

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
5 February 2009 (05.02.2009)

PCT

(10) International Publication Number
WO 2009/016249 A2

(51) International Patent Classification:

C12N 15/82 (2006.01) *C12N 15/29* (2006.01)
C12N 9/02 (2006.01) *C07K 14/415* (2006.01)
C12N 15/53 (2006.01) *A01H 5/00* (2006.01)

(21) International Application Number:

PCT/EP2008/060106

(22) International Filing Date: 1 August 2008 (01.08.2008)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/953,562 2 August 2007 (02.08.2007) US

(71) Applicant (*for all designated States except US*): **BASF PLANT SCIENCE GMBH** [DE/DE]; 67056 Ludwigshafen (DE).

(72) Inventor; and

(75) Inventor/Applicant (*for US only*): **SHIRLEY, Amber** [US/US]; 123 Finsbury Street, Durham, NC 27703 (US).

(74) Agent: **KRIEGER, Stephan**; BASF SE, Carl-Bosch-Strasse, GVX - C 6, 67056 Ludwigshafen (DE).

(81) Designated States (*unless otherwise indicated, for every*

kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every*

kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- *without international search report and to be republished upon receipt of that report*
- *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

(54) Title: TRANSGENIC PLANTS WITH INCREASED STRESS TOLERANCE AND YIELD

(57) Abstract: Polynucleotides are disclosed which are capable of enhancing a growth, yield under water-limited conditions, and/or increased tolerance to an environmental stress of a plant transformed to contain such polynucleotides. Also provided are methods of using such polynucleotides and transgenic plants and agricultural products, including seeds, containing such polynucleotides as transgenes.



WO 2009/016249 A2

TRANSGENIC PLANTS WITH INCREASED STRESS TOLERANCE AND YIELD

This application claims priority benefit of U.S. provisional patent application Serial Number 60/953562, filed August 2, 2007, the contents of which are hereby incorporated by reference.

5

FIELD OF THE INVENTION

[0001] This invention relates generally to transgenic plants which overexpress nucleic acid sequences encoding polypeptides capable of conferring increased stress tolerance and consequently, increased plant growth and crop yield, under normal or abiotic stress conditions.

10 Additionally, the invention relates to novel isolated nucleic acid sequences encoding polypeptides that confer upon a plant increased tolerance under abiotic stress conditions, and/or increased plant growth and/or increased yield under normal or abiotic stress conditions.

BACKGROUND OF THE INVENTION

15 [0002] Abiotic environmental stresses, such as drought, salinity, heat, and cold, are major limiting factors of plant growth and crop yield. Crop yield is defined herein as the number of bushels of relevant agricultural product (such as grain, forage, or seed) harvested per acre. 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.

20 [0003] Water availability is an important aspect of the abiotic stresses and their effects on plant growth. Continuous exposure to drought conditions causes major alterations in the plant metabolism which ultimately lead to cell death and consequently to yield losses. 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
25 freezing temperatures, plant cells lose water as a result of ice formation within the plant. Accordingly, crop damage from drought, heat, salinity, and cold stress, is predominantly due to dehydration.

[0004] Because plants are typically exposed to conditions of reduced water availability during their life cycle, most plants have evolved protective mechanisms against desiccation
30 caused by abiotic stresses. However, if the severity and duration of desiccation conditions are too great, the effects on development, growth, plant size, and yield of most crop plants are profound. Developing plants efficient in water use is therefore a strategy that has the potential to significantly improve human life on a worldwide scale.

[0005] Traditional plant breeding strategies are relatively slow and require abiotic stress-
35 tolerant founder lines for crossing with other germplasm to develop new abiotic stress-resistant lines. Limited germplasm resources for such founder lines and incompatibility in crosses between distantly related plant species represent significant problems encountered in conventional breeding. Breeding for tolerance has been largely unsuccessful.

[0006] Many agricultural biotechnology companies have attempted to identify genes that
40 could confer tolerance to abiotic stress responses, in an effort to develop transgenic abiotic stress-tolerant crop plants. Although some genes that are involved in stress responses or water use efficiency in plants have been characterized, the characterization and cloning of plant genes that confer stress tolerance and/or water use efficiency remains largely incomplete and fragmented. To date, success at developing transgenic abiotic stress-tolerant crop plants has

been limited, and no such plants have been commercialized.

[0007] In order to develop transgenic abiotic stress-tolerant crop plants, it is necessary to assay a number of parameters in model plant systems, greenhouse studies of crop plants, and in field trials. For example, water use efficiency (WUE), is a parameter often correlated with drought tolerance. Studies of a plant's response to desiccation, osmotic shock, and temperature extremes are also employed to determine the plant's tolerance or resistance to abiotic stresses. When testing for the impact of the presence of a transgene on a plant's stress tolerance, the ability to standardize soil properties, temperature, water and nutrient availability and light intensity is an intrinsic advantage of greenhouse or plant growth chamber environments compared to the field.

[0008] WUE has been defined and measured in multiple ways. One approach is to calculate the ratio of whole plant dry weight, to the weight of water consumed by the plant throughout its life. Another variation is to use a shorter time interval when biomass accumulation and water use are measured. Yet another approach is to use measurements from restricted parts of the plant, for example, measuring only aerial growth and water use. WUE also has been defined as the ratio of CO₂ uptake to water vapor loss from a leaf or portion of a leaf, often measured over a very short time period (e.g. seconds/minutes). The ratio of ¹³C/¹²C fixed in plant tissue, and measured with an isotope ratio mass-spectrometer, also has been used to estimate WUE in plants using C₃ photosynthesis.

[0009] An increase in WUE is informative about the relatively improved efficiency of growth and water consumption, but this information taken alone does not indicate whether one of these two processes has changed or both have changed. In selecting traits for improving crops, an increase in WUE due to a decrease in water use, without a change in growth would have particular merit in an irrigated agricultural system where the water input costs were high. An increase in WUE driven mainly by an increase in growth without a corresponding jump in water use would have applicability to all agricultural systems. In many agricultural systems where water supply is not limiting, an increase in growth, even if it came at the expense of an increase in water use (i.e. no change in WUE), could also increase yield. Therefore, new methods to increase both WUE and biomass accumulation are required to improve agricultural productivity.

[0010] Concomitant with measurements of parameters that correlate with abiotic stress tolerance are measurements of parameters that indicate the potential impact of a transgene on crop yield. For forage crops like alfalfa, silage corn, and hay, the plant biomass correlates with the total yield. For grain crops, however, other parameters have been used to estimate yield, such as plant size, as measured by total plant dry weight, above-ground dry weight, above-ground fresh weight, leaf area, stem volume, plant height, rosette diameter, leaf length, root length, root mass, tiller number, and leaf number. Plant size at an early developmental stage will typically correlate with plant size later in development. A larger plant with a greater leaf area can typically absorb more light and carbon dioxide than a smaller plant and therefore will likely gain a greater weight during the same period. This is in addition to the potential continuation of the micro-environmental or genetic advantage that the plant had to achieve the larger size initially. There is a strong genetic component to plant size and growth rate, and so for a

range of diverse genotypes plant size under one environmental condition is likely to correlate with size under another. In this way a standard environment is used to approximate the diverse and dynamic environments encountered at different locations and times by crops in the field.

[0011] Harvest index, the ratio of seed yield to above-ground dry weight, is relatively stable under many environmental conditions and so a robust correlation between plant size and grain yield is possible. Plant size and grain yield are intrinsically linked, because the majority of grain biomass is dependent on current or stored photosynthetic productivity by the leaves and stem of the plant. Therefore, selecting for plant size, even at early stages of development, has been used as to screen for plants that may demonstrate increased yield when exposed to field testing. As with abiotic stress tolerance, measurements of plant size in early development, under standardized conditions in a growth chamber or greenhouse, are standard practices to measure potential yield advantages conferred by the presence of a transgene.

[0012] There is a need, therefore, to identify additional genes expressed in stress tolerant plants and/or plants that are efficient in water use that have the capacity to confer stress tolerance and/or increased water use efficiency to the host plant and to other plant species. Newly generated stress tolerant plants and/or plants with increased water use efficiency 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

[0013] The present inventors have discovered that transforming a plant with certain polynucleotides results in enhancement of the plant's growth and response to environmental stress, and accordingly the yield of the agricultural products of the plant is increased, when the polynucleotides are present in the plant as transgenes. The polynucleotides capable of mediating such enhancements have been isolated from *Brassica napus*, *Oryza sativa*, *Glycine max*, *Triticum aestivum*, *Hordeum vulgare*, *Zea mays*, and *Linum usitatissimum* and are listed in Table 1, and the sequences thereof are set forth in the Sequence Listing as indicated in Table 1.

Table 1

Gene ID	Organism	Polynucleotide SEQ ID NO	Amino acid SEQ ID NO
BN51364980	<i>B. napus</i>	1	2
OS34096188	<i>O. sativa</i>	3	4
OS32583643	<i>O. sativa</i>	5	6
GM53626178	<i>G. max</i>	7	8
TA56540264	<i>T. aestivum</i>	9	10
BN45206322	<i>B. napus</i>	11	12
GM48923793	<i>G. max</i>	13	14
TA55969932	<i>T. aestivum</i>	15	16
BN47310186	<i>B. napus</i>	17	18
BN51359456	<i>B. napus</i>	19	20

Gene ID	Organism	Polynucleotide SEQ ID NO	Amino acid SEQ ID NO
HV62552639	H. vulgare	21	22
ZM61995511	Z. mays	23	24
LU61567101	L. usitatissimum	25	26
LU61893412	L. usitatissimum	27	28
OS39781852	O. sativa	29	30
OS34701560	O. sativa	31	32
OS36821256	O. sativa	33	34
GM51659494	G. max	35	36
GM49780101	G. max	37	38
GM59637305	G. max	39	40
TA55974113	T. aestivum	41	42

[0014] In one embodiment, the invention provides a transgenic plant transformed with an expression cassette comprising an isolated polynucleotide encoding a methionine sulfoxide reductase family protein selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, and SEQ ID NO:10, or a SelR protein domain.

[0015] In another embodiment, the invention provides a transgenic plant transformed with an expression cassette comprising an isolated polynucleotide encoding a homeodomain leucine zipper protein having a sequence as set forth in SEQ ID NO:12, SEQ ID NO:14, or SEQ ID NO: 16.

[0016] In another embodiment, the invention provides a transgenic plant transformed with an expression cassette comprising an isolated polynucleotide encoding a zinc finger protein containing an A20 domain in combination with an AN1 domain selected from the group consisting of SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, and SEQ ID NO:44, or both AN1-like and A20-like zinc finger protein domains.

[0017] In a further embodiment, the invention concerns a seed produced by the transgenic plant of the invention, wherein the seed is true breeding for a transgene comprising the polynucleotide described above. Plants derived from the seed of the invention demonstrate increased tolerance to an environmental stress, and/or increased plant growth, and/or increased yield, under normal or stress conditions as compared to a wild type variety of the plant.

[0018] In a still another aspect, the invention concerns products produced by or from the transgenic plants of the invention, their plant parts, or their seeds, such as a foodstuff, feedstuff, food supplement, feed supplement, cosmetic or pharmaceutical.

[0019] The invention further provides the isolated polynucleotides identified in Table 1 below, and polypeptides identified in Table 1. The invention is also embodied in recombinant vector comprising an isolated polynucleotide of the invention.

[0020] In yet another embodiment, the invention concerns a method of producing the aforesaid transgenic plant, wherein the method comprises transforming a plant cell with an ex-

pression vector comprising an isolated polynucleotide of the invention, and generating from the plant cell a transgenic plant that expresses the polypeptide encoded by the polynucleotide. Expression of the polypeptide in the plant results in increased tolerance to an environmental stress, and/or growth, and/or yield under normal and/or stress conditions as compared to a wild type variety of the plant.

[0021] In still another embodiment, the invention provides a method of increasing a plant's tolerance to an environmental stress, and/or growth, and/or yield. The method comprises the steps of transforming a plant cell with an expression cassette comprising an isolated polynucleotide of the invention, and generating a transgenic plant from the plant cell, wherein the transgenic plant comprises the polynucleotide.

BRIEF DESCRIPTION OF THE DRAWINGS

[0022] Figure 1 is an alignment of BN51364980, OS34096188, OS32583643, GM53626178, TA56540264 with a known methionine sulfoxide reductase family protein.

[0023] Figure 2 is an alignment of BN45206322, GM48923793, and TA55969932 with a known homeodomain leucine zipper protein.

[0024] Figure 3 is an alignment of BN47310186, BN51359456, HV62552639, ZM61995511, LU61567101, LU61893412, OS39781852, OS34701560, OS36821256, GM51659494, GM49780101, GM59637305, and TA55974113 with a known A20 and AN1 domain containing zinc finger protein.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0025] Throughout this application, various publications are referenced. The disclosures of all of these publications and those references cited within those publications in their entireties are hereby incorporated by reference into this application in order to more fully describe the state of the art to which this invention pertains. The terminology used herein is for the purpose of describing specific embodiments only and is not intended to be limiting. As used herein, "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 used.

[0026] In one embodiment, the invention provides a transgenic plant that overexpresses an isolated polynucleotide identified in Table 1, or a homolog thereof. The transgenic plant of the invention demonstrates an increased tolerance to an environmental stress as compared to a wild type variety of the plant. The overexpression of such isolated nucleic acids in the plant may optionally result in an increase in plant growth or in yield of associated agricultural products, under normal or stress conditions, as compared to a wild type variety of the plant. Without wishing to be bound by any theory, the increased tolerance to an environmental stress, increased growth, and/or increased yield of a transgenic plant of the invention is believed to result from an increase in water use efficiency of the plant.

[0027] As defined herein, a "transgenic plant" is a plant that has been altered using recombinant DNA technology to contain an isolated nucleic acid which would otherwise not be present in the plant. As used herein, the term "plant" includes a whole plant, plant cells, and plant parts. 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. The transgenic plant of the invention may be male sterile or male fertile, and may further include transgenes other than those that comprise the isolated polynucleotides described herein.

[0028] As used herein, the term "variety" refers to a group of plants within a species that share constant characteristics 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 isolated polynucleotides introduced into a plant variety. As also used herein, the term "wild type variety" refers to a group of plants that are analyzed for comparative purposes as a control plant, wherein the wild type variety plant is identical to the transgenic plant (plant transformed with an isolated polynucleotide in accordance with the invention) with the exception that the wild type variety plant has not been transformed with an isolated polynucleotide of the invention.

