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(54) Title: GENES FOR IMPROVING NUTRIENT UPTAKE AND ABIOTIC STRESS TOLERANCE IN PLANTS

(57) Abstract: The present disclosure provides methods to increase crop yield utilizing transgenic genes controlling plant growth and yield. The specific genes can be used to increase tissue growth and abiotic stress tolerance. Plants, plant progeny, seeds and tissues created by these methods are also described. Polynucleotides encoding the sequences are provided for expression in a plant of interest. Expression cassettes, plants, plant cells, plant parts and seeds comprising the sequences of the disclosure are further provided. In specific embodiments, the polynucleotide is operably linked to a constitutive promoter.



GENES FOR IMPROVING NUTRIENT UPTAKE AND ABIOTIC STRESS TOLERANCE IN PLANTS

5 FIELD OF THE DISCLOSURE

The disclosure relates generally to compositions and methods for increasing crop yield.

BACKGROUND

10 The domestication of many plants has correlated with dramatic increases in yield. Most phenotypic variation occurring in natural populations is continuous and is effected by multiple gene influences. The identification of specific genes responsible for the dramatic differences in yield, in domesticated plants, has become an important focus of agricultural research.

15 One group of genes affecting yield are the nitrogen utilization efficiency (NUE) genes. These genes have utility for improving the use of nitrogen in crop plants, especially maize. The genes can be used to alter the genetic composition of the plants rendering them more productive with current fertilizer application standards, or maintaining their productive rates with significantly reduced fertilizer input. Increased nitrogen use efficiency can result from
20 enhanced uptake and assimilation of nitrogen fertilizer and/or the subsequent remobilization and reutilization of accumulated nitrogen reserves. Plants containing these genes can therefore be used for the enhancement of yield. Improving the nitrogen use efficiency in corn would increase corn harvestable yield per unit of input nitrogen fertilizer, both in developing nations where access to nitrogen fertilizer is limited and in developed nations
25 were the level of nitrogen use remains high. Nitrogen utilization improvement also allows decreases in on-farm input costs, decreased use and dependence on the non-renewable energy sources required for nitrogen fertilizer production, and decreases the environmental impact of nitrogen fertilizer manufacturing and agricultural use.

Two kinds of genes have been found in plants that regulate plant growth and
30 development. Some genes can enhance plant growth while others suppress plant growth. For example, during leaf development, growth enhancing genes are active to keep young leaves growing. When the leaf reaches full-size, the growth suppressing genes are activated to stop the leaf from further growth.

Insufficient water for optimum growth and development of crop plants is a major
35 obstacle to consistent or increased food production worldwide. Population growth, climate change, irrigation-induced soil salinity, and loss of productive agricultural land to development are among the factors contributing to a need for crop plants which can tolerate

drought. Drought stress often results in reduced yield. In maize, this yield loss results in large part from plant failure to set and fill seed in the apical portion of the ear, a phenomenon known as tip kernel abortion.

Plants are restricted to their habitats and must adjust to the prevailing environmental conditions of their surroundings. To cope with abiotic stressors in their habitats, higher plants use a variety of adaptations and plasticity with respect to gene regulation, morphogenesis and metabolism. Adaptation and defense strategies may involve the activation of genes encoding proteins important in the acclimation or defense towards different stressors including drought. Understanding and leveraging the mechanisms of abiotic stress tolerance will have a significant impact on crop productivity.

Methods are needed to enhance drought stress tolerance and to maintain or increase yield under drought conditions.

Crop yield improvements have long been sought and are an age-old problem. Crop yield enhancement has been achieved in the past, by various means, some known, most not. Continued crop yield enhancement will be challenging, demanding specific physiological improvements, such as abiotic stress, and involving more targeted specific approaches, that is, by manipulation of known sets of genes and including both transgenic and breeding approaches. Water limitations globally are the main limitation of crop yield. No prior solution is found to be sufficient to solve the problem of limited crop yield, and thus it remains an unsolved or unfulfilled problem warranting further investigation. This disclosure identifies a set of specific genes that can boost crop yield. It is expected that the main approach for crop yield improvements with these genes is via a judicious ectopic expression, and/or specific native or *induced allele selections that could also achieve the yield enhancing effects. Some genes may* require reduced expression or expression targeted to specific tissue(s) or developmental profiles.

The present disclosure provides methods to increase crop yield utilizing the disclosed genes controlling plant growth and yield. Plants, plant progeny, seeds and tissues created by these methods are also described.

BRIEF SUMMARY

The disclosure relates generally to compositions and methods for increasing crop yield. Certain embodiments provide methods for enhancing growth of harvestable organs. Certain embodiments provide methods for suppressing growth of non-harvestable organs such as male flower and pollen. Certain embodiments comprise pairs of growth enhancement components and growth suppression components in which the phenotype of the plants is modified to increase harvest index and subsequently crop yield. Certain

embodiments provide constructs and methods useful for restructure of plant growth and development through manipulating organ size through cell size or cell numbers.

The present disclosure presents methods to alter the genetic composition of crop plants, especially maize, so that such crops can be more productive with current fertilizer applications and/or as productive with significantly reduced fertilizer input. The utility of this disclosure is then both yield enhancement and reduced fertilizer costs with corresponding reduced impact to the environment. The genetic enhancement of the crop plant's intrinsic genetics in order to enhance nitrogen use efficiency has not been achieved by scientists in the past in any commercially viable sense. This disclosure uniquely uses a highly selected set of maize plants that has been shown to differ in aspects of nitrogen utilization. The plants were then subjected to experiments in mRNA profiling and data analysis to yield a set of genes that are useful for modification of crop plants, especially maize for enhancing nitrogen use efficiency.

Compositions and methods for controlling plant growth for increasing yield in a plant are provided. The compositions include specific gene sequences from sorghum, maize, *Arabidopsis thaliana* and *Pichia angusta*. Compositions of the disclosure comprise amino acid sequences and nucleotide sequences selected from SEQ ID NOS: 1-5105 as well as variants and fragments thereof.

Polynucleotides encoding the sequences are provided in DNA constructs for expression in a plant of interest. Expression cassettes, plants, plant cells, plant parts and seeds comprising the sequences of the disclosure are further provided. In one aspect, the polynucleotide is operably linked to a constitutive promoter. In another aspect, the polynucleotide is operably linked to a tissue-specific/tissue-preferential promoter.

Methods for modulating the level of a yield improvement sequence in a plant or plant part is provided. The methods comprise introducing into a plant or plant part a heterologous polynucleotide comprising a yield improvement sequence of the disclosure. The level of yield improvement polypeptide can be increased or decreased. Such method can be used to increase the yield in plants; in one embodiment, the method is used to increase grain yield in cereals.

Methods are provided for increasing abiotic stress in plants. More particularly, the methods of the disclosure find use in agriculture for increasing abiotic stress in dicot and monocot plants. The methods comprise introducing into a plant cell a polynucleotide that encodes a polypeptide operably linked to a promoter that drives expression in a plant.

Methods are further provided for maintaining or increasing yield in plants under drought conditions. Also provided are transformed plants, plant tissues, plant cells and seeds thereof.

DETAILED DESCRIPTION

Methods are provided for increasing stress tolerance, particularly abiotic stress tolerance, in plants. These methods find use, for example, in increasing tolerance to drought stress and maintaining or increasing yield during drought conditions, particularly in agricultural plants.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. Unless mentioned otherwise, the techniques employed or contemplated herein are standard methodologies well known to one of ordinary skill in the art. The materials, methods and examples are illustrative only and not limiting. The following is presented by way of illustration and is not intended to limit the scope of the disclosure.

The present disclosures now will be described more fully hereinafter with reference to the accompanying drawings, in which some, but not all embodiments of the disclosure are shown. Indeed, these disclosures may be embodied in many different forms and should not be construed as limited to the embodiments set forth herein; rather, these embodiments are provided so that this disclosure will satisfy applicable legal requirements. Like numbers refer to like elements throughout.

Many modifications and other embodiments of the disclosures set forth herein will come to mind to one skilled in the art to which these disclosures pertain having the benefit of the teachings presented in the foregoing descriptions and the associated drawings. Therefore, it is to be understood that the disclosures are not to be limited to the specific embodiments disclosed and that modifications and other embodiments are intended to be included within the scope of the appended claims. Although specific terms are employed herein, they are used in a generic and descriptive sense only and not for purposes of limitation.

The practice of the present disclosure will employ, unless otherwise indicated, conventional techniques of botany, microbiology, tissue culture, molecular biology, chemistry, biochemistry and recombinant DNA technology, which are within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Langenheim and Thimann, *BOTANY: PLANT BIOLOGY AND ITS RELATION TO HUMAN AFFAIRS*, John Wiley (1982); *CELL CULTURE AND SOMATIC CELL GENETICS OF PLANTS*, vol. 1, Vasil, ed. (1984); Stanier, *et al.*, *THE MICROBIAL WORLD*, 5th ed., Prentice-Hall (1986); Dhringra and Sinclair, *BASIC PLANT PATHOLOGY METHODS*, CRC Press (1985); Maniatis, *et al.*, *MOLECULAR CLONING: A LABORATORY MANUAL* (1982); *DNA CLONING*, vols. I and II, Glover, ed. (1985); *OLIGONUCLEOTIDE SYNTHESIS*, Gait, ed. (1984); *NUCLEIC ACID HYBRIDIZATION*, Hames and Higgins, eds. (1984); and the series *METHODS IN ENZYMOLOGY*, Colowick and Kaplan, eds, Academic Press, Inc., San Diego, CA.

Units, prefixes and symbols may be denoted in their SI accepted form. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively. Numeric ranges are inclusive of the numbers defining the range. Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes. The terms defined below are more fully defined by reference to the specification as a whole.

In describing the present disclosure, the following terms will be employed and are intended to be defined as indicated below.

By "microbe" is meant any microorganism (including both eukaryotic and prokaryotic microorganisms), such as fungi, yeast, bacteria, actinomycetes, algae and protozoa, as well as other unicellular structures.

By "amplified" is meant the construction of multiple copies of a nucleic acid sequence or multiple copies complementary to the nucleic acid sequence using at least one of the nucleic acid sequences as a template. Amplification systems include the polymerase chain reaction (PCR) system, ligase chain reaction (LCR) system, nucleic acid sequence based amplification (NASBA, Cangene, Mississauga, Ontario), Q-Beta Replicase systems, transcription-based amplification system (TAS), and strand displacement amplification (SDA). See, e.g., DIAGNOSTIC MOLECULAR MICROBIOLOGY: PRINCIPLES AND APPLICATIONS, Persing, *et al.*, eds., American Society for Microbiology, Washington, DC (1993). The product of amplification is termed an amplicon.

The term "conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refer to those nucleic acids that encode identical or conservatively modified variants of the amino acid sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations" and represent one species of conservatively modified variation. Every nucleic acid sequence herein that encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of ordinary skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine; one exception is *Micrococcus rubens*, for which GTG is the methionine codon (Ishizuka, *et al.*, (1993) *J. Gen. Microbiol.* 139:425-32) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid, which encodes a polypeptide

of the present disclosure, is implicit in each described polypeptide sequence and incorporated herein by reference.

