked sequences like translational enhancers such as the overdrive-sequence containing the 5'-untranslated leader sequence from tobacco mosaic virus enhancing the polypeptide per RNA ratio (Gallie et al., 1987, Nucl. Acids Research 15:8693-8711). Examples of plant expression vectors include those detailed in: Becker, D. et al., 1992, New plant binary vectors with selectable markers located proximal to the left border, Plant Mol. Biol. 20: 1195-1197; and Bevan, M.W., 1984, Binary Agrobacterium vectors for plant transformation, Nucl. Acid. Res. 12:8711-8721; and Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol. 1, Engineering and Utilization, eds.: Kung and R. Wu, Academic Press, 1993, S. 15-38.

[0101] Plant gene expression should be operatively linked to an appropriate promoter conferring gene expression in a timely, cell or tissue specific manner. Promoters useful in the expression cassettes of the invention include any promoter that is capable of initiating transcription in a plant cell. Such promoters include, but are not limited to, those that can be obtained from plants, plant viruses, and bacteria that contain genes that are expressed in plants, such as *Agrobacterium* and *Rhizobium.*

[0102] The promoter may be constitutive, inducible, developmental stage-preferred, cell type-preferred, tissue-preferred, or organ-preferred. Constitutive promoters are active under most conditions. Examples of constitutive promoters include the CaMV 19S and 35 S promoters (Odell et al., 1985, Nature 313:810-812), the sX CaMV 35S promoter (Kay et al., 1987, Science 236:1299-1302) the Sep1 promoter, the rice actin promoter (McElroy et al., 1990, Plant Cell 2: 163-171), the *Arabidopsis* actin promoter, the ubiquitan promoter (Christensen et al., 1989, Plant Molec Biol 18:675-689); pEmu (Last et al., 1991, Theor Appl Genet 81:581-588), the figwort mosaic virus 35S promoter, the Smas promoter (Velten et al., 1984, EMBO J 3:2723-2730), the GRP1-8 promoter, the cinnamyl alcohol dehydrogenase promoter (U.S. Patent No. 5,683,439), promoters from the T-DNA of Agrobacterium, such as mannopine synthase, nopaline synthase, and octopine synthase, the small subunit of ribulose biphosphate carboxylase (ssuRUBISCO) promoter, and the like.

[0103] Inducible promoters are active under certain environmental conditions, such as the presence or absence of a nutrient or metabolite, heat or cold, light, pathogen attack, anaerobic conditions, and the like. For example, the hsp80 promoter from *Brassica* is induced by heat shock; the PPDK promoter is induced by light; the PR-1 promoter from tobacco, *Arabidopsis,* and maize are inducible by infection with a pathogen; and the Adh1 promoter is induced by hypoxia and cold stress. Plant gene expression can also be facilitated via an inducible promoter (For a review, see Gatz, 1997, Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:89-108). Chemically inducible promoters are especially suitable if gene expression is wanted to occur in a time specific manner. Examples of such promoters are a salicylic acid inducible promoter (PCT Application No. WO 95/19443), a tetracycline inducible promoter (Gatz et al., 1992, Plant J. 2:397-404), and an ethanol inducible promoter (PCT Application No. WO 93/21334).

[0104] [0100] In one preferred embodiment of the present invention, the inducible promoter is a stress-inducible promoter. Stress inducible promoters include, but are not limited to, Cor78 (Chak et al., 2000, Planta 210:875-883; Hovath et al., 1993, Plant Physiol. 103:1047-1053), Cor15a (Artus et al., 1996, PNAS 93(23):13404-09), Rci2A (Medina et al., 2001, Plant Physiol. 125:1655-66; Nylander et al., 2001, Plant Mol. Biol. 45:341-52; Navarre and Goffeau, 2000, EMBO J. 19:2515-24; Capel et al., 1997, Plant Physiol. 115:569-76), Rd22 (Xiong et al., 2001, Plant Cell 13:2063-83; Abe et al., 1997, Plant Cell 9:1859-68; Iwasaki et al., 1995, Mol. Gen. Genet. 247:391-8), cDet6 (Lang and Palve, 1992, Plant Mol. Biol. 20:951-62), ADH1 (Hoeren et al., 1998, Genetics 149:479-90), KAT1 (Nakamura et al., 1995, Plant Physiol. 109:371-4), KST1 (Müller-Röber et al., 1995, EMBO 14:2409-16), Rha1 (Terryn et al., 1993, Plant Cell 5:1761-9; Terryn et al., 1992, FEBS Lett. 299(3):287-90), ARSK1 (Atkinson et al., 1997, GenBank Accession # L22302, and PCT Application No. WO 97/20057), PtxA (Plesch et al., GenBank Accession # X67427), SbHRGP3 (Ahn et al., 1996, Plant Cell 8:1477-90), GH3 (Liu et al., 1994, Plant Cell 6:645-57), the pathogen inducible PRP1-gene promoter (Ward et al., 1993, Plant. Mol. Biol. 22:361-366), the heat inducible hsp80-promoter from tomato (U.S. Patent No. 5187267), cold inducible alpha-amylase promoter from potato (PCT Application No. WO 96/12814), or the wound-inducible pinII-promoter (European Patent No. 375091). For other examples of drought, cold, and salt-inducible promoters, such as the RD29A promoter, see Yamaguchi-Shinozalei et al., 1993, Mol. Gen. Genet. 236:331-340.

[0105] [0101] Developmental stage-preferred promoters are preferentially expressed at certain stages of development. Tissue and organ preferred promoters include those that are preferentially expressed in certain tissues or organs, such as leaves, roots, seeds, or xylem. Examples of tissue preferred and organ preferred promoters include, but are not limited to fruit-preferred, ovule-preferred, male tissue-preferred, seed-preferred, integument-preferred, tuber-preferred, stalk-preferred, pericarp-preferred, and leaf-preferred, stigma-preferred, pollen-preferred, anther-preferred, a petal-preferred, sepal-preferred, pedicel-preferred, silique-preferred, stem-preferred, root-preferred promoters, and the like. Seed preferred promoters are preferentially expressed during seed development and/or germination. For example, seed preferred promoters can be embryo-preferred, endosperm preferred, and seed coat-preferred. See Thompson et al., 1989, BioEssays 10:108. Examples of seed preferred promoters include, but are not limited to, cellulose synthase (celA), Cim1, gamma-zein, globulin-1, maize 19 kD zein (cZ19B1), and the like.

[0106] [0102] Other suitable tissue-preferred or organ-preferred promoters include the napin-gene promoter from rapeseed (U.S. Patent No. 5,608,152), the USP-promoter from Vicia faba (Baeumlein et al., 1991, Mol Gen Genet. 225 (3):459-67), the oleosin-promoter from *Arabidopsis* (PCT Application No. WO 98/45461), the phaseolin-promoter from *Phaseolus vulgaris* (U.S. Patent No. 5,504,200), the Bce4-promoter from *Brassica* (PCT Application No. WO 91/13980), or the legumin B4 promoter (LeB4; Baeumlein et al., 1992, Plant Journal, 2(2):233-9) as well as promoters conferring seed specific expression in monocot plants like maize, barley, wheat, rye, rice, etc. Suitable promoters to note are the 1pt2 or 1pt1-gene promoter from barley (PCT Application No. WO 95/15389 and PCT Application No. WO 95/23230) or those described in PCT Application No. WO 99/16890 (promoters from the barley hordein-gene, rice glutelin gene, rice oryzin gene, rice prolamin gene, wheat gliadin gene, wheat glutelin gene, oat glutelin gene, Sorghum kasirin-gene, and rye secalin gene).

[0107] [0103] Other promoters useful in the expression cassettes of the invention include, but are not limited to, the major chlorophyll a/b binding protein promoter, histone promoters, the Ap3 promoter, the β-conglycin promoter, the napin promoter, the soybean lectin promoter, the maize 15kD zein promoter, the 22kD zein promoter, the 27kD zein promoter, the g-zein promoter, the waxy, shrunken 1, shrunken 2 and bronze promoters, the Zm13 promoter (U.S. Patent No. 5,086,169), the maize polygalacturonase promoters (PG) (U.S. Patent Nos. 5,412,085 and 5,545,546), and the SGB6 promoter (U.S. Patent No. 5,470,359), as well as synthetic or other natural promoters.

[0108] Additional flexibility in controlling heterologous gene expression in plants may be obtained by using DNA binding domains and response elements from heterologous sources *(i.e.,* DNA binding domains from non-plant sources). An example of such a heterologous DNA binding domain is the LexA DNA binding domain (Brent and Ptashne, 1985, Cell 43:729-736).

[0109] The invention further provides a recombinant expression vector comprising a PKSRP DNA molecule of the invention cloned into the expression vector in an antisense orientation. That is, the DNA molecule is operatively linked to a regulatory sequence in a manner that allows for expression (by transcription of the DNA molecule) of an RNA molecule that is antisense to a PKSRP mRNA. Regulatory sequences operatively linked to a nucleic acid molecule cloned in the antisense orientation can be chosen which direct the continuous expression of the antisense RNA molecule in a variety of cell types. For instance, viral promoters and/or enhancers, or regulatory sequences can be chosen which direct constitutive, tissue specific, or cell type specific expression of antisense RNA. The antisense expression vector can be in the form of a recombinant plasmid, phagemid, or attenuated virus wherein antisense nucleic acids are produced under the control of a high efficiency regulatory region. The activity of the regulatory region can be determined by the cell type into which the vector is introduced. For a discussion of the regulation of gene expression using antisense genes, see Weintraub, H. et al., 1986, Antisense RNA as a molecular tool for genetic analysis, Reviews - Trends in Genetics, Vol. 1(1), and Mol et al., 1990, FEBS Letters 268:427-430.

[0110] Another aspect of the invention pertains to host cells into which a recombinant expression vector of the invention has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but they also apply to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term as used herein. A host cell can be any prokaryotic or eukaryotic cell. For example, a PKSRP can be expressed in bacterial cells such as C. *glutamicum,* insect cells, fungal cells, or mammalian cells (such as Chinese hamster ovary cells (CHO) or COS cells), algae, ciliates, plant cells, fungi, or other microorganisms like C. *glutamicum.* Other suitable host cells are known to those skilled in the art.

[0111] A host cell of the invention, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (i.e., express) a PKSRP. Accordingly, the invention further provides methods for producing PKSRPs using the host cells of the invention. In one embodiment, the method comprises culturing the host cell of invention (into which a recombinant expression vector encoding a PKSRP has been introduced, or into which genome has been introduced a gene encoding a wild-type or altered PKSRP) in a suitable medium until PKSRP is produced. In another embodiment, the method further comprises isolating PKSRPs from the medium or the host cell.

[0112] Another aspect of the invention pertains to isolated PKSRPs, and biologically active portions thereof. An "iso-

lated" or "purified" polypeptide or biologically active portion thereof is free of some of the cellular material when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of PKSRP in which the polypeptide is separated from some of the cellular components of the cells in which it is naturally or recombinantly produced. In one embodiment, the language "substantially free of cellular material" includes preparations of a PKSRP having less than about 30% (by dry weight) of non-PKSRP material (also referred to herein as a "contaminating polypeptide"), more preferably less than about 20% of non-PKSRP material, still more preferably less than about 10% of non-PKSRP material, and most preferably less than about 5% non-PKSRP material.

[0113] When the PKSRP or biologically active portion thereof is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the polypeptide preparation. The language "substantially free of chemical precursors or other chemicals" includes preparations of PKSRP in which the polypeptide is separated from chemical precursors or other chemicals that are involved in the synthesis of the polypeptide. In one embodiment, the language "substantially free of chemical precursors or other chemicals" includes preparations of a PKSRP having less than about 30% (by dry weight) of chemical precursors or non-PKSRP chemicals, more preferably less than about 20% chemical precursors or non-PKSRP chemicals, still more preferably less than about 10% chemical precursors or non-PKSRP chemicals, and most preferably less than about 5% chemical precursors or non-PKSRP chemicals. In preferred embodiments, isolated polypeptides, or biologically active portions thereof, lack contaminating polypeptides from the same organism from which the PKSRP is derived. Typically, such polypeptides are produced by recombinant expression of, for example, a *Physcomitrella patens, Brassica napus, Glycine max,* or *Oryza sativa* PKSRP in plants other than *Physcomitrella patens, Brassica napus, Glycine max,* or *Oryza sativa,* or microorganisms such as C. *glutamicum,* ciliates, algae or fungi.

[0114] The nucleic acid molecules, polypeptides, polypeptide homologs, fusion polypeptides, primers, vectors, and host cells described herein can be used in one or more of the following methods: identification of *Physcomitrella patens, Brassica napus, Glycine max,* or *Oryza sativa* and related organisms; mapping of genomes of organisms related to *Physcomitrella patens, Brassica napus, Glycine max,* or *Oryza sativa;* identification and localization of *Physcomitrella patens, Brassica napus, Glycine max,* or *Oryza sativa* sequences of interest; evolutionary studies; determination of PKSRP regions required for function; modulation of a PKSRP activity; modulation of the metabolism of one or more cell functions; modulation of the transmembrane transport of one or more compounds; modulation of stress resistance; and modulation of expression of PKSRP nucleic acids.

[0115] The moss *Physcomitrella patens* represents one member of the mosses. It is related to other mosses such as *Ceratodon purpureus* which is capable of growth in the absence of light. Mosses like *Ceratodon* and *Physcomitrella* share a high degree of sequence identity on the DNA sequence and polypeptide level allowing the use of heterologous screening of DNA molecules with probes evolving from other mosses or organisms, thus enabling the derivation of a consensus sequence suitable for heterologous screening or functional annotation and prediction of gene functions in third species. The ability to identity such functions can therefore have significant relevance, e.g., prediction of substrate specificity of enzymes. Further, these nucleic acid molecules may serve as reference points for the mapping of moss genomes, or of genomes of related organisms.

[0116] The PKSRP nucleic acid molecules of the invention have a variety of uses. Most importantly, the nucleic acid and amino acid sequences of the present invention can be used to transform plants, thereby inducing tolerance to stresses such as drought, high salinity and cold or lodging. The present invention therefore provides a transgenic plant transformed by a PKSRP nucleic acid, wherein expression of the nucleic acid sequence in the plant results in increased tolerance to environmental stress or increased resistance to lodging as compared to a wild type variety of the plant. The transgenic plant can be a monocot or a dicot. The invention further provides that the transgenic plant can be selected from maize, wheat, rye, oat, triticale, rice, barley, soybean, peanut, cotton, rapeseed, canola, manihot, pepper, sunflower, tagetes, solanaceous plants, potato, tobacco, eggplant, tomato, Vicia species, pea, alfalfa, coffee, cacao, tea, Salix species, oil palm, coconut, perennial grass, and forage crops, for example.

[0117] [00104] In particular, the present invention describes using the expression of PK-11 of Physcomitrella patens; engineer drought-tolerant; salt-tolerant, cold-tolerant, and/or lodging-resistant plants. This strategy has herein been demonstrated for Arabidopsis thaliana, Rapeseed/Canola, soybeans, corn, and wheat, but its application is not restricted to these plants. Accordingly, the invention provides a transgenic plant containing a PKSRP such as PK-11 as defined in SEQ ID NO:12, wherein the plant has an increased tolerance to an environmental stress selected from drought, increased salt, decreased or increased temperature. In preferred embodiments, the environmental, stress is drought or decreased temperature.