[0029] As defined herein, the term "nucleic acid" and "polynucleotide" are interchangeable and 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. 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). For example, a cloned nucleic acid is considered isolated. 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 transformation. 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. While it may optionally encompass untranslated sequence located at both the 3' and 5' ends of the coding region of a gene, it may be preferable to remove the sequences which naturally flank the coding region in its naturally occurring replicon.

[0030] As used herein, the term "environmental stress" refers to a sub-optimal condition associated with salinity, drought, nitrogen, temperature, metal, chemical, pathogenic, or oxidative stresses, or any combination thereof. The terms "water use efficiency" and "WUE" refer to the amount of organic matter produced by a plant divided by the amount of water used by the plant in producing it, i.e., the dry weight of a plant in relation to the plant's water use. As used herein, the term "dry weight" refers to everything in the plant other than water, and includes, for example, carbohydrates, proteins, oils, and mineral nutrients.

[0031] Any plant species may be transformed to create a transgenic plant in accordance with the invention. The transgenic plant of the invention may be a dicotyledonous plant or a monocotyledonous plant. For example and without limitation, transgenic plants of the invention

may be derived from any of the following dicotyledonous plant families: Leguminosae, including plants such as pea, alfalfa and soybean; Umbelliferae, including plants such as carrot and celery; Solanaceae, including the plants such as tomato, potato, aubergine, tobacco, and pepper; Cruciferae, particularly the genus Brassica, which includes plant such as oilseed rape, beet, cabbage, cauliflower and broccoli); and Arabidopsis thaliana; Compositae, which includes plants such as lettuce; Malvaceae, which includes cotton; Fabaceae, which includes plants such as peanut, and the like. Transgenic plants of the invention may be derived from monocotyledonous plants, such as, for example, wheat, barley, sorghum, millet, rye, triticale, maize, rice, oats and sugarcane. Transgenic plants of the invention are also embodied as trees such as apple, pear, quince, plum, cherry, peach, nectarine, apricot, papaya, mango, and other woody species including coniferous and deciduous trees such as poplar, pine, sequoia, cedar, oak, and the like. Especially preferred are Arabidopsis thaliana, Nicotiana tabacum, oilseed rape, soybean, corn (maize), wheat, linseed, potato and tagetes.

[0032] As shown in Table 1, one embodiment of the invention is a transgenic plant transformed with an expression cassette comprising an isolated polynucleotide encoding a methionine sulfoxide reductase family protein. Methionine sulfoxide reductases (MSRs) catalyze the thioredoxin-dependent reduction of methionine sulfoxide (MetSO) to the correct methionine residue. Methionine is highly susceptible to oxidative damage, and methionine oxidation results in modification of the activity and conformation of many proteins.

[0033] There are two types of MSRs, type A and type B; however, these two types are unrelated in both sequence and structure. The MSRB enzyme selectively catalytically reduces the MetSO R enantiomer. MSRB type proteins contain four conserved cysteine residues in two CxxC motifs, where x can be any amino acid. These CxxC motifs are potentially involved in zinc fixation.

[0034] There are described examples from several plant species where environmental stress conditions result in increased reactive oxygen species (ROS) levels and resulting oxidative damage leads to modification in MSR gene expression. MSRs themselves are good candidates for direct antioxidants since cyclic oxidation and reduction of methionine residues could function as an efficient pathway to remove ROS in cells. In eukaryotes, senescence and a host of diseases are triggered by methionine oxidation resulting in the disruption of protein structure and function. The substrates of MSR proteins are largely unknown. To date, the first plant MSR substrate to have been identified is the small, plastidic heat shock protein Hsp21. Hsp21 contains a conserved N-terminal region that is highly enriched in methionine residues. This methionine region must be maintained in the reduced form in order to maintain the chaperone-like activity of Hsp21.

[0035] The transgenic plant of this embodiment may comprise any polynucleotide encoding a methionine sulfoxide reductase family protein. Preferably, the transgenic plant of this embodiment comprises a polynucleotide encoding a SelR domain having a sequence comprising amino acids 77 to 199 of SEQ ID NO:2; amino acids 79 to 200 of SEQ ID NO: 4; amino acids 91 to 213 of SEQ ID NO: 6; amino acids 79 to 200 of SEQ ID NO: 8; amino acids 80 to 201 of SEQ ID NO: 10. More preferably, the transgenic plant of this embodiment comprises a polynucleotide encoding a methionine sulfoxide reductase family protein having a sequence

comprising amino acids 1 to 205 of SEQ ID NO:2; amino acids 1 to 204 of SEQ ID NO: 4; amino acids 1 to 214 of SEQ ID NO: 6; amino acids 1 to 202 of SEQ ID NO: 8; amino acids 1 to 206 of SEQ ID NO: 10.

[0036] In another embodiment, the invention provides a transgenic plant transformed with an expression cassette comprising an isolated polynucleotide encoding a homeodomain leucine zipper protein. Homeodomain leucine zipper (HDZip) proteins belong to a family of transcription factors that interact as dimers via a leucine zipper domain and bind DNA in a sequence specific manner via their homeodomains. Based upon sequence, the HDZip family proteins are divided into four classes. The Class I HDZip proteins are suggested to regulate plant response to ABA and may have regulatory roles related to ABA signalling. The Class I HDZip protein members can form heterodimers in vitro; therefore, this class may constitute an interacting network of proteins that mediates responses to environmental stimuli and/or integrates signals to regulate similar sets of target genes.

[0037] The transgenic plant of this embodiment may comprise any polynucleotide encoding a homeodomain leucine zipper protein. Preferably, the transgenic plant of this embodiment comprises a polynucleotide encoding a homeobox domain having a sequence comprising amino acids 62 to 116 of SEQ ID NO:12; amino acids 83 to 137 of SEQ ID NO: 14; amino acids 76 to 130 of SEQ ID NO: 16 or a homeobox associated leucine zipper domain having a sequence comprising amino acids 117 to 161 of SEQ ID NO: 12; amino acids 138 to 182 of SEQ ID NO: 14; amino acids 131 to 175 of SEQ ID NO: 16. More preferably, the transgenic plant of this embodiment comprises a polynucleotide encoding a homeodomain leucine zipper protein having a sequence comprising amino acids 1 to 310 of SEQ ID NO:12; amino acids 1 to 331 of SEQ ID NO: 14; amino acids 1 to 340 of SEQ ID NO: 16.

[0038] In another embodiment, the invention provides a transgenic plant transformed with an expression cassette comprising an isolated polynucleotide encoding a A20 and AN1 domain containing zinc finger protein. The A20 and AN1 domain containing zinc finger proteins are found in all eukaryotes. These proteins are characterized by the presence of an A20 zinc finger domain containing multiple Cysteine₂/Cysteine₂ finger motifs and an AN1 zinc finger domain. The AN1 domain is usually found in proteins containing the A20 zinc finger domain. The function of these proteins is well characterized in animal systems, but little is known about the function of these proteins in plants.

[0039] The rice OsiSAP1 protein was identified as the first plant protein having both A20 and AN1 zinc finger domains. This protein was found to be associated with multiple stresses. The OsiSAP1 gene is induced in response to environmental stresses such as cold, salt, drought, submergence, wounding, and heavy metals. An ortholog from bean is also known to be inducible when eliciter treated and in response to wounding. When overexpressed in tobacco, OsiSAP1 engenders abiotic stress tolerance. OsiSAP1 does not have a typical nuclear localization signal and thus is believed to function via the zinc finger domains for protein-protein interaction.

[0040] The transgenic plant of this embodiment may comprise any polynucleotide encoding a zinc finger protein containing an A20 domain in combination with an AN1 domain. Preferably, the transgenic plant of this embodiment comprises a polynucleotide encoding an

A20-like zinc finger having a sequence comprising amino acids 15 to 39 of SEQ ID NO:18; amino acids 13 to 37 of SEQ ID NO: 20; amino acids 15 to 39 of SEQ ID NO: 22; amino acids 14 to 38 of SEQ ID NO: 24; amino acids 14 to 38 of SEQ ID NO: 26; amino acids 40 to 64 of SEQ ID NO: 28; amino acids 15 to 39 of SEQ ID NO: 30; amino acids 19 to 43 of SEQ ID NO: 32; amino acids 13 to 37 of SEQ ID NO: 34; amino acids 19 to 43 of SEQ ID NO: 36; amino acids 18 to 42 of SEQ ID NO: 38; amino acids 15 to 39 of SEQ ID NO: 40; amino acids 15 to 39 of SEQ ID NO: 42; amino acids 19 to 43 of SEQ ID NO: 44 and an AN1-like zinc finger domain having a sequence comprising amino acids 118 to 158 of SEQ ID NO: 18; amino acids 128 to 168 of SEQ ID NO: 20; amino acids 95 to 135 of SEQ ID NO: 22; amino acids 112 to 152 of SEQ ID NO: 24; amino acids 115 to 155 of SEQ ID NO: 26; amino acids 179 to 219 of SEQ ID NO: 28; amino acids 110 to 150 of SEQ ID NO: 30; amino acids 105 to 145 of SEQ ID NO: 32; amino acids 105 to 145 of SEQ ID NO: 34; amino acids 111 to 151 of SEQ ID NO: 36; amino acids 102 to 142 of SEQ ID NO: 38; amino acids 113 to 153 of SEQ ID NO: 40; amino acids 115 to 155 of SEQ ID NO: 42; amino acids 106 to 146 of SEQ ID NO: 44. More preferably, the transgenic plant of this embodiment comprises a polynucleotide encoding a A20 and AN1 domain containing zinc finger protein having a sequence comprising amino acids 1 to 177 of SEQ ID NO:18; amino acids 1 to 187 of SEQ ID NO: 20; amino acids 1 to 154 of SEQ ID NO: 22; amino acids 1 to 171 of SEQ ID NO: 24; amino acids 1 to 174 of SEQ ID NO: 26; amino acids 1 to 239 of SEQ ID NO: 28; amino acids 1 to 169 of SEQ ID NO: 30; amino acids 1 to 164 of SEQ ID NO: 32; amino acids 1 to 164 of SEQ ID NO: 34; amino acids 1 to 170 of SEQ ID NO: 36; amino acids 1 to 161 of SEQ ID NO: 38; amino acids 1 to 172 of SEQ ID NO: 40; amino acids 1 to 174 of SEQ ID NO: 42, amino acids 1 to 165 of SEQ ID NO: 44.

[0041] The invention further provides a seed produced by a transgenic plant expressing polynucleotide listed in Table 1, wherein the seed contains the polynucleotide, and wherein the plant is true breeding for increased growth and/or yield under normal or stress conditions and/or increased tolerance to an environmental stress as compared to a wild type variety of the plant. The invention also provides a product produced by or from the transgenic plants expressing the polynucleotide, their plant parts, or their seeds. The product can be obtained using various methods well known in the art. As used herein, the word "product" includes, but not limited to, a foodstuff, feedstuff, a food supplement, feed supplement, cosmetic or pharmaceutical. Foodstuffs are regarded as compositions used for nutrition or for supplementing nutrition. Animal feedstuffs and animal feed supplements, in particular, are regarded as foodstuffs. The invention further provides an agricultural product produced by any of the 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.

[0042] In a preferred embodiment, an isolated polynucleotide of the invention comprises a polynucleotide having a sequence selected from the group consisting of the polynucleotide sequences listed in Table 1. These polynucleotides may comprise sequences of the coding region, as well as 5' untranslated sequences and 3' untranslated sequences.

[0043] A polynucleotide of the invention can be isolated using standard molecular biology techniques and the sequence information provided herein. Synthetic oligonucleotide primers for polymerase chain reaction amplification can be designed based upon the nucleotide se-

quence shown in Table 1. 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 the nucleotide sequences listed in Table 1 can be prepared by standard synthetic techniques, e.g., using an automated DNA synthesizer.

[0044] "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, analogs, and orthologs, as defined below. 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. The term homolog further encompasses nucleic acid molecules that differ from one of the nucleotide sequences shown in Table 1 due to degeneracy of the genetic code and thus encode the same polypeptide. 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).

[0045] To determine the percent sequence identity of two amino acid sequences (e.g., one of the polypeptide sequences of Table 1 and a homolog 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 is occupied by the same amino acid residue as the corresponding position in the other sequence then the molecules are identical at that position. The same type of comparison can be made between two nucleic acid sequences.

[0046] Preferably, the isolated amino acid homologs, analogs, and orthologs of the polypeptides of the present invention are at least about 50-60%, preferably at least about 60-70%, and more preferably at least about 70-75%, 75-80%, 80-85%, 85-90%, or 90-95%, and most preferably at least about 96%, 97%, 98%, 99%, or more identical to an entire amino acid sequence identified in Table 1. In another preferred embodiment, an isolated nucleic acid homolog of the invention comprises a nucleotide sequence which is at least about 40-60%, preferably at least about 60-70%, more preferably at least about 70-75%, 75-80%, 80-85%, 85-90%, or 90-95%, and even more preferably at least about 95%, 96%, 97%, 98%, 99%, or more identical to a nucleotide sequence shown in Table 1.

[0047] The percent sequence identity between two nucleic acid or polypeptide sequences may be determined using, for example, the Vector NTI 9.0 (PC) software package (Invitrogen, 1600 Faraday Ave., Carlsbad, CA92008). When Vector NTI is used, a gap opening penalty of 15 and a gap extension penalty of 6.66 may be used for determining the percent identity of two nucleic acids and a gap opening penalty of 10 and a gap extension penalty of 0.1 may be used for determining the percent identity of two polypeptides. All other Vector NTI parameters may be set at the default settings. For purposes of a multiple alignment (Clustal W algorithm) using Vector NTI, the gap opening penalty is 10, and the gap extension penalty is

0.05 with blosum62 matrix. Alternatively, Align 2.0 (Myers and Miller (1989) CABIOS 4, 11-17) may be used, with all parameters set to default settings. 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.