As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a “conservatively modified variant” when the alteration results in the substitution of an amino acid with a chemically similar amino acid. Thus, any number of amino acid residues selected from the group of integers consisting of from 1 to 15 can be so altered. Thus, for example, 1, 2, 3, 4, 5, 7 or 10 alterations can be made. Conservatively modified variants typically provide similar biological activity as the unmodified polypeptide sequence from which they are derived. For example, substrate specificity, enzyme activity, or ligand/receptor binding is generally at least 30%, 40%, 50%, 60%, 70%, 80% or 90%, preferably 60-90% of the native protein for its native substrate. Conservative substitution tables providing functionally similar amino acids are well known in the art.

The following six groups each contain amino acids that are conservative substitutions for one another:

- 1) Alanine (A), Serine (S), Threonine (T);
- 2) Aspartic acid (D), Glutamic acid (E);
- 3) Asparagine (N), Glutamine (Q);
- 4) Arginine (R), Lysine (K);
- 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
- 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

See also, Creighton, PROTEINS, W.H. Freeman and Co. (1984).

As used herein, “consisting essentially of” means the inclusion of additional sequences to an object polynucleotide where the additional sequences do not selectively hybridize, under stringent hybridization conditions, to the same cDNA as the polynucleotide and where the hybridization conditions include a wash step in 0.1X SSC and 0.1% sodium dodecyl sulfate at 65°C.

By “encoding” or “encoded,” with respect to a specified nucleic acid, is meant comprising the information for translation into the specified protein. A nucleic acid encoding a protein may comprise non-translated sequences (e.g., introns) within translated regions of the nucleic acid, or may lack such intervening non-translated sequences (e.g., as in cDNA). The information by which a protein is encoded is specified by the use of codons. Typically, the amino acid sequence is encoded by the nucleic acid using the “universal” genetic code. However, variants of the universal code, such as is present in some plant, animal and fungal mitochondria, the bacterium *Mycoplasma capricolum* (Yamao, *et al.*, (1985) *Proc. Natl.*

Acad. Sci. USA 82:2306-9) or the ciliate *Macronucleus*, may be used when the nucleic acid is expressed using these organisms.

When the nucleic acid is prepared or altered synthetically, advantage can be taken of known codon preferences of the intended host where the nucleic acid is to be expressed.

5 For example, although nucleic acid sequences of the present disclosure may be expressed in both monocotyledonous and dicotyledonous plant species, sequences can be modified to account for the specific codon preferences and GC content preferences of monocotyledonous plants or dicotyledonous plants as these preferences have been shown to differ (Murray, *et al.*, (1989) *Nucleic Acids Res.* 17:477-98, herein incorporated by
10 reference). Thus, the maize preferred codon for a particular amino acid might be derived from known gene sequences from maize. Maize codon usage for 28 genes from maize plants is listed in Table 4 of Murray, *et al.*, *supra*.

As used herein, "heterologous" in reference to a nucleic acid is a nucleic acid that originates from a foreign species, or, if from the same species, is substantially modified from
15 its native form in composition and/or genomic locus by deliberate human intervention. For example, a promoter operably linked to a heterologous structural gene is from a species different from that from which the structural gene was derived or, if from the same species, one or both are substantially modified from their original form. A heterologous protein may originate from a foreign species or, if from the same species, is substantially modified from
20 its original form by deliberate human intervention.

By "host cell" is meant a cell, which contains a vector and supports the replication and/or expression of the expression vector. Host cells may be prokaryotic cells such as *E. coli*, or eukaryotic cells such as yeast, insect, plant, amphibian or mammalian cells. Preferably, host cells are monocotyledonous or dicotyledonous plant cells, including but not
25 limited to maize, sorghum, sunflower, soybean, wheat, alfalfa, rice, cotton, canola, barley, millet and tomato. A particularly preferred monocotyledonous host cell is a maize host cell.

The term "hybridization complex" includes reference to a duplex nucleic acid structure formed by two single-stranded nucleic acid sequences selectively hybridized with each other.

30 The term "introduced" in the context of inserting a nucleic acid into a cell, means "transfection" or "transformation" or "transduction" and includes reference to the incorporation of a nucleic acid into a eukaryotic or prokaryotic cell where the nucleic acid may be incorporated into the genome of the cell (e.g., chromosome, plasmid, plastid or mitochondrial DNA), converted into an autonomous replicon, or transiently expressed (e.g.,
35 transfected mRNA).

The terms "isolated" refers to material, such as a nucleic acid or a protein, which is substantially or essentially free from components which normally accompany or interact with

it as found in its naturally occurring environment. The isolated material optionally comprises material not found with the material in its natural environment. Nucleic acids, which are “isolated”, as defined herein, are also referred to as “heterologous” nucleic acids. Unless otherwise stated, the term “yield improvement nucleic acid” means a nucleic acid comprising a polynucleotide (“yield improvement polynucleotide”) encoding a yield improvement polypeptide. The term “Growth Enhancement gene” means a gene that when expressed can increase cell numbers, cell size and dry matter accumulation, resulting in increased organ size, numbers and dry weight. On the opposite, the term “Growth suppression gene” means a gene when expressed can decrease or inhibit cell numbers, cell size and dry matter accumulation, resulting in decreased organ size, numbers and dry weight. The term “yield improvement gene” may include both “Growth Enhancer gene” and “Growth suppressor gene”.

As used herein, “nucleic acid” includes reference to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogues having the essential nature of natural nucleotides in that they hybridize to single-stranded nucleic acids in a manner similar to naturally occurring nucleotides (e.g., peptide nucleic acids).

By “nucleic acid library” is meant a collection of isolated DNA or RNA molecules, which comprise and substantially represent the entire transcribed fraction of a genome of a specified organism. Construction of exemplary nucleic acid libraries, such as genomic and cDNA libraries, is taught in standard molecular biology references such as Berger and Kimmel, GUIDE TO MOLECULAR CLONING TECHNIQUES, from the series METHODS IN ENZYMOLOGY, vol. 152, Academic Press, Inc., San Diego, CA (1987); Sambrook, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2nd ed., vols. 1-3 (1989); and CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Ausubel, *et al.*, eds, Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc. (1994 Supplement).

As used herein “operably linked” includes reference to a functional linkage between a first sequence, such as a promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. Generally, operably linked means that the nucleic acid sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame.

As used herein, the term “plant” includes reference to whole plants, plant organs (e.g., leaves, stems, roots, etc.), seeds and plant cells and progeny of same. Plant cell, as used herein includes, without limitation, seeds suspension cultures, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen and

microspores. The class of plants, which can be used in the methods of the disclosure, is generally as broad as the class of higher plants amenable to transformation techniques, including both monocotyledonous and dicotyledonous plants including species from the genera: *Cucurbita*, *Rosa*, *Vitis*, *Juglans*, *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*,
5 *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Geranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Datura*, *Hyoscyamus*, *Lycopersicon*, *Nicotiana*, *Solanum*, *Petunia*, *Digitalis*, *Majorana*, *Ciahorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Heterocallis*, *Nemesis*, *Pelargonium*, *Panieum*, *Pennisetum*, *Ranunculus*, *Senecio*, *Salpiglossis*, *Cucumis*, *Browaalia*, *Glycine*, *Pisum*, *Phaseolus*, *Lolium*, *Oryza*,
10 *Avena*, *Hordeum*, *Secale*, *Allium* and *Triticum*. A particularly preferred plant is *Zea mays*.

As used herein, "yield" includes reference to bushels per acre of a grain crop at harvest, as adjusted for grain moisture (15% typically). Grain moisture is measured in the grain at harvest. The adjusted test weight of grain is determined to be the weight in pounds per bushel, adjusted for grain moisture level at harvest.

As used herein, "polynucleotide" includes reference to a deoxyribopolynucleotide, ribopolynucleotide or analogs thereof that have the essential nature of a natural ribonucleotide in that they hybridize, under stringent hybridization conditions, to substantially the same nucleotide sequence as naturally occurring nucleotides and/or allow translation into the same amino acid(s) as the naturally occurring nucleotide(s). A polynucleotide can
20 be full-length or a subsequence of a native or heterologous structural or regulatory gene. Unless otherwise indicated, the term includes reference to the specified sequence as well as the complementary sequence thereof. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "polynucleotides" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as
25 tritylated bases, to name just two examples, are polynucleotides as the term is used herein. It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term polynucleotide as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and
30 cells, including *inter alia*, simple and complex cells.

The terms "polypeptide," "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers.

As used herein "promoter" includes reference to a region of DNA upstream from the start of transcription and involved in recognition and binding of RNA polymerase and other proteins to initiate transcription. A "plant promoter" is a promoter capable of initiating

transcription in plant cells. Exemplary plant promoters include, but are not limited to, those that are obtained from plants, plant viruses and bacteria which comprise genes expressed in plant cells such *Agrobacterium* or *Rhizobium*. Examples are promoters that preferentially initiate transcription in certain tissues, such as leaves, roots, seeds, fibres, xylem vessels, tracheids or sclerenchyma. Such promoters are referred to as “tissue preferred.” A “cell type” specific promoter primarily drives expression in certain cell types in one or more organs, for example, vascular cells in roots or leaves. An “inducible” or “regulatable” promoter is a promoter, which is under environmental control. Examples of environmental conditions that may affect transcription by inducible promoters include anaerobic conditions or the presence of light. Another type of promoter is a developmentally regulated promoter, for example, a promoter that drives expression during pollen development. Tissue preferred, cell type specific, developmentally regulated, and inducible promoters constitute the class of “non-constitutive” promoters. A “constitutive” promoter is a promoter, which is active under most environmental conditions.

The term “yield improvement polypeptide” refers to one or more amino acid sequences. The term is also inclusive of fragments, variants, homologs, alleles or precursors (e.g., preproteins or proproteins) thereof. A “yield improvement protein” comprises a yield improvement polypeptide. Unless otherwise stated, the term “yield improvement nucleic acid” means a nucleic acid comprising a polynucleotide (“yield improvement polynucleotide”) encoding a yield improvement polypeptide.

As used herein “recombinant” includes reference to a cell or vector, that has been modified by the introduction of a heterologous nucleic acid or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found in identical form within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all as a result of deliberate human intervention. The term “recombinant” as used herein does not encompass the alteration of the cell or vector by naturally occurring events (e.g., spontaneous mutation, natural transformation/transduction/transposition) such as those occurring without deliberate human intervention.

As used herein, a “recombinant expression cassette” is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements, which permit transcription of a particular nucleic acid in a target cell. The recombinant expression cassette can be incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus or nucleic acid fragment. Typically, the recombinant expression cassette portion of an expression vector includes, among other sequences, a nucleic acid to be transcribed and a promoter.

The terms "residue" or "amino acid residue" or "amino acid" are used interchangeably herein to refer to an amino acid that is incorporated into a protein, polypeptide, or peptide (collectively "protein"). The amino acid may be a naturally occurring amino acid and, unless otherwise limited, may encompass known analogs of natural amino acids that can function in a similar manner as naturally occurring amino acids.