[0118] Accordingly, the invention provides a method of producing a transgenic plant with a PKSRP coding nucleic acid, wherein expression of the nucleic acid in the plant results in increased tolerance to environmental stress as compared to a wild type variety of the plant comprising: (a) introducing into a plant cell an expression vector comprising a PKSRP nucleic acid, and (b) generating from the plant cell a transgenic plant with an increased tolerance to environ-

mental stress as compared to a wild type variety of the plant. Also included within the present invention are methods of increasing a plant's resistance to lodging, comprising transforming a plant cell with an expression cassette comprising a nucleic acid encoding a PKSRP and generating a transgenic plant from the transformed plant cell. The plant cell includes, but is not limited to, a protoplast, gamete producing cell, and a cell that regenerates into a whole plant. As used herein, the term "transgenic" refers to any plant, plant cell, callus, plant tissue, or plant part, that contains all or part of at least one recombinant polynucleotide. In many cases, all or part of the recombinant polynucleotide is stably integrated into a chromosome or stable extra-chromosomal element, so that it is passed on to successive generations. In preferred embodiments, the PKSRP nucleic acid encodes a protein comprising SEQ ID NO:12,

[0119] The present invention also provides a method of modulating a plant's tolerance to an environmental stress comprising, modifying the expression of a PKSRP coding nucleic acid in the plant. The plant's tolerance to the environmental stress can be increased or decreased as achieved by increasing or decreasing the expression of a PKSRP, respectively. Preferably, the plant's tolerance to the environmental stress is increased by increasing expression of a PKSRP. Expression of a PKSRP can be modified by any method known to those of skill in the art. The methods of increasing expression of PKSRPs can be used wherein the plant is either transgenic or not transgenic. In cases when the plant is transgenic, the plant can be transformed with a vector containing any of the above described PKSRP coding nucleic acids, or the plant can be transformed with a promoter that directs expression of native PKSRP in the plant, for example. The invention provides that such a promoter can be tissue specific, developmentally regulated, or stress-inducible. Alternatively, non-transgenic plants can have native PKSRP expression modified by inducting a native promoter. The expression of PK-1 as defined in SEQ ID NO:11, in target plants can be accomplished by, but is not limited to, one of the following examples: (a) constitutive promoter, (b) stress-inducible promoter, (c) chemical-induced promoter, and (d) engineered promoter overexpression with, for example, zinc-finger derived transcription factors (Greisman and Pabo, 1997, Science 275:657).

[0120] In a preferred embodiment, transcription of the PKSRP is modulated using zinc-finger derived transcription factors (ZFPs) as described in Greisman and Pabo, 1997, Science 275:657 and manufactured by Sangamo Biosciences, Inc. These ZFPs comprise both a DNA recognition domain and a functional domain that causes activation or repression of a target nucleic acid such as a PKSRP nucleic acid. Therefore, activating and repressing ZFPs can be created that specifically recognize the PKSRP promoters described above and used to increase or decrease PKSRP expression in a plant, thereby modulating the stress tolerance of the plant. Also described herein is the identification of the homologs of PK-11 as defined in SEQ ID NO:11,

in a target plant as well as the homolog's promoter. The invention also provides a method of increasing expression of a gene of interest within a host cell as compared to a wild type variety of the host cell, wherein the gene of interest is transcribed in response to a PKSRP, comprising: (a) transforming the host cell with an expression vector comprising a PKSRP coding nucleic acid, and (b) expressing the PKSRP within the host cell, thereby increasing the expression of the gene transcribed in response to the PKSRP, as compared to a wild type variety of the host cell,

[0121] In addition to introducing the PKSRP nucleic acid sequences into transgenic plants, these sequences can also be used to identify an organism as being *Physcomitrella patens, Brassica napus, Glycine max, Oryza sativa,* or a close relative thereof. Also, they may be used to identify the presence of *Physcomitrella patens, Brassica napus, Glycine max, Oryza sativa,* or a relative thereof in a mixed population of microorganisms. The invention provides the nucleic acid sequences of a number of *Physcomitrella patens, Brassica napus, Glycine max,* and *Oryza sativa* genes; by probing the extracted genomic DNA of a culture of a unique or mixed population of microorganisms under stringent conditions with a probe spanning a region of a *Physcomitrella patens, Brassica napus, Glycine max,* or *Oryza sativa* gene which is unique to this organism, one can ascertain whether this organism is present.

[0122] Further, the nucleic acid and polypeptide molecules of the invention may serve as markers for specific regions of the genome. This has utility not only in the mapping of the genome, but also in functional studies of *Physcomitrella patens, Brassica napus, Glycine max,* and *Oryza sativa* polypeptides. For example, to identify the region of the genome to which a particular *Physcomitrella patens* DNA-binding polypeptide binds, the *Physcomitrella patens* genome could be digested, and the fragments incubated with the DNA-binding polypeptide. Those fragments that bind the polypeptide may be additionally probed with the nucleic acid molecules of the invention, preferably with readily detectable labels. Binding of such a nucleic acid molecule to the genome fragment enables the localization of the fragment to the genome map of *Physcomitrella platens,* and, when performed multiple times with different enzymes, facilitates a rapid determination of the nucleic acid sequence to which the polypeptide binds. Further, the nucleic acid molecules of the invention may be sufficiently identical to the sequences of related species such that these nucleic acid molecules may serve as markers for the construction of a genomic map in related mosses.

[0123] The PKSRP nucleic acid molecules of the invention are also useful for evolutionary and polypeptide structural studies. The metabolic and transport processes in which the molecules of the invention participate are utilized by a wide variety of prokaryotic and eukaryotic cells; by comparing the sequences of the nucleic acid molecules of the present invention to those encoding similar enzymes from other organisms, the evolutionary relatedness of the organisms can be assessed. Similarly, such a comparison permits an assessment of which regions of the sequence are conserved and

which are not, which may aid in determining those regions of the polypeptide that are essential for the functioning of the enzyme. This type of determination is of value for polypeptide engineering studies and may give an indication of what the polypeptide can tolerate in terms of mutagenesis without losing function.

[0124] Manipulation of the PKSRP nucleic acid molecules of the invention may result in the production of PKSRPs having functional differences from the wild-type PKSRPs. These polypeptides may be improved in efficiency or activity, may be present in greater numbers in the cell than is usual, or may be decreased in efficiency or activity.

[0125] There are a number of mechanisms by which the alteration of a PKSRP of the invention may directly affect stress response and/or stress tolerance. In the case of plants expressing PKSRPs, increased transport can lead to improved salt and/or solute partitioning within the plant tissue and organs. By either increasing the number or the activity of transporter molecules which export ionic molecules from the cell, it may be possible to affect the salt tolerance of the cell.

[0126] The effect of the genetic modification in plants, *C. glutamicum,* fungi, algae, or ciliates on stress tolerance can be assessed by growing the modified microorganism or plant under less than suitable conditions and then analyzing the growth characteristics and/or metabolism of the plant. Such analysis techniques are well known to one skilled in the art, and include dry weight, wet weight, polypeptide synthesis, carbohydrate synthesis, lipid synthesis, evapotranspiration rates, general plant and/or crop yield, flowering, reproduction, seed setting, root growth, respiration rates, photosynthesis rates, etc. (Applications of HPLC in Biochemistry in: Laboratory Techniques in Biochemistry and Molecular Biology, vol. 17; Rehm et al., 1993 Biotechnology, vol. 3, Chapter III: Product recovery and purification, page 469-714, VCH: Weinheim; Belter, P.A. et al., 1988, Bioseparations: downstream processing for biotechnology, John Wiley and Sons; Kennedy, J.F. and Cabral, J.M.S., 1992, Recovery processes for biological materials, John Wiley and Sons; Shaeiwitz, J.A. and Henry, J.D., 1988, Biochemical separations, in: Ulmann's Encyclopedia of Industrial Chemistry, vol. B3, Chapter 11, page 1-27, VCH: Weinheim; and Dechow, F.J., 1989, Separation and purification techniques in biotechnology, Noyes Publications).

[0127] For example, yeast expression vectors comprising the nucleic acids disclosed herein, or fragments thereof, can be constructed and transformed into *Saccharomyces cerevisiae* using standard protocols. The resulting transgenic cells can then be assayed for fail or alteration of their tolerance to drought, salt, and temperature stress. Similarly, plant expression vectors comprising the nucleic acids disclosed herein, or fragments thereof, can be constructed and transformed into an appropriate plant cell such as *Arabidopsis,* soy, rape, maize, wheat, *Medicago truncatula,* etc., using standard protocols. The resulting transgenic cells and/or plants derived therefrom can then be assayed for fail or alteration of their tolerance to drought, salt, temperature stress, and lodging.

[0128] The engineering of one or more PKSRP genes of the invention may also results in PKSRPs having altered activities which indirectly impact the stress response and/or stress tolerance of algae, plants, ciliates, or fungi, or other microorganism like *C. glutamicum.* For example, the normal biochemical processes of metabolism result in the production of a variety of products (e.g., hydrogen peroxide and other reactive oxygen species) which may actively interfere with these same metabolic processes. For example, peroxynitrite is known to nitrate tyrosine side chains, thereby inactivating some enzymes having tyrosine in the active site (Groves, J.T., 1999, Curr. Opin. Chem. Biol. 3(2):226-235). While these products are typically excreted, cells can be genetically altered to transport more products than is typical for a wild-type cell. By optimizing the activity of one or more PKSRP of the invention which are involved in the export of specific molecules, such as salt molecules, it may be possible to improve the stress tolerance of the cell.

[0129] Additionally, the sequences disclosed herein, or fragments thereof, can be used to generate knockout mutations in the genomes of various organism, such as bacteria, mammalian cells, yeast cells, and plant cells (Girke, T., 1998, The Plant Journal 15:39-48). The resultant knockout cells can then be evaluated for their ability or capacity to tolerate various stress conditions, their response to various stress conditions, and the effect on the phenotype and/or genotype of the mutation. For other methods of gene inactivation, see U.S. Patent No. 6,004,804 "Non-Chimeric Mutational Vectors" and Puttaraju et al., 1999, Spliceosome-mediated RNA trans-splicing as a tool for gene therapy, Nature Biotechnology 17:246-252.

[0130] The aforementioned mutagenesis strategies for PKSRPs resulting in increased stress resistance are not meant to be limiting; variations on these strategies will be readily apparent to one skilled in the art. Using such strategies, and incorporating the mechanisms disclosed herein, the nucleic acid and polypeptide molecules of the invention may be utilized to generate algae, ciliates, plants, fungi, or other microorganisms like *C. glutamicum* expressing mutated PKSRP nucleic acid and polypeptide molecules such that the stress tolerance is improved.

[0131] The present invention also provides antibodies that specifically bind to a PKSRP, or a portion thereof each having at least 70% sequence identity with a polypeptide as defined in SEQ ID NO: 12, as encoded by a nucleic acid described herein. Antibodies can be made by many well-known methods (See; e.g. Harlow and Lane, "Antibodies; A Laboratory Manual," Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, (1988)). Briefly, purified antigen can be injected into an animal in an amount and in intervals sufficient to elicit an immune response. Antibodies can either be purified directly, or spleen cells can be obtained from the animal. The cells can then fused with an immortal cell line and screened for antibody secretion. The antibodies can be used to screen nucleic acid clone libraries for cells secreting the antigen. Those positive clones can then be sequenced. See, for example, Kelly et al., 1992, Bio/Technology

10:163-167; Bebbington et al., 1992, Bio/Technology 10:169-175.

[0132] The phrases "selectively binds" and "specifically binds" with the polypeptide refer to a binding reaction that is determinative of the presence of the polypeptide in a heterogeneous population of polypeptides and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bound to a particular polypeptide do not bind in a significant amount to other polypeptides present in the sample. Selective binding of an antibody under such conditions may require an antibody that is selected for its specificity for a particular polypeptide. A variety of immunoassay formats may be used to select antibodies that selectively bind with a particular polypeptide. For example, solid-phase ELISA immunoassays are routinely used to select antibodies selectively immunoreactive with a polypeptide. See Harlow and Lane, "Antibodies, A Laboratory Manual," Cold Spring Harbor Publications, New York, (1988), for a description of immunoassay formats and conditions that could be used to determine selective binding.

[0133] In some instances, it is desirable to prepare monoclonal antibodies from various hosts. A description of techniques for preparing such monoclonal antibodies may be found in Stites et al., eds., "Basic and Clinical Immunology," (Lange Medical Publications, Los Altos, Calif., Fourth Edition) and references cited therein, and in Harlow and Lane, "Antibodies, A Laboratory Manual," Cold Spring Harbor Publications, New York, (1988).

EXAMPLES

Example 1

Growth of Physcomitrella patens cultures

[0134] For this study, plants of the species *Physcomitrella patens* (Hedw.) B.S.G. from the collection of the genetic studies section of the University of Hamburg were used. They originate from the strain 16/14 collected by H.L.K. Whitehouse in Gransden Wood, Huntingdonshire (England), which was subcultured from a spore by Engel (1968, Am. J. Bot. 55, 438-446). Proliferation of the plants was carried out by means of spores and by means of regeneration of the gametophytes. The protonema developed from the haploid spore as a chloroplast-rich chloronema and chloroplast-low caulonema, on which buds formed after approximately 12 days. These grew to give gametophores bearing antheridia and archegonia. After fertilization, the diploid sporophyte with a short seta and the spore capsule resulted, in which the meiospores matured.

[0135] Culturing was carried out in a climatic chamber at an air temperature of 25˚C and light intensity of 55 micromols^{-1}m^{2} (white light; Philips TL 65W/25 fluorescent tube) and a light/dark change of 16/8 hours. The moss was either modified in liquid culture using Knop medium according to Reski and Abel (1985, Planta 165:354-358) or cultured on Knop solid medium using 1% oxoid agar (Unipath, Basingstoke, England). The protonemas used for RNA and DNA isolation were cultured in aerated liquid cultures. The protonemas were comminuted every 9 days and transferred to fresh culture medium.

Example 2

Total DNA isolation from plants

[0136] The details for the isolation of total DNA relate to the working up of one gram fresh weight of plant material. The materials used include the following buffers: CTAB buffer: 2% (w/v) N-cethyl-N,N,N-trimethylammonium bromide (CTAB); 100 mM Tris HCl pH 8.0; 1.4 M NaCl; 20 mM EDTA; N-Laurylsarcosine buffer: 10% (w/v) N-laurylsarcosine; 100 mM Tris HCl pH 8.0; 20 mM EDTA.