5 [0048] Nucleic acid molecules corresponding to homologs, analogs, and orthologs of the polypeptides listed in Table 1 can be isolated based on their identity to said polypeptides, using the polynucleotides encoding the respective polypeptides or primers based thereon, as hybridization probes according to standard hybridization techniques under stringent hybridization conditions. As used herein with regard to hybridization for DNA to a DNA blot, the term "stringent conditions" refers to hybridization overnight at 60°C in 10X Denhart's 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, in a preferred embodiment, the phrase "stringent conditions" refers to hybridization in a 6X SSC solution at 65°C. In another embodiment, "highly stringent conditions" refers to hybridization overnight at 65°C in 10X Denhart's 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; well known in the art (see, for example, Current Protocols in Molecular Biology, Chapter 2, Ausubel et al., eds., Greene Publishing and Wiley-Interscience, New York, 1995; and Tijssen, 1993, Laboratory Techniques in Biochemistry and Molecular Biology: Hybridization with Nucleic Acid Probes, Part I, Chapter 2, Elsevier, New York, 1993). Preferably, an isolated nucleic acid molecule of the invention that hybridizes under stringent or highly stringent conditions to a nucleotide sequence listed in Table 1 corresponds to a naturally occurring nucleic acid molecule.

[0049] There are a variety of methods that can be used to produce libraries of potential 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 sequences. Methods for synthesizing degenerate oligonucleotides are known in the art (See, e.g., Narang, 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).

[0050] Additionally, optimized nucleic acids can be created. Preferably, an optimized nucleic acid encodes a polypeptide that has a function similar to those of the polypeptides listed in Table 1 and/or modulates a plant's growth and/or yield under normal and/or water-limited conditions and/or tolerance to an environmental stress, and more preferably increases a plant's growth and/or yield under normal and/or water-limited conditions and/or 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 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; 4) to eliminate sequences that cause destabilization, inappropriate polyadenylation, degradation and termination of RNA, or that form secondary structure hairpins or RNA splice sites; or 5) elimination of antisense open reading frames. Increased expression of nucleic acids in plants can be achieved by utilizing the distribution frequency of codon usage in plants in general or in 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.

[0051] An isolated polynucleotide of the invention 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 nucleic acids of this invention also preferably have CG and TA doublet avoidance indices closely approximating those of the chosen host plant. More preferably, these indices deviate from that of the host by no more than about 10-15%.

[0052] The invention further provides an isolated recombinant expression vector comprising a polynucleotide as described above, wherein expression of the vector in a host cell results in the plant's increased growth and/or yield under normal or water-limited conditions and/or increased tolerance to environmental stress as compared to a wild type variety of the host cell. 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 a bacterial or plant 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 well known in the art. 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 encoded by nucleic acids as described herein.

[0053] Plant gene expression should be operatively linked to an appropriate promoter conferring gene expression in a timely, cell specific, or tissue specific manner. Promoters use-

ful 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*.

5 [0054] 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 35S promoters (Odell et al., 1985, *Nature* 313:810-812), the sX CaMV 35S promoter (Kay et al., 1987, *Science* 236:1299-1302) the Sep1 promoter, the rice actin promoter (McElroy et al., 1990, *Plant Cell* 10 2:163-171), the Arabidopsis actin promoter, the ubiquitin promoter (Christensen et al., 1989, *Plant Molec. Biol.* 18:675-689), pEmu (Last et al., 1991, *Theor. Appl. Genet.* 81:581-588), the figwort mosaic virus 35S promoter, the Smas promoter (Velten et al., 1984, *EMBO J* 3:2723-2730), the super promoter (U.S. Patent No. 5, 955,646), the GRP1-8 promoter, the cinnamyl alcohol dehydrogenase promoter (U.S. Patent No. 5,683,439), promoters from the T-DNA of 15 *Agrobacterium*, such as mannopine synthase, nopaline synthase, and octopine synthase, the small subunit of ribulose biphosphate carboxylase (ssuRUBISCO) promoter, and the like.

[0055] Inducible promoters are preferentially 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 20 induced by heat shock; the PPKK promoter is induced by light; the PR-1 promoters 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 25 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).

[0056] In one preferred embodiment of the present invention, the inducible promoter is a 30 stress-inducible promoter. For the purposes of the invention, stress-inducible promoters are preferentially active under one or more of the following stresses: sub-optimal conditions associated with salinity, drought, nitrogen, temperature, metal, chemical, pathogenic, and oxidative stresses. 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., 35 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* 40 149:479-90), KAT1 (Nakamura et al., 1995, *Plant Physiol.* 109:371-4), KST1 (Müller-Röber et al., 1995, *EMBO* 14:2409-16), Rha1 (Terry et al., 1993, *Plant Cell* 5:1761-9; Terry 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-Shinozaki et al., 1993, Mol. Gen. Genet. 236:331-340.

[0057] 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, leaf-preferred, stigma-preferred, pollen-preferred, anther-preferred, 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.

[0058] 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 lpt2 or lpt1-gene promoter from barley (PCT Application No. WO 95/15389 and PCT Application No. WO 95/23230) or those described in PCT Application No. WO 99/16890 (promoters from the barley hordein-gene, rice glutelin gene, rice oryzin gene, rice prolamin gene, wheat gliadin gene, wheat glutelin gene, oat glutelin gene, Sorghum kasirin-gene, and rye secalin gene).

[0059] 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.

[0060] 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).

[0061] In a preferred embodiment of the present invention, the polynucleotides listed in Table 1 are expressed in plant cells from higher plants (e.g., the spermatophytes, such as crop plants). A polynucleotide may be "introduced" into a plant cell by any means, including transfection, transformation or transduction, electroporation, particle bombardment, agroinfection, and the like. Suitable methods for transforming or transfecting plant cells are disclosed, for example, using particle bombardment as set forth in U.S. Pat. Nos. 4,945,050; 5,036,006; 5,100,792; 5,302,523; 5,464,765; 5,120,657; 6,084,154; and the like. More preferably, the transgenic corn seed of the invention may be made using Agrobacterium transformation, as described in U.S. Pat. Nos. 5,591,616; 5,731,179; 5,981,840; 5,990,387; 6,162,965; 6,420,630, U.S. patent application publication number 2002/0104132, and the like. Transformation of soybean can be performed using for example a technique described in European Patent No. EP 0424047, U.S. Patent No. 5,322,783, European Patent No. EP 0397 687, U.S. Patent No. 5,376,543, or U.S. Patent No. 5,169,770. A specific example of wheat transformation can be found in PCT Application No. WO 93/07256. Cotton may be transformed using methods disclosed in U.S. Pat. Nos. 5,004,863; 5,159,135; 5,846,797, and the like. Rice may be transformed using methods disclosed in U.S. Pat. Nos. 4,666,844; 5,350,688; 6,153,813; 6,333,449; 6,288,312; 6,365,807; 6,329,571, and the like. Other plant transformation methods are disclosed, for example, in U.S. Pat. Nos. 5,932,782; 6,153,811; 6,140,553; 5,969,213; 6,020,539, and the like. Any plant transformation method suitable for inserting a transgene into a particular plant may be used in accordance with the invention.

[0062] According to the present invention, the introduced polynucleotide may be maintained in the plant cell stably if it is incorporated into a non-chromosomal autonomous replicon or integrated into the plant chromosomes. Alternatively, the introduced polynucleotide may be present on an extra-chromosomal non-replicating vector and may be transiently expressed or transiently active.

[0063] Another aspect of the invention pertains to an isolated polypeptide having a sequence selected from the group consisting of the polypeptide sequences listed in Table 1. An "isolated" or "purified" polypeptide 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 a polypeptide 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 polypeptide of the invention having less than about 30% (by dry weight) of contaminating polypeptides, more preferably less than about 20% of contaminating polypeptides, still more preferably less than about 10% of contaminating polypeptides, and most preferably less than about 5% contaminating polypeptides.

[0064] 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 are abundant and well known to one skilled in the art.

[0065] The invention is also embodied in a method of producing a transgenic plant comprising at least one polynucleotide listed in Table 1, wherein expression of the polynucleotide in the plant results in the plant's increased growth and/or yield under normal or water-limited conditions and/or increased tolerance to an environmental stress as compared to a wild type variety of the plant comprising the steps of: (a) introducing into a plant cell an expression vector comprising at least one polynucleotide listed in Table 1, and (b) generating from the plant cell a transgenic plant that expresses the polynucleotide, wherein expression of the polynucleotide in the transgenic plant results in the plant's increased growth and/or yield under normal or water-limited conditions and/or increased tolerance to environmental stress as compared to a wild type variety of the plant. The plant cell may be, 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 at least one recombinant polynucleotide listed in Table 1. In many cases, the recombinant polynucleotide is stably integrated into a chromosome or stable extra-chromosomal element, so that it is passed on to successive generations.

[0066] The present invention also provides a method of increasing a plant's growth and/or yield under normal or water-limited conditions and/or increasing a plant's tolerance to an environmental stress comprising the steps of increasing the expression of at least one polynucleotide listed in Table 1 in the plant. Expression of a protein can be increased by any method known to those of skill in the art.

[0067] The effect of the genetic modification on plant growth and/or yield and/or stress tolerance can be assessed by growing the modified plant under normal and/or 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, metabolite composition, etc., using methods known to those of skill in biotechnology.

[0068] The invention is further illustrated by the following examples, which are not to be construed in any way as imposing limitations upon the scope thereof.

EXAMPLE 1

Cloning of full-length cDNAs

[0069] The full-length DNA sequence of the *Physcomitrella patens* EST65 methionine sulfoxide reductase family protein (SEQ ID NO:43) was blasted against proprietary databases of canola, soybean, rice, maize, linseed, sunflower, barley, and wheat cDNAs at an e value of e^{-10} (Altschul et al., 1997, Nucleic Acids Res. 25: 3389-3402). All the contig hits were analyzed for the putative full length sequences, and the longest clones representing the putative full length contigs were fully sequenced. One homolog from canola, two homologs from rice, one homolog from soybean, and one homolog from wheat were identified. The degrees of amino acid iden-

tity and similarity of these sequences to the respective closest known public sequences are indicated in Tables 2 through 6 (Align 2.0).

Table 2

5 Comparison of BN51364980 (SEQ ID NO:2) to known methionine sulfoxide reductases

Public Database Accession #	Species	Sequence Identity (%)
NP_564640	Arabidopsis thaliana	82.00%
AAM65202	A. thaliana	66.70%
BAD35399	O. sativa	56.10%
NP_001057620	O. sativa	55.80%
ZP_01592095	Geobacter lovleyi SZ	42.40%

Table 3

Comparison of OS34096188 (SEQ ID NO:4) to known methionine sulfoxide reductases

Public Database Accession #	Species	Sequence Identity (%)
ABE84787	Medicago truncatula	72.60%
NP_567639	A. thaliana	61.90%
AAM62876	A. thaliana	61.00%
NP_567271	A.thaliana	58.70%
EAY98001	O. sativa	57.80%

10 Table 4

Comparison of OS32583643 (SEQ ID NO:6) to known methionine sulfoxide reductases

Public Database Accession #	Species	Sequence Identity (%)
NP_001057620	O. sativa	99.10%
BAD35399	O. sativa	74.80%
NP_564640	A. thaliana	59.60%
AAM65202	A.thaliana	53.70%
YP_846684	Syntrophobacter fumaroxidans MPOB	44.20%

Table 5

Comparison of GM53626178 (SEQ ID NO:8) to known methionine sulfoxide reductases

Public Data- base Accession #	Species	Sequence Identity (%)
ABE84787	M. truncatula	72.80%
NP_567639	A. thaliana	60.90%
AAM62876	A. thaliana	60.90%
EAY98001	O. sativa	59.10%
AAO72582	O. sativa	57.60%

Table 6

5 Comparison of TA56540264 (SEQ ID NO:10) to known methionine sulfoxide reductases

Public Data- base Accession #	Species	Sequence Identity (%)
EAY98001	O. sativa	72.90%
NP_001055501	O. sativa	69.50%
ABE84787	M. truncatula	65.50%
NP_567639	A. thaliana	60.60%
AAM62876	A. thaliana	59.60%

[0070] The full-length DNA sequence of the *P. patens* EST12 homeodomain leucine zipper protein (SEQ ID NO:44) was blasted against proprietary databases of canola, soybean, rice, maize, linseed, sunflower, barley, and wheat cDNAs at an e value of e^{-10} (Altschul et al., 1997, Nucleic Acids Res. 25: 3389-3402). All the contig hits were analyzed for the putative full length sequences, and the longest clones representing the putative full length contigs were fully sequenced. One homolog from canola, one homolog from soybean, and one homolog from wheat were identified. The degrees of amino acid identity and similarity of these sequences to the respective closest known public sequence are indicated in Tables 7 through 9 (Align 2.0).

15 Table 7

Comparison of BN45206322 (SEQ ID NO:12) to known homeodomain leucine zipper proteins

Public Data- base Accession #	Species	Sequence Identity (%)
AAR04932	B. napus	95.20%
AAF73482	Brassica rapa	91.30%
AAD41726	A. thaliana	81.20%
NP_195716	A. thaliana	69.90%
AAK96762	A. thaliana	69.60%

Table 8

Comparison of GM48923793 (SEQ ID NO:14) to known homeodomain leucine zipper proteins

Public Database Accession #	Species	Sequence Identity (%)
AAX98670	G.e max	56.00%
AAK84886	Phaseolus vulgaris	51.60%
CAA64417	Solanum lycopersicum	47.40%
BAA05624	Daucus carota	46.70%
AAF01765	G. max	44.00%

Table 9

5 Comparison of TA55969932 (SEQ ID NO: 16) to known homeodomain leucine zipper proteins

Public Data-base Accession #	Species	Sequence Identity (%)
NP_001048008	O. sativa	74.00%
EAY87390	O. sativa	73.80%
NP_001061807	O. sativa	49.90%
AAD37698	O. sativa	49.60%
AAS83417	O. sativa	46.30%

[0071] The full-length DNA sequence of the *P. patens* EST307 A20 and AN1 domain containing zinc finger protein (SEQ ID NO:45) was blasted against proprietary databases of canola, soybean, rice, maize, linseed, sunflower, barley, and wheat cDNAs at an e value of e^{-10} (Altschul et al., 1997, Nucleic Acids Res. 25: 3389-3402). All the contig hits were analyzed for the putative full length sequences, and the longest clones representing the putative full length contigs were fully sequenced. Two homologs from canola, one homolog from barley, two homologs from corn, two homologs from linseed, three homolog from soybean, three homologs from rice, and one homolog from wheat were identified. The degrees of amino acid identity and similarity of these sequences to the respective closest known public sequences are indicated in Tables 10 through 22 (Align 2.0).