The term "selectively hybridizes" includes reference to hybridization, under stringent hybridization conditions, of a nucleic acid sequence to a specified nucleic acid target sequence to a detectably greater degree (e.g., at least 2-fold over background) than its hybridization to non-target nucleic acid sequences and to the substantial exclusion of non-target nucleic acids. Selectively hybridizing sequences typically have about at least 40% sequence identity, preferably 60-90% sequence identity and most preferably 100% sequence identity (i.e., complementary) with each other.

The terms "stringent conditions" or "stringent hybridization conditions" include reference to conditions under which a probe will hybridize to its target sequence, to a detectably greater degree than other sequences (e.g., at least 2-fold over background). Stringent conditions are sequence-dependent and will be different in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences can be identified which can be up to 100% complementary to the probe (homologous probing). Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing). Optimally, the probe is approximately 500 nucleotides in length, but can vary greatly in length from less than 500 nucleotides to equal to the entire length of the target sequence.

Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (e.g., 10 to 50 nucleotides) and at least about 60°C for long probes (e.g., greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide or Denhardt's. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1 M NaCl, 1% SDS at 37°C and a wash in 0.5X to 1X SSC at 55 to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C and a wash in 0.1X SSC at 60 to 65°C. Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m can be approximated from the equation of

Meinkoth and Wahl, (1984) *Anal. Biochem.* 138:267-84: $T_m = 81.5^{\circ}\text{C} + 16.6 (\log M) + 0.41 (\%GC) - 0.61 (\% \text{ form}) - 500/L$; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution and L is the length of the hybrid in base pairs. The T_m is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1°C for each 1% of mismatching; thus, T_m , hybridization and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with $\geq 90\%$ identity are sought, the T_m can be decreased 10°C . Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3 or 4°C lower than the thermal melting point (T_m); moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9 or 10°C lower than the thermal melting point (T_m); low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15 or 20°C lower than the thermal melting point (T_m). Using the equation, hybridization and wash compositions, and desired T_m , those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a T_m of less than 45°C (aqueous solution) or 32°C (formamide solution) it is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen, LABORATORY TECHNIQUES IN BIOCHEMISTRY AND MOLECULAR BIOLOGY--HYBRIDIZATION WITH NUCLEIC ACID PROBES, part I, chapter 2, "Overview of principles of hybridization and the strategy of nucleic acid probe assays," Elsevier, New York (1993) and CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, chapter 2, Ausubel, *et al.*, eds, Greene Publishing and Wiley-Interscience, New York (1995). Unless otherwise stated, in the present application high stringency is defined as hybridization in 4X SSC, 5X Denhardt's (5 g Ficoll, 5 g polyvinylpyrrolidone, 5 g bovine serum albumin in 500ml of water), 0.1 mg/ml boiled salmon sperm DNA, and 25 mM Na phosphate at 65°C , and a wash in 0.1X SSC, 0.1% SDS at 65°C .

As used herein, "transgenic plant" includes reference to a plant, which comprises within its genome a heterologous polynucleotide. Generally, the heterologous polynucleotide is stably integrated within the genome such that the polynucleotide is passed on to successive generations. The heterologous polynucleotide may be integrated into the genome alone or as part of a recombinant expression cassette. "Transgenic" is used herein to include any cell, cell line, callus, tissue, plant part or plant, the genotype of which has been altered by the presence of heterologous nucleic acid including those transgenics

initially so altered as well as those created by sexual crosses or asexual propagation from the initial transgenic. The term “transgenic” as used herein does not encompass the alteration of the genome (chromosomal or extra-chromosomal) by conventional plant breeding methods or by naturally occurring events such as random cross-fertilization, non-recombinant viral infection, non-recombinant bacterial transformation, non-recombinant transposition or spontaneous mutation.

As used herein, “vector” includes reference to a nucleic acid used in transfection of a host cell and into which can be inserted a polynucleotide. Vectors are often replicons. Expression vectors permit transcription of a nucleic acid inserted therein.

The following terms are used to describe the sequence relationships between two or more nucleic acids or polynucleotides or polypeptides: (a) “reference sequence,” (b) “comparison window,” (c) “sequence identity,” (d) “percentage of sequence identity” and (e) “substantial identity.”

As used herein, “reference sequence” is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset or the entirety of a specified sequence; for example, as a segment of a full-length cDNA or gene sequence or the complete cDNA or gene sequence.

As used herein, “comparison window” means includes reference to a contiguous and specified segment of a polynucleotide sequence, wherein the polynucleotide sequence may be compared to a reference sequence and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Generally, the comparison window is at least 20 contiguous nucleotides in length, and optionally can be 30, 40, 50, 100 or longer. Those of skill in the art understand that to avoid a high similarity to a reference sequence due to inclusion of gaps in the polynucleotide sequence a gap penalty is typically introduced and is subtracted from the number of matches.

Methods of alignment of nucleotide and amino acid sequences for comparison are well known in the art. The local homology algorithm (BESTFIT) of Smith and Waterman, (1981) *Adv. Appl. Math* 2:482, may conduct optimal alignment of sequences for comparison; by the homology alignment algorithm (GAP) of Needleman and Wunsch, (1970) *J. Mol. Biol.* 48:443-53; by the search for similarity method (Tfasta and Fasta) of Pearson and Lipman, (1988) *Proc. Natl. Acad. Sci. USA* 85:2444; by computerized implementations of these algorithms, including, but not limited to: CLUSTAL in the PC/Gene program by Intelligenetics, Mountain View, California, GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package®, Version 8 (available from Genetics Computer Group (GCG® programs (Accelrys, Inc., San Diego, CA)). The CLUSTAL program is well

described by Higgins and Sharp, (1988) *Gene* 73:237-44; Higgins and Sharp, (1989) *CABIOS* 5:151-3; Corpet, *et al.*, (1988) *Nucleic Acids Res.* 16:10881-90; Huang, *et al.*, (1992) *Computer Applications in the Biosciences* 8:155-65 and Pearson, *et al.*, (1994) *Meth. Mol. Biol.* 24:307-31. The preferred program to use for optimal global alignment of multiple sequences is PileUp (Feng and Doolittle, (1987) *J. Mol. Evol.*, 25:351-60 which is similar to the method described by Higgins and Sharp, (1989) *CABIOS* 5:151-53 and hereby incorporated by reference). The BLAST family of programs which can be used for database similarity searches includes: BLASTN for nucleotide query sequences against nucleotide database sequences; BLASTX for nucleotide query sequences against protein database sequences; BLASTP for protein query sequences against protein database sequences; TBLASTN for protein query sequences against nucleotide database sequences; and TBLASTX for nucleotide query sequences against nucleotide database sequences. See CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, Chapter 19, Ausubel, *et al.*, eds., Greene Publishing and Wiley-Interscience, New York (1995).

GAP uses the algorithm of Needleman and Wunsch, *supra*, to find the alignment of two complete sequences that maximizes the number of matches and minimizes the number of gaps. GAP considers all possible alignments and gap positions and creates the alignment with the largest number of matched bases and the fewest gaps. It allows for the provision of a gap creation penalty and a gap extension penalty in units of matched bases. GAP must make a profit of gap creation penalty number of matches for each gap it inserts. If a gap extension penalty greater than zero is chosen, GAP must, in addition, make a profit for each gap inserted of the length of the gap times the gap extension penalty. Default gap creation penalty values and gap extension penalty values in Version 10 of the Wisconsin Genetics Software Package® are 8 and 2, respectively. The gap creation and gap extension penalties can be expressed as an integer selected from the group of integers consisting of from 0 to 100. Thus, for example, the gap creation and gap extension penalties can be 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50 or greater.

GAP presents one member of the family of best alignments. There may be many members of this family, but no other member has a better quality. GAP displays four figures of merit for alignments: Quality, Ratio, Identity and Similarity. The Quality is the metric maximized in order to align the sequences. Ratio is the quality divided by the number of bases in the shorter segment. Percent Identity is the percent of the symbols that actually match. Percent Similarity is the percent of the symbols that are similar. Symbols that are across from gaps are ignored. A similarity is scored when the scoring matrix value for a pair of symbols is greater than or equal to 0.50, the similarity threshold. The scoring matrix used in Version 10 of the Wisconsin Genetics Software Package® is BLOSUM62 (see, Henikoff and Henikoff, (1989) *Proc. Natl. Acad. Sci. USA* 89:10915).

Unless otherwise stated, sequence identity/similarity values provided herein refer to the value obtained using the BLAST 2.0 suite of programs using default parameters (Altschul, *et al.*, (1997) *Nucleic Acids Res.* 25:3389-402).

As those of ordinary skill in the art will understand, BLAST searches assume that proteins can be modeled as random sequences. However, many real proteins comprise regions of nonrandom sequences, which may be homopolymeric tracts, short-period repeats or regions enriched in one or more amino acids. Such low-complexity regions may be aligned between unrelated proteins even though other regions of the protein are entirely dissimilar. A number of low-complexity filter programs can be employed to reduce such low-complexity alignments. For example, the SEG (Wooten and Federhen, (1993) *Comput. Chem.* 17:149-63) and XNU (Claverie and States, (1993) *Comput. Chem.* 17:191-201) low-complexity filters can be employed alone or in combination.

As used herein, "sequence identity" or "identity" in the context of two nucleic acid or polypeptide sequences includes reference to the residues in the two sequences, which are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g., charge or hydrophobicity) and therefore do not change the functional properties of the molecule. Where sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences, which differ by such conservative substitutions, are said to have "sequence similarity" or "similarity." Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, e.g., according to the algorithm of Meyers and Miller, (1988) *Computer Applic. Biol. Sci.* 4:11-17, e.g., as implemented in the program PC/GENE (Intelligenetics, Mountain View, California, USA).

As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or

amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

The term "substantial identity" of polynucleotide sequences means that a polynucleotide comprises a sequence that has between 50-100% sequence identity, preferably at least 50% sequence identity, preferably at least 60% sequence identity, preferably at least 70%, more preferably at least 80%, more preferably at least 90% and most preferably at least 95%, compared to a reference sequence using one of the alignment programs described using standard parameters. One of skill will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like. Substantial identity of amino acid sequences for these purposes normally means sequence identity of between 55-100%, preferably at least 55%, preferably at least 60%, more preferably at least 70%, 80%, 90% and most preferably at least 95%.

Another indication that nucleotide sequences are substantially identical is if two molecules hybridize to each other under stringent conditions. The degeneracy of the genetic code allows for many amino acids substitutions that lead to variety in the nucleotide sequence that code for the same amino acid, hence it is possible that the DNA sequence could code for the same polypeptide but not hybridize to each other under stringent conditions. This may occur, e.g., when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. One indication that two nucleic acid sequences are substantially identical is that the polypeptide, which the first nucleic acid encodes, is immunologically cross reactive with the polypeptide encoded by the second nucleic acid.