[0137] The plant material was triturated under liquid nitrogen in a mortar to give a fine powder and transferred to 2 ml Eppendorf vessels. The frozen plant material was then covered with a layer of 1 ml of decomposition buffer (1 ml CTAB buffer, 100 µl of N-laurylsarcosine buffer, 20 µl of β-mercaptoethanol, and 10 µl of proteinase K solution, 10 mg/ml) and incubated at 60˚C for one hour with continuous shaking. The homogenate obtained was distributed into two Eppendorf vessels (2 ml) and extracted twice by shaking with the same volume of chloroform/isoamyl alcohol (24:1). For phase separation, centrifugation was carried out at 8000 x g and room temperature for 15 minutes in each case. The DNA was then precipitated at -70˚C for 30 minutes using ice-cold isopropanol. The precipitated DNA was sedimented at 4˚C and 10,000 x g for 30 minutes and resuspended in 180 µl of TE buffer (Sambrook et al., 1989, Cold Spring Harbor Laboratory Press: ISBN 0-87969-309-6). For further purification, the DNA was treated with NaCl (1.2 M final concentration) and precipitated again at -70˚C for 30 minutes using twice the volume of absolute ethanol. After a washing step with 70% ethanol, the DNA was dried and subsequently taken up in 50 µl of H_2O + RNAse (50 mg/ml final concentration). The DNA was dissolved overnight at 4˚C and the RNAse digestion was subsequently carried out at 37˚C for 1 hour. Storage of the DNA took place at 4˚C.

Example 3

Isolation of total RNA and poly-(A)+ RNA and cDNA library construction from Physcomitrella patens

[0138] For the investigation of transcripts, both total RNA and poly-$(A)^+$ RNA were isolated. The total RNA was obtained from wild-type 9 day old protonemata following the GTC-method (Reski et al., 1994, Mol. Gen. Genet., 244:352-359). The Poly(A)+ RNA was isolated using Dyna Beads^R (Dynal, Oslo, Norway) following the instructions of the manufacturer's protocol. After determination of the concentration of the RNA or of the poly(A)+ RNA, the RNA was precipitated by addition of 1/10 volumes of 3 M sodium acetate pH 4.6 and 2 volumes of ethanol and stored at -70˚C.

[0139] For cDNA library construction, first strand synthesis was achieved using Murine Leukemia Virus reverse transcriptase (Roche, Mannheim, Germany) and oligo-d(T)-primers, second strand synthesis by incubation with DNA polymerase I, Klenow enzyme and RNAseH digestion at 12˚C (2 hours), 16˚C (1 hour), and 22˚C (1 hour). The reaction was stopped by incubation at 65˚C (10 minutes) and subsequently transferred to ice. Double stranded DNA molecules were blunted by T4-DNA-polymerase (Roche, Mannheim) at 37˚C (30 minutes). Nucleotides were removed by phenol/chloroform extraction and Sephadex G50 spin columns. EcoRI adapters (Pharmacia, Freiburg, Germany) were ligated to the cDNA ends by T4-DNA-ligase (Roche, 12˚C, overnight) and phosphorylated by incubation with polynucleotide kinase (Roche, 37˚C, 30 minutes). This mixture was subjected to separation on a low melting agarose gel. DNA molecules larger than 300 base pairs were eluted from the gel, phenol extracted, concentrated on Elutip-D-columns (Schleicher and Schuell, Dassel, Germany), and were ligated to vector arms and packed into lambda ZAPII phages or lambda ZAP-Express phages using the Gigapack Gold Kit (Stratagene, Amsterdam, Netherlands) using material and following the instructions of the manufacturer.

Example 4

Sequencing and function annotation of Physcomitrella patens ESTs

[0140] cDNA libraries as described in Example 3 were used for DNA sequencing according to standard methods, and in particular, by the chain termination method using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin-Elmer, Weiterstadt, Germany). Random Sequencing was carried out subsequent to preparative plasmid recovery from cDNA libraries via *in vivo* mass excision, retransformation, and subsequent plating of DH10B on agar plates (material and protocol details from Stratagene, Amsterdam, Netherlands). Plasmid DNA was prepared from overnight grown *E. coli* cultures grown in Luria-Broth medium containing ampicillin (See Sambrook et al., 1989, Cold Spring Harbor Laboratory Press: ISBN 0-87969-309-6) on a Qiagene DNA preparation robot (Qiagen, Hilden) according to the manufacturer's protocols. Sequencing primers with the following nucleotide sequences were used:

5'-CAGGAAACAGCTATGACC-3'	SEQ ID NO:31
5 '-CTAAAGGGAACAAAAGCTG-3'	SEQ IDNO:32
5'-TGTAAAACGACGGCCAGT-3'	SEQ ID NO:33

[0141] Sequences were processed and annotated using the software package EST-MAX commercially provided by Bio-Max (Munich, Germany). The program incorporates practically all bioinformatics methods important for functional and structural characterization of polypeptide sequences. The most important algorithms incorporated in EST-MAX are: FASTA (Very sensitive sequence database searches with estimates of statistical significance; Pearson W.R., 1990, Rapid and sensitive sequence comparison with FASTP and FASTA, Methods Enzymol. 183:63-98); BLAST (Very sensitive sequence database searches with estimates of statistical significance; Altschul S.F. et al., Basic local alignment search tool, Journal of Molecular Biology 215:403-10); PREDATOR (High-accuracy secondary structure prediction from single and multiple sequences, Frishman, D. and Argos; P., 1997, 75% accuracy in polypeptide secondary structure prediction, Polypeptides, 27:329-335); CLUSTALW (Multiple sequence alignment; Thompson, J.D. et al., 1994, CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research, 22:4673-4680); TMAP (Transmembrane region prediction from multiply aligned sequences; Persson, B. and Argos, P., 1994, Prediction of transmembrane segments in polypeptides utilizing multiple sequence alignments, J. Mol. Biol. 237:182-192); ALOM2 (Transmembrane region prediction from single sequences; Klein, P. et al., Prediction of polypeptide function from sequence properties: A discriminate analysis of a database. Biochim. Biophys. Acta 787:221-226 (1984). Version 2 by Dr. K. Nakai); PROSEARCH (Detection of PROSITE polypeptide sequence patterns; Kolakowski L.F. Jr. ,et al., 1992, ProSearch: fast searching of polypeptide sequences with regular expression patterns related to polypeptide structure and function, Biotechniques 13:919-921); BLIMPS (Similarity searches against a database of ungapped blocks; J.C. Wallace and Henikoff S., 1992);

and PATMAT (A searching and extraction program for sequence, pattern and block queries and databases, CABIOS 8:249-254. Written by Bill Alford.).

Example 5

Identification of Physcomitrella patens ORFs corresponding to PK-11

[0142] The *Physcomitrella patens* partial cDNA (EST-) for partial PK-11 (SEQ ID NO:10) was identified in the *Physcomitrella patens* EST sequencing program using the program EST-MAX through BLAST analysis. This particular clone, which was found to encode a Protein Kinase, was chosen for further analyses.

Example 6

Cloning of the full-length Physcomitrella patens CDNA encoding for PK-11

To isolate the clone encoding

[0143] PK-I1 (SEQ ID NO:11) from *Physcomitrella patens,* cDNA libraries were created with SMART RACE cDNA Amplification kit (Clontech Laboratories) following the manufacturer's instructions. Total RNA isolated as described in Example 3 was used as the template. The cultures were treated prior to RNA isolation as follows: Salt Stress: 2, 6, 12, 24, 48 hours with 1-M NaCl-supplemented medium; Cold Stress: 4˚C for the same time points as for salt; Drought Stress: cultures were incubated on dry filter paper for the same - time points as for salt.

5' RACE Protocol

[0144] The EST sequence of 11 (SEQ ID NO:10) identified from the database search as described in Example 5 was used to design oligos for RACE (See Table 1). The extended sequence for this gene was obtained by performing Rapid Amplification of cDNA Ends polymerase chain reaction (RACE PCR) using the Advantage 2 PCR kit (Clontech Laboratories) and the SMART RACE cDNA amplification kit (Clontech Laboratories) using a Biometra T3 Thermocycler following the manufacturer's instructions. The sequence obtained from the RACE reaction corresponded to full-length coding region of design oligos for full-length cloning of the respective gene (See below Full-Length Amplification).

Full-length Amplification

[0145] The full-length clone for PK-11 (SEQ ID NO:11) was isolated by repeating the RACE method but using the gene-specific primers as given in Table 1.

[0146] The amplified fragment was extracted from agarose gel with a QIAquick Gel Extraction Kit (Qiagen) and ligated into the TOPO pCR 2.1 vector (Invitrogen) following manufacturer's instructions. The recombinant vector was transformed into Top10 cells (Invitrogen) using standard conditions (Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual. 2nd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). Transformed cells were selected for on LB agar containing 100 μg/ml carbenicillin, 0.8 mg X-gal (5-bromo-4-chloro-3-indolyl-(3-D-galactoside), and 0.8 mg IPTG (isopropylthio-[3-D-galactoside) grown overnight at 37˚C. White colonies were selected and used to inoculate 3 ml of liquid LB containing 100 μg/ml ampicillin and grown overnight at 37˚C. Plasmid DNA was extracted using the QIAprep Spin Miniprep Kit (Qiagen) following manufacturer's instructions. Analyses of subsequent clones and restriction mapping was performed according to standard molecular biology techniques (Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.).

Table 1

Scheme and Primers Used for Cloning of the Full-Length Clone				
PK-11	XmaI/SacI	5' RACE and RT-PCR for FL clone	RC253: 5'GCAGCGGTAT ATCCTTGCTCCT CATC3'RC520:5' CGATGTGAGAC GCCCTTGCTGTG GCA3'RC721:5'G CAACGACTTGC CAGAACCTCGT GC3' (SEQ ID NO:41)	RC1158: 5'ATCCCGGGTTT CTGGAATAGCTC AGAAGCGT3'RC1 159:5'CGGAGCTC GATGCAGCGGTA TATCCTTGCTCCT 3' (SEQ ID NO:42)

[0147] After completion of the hybridizations, a profile was generated for each spot (representing a cDNA insert), as to which of the 288 ^{33}P radiolabeled 7-mer oligonucleotides bound to that particular spot (cDNA insert), and to what degree. This profile is defined as the signature generated from that clone. Each clone's signature was compared with all other signatures generated from the same organism to identify clusters of related signatures. This process "sorts" all of the clones from an organism into clusters before sequencing.

Gene Isolation

[0148] The clones were sorted into various clusters based on their having identical or similar hybridization signatures. A cluster should be indicative of the expression of an individual gene or gene family. A by-product of this analysis is an expression profile for the abundance of each gene in a particular library. One-path sequencing from the 5' end was used to predict the function of the particular clones by similarity and motif searches in sequence databases.

[0149] The full-length DNA sequence of the *Physcomitrella patens* PK-3 (SEQ ID NO: 8) or PK-10 (SEQ ID NO:11) was blasted against proprietary contig databases of canola, rice, and soybean at E value of E-10. (Altschul, Stephen et al. Gapped BLAST and PSI_BLAST: a new generation of protein database search program. Nucleic Acids Res. 25: 3389-3402).

Example 7

Engineering stress-tolerant Arabidopsis plants by over-expressing a PKSRP gene

Binary vector construction: pBPS-JH001

[0150] The plasmid construct pLMNC53 (Mankin, 2000, Ph.D. thesis, University of North Carolina) was digested with HindIII (Roche) and blunt-end filled with Klenow enzyme and 0.1 mM dNTPs according to manufacturer's instructions. This fragment was purified by agarose gel and extracted via the QIAquick Gel Extraction kit (Qiagen) according to manufacturer's instructions. The purified fragment was then digested with EcoRI (Roche), purified by agarose gel, and extracted via the QIAquick Gel Extraction kit (Qiagen) according to manufacturer's instructions. The resulting 1.4 kilobase fragment, the gentamycin cassette, included the nos promoter, aacCI gene, and the g7 terminator.

[0151] The vector pBlueScript was digested with EcoRI and SmaI (Roche) according to manufacturer's instructions, and the resulting fragment was extracted from agarose gel with a QIAquick Gel Extraction Kit (Qiagen) according to manufacturer's instructions. The digested pBlueScript vector and the gentamycin cassette fragments were ligated with T4 DNA Ligase (Roche) according to manufacturer's instructions, joining the two respective EcoRI sites and joining the blunt-ended HindIII site with the SmaI site.

[0152] The recombinant vector (pGMBS) was transformed into Top10 cells (Invitrogen) using standard conditions. Transformed cells were selected for on LB agar containing 100 μg/ml carbenicillin, 0.8 mg X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) and 0.8 mg IPTG (isopropylthio-β-D-galactoside), grown overnight at 37˚C. White colonies were selected and used to inoculate 3 ml of liquid LB containing 100 μg/ml ampicillin and grown overnight at 37˚C. Plasmid DNA was extracted using the QIAprep Spin Miniprep Kit (Qiagen) following manufacturer's instructions. Analyses of subsequent clones and restriction mapping were performed according to standard molecular biology techniques (Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual. 2nd Edition, Cold Spring Harbor Laboratory Press, Cold

Spring Harbor, Any).

[0153] Both the pGMBS vector and p1bxSuperGUS vector were digested with XbaI and KpnI (Roche) according to manufacturer's instructions, excising the gentamycin cassette from pGMBS and producing the backbone from the p1bxSuperGUS vector. The resulting fragments were extracted from agarose gel with a QIAquick Gel Extraction Kit (Qiagen) according to manufacturer's instructions. These two fragments were ligated with T4 DNA ligase (Roche) according to manufacturer's instructions.

[0154] The resulting recombinant vector (pBPS-JH001) was transformed into Top10 cells (Invitrogen) using standard conditions. Transformed cells were selected for on LB agar containing 100 μg/ml carbenicillin, 0.8 mg X-gal (5-bromo-4-chloro-3-indolyl-(3-D-galactoside) and 0.8 mg IPTG (isopropylthio-β-D-galactoside), grown overnight at 37˚C. White colonies were selected and used to inoculate 3 ml of liquid LB containing 100 μg/ml ampicillin and grown overnight at 37˚C. Plasmid DNA was extracted using the QIAprep Spin Miniprep Kit (Qiagen) following manufacturer's instructions. Analyses of subsequent clones and restriction mapping were performed according to standard molecular biology techniques (Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY).

Binary vector construction: pBPS-SC022

[0155] The plasmid construct pACGH101 was digested with PstI (Roche) and FseI (NEB) according to manufacturers' instructions. The fragment was purified by agarose gel and extracted via the Qiaex II DNA Extraction kit (Qiagen). This resulted in a vector fragment with the *Arabidopsis* Actin2 promoter with internal intron and the OCS3 terminator.