Table 10

Comparison of BN47310186 (SEQ ID NO: 18) to known A20 and AN1 domain containing zinc finger proteins

Public Data-base Accession #	Species	Sequence Identity (%)
NP_564585	A. thaliana	88.70%
AAN71995	A. thaliana	88.10%
ABL67658	Citrus cv. Shiranuhi	59.40%
AAQ84334	O. sativa	56.00%
AAD38146	Prunus armeniaca	55.20%

5

Table 11

Comparison of BN51359456 (SEQ ID NO: 20) to known A20 and AN1 domain containing zinc finger proteins

Public Data-base Accession #	Species	Sequence Identity (%)
NP_190848	A. thaliana	71.60%
AAK68811	A. thaliana	71.10%
NP_565844	A. thaliana	66.00%
ABE93196	M. truncatula	51.10%
AAN71995	A. thaliana	47.10%

10

Table 12

Comparison of HV62552639 (SEQ ID NO: 22) to known A20 and AN1 domain containing zinc finger proteins

Public Data-base Accession #	Species	Sequence Identity (%)
NP_001055132	O. sativa	70.10%
AAR96005	Musa acuminata	51.80%
AAA33773	P. vulgaris	42.60%
EAZ09556	O. sativa	40.80%
EAZ45178	O. sativa	39.40%

15

Table 13

Comparison of ZM61995511 (SEQ ID NO: 24) to known A20 and AN1 domain containing zinc finger proteins

Public Data- base Accession #	Species	Sequence Identity (%)
AAQ84334	O. sativa	79.50%
AAX14637	Z. mays	77.00%
EAZ01657	O. sativa	71.70%
ABL67658	Citrus cv. Shiranuhi	69.50%
NP_001046186	O. sativa	65.90%

5

Table 14

Comparison of LU61567101 (SEQ ID NO: 26) to known A20 and AN1 domain containing zinc finger proteins

Public Data- base Accession #	Species	Sequence Identity (%)
CAE73100	Caenorhabditis briggsae	29.30%
NP_190848	A. thaliana	29.20%
XP_001357850	Drosophila pseudoobscura	29.20%
EAY92150	O. sativa	29.10%
ABL97956	B. rapa	28.90%

10

Table 15

Comparison of LU61893412 (SEQ ID NO: 28) to known A20 and AN1 domain containing zinc finger proteins

Public Data- base Accession #	Species	Sequence Identity (%)
ABL67658	Citrus cv. Shi- ranuhi	67.40%
AAD38146	P. armeniaca	64.60%
AAQ84334	O. sativa	61.70%
ABN08135	M. truncatula	61.50%
AAN71995	A. thaliana	61.00%

Table 16

Comparison of OS39781852 (SEQ ID NO: 30) to known A20 and AN1 domain containing zinc finger proteins

Public Database Accession #	Species	Sequence Identity (%)
EAZ45178	<i>O. sativa</i>	99.40%
EAZ09556	<i>O. sativa</i>	99.40%
NP_001063521	<i>O. sativa</i>	65.90%
ABI23728	<i>Chrysanthemum x morifolium</i>	58.80%
AAA33773	<i>P. vulgaris</i>	47.30%

5

Table 17

Comparison of OS34701560 (SEQ ID NO: 32) to known A20 and AN1 domain containing zinc finger proteins

Public Database Accession #	Species	Sequence Identity (%)
NP_565844	<i>A. thaliana</i>	58.80%
ABE93196	<i>M. truncatula</i>	56.40%
NP_190848	<i>A. thaliana</i>	55.60%
AAK68811	<i>A. thaliana</i>	55.60%
ABL67658	<i>Citrus cv. Shiranuhi</i>	48.30%

10

Table 18

Comparison of OS36821256 (SEQ ID NO: 34) to known A20 and AN1 domain containing zinc finger proteins

Public Database Accession #	Species	Sequence Identity (%)
EAZ45178	<i>O. sativa</i>	63.00%
EAZ09556	<i>O. sativa</i>	63.00%
ABI23728	<i>Chrysanthemum x morifolium</i>	51.10%
AAA33773	<i>P. vulgaris</i>	43.40%
AAX14637	<i>Z. mays</i>	43.30%

Table 19

Comparison of GM51659494 (SEQ ID NO: 36) to known A20 and AN1 domain containing zinc finger proteins

Public Data- base Accession #	Species	Sequence Identity (%)
AAA33773	<i>P. vulgaris</i>	58.00%
EAZ09556	<i>O. sativa</i>	54.20%
EAZ45178	<i>O. sativa</i>	54.20%
NP_566429	<i>A. thaliana</i>	51.50%
ABI23728	<i>Chrysanthemum</i> <i>x morifolium</i>	44.40%

5

Table 20

Comparison of GM49780101 (SEQ ID NO: 38) to known A20 and AN1 domain containing zinc finger proteins

Public Data- base Accession #	Species	Sequence Identity (%)
ABN08135	<i>M. truncatula</i>	76.70%
ABL67658	Citrus cv. Shi- ranuhi	66.30%
AAD38146	<i>P. armeniaca</i>	64.60%
AAQ84334	<i>O. sativa</i>	64.40%
AAX14637	<i>Z. mays</i>	61.90%

10

Table 21

Comparison of GM59637305 (SEQ ID NO: 40) to known A20 and AN1 domain containing zinc finger proteins

Public Data- base Accession #	Species	Sequence Identity (%)
AAD38146	<i>P. armeniaca</i>	69.30%
ABL67658	Citrus cv. Shi- ranuhi	68.60%
AAQ84334	<i>O. sativa</i>	65.90%
AAX14637	<i>Z. mays</i>	64.00%
NP_564585	<i>A. thaliana</i>	63.70%

Table 22

Comparison of TA55974113 (SEQ ID NO: 42) to known A20 and AN1 domain containing zinc finger proteins

Public Data- base Accession #	Species	Sequence Identity (%)
EAZ09556	<i>O. sativa</i>	73.20%
EAZ45178	<i>O. sativa</i>	72.60%
ABI23728	<i>Chrysanthemum</i> <i>x morifolium</i>	54.20%
NP_001063521	<i>O. sativa</i>	49.00%
AAA33773	<i>P. vulgaris</i>	46.10%

5

EXAMPLE 2

Water stress-tolerant *Arabidopsis* plants

[0072] The polynucleotides of Table 1 are ligated into a binary vector containing a selectable marker. The resulting recombinant vector contains the corresponding gene in the sense orientation under a constitutive promoter. The recombinant vectors are transformed into an *Agrobacterium tumefaciens* strain according to standard conditions. *A. thaliana* ecotype Col-0 or C24 are grown and transformed according to standard conditions. T1 and T2 plants are screened for resistance to the selection agent conferred by the selectable marker gene. T3 seeds are used in greenhouse or growth chamber experiments.

15 [0073] Approximately 3-5 days prior to planting, seeds are refrigerated for stratification. Seeds are then planted, fertilizer is applied and humidity is maintained using transparent domes. For the "biomass" assay, plants are grown in a greenhouse at 22°C with photoperiod of 16 hours light/8 hours dark and watered twice a week. For the "cycling drought" assay, plants are grown in a growth chamber at 22°C with 55% relative humidity with photoperiod was set at 16h light /8 h dark and watered on days 0, 18, and 25 after sowing.

20 [0074] At 19 and 22 days, plant area, leaf area, biomass, color distribution, color intensity, and growth rate for each plant are measured using using a commercially available imaging system. Biomass is calculated as the total plant leaf area at the last measuring time point. Growth rate is calculated as the plant leaf area at the last measuring time point minus the plant leaf area at the first measuring time point divided by the plant leaf area at the first measuring time point. Health index is calculated as the dark green leaf area divided by the total plant leaf area. Table 23 presents the biomass, growth rate, and health index for independent transformation events (lines) of transgenic plants overexpressing polynucleotides represented as SEQ ID NOs:5, 7, and 13. The percent change of a line compared to pooled wild-type controls was calculated, and the significant (p) value was calculated using a t-test. An event was called positive if percent change was greater than 0 and $p < 0.1$, not significant (NS) if $p > 0.1$ regardless of percent change, and negative if percent change was less than 0 and $p < 0.1$.

Table 23

Gene ID	Result category	Number of events called in each result category per assay		
		Biomass	Growth Rate	Health Index
OS32583643 SEQ ID NO: 5	Positive	1	2	2
	NS	8	5	6
	negative	1	3	2
GM53626178 SEQ ID NO: 7	Positive	6	2	1
	NS	3	6	7
	negative	1	2	2
GM48923793 SEQ ID NO: 13	Positive	3	1	4
	NS	2	2	4
	negative	5	7	2

EXAMPLE 3

Nitrogen stress tolerant Arabidopsis plants

- 5 [0075] The polynucleotides of Table 1 are ligated into a binary vector containing a selectable marker. The resulting recombinant vector contains the corresponding gene in the sense orientation under a constitutive promoter. The recombinant vectors are transformed into an *A. tumefaciens* strain according to standard conditions. *A. thaliana* ecotype Col-0 or C24 are grown and transformed according to standard conditions. T1 and T2 plants are screened for resistance to the selection agent conferred by the selectable marker gene.

- 10 [0076] Plants are grown in flats using a substrate that contains no organic components. Each flat is wet with water before seedlings resistant to the selection agent are transplanted onto substrate. Plants are grown in a growth chamber set to 22°C with a 55% relative humidity with photoperiod set at 16h light/ 8h dark. A controlled low or high nitrogen nutrient solution is added to waterings on Days 12, 15, 22 and 29. Watering without nutrient solution occurs on Days 18, 25, and 32. Images of all plants in a tray are taken on days 26, 30, and 33 using a commercially available imaging system. At each imaging time point, biomass and plant phenotypes for each plant are measured including plant area, leaf area, biomass, color distribution, color intensity, and growth rate.

20

EXAMPLE 4

Stress-tolerant Rapeseed/Canola plants

- 25 [0077] Canola cotyledonary petioles of 4 day-old young seedlings are used as explants for tissue culture and transformed according to EP1566443. The commercial cultivar Westar (Agriculture Canada) is the standard variety used for transformation, but other varieties can be used. *A. tumefaciens* GV3101:pMP90RK containing a binary vector is used for canola transformation. The standard binary vector used for transformation is pSUN (WO02/00900), but many different binary vector systems have been described for plant transformation (e.g. An, G. in *Agrobacterium Protocols, Methods in Molecular Biology* vol 44, pp 47-62, Gartland KMA and

MR Davey eds. Humana Press, Totowa, New Jersey). A plant gene expression cassette comprising a selection marker gene and a plant promoter regulating the transcription of the cDNA encoding the polynucleotide is employed. Various selection marker genes can be used including the mutated acetohydroxy acid synthase (AHAS) gene disclosed in US Pat. Nos. 5,767,366 and 6,225,105. A suitable promoter is used to regulate the trait gene to provide constitutive, developmental, tissue or environmental regulation of gene transcription.

[0078] Canola seeds are surface-sterilized in 70% ethanol for 2 min, incubated for 15 min in 55°C warm tap water and then in 1.5% sodium hypochlorite for 10 minutes, followed by three rinses with sterilized distilled water. Seeds are then placed on MS medium without hormones, containing Gamborg B5 vitamins, 3% sucrose, and 0.8% Oxoidagar. Seeds are germinated at 24°C for 4 days in low light (< 50 µMol/m²s, 16 hours light). The cotyledon petiole explants with the cotyledon attached are excised from the in vitro seedlings, and inoculated with Agrobacterium by dipping the cut end of the petiole explant into the bacterial suspension. The explants are then cultured for 3 days on MS medium including vitamins containing 3.75 mg/l BAP, 3% sucrose, 0.5 g/l MES, pH 5.2, 0.5 mg/l GA3, 0.8% Oxoidagar at 24°C, 16 hours of light. After three days of co-cultivation with Agrobacterium, the petiole explants are transferred to regeneration medium containing 3.75 mg/l BAP, 0.5 mg/l GA3, 0.5 g/l MES, pH 5.2, 300 mg/l timentin and selection agent until shoot regeneration. As soon as explants start to develop shoots, they are transferred to shoot elongation medium (A6, containing full strength MS medium including vitamins, 2% sucrose, 0.5% Oxoidagar, 100 mg/l myo-inositol, 40 mg/l adenine sulfate, 0.5 g/l MES, pH 5.8, 0.0025 mg/l BAP, 0.1 mg/l IBA, 300 mg/l timentin and selection agent).

[0079] Samples from both in vitro and greenhouse material of the primary transgenic plants (T0) are analyzed by qPCR using TaqMan probes to confirm the presence of T-DNA and to determine the number of T-DNA integrations.

[0080] Seed is produced from the primary transgenic plants by self-pollination. The second-generation plants are grown in greenhouse conditions and self-pollinated. The plants are analyzed by qPCR using TaqMan probes to confirm the presence of T-DNA and to determine the number of T-DNA integrations. Homozygous transgenic, heterozygous transgenic and azygous (null transgenic) plants are compared for their stress tolerance, for example, in assays similar to those described in Examples 2 and 3, and for yield, both in the greenhouse and in field studies.

EXAMPLE 5

Screening for stress-tolerant rice plants

[0081] Transgenic rice plants comprising a polynucleotide of Table 1 are generated using known methods. Approximately 15 to 20 independent transformants (T0) are generated. The primary transformants are transferred from tissue culture chambers to a greenhouse for growing and harvest of T1 seeds. Five events of the T1 progeny segregated 3:1 for presence/absence of the transgene are retained. For each of these events, 10 T1 seedlings containing the transgene (hetero- and homozygotes), and 10 T1 seedlings lacking the transgene (nullizygotes) are selected by visual marker screening. The selected T1 plants are transferred

to a greenhouse. Each plant receives a unique barcode label to link unambiguously the phenotyping data to the corresponding plant. The selected T1 plants are grown on soil in 10 cm diameter pots under the following environmental settings: photoperiod = 11.5 h, daylight intensity = 30,000 lux or more, daytime temperature = 28°C or higher, night time temperature = 22°C, relative humidity = 60-70%. Transgenic plants and the corresponding nullizygotes are grown side-by-side at random positions. From the stage of sowing until the stage of maturity, the plants are passed several times through a digital imaging cabinet. At each time point digital, images (2048x1536 pixels, 16 million colours) of each plant are taken from at least 6 different angles.