The terms "substantial identity" in the context of a peptide indicates that a peptide comprises a sequence with between 55-100% sequence identity to a reference sequence preferably at least 55% sequence identity, preferably 60% preferably 70%, more preferably 80%, most preferably at least 90% or 95% sequence identity to the reference sequence over a specified comparison window. Preferably, optimal alignment is conducted using the homology alignment algorithm of Needleman and Wunsch, *supra*. An indication that two peptide sequences are substantially identical is that one peptide is immunologically reactive with antibodies raised against the second peptide. Thus, a peptide is substantially identical to a second peptide, for example, where the two peptides differ only by a conservative substitution. In addition, a peptide can be substantially identical to a second peptide when they differ by a non-conservative change if the epitope that the antibody recognizes is substantially identical. Peptides, which are "substantially similar" share sequences as, noted

above except that residue positions, which are not identical, may differ by conservative amino acid changes.

The disclosure describes yield improvement polynucleotides and polypeptides. The novel nucleotides and proteins of the disclosure have an expression pattern which indicates that they regulate cell number and thus play an important role in plant development. The polynucleotides are expressed in various plant tissues. The polynucleotides and polypeptides thus provide an opportunity to manipulate plant development to alter seed and vegetative tissue development, timing or composition. This may be used to create a sterile plant, a seedless plant or a plant with altered endosperm composition.

Nucleic Acids

The present disclosure provides, *inter alia*, isolated nucleic acids of RNA, DNA and analogs and/or chimeras thereof, comprising a yield improvement polynucleotide.

The present disclosure also includes polynucleotides optimized for expression in different organisms. For example, for expression of the polynucleotide in a maize plant, the sequence can be altered to account for specific codon preferences and to alter GC content as according to Murray, *et al*, *supra*. Maize codon usage for 28 genes from maize plants is listed in Table 4 of Murray, *et al*, *supra*.

The yield improvement nucleic acids of the present disclosure comprise isolated yield improvement polynucleotides which are inclusive of:

- (a) a polynucleotide encoding a yield improvement polypeptide and conservatively modified and polymorphic variants thereof;
- (b) a polynucleotide having at least 70% sequence identity with polynucleotides of (a) or (b);
- (c) complementary sequences of polynucleotides of (a) or (b).

The following table, Table 1, lists the specific identities of the polynucleotides and polypeptides and disclosed herein.

TABLE 1

RR10	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 1 SEQ ID NO: 2 SEQ ID NO: 3405
PTK1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3 SEQ ID NO: 4 SEQ ID NO: 3406
ARGOS	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 5 SEQ ID NO: 6 SEQ ID NO: 3407
ARPK	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 7 SEQ ID NO: 8 SEQ ID NO: 3408
BHLH	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 9 SEQ ID NO: 10 SEQ ID NO: 3409
COXVIIa	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 11 SEQ ID NO: 12 SEQ ID NO: 3410
GIRLPK	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 13 SEQ ID NO: 14 SEQ ID NO: 3411
SEU1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 15 SEQ ID NO: 16 SEQ ID NO: 3412
ERECTA	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 17 SEQ ID NO: 18 SEQ ID NO: 3413
PHDF	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 19 SEQ ID NO: 20 SEQ ID NO: 3414
TFL6	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 21 SEQ ID NO: 22 SEQ ID NO: 3415
TFL8	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 23 SEQ ID NO: 24 SEQ ID NO: 3416
FIE2	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 25 SEQ ID NO: 26 SEQ ID NO: 3417
MEZ2	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 27 SEQ ID NO: 28 SEQ ID NO: 3418
TPS1HA	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 29 SEQ ID NO: 30 SEQ ID NO: 3419
SVP4	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 31 SEQ ID NO: 32 SEQ ID NO: 3420
SILKY1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 33 SEQ ID NO: 34 SEQ ID NO: 3421
EBNA2	Sorghum bicolor	Polynucleotide Polypeptide	SEQ ID NO: 35 SEQ ID NO: 36

		Genomic	SEQ ID NO: 3422
MYB	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 37 SEQ ID NO: 38 SEQ ID NO: 3423
ALF1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 39 SEQ ID NO: 40 SEQ ID NO: 3424
ALF2	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 41 SEQ ID NO: 42 SEQ ID NO: 3425
NDK4	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 43 SEQ ID NO: 44 SEQ ID NO: 3426
PPBP1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 45 SEQ ID NO: 46 SEQ ID NO: 3427
SPS	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 47 SEQ ID NO: 48 SEQ ID NO: 3428
SIG2B	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 49 SEQ ID NO: 50 SEQ ID NO: 3429
HAP32	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 51 SEQ ID NO: 52 SEQ ID NO: 3430
ARGOS3	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 53 SEQ ID NO: 54 SEQ ID NO: 3431
ARP6	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 55 SEQ ID NO: 56 SEQ ID NO: 3432
HAP3L3	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 57 SEQ ID NO: 58 SEQ ID NO: 3433
CBFA2	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 59 SEQ ID NO: 60 SEQ ID NO: 3434
TFL10	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 61 SEQ ID NO: 62 SEQ ID NO: 3435
TFL13	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 63 SEQ ID NO: 64 SEQ ID NO: 3436
SIG2A	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 65 SEQ ID NO: 66 SEQ ID NO: 3437
ALAAT	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 67 SEQ ID NO: 68 SEQ ID NO: 3438
FBA1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 69 SEQ ID NO: 70 SEQ ID NO: 3439
SVP3	Sorghum bicolor	Polynucleotide Polypeptide	SEQ ID NO: 71 SEQ ID NO: 72

		Genomic	SEQ ID NO: 3440
CNR1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 73 SEQ ID NO: 74 SEQ ID NO: 3441
POL	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 75 SEQ ID NO: 76 SEQ ID NO: 3442
GIP	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 77 SEQ ID NO: 78 SEQ ID NO: 3443
FT6	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 79 SEQ ID NO: 80 SEQ ID NO: 3444
NADHTR	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 81 SEQ ID NO: 82 SEQ ID NO: 3445
RVDH	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 83 SEQ ID NO: 84 SEQ ID NO: 3446
SENC	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 85 SEQ ID NO: 86 SEQ ID NO: 3447
FT4	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 87 SEQ ID NO: 88 SEQ ID NO: 3448
SBP8	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 89 SEQ ID NO: 90 SEQ ID NO: 3449
NRP1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 91 SEQ ID NO: 92 SEQ ID NO: 3450
TFL16	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 93 SEQ ID NO: 94 SEQ ID NO: 3451
PP2C	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 95 SEQ ID NO: 96 SEQ ID NO: 3452
NUCPU3	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 97 SEQ ID NO: 98 SEQ ID NO: 3453
DTP7	Arabidopsis thaliana	Polynucleotide Polypeptide Genomic	SEQ ID NO: 99 SEQ ID NO: 100 SEQ ID NO: 3454
ARGOS6	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 101 SEQ ID NO: 102 SEQ ID NO: 3455
ARGOS8	Zea mays	Polynucleotide Polypeptide Genomic	SEQ ID NO: 103 SEQ ID NO: 104 SEQ ID NO: 3456
ARP7	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 105 SEQ ID NO: 106 SEQ ID NO: 3457
ARGOS9	Sorghum bicolor	Polynucleotide Polypeptide	SEQ ID NO: 107 SEQ ID NO: 108

		Genomic	SEQ ID NO: 3458
NUCPU7	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 109 SEQ ID NO: 110 SEQ ID NO: 3459
EBP1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 111 SEQ ID NO: 112 SEQ ID NO: 3460
LRR	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 113 SEQ ID NO: 114 SEQ ID NO: 3461
TFL26	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 115 SEQ ID NO: 116 SEQ ID NO: 3462
SINA	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 117 SEQ ID NO: 118 SEQ ID NO: 3463
ALP	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 119 SEQ ID NO: 120 SEQ ID NO: 3464
GSH1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 121 SEQ ID NO: 122 SEQ ID NO: 3465
FBA1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 123 SEQ ID NO: 124 SEQ ID NO: 3466
BZFP1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 125 SEQ ID NO: 126 SEQ ID NO: 3467
TFL1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 127 SEQ ID NO: 128 SEQ ID NO: 3468
TFL2	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 129 SEQ ID NO: 130 SEQ ID NO: 3469
TFL3	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 131 SEQ ID NO: 132 SEQ ID NO: 3470
YECPU1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 133 SEQ ID NO: 134 SEQ ID NO: 3471
DZFP1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 135 SEQ ID NO: 136 SEQ ID NO: 3472
YECPU2	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 137 SEQ ID NO: 138 SEQ ID NO: 3473
BPIRP1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 139 SEQ ID NO: 140 SEQ ID NO: 3474
EREFTs	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 141 SEQ ID NO: 142 SEQ ID NO: 3475
YECPU3	Sorghum bicolor	Polynucleotide Polypeptide	SEQ ID NO: 143 SEQ ID NO: 144

		Genomic	SEQ ID NO: 3476
PC4	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 145 SEQ ID NO: 146 SEQ ID NO: 3477
D9D8	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 147 SEQ ID NO: 148 SEQ ID NO: 3478
ARG4	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 149 SEQ ID NO: 150 SEQ ID NO: 3479
PKL1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 151 SEQ ID NO: 152 SEQ ID NO: 3480
SERK2	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 153 SEQ ID NO: 154 SEQ ID NO: 3481
WSPL1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 155 SEQ ID NO: 156 SEQ ID NO: 3482
ZFP1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 157 SEQ ID NO: 158 SEQ ID NO: 3483
AP2L1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 159 SEQ ID NO: 160 SEQ ID NO: 3484
HMG	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 161 SEQ ID NO: 162 SEQ ID NO: 3485
CNGC	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 163 SEQ ID NO: 164 SEQ ID NO: 3486
MEIIS5	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 165 SEQ ID NO: 166 SEQ ID NO: 3487
NDBP	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 167 SEQ ID NO: 168 SEQ ID NO: 3488
RGDI	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 169 SEQ ID NO: 170 SEQ ID NO: 3489
SBPPDK	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 171 SEQ ID NO: 172 SEQ ID NO: 3490
SAMPU2	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 173 SEQ ID NO: 174 SEQ ID NO: 3491
CNR06RNAi	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 175 SEQ ID NO: 176 SEQ ID NO: 3492
VRS1RNAi	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 177 SEQ ID NO: 178 SEQ ID NO: 3493
FBL2	Sorghum bicolor	Polynucleotide Polypeptide	SEQ ID NO: 179 SEQ ID NO: 180