[0156] Primers for PCR amplification of the NPTII gene were designed [5'NPT-Pst: GCG-CTG-CAG-ATT-TCA-TTT-GGA-GAG-GAC-ACG (SEQ ID NO:39); 3'NPT-Fse: CGC-GGC-CGG-CCT-CAG-AAG-AAC-TCG-TCA-AGA-AGG-CG (SEQ ID NO:40)]. The 0.9 kilobase NPTII gene was amplified via PCR from pCambia 2301 plasmid DNA using the following parameters: 94˚C 60sec, {94˚C 60sec, 61˚C (-0.1˚C per cycle) 60sec, 72˚C 2min} x 25 cycles, 72˚C 10min on Biometra T-Gradient machine. The amplified product was purified via the Qiaquick PCR Extraction kit (Qiagen) following manufacturer's instructions. The PCR DNA was then subcloned into the pCR-BluntII TOPO vector (Invitrogen) following manufacturer's instructions (NPT-Topo construct). These ligations were transformed into Top10 cells (Invitrogen) and grown on LB plates with 50 μg/ml kanamycin sulfate overnight at 37˚C. Colonies were then used to inoculate 2 ml LB media with 50 μg/ml kanamycin sulfate and grown overnight at 37˚C. Plasmid DNA was recovered using the Qiaprep Spin Miniprep kit (Qiagen) and sequenced in both the 5' and 3' directions using standard conditions. Subsequent analysis of the sequence data using VectorNTI software revealed that there were not any PCR errors introduced in the NPTII gene sequence.

[0157] The NPT-Topo construct was then digested with PstI (Roche) and FseI (NEB) according to manufacturers' instructions. The 0.9 kilobase fragment was purified on agarose gel and extracted by Qiaex II DNA Extraction kit (Qiagen). The Pst/Fse insert fragment from NPT-Topo and the Pst/Fse vector fragment from pACGH101 were then ligated together using T4 DNA Ligase (Roche) following manufacturer's instructions. The ligation reaction was then transformed into Top10 cells (Invitrogen) under standard conditions, creating pBPS-sc019 construct. Colonies were selected on LB plates with 50 μg/ml kanamycin sulfate and grown overnight at 37˚C. These colonies were then used to inoculate 2 ml LB media with 50 μg/ml kanamycin sulfate and grown overnight at 37˚C. Plasmid DNA was recovered using the Qiaprep Spin Miniprep kit (Qiagen) following the manufacturer's instructions.

[0158] The pBPS-SC019 construct was digested with KpnI and BsaI (Roche) according to manufacturer's instructions. The fragment was purified via agarose gel and then extracted via the Qiaex II DNA Extraction kit (Qiagen) as per its instructions, resulting in a 3 kilobase Act-NPT cassette, which included the *Arabidopsis* Actin2 promoter with internal intron, the NPTII gene, and the OCS3 terminator.

[0159] The pBPS-JH001 vector was digested with SpeI and ApaI (Roche) and blunt-end filled with Klenow enzyme and 0.1 mM dNTPs (Roche) according to manufacturer's instructions. This produced a 10.1 kilobase vector fragment minus the Gentamycin cassette, which was recircularized by self-ligating with T4 DNA Ligase (Roche), and transformed into Top10 cells (Invitrogen) via standard conditions. Transformed cells were selected for on LB agar containing 50 μg/ml kanmycin sulfate and grown overnight at 37˚C. Colonies were then used to inoculate 2 ml of liquid LB containing 50μg/ml kanamycin sulfate and grown overnight at 37˚C. Plasmid DNA was extracted using the QIAprep Spin Miniprep Kit (Qiagen) following manufacturer's instructions. The recircularized plasmid was then digested with KpnI (Roche) and extracted from agarose gel via the Qiaex II DNA Extraction kit (Qiagen) according to manufacturers' instructions.

[0160] The Act-NPT Kpn-cut insert and the Kpn-cut pBPS-JH001 recircularized vector were then ligated together using T4 DNA Ligase (Roche) and transformed into Top10 cells (Invitrogen) according to manufacturers' instructions. The resulting construct, pBPS-SC022, now contained the Super Promoter, the GUS gene, the NOS terminator, and the Act-NPT cassette. Transformed cells were selected for on LB agar containing 50μg/ml kanmycin sulfate and grown overnight at 37˚C. Colonies were then used to inoculate 2 ml of liquid LB containing 50μg/ml kanamycin sulfate and grown overnight at 37˚C. Plasmid DNA was extracted using the QIAprep Spin Miniprep Kit (Qiagen) following manufac-

turer's instructions. After confirmation of ligation success via restriction digests, pBPS-sc022 plasmid DNA was further propagated and recovered using the Plasmid Midiprep Kit (Qiagen) following the manufacturer's instructions.

[0161] Analyses of clones by restriction mapping was performed according to standard molecular biology techniques (Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory).

Subcloning of PK-11 into the binary vector.

[0162] The fragment containing the *Physcomitrella patens* polypeptide kinase was subcloned from the recombinant PCR2.1 TOPO vectors by double digestion with restriction enzymes (See Table 2) according to manufacturer's instructions. The subsequent fragment was excised from agarose gel with a QIAquick Gel Extraction Kit (Qiagen) according to manufacturer's instructions and ligated into the binary vector pBPS-SC022 which was cleaved with appropriate enzymes (See Table 2) and dephosphorylated prior to ligation. The resulting recombinant vector (See Table 2) contained the corresponding Polypeptide Kinase in the sense orientation under the constitutive super promoter.

[0163] Listed are the names of the various constructs of the *Physcomitrella patens* Polypeptide Kinases used for plant transformation

Table 2

Gene	Binary Vector	Enzymes Used to Generate Gene Fragment	Enzymes Used to Restrict the Binary Vector	Binary Vector Construct
PK-11	pBPS-SC022	XmaI/SacI	XmaI/SacI	pBPS-LVM230

Agrobacterium Transformation

[0164] The recombinant vector was transformed into *Agrobacterium: tumefaciens* C58C1 and PMP90 according to standard conditions (Hoefgen and Willmitzer, 1990),

Plant Transformation

[0165] *Arabidopsis thaliana* ecotype C24 were grown and transformed according to standard conditions (Bechtold, 1993, Acad. Sci. Paris. 316:1194-1199; Bent et al., 1994, Science 265:1856-1860).

Screening of Transformed Plants

[0166] T1 seeds were sterilized according to standard protocols (Xiong et al., 1999, Plant Molecular Biology Reporter 17: 159-170). Seeds were plated one Murashige and Skoog media (MS) pH 5.7 with KOH (Sigma-Aldrich), 0.6% agar and supplemented with 1% sucrose, 2 μg/ml benomyl (Sigma-Aldrich), and 150 μg/ml gentamycin (Sigma-Aldrich) (pBPS-JH001 transformants) or 50 μg/ml kanamycin (pBPS-SC022 transformants). Seeds on plates were vernalized for four days at 4˚C. The seeds were germinated in a climatic chamber at an air temperature of 22˚C and light intensity of 40 micromols^{-1m-2} (white light; Philips TL 65W/25 fluorescent tube) and 16 hours light and 8 hours dark day length cycle. Transformed seedlings were selected after 14 days and transferred to ½ MS media pH 5.7 with KOH 0.6% agar plates supplemented with 1% sucrose, 0.5 g/L MES (Sigma-Aldrich), and 2 μg/ml benomyl (Sigma-Aldrich) and allowed to recover for five to seven days.

Drought Tolerance Screening

[0167] T1 seedlings were transferred to dry, sterile filter paper in a petri dish and allowed to desiccate for two hours at 80% RH (relative humidity) in a Sanyo Growth Cabinet MLR-350H, micromols-^{1m2} (white light; Philips TL 65W/25 fluorescent tube). The RH was then decreased to 60%, and the seedlings were desiccated further for eight hours. Seedlings were then removed and placed on ½ MS 0.6% agar plates supplemented with 2 μg/ml benomyl (Sigma-Aldrich) and 0.5 g/L MES (Sigma-Aldrich) and scored after five days.

[0168] Transgenic plants overexpressing the PKSRP are screened for their improved drought tolerance, demonstrating that transgene expression confers drought tolerance.

"In-Soil" Drought Tolerance Screening

[0169] T1 seeds were sterilized in 100% bleach, 0.01% TritonX for five minutes two times and rinsed five times with sterile ddH2O The sterile seeds were plated onto selection plates (1/2 MS, 0.6% phytagar, 0.5 g/L MES, 1% sucrose, 2. μg/ml benamyl, 50 μg/ml kanamycin, 0.6% agar). Plates were incubated at 4˚C for 4 days in the dark.

[0170] Plates were then moved for to 22˚C under continuous light for 10 days for termination and concomitant selection for transgenic plants. Seedlings were transplanted at the 4-5-leaf stage into 5.5 cm diameter pots filled with loosely packed soil (Metromix 360, Scotts) wetted with 1 g/L 20-20-20 fertilizer (Peters Professional, Scotts). Pots were placed randomly on trays with 5 control plants (transformed lines with empty vector) in each tray. Trays were placed randomly in the growth chamber.

[0171] Plants were grown (22˚C, continuous light) for approximately seven days, watering as needed. Watering was stopped at the time when the majority of the plants was about to bolt, and this point was denoted day "0" of the assay. After this day, trays were turned 180˚ every other day to minimize local drying patterns. The assay was stopped approximately at day 12-19, depending on the speed of drying of the pots containing the controls. Pots were then watered and survival rates were determined after 5 days.

[0172] PK 11 over-expressing *Arabidopsis thaliana* plants showed a 65% survival rate (11 survivors from 17 stressed plants) to the stress screening. This survival rate is significantly higher, 99% confidence interval, than that of the control. It is noteworthy that these analyses were performed with T1 plants. The results should be better when a homozygous, strong expresser is found.

[0173]

Table 3

Summary of the drought stress tests			
Gene Name	Drought Test Summay		
	Number of survivors	Total Number of plants	Percentage of survivors
PpPK-11	11	17	65%
HS = significant difference with 99% confidence interval on a z-test			

Freezing Tolerance Screening

[0174] Seedlings are moved to petri dishes containing % MS 0.6% agar supplemented with 2% sucrose and 2 μg/ml benomyl. After four days, the seedlings are incubated at 4˚C for 1 hour and then covered with shaved ice. The seedlings are then placed in an Environmental Specialist ES2000 Environmental Chamber and incubated for 3.5 hours beginning at -1.0˚C, decreasing -1˚C each hour. The seedlings are then incubated at -5.0˚C for 24 hours and then allowed to thaw at 5˚C for 12 hours. The water is poured off and the seedlings are scored after 5 days.

Salt Tolerance Screening

[0175] Seedlings are transferred to filter paper soaked in ½ MS and placed on ½ MS 0.6% agar supplemented with 2 μg/ml benomyl the night before the salt tolerance screening. For the salt tolerance screening, the filter paper with the seedlings is moved to stacks of sterile filter paper, soaked in 50 mM NaCl, in a petri dish. After two hours, the filter paper with the seedlings is moved to stacks of sterile filter paper, soaked with 200 mM NaCl, in a petri dish. After two hours, the filter paper with the seedlings is moved to stacks of sterile filter paper, soaked in 600 mM NaCl, in a petri dish. After 10 hours, the seedlings are moved to petri dishes containing ½ MS 0.6% agar supplemented with 2 μg/ml benomyl. The seedlings are scored after 5 days. The transgenic plants are screened for their improved salt tolerance demonstrating that transgene expression confers salt tolerance.

Example 8

Engineering stress-tolerant soybean plants by over-expressing the - PK-11 gene

[0176] The construct nRPS-LVM230 is used to transform soybean as described below.

[0177] Seeds of soybean are surface sterilized with 70% ethanol for 4 minutes at room temperature with continuous shaking, followed by 20% (v/v) Clorox supplemented with 0.05% (v/v) Tween for 20 minutes with continuous shaking. Then, the seeds are rinsed 4 times with distilled water and placed on moistened sterile filter paper in a Petri dish at room temperature for 6 to 39 hours. The seed coats are peeled off, and cotyledons are detached from the embryo axis. The embryo axis is examined to make sure that the meristematic region is not damaged. The excised embryo axes are collected in a half-open sterile Petri dish and air-dried to a moisture content less than 20% (fresh weight) in a sealed Petri dish until further use.

[0178] . *Agrobacterium tumefaciens* culture is prepared from a single colony in LB solid medium plus appropriate antibiotics (e.g. 100 mg/l streptomycin, 50 mg/l kanamycin) followed by growth of the single colony in liquid LB medium to an optical density at 600 nm of 0.8. Then, the bacteria culture is pelleted at 7000 rpm for 7 minutes at room temperature, and resuspended in MS (Murashige and Skoog, 1962) medium supplemented with 100 μM acetosyringone. Bacteria cultures are incubated in this pre-induction medium for 2 hours at room temperature before use. The axis of soybean zygotic seed embryos at approximately 15% moisture content are imbibed for 2 hoon at room temperature with the pre-induced *Agrobacterium* suspension culture. The embryos are removed from the imbibition culture and transferred to Petri dishes containing solid MS medium supplemented with 2% sucrose and incubated for 2 days, in the dark at room temperature.

[0179] Alternatively, the embryos are placed on top of moistened (liquid MS medium) sterile filter paper in a Petri dish and incubated under the same conditions described above. After this period, the embryos are transferred to either solid or liquid MS medium supplemented with 500 mg/L carbenicillin or 300mg/L cefotaxime to kill the agrobacteria. The liquid medium is used to moisten the sterile filter paper. The embryos are incubated during 4 weeks at 25˚C, under 150 μmol $m^{-2}sec^{-1}$ and 12 hours photoperiod. Once the seedlings have produced roots, they are transferred to sterile metromix soil. The medium of the *in vitro* plants is washed off before transferring the plants to soil. The plants are kept under a plastic cover for 1 week to favor the acclimatization process. Then the plants are transferred to a growth room where they are incubated at 25˚C, under 150 μmol $m^{-2}sec^{-1}$ light intensity and 12 hours photoperiod for about 80 days.

[0180] The transgenic plants are then screened for their improved drought, salt, and/or cold tolerance according to the screening method described in Example 7, demonstrating that transgene expression confers stress tolerance.

Example 9

Engineering stress-tolerant Rapeseed/Canola plants by over-expressing the PK-11 gene

[0181] The constructs pBPS-LVM230 is used to transform rapeseed/canola as described below.

[0182] The method of plant transformation described herein is applicable to *Brassica* and other crops. Seeds of canola are surface sterilized with 70% ethanol for 4 minutes at room temperature with continuous shaking, followed by 20% (v/v) Clorox supplemented with 0.05 % (v/v) Tween for 20 minutes, at room temperature with continuous shaking. Then, the seeds are rinsed 4 times with distilled water and placed on moistened sterile filter paper in a Petri dish at room temperature for 18 hours. Then the seed coats are removed, and the seeds are air dried overnight in a half-open sterile Petri dish. During this period, the seeds lose approximately 85% of its water content The seeds are then stored at room temperature in a sealed Petri dish until further use. DNA constructs and embryo imbibition are as described in Example 10. Samples of the primary transgenic plants (TO) are analyzed by PCT to confirm the presence of T-DNA. These results are confirmed by Southern hybridization in which DNA is electrophoresed on a 1% agarose gel and transferred to a positively charged nylon membrane (Roche Diagnostics). The PCR DIG Probe Synthesis Kit (Roche Diagnostics) is used to prepare a digoxigenin-labelled probe by PCR and used as recommended by the manufacturer.

[0183] The transgenic plants are then screened for their improved stress tolerance according to the screening method described in Example 7, demonstrating that transgene expression confers stress tolerance.