[0082] The data obtained in the first experiment with T1 plants are confirmed in a second experiment with T2 plants. Lines that have the correct expression pattern are selected for further analysis. Seed batches from the positive plants (both hetero- and homozygotes) in T1 are screened by monitoring marker expression. For each chosen event, the heterozygote seed batches are then retained for T2 evaluation. Within each seed batch, an equal number of positive and negative plants are grown in the greenhouse for evaluation.

[0083] Transgenic plants are screened for their improved growth and/or yield and/or stress tolerance, for example, using assays similar to those described in Examples 2 and 3, and for yield, both in the greenhouse and in field studies.

EXAMPLE 6

Stress-tolerant soybean plants

[0084] The polynucleotides of Table 1 are transformed into soybean using the methods described in commonly owned copending international application number WO 2005/121345, the contents of which are incorporated herein by reference.

[0085] The transgenic plants generated are then screened for their improved growth under water-limited conditions and/or drought, salt, and/or cold tolerance, for example, using assays similar to those described in Examples 2 and 3, and for yield, both in the greenhouse and in field studies.

EXAMPLE 7

Stress-tolerant wheat plants

[0086] The polynucleotides of Table 1 are transformed into wheat using 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 growth and/or yield under water-limited conditions and/or stress tolerance, for example, in assays similar to those described in Examples 2 and 3, and for yield, both in the greenhouse and in field studies.

EXAMPLE 8

Stress-tolerant corn plants

[0087] The polynucleotides of Table 1 are transformed into immature embryos of corn

using *Agrobacterium*. After imbibition, embryos are transferred to medium without selection agent. Seven to ten days later, embryos are transferred to medium containing selection agent and grown for 4 weeks (two 2-week transfers) to obtain transformed callus cells. Plant regeneration is initiated by transferring resistant calli to medium supplemented with selection agent and grown under light at 25-27°C for two to three weeks. Regenerated shoots are then transferred to rooting box with medium containing selection agent. Plantlets with roots are transferred to potting mixture in small pots in the greenhouse and after acclimatization are then transplanted to larger pots and maintained in greenhouse till maturity.

[0088] Each of these plants is uniquely labeled, sampled and analyzed for transgene copy number. Transgene positive and negative plants are marked and paired with similar sizes for transplanting together to large pots. This provides a uniform and competitive environment for the transgene positive and negative plants. The large pots are watered to a certain percentage of the field water capacity of the soil depending on the severity of water-stress desired. The soil water level is maintained by watering every other day. Plant growth and physiology traits such as height, stem diameter, leaf rolling, plant wilting, leaf extension rate, leaf water status, chlorophyll content and photosynthesis rate are measured during the growth period. After a period of growth, the above ground portion of the plants is harvested, and the fresh weight and dry weight of each plant are taken. A comparison of the drought tolerance phenotype between the transgene positive and negative plants is then made.

[0089] The pots are covered with caps that permit the seedlings to grow through but minimize water loss. Each pot is weighed periodically and water added to maintain the initial water content. At the end of the experiment, the fresh and dry weight of each plant is measured, the water consumed by each plant is calculated and WUE of each plant is computed. Plant growth and physiology traits such as WUE, height, stem diameter, leaf rolling, plant wilting, leaf extension rate, leaf water status, chlorophyll content and photosynthesis rate are measured during the experiment. A comparison of WUE phenotype between the transgene positive and negative plants is then made.

[0090] These pots are kept in an area in the greenhouse that has uniform environmental conditions, and cultivated optimally. Each of these plants is uniquely labeled, sampled and analyzed for transgene copy number. The plants are allowed to grow under these conditions until they reach a predefined growth stage. Water is then withheld. Plant growth and physiology traits such as height, stem diameter, leaf rolling, plant wilting, leaf extension rate, leaf water status, chlorophyll content and photosynthesis rate are measured as stress intensity increases. A comparison of the desiccation tolerance phenotype between transgene positive and negative plants is then made.

[0091] Segregating transgenic corn seeds for a transformation event are planted in small pots for testing in a cycling drought assay. These pots are kept in an area in the greenhouse that has uniform environmental conditions, and cultivated optimally. Each of these plants is uniquely labeled, sampled and analyzed for transgene copy number. The plants are allowed to grow under these conditions until they reach a predefined growth stage. Plants are then repeatedly watered to saturation at a fixed interval of time. This water/drought cycle is repeated for the duration of the experiment. Plant growth and physiology traits such as height, stem di-

ameter, leaf rolling, leaf extension rate, leaf water status, chlorophyll content and photosynthesis rate are measured during the growth period. At the end of the experiment, the plants are harvested for above-ground fresh and dry weight. A comparison of the cycling drought tolerance phenotype between transgene positive and negative plants is then made.

- 5 [0092] In order to test segregating transgenic corn for drought tolerance under rain-free conditions, managed-drought stress at a single location or multiple locations is used. Crop water availability is controlled by drip tape or overhead irrigation at a location which has less than 10cm rainfall and minimum temperatures greater than 5°C expected during an average 5 month season, or a location with expected in-season precipitation intercepted by an automated “rain-out shelter” which retracts to provide open field conditions when not required. Standard ag-
10 nomic practices in the area are followed for soil preparation, planting, fertilization and pest control. Each plot is sown with seed segregating for the presence of a single transgenic insertion event. A Taqman transgene copy number assay is used on leaf samples to differentiate the transgenics from null-segregant control plants. Plants that have been genotyped in this manner
15 are also scored for a range of phenotypes related to drought-tolerance, growth and yield. These phenotypes include plant height, grain weight per plant, grain number per plant, ear number per plant, above ground dry-weight, leaf conductance to water vapor, leaf CO₂ uptake, leaf chlorophyll content, photosynthesis-related chlorophyll fluorescence parameters, water use efficiency, leaf water potential, leaf relative water content, stem sap flow rate, stem hydraulic
20 conductivity, leaf temperature, leaf reflectance, leaf light absorptance, leaf area, days to flowering, anthesis-silking interval, duration of grain fill, osmotic potential, osmotic adjustment, root size, leaf extension rate, leaf angle, leaf rolling and survival. All measurements are made with commercially available instrumentation for field physiology, using the standard protocols provided by the manufacturers. Individual plants are used as the replicate unit per event.
- 25 [0093] In order to test non-segregating transgenic corn for drought tolerance under rain-free conditions, managed-drought stress at a single location or multiple locations is used. Crop water availability is controlled by drip tape or overhead irrigation at a location which has less than 10cm rainfall and minimum temperatures greater than 5°C expected during an average 5 month season, or a location with expected in-season precipitation intercepted by an automated
30 “rain-out shelter” which retracts to provide open field conditions when not required. Standard agronomic practices in the area are followed for soil preparation, planting, fertilization and pest control. Trial layout is designed to pair a plot containing a non-segregating transgenic event with an adjacent plot of null-segregant controls. A null segregant is progeny (or lines derived from the progeny) of a transgenic plant that does not contain the transgene due to Mendelian
35 segregation. Additional replicated paired plots for a particular event are distributed around the trial. A range of phenotypes related to drought-tolerance, growth and yield are scored in the paired plots and estimated at the plot level. When the measurement technique could only be applied to individual plants, these are selected at random each time from within the plot. These phenotypes include plant height, grain weight per plant, grain number per plant, ear number per
40 plant, above ground dry-weight, leaf conductance to water vapor, leaf CO₂ uptake, leaf chlorophyll content, photosynthesis-related chlorophyll fluorescence parameters, water use efficiency, leaf water potential, leaf relative water content, stem sap flow rate, stem hydraulic conductivity,

leaf temperature, leaf reflectance, leaf light absorptance, leaf area, days to flowering, anthesis-silking interval, duration of grain fill, osmotic potential, osmotic adjustment, root size, leaf extension rate, leaf angle, leaf rolling and survival. All measurements are made with commercially available instrumentation for field physiology, using the standard protocols provided by the manufacturers. Individual plots are used as the replicate unit per event.

[0094] To perform multi-location testing of transgenic corn for drought tolerance and yield, five to twenty locations encompassing major corn growing regions are selected. These are widely distributed to provide a range of expected crop water availabilities based on average temperature, humidity, precipitation and soil type. Crop water availability is not modified beyond standard agronomic practices. Trial layout is designed to pair a plot containing a non-segregating transgenic event with an adjacent plot of null-segregant controls. A range of phenotypes related to drought-tolerance, growth and yield are scored in the paired plots and estimated at the plot level. When the measurement technique could only be applied to individual plants, these are selected at random each time from within the plot. These phenotypes included plant height, grain weight per plant, grain number per plant, ear number per plant, above ground dry-weight, leaf conductance to water vapor, leaf CO₂ uptake, leaf chlorophyll content, photosynthesis-related chlorophyll fluorescence parameters, water use efficiency, leaf water potential, leaf relative water content, stem sap flow rate, stem hydraulic conductivity, leaf temperature, leaf reflectance, leaf light absorptance, leaf area, days to flowering, anthesis-silking interval, duration of grain fill, osmotic potential, osmotic adjustment, root size, leaf extension rate, leaf angle, leaf rolling and survival. All measurements are made with commercially available instrumentation for field physiology, using the standard protocols provided by the manufacturers. Individual plots are used as the replicate unit per event.

APPENDIX

cDNA sequence of BN51364980 from canola (SEQ ID NO: 1):

5 atggcttcttctagttgtttaccattcagtcacgtttcgtctcagcgagaacaaagctcgattcaatctccaaaccgagtcctccggattc
gcttgctgcttctcttataaaaccagaaacttgaatctctctgttcttctcgggtgttccatgggttcccttaactcttctcagaaatcagacaac
gtccaagaagctgcaaagagtgactttgcttcaataagtgaaggtagtggaagaaacggctaacaccagaacagtattacatcac
cagacagaagggaacagagagagcttactggtgagtattggaatacaaagaccccaggagtatacaaatgtatctgttgcgaca
cgccactgtttgactcatcaacaaagcttgatagtgaaccgggtggccatcgattaccaacctattggaacaatgtgaagtcaaa
10 gctggacctctctatcatcttcatgcttagacaagaagtatctgtgctgtttgtaacgcccatttgggtcatgtcttcgatgacgggccag
accaaccggaacgatattgcctcaacagtgctgctctgaaacttgagtcattggag agaacaagagaatga

The BN51364980 cDNA is translated into the following amino acid sequence (SEQ ID NO:2):

15 massscftiqsrfsartkldsiskspslsgfacrsltkprnlslvllrcsmgsfnssqksdnlvqeaaksdfasisegewkkrltpeqyyitr
qkgteraftgeywnktkpgvykciccdtpldfsstkldsgtgwpsyyqpignnvkskldsiifmprqevicavcnahlgvhfddgprptg
kryclnsaalkleslertre

cDNA sequence of OS34096188 from rice (SEQ ID NO:3):

20 atgggcttcaatatctgagaaccacttccatctccactcctatctcttctccaaatccaaacccattttctcaactcttctcgttcttctccttc
caccattttcccccaaagtccgttactcccaccactcttttcgtttctgccaccccttcttcaactctccatcccaagcttggtttctggttg
gattgtggccatggccgcacctggctctctccgcaaatccgaggaagagtggcgcgcaattctctcccctgaacagtttcggatctca
ggcaaaagggcaccgagttccctggaacaggagagtatgacaagttctatgaagagggagttacaactgtgctggttggtgggactc
25 cactctacaggtccataacaaaattcaattctggttggtggtggccagccttctatgaggggattcccgagccataaatcgcaatccg
gatcctgatgggatgaggacagaaataacgtgtgctgcttggtgggggacatctaggtcacgtctttaaaggagaaggattccaacac
ccactaacgaacgccattgtgtcaatagcatttcgctgaaatttcgcccagccaattcttattcttaa

The OS34096188 cDNA is translated into the following amino acid sequence (SEQ ID NO:4):

30 mgfnilrttsistpisskskpfistllrsspstifppksvpttllfvsatpfftlhpklgfrggivamaapgslrkseewrailspeqfrilrqgte
fpgtgeydkfyeegvncagcgtplyrsitkfnsgcgwpaifyegipgainrnpdpdgmrtaitcaacgghlghvfkggegftptnerhc
vnsislkfpansys

35 cDNA sequence of OS32583643 from rice (SEQ ID NO:5):

[illegible]

gctgtgtgctgtctgtgacgctcatctggggcacgtgttgatgatgggccacgaccaacagggaaaagatactgtatcaatagcgcat
ctctcaagctgaagaagacccagtag

The OS32583643 cDNA is translated into the following amino acid sequence (SEQ ID NO:6):

5

mamrqyaaataasssfrarprarpsclpaaalplapccgvawsrasyrrasvramgaasssssssssspspggqaqaqaqgkp
nystsldeewrkrltkdqyyitrqkgeraftgeywntktpgiyhcvccdtplfessstkfdsgtgwpsyyqpigdnvkckldmsiifmprt
evlcavcdahlghvfdgprptgkrycinsaslkklktq

10 cDNA sequence of GM53626178 from soybean (SEQ ID NO:7):

atgggattgagtattctgagaagcacttccatttccactcctatctcttctccaaatccaaacccattttctcaactcttgcttcattcttctg
cctccatttccccacaaagtgtgttactcccaccactctttctgttctgccacccccttctcaccgcctcaccgaagcgcggttctgtg
tgggattgtggccatggccgctgtgctcgcgcaaatcagaggaagagtgccgcgcagttctctccctgaacagttctgtattct
15 caggcaaaagggcaccgagttccctggaacaggagagtatgacaagttcttgatgaggagtttacaactgtgctggttgtgggac
acctcttacaggtccttaacaaaattcaattctggttgtggctggcagccttctatgaggggattcctggagccataaatcgcaatccg
gacctgatgggatgaggacagaaataacgtgtgctgcttgtgggggacatctaggtcacgtctttaaaggagaaggattccaacgc
ccactaacgaacgccattgtgtcaatagcatttactgaaatttgcgccagccaattcttaa