		Genomic	SEQ ID NO: 3494
UCP1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 181 SEQ ID NO: 182 SEQ ID NO: 3495
CNR02RNAi	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 183 SEQ ID NO: 184 SEQ ID NO: 3496
TTL1RNAi	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 185 SEQ ID NO: 186 SEQ ID NO: 3497
PPDK	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 187 SEQ ID NO: 188 SEQ ID NO: 3498
LEC1LIKERNai	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 189 SEQ ID NO: 190 SEQ ID NO: 3499
GW21	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 191 SEQ ID NO: 192 SEQ ID NO: 3500
GW22	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 193 SEQ ID NO: 194 SEQ ID NO: 3501
PCYS1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 195 SEQ ID NO: 196 SEQ ID NO: 3502
SUMO E3	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 197 SEQ ID NO: 198 SEQ ID NO: 3503
M14	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 199 SEQ ID NO: 200 SEQ ID NO: 3504
EDCP647011	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 201 SEQ ID NO: 202 SEQ ID NO: 3505
SPL1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 203 SEQ ID NO: 204 SEQ ID NO: 3506
ADA2	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 205 SEQ ID NO: 206 SEQ ID NO: 3507
LOBDP1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 207 SEQ ID NO: 208 SEQ ID NO: 3508
YEC P4	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 209 SEQ ID NO: 210 SEQ ID NO: 3509
SAUER2	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 211 SEQ ID NO: 212 SEQ ID NO: 3510
ET3	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 213 SEQ ID NO: 214 SEQ ID NO: 3511
PWWPDPL1	Sorghum bicolor	Polynucleotide Polypeptide	SEQ ID NO: 215 SEQ ID NO: 216

		Genomic	SEQ ID NO: 3512
GSH1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 217 SEQ ID NO: 218 SEQ ID NO: 3513
NAC6	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 219 SEQ ID NO: 220 SEQ ID NO: 3514
M8	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 221 SEQ ID NO: 222 SEQ ID NO: 3515
CIPK1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 223 SEQ ID NO: 224 SEQ ID NO: 3516
TD1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 225 SEQ ID NO: 226 SEQ ID NO: 3517
ER1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 227 SEQ ID NO: 228 SEQ ID NO: 3518
YABBY14	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 229 SEQ ID NO: 230 SEQ ID NO: 3519
PCRTC	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 231 SEQ ID NO: 232 SEQ ID NO: 3520
CSZ1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 233 SEQ ID NO: 234 SEQ ID NO: 3521
ZIMFP	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 235 SEQ ID NO: 236 SEQ ID NO: 3522
WDRP	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 237 SEQ ID NO: 238 SEQ ID NO: 3523
LEA	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 239 SEQ ID NO: 240 SEQ ID NO: 3524
HSP	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 241 SEQ ID NO: 242 SEQ ID NO: 3525
GmSRP	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 243 SEQ ID NO: 244 SEQ ID NO: 3526
LTP	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 245 SEQ ID NO: 246 SEQ ID NO: 3527
IRDR	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 247 SEQ ID NO: 248 SEQ ID NO: 3528
KN1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 249 SEQ ID NO: 250 SEQ ID NO: 3529
INCW2	Sorghum bicolor	Polynucleotide Polypeptide	SEQ ID NO: 251 SEQ ID NO: 252

		Genomic	SEQ ID NO: 3530
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Sb01g004490	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 255 SEQ ID NO: 256 SEQ ID NO: 3532
YEP1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 257 SEQ ID NO: 258 SEQ ID NO: 3533
YEP31	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 259 SEQ ID NO: 260 SEQ ID NO: 3534
LRR3	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 261 SEQ ID NO: 262 SEQ ID NO: 3535
UP	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 263 SEQ ID NO: 264 SEQ ID NO: 3536
GRF5	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 265 SEQ ID NO: 266 SEQ ID NO: 3537
HSD1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 267 SEQ ID NO: 268 SEQ ID NO: 3538
SDH	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 269 SEQ ID NO: 270 SEQ ID NO: 3539
SUT1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 271 SEQ ID NO: 272 SEQ ID NO: 3540
SPP1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 273 SEQ ID NO: 274 SEQ ID NO: 3541
SCL	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 275 SEQ ID NO: 276 SEQ ID NO: 3542
GRP5	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 277 SEQ ID NO: 278 SEQ ID NO: 3543
BA1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 279 SEQ ID NO: 280 SEQ ID NO: 3544
Bif2	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 281 SEQ ID NO: 282 SEQ ID NO: 3545
Sb03g032340	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 283 SEQ ID NO: 284 SEQ ID NO: 3546
MIPS1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 285 SEQ ID NO: 286 SEQ ID NO: 3547
TOR	Sorghum bicolor	Polynucleotide Polypeptide	SEQ ID NO: 287 SEQ ID NO: 288

		Genomic	SEQ ID NO: 3548
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Sb04g029890	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 291 SEQ ID NO: 292 SEQ ID NO: 3550
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Sb07g024970	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 301 SEQ ID NO: 302 SEQ ID NO: 3555
Sb07g025220	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 303 SEQ ID NO: 304 SEQ ID NO: 3556
Sb07g024890	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 305 SEQ ID NO: 306 SEQ ID NO: 3557
Sb05g022280	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 307 SEQ ID NO: 308 SEQ ID NO: 3558
Sb07g026630	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 309 SEQ ID NO: 310 SEQ ID NO: 3559
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Sb10g006910	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 315 SEQ ID NO: 316 SEQ ID NO: 3562
Sb06g033870	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 317 SEQ ID NO: 318 SEQ ID NO: 3563
Sb03g034260	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 319 SEQ ID NO: 320 SEQ ID NO: 3564
Sb06g033840	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 321 SEQ ID NO: 322 SEQ ID NO: 3565
Sb04g006250	Sorghum bicolor	Polynucleotide Polypeptide	SEQ ID NO: 323 SEQ ID NO: 324

		Genomic	SEQ ID NO: 3566
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Sb01g023740	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 327 SEQ ID NO: 328 SEQ ID NO: 3568
Sb10g029510	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 329 SEQ ID NO: 330 SEQ ID NO: 3569
Sb04g003690	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 331 SEQ ID NO: 332 SEQ ID NO: 3570
Sb10g027830	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 333 SEQ ID NO: 334 SEQ ID NO: 3571
Sb10g027790	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 335 SEQ ID NO: 336 SEQ ID NO: 3572
Sb04g036060	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 337 SEQ ID NO: 338 SEQ ID NO: 3573
Sb06g001970	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 339 SEQ ID NO: 340 SEQ ID NO: 3574
Sb01g011700	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 341 SEQ ID NO: 342 SEQ ID NO: 3575
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Sb09g029240	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 351 SEQ ID NO: 352 SEQ ID NO: 3580
Sb09g002810	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 353 SEQ ID NO: 354 SEQ ID NO: 3581
Sb01g049650	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 355 SEQ ID NO: 356 SEQ ID NO: 3582
Sb01g032250	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 357 SEQ ID NO: 358 SEQ ID NO: 3583
Sb07g003690	Sorghum bicolor	Polynucleotide Polypeptide	SEQ ID NO: 359 SEQ ID NO: 360

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Sb01g006100	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 373 SEQ ID NO: 374 SEQ ID NO: 3591
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GLN1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 377 SEQ ID NO: 378 SEQ ID NO: 3593
NR1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 379 SEQ ID NO: 380 SEQ ID NO: 3594
Sb10g026570	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 381 SEQ ID NO: 382 SEQ ID NO: 3595
Sb01g028950	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 383 SEQ ID NO: 384 SEQ ID NO: 3596
NLM6	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 385 SEQ ID NO: 386 SEQ ID NO: 3597
NRT1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 387 SEQ ID NO: 388 SEQ ID NO: 3598
NAC100	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 389 SEQ ID NO: 390 SEQ ID NO: 3599
PIP1E	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 391 SEQ ID NO: 392 SEQ ID NO: 3600
Sb05g004100	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 393 SEQ ID NO: 394 SEQ ID NO: 3601
AMP1	Sorghum bicolor	Polynucleotide Polypeptide	SEQ ID NO: 395 SEQ ID NO: 396

		Genomic	SEQ ID NO: 3602
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MPK3	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 399 SEQ ID NO: 400 SEQ ID NO: 3604
ERD9	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 401 SEQ ID NO: 402 SEQ ID NO: 3605
Sb09g013790	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 403 SEQ ID NO: 404 SEQ ID NO: 3606
SEL1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 405 SEQ ID NO: 406 SEQ ID NO: 3607
AKHSDH	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 407 SEQ ID NO: 408 SEQ ID NO: 3608
Sb09g001560	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 409 SEQ ID NO: 410 SEQ ID NO: 3609
MAT2	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 411 SEQ ID NO: 412 SEQ ID NO: 3610
GLN1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 413 SEQ ID NO: 414 SEQ ID NO: 3611
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Sb03g040180	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 417 SEQ ID NO: 418 SEQ ID NO: 3613
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Sb01g003680	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 429 SEQ ID NO: 430 SEQ ID NO: 3619
Sb01g042740	Sorghum bicolor	Polynucleotide Polypeptide	SEQ ID NO: 431 SEQ ID NO: 432

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Sb01g042450	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 443 SEQ ID NO: 444 SEQ ID NO: 3626
Sb02g037580	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 445 SEQ ID NO: 446 SEQ ID NO: 3627
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Sb07g005200	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 461 SEQ ID NO: 462 SEQ ID NO: 3635
Sb01g017230	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 463 SEQ ID NO: 464 SEQ ID NO: 3636
Sb01g047140	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 465 SEQ ID NO: 466 SEQ ID NO: 3637
Sb02g010760	Sorghum bicolor	Polynucleotide Polypeptide	SEQ ID NO: 467 SEQ ID NO: 468

		Genomic	SEQ ID NO: 3638
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Sb04g030600	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 471 SEQ ID NO: 472 SEQ ID NO: 3640
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Sb03g006420	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 501 SEQ ID NO: 502 SEQ ID NO: 3655
Sb01g002960	Sorghum bicolor	Polynucleotide Polypeptide	SEQ ID NO: 503 SEQ ID NO: 504

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RHS1	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 533 SEQ ID NO: 534 SEQ ID NO: 3671
RHS2	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 535 SEQ ID NO: 536 SEQ ID NO: 3672
RHS3	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 537 SEQ ID NO: 538 SEQ ID NO: 3673
RHS4	Arabidopsis thaliana	Polynucleotide Polypeptide	SEQ ID NO: 539 SEQ ID NO: 540