Example 10

Engineering stress-tolerant corn plants by over-expressing the PK-11 gene

[0184] The construct pBPS-LVM230 is used to transform corn as described below.

[0185] Transformation of maize (*Zea Mays L.*) is performed with the method described by Ishida et .al., 1996, Nature Biotech. 14745-50. Immature embryos are co-cultivated with *Agrobacterium tumefaciens* that carry "super binary" vectors, and transgenic plants are recovered through organogenesis. This procedure provides a transformation efficiency of between 2.5% and 20%. The transgenic plants are then screened for their improved drought, salt, and/or cold tolerance according to the screening method described in Example 7, demonstrating that transgene expression, confers stress tolerance.

Example 11

Engineering stress-tolerant wheat plants by over-expressing the PK-11 gene

[0186] The construct pBPS-LVM230 is used to transform wheat as described below.

[0187] Transformation of wheat is performed with the method described by Ishida et al., 1996, Nature Biotech. 14745-50. Immature embryos are co-cultivated with *Agrobacterium tumefaciens* that carry "super binary" vectors, and transgenic plants are recovered through organogenesis. This procedure provides a transformation efficiency between 2.5% and 20%. The transgenic plants are then screened for their improved stress tolerance according to the screening method described in Example 7, demonstrating that transgene expression confers stress tolerance.

Example 12

Identification of Identical and Heterologous Genes

[0188] Gene sequences can be used to identify identical or heterologous genes from cDNA or genomic libraries. Identical genes (e. g. full-length cDNA clones) can be isolated via nucleic acid hybridization using for example cDNA libraries. Depending on the abundance of the gene of interest, 100,000 up to 1,000,000 recombinant bacteriophages are plated and transferred to nylon membranes. After denaturation with alkali, DNA is immobilized on the membrane by e.g. W cross linking. Hybridization is carried out at high stringency conditions. In aqueous solution, hybridization and washing is performed at an ionic strength of 1 M NaCl and a temperature of 68˚C. Hybridization probes are generated by e.g. radioactive (^{32}P) nick transcription labeling (High Prime, Roche, Mannheim, Germany). Signals are detected by autoradiography.

[0189] Partially identical or heterologous genes that are related but not identical can be identified in a manner analogous to the above-described procedure using low stringency hybridization and washing conditions. For aqueous hybridization, the ionic strength is normally kept at 1 M NaCl while the temperature is progressively lowered from 68 to 42˚C.

[0190] Isolation of gene sequences with homology (or sequence identity/similarity) only in a distinct domain of (for example 10-20 amino acids) can be carried out by using synthetic radio labeled oligonucleotide probes. Radiolabeled oligonucleotides are prepared by phosphorylation of the 5-prime end of two complementary oligonucleotides with T4 polynucleotide kinase. The complementary oligonucleotides are annealed and ligated to form concatemers. The double stranded concatemers are than radiolabeled by, for example, nick transcription. Hybridization is normally performed at low stringency conditions using high oligonucleotide concentrations.

Oligonucleotide hybridization solution:
6 x SSC
0.01 M sodium phosphate
1 mM EDTA (pH 8)
0.5 % SDS
100 μg/ml denatured salmon sperm DNA
0.1 % nonfat dried milk

[0191] During hybridization, temperature is lowered stepwise to 5-10˚C below the estimated oligonucleotide T_m or down to room temperature followed by washing steps and autoradiography. Washing is performed with low stringency such as 3 washing steps using 4 x SSC. Further details are described by Sambrook, J. et al., 1989, "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor Laboratory Press or Ausubel, F.M. et al., 1994, "Current Protocols in Molecular Biology," John Wiley & Sons.

Example 13

Identification of Identical Genes by Screening Expression Libraries with Antibodies

[0192] c-DNA clones can be used to produce recombinant polypeptide for example in *E. coli* (e.g. Qiagen QIAexpress pQE system). Recombinant polypeptides are then normally affinity purified via Ni-NTA affinity chromatography (Qiagen). Recombinant polypeptides are then used to produce specific antibodies for example by using standard techniques for rabbit immunization. Antibodies are affinity purified using a Ni-NTA column saturated with the recombinant antigen as described by Gu et al., 1994, BioTechniques 17:257-262. The antibody can than be used to screen expression cDNA libraries to identify identical or heterologous genes via an immunological screening (Sambrook, J. et al., 1989, "Molecular Cloning: A Laboratory Manual," Cold Spring Harbor Laboratory Press or Ausubel, F.M. et al., 1994, "Current Protocols in Molecular Biology", John Wiley & Sons).

Example 14

In vivo Mutagenesis

[0193] *In vivo* mutagenesis of microorganisms can be performed by passage of plasmid (or other vector) DNA through *E. coli* or other microorganisms (e.g. *Bacillus* spp. or yeasts such as *Saccharomyces cerevisiae)* which are impaired in their capabilities to maintain the integrity of their genetic information. Typical mutator strains have mutations in the genes for the DNA repair system (e.g., mutHLS, mutD, mutT, etc.; for reference, see Rupp, W.D., 1996, DNA repair mechanisms, in: Escherichia coli and Salmonella, p. 2277-2294, ASM: Washington.) Such strains are well known to those skilled in the art. The use of such strains is illustrated, for example, in Greener, A. and Callahan, M., 1994, Strategies 7: 32-34. Transfer of mutated DNA molecules into plants is preferably done after selection and testing in microorganisms. Transgenic plants are generated according to various examples within the exemplification of this document.

Example 15

In vitro Analysis of the Function of Physcomitrella Genes in Transgenic Organisms

[0194] The determination of activities and kinetic parameters of enzymes is well established in the art. Experiments to determine the activity of any given altered enzyme must be tailored to the specific activity of the wild-type enzyme, which is well within the ability of one skilled in the art. Overviews about enzymes in general, as well as specific details concerning structure, kinetics, principles, methods, applications and examples for the determination of many enzyme activities may be found, for example, in the following references: Dixon, M., and Webb, E.C., 1979, Enzymes. Longmans: London; Fersht, 1985, Enzyme Structure and Mechanism. Freeman: New York; Walsh, 1979, Enzymatic Reaction Mechanisms. Freeman: San Francisco; Price, N.C., Stevens, L.,1982, Fundamentals of Enzymology. Oxford Univ. Press: Oxford; Boyer, P.D., ed., 1983, The Enzymes, 3rd ed. Academic Press: New York; Bisswanger, H., 1994, Enzymkinetik, 2nd ed. VCH: Weinheim (ISBN 3527300325); Bergmeyer, H.U., Bergmeyer, J., Graβ31, M., eds., 1983-1986, Methods of Enzymatic Analysis, 3rd ed., vol. I-XII, Verlag Chemie: Weinheim; and Ullmann's Encyclopedia of Industrial Chemistry, 1987, vol. A9, Enzymes. VCH: Weinheim, p. 352-363.

[0195] The activity of proteins which bind to DNA can be measured by several well-established methods, such as DNA band-shift assays (also called gel retardation assays). The effect of such proteins on the expression of other molecules can be measured using reporter gene assays (such as that described in Kolmar, H. et al., 1995, EMBO J. 14: 3895-3904 and references cited therein). Reporter gene test systems are well known and established for applications in both prokaryotic and eukaryotic cells, using enzymes such as β-galactosidase, green fluorescent protein, and several others.

[0196] The determination of activity of membrane-transport proteins can be performed according to techniques such as those described in Gennis, R.B., 1989, Pores, Channels and Transporters, in Biomembranes, Molecular Structure and Function, pp. 85-137, 199-234 and 270-322, Springer: Heidelberg.

Example 16

Purification of the Desired Product from Transformed Organisms

[0197] Recovery of the desired product from plant material (i.e., *Physcomitrella patens* or *Arabidopsis thaliana),* fungi, algae, ciliates, *C. glutamicum* cells, or other bacterial cells transformed with the nucleic acid sequences described herein, or the supernatant of the above-described cultures can be performed by various methods well known in the art. If the desired product is not secreted from the cells, the cells can be harvested from the culture by low-speed centrifugation, and the cells can be lysed by standard techniques, such as mechanical force or sonification. Organs of plants can be separated mechanically from other tissue or organs. Following homogenization, cellular debris is removed by centrifugation, and the supernatant fraction containing the soluble proteins is retained for further purification of the desired compound. If the product is secreted from desired cells, then the cells are removed from the culture by low-speed centrifugation, and the supernatant fraction is retained for further purification.

[0198] The supernatant fraction from either purification method is subjected to chromatography with a suitable resin, in which the desired molecule is either retained on a chromatography resin while many of the impurities in the sample are not, or where the impurities are retained by the resin while the sample is not. Such chromatography steps may be repeated as necessary, using the same or different chromatography resins. One skilled in the art would be well-versed in the selection of appropriate chromatography resins and in their most efficacious application for a particular molecule to be purified. The purified product may be concentrated by filtration or ultrafiltration, and stored at a temperature at which the stability of the product is maximized.

[0199] There is a wide array of purification methods known to the art and the preceding method of purification is not meant to be limiting. Such purification techniques are described, for example, in Bailey, J.E. & Ollis, D.F., 1986, Biochemical Engineering Fundamentals, McGraw-Hill: New York. Additionally, the identity and purity of the isolated compounds may be assessed by techniques standard in the art. These include high-performance liquid chromatography (HPLC), spectroscopic methods, staining methods, thin layer chromatography, NIRS, enzymatic assay, or microbiologically. Such analysis methods are reviewed in: Patek et al., 1994, Appl. Environ. Microbiol. 60:133-140; Malakhova et al., 1996, Biotekhnologiya 11:27-32; Schmidt et al., 1998, Bioprocess Engineer 19:67-70; Ulmann's Encyclopedia of Industrial Chemistry, 1996, vol. A27, VCH: Weinheim, p. 89-90, p. 521-540, p. 540-547, p. 559-566, 575-581, and p. 581-587; Michal, G., 1999, Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology, John Wiley and Sons; Fallon, A. et al., 1987, Applications of HPLC in Biochemistry in: Laboratory Techniques in Biochemistry and Molecular Biology, vol. 17.

APPENDIX

[0200]

Nucleotide sequence of the partial PK-11 from *Physcomitrella patens* (SEQ ID NO: 10)

GGCACGAGATTTGGTTGCAAAATAGGTAACTACAACTTAAGAAGAAAAACAATC
TCTCTCTTTCCCCACACAAGATACAACTTCGCTTTTTCCATCACTTACACCAGAAA
GCCCAAAGTAGGGTAGATTGTCACACATCGCTATGATCCCAATTAAGCATCTACT
ACTTTTCATCAGATCAGCAAACTACCAATCATAGAAACTAGGTGATGAATATTAC
GATACTTTCAGGTTCAATGCGAAATCCAAGGTTAACAGTAATGAATGTATTCAAG
CTCTGTACATGCATTAATTTTATGCTACCAGTAGAAAACTTCATTTGACGATGCA
GCGGTATATCCTTGCTCCTCATCCAAGTTAATGCTGGAGCAGAATTATTTATTCTT
AACTACAGATGGCGTCAATGTCTTAACTTCGTCCTTATCAACTGCCGTTGTCCAT
ATGATATCTTTCAACCGGGTACAAAAGCTTTTATAGAACTTGTGATATTCCAAAG
TATCACCCGCAGCCTTCCGCACTTCAATCATGAATAAAGAAGGAGCCACCTCGTA
CACTTCGATAGCCCAGCCAAGTGCTGTGATATTCTGCCTCACTACTGCCTCGAGC

Nucleotide sequence of the full-length PK-11 from *Physcomitrella patens* (SEQ ID NO:11)

ATCCCGGGTGTCGGAATTCGGTCACAATGAGCTAGTGTGTTGTTTGATTGTGGCC
TCAGCTGGAGAGGCTTTGGTATCGTTAGCAGCGAGTGACGCTGTTGAAGGATTGT
ATCCATCCACAAGCGAGAAGCCTTGCCTAATTTTTGGGAGGGAAAGGTGGTTCTC
ACATGAGAGGAGCAGTTGTCGATGCCCCAATGAAGGGTGACAGGAGAGCATGCA
TTTTGGGAGGAATGGGAAGACCTAATGGTGGAACCATCTTGTACGTGTTGGTGAT
TTCATTCATTGCTTTGGTGAATGGAGCCACCGATCCGAACGATGTGTCTGCTTTG
AATACTATGTTCACTGGCTTCAACAGCGATCCTAAGCTCACGAACTGGGTGCAAA
ACGCGGGTGATCCCTGCGGAACCAACTGGCTGGGCGTTACTTGTGATGGGACCTT
CGTCACCTCAATCAAGCTATCCAACATGGGACTGAATGGGAAGGTGGAGGGATG
GGTGTTGCAGAAGTTTCAACACCTCTCTGTGCTTGACCTTAGCCATAATAATCTT
GCTAGCGGAATTCCTGAGATGTTTCCTCCCAAGTTGACTGAACTAGATTTGTCTT

ACAACCAGCTCACGGGTAGTTTTCCTTATTTGATAATCAACATCCCTACTTTGAC
AAGCATAAAACTGAATAACAACAAGCTGAGTGGAACGCTCGATGGGCAGGTTTT
CAGTAAACTCACAAACTTAATCACCCTCGATATTTCCAACAACGCAATTACAGGG
CCGATTCCCGAGGGCATGGGTGACATGGTCAGCCTAAGATTTTTGAACATGCAA
AATAATAAGCTGACTGGACCAATCCCAGACACATTGGCTAATATTCCATCTCTAG
AAACATTGGACGTATCTAACAACGCGCTTACTGGCTTTCTCCCACCAAACCTGAA
CCCAAAGAATTTCAGATATGGAGGCAATCCACTCAACACCCAAGCCCCTCCTCC
ACCACCGTTTACACCACCGCCACCTTCAAAGAATCCAAAGCCTATTCCTCCTCCA
CCCCACCCTGGTAGCCGAACACCAGATACTGCTCCTAAGGCTGAAGGCGGCATC
GTATCAGGCGCAGCAATTGCTGGGATTGTCGTGGGAGCAATTTTGGTGCTTGCAG
CAATTTTCATAGCTGTATGGTTCTTTGTCGTCCGTAAAAGATCTGAGCTTACCAA
ACCTTTGGATTTAGAGGCTAATCACAGCAGCCGACGCACTTGGTTTCTGCCACTT
ATTCCAGCTGGTAAAGAAAAACCACCTAAAATGAAAGTGTTTGAAGCAGATACA
TTTGAGAAGGAAGTGGAAGAGCCGAAGATCAAGGCCTTACCTCCATTGAAGTCA
CTTAAAGTACCTCCAGCATTGAAGGTTGAGGAAGCTACCTACAAGGTTGAAAGT
GAAGGGAAGGTGAACAAGAGCAACATTACAGCAAGAGAGTTTTCCGTCGCAGA
ACTTCAGGCGGCTACGGACAGTTTCTCAGAGGATAATTTACTTGGCGAAGGTTCG
CTTGGTTGTGTTTACCGCGCGGAGTTCCCCGACGGTGAGGTTCTAGCTGTCAAGA
AACTTGATACAACAGCCTCCATGGTTCGGAATGAAGATGATTTCTTGAGCGTTGT
CGATGGCTTGGCCCGGCTACAACATACCAATTCTAATGAACTCGTAGGCTACTGT
GCCGAGCATGGGCAACGACTTCTGGTCTACAAGTTCATCAGTCGAGGGACACTC
CATGAACTGCTTCATGGCTCAGCCGATAGCCCCAAGGAGTTGTCATGGAATGTCC
GTGTGAAGATTGCACTTGGTTGTGCGCGGGCTCTTGAGTATTTCCATGAAATCGT
TTCGCAGCCGGTTGTGCACCGCAACTTTAGATCCTCAAACATTCTTTTGGATGAT
GAGCTGAACCCACATGTGTCGGATTGTGGTTTGGCTGCTTTTACCCCATCCAGTG
CTGAACGGCAGGTCTCTGCCCAAGTGTTGGGATCTTTTGGACACAGTCCCCCTGA
ATTCAGCACATCTGGAATGTATGATGTGAAAAGCGACGTTTATAGCTTTGGTGTT
GTGATGCTTGAGCTTATGACAGGACGCAAGCCTTTAGACAGCTCAAGACCAAGA
TCCGAGCAAAACCTGGTGCGATGGGCAACACCACAACTGCATGATATTGATGCA
CTCGCAAGAATGGTGGATCCAGCGTTAGAGGGTGCTTACCCTGCCAAGTCCCTCT
CCCGGTTCGCCGACATCGTTGCCTTGTGTGTCCAGCCCGAACCCGAATTCCGACC
TCCTATATCTGAAGTAGTGCAGTCCCTGGTAAGGCTTATGCAGCGTGCAGCTTTA
AGTAAACGCCGGCATGAGTACAACGCAGGCGTTCCTCAGACTGATATGGAGGAC
CCTAGTGATTACTTGTGACAGAAGTAAGTATCCTGGTCGATACTTCCCAATTTCA