20 The GM53626178 cDNA is translated into the following amino acid sequence (SEQ ID NO:8):

mglslrststispiisskskipfstlvrssfasisptkcvtpthfvsatpfftasprkgrfggivamaaagslrkseewravlspeqfrilrqk
gtefpgtgeydkffdegvyncagcgtplyrsltkfnsgcgwpafyegipgainrnpdpdgmrtetcaacgghlghvfkgegfptptne
rhcvnsislkfapans

25

cDNA sequence of TA56540264 from wheat (SEQ ID NO:9):

atggcgtcgccccacgcccacccggccacgcggccccctctcatcgctcccgctccctcctcgccccgctcctcctccgcccaccg
ccgcccgcgtcgccccgccccgctccctctcctctcgtgctcgcggtcgcgggcgcgggcctactgccagccggacgacggt
30 tgccggcgccggtggtggtatgtcgtcgcgggcgcccacgccccgggcccgtgcagaagtgcgaggaggagtgaggagccgtcc
tcacgcccggagcagttccgcatcctccgccaagggcaccgagtatcctggaacaggtgaatatgacaagttcttcagtggggtta
tttacggatgtgctggctgtggaacccccctgtacaaatcatctacgaagtcaactcagggtgtggttggccagcattctatgaaggattt
cctggagccataaaacggacggcggtatcctgatgggagggcaattgagatcacatgtgctgcttgaaggacatctggggcatgtg
ttcaaaggggaggggttcaacacgccgactgatgagcgacactcggtcaacagtatctactcaagttcgttccggcctctgaagag
35 gctagtga

The TA56540264 cDNA is translated into the following amino acid sequence (SEQ ID NO:10):

40

masphahpatrplsslpillarsssaataaassarpaslsrscsraraycpagrrlpavvamssaaptpgpvqkseeweavlt
peqfrilrrkgtteypgtgeydkffsegiygcagcgtplyksstkfnsngcgwpafyegfpgaikrtadpdgrrietcaaceghlghvfkge
gfntptderhcvnsislkfvaseas

cDNA sequence of BN45206322 from canola (SEQ ID NO:11):

atgatgaagagattaagcagttcagattcagtggtggtctcatctcttattgtcccactactccacagatcagccgaatccaagaaga
tgcgggagagaatttcagtcgatgctcgaaggttacgaggaggaagaagaagaagccataaccgaggaaagaggacaaaccg
5 gtttagccgagaagaagagacggttaaacattaaccaagttaaagccttgagaaaaatttcagttagagaacaagcttgagcctg
agaggaaagtgaagttagctcaagaacttggtctccaacctcgtaagtagctgtttggttcagaaccgccgtgcgcggtggaagac
aaaacagcttgagaaagattacggtgttctcaaaacgcaatacgattctctccgccataactttgattccctccgccgtgaaaatgaatc
tcttctcaagagatcggtaaaactaaaagctaagcttaacggagaagaagaaggagatgatgttgatgaagaagagaacaacttg
cgacgatggagagtgtgtttccgtcaaggaagaagaagtttcgttgcggagcagatcacagagccgccgtcttctcctccgcagct
10 tctagagcattccgacagttcaattaccggagttcaccgacctccgcgaccttctccgttaaaggccgaggcttctcctccgcgcc
gctggatcgctcgacagtagcagattcgagcgccgtgtgaacgaggaaagtagctctaacgttacggcggtccggcgacggttccc
ggcggcagtttcttcagtttgtgaaatggagcagacggaggatcacgacgacttctgagtgagagaagaagcgtgcgggttttctc
cgatgaacagccaccgtctctgcactggtattccaccgttgatcagtggaactga

15 The BN45206322 cDNA is translated into the following amino acid sequence (SEQ ID NO:12):

mmkrlsssdsvggllslcpttstdqpnprrcgrefqsmlegyeaaaaaiteergqtglaekrrlninqvkaleknfelenkleperkv
klaqelglqprqvavwfqnrrarwtkqlekdygvlktqydsrlrhndslrrenesllqeigklkaklngeeegddvdeennlatmesd
vsvkeeevslpeqiteppsspqllehdsfnysftldrlplkaaassvaaagssdsdssavlnesssnvtaapatvpggslqf
20 vkmeqtedhddflsgeeacgffsdeqppslhwystvdqwn

cDNA sequence of GM48923793 from soybean (SEQ ID NO:13):

atggcgggtagtggaagtgcctttccaacatcactagctttctcgcacccaacaaccctcttcaacctctcgattcttctcttctctc
25 tgcaccttctctgctcttctcctcggttcgagatccatgatgatgtttgatggagaaggaggaaggggtgaacggctccttctccgcg
cgtttgacatggacgacaatggggatgagtgcatggacgagtagcttcatcaaccgagaagaagcgacgtctctcgcgagccag
gttcagtttctagagaagagcttcgaggaggagaacaagctgaacccgagagaaagaccaaactagccaaagaccttggttgca
gccacggcaagtgctatttggtccagaaccgtagagctcgggtgaagaacaacagctggagaaggattacgagactctgcatg
caagttttgagagtcacaagtccaactatgactgtcttcaaggagaaagacaagttaaaagctgaggtggcgagcctcactgagaa
30 ggtgcttgaagagggaaacaagaggggcacatgaagcaggctgaaagtgaagtgaagaacaaaaggattattgcatttgca
ggaacaggaaccacccagaggctttactgcaatcagtttcggaggggagaaggatccaaagtcttctgtcgttgggggtgtaaa
caggaagatatcagttcagcaaggagtacattttgattcagatagtcacattacaccgatggagttcactctgcgctgctagagca
tggtgattcttctatgtgttgagcctgatcaatcagatatgtcacaagatgaagaagataacctcagcaagagtctctacccttctacc
tcttcccaaactgaagaagatgtggattactccgacccacctgaaagtcttgaattttggatttctgaggaagatcatgtccttgga
35 cctgggcttactactaa

The GM48923793 cDNA is translated into the following amino acid sequence (SEQ ID NO:14):

magsgsafsnitsflrtqqpssqpldssflsapssapflgsrsmmsfdgeggkgcngsfrafdmddngdecmdyfhqpekkrrl
40 sasqvqfleksfeenkleperktklakdlglqprqvaiwfqnrrarwnkqlekdyetlhasfeslksnydcillekdiklaevasltek
vlargkqeghmkaesesetkglhlhqeppqrlllqsvsegegskvssvvggckqedissarsdildsdsphytdgvhsalleh
gdssyvfepdqsdmsqdeednlkslypsylfpkleedvdysdppesscnfgfpeedhvlwtwayy

cDNA sequence of TA55969932 from wheat (SEQ ID NO:15):

5 atggagcccgccggctcatcttcaacacgtcgggtccggcaacggacagatgcttcatggactgcggcgccggcgccatcgc
cggcgccggccggcatgttccatcgaggggtgagaccggctcctcggcgccatggaagaagggcgccgctgaagcgcccttctc
acctcgccggatgacatgctggaggaggagtactacgacgagcagctcccgagaagaagcgccgctcacgccggagcaggt
ccacctgctggagaggagcttcgaggaggagaacaagctggagccggagaggaagacggagctggcccgcaagctcgggctg
cagccacggcaggtggccgtctggttcagaaccgccgcgccgggtggaagacaaagacgctggagcgcgacttcgaccgcctc
aaggcgctcttcgacgccctccgcgccgaccacgacgcgctcctccaggacaaccaccggctccgggtcacaggtggtaacgttga
10 ccgagaagatgcaagataaggaggcgccggaaggcagcttcgggtgcagccgccgacgcctcggagccggagcaggcgccgg
cggaggcggaaggctccttgccgacgccgaggagcaggccgcggcagcggaggcgttcgaggtggtgcagcagcagctgcac
gtgaaggacgaggagaggctgagcccggggagcggcgggagcgcgggtgctggacgcgagggacgcgctgctcgggagcggga
tgcggcctcgccggcgtggtggacagcagcgtggactcgtactgctcccgggggcgccggcgccgacgagtaccacgagtgcg
tggtagggcccggtggcgggcgccatccagtcggaggaggacgacggcgccggcagcagcaggggtgcagctactaccccgac
15 gacgccgcgctcttctcgccgccgcgaagggcacggccaccatcgcacggacgacgacgatcagcaggacgacggccagat
cagctactggatgtggaactag

The TA55969932 cDNA is translated into the following amino acid sequence (SEQ ID NO:16):

20 mepgrlfnstsgsgngqmlfmdcgaggiagaagmfhrgrvpvlggmeeergvkrpfftsppdmleeeydeqlpekkrrltpeqvh
llersfeeenkleperktelarklglqrqvavwfqnrrarwkktlerdfdrkasfdalradhdallqdnhrslsqvvtltkmqdkape
gsfgaaaadasepeqaaaekasladaeeqaaaaeafevvqqqlhvkdeerlspgsggsavldardallgsgcglagvvdssvds
ycfpaggaggyhecvvgpvaggiqseeddgagsdegcsyypddaavfaaaqghghrtddddqddgqisywmwn

25 cDNA sequence of BN47310186 from canola (SEQ ID NO:17):

atggaccacgacaaaacaggatgccaaagccacctgaaggtcccaagctatgcatcaacaactgcggtttcttcggaagcgctgc
cacaatgaacatgtgttccaagtgatcacaaggctatcctgtttcaacaggaacaggggctaggttgcatctgcagtgctggtgttac
atcatcatccagcaacatcttaaaggaaaccttctgctgctaccgcgctggtgatgctgaaaccaaaccgttgagccggtggtgtctc
30 tgtacagccatcttctgtccaagttgccgagaggtagtagctccagaagccgctgcagcaaaactaaaggaaggaccaagccgat
gtgctacttgcaataaacgggttggtctgactggattcaaatgctgctgtggtgacctcttctgcgggacgcaccggttatgcagacataca
caactgctccttcaattacatgccgctgcgcaagaagctatagctaaagcaaaccgggtgtgaaggcagagaagcttgacaaaat
ctga

35 The BN47310186 cDNA is translated into the following amino acid sequence (SEQ ID NO:18):

mdhdktgcspppegpklcinncgffgsaatmnmcskchkailfqeqgarfasavsggtssssnilketfaatalvdaetksvepva
vsvqpssvqvaaevvapeaaaaklkegpsrcatcnkrvgltgfkrcgdlfcgthryadihncsfnyhaaaqeaikanpvvkaekl
dki

40

cDNA sequence of BN51359456 from canola (SEQ ID NO:19):

atggcgggaagagcatcgatgccagacgccggaaggccaccgtctctgtgctaacaactgcggcttctcggcagctccgccacat
gaatctatgctccaactgctacggcgatctctgaccttaagcaacagcaagcttccatgaaatccaccgtcgaatcctctctcgcgct
atctctccgtcgtcagagatcggtctatgcaatccaccgttgaatcctctctcgcgacgtatctctccatcaccggagaccattcca
tctctctccaatgatccagcctctcggtcgaaacccatcagctgaattggaggtaacggcgacgaagacggtgactccgccgcccgg
5 agcagcagcagaaacggccgaatcgggtgcacgacgtgtaggaaacgggtcgggtgaccgggttcaagtgcgggtgcgggacg
acttttgcggggctcacaggtagccggagggtccatggatgcaccttcgatttcaaatacgccgggtcggaagagatcgccaaggcg
aaccactcgtcaaagcggcgaagcttcagaagattga

The BN51359456 cDNA is translated into the following amino acid sequence (SEQ ID NO:20):

maeehrcqtpeghrlcanncgflgssatmnlcsncygdllkqqasmkstvesslsavspssseigsmqstvesslsdvspssp
etisisspmiqlvrnpsaelevtatktvtppeqqkrpnrttckrvglgtfkcrcgttfcgahrypevhgctfdksagreeiaknpl
vkaaklqki

cDNA sequence of HV62552639 from barley (SEQ ID NO:21):

atggcccaggagagttgtgatctcaacaaggacgaggccgagatcctgaagccatcctctccacaccttcgcctccttcgccagcc
acaccaccaccaccaaccgctcaaataccagaaccacaacctccacactcaccaccacaaccaccggcagctcaattctgtcca
ggccctgcgaggtgttcccatagagacttcaaaaaggaggaaacatgctgatgcggtgtcaatggccattgtggttagccattgtcg
20 tctgtgctgttcgttaaccgttgcaacgtgtgccgaagagagttggttgaccgggttccgttgccggtgtgagaagctctttgtccgcg
caccggcattcagaaagccacgactgctcatttgattataaaactgtgggtcgggaggagattgcccgggcaaaccctctgatcagg
gctgccaagatcattaggatatga

The HV62552639 cDNA is translated into the following amino acid sequence (SEQ ID NO:22):

maqescdlnkdeaeilkpssstpsppspatpppptaqi pepqpphsp pppaaqflsrpcevvp ietsskrkhadavsm aivvep
lssvlfvnrcnvrkrvgltgfrcrceklfcprhrsheshdcfsdyktvgreeiaranpliraakiiri

cDNA sequence of ZM61995511 from corn (SEQ ID NO:23):

atggaacacaaggaggcggggtgccagcagccggaggggcccaatcctatgcatcaataactgcggcttcttcggcagtgctgcga
cgatgaacatgtgtccaagtgccacaaggagatgataacgaagcaggagcaggcccagctgggtgcctccccatcgatagcat
tgtcaatggcggtgacggcgggaaaggacctgtaattgctgcatctgtaaattgtgcagttcctcaagttgagcagaagactattgtgt
gcagcccattgctgtagctgaaaccagcgaggctgctgctgtaatccccaaggccaaggaaggcccagaccgggtgcgcggcctgc
35 aggaagcgtgttgggtgacgggatttagctgccgatgcgggaacatgtactgttcggtgcaccgctactccgacaaacatgactgtc
agttcgactatcggactgcagcaagggacgcgattgccaaaggccaatcctgtggtgagggcggagaagctcgacaagatctga