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RHS6	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 543 SEQ ID NO: 544 SEQ ID NO: 3676
RHS7	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 545 SEQ ID NO: 546 SEQ ID NO: 3677
RHS8	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 547 SEQ ID NO: 548 SEQ ID NO: 3678
RHS9	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 549 SEQ ID NO: 550 SEQ ID NO: 3679
Sb03g029150	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 551 SEQ ID NO: 552 SEQ ID NO: 3680
RHS10	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 553 SEQ ID NO: 554 SEQ ID NO: 3681
Sb01g015140	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 555 SEQ ID NO: 556 SEQ ID NO: 3682
RHS11	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 557 SEQ ID NO: 558 SEQ ID NO: 3683
RHS12	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 559 SEQ ID NO: 560 SEQ ID NO: 3684
Sb03g006140	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 561 SEQ ID NO: 562 SEQ ID NO: 3685
Sb07g019540	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 563 SEQ ID NO: 564 SEQ ID NO: 3686
RHS13	Arabidopsis thaliana	Polynucleotide Polypeptide Genomic	SEQ ID NO: 565 SEQ ID NO: 566 SEQ ID NO: 3687
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RHS15	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 571 SEQ ID NO: 572 SEQ ID NO: 3690
RHS16	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 573 SEQ ID NO: 574 SEQ ID NO: 3691
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RHS17	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 579 SEQ ID NO: 580 SEQ ID NO: 3694
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Sb04g008160	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 2093 SEQ ID NO: 2094 SEQ ID NO: 4451
Sb04g008360	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 2095 SEQ ID NO: 2096 SEQ ID NO: 4452
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Sb04g008760	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 2099 SEQ ID NO: 2100 SEQ ID NO: 4454
Sb04g008770	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 2101 SEQ ID NO: 2102 SEQ ID NO: 4455
Sb04g009090	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 2103 SEQ ID NO: 2104 SEQ ID NO: 4456
Sb04g009760	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 2105 SEQ ID NO: 2106 SEQ ID NO: 4457
Sb04g010290	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 2107 SEQ ID NO: 2108 SEQ ID NO: 4458
Sb04g010650	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 2109 SEQ ID NO: 2110 SEQ ID NO: 4459
Sb04g010980	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 2111 SEQ ID NO: 2112 SEQ ID NO: 4460
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Sb04g012170	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 2119 SEQ ID NO: 2120 SEQ ID NO: 4464
Sb04g012920	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 2121 SEQ ID NO: 2122 SEQ ID NO: 4465
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Sb04g029410	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 2229 SEQ ID NO: 2230 SEQ ID NO: 4519
Sb04g029660	Sorghum bicolor	Polynucleotide Polypeptide	SEQ ID NO: 2231 SEQ ID NO: 2232

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Sb10g006150	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3209 SEQ ID NO: 3210 SEQ ID NO: 5009
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Sb10g003240	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3213 SEQ ID NO: 3214 SEQ ID NO: 5011
Sb10g003300	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3215 SEQ ID NO: 3216 SEQ ID NO: 5012
Sb10g003860	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3217 SEQ ID NO: 3218 SEQ ID NO: 5013
Sb10g004560	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3219 SEQ ID NO: 3220 SEQ ID NO: 5014
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Sb10g006250	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3231 SEQ ID NO: 3232 SEQ ID NO: 5020
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Sb10g006470	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3235 SEQ ID NO: 3236 SEQ ID NO: 5022
Sb10g013160	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3237 SEQ ID NO: 3238 SEQ ID NO: 5023
Sb10g006910	Sorghum bicolor	Polynucleotide Polypeptide	SEQ ID NO: 3239 SEQ ID NO: 3240

		Genomic	SEQ ID NO: 5024
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Sb10g010300	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3271 SEQ ID NO: 3272 SEQ ID NO: 5040
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Sb10g010750	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3281 SEQ ID NO: 3282 SEQ ID NO: 5045
Sb10g011210	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3283 SEQ ID NO: 3284 SEQ ID NO: 5046
Sb10g011760	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3285 SEQ ID NO: 3286 SEQ ID NO: 5047
Sb10g012730	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3287 SEQ ID NO: 3288 SEQ ID NO: 5048
Sb10g012770	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3289 SEQ ID NO: 3290 SEQ ID NO: 5049
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Sb10g016843	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3297 SEQ ID NO: 3298 SEQ ID NO: 5053
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Sb10g021590	Sorghum bicolor	Polynucleotide Polypeptide	SEQ ID NO: 3311 SEQ ID NO: 3312

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Sb10g024120	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3335 SEQ ID NO: 3336 SEQ ID NO: 5072
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Sb10g025070	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3343 SEQ ID NO: 3344 SEQ ID NO: 5076
Sb10g133250	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3345 SEQ ID NO: 3346 SEQ ID NO: 5077
Sb10g025240	Sorghum bicolor	Polynucleotide Polypeptide	SEQ ID NO: 3347 SEQ ID NO: 3348

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		Genomic	SEQ ID NO: 5096
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Sb10g030240	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3391 SEQ ID NO: 3392 SEQ ID NO: 5100
Sb10g031070	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3393 SEQ ID NO: 3394 SEQ ID NO: 5101
Sb10g031300	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3395 SEQ ID NO: 3396 SEQ ID NO: 5102
Sb1676s002010	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3397 SEQ ID NO: 3398 SEQ ID NO: 5103
Sb2674s002010	Sorghum bicolor	Polynucleotide Polypeptide Genomic	SEQ ID NO: 3399 SEQ ID NO: 3400 SEQ ID NO: 5104
dpzm00g103644	Zea mays	Polynucleotide Polypeptide	SEQ ID NO: 3401 SEQ ID NO: 3402
sbiMIR156B	Sorghum bicolor	Polynucleotide Genomic	SEQ ID NO: 3403 SEQ ID NO: 5105
ADH1YNT1PA	Pichia angusta	Polynucleotide	SEQ ID NO: 3404

Construction of Nucleic Acids

The isolated nucleic acids of the present disclosure can be made using (a) standard recombinant methods, (b) synthetic techniques or combinations thereof. In some embodiments, the polynucleotides of the present disclosure will be cloned, amplified or otherwise constructed from a fungus or bacteria.

The nucleic acids may conveniently comprise sequences in addition to a polynucleotide of the present disclosure. For example, a multi-cloning site comprising one or more endonuclease restriction sites may be inserted into the nucleic acid to aid in isolation of the polynucleotide. Also, translatable sequences may be inserted to aid in the isolation of the translated polynucleotide of the present disclosure. For example, a hexa-histidine marker sequence provides a convenient means to purify the proteins of the present

disclosure. The nucleic acid of the present disclosure - excluding the polynucleotide sequence - is optionally a vector, adapter or linker for cloning and/or expression of a polynucleotide of the present disclosure. Additional sequences may be added to such cloning and/or expression sequences to optimize their function in cloning and/or expression, to aid in isolation of the polynucleotide or to improve the introduction of the polynucleotide into a cell. Typically, the length of a nucleic acid of the present disclosure less the length of its polynucleotide of the present disclosure is less than 20 kilobase pairs, often less than 15 kb, and frequently less than 10 kb. Use of cloning vectors, expression vectors, adapters, and linkers is well known in the art. Exemplary nucleic acids include such vectors as: M13, lambda ZAP Express, lambda ZAP II, lambda gt10, lambda gt11, pBK-CMV, pBK-RSV, pBluescript II, lambda DASH II, lambda EMBL 3, lambda EMBL 4, pWE15, SuperCos 1, SurfZap, Uni-ZAP, pBC, pBS+/-, pSG5, pBK, pCR-Script, pET, pSPUTK, p3'SS, pGEM, pSK+/-, pGEX, pSPORTI and II, pOPRSVI CAT, pOPI3 CAT, pXT1, pSG5, pPbac, pMbac, pMC1neo, pOG44, pOG45, pFRT β GAL, pNEO β GAL, pRS403, pRS404, pRS405, pRS406, pRS413, pRS414, pRS415, pRS416, lambda MOSSlox and lambda MOSElox. Optional vectors for the present disclosure, include but are not limited to, lambda ZAP II and pGEX. For a description of various nucleic acids see, e.g., Stratagene Cloning Systems, Catalogs 1995, 1996, 1997 (La Jolla, CA); and, Amersham Life Sciences, Inc, Catalog '97 (Arlington Heights, IL).

Synthetic Methods for Constructing Nucleic Acids

The isolated nucleic acids of the present disclosure can also be prepared by direct chemical synthesis by methods such as the phosphotriester method of Narang, *et al.*, (1979) *Meth. Enzymol.* 68:90-9; the phosphodiester method of Brown, *et al.*, (1979) *Meth. Enzymol.* 68:109-51; the diethylphosphoramidite method of Beaucage, *et al.*, (1981) *Tetra. Letts.* 22(20):1859-62; the solid phase phosphoramidite triester method described by Beaucage, *et al.*, *supra*, e.g., using an automated synthesizer, e.g., as described in Needham-VanDevanter, *et al.*, (1984) *Nucleic Acids Res.* 12:6159-68 and the solid support method of US Patent Number 4,458,066. Chemical synthesis generally produces a single stranded oligonucleotide. This may be converted into double stranded DNA by hybridization with a complementary sequence or by polymerization with a DNA polymerase using the single strand as a template. One of skill will recognize that while chemical synthesis of DNA is limited to sequences of about 100 bases, longer sequences may be obtained by the ligation of shorter sequences.

UTRs and Codon Preference

In general, translational efficiency has been found to be regulated by specific sequence elements in the 5' non-coding or untranslated region (5' UTR) of the RNA. Positive sequence motifs include translational initiation consensus sequences (Kozak, (1987) *Nucleic Acids Res.* 15:8125) and the 5' 7-methyl GpppG RNA cap structure (Drummond, *et al.*, (1985) *Nucleic Acids Res.* 13:7375). Negative elements include stable intramolecular 5' UTR stem-loop structures (Muesing, *et al.*, (1987) *Cell* 48:691) and AUG sequences or short open reading frames preceded by an appropriate AUG in the 5' UTR (Kozak, *supra*, Rao, *et al.*, (1988) *Mol. and Cell. Biol.* 8:284). Accordingly, the present disclosure provides 5' and/or 3' UTR regions for modulation of translation of heterologous coding sequences.

Further, the polypeptide-encoding segments of the polynucleotides of the present disclosure can be modified to alter codon usage. Altered codon usage can be employed to alter translational efficiency and/or to optimize the coding sequence for expression in a desired host or to optimize the codon usage in a heterologous sequence for expression in maize. Codon usage in the coding regions of the polynucleotides of the present disclosure can be analyzed statistically using commercially available software packages such as "Codon Preference" available from the University of Wisconsin Genetics Computer Group. See, Devereaux, *et al.*, (1984) *Nucleic Acids Res.* 12:387-395; or MacVector 4.1 (Eastman Kodak Co., New Haven, Conn.). Thus, the present disclosure provides a codon usage frequency characteristic of the coding region of at least one of the polynucleotides of the present disclosure. The number of polynucleotides (3 nucleotides per amino acid) that can be used to determine a codon usage frequency can be any integer from 3 to the number of polynucleotides of the present disclosure as provided herein. Optionally, the polynucleotides will be full-length sequences. An exemplary number of sequences for statistical analysis can be at least 1, 5, 10, 20, 50 or 100.

Sequence Shuffling

The present disclosure provides methods for sequence shuffling using polynucleotides of the present disclosure, and compositions resulting therefrom. Sequence shuffling is described in PCT Publication Number 1996/19256. See also, Zhang, *et al.*, (1997) *Proc. Natl. Acad. Sci. USA* 94:4504-9 and Zhao, *et al.*, (1998) *Nature Biotech* 16:258-61. Generally, sequence shuffling provides a means for generating libraries of polynucleotides having a desired characteristic, which can be selected or screened for. Libraries of recombinant polynucleotides are generated from a population of related sequence polynucleotides, which comprise sequence regions, which have substantial sequence identity and can be homologously recombined in vitro or in vivo. The population of

sequence-recombined polynucleotides comprises a subpopulation of polynucleotides which possess desired or advantageous characteristics and which can be selected by a suitable selection or screening method. The characteristics can be any property or attribute capable of being selected for or detected in a screening system and may include properties of: an encoded protein, a transcriptional element, a sequence controlling transcription, RNA processing, RNA stability, chromatin conformation, translation or other expression property of a gene or transgene, a replicative element, a protein-binding element, or the like, such as any feature which confers a selectable or detectable property. In some embodiments, the selected characteristic will be an altered K_m and/or K_{cat} over the wild-type protein as provided herein. In other embodiments, a protein or polynucleotide generated from sequence shuffling will have a ligand binding affinity greater than the non-shuffled wild-type polynucleotide. In yet other embodiments, a protein or polynucleotide generated from sequence shuffling will have an altered pH optimum as compared to the non-shuffled wild-type polynucleotide. The increase in such properties can be at least 110%, 120%, 130%, 140% or greater than 150% of the wild-type value.