AGCATAGAGAACCTCCCGCGCGTCTACTCCCACTTGATTTTCAAAGCTGGCGAAA
AGTGGCCAAATTTGTGGATTTGTGACACCTTGCAACTAAATCGGGGAGATATTCA
GCTTCTTTGCAATTCCAGACCATGATGGCACAGACTTTGGCTTGCATCCTCCTCAT
TATTACTGAAGCTTTTGCTTCTAATGGCGGATTACTGATTATGGATGACTATCCC
GTTTCCAGGCAGACGTGAAGAGAAGTGTTGGCTTCCGAAGTTGTTAAATTGTATC
GACGGCTGAAAGCTTTTTTAAGAGCTTACTTCTGGGTCCTAGTTAGTGATATTAA
GGTCCCTGTGCCTTAAGAGTAATGTGCAATTCCTGTTGTGTTGCAAACTCGGGTA
ACGCTTTGTCTTGTAGTTTTGGCACATTACAAGGTTAGTTCGACAGTGAACTCAC
AATTTGAACAGATTAGTTAGGGAGTGTAACTCTAGCAAAAGTTGATTCCTTGTGG
TTACCCAATTTTTTGAATGTGAACTCCCACTCATTGGTGTGATGGAGTACATGAT
TCGCACGAGCTCGC

Deduced amino acid sequence of PK-11 from *Physcomitrella patens* (SEQ ID NO: 12)

MRGAVVDAPMKGDRRACILGGMGRPNGGTILYVLVISFIALVNGATDPNDVSALNT
MFTGFNSDPKLTNWVQNAGDPCGTNWLGVTCDGTFVTSIKLSNMGLNGKVEGWVL
QKFQHLSVLDLSHNNLASGIPEMFPPKLTELDLSYNQLTGSFPYLIINIPTLTSIKLNNN
KLSGTLDGQVFSKLTNLITLDISNNAITGPIPEGMGDMVSLRFLNMQNNKLTGPIPDTL
ANIPSLETLDVSNNALTGFLPPNLNPKNFRYGGNPLNTQAPPPPPFTPPPPSKNPKPIPP
PPHPGSRTPDTAPKAEGGIVSGAAIAGIVVGAILVLAAIFIAVWFFVVRKRSELTKPLD
LEANHSSRRTWFLPLIPAGKEKPPKMKVFEADTFEKEVEEPKIKALPPLKSLKVPPAL
KVEEATYKVESEGKVNKSNITAREFSVAELQAATDSFSEDNLLGEGSLGCVYRAEFP
DGEVLAVKKLDTTASMVRNEDDFLSVVDGLARLQHTNSNELVGYCAEHGQRLLVY
KFISRGTLHELLHGSADSPKELSWNVRVKIALGCARALEYFHEIVSQPVVHRNFRSSNI
LLDDELNPHVSDCGLAAFTPSSAERQVSAQVLGSFGHSPPEFSTSGMYDVKSDVYSF
GVVMLELMTGRKPLDSSRPRSEQNLVRWATPQLHDIDALARMVDPALEGAYPAKSL
SRFADIVALCVQPEPEFRPPISEVVQSLVRLMQRAALSKRRHEYNAGVPQTDMEDPS
DYL*

Claims

1. an isolated nucleic acid, wherein the nucleic acid comprises a polynucleotide encoding a polypeptide having at least 70% sequence identity with a polypeptide as defined in SEQ ID NO: 12, wherein the nucleic acid encodes a polypeptide that functions as a modulator of a plant stress response and wherein the stress is selected from one or more of the group consisting of high salinity, drought, and low temperature.

2. The isolated nucleic acid of claim 1, wherein the nucleic acid comprises a polynucleotide selected from the group consisting of:

a) the polynucleotide as defined in SEQ ID NO:11,
b) a polynucleotide encoding the polypeptide as defined in SEQ ID NO:12, and
c) a polynucleotide complementary to a polynucleotide of any of a) or b) above.

3. A vector comprising a nucleic acid of Claim 1 or 2.

4. A transgenic plant cell being transformed with and/or comprising the vector of claim 3.

5. A transgenic plant comprising a plant cell of Claim 4.

6. The plant of Claim 5, wherein the plant is a monocot, or a dicot.

7. The plant of Claim 5, wherein the nucleic acid encodes a polypeptide that functions as a modulator of a plant stress response, and wherein the stress is selected from one or more of the group consisting of high salinity, drought, and low temperature.

8. A plant seed produced by the plant of Claim 5, wherein the plant seed comprises the PKSRP coding nucleic acid as defined in any one of claims 1-3.

9. The seed of Claim 8, wherein the seed is true breeding for an increased tolerance to environmental stress as compared to a wild type variety of the seed, and wherein the stress is selected from one or more of the group consisting of high salinity, drought, and low temperature.

10. The seed of Claim 8, wherein the nucleic acid encodes a polypeptide that functions as a modulator of a plant stress response, and wherein the stress is selected from one or more of the group consisting of high salinity, drought, and low temperature.

11. A method of producing a transgenic plant containing a nucleic acid wherein the plant has an increased tolerance to an environmental stress as compared to a wild type variety of the plant comprising, transforming a plant cell with an expression vector comprising the nucleic acid and generating from the plant cell the transgenic plant, wherein the nucleic acid encodes a polypeptide that functions as a modulator of a plant stress response, and wherein the nucleic acid is selected from the group consisting of:

a) the polynucleotide as defined in SEQ ID NO:11,
b) a polynucleotide encoding the polypeptide as defined in SEQ ID NO:12,
c) a polynucleotide encoding a polypeptide having at least 70% sequence identity with a polypeptide as defined in SEQ ID NO:12,
d) a polynucleotide complementary to a polynucleotide of any of a) through c) above, and wherein the environmental stress is selected from one or more of the group consisting of high salinity, drought, and low temperature.

12. The method of Claim 11, wherein the nucleic acid comprises a polynucleotide encoding a polypeptide having at least 99% sequence identity with the polypeptide as defined in SEQ ID NO:12, wherein the nucleic acid encodes a polypeptide that functions as a modulator of a plant stress response, and wherein the stress is selected from one or more of the .group consisting of high salinity, drought, and low temperature.

13. The method of Claim 11, wherein the plant is a monocot or a dicot.

14. The method of Claim 11, wherein the plant is selected from the group consisting of maize, wheat, rye, oat, triticale, rice, barley, soybean, peanut, cotton, rapeseed, canola, manihot, pepper, sunflower, tagetes, solanaceous plants, potato, tobacco, eggplant, tomato, Vicia species, pea, alfalfa, coffee, cacao, tea, Salix species, oil palm, coconut, perennial grass, and a forage crop.

15. The method of Claim 11, wherein the plant's stress tolerance is increased by increasing expression of the nucleic acid in the plant.

16. The method of Claim 11, wherein the nucleic acid encodes a polypeptide that functions as a protein kinase.

17. The method of Claim 11, wherein the expression vector comprises a promoter operatively linked to the nucleic acid.

18. The method of Claim 17, wherein the promoter is tissue specific or developmentally regulated, or stress-inducible.

Patentansprüche

1. Isolierte Nukleinsäure, wobei die Nukleinsäure ein Polynukleotid umfasst, das für ein Polypeptid mit mindestens 70% Sequenzidentität zu einem Polypeptid gemäß SEQ ID Nr.:12 codiert, wobei die Nukleinsäure für ein Polypeptid codiert, das als Modulator einer pflanzlichen Stressreaktion agiert und wobei der Stress aus einem oder mehreren der Gruppe bestehend aus Versalzung, Trockenheit und Kälte ausgewählt ist.

2. Isolierte Nukleinsäure nach Anspruch 1, wobei die Nukleinsäure ein Polynukleotid, ausgewählt aus der Gruppe bestehend aus:

a) dem Polynukleotid nach SEQ ID Nur.:11,
b) einem Polynukleotid, das für das Polypeptid nach SEQ ID Nur.:12 codiert, und
c) einem Polynukleotid, das zu einem Polynukleotid gemäß einem der Punkte a) oder b) oben komplementär ist,

umfasst.

3. Vektor, umfassend eine Nukleinsäure nach Anspruch 1 oder 2.

4. Transgene Pflanzenzelle, die mit dem Vektor nach Anspruch 3 transformiert ist und/oder den Vektor nach Anspruch 3 umfasst.

5. Transgene Pflanze, umfassend eine Pflanzenzelle nach Anspruch 4.

6. Pflanze nach Anspruch 5, wobei es sich bei der Pflanze um eine monokotyle oder eine dikotyle Pflanze handelt.

7. Pflanze nach Anspruch 5, wobei die Nukleinsäure für ein Polypeptid codiert, das als Modulator einer pflanzlichen Stressreaktion agiert und wobei der Stress aus einem oder mehreren der Gruppe bestehend aus Versalzung, Trockenheit und Kälte ausgewählt ist.

8. Pflanzensamen, der von der Pflanze nach Anspruch 5 erzeugt wird, wobei der Pflanzensamen die PKSRP-Codiernukleinsäure nach einem der Ansprüche 1-3 umfasst.

9. Samen nach Anspruch 8, wobei der Samen für eine erhöhte Toleranz gegenüber Umweltstress im Vergleich zu einer Wildtypsorte des Samens reinerbig ist und wobei der Stress aus einem oder mehreren der Gruppe bestehend aus Versalzung, Trockenheit und Kälte ausgewählt ist.

10. Samen nach Anspruch 8, wobei die Nukleinsäure für ein Polypeptid codiert, das als Modulator einer pflanzlichen Stressreaktion agiert und wobei der Stress aus einem oder mehreren der Gruppe bestehend aus Versalzung, Trockenheit und Kälte ausgewählt ist.

11. Verfahren zur Herstellung einer transgenen Pflanze, die eine Nukleinsäure enthält, wobei die Pflanze eine erhöhte Toleranz gegenüber einem Umweltstress im Vergleich zu einer Wildtypsorte der Pflanze aufweist, wobei das Verfahren umfasst, dass man eine Pflanzenzelle mit einem Expressionsvektor, der die Nukleinsäure umfasst, transformiert und aus der Pflanzenzelle die transgene Pflanze erzeugt, wobei die Nukleinsäure für ein Polypeptid codiert, das als Modulator einer pflanzlichen Stressreaktion agiert, und wobei die Nukleinsäure aus der Gruppe bestehend aus:

a) dem Polynukleotid nach SEQ ID Nur.:11,
b) einem Polynukleotid, das für das Polypeptid nach SEQ ID Nr.:12 codiert,
c) einem Polynukleotid, das für ein Polypeptid mit mindestens 70% Sequenzidentität zu einem Polypeptid gemäß SEQ ID Nr.:12 codiert,
d) einem Polynukleotid, das zu einem Polynukleotid nach einem der Punkte a) bis c) oben komplementär ist, und wobei der Umweltstress aus einem oder mehreren der Gruppe bestehend aus Versalzung, Trockenheit und Kälte ausgewählt ist,

ausgewählt ist.

12. Verfahren nach Anspruch 11, wobei die Nukleinsäure ein Polynukleotid umfasst, das für ein Polypeptid mit mindestens 99% Sequenzidentität zu dem Polypeptid gemäß SEQ ID Nr.:12 codiert, wobei die Nukleinsäure für ein Polypeptid codiert, das als Modulator einer pflanzlichen Stressreaktion agiert und wobei der Stress aus einem oder mehreren der Gruppe bestehend aus Versalzung, Trockenheit und Kälte ausgewählt ist.

13. Verfahren nach Anspruch 11, wobei es sich bei der Pflanze um eine monokotyle oder eine dikotyle Pflanze handelt.

14. Verfahren nach Anspruch 11, wobei die Pflanze aus der Gruppe bestehend aus Mais, Weizen, Roggen, Hafer, Triticale, Reis, Gerste, Sojabohne, Erdnuss, Baumwolle, Raps, Canola, Maniok, Pfeffer, Sonnenblume, Tagetes, Solanaceen-Pflanzen, Kartoffel, Tabak, Aubergine, Tomate, Vicia-Arten, Erbse, Luzerne, Kaffee, Kakao, Tee, Salix-Arten, Ölpalme, Kokosnuss, ausdauerndem Gras und einer Feldfutterfrucht ausgewählt ist.

15. Verfahren nach Anspruch 11, wobei die Stresstoleranz der Pflanze durch Erhöhen der Expression der Nukleinsäure in der Pflanze erhöht wird.

16. Verfahren nach Anspruch 11, wobei die Nukleinsäure für ein Polypeptid codiert, das als Proteinkinase agiert.

17. Verfahren nach Anspruch 11, wobei der Expressionsvektor einen Promoter umfasst, der mit der Nukleinsäure operativ verknüpft ist.

18. Verfahren nach Anspruch 17, wobei der Promoter gewebespezifisch oder entwicklungsreguliert oder stressinduzierbar ist.

Revendications

1. Acide nucléique isolé, dans lequel l'acide nucléique comprend un polynucléotide codant pour un polypeptide qui a une identité de séquence d'au moins 70% avec un polypeptide tel que défini dans la SEQ ID n : 12, dans lequel l'acide nucléique code pour un polypeptide qui fonctionne comme modulateur de la réponse d'une plante au stress et dans lequel le stress est choisi parmi un ou plusieurs élément(s) du groupe constitué par une salinité élevée, la sécheresse et une température basse.