The ZM61995511 cDNA is translated into the following amino acid sequence (SEQ ID NO:24):

mehkeagcqpegpilcinncgffgsaatmnmcskchkemitkqeqaqlaaspidsivnggdggkpviaasvnavvpqveqk
tivqpmlvaetseaaavipkakegpdrcaacrkrvgltgfscrcgnmycsvhrysdkhdcqfdyrtaardaiakanpvvraekldki

cDNA sequence of LU61567101 from linseed (SEQ ID NO:25):

atggctccttcaccttgctccacggctgcacggccaattgccccgctgccactcttacggacaccccatcttcgggaactcagatctc
gccgctggcggcagcgatagctccacgtcgggtgttgaaaagtaggatccgctcgtgattcagtcgcctgcgaagaatcacgcgttcg
5 gccaagcttggtgcccgggttttccctcgagctcctcccccttcgcccgcacaaagttcgccccaaagatggcgaggggaaaggacc
gctgaagccgatcgagaagcagccgtcgaagaagcgtccgttctgcttctcccgacgagacgattgacgcgacgggtcctccgtc
caccaaaccgttcggttcgttcgttccgtctgtgtcacggacgccgacgaggccagggtgaaggcgaaccgcgagttctcgctccg
gtatcccgcgaacgtggcttcgatccgactgacatgaccttcgtaacgccgcccgcgctcgggtaatgcgagggaggaagcgaa
gaagtggcgcggcagttgcaagaagcgcgtggggctgttaggggtcaagtgcaggtgtacgaagttcttctgtgggaagcatcggat
10 cctgaggagcatggtgtacgttcgatcatgtggcgttcgggagggcgattatcgagaaacagaatcctgttctcgagaccgacaagc
tggtggacagaatctga

The LU61567101 cDNA is translated into the following amino acid sequence (SEQ ID NO:26):

15 mapspcvhgctancprchsyghpifgnsdlaaggsdtstsvfgkvgsvviqspaknhafgqacgpvpsssspfrikfpgkdgeg
kgplkpiekqpskkrpfcspdetidatvppstkpfgsfrsvctdadearknareffapvsrkrghdptdmtfgnaaaaaanareeak
kwcgscckrvllgfkrcrctkfcgkhrypeehgctfdhvfgrriekqnpvletdklvdri

cDNA sequence of LU61893412 from linseed (SEQ ID NO:27):

20 atggaccatgacgaggcaggctgccaggctccttcgatcatcctattctgtgcgttaacaattgcggcttcttcggaagtgtgccacc
atgaacatgtgtcaaagtgccacaaggatagctgctaaaccaagagcaatccaagcttctgcttcacggcagcaagtatcctc
aacggatcgtgatgagcctcggaagggaactcgttattgtgctaagaccaattcggtagaaccaagaccatctccgtccaacca
tcttctgctcaagtgtgaagagagtatcgaaatgaagctgccaaaagaagggccagtaggtgcaacacttgcaacaaacgtgtc
25 ggtttgaccggattcaaagtgcgtgcgagaacatgttctgcgcaaaccatcgctactcggaacaagcacaattgccccttgattaccg
cactgctggccgtgaagctatctcaaaggccaatccttgggtgaaggcggagaaagctcgacaaaatctga

The LU61893412 cDNA is translated into the following amino acid sequence (SEQ ID NO:28):

30 mdhdeagcqapsdhpilcvnncgffgsaatmnmsckchkdtmInqeqsklaassaasilngssmslgrlviaaktnsveptis
vqpssassaeesiemklpkempsrcntcnkrvltgfkrcenmfcanhrysdkhncpfdyrtagreaiskanplvkaekldki

cDNA sequence of OS39781852 from rice (SEQ ID NO:29):

35 atggcgcagcgcgacaagaaggatcaggagccgacggagctcagggcgccggagatcacgctgtgcgccaacagctgcggatt
cccgggcaaccgggccacgcagaacctctgccagaactgcttctggcggccacggcgtccacctcgctcggcttcttctgtcgtcac
cgggtctcgacaagcagccgcccaggccggcgccgctggtgagcctcaggctcctctcccaccgcctgtggaggagatggc
ctccgcgctcgcgacggcgccggcgccggtcgccaagacgtcggcgggtgaaccgggtgtccagggtccggaagcgtgtcggcctc
accgggttcgggtgccggtgcggccacctgttctgcggcgagcaccgggtactccgaccgccacgggtgcagctacgactacaagtc
40 ggcggcgaggggacgccatcgccagggaacccgggtggtgcgcgcggccaagatcgtaggttctga

The OS39781852 cDNA is translated into the following amino acid sequence (SEQ ID NO:30):

maqrddkdeptelrapeitlcanscgfpgnpatqnlcqnclaatstsspslsspvldkqpprpaaplvepqaplpvpveemas
alatapapvaksavnrcsrckrvglgtgfrcrghlfcgehrysdhrhgcsydyksaardaiardnpvraakivrf

5 cDNA sequence of OS34701560 from rice (SEQ ID NO:31):

atggccgaagaacaccgatgccaagctcccgaaggacacagactctgctccaacaactgcggtttcttgtagccccgccaccatg
aatctctgttccaaatgctacagagacatccgttgaaggaagaagaacaagccaaaaccaaaccacaatcgaaaccgctcttca
ggatcttctccgccaccgtcaccgcaaccgcccgtgctcctcctcgtggaatcccctcggcgccggtgaatccctccctcaacca
10 ccggtgctgattcgccgatatagccgcaccggtcaggcgaaccggtgcggcgctgtaggaagcgctgggggtgacaggggtc
aagtgcagggtcggaacaacgtttgtgggagccacaggtaccccgagaaacacgcgtgtggctcgattcaaggcgtggggag
agaggagatagcacgggcgaatcccgtgatcaaaggcgagaagctacggaggattaa

15 The OS34701560 cDNA is translated into the following amino acid sequence (SEQ ID NO:32):

maeehrcqapeghrlcsnncgffgspatmnlcskcyrdirlkeeeqaktkstietalsgsssatvtatavvassvespsapveslpqp
pvlipdiaapvqanrcgacrkrvglgtgfrcrghlfcgehrysdhrhgcsydyksaardaiardnpvraakivrf

20 cDNA sequence of OS36821256 from rice (SEQ ID NO:33):

atggcgagagggagaagaaggtggaggagccgacggagctgagggcgccggagatgacgctctgcgccaacagctgcgggt
tcccgggcaacccggcgaccaacaacctctgccagaactgcttcttggtgctcggcgctcttcttcttctccgcccgtgcctcgcc
gtcgacgacgtcgttgccggtgttccggtggtggagaagccgaggcaggccgtacagtcgtcgccggcgccggtggcgctgg
tggttgagcggccgacggcggggcccgtggagtcgtcgtcgaaggcgtcgaggtcgtcgtcggtcaaccgatgccacagctgccg
25 gaggcgggtgggcctgaccgggttccggtgccgctgcggcgagctctactcgggcgcgccaccggtactccgaccgccacgactgc
agcttcgactacaagtcggcgggcgaggagccatcgccagggagaaccccgctcgtccgcccgaagatcggttaggttctaa

The OS36821256 cDNA is translated into the following amino acid sequence (SEQ ID NO:34):

30 maqrekkveptelrapemtlcanscgfpgnpatnnlcqnclaaasassssssaaaspstslpvfpvvekprqavqssaaaaval
vverptagpvsssskasrsssvnrchscrrrvglgtgfrcrghlfcgehrysdhrhgcsydyksaardaiarenpvraakivrf

cDNA sequence of GM51659494 from soybean (SEQ ID NO:35):

35 atggctcagaaaaccgagaaagaagaaccgactcaaagttccggaacgattacgctttgctcaacaactgcggcgtcaccg
gaaaccctgccacgaataacatgtgccagaagtgttactgcttaccgccaccacttccggcgccggaggtgccggaatagctt
ctccggcgaccagatccggcgtctccgcgctcctcagaagagatctttctgaagagccctcgccggtggcggtatcctcttctcg
gaccagacgacgccgtcgaggcgaagcgctggtaaccgctgctccggatgccggcggaaggctcgactaccggattccgg
tgccgggtgcggcgagctcttctgcgcccagaccggtactccgaccgccacgactgcagctatgactacaaagccgcccgaagag
40 aagccatcgcgagggagaatccggtgatcagagctgcgaagatcgtaaaagtctga

The GM51659494 cDNA is translated into the following amino acid sequence (SEQ ID NO:36):

maqktekeetdfkvpetitlcvnncgvtgnpatnncqkcfstattsaggagiaspatrsgvsarpqkrsfpeepspvadppss
dqtppseakrvvnrcsgcrkrvgltgfrcrclgelfcaehrysdhrhdcydykaagreaiarenpviraakivkv

5 cDNA sequence of GM49780101 from soybean (SEQ ID NO:37):

atggagcctcatgatgagactggatgccaggctcctgaacgccccattcttgcatataataattgtggcttcttggaagagcagctacca
tgaacatgtgtccaagtgttacaaggacatgctgtgaagcaggagcaggacaaattgcagcatcatccgtgaaaacattgtgaat
ggcagttccaatggcaatggaaagcaggctgtggctactgggtgctgtgtgacaaagtgaagctgtggaggtcaagattgtctgtgt
10 cagagttctgtggattcgtcctccggtgatagtttgagatgaaagccaagactggcccagtagatgtgctacatgccggaacgtgtt
ggtttaactggttcagctgcaaatgtggcaacctctctgtgcaatgcatcgctattctgataaacatgattgcccttttgattataggactgt
tggtcaggatgccatagctaaagccaaccccataattaaggcagataagctcgacaaaatctag

15 The GM49780101 cDNA is translated into the following amino acid sequence (SEQ ID NO:38):

mephdetgcqaperpilcinncgffgraatmnmcskcykdmllkqeqdkfaassvenivngssngngkqavatgavavqveave
vkivcaqssvdsssgdslemkaktgprscatcrkrvgltgfsckcgnlfcamhrysdkhdcpfdyrtvgqdaikanpiikadklki

20 cDNA sequence of GM59637305 from soybean (SEQ ID NO:39):

atggaccatgacaagactgggtgccaagctcctcctgaaggctctatattgtgcatcaacaactgtgggttttgaagtgcagctacc
atgaacatgtgttctaaatgccacaaagacataattgtgaaacaggagcaggccaagcttgacagcatcatccattgggaatattatga
atgggtcatcaagcagcactgaaaaggaacctgtgtgtgctgctgctgataattgatatccagttattccagtagagcctaaaactgt
ctctgtgcaacctttatttgggtcaggtccagaggggagtggtgaggcaaagccgaaggatggacaaaaacgttgacagcagctgcaa
25 caagcgagttggttgacagggttaattgtcgatgtgggtgacctttttgtgctgtacatcgctactcgacaagcataattgcccatgtgatt
accgcactgccgctcaagatgctatagctaaagcaaaccagttgcaaggctgaaaagcttgataagatctaa

The GM59637305 cDNA is translated into the following amino acid sequence (SEQ ID NO:40):

30 mdhdkgtgcqappegpilcinncgffgsaatmnmcskchkdillkqeqaklaassignimngsssstekpvaanaanidipvipve
pktvsvqplfgsgpegsveakpkdgpkrccscnkrvgltgfnrcrgdlfclctslldkhncpfdyrtaaqdaikanpvvkaekldki

cDNA sequence of TA55974113 from wheat (SEQ ID NO:41):

35 atggcgcagcgggatcacaagcaggaggagcccacggagctgcgggcgccggagatcacgctctgcgccaacagctgcggctt
cccggggcaacccggccacgcagaacctctgccagaactgcttcttgccgggccggcgctccacgtcgccgtcttctcctcctcctcct
cctctctctgcggggcggtgctcgcgccgacccccgcatcgacaggccgagggcgccgctggaggcgagctggcacgcccc
gccgtcgacctgtctcggcgacggaggcgaagccggcgaggacgtcggtgaaccggtgctccagctgccggaagcgctgggg
ctgacgggggtccgggtgccgggtcgggcgacatgttctcgggcgagcaccgggtactcggaacgggcacgggtgcagctacgactacaa
40 ggccgcccggcaggagcggcatcgccagggaacccccgctgctgcgcgccccaagatcgtaggttctga

The TA55974113 cDNA is translated into the following amino acid sequence (SEQ ID NO:42):

maqrldhkqeeptelrapeitlcanscgfpgnpatqnlcqnclflagpastpsssssssslpgvsaptpvidrprpableaelarpav
dlapateakpartsvnrcsscrkrvgtgfrcrcgdmfcgehrysdhrgcsydykaaardaiardnpvvraakivrf

5 The EST65 amino acid sequence (SEQ ID NO:43):

mvaesvlvcrssvvgaglsfvggegakresagpggrsvflgaqvqkmgagmsarsdvrpaavpkasgdvseqtdyktfsdeewk
krlsqqqfyvarkkgtterpftgeywntktagtylcvcccktplfssktkfdsgtgwpsyydtigdnvkshmdwsipfmpртеvvcavcda
hlghvfddgprptgkrycinsaaidlkaekqeern

10

The EST12 amino acid sequence (SEQ ID NO:44):

mvvpslpafggqnamlrrnidnntdtlislqgscsprvsmqqvprsseslenmmgacgqklpyfssfdgpsveeqedvdegidef
ahhvekkrrlsleqvrslernfevenkleperkmqlakelglprqvavwfnrrarwtkqlehdyetlkkaydrkadfeavtldtnalk
15 aevsrlkgisnddvkpaefvqgkcdtshpaspasersdivssnrntptihvdpvapeeagahlmssdsnssevmdadsprtsh
tsasrstlstsvvpdeglgvaqyphfspenfvgnmpeicadqslasqvkleihsfnpdqtflllpnwwdwa

The EST307 amino acid sequence (SEQ ID NO:45):

20 matervsqettsqapegpvmcknlcgffgsqatmglskcyretvmqakmtalaeqatqaaqatsataaavqppapvhetklce
vertmivphqsssyqqdlvtpaaaapqavkssiaapsrpepnrcgscrkrvgtgfkrcrgnlycalhrysdkhtctydykaagqei
akanplvvaekvvkf

CLAIMS

1. A transgenic plant transformed with an expression cassette comprising an isolated polynucleotide encoding a polypeptide having a sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:6, SEQ ID NO:8, and SEQ ID NO:10.
5
2. A transgenic plant transformed with an expression cassette comprising an isolated polynucleotide encoding a polypeptide having a sequence selected from the group consisting of SEQ ID NO:12, SEQ ID NO:14, and SEQ ID NO:16.
10
3. A transgenic plant transformed with an expression cassette comprising an isolated polynucleotide a polypeptide having a sequence selected from the group consisting of SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:34, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, and SEQ ID NO:44.
15
4. An isolated polynucleotide having a sequence selected from the group consisting of the polynucleotide sequences set forth in Table 1.
20
5. An isolated polypeptide having a sequence selected from the group consisting of the polypeptide sequences set forth in Table 1.
6. A method of producing a transgenic plant comprising at least one polynucleotide listed in Table 1, wherein expression of the polynucleotide in the plant results in the plant's increased growth and/or yield under normal or water-limited conditions and/or increased tolerance to an environmental stress as compared to a wild type variety of the plant comprising the steps of:
25
(a) introducing into a plant cell an expression vector comprising at least one polynucleotide listed in Table 1, and
30
(b) generating from the plant cell a transgenic plant that expresses the polynucleotide, wherein expression of the polynucleotide in the transgenic plant results in the plant's increased growth and/or yield under normal or water-limited conditions and/or increased tolerance to environmental stress as compared to a wild type variety of the plant.
35
7. A method of increasing a plant's growth and/or yield under normal or water-limited conditions and/or increasing a plant's tolerance to an environmental stress comprising the steps of increasing the expression of at least one polynucleotide listed in Table 1 in the plant.