Recombinant Expression Cassettes

The present disclosure further provides recombinant expression cassettes comprising a nucleic acid of the present disclosure. A nucleic acid sequence coding for the desired polynucleotide of the present disclosure, for example a cDNA or a genomic sequence encoding a polypeptide long enough to code for an active protein of the present disclosure, can be used to construct a recombinant expression cassette which can be introduced into the desired host cell. A recombinant expression cassette will typically comprise a polynucleotide of the present disclosure operably linked to transcriptional initiation regulatory sequences which will direct the transcription of the polynucleotide in the intended host cell, such as tissues of a transformed plant.

For example, plant expression vectors may include (1) a cloned plant gene under the transcriptional control of 5' and 3' regulatory sequences and (2) a dominant selectable marker. Such plant expression vectors may also contain, if desired, a promoter regulatory region (e.g., one conferring inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific/selective expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site and/or a polyadenylation signal.

A plant promoter fragment can be employed which will direct expression of a polynucleotide of the present disclosure in all tissues of a regenerated plant. Such promoters are referred to herein as "constitutive" promoters and are active under most environmental conditions and states of development or cell differentiation. Examples of

constitutive promoters include the 1'- or 2'- promoter derived from T-DNA of *Agrobacterium tumefaciens*, the Smas promoter, the cinnamyl alcohol dehydrogenase promoter (US Patent Number 5,683,439), the Nos promoter, the rubisco promoter, the GRP1-8 promoter, the 35S promoter from cauliflower mosaic virus (CaMV), as described in Odell, *et al.*, (1985) *Nature* 313:810-2; rice actin (McElroy, *et al.*, (1990) *Plant Cell* 163-171); ubiquitin (Christensen, *et al.*, (1992) *Plant Mol. Biol.* 12:619-632 and Christensen, *et al.*, (1992) *Plant Mol. Biol.* 18:675-89); pEMU (Last, *et al.*, (1991) *Theor. Appl. Genet.* 81:581-8); MAS (Velten, *et al.*, (1984) *EMBO J.* 3:2723-30) and maize H3 histone (Lepetit, *et al.*, (1992) *Mol. Gen. Genet.* 231:276-85 and Atanassova, *et al.*, (1992) *Plant Journal* 2(3):291-300); ALS promoter, as described in PCT Application Number WO 1996/30530; GOS2 (US Patent Number 6,504,083) and other transcription initiation regions from various plant genes known to those of skill. For the present disclosure ubiquitin is the preferred promoter for expression in monocot plants.

Alternatively, the plant promoter can direct expression of a polynucleotide of the present disclosure in a specific tissue or may be otherwise under more precise environmental or developmental control. Such promoters are referred to here as "inducible" promoters (Rab17, RAD29). Environmental conditions that may affect transcription by inducible promoters include pathogen attack, anaerobic conditions or the presence of light. Examples of inducible promoters are the Adh1 promoter, which is inducible by hypoxia or cold stress, the Hsp70 promoter, which is inducible by heat stress and the PPDK promoter, which is inducible by light.

Examples of promoters under developmental control include promoters that initiate transcription only, or preferentially, in certain tissues, such as leaves, roots, fruit, seeds or flowers. The operation of a promoter may also vary depending on its location in the genome. Thus, an inducible promoter may become fully or partially constitutive in certain locations.

If polypeptide expression is desired, it is generally desirable to include a polyadenylation region at the 3'-end of a polynucleotide coding region. The polyadenylation region can be derived from a variety of plant genes or from T-DNA. The 3' end sequence to be added can be derived from, for example, the nopaline synthase or octopine synthase genes or alternatively from another plant gene or less preferably from any other eukaryotic gene. Examples of such regulatory elements include, but are not limited to, 3' termination and/or polyadenylation regions such as those of the *Agrobacterium tumefaciens* nopaline synthase (nos) gene (Bevan, *et al.*, (1983) *Nucleic Acids Res.* 12:369-85); the potato proteinase inhibitor II (PINII) gene (Keil, *et al.*, (1986) *Nucleic Acids Res.* 14:5641-50 and An, *et al.*, (1989) *Plant Cell* 1:115-22) and the CaMV 19S gene (Mogen, *et al.*, (1990) *Plant Cell* 2:1261-72).

An intron sequence can be added to the 5' untranslated region or the coding sequence of the partial coding sequence to increase the amount of the mature message that accumulates in the cytosol. Inclusion of a spliceable intron in the transcription unit in both plant and animal expression constructs has been shown to increase gene expression at both the mRNA and protein levels up to 1000-fold (Buchman and Berg, (1988) *Mol. Cell Biol.* 8:4395-4405; Callis, *et al.*, (1987) *Genes Dev.* 1:1183-200). Such intron enhancement of gene expression is typically greatest when placed near the 5' end of the transcription unit. Use of maize introns Adh1-S intron 1, 2 and 6, the Bronze-1 intron are known in the art. See generally, THE MAIZE HANDBOOK, Chapter 116, Freeling and Walbot, eds., Springer, New York (1994).

Plant signal sequences, including, but not limited to, signal-peptide encoding DNA/RNA sequences which target proteins to the extracellular matrix of the plant cell (Dratewka-Kos, *et al.*, (1989) *J. Biol. Chem.* 264:4896-900), such as the *Nicotiana plumbaginifolia* extension gene (DeLoose, *et al.*, (1991) *Gene* 99:95-100); signal peptides which target proteins to the vacuole, such as the sweet potato sporamin gene (Matsuka, *et al.*, (1991) *Proc. Natl. Acad. Sci. USA* 88:834) and the barley lectin gene (Wilkins, *et al.*, (1990) *Plant Cell*, 2:301-13); signal peptides which cause proteins to be secreted, such as that of PR1b (Lind, *et al.*, (1992) *Plant Mol. Biol.* 18:47-53) or the barley alpha amylase (BAA) (Rahmatullah, *et al.*, (1989) *Plant Mol. Biol.* 12:119, and hereby incorporated by reference) or signal peptides which target proteins to the plastids such as that of rapeseed enoyl-Acp reductase (Verwaert, *et al.*, (1994) *Plant Mol. Biol.* 26:189-202) are useful in the disclosure.

The vector comprising the sequences from a polynucleotide of the present disclosure will typically comprise a marker gene, which confers a selectable phenotype on plant cells. Usually, the selectable marker gene will encode antibiotic resistance, with suitable genes including genes coding for resistance to the antibiotic spectinomycin (e.g., the *aada* gene), the streptomycin phosphotransferase (SPT) gene coding for streptomycin resistance, the neomycin phosphotransferase (NPTII) gene encoding kanamycin or geneticin resistance, the hygromycin phosphotransferase (HPT) gene coding for hygromycin resistance, genes coding for resistance to herbicides which act to inhibit the action of acetolactate synthase (ALS), in particular the sulfonyleurea-type herbicides (e.g., the acetolactate synthase (ALS) gene containing mutations leading to such resistance in particular the S4 and/or Hra mutations), genes coding for resistance to herbicides which act to inhibit action of glutamine synthase, such as phosphinothricin or basta (e.g., the *bar* gene) or other such genes known in the art. The *bar* gene encodes resistance to the herbicide basta and the ALS gene encodes resistance to the herbicide chlorsulfuron.

Typical vectors useful for expression of genes in higher plants are well known in the art and include vectors derived from the tumor-inducing (Ti) plasmid of *Agrobacterium*

tumefaciens described by Rogers, *et al.*, (1987) *Meth. Enzymol.* 153:253-77. These vectors are plant integrating vectors in that on transformation, the vectors integrate a portion of vector DNA into the genome of the host plant. Exemplary *A. tumefaciens* vectors useful herein are plasmids pKYLX6 and pKYLX7 of Schardl, *et al.*, (1987) *Gene* 61:1-11 and Berger, *et al.*, (1989) *Proc. Natl. Acad. Sci. USA*, 86:8402-6. Another useful vector herein is plasmid pBI101.2 that is available from CLONTECH Laboratories, Inc. (Palo Alto, CA).

Expression of Proteins in Host Cells

Using the nucleic acids of the present disclosure, one may express a protein of the present disclosure in a recombinantly engineered cell such as bacteria, yeast, insect, mammalian or preferably plant cells. The cells produce the protein in a non-natural condition (e.g., in quantity, composition, location and/or time), because they have been genetically altered through human intervention to do so.

It is expected that those of skill in the art are knowledgeable in the numerous expression systems available for expression of a nucleic acid encoding a protein of the present disclosure. No attempt to describe in detail the various methods known for the expression of proteins in prokaryotes or eukaryotes will be made.

In brief summary, the expression of isolated nucleic acids encoding a protein of the present disclosure will typically be achieved by operably linking, for example, the DNA or cDNA to a promoter (which is either constitutive or inducible), followed by incorporation into an expression vector. The vectors can be suitable for replication and integration in either prokaryotes or eukaryotes. Typical expression vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the DNA encoding a protein of the present disclosure. To obtain high level expression of a cloned gene, it is desirable to construct expression vectors which contain, at the minimum, a strong promoter, such as ubiquitin, to direct transcription, a ribosome binding site for translational initiation and a transcription/translation terminator. Constitutive promoters are classified as providing for a range of constitutive expression. Thus, some are weak constitutive promoters and others are strong constitutive promoters. Generally, by "weak promoter" is intended a promoter that drives expression of a coding sequence at a low level. By "low level" is intended at levels of about 1/10,000 transcripts to about 1/100,000 transcripts to about 1/500,000 transcripts. Conversely, a "strong promoter" drives expression of a coding sequence at a "high level" or about 1/10 transcripts to about 1/100 transcripts to about 1/1,000 transcripts.

One of skill would recognize that modifications could be made to a protein of the present disclosure without diminishing its biological activity. Some modifications may be made to facilitate the cloning, expression or incorporation of the targeting molecule into a

fusion protein. Such modifications are well known to those of skill in the art and include, for example, a methionine added at the amino terminus to provide an initiation site or additional amino acids (e.g., poly His) placed on either terminus to create conveniently located restriction sites or termination codons or purification sequences.