2. Acide nucléique isolé selon la revendication 1, dans laquelle l'acide nucléique comprend un polynucléotide choisi dans le groupe constitué par:

a) le polynucléotide tel que défini dans la SEQ ID n° : 11 ;
b) un polynucléotide codant pour le polypeptide tel que défini dans la SEQ ID n° : 12 ; et
c) un polynucléotide complémentaire d'un polynucléotide selon l'un quelconque des paragraphes a) ou b) ci-dessus.

3. Vecteur comprenant un acide nucléique selon la revendication 1 ou la 2.

4. Cellule végétale transgénique étant transformée avec le vecteur selon la revendication 3 et/ou comprenant celui-ci.

5. Plante transgénique comprenant une cellule végétale selon la revendication 4.

6. Plante selon la revendication 5, dans laquelle la plante est une monocotylédone ou une dicotylédone.

7. Plante selon la revendication 5, dans laquelle l'acide nucléique code pour un polypeptide qui fonctionne comme modulateur de la réponse d'une plante au stress et dans laquelle le stress est choisi parmi un ou plusieurs élément (s) du groupe constitué par une salinité élevée, la sécheresse et une température basse.

8. Graine de plante produite par la plante selon la revendication 5, dans laquelle la graine de plante comprend l'acide nucléique codant de PKSRP, tel que défini dans l'une quelconque des revendications 1 à 3.

9. Graine selon la revendication 8, dans laquelle la graine est génétiquement pure pour une tolérance accrue à un

stress environnemental, en comparaison avec une variété de type sauvage de la graine, et dans laquelle le stress est choisi parmi un ou plusieurs élément(s) du groupe constitué par une salinité élevée, la sécheresse et une température basse.

10. Graine selon la revendication 8, dans laquelle l'acide nucléique code pour un polypeptide qui fonctionne comme modulateur de la réponse d'une plante au stress et dans laquelle le stress est choisi parmi un ou plusieurs élément (s) du groupe constitué par une salinité élevée, la sécheresse et une température basse.

11. Procédé de production d'une plante transgénique contenant un acide nucléique, dans lequel la plante a une tolérance accrue à un stress environnemental en comparaison avec une variété de type sauvage de la plante, comprenant les étapes consistant à : transformer une cellule végétale avec un vecteur d'expression comprenant l'acide nucléique ; et générer la plante transgénique à partir de la cellule végétale, dans lequel l'acide nucléique code pour un polypeptide qui fonctionne comme modulateur de la réponse d'une plante au stress et dans lequel l'acide nucléique est choisi dans le groupe constitué par :

a) le polynucléotide tel que défini dans la SEQ ID n° : 11 ;
b) un polynucléotide codant pour le polypeptide tel que défini dans la SEQ ID n° : 12 ;
c) un polynucléotide codant pour un polypeptide qui a une identité de séquence d'au moins 70% avec un polypeptide tel que défini dans la SEQ ID n° : 12 ;
d) un polynucléotide complémentaire d'un polynucléotide selon l'un quelconque des paragraphes a) à c) ci-dessus, et dans lequel le stress environnemental est choisi parmi un ou plusieurs élément(s) du groupe constitué par une salinité élevée, la sécheresse et une température basse.

12. Procédé selon la revendication 11, dans lequel l'acide nucléique comprend un polynucléotide codant pour un polypeptide, qui a une identité de séquence d'au moins 99% avec le polypeptide tel que défini dans la SEQ ID n° . 12, et dans lequel l'acide nucléique code pour un polypeptide qui fonctionne comme modulateur de la réponse d'une plante au stress et dans lequel le stress est choisi parmi un ou plusieurs élément(s) du groupe constitué par une salinité élevée, la sécheresse et une température basse.

13. Procédé selon la revendication 11, dans lequel la plante est une monocotylédone ou une dicotylédone.

14. Procédé selon la revendication 11, dans lequel la plante est choisie dans le groupe constitué par le maïs, le blé, le seigle, l'avoine, un triticale, le riz, l'orge, le soja, une arachide, le coton, le colza, le colza canola, le manioc, le poivre, le tournesol, une tagète, des plantes solanacées, la pomme de terre, le tabac, l'aubergine, la tomate, l'espèce Vicia, lé pois, la luzerne, le café, le cacao, le thé, l'espèce Salix, le palmier à huile, la noix de coco, les herbes vivaces et une culture fourragère.

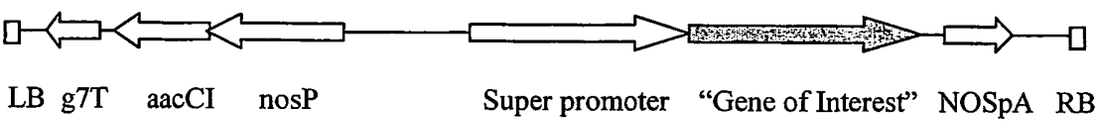
15. Procédé selon la revendication 11, dans lequel la tolérance au stress de la plante est accrue en augmentant l'expression de l'acide nucléique dans la plante.

16. Procédé selon la revendication 11, dans lequel l'acide nucléique code pour un polypeptide qui fonctionne comme une protéine kinase.

17. Procédé selon la revendication 11, dans lequel le vecteur d'expression comprend un promoteur étant lié, de manière opérationnelle, à l'acide nucléique.

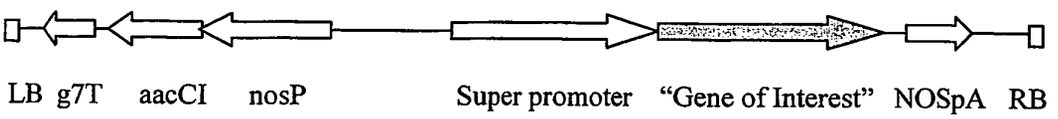
18. Procédé selon la revendication 17, dans lequel le promoteur est spécifique d'un tissu, régulé d'un point de vue du développement ou bien inductible par un stress.

FIGURE 1



pBPS-JH001

FIGURE 2



pBPS-SC022

REFERENCES CITED IN THE DESCRIPTION

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Patent documents cited in the description

- EP 0359472 A **[0064]**
- EP 0385962 A **[0064]**
- WO 9116432 A **[0064]**
- US 5380831 A **[0064]**
- US 5436391 A **[0064]**
- US 4987071 A **[0073]**
- US 5116742 A, Cech **[0073]**
- US 6025167 A **[0073]**
- US 5773260 A **[0073]**
- US 5496698 A **[0073]**
- US 4130641 A **[0075]**
- US 4024222 A **[0075]**
- US 4283393 A **[0075]**
- US 5795715 A **[0075]**
- US 4801340 A **[0076]**
- US 5034323 A **[0076]**
- US 5231020 A **[0076]**
- US 5283184 A **[0076]**
- WO 9801572 A **[0083]**
- US 4873316 A **[0091]**
- EP 264166 A **[0091]**
- EP 0424047 A **[0095]**
- US 5322783 A **[0095]**
- EP 0397687 A **[0095]**
- US 5376543 A **[0095]**
- US 5169770 A **[0095]**
- US 5990387 A **[0095]**
- WO 9307256 A **[0095]**
- US 5683439 A **[0102]**
- WO 9519443 A **[0103]**
- WO 9321334 A **[0103]**
- WO 9720057 A **[0104]**
- US 5187267 A **[0104]**
- WO 9612814 A **[0104]**
- EP 375091 A **[0104]**
- US 5608152 A **[0106]**
- WO 9845461 A **[0106]**
- US 5504200 A **[0106]**
- WO 9113980 A **[0106]**
- WO 9515389 A **[0106]**
- WO 9523230 A **[0106]**
- WO 9916890 A **[0106]**
- US 5086169 A **[0107]**
- US 5412085 A **[0107]**
- US 5545546 A **[0107]**
- US 5470359 A **[0107]**
- US 6004804 A **[0129]**

Non-patent literature cited in the description

- **Van der Gear et al.** *Annu. Rev. Cell Biol.,* 1994, vol. 10, 251-337 **[0007]**
- **Becraft, PW.** *Trends Plant Sci.,* 1998, vol. 3, 384-388 **[0007]**
- **Knight et al.** *Nature,* 1991, vol. 352, 524 **[0008]**
- **Schroeder, J.I. ; Thuleau, P.** *Plant Cell,* 1991, vol. 3, 555 **[0008]**
- **Bush, D.S.** *Annu. Rev. Plant Phys. Plant Mol. Biol.,* 1995, vol. 46, 95 **[0008]**
- **Urao, T. et al.** *Mol. Gen. Genet.,* 1994, vol. 244, 331 **[0008]**
- **Hajdukiewicz et al.** *Plant Molec. Biol.,* 1994, vol. 25, 989-94 **[0026]**
- **Becker et al.** *Plant Molec. Biol.,* 1992, vol. 20, 1195-97 **[0026]**
- **Jefferson et al.** *EMBO J.,* 1987, vol. 6, 3901-7 **[0026]**
- **Hajdukiewicz et al.** *Plant Molec. Biol.,* 1994, vol. 25, 989-98 **[0027]**
- **An et al.** *Plant J.,* 1996, vol. 10, 107-21 **[0027]**
- **Weigel et al.** *Plant Physiol,* 2000, vol. 122, 1003-13 **[0027]**
- **Chirgwin et al.** *Biochemistry,* 1979, vol. 18, 5294-5299 **[0039]**
- Current Protocols in Molecular Biology. John Wiley & Sons, 1992 **[0045]**
- **Narang, S.A.** *Tetrahedron,* 1983, vol. 39, 3 **[0048]**
- **Itakura et al.** *Annu. Rev. Biochem.,* 1984, vol. 53, 323 **[0048]**
- **Itakura et al.** *Science,* 1984, vol. 198, 1056 **[0048]**
- **Ike et al.** *Nucleic Acid Res.,* 1983, vol. 11, 477 **[0048]**
- **Arkin ; Yourvan.** *PNAS,* 1992, vol. 89, 7811-7815 **[0050]**
- **Delgrave et al.** *Polypeptide Engineering,* 1993, vol. 6 (3), 327-331 **[0050]**
- **Meinkoth ; Wahl.** *Anal. Biochem.,* 1984, vol. 138, 267-284 **[0057]**
- Current Protocols in Molecular Biology. Greene Publishing and Wiley-Interscience, 1995 **[0057]**
- **Tijssen.** Laboratory Techniques in Biochemistry and Molecular Biology: Hybridization with Nucleic Acid Probes. Elsevier **[0057]**

- **Tatusov, R.L. et al.** *Science,* 1997, vol. 278 (5338), 631-637 **[0059]**
- **Perlack et al.** *Proc. Natl. Acad. Sci. USA,* 1991, vol. 88, 3324-3328 **[0064]**
- **Murray et al.** *Nucleic Acids Res.,* 1989, vol. 17, 477-498 **[0064]**
- **Gaultier et al.** *Nucleic Acids. Res.,* 1987, vol. 15, 6625-6641 **[0071]**
- **Inoue et al.** *Nucleic Acids Res.,* 1987, vol. 15, 6131-6148 **[0071]**
- **Inoue et al.** *FEBS Lett.,* 1987, vol. 215, 327-330 **[0071]**
- **Haselhoff ; Gerlach.** *Nature,* 1988, vol. 334, 585-591 **[0073]**
- **Bartel, D. ; Szostak, J.W.** *Science,* 1993, vol. 261, 1411-1418 **[0073]**
- **Moser et al.** *Science,* 1987, vol. 238, 645-650 **[0076]**
- **Cooney et al.** *Science,* 1988, vol. 241, 456-459 **[0076]**
- **Napoli et al.** *The Plant Cell,* 1990, vol. 2, 279-289 **[0076]**
- **Van der Kroll et al.** *The Plant Cell,* 1990, vol. 2, 291-299 **[0076]**
- **Smith et al.** *Mol. Gen. Genetics,* 1990, vol. 224, 477-481 **[0076]**
- **Helene, C.** *Anticancer Drug Des.,* 1991, vol. 6 (6), 569-84 **[0078]**
- **Helene, C. et al.** *Ann. N.Y. Acad. Sci.,* 1992, vol. 660, 27-36 **[0078]**
- **Maher, L.J.** *Bioassays,* 1992, vol. 14 (12), 807-15 **[0078]**
- **Ausubel et al.** Current Protocols in Molecular Biology. Wiley, 1988 **[0080]**
- **Bormann, E.R. et al.** *Mol. Microbiol.,* 1992, vol. 6, 317-326 **[0080]**
- **Goeddel.** Gene Expression Technology: Methods in Enzymology. Academic Press, 1990, vol. 185 **[0082] [0083]**
- **Gruber ; Crosby.** Methods in Plant Molecular Biology and Biotechnology. CRC Press, 89-108 **[0082]**
- **Romanos, M.A. et al.** Foreign gene expression in yeast: a review. *Yeast,* 1992, vol. 8, 423-488 **[0083]**
- Heterologous gene expression in filamentous fungi. **van den Hondel, C.A.M.J.J. et al.** More Gene Manipulations in Fungi. Academic Press, 396-428 **[0083]**
- Gene transfer systems and vector development for filamentous fungi. **van den Hondel, C.A.M.J.J. ; Punt, P.J. et al.** Applied Molecular Genetics of Fungi. Cambridge University Press, 1-28 **[0083]**
- **Falciatore et al.** *Marine Biotechnology,* 1999, vol. 1 (3), 239-251 **[0083] [0093]**
- **Schmidt, R. ; Willmitzer, L.** High efficiency Agrobacterium tumefaciens-mediated transformation of Arabidopsis thaliana leaf and cotyledon explants. *Plant Cell Rep.,* 1988, 583-586 **[0083]**
- Plant Molecular Biology and Biotechnology. C Press, 1993, 71-119 **[0083]**
- **F.F. White ; B. Jenes et al.** Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization. Academic Press, 1993, vol. 1, 128-43 **[0083]**
- **Potrykus.** *Annu. Rev. Plant Physiol. Plant Molec. Biol.,* 1991, vol. 42, 205-225 **[0083]**
- **Smith, D.B. ; Johnson, K.S.** Gene. Pharmacia Biotech Inc, 1988, vol. 67, 31-40 **[0085]**
- **Amann et al.** *Gene,* 1988, vol. 69, 301-315 **[0086]**
- **Studier et al.** Gene Expression Technology: Methods in Enzymology. Academic Press, 1990, vol. 185, 60-89 **[0086]**
- **Gottesman, S.** *Gene Expression Technology: Methods in Enzymology,* 1990, vol. 185, 119-128 **[0087]**
- **Wada et al.** *Nucleic Acids Res.,* 1992, vol. 20, 2111-2118 **[0087]**
- **Baldari et al.** *EMBO J.,* 1987, vol. 6, 229-234 **[0088]**
- **Kurjan ; Herskowitz.** *Cell,* 1982, vol. 30, 933-943 **[0088]**
- **Schultz et al.** *Gene,* 1987, vol. 54, 113-123 **[0088]**
- Gene transfer systems and vector development for filamentous fungi. **van den Hondel, C.A.M.J.J. ; Punt, P.J. et al.** Applied Molecular Genetics of Fungi. Cambridge University Press, 1991, 1-28 **[0088]**
- **Smith et al.** *Mol. Cell Biol.,* 1983, vol. 3, 2156-2165 **[0089]**
- **Lucklow ; Summers.** *Virology,* 1989, vol. 170, 31-39 **[0089]**
- **Seed, B.** *Nature,* 1987, vol. 329, 840 **[0090]**
- **Kaufman et al.** *EMBO J.,* 1987, vol. 6, 187-195 **[0090]**
- **Sambrook, J. ; Fritsh, E. F. ; Maniatis, T.** Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, 1989 **[0090]**
- **Pinkert et al.** *Genes Dev.,* 1987, vol. 1, 268-277 **[0091]**
- **Calame ; Eaton.** *Adv. Immunol.,* 1988, vol. 43, 235-275 **[0091]**
- **Winoto ; Baltimore.** *EMBO J.,* 1989, vol. 8, 729-733 **[0091]**
- **Banerji et al.** *Cell,* 1983, vol. 33, 729-740 **[0091]**
- **Queen ; Baltimore.** *Cell,* 1983, vol. 33, 741-748 **[0091]**
- **Byrne ; Ruddle.** *PNAS,* 1989, vol. 86, 5473-5477 **[0091]**
- **Edlund et al.** *Science,* 1985, vol. 230, 912-916 **[0091]**
- **Kessel ; Gruss.** *Science,* 1990, vol. 249, 374-379 **[0091]**
- **Campes ; Tilghman.** *Genes Dev.,* 1989, vol. 3, 537-546 **[0091]**
- **Sambrook et al.** Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, 1989 **[0094]**
- Methods in Molecular Biology. Humana Press, 1995, vol. 44 **[0094]**
- **Koncz ; Schell.** *Mol. Gen. Genet.,* 1986, vol. 204, 383-396 **[0095]**