Figure 2 of 3

SEQ ID NO: 12	(1)	-----MMKRLSSDSVGGGLISLCPTTSTDQPNR-----RCGREFQSMLEGY
SEQ ID NO: 44	(1)	MVPSLPAGGQNAMLRRNIDNNTDTLISLLQGSCSPRVSMQQVPRSSSELENMMG---ACGQKLPYFSSFDGSPS
SEQ ID NO: 14	(1)	-----MAGSGSAFNSITS---FLRTQQPSSQPLDSSLFLSAPSSAPFLGSRSMMSFDDGEGKGCNGSFFRA
SEQ ID NO: 16	(1)	-----MEPGRLIIFNTSGSGNGQMLFMDCGAGGAGAGAAAGMFHRGVRPVLGGME-----E-GRGVKRPF
SEQ ID NO: 12	(43)	EEEEEEAITEERGQTLAEKKRRRLNINQVKALEKNFELENKLEPERKVKLAQELGLQPRQVAVWFQNNRRARWKTK
SEQ ID NO: 44	(73)	VEEQEDVDEGIDEFAHHVEKKRRRLSLEQVRSLEKNFELENKLEPERKMQLAQELGLRPRQVAVWFQNNRRARWKTK
SEQ ID NO: 14	(64)	FDMDDNGDECMDEYFHQPEKKRRRLSASQVQFLEKSFEEENKLEPERKTKLAKDLGLQPRQVAIWQNNRRARWKTK
SEQ ID NO: 16	(57)	FTSPDDMLEEEYYDEQLPEKKRRRLTPEQVHLLERSFEEENKLEPERKTELARKLGLQPRQVAVWFQNNRRARWKTK
SEQ ID NO: 12	(118)	QLEKDYGVLTQYDSLRFNFDLSLRRENESILLQEI GK LKAKLNGE -EEGDDVDDEEENNLATMESDVSVKEEE---V
SEQ ID NO: 44	(148)	QLEHDYETLKKAYDRLLKADFEAVTLDTNALKAEVSRLLKGISNDDVKPAEFVQKGKCDTTSHPASPAQSERSD---I
SEQ ID NO: 14	(139)	QLEKDYETLHASFESLKSNYDCLLKEKDKLKAEVASLTEKVLARGKQEGHMKQAESESEETKGLLHLQEQEPPQR
SEQ ID NO: 16	(132)	TLERDFDRLKASFDALRADHDALLQDNHRLRSQVVTLTETKMQDKEAPEGSFAGAADASEPEQAAAEAKASLADAE
SEQ ID NO: 12	(189)	SLPEQITEPPSSPPQLLEHSDSFNYRSFTDLRLDPLPLKAAASSVAAAAGSSDSSAVLNEESSSNVTAAPATVP
SEQ ID NO: 44	(220)	VSSNRRTPTTIHVDPVAPEEAGAHLTMSSDSNSSEVMDADSPTSHTSASRSTLSTSVVQPDGGLGVAQYPHFSP
SEQ ID NO: 14	(214)	LLLSVSEGESEKSVSSVVGCKQEDISSARSDILDSDPHYTDGVHSALLEHGDSSYVFEPDQSDMSQDEEDNLS
SEQ ID NO: 16	(207)	EQAAAAEAFEVVQQLHVKDEERLSPGSGSAVLDARDALLGSGCGLAGVVDSVSDSYCFPGGAGGDEYHECVVG
SEQ ID NO: 12	(264)	--GGSFLQFVKMEQTEHDHDDFLSGEEACGFFSDEQPPSLHWYSTVDQWN-----
SEQ ID NO: 44	(295)	---ENFVGPNMPEICADQSLASQVKLEEIHSFNPDQTFLLLPNWDWA-----
SEQ ID NO: 14	(289)	--KSLYPSYLPFKLEEDVDYSDPPSSCNFGFPPEEDHVLWTWAY-----
SEQ ID NO: 16	(282)	PVAGGIQSEEDDAGSDEGCSYYPDDAAVFFAAQQAQGHGHRHTDDDDQDDGQI SYWMWN-

Figure 3 of 3

SEQ ID NO:18	-----MDHDKTGCGQSPPEGPKLCINNCGFFGSAATMNMCSKCHKAILFQQEQGARFA---	(1)
SEQ ID NO:20	-----MAEEHRCQTP-EGHRLCANNCGFLGSSATMNLCSNCYGDLCIKQQ-QASMK---	(1)
SEQ ID NO:34	-----MAEEHRCQAP-EGHRLCSNNCGFFGSPATMNLCSKCYRDIRLKEEQAKTK---	(1)
SEQ ID NO:38	-----MAQTEKEETDFKVP-ETITLCVNNCGVTGNPATNNMCQKCFATASTATSGAGGAG---	(1)
SEQ ID NO:36	-----MAQREKKVEEPTELRAPEMTLCANSCGFFGNPATNNLCQNCFLAASASS-SSS---	(1)
SEQ ID NO:32	-----MAQRDKKDQEPTELRAPEITLCANSCGFFGNPATQNLQNCFLAATASTSSPSSL---	(1)
SEQ ID NO:44	-----MAQRDHKEEPTELRAPEITLCANSCGFFGNPATQNLQNCFLAGPASTS-PSSSS---	(1)
SEQ ID NO:22	-----MAQESCDLNKDEAEILKPSSTPSPSPATPPPTA-----	(1)
SEQ ID NO:28	MAPSPCVHGTANCPRCHSYGHPIFGNSDLAAGSDTSTSVFGKVGSVVIQSPAKNHAFGACGVPFPSSSPFR	(1)
SEQ ID NO:45	-----MATERVSEETTSQAP-EGPVMKNLGCFFGSAATMGLCSKCYRETMQAKMTALAE---	(1)
SEQ ID NO:42	-----MDHDKTGCGQAPPEGPILCINNCGFFGSAATMNMCSKCHKDILLKQEQAKLAA---	(1)
SEQ ID NO:30	-----MDHDEAGCQAPSDHPILCVNNCGFFGSAATMNMCSKCHKDMLNQEQSKLAA---	(1)
SEQ ID NO:40	-----MEPHDETGCQAP-ERPILCINNCGFFGSAATMNMCSKCHKDMLLKQEQDKFAA---	(1)
SEQ ID NO:26	-----MEHKEAGCQAP-EGPILCINNCGFFGSAATMNMCSKCHKEMITKQDQAKLAA---	(1)
SEQ ID NO:24	-----MEHKEAGCQQP-EGPILCINNCGFFGSAATMNMCSKCHKEMITKQEQQAKLAA---	(1)
SEQ ID NO:18	-----SAVSGGTS-----SSSNILKE-----TFAATALVDAETKS-----VEPVAVS	(53)
SEQ ID NO:20	-----STVESLSAV-----SPPSSEIGSMQSTVESLSDVSPSPETISISSPMIQPLV	(50)
SEQ ID NO:34	-----STIETALSGS-----S-----SATVTATAVAVSSVESPSAP-----VESLP	(51)
SEQ ID NO:38	-----IASPATRSG-----VSARPPQKRFP-----EESPQVAD	(56)
SEQ ID NO:36	-----AAASPSTTS-----LP-----VFPVVEKPRQ-----AVQSSAAA	(54)
SEQ ID NO:32	-----SPVLDKQP-----RP-----AAPLVEPQAP-----LPPVEEM	(57)
SEQ ID NO:44	-----SSSSSLPGV-----SA-----PTPVIDRPRP-----APLEAELA	(56)
SEQ ID NO:22	-----QIPEPQP-----HSPQPAAQFLSRPCEVVPPIET	(37)
SEQ ID NO:28	RIKFGPKDGEKGPLKPIEKQPSKKRPFCSFDETIDATVPPSTKPFGRSVCVTDAEARLKANREFFAPVSR	(76)
SEQ ID NO:45	-----QATQAAQATS-----ATAAAVQPPAPVHETKLTCEVERTMIV-----PHQSSSYQ	(56)
SEQ ID NO:42	-----SSIGNIMNGS-----SSSTEKEP-----VVAANANIDIPVIP-----VEPKTVSV	(53)
SEQ ID NO:30	-----SSAASILNGS-----SMSLIGREL-----VIAAKTNS-----VEPKTISV	(53)
SEQ ID NO:40	-----SSVENIVNGS-----SNGNGKO-----AVATGAVAVQVEA-----VEVKIVCA	(53)
SEQ ID NO:26	-----SSIDSIVNG-----SDAYME-----PVVAGSNTVVAVAQ-----VELQTMNV	(52)
SEQ ID NO:24	-----SPIDSIVNG-----GDGKG-----PVIAAS-VNVAVPQ-----VEQKTI-V	(52)
SEQ ID NO:18	QPSVQV-AAEVVAPEAAAAKKEGPRSCATCNKRVLGTGFKRCRCDLFCGTHRYADIHNCSFNHYHAAQAIAK	(91)
SEQ ID NO:20	RNPSAELEVTA TKTVTPPEQQQKRPNRCTTCRKRVLGTGFKRCRCGTTCGCAHRYPEVHGCTFDKFSAGREEIAK	(100)
SEQ ID NO:34	QPP-----VLI SPDIAAPVQANRCGACRKRVLGTGFKRCRCGTTCGSHRYPEKHACGDFDKAVGREEIAR	(87)
SEQ ID NO:38	PPS-----SDQTTPEAKRVVNRCSGCRKRVLGTGFKRCRGELFCAEHRYSDRHDCCSYDYKAAAGREAIAR	(84)
SEQ ID NO:36	AVALVVERPTAGPVESSSKASRSSVNRCCHRRRVVLGTGFKRCRGELYCGAHRYSDRHDCCSFYKSAARDAIAR	(83)
SEQ ID NO:32	ASALATAPAPVAKTS-----AVNRCSCRKRVLGTGFKRCRGHLFCGEHRYSDRHGCSYDYKSAARDAIAR	(86)
SEQ ID NO:44	RPVADLAPATEAKPART-----SVNRCSSCRKRVLGTGFKRCRCDMFCGEHRYSDRHGCSYDYKAAARDAIAR	(85)
SEQ ID NO:22	SKKR-KHADAVSMAI VPELSSVLFNRCNVCRKRVLGTGFKRCRCCKLFPCPRHRHSESHDCSFYKTVGREEIAR	(68)
SEQ ID NO:28	KRGFDPTDMT FGNAAAAANAREEAKKWCSCKKRVLGLGFKRCRCCKTFCCGKHHRYPEEHGCTFDHVAFGRI IEK	(151)
SEQ ID NO:45	QDLVTPAAAPQAVKSSIAAPSRPEPNRCGSKRVLGTGFKRCRGNLYCALHRYSDKHTCTYDYKAAAGQEAIAK	(101)
SEQ ID NO:42	QPL-----FGSGPEGSEAKP-KDGPKRCSNKRVLGTGFNCRCGDLFLCCTSLLDKHNCFDYRTAAQDAIAK	(93)

Figure 3 continued

SEQ ID NO: 30	(87)	QPS-----SASSAEESIEMKLPKEGPSRCNTCNKRVGLTGFKCRCENMFCAHRYSDKHNCPFDYRTAGREAIISK
SEQ ID NO: 40	(91)	QSS-----VDSSSGDSLEMKAKTGPSRCATCRKRVGLTGFSCKCGNLFCAHRYSDKHDCPFDYRTVGQDAIAK
SEQ ID NO: 26	(89)	QQPADVAGPSEGVAAISKGGK--VGPNRCSACRKRVLGTGFNCRGNYCALHRYSDKHDCCKFDYRTAARDAIAK
SEQ ID NO: 24	(87)	VQPM LVAETSEAAAVIPKAK--EGPDRCAACRKRVLGTGFSCRCGNMYCSVHRYSDKHDCQFDYRTAARDAIAK
SEQ ID NO: 18	(165)	ANP VVKA EKLDKI--
SEQ ID NO: 20	(175)	ANPLVKA AKLQKI--
SEQ ID NO: 34	(152)	ANPVIKGEKLRI--
SEQ ID NO: 38	(149)	ENPVIRAAKIVKV--
SEQ ID NO: 36	(158)	ENP VVRA AKIVRF--
SEQ ID NO: 32	(152)	DNP VVRA AKIVRF--
SEQ ID NO: 44	(153)	DNP VVRA AKIVRF--
SEQ ID NO: 22	(142)	ANPLIRAAKIIIRI--
SEQ ID NO: 28	(226)	QNPVLETDKLVDRI--
SEQ ID NO: 45	(176)	ANPLVVAEKVVKF--
SEQ ID NO: 42	(162)	ANP VVKA EKLDKI--
SEQ ID NO: 30	(157)	ANPLVKA EKLDKI--
SEQ ID NO: 40	(160)	ANPIIKADKLDKI--
SEQ ID NO: 26	(162)	ANP VVKADKLDKI--
SEQ ID NO: 24	(159)	ANP VVRA EKLDKI--