5

Expression in Prokaryotes

Prokaryotic cells may be used as hosts for expression. Prokaryotes most frequently are represented by various strains of *E. coli*; however, other microbial strains may also be used. Commonly used prokaryotic control sequences which are defined herein to include
10 promoters for transcription initiation, optionally with an operator, along with ribosome binding site sequences, include such commonly used promoters as the beta lactamase (penicillinase) and lactose (lac) promoter systems (Chang, *et al.*, (1977) *Nature* 198:1056), the tryptophan (trp) promoter system (Goeddel, *et al.*, (1980) *Nucleic Acids Res.* 8:4057) and the lambda derived P_L promoter and N-gene ribosome binding site (Shimatake, *et al.*,
15 (1981) *Nature* 292:128). The inclusion of selection markers in DNA vectors transfected in *E. coli* is also useful. Examples of such markers include genes specifying resistance to ampicillin, tetracycline or chloramphenicol.

The vector is selected to allow introduction of the gene of interest into the appropriate host cell. Bacterial vectors are typically of plasmid or phage origin. Appropriate bacterial
20 cells are infected with phage vector particles or transfected with naked phage vector DNA. If a plasmid vector is used, the bacterial cells are transfected with the plasmid vector DNA. Expression systems for expressing a protein of the present disclosure are available using *Bacillus sp.* and *Salmonella* (Palva, *et al.*, (1983) *Gene* 22:229-35; Mosbach, *et al.*, (1983) *Nature* 302:543-5). The pGEX-4T-1 plasmid vector from Pharmacia is the preferred *E. coli*
25 expression vector for the present disclosure.

Expression in Eukaryotes

A variety of eukaryotic expression systems such as yeast, insect cell lines, plant and mammalian cells, are known to those of skill in the art. As explained briefly below, the
30 present disclosure can be expressed in these eukaryotic systems. In some embodiments, transformed/transfected plant cells, as discussed *infra*, are employed as expression systems for production of the proteins of the instant disclosure.

Synthesis of heterologous proteins in yeast is well known. Sherman, *et al.*, (1982) METHODS IN YEAST GENETICS, Cold Spring Harbor Laboratory is a well recognized work
35 describing the various methods available to produce the protein in yeast. Two widely utilized yeasts for production of eukaryotic proteins are *Saccharomyces cerevisiae* and *Pichia pastoris*. Vectors, strains and protocols for expression in *Saccharomyces* and *Pichia* are

known in the art and available from commercial suppliers (e.g., Invitrogen). Suitable vectors usually have expression control sequences, such as promoters, including 3-phosphoglycerate kinase or alcohol oxidase and an origin of replication, termination sequences and the like as desired.

5 A protein of the present disclosure, once expressed, can be isolated from yeast by lysing the cells and applying standard protein isolation techniques to the lysates or the pellets. The monitoring of the purification process can be accomplished by using Western blot techniques or radioimmunoassay of other standard immunoassay techniques.

10 The sequences encoding proteins of the present disclosure can also be ligated to various expression vectors for use in transfecting cell cultures of, for instance, mammalian, insect or plant origin. Mammalian cell systems often will be in the form of monolayers of cells although mammalian cell suspensions may also be used. A number of suitable host cell lines capable of expressing intact proteins have been developed in the art, and include the HEK293, BHK21 and CHO cell lines. Expression vectors for these cells can include
15 expression control sequences, such as an origin of replication, a promoter (e.g., the CMV promoter, a HSV *tk* promoter or *pgk* (phosphoglycerate kinase) promoter), an enhancer (Queen, *et al.*, (1986) *Immunol. Rev.* 89:49) and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites (e.g., an SV40 large T Ag poly A addition site) and transcriptional terminator sequences. Other animal cells
20 useful for production of proteins of the present disclosure are available, for instance, from the American Type Culture Collection Catalogue of Cell Lines and Hybridomas (7th ed., 1992).

Appropriate vectors for expressing proteins of the present disclosure in insect cells are usually derived from the SF9 baculovirus. Suitable insect cell lines include mosquito
25 larvae, silkworm, armyworm, moth, and *Drosophila* cell lines such as a Schneider cell line (see, e.g., Schneider, (1987) *J. Embryol. Exp. Morphol.* 27:353-65).

As with yeast, when higher animal or plant host cells are employed, polyadenylation or transcription terminator sequences are typically incorporated into the vector. An example of a terminator sequence is the polyadenylation sequence from the bovine growth hormone
30 gene. Sequences for accurate splicing of the transcript may also be included. An example of a splicing sequence is the VP1 intron from SV40 (Sprague, *et al.*, (1983) *J. Virol.* 45:773-81). Additionally, gene sequences to control replication in the host cell may be incorporated into the vector such as those found in bovine papilloma virus type-vectors (Saveria-Campo, "Bovine Papilloma Virus DNA a Eukaryotic Cloning Vector," in DNA CLONING: A
35 PRACTICAL APPROACH, vol. II, Glover, ed., IRL Press, Arlington, VA, pp. 213-38 (1985)).

In addition, the gene for yield improvement placed in the appropriate plant expression vector can be used to transform plant cells. The polypeptide can then be isolated from plant

callus or the transformed cells can be used to regenerate transgenic plants. Such transgenic plants can be harvested, and the appropriate tissues (seed or leaves, for example) can be subjected to large scale protein extraction and purification techniques.

5 Plant Transformation Methods

Numerous methods for introducing foreign genes into plants are known and can be used to insert a yield improvement polynucleotide into a plant host, including biological and physical plant transformation protocols. See, e.g., Miki, *et al.*, "Procedure for Introducing Foreign DNA into Plants," in METHODS IN PLANT MOLECULAR BIOLOGY AND
 10 BIOTECHNOLOGY, Glick and Thompson, eds., CRC Press, Inc., Boca Raton, pp. 67-88 (1993). The methods chosen vary with the host plant, and include chemical transfection methods such as calcium phosphate, microorganism-mediated gene transfer such as *Agrobacterium* (Horsch, *et al.*, (1985) *Science* 227:1229-31), electroporation, micro-injection and biolistic bombardment.

15 Expression cassettes and vectors and *in vitro* culture methods for plant cell or tissue transformation and regeneration of plants are known and available. See, e.g., Gruber, *et al.*, "Vectors for Plant Transformation," in METHODS IN PLANT MOLECULAR BIOLOGY AND BIOTECHNOLOGY, *supra*, pp. 89-119.

The isolated polynucleotides or polypeptides may be introduced into the plant by one
 20 or more techniques typically used for direct delivery into cells. Such protocols may vary depending on the type of organism, cell, plant or plant cell, i.e., monocot or dicot, targeted for gene modification. Suitable methods of transforming plant cells include microinjection (Crossway, *et al.*, (1986) *Biotechniques* 4:320-334 and US Patent Number 6,300,543), electroporation (Riggs, *et al.*, (1986) *Proc. Natl. Acad. Sci. USA* 83:5602-5606), direct gene
 25 transfer (Paszowski, *et al.*, (1984) *EMBO J.* 3:2717-2722) and ballistic particle acceleration (see, for example, Sanford, *et al.*, US Patent Number 4,945,050; WO 1991/10725 and McCabe, *et al.*, (1988) *Biotechnology* 6:923-926). Also see, Tomes, *et al.*, Direct DNA Transfer into Intact Plant Cells Via Microprojectile Bombardment. pp.197-213 in *Plant Cell, Tissue and Organ Culture, Fundamental Methods* eds. Gamborg and Phillips, Springer-
 30 Verlag Berlin Heidelberg New York, 1995; US Patent Number 5,736,369 (meristem); Weissinger, *et al.*, (1988) *Ann. Rev. Genet.* 22:421-477; Sanford, *et al.*, (1987) *Particulate Science and Technology* 5:27-37 (onion); Christou, *et al.*, (1988) *Plant Physiol.* 87:671-674 (soybean); Datta, *et al.*, (1990) *Biotechnology* 8:736-740 (rice); Klein, *et al.*, (1988) *Proc. Natl. Acad. Sci. USA* 85:4305-4309 (maize); Klein, *et al.*, (1988) *Biotechnology* 6:559-563
 35 (maize); WO 1991/10725 (maize); Klein, *et al.*, (1988) *Plant Physiol.* 91:440-444 (maize); Fromm, *et al.*, (1990) *Biotechnology* 8:833-839 and Gordon-Kamm, *et al.*, (1990) *Plant Cell* 2:603-618 (maize); Hooydaas-Van Slogteren and Hooykaas, (1984) *Nature* (London)

311:763-764; Bytebier, *et al.*, (1987) *Proc. Natl. Acad. Sci. USA* 84:5345-5349 (Liliaceae); De Wet, *et al.*, (1985) In *The Experimental Manipulation of Ovule Tissues*, ed. Chapman, *et al.*, pp. 197-209; Longman, NY (pollen); Kaeppler, *et al.*, (1990) *Plant Cell Reports* 9:415-418 and Kaeppler, *et al.*, (1992) *Theor. Appl. Genet.* 84:560-566 (whisker-mediated transformation); US Patent Number 5,693,512 (sonication); D'Halluin, *et al.*, (1992) *Plant Cell* 4:1495-1505 (electroporation); Li, *et al.*, (1993) *Plant Cell Reports* 12:250-255 and Christou and Ford, (1995) *Annals of Botany* 75:407-413 (rice); Osjoda, *et al.*, (1996) *Nature Biotech.* 14:745-750; Agrobacterium mediated maize transformation (US Patent Number 5,981,840); silicon carbide whisker methods (Frame, *et al.*, (1994) *Plant J.* 6:941-948); laser methods (Guo, *et al.*, (1995) *Physiologia Plantarum* 93:19-24); sonication methods (Bao, *et al.*, (1997) *Ultrasound in Medicine & Biology* 23:953-959; Finer and Finer, (2000) *Lett Appl Microbiol.* 30:406-10; Amoah, *et al.*, (2001) *J Exp Bot* 52:1135-42); polyethylene glycol methods (Krens, *et al.*, (1982) *Nature* 296:72-77); protoplasts of monocot and dicot cells can be transformed using electroporation (Fromm, *et al.*, (1985) *Proc. Natl. Acad. Sci. USA* 82:5824-5828) and microinjection (Crossway, *et al.*, (1986) *Mol. Gen. Genet.* 202:179-185), all of which are herein incorporated by reference.

Agrobacterium-mediated Transformation

The most widely utilized method for introducing an expression vector into plants is based on the natural transformation system of *Agrobacterium*. *A. tumefaciens* and *A. rhizogenes* are plant pathogenic soil bacteria, which genetically transform plant cells. The Ti and Ri plasmids of *A. tumefaciens* and *A. rhizogenes*, respectively, carry genes responsible for genetic transformation of plants. See, e.g., Kado, (1991) *Crit. Rev. Plant Sci.* 10:1. Descriptions of the *Agrobacterium* vector systems and methods for *Agrobacterium*-mediated gene transfer are provided in Gruber, *et al.*, *supra*; Miki, *et al.*, *supra*; and Moloney, *et al.*, (1989) *Plant Cell Reports* 8:238.

Similarly, the gene can be inserted into the T-DNA region of a Ti or Ri plasmid derived from *A. tumefaciens* or *A. rhizogenes*, respectively. Thus, expression