- **Deblaere et al.** *Nucl. Acids Res.,* 1994, vol. 13, 4777-4788 **[0095]**
- **Gelvin, Stanton B. ; Schilperoort, Robert A.** Plant Molecular Biology Manual. Kluwer Academic Publ, 1995 **[0095]**
- **Glick, Bernard R. ; Thompson, John E.** Methods in Plant Molecular Biology and Biotechnology. CRC Press, 1993, 360 **[0095]**
- **Moloney et al.** *Plant cell Report,* 1989, vol. 8, 238-242 **[0095]**
- **De Block et al.** *Plant Physiol.,* 1989, vol. 91, 694-701 **[0095]**
- **Mlynarova et al.** *Plant Cell Report,* 1994, vol. 13, 282-285 **[0095]**
- **Freeling ; Walbot.** The maize handbook. Springer Verlag, 1993 **[0095]**
- **Cole-Strauss et al.** *Nucleic Acids Research,* 1999, vol. 27 (5), 1323-1330 **[0097]**
- **Kmiec.** *Gene therapy American Scientist,* 1999, vol. 87 (3), 240-247 **[0097]**
- **Thomas, K.R. ; Capecchi, M.R.** *Cell,* 1987, vol. 51, 503 **[0098]**
- **Strepp et al.** *PNAS,* 1998, vol. 95 (8), 4368-4373 **[0098]**
- **Gielen et al.** *EMBO J.,* 1984, vol. 3, 835 **[0100]**
- **Gallie et al.** *Nucl. Acids Research,* 1987, vol. 15, 8693-8711 **[0100]**
- **Becker, D. et al.** New plant binary vectors with selectable markers located proximal to the left border. *Plant Mol. Biol.,* 1992, vol. 20, 1195-1197 **[0100]**
- **Bevan, M.W.** Binary Agrobacterium vectors for plant transformation. *Nucl. Acid. Res.,* 1984, vol. 12, 8711-8721 **[0100]**
- Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol. 1, Engineering and Utilization. Academic Press, 1993, vol. 1, 15-38 **[0100]**
- **Odell et al.** *Nature,* 1985, vol. 313, 810-812 **[0102]**
- **Kay et al.** *Science,* 1987, vol. 236, 1299-1302 **[0102]**
- **McElroy et al.** *Plant Cell,* 1990, vol. 2, 163-171 **[0102]**
- **Christensen et al.** *Plant Molec Biol,* 1989, vol. 18, 675-689 **[0102]**
- **Last et al.** *Theor Appl Genet,* 1991, vol. 81, 581-588 **[0102]**
- **Velten et al.** *EMBO J,* 1984, vol. 3, 2723-2730 **[0102]**
- **Gatz.** *Annu. Rev. Plant Physiol. Plant Mol. Biol.,* 1997, vol. 48, 89-108 **[0103]**
- **Gatz et al.** *Plant J.,* 1992, vol. 2, 397-404 **[0103]**
- **Chak et al.** *Planta,* 2000, vol. 210, 875-883 **[0104]**
- **Hovath et al.** *Plant Physiol.,* 1993, vol. 103, 1047-1053 **[0104]**
- **Artus et al.** *PNAS,* 1996, vol. 93 (23), 13404-09 **[0104]**
- **Medina et al.** *Plant Physiol.,* 2001, vol. 125, 1655-66 **[0104]**
- **Nylander et al.** *Plant Mol. Biol.,* 2001, vol. 45, 341-52 **[0104]**
- **Navarre ; Goffeau.** *EMBO J.,* 2000, vol. 19, 2515-24 **[0104]**
- **Capel et al.** *Plant Physiol.,* 1997, vol. 115, 569-76 **[0104]**
- **Xiong et al.** *Plant Cell,* 2001, vol. 13, 2063-83 **[0104]**
- **Abe et al.** *Plant Cell,* 1997, vol. 9, 1859-68 **[0104]**
- **Iwasaki et al.** *Mol. Gen. Genet.,* 1995, vol. 247, 391-8 **[0104]**
- **Lang ; Palve.** *Plant Mol. Biol.,* 1992, vol. 20, 951-62 **[0104]**
- **Hoeren et al.** *Genetics,* 1998, vol. 149, 479-90 **[0104]**
- **Nakamura et al.** *Plant Physiol.,* 1995, vol. 109, 371-4 **[0104]**
- **Müller-Röber et al.** *EMBO,* 1995, vol. 14, 2409-16 **[0104]**
- **Terryn et al.** *Plant Cell,* 1993, vol. 5, 1761-9 **[0104]**
- **Terryn et al.** *FEBS Lett.,* vol. 299 (3), 287-90 **[0104]**
- **Ahn et al.** *Plant Cell,* 1996, vol. 8, 1477-90 **[0104]**
- **Liu et al.** *Plant Cell,* 1994, vol. 6, 645-57 **[0104]**
- **Ward et al.** *Plant. Mol. Biol.,* 1993, vol. 22, 361-366 **[0104]**
- **Yamaguchi-Shinozalei et al.** *Mol. Gen. Genet.,* 1993, vol. 236, 331-340 **[0104]**
- **Thompson et al.** *BioEssays,* 1989, vol. 10, 108 **[0105]**
- **Baeumlein et al.** *Mol Gen Genet.,* 1991, vol. 225 (3), 459-67 **[0106]**
- **Baeumlein et al.** *Plant Journal,* 1992, vol. 2 (2), 233-9 **[0106]**
- **Brent ; Ptashne.** *Cell,* 1985, vol. 43, 729-736 **[0108]**
- **Weintraub, H. et al.** Antisense RNA as a molecular tool for genetic analysis. *Reviews - Trends in Genetics,* 1986, vol. 1 (1 **[0109]**
- **Mol et al.** *FEBS Letters,* 1990, vol. 268, 427-430 **[0109]**
- **Greisman ; Pabo.** *Science,* 1997, vol. 275, 657 **[0119]**
- **Greisman ; Pabo.** Science. Sangamo Biosciences, Inc, 1997, vol. 275, 657 **[0120]**
- Applications of HPLC in Biochemistry in: Laboratory Techniques in Biochemistry and Molecular Biology. **Rehm et al.** Biotechnology. VCH, 1993, vol. 3, 17, 469-714 **[0126]**
- **Belter, P.A. et al.** Bioseparations: downstream processing for biotechnology. John Wiley and Sons, 1988 **[0126]**
- **Kennedy, J.F. ; Cabral, J.M.S.** Recovery processes for biological materials. John Wiley and Sons, 1992 **[0126]**
- Biochemical separations. **Shaeiwitz, J.A. ; Henry, J.D.** Ulmann's Encyclopedia of Industrial Chemistry. VCH, 1988, vol. B3, 1-27 **[0126]**
- **Dechow, F.J.** Separation and purification techniques in biotechnology. Noyes Publications, 1989 **[0126]**
- **Groves, J.T.** *Curr. Opin. Chem. Biol.,* 1999, vol. 3 (2), 226-235 **[0128]**

- **Girke, T.** *The Plant Journal,* 1998, vol. 15, 39-48 **[0129]**
- **Puttaraju et al.** Spliceosome-mediated RNA trans-splicing as a tool for gene therapy. *Nature Biotechnology,* 1999, vol. 17, 246-252 **[0129]**
- **Harlow ; Lane.** Antibodies; A Laboratory Manual. Cold Spring Harbor Laboratory, 1988 **[0131]**
- **Kelly et al.** *Bio/Technology,* 1992, vol. 10, 163-167 **[0131]**
- **Bebbington et al.** *Bio/Technology,* 1992, vol. 10, 169-175 **[0131]**
- **Harlow ; Lane.** Antibodies, A Laboratory Manual. Cold Spring Harbor Publications, 1988 **[0132] [0133]**
- Basic and Clinical Immunology. Lange Medical Publications **[0133]**
- **H.L.K. Whitehouse.** *Gransden Wood* **[0134]**
- **Engel.** *Am. J. Bot.,* 1968, vol. 55, 438-446 **[0134]**
- **Reski ; Abel.** *Planta,* 1985, vol. 165, 354-358 **[0135]**
- **Reski et al.** *Mol. Gen. Genet.,* 1994, vol. 244, 352-359 **[0138]**
- **Pearson W.R.** Rapid and sensitive sequence comparison with FASTP and FASTA. *Methods Enzymol.,* 1990, vol. 183, 63-98 **[0141]**
- **Altschul S.F. et al.** Basic local alignment search tool. *Journal of Molecular Biology,* vol. 215, 403-10 **[0141]**
- **Frishman, D ; Argos; P.** 75% accuracy in polypeptide secondary structure prediction. *Polypeptides,* 1997, vol. 27, 329-335 **[0141]**
- **Thompson, J.D. et al.** CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. *Nucleic Acids Research,* 1994, vol. 22, 4673-4680 **[0141]**
- **Persson, B. ; Argos, P.** Prediction of transmembrane segments in polypeptides utilizing multiple sequence alignments. *J. Mol. Biol.,* 1994, vol. 237, 182-192 **[0141]**
- **Klein, P. et al.** Prediction of polypeptide function from sequence properties: A discriminate analysis of a database. *Biochim. Biophys. Acta,* 1984, vol. 787, 221-226 **[0141]**
- **Kolakowski L.F. Jr.** ProSearch: fast searching of polypeptide sequences with regular expression patterns related to polypeptide structure and function. *Biotechniques,* 1992, vol. 13, 919-921 **[0141]**
- **Bill Alford.** *CABIOS,* vol. 8, 249-254 **[0141]**
- **Sambrook et al.** Molecular Cloning, A Laboratory Manual. Cold Spring Harbor Laboratory Press, 1989 **[0146] [0152] [0154]**
- **Stephen et al.** Gapped BLAST and PSI_BLAST: a new generation of protein database search program. *Nucleic Acids Res.,* vol. 25, 3389-3402 **[0149]**
- **Sambrook et al.** Molecular Cloning, A Laboratory Manual. Cold Spring Harbor Laboratory, 1989 **[0161]**
- **Bechtold.** *Acad. Sci. Paris.,* 1993, vol. 316, 1194-1199 **[0165]**
- **Bent et al.** *Science,* 1994, vol. 265, 1856-1860 **[0165]**
- **Xiong et al.** *Plant Molecular Biology Reporter,* 1999, vol. 17, 159-170 **[0166]**
- **Ishida.** *Nature Biotech.,* 1996, 14745-50 **[0185]**
- **Ishida et al.** *Nature Biotech.,* 1996, 14745-50 **[0187]**
- **Sambrook, J. et al.** Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, 1989 **[0191] [0192]**
- **Ausubel, F.M. et al.** Current Protocols in Molecular Biology. John Wiley & Sons, 1994 **[0191] [0192]**
- **Gu et al.** *BioTechniques,* 1994, vol. 17, 257-262 **[0192]**
- **Rupp, W.D.** *DNA repair mechanisms, in: Escherichia coli and Salmonella,* 1996, 2277-2294 **[0193]**
- **Greener, A. ; Callahan, M.** *Strategies,* 1994, vol. 7, 32-34 **[0193]**
- **Dixon, M. ; Webb, E.C.** *Enzymes,* 1979 **[0194]**
- **Fersht.** Enzyme Structure and Mechanism. Freeman, 1985 **[0194]**
- **Walsh.** Enzymatic Reaction Mechanisms. Freeman, 1979 **[0194]**
- **Price, N.C. ; Stevens, L.** Fundamentals of Enzymology. Oxford Univ. Press, 1982 **[0194]**
- The Enzymes. Academic Press, 1983 **[0194]**
- **Bisswanger, H.** Enzymkinetik. VCH, 1994 **[0194]**
- Methods of Enzymatic Analysis. Verlag Chemie, 1983, vol. I-XII **[0194]**
- Ullmann's Encyclopedia of Industrial Chemistry. VCH, 1987, vol. A9, 352-363 **[0194]**
- **Kolmar, H. et al.** *EMBO J.,* 1995, vol. 14, 3895-3904 **[0195]**
- **Gennis, R.B.** Pores, Channels and Transporters, in Biomembranes, Molecular Structure and Function. Springer, 1989, 85-137199-234270-322 **[0196]**
- **Bailey, J.E. ; Ollis, D.F.** Biochemical Engineering Fundamentals. McGraw-Hill, 1986 **[0199]**
- **Patek et al.** *Appl. Environ. Microbiol.,* 1994, vol. 60, 133-140 **[0199]**
- **Malakhova et al.** *Biotekhnologiya,* 1996, vol. 11, 27-32 **[0199]**
- **Schmidt et al.** *Bioprocess Engineer,* 1998, vol. 19, 67-70 **[0199]**
- Ulmann's Encyclopedia of Industrial Chemistry. VCH, 1996, vol. A27, 89-90521-540540-547559-566575-581581-587 **[0199]**
- **Michal, G.** Biochemical Pathways: An Atlas of Biochemistry and Molecular Biology. John Wiley and Sons, 1999 **[0199]**
- **Fallon, A. et al.** *Applications of HPLC in Biochemistry in: Laboratory Techniques in Biochemistry and Molecular Biology,* 1987, vol. 17 **[0199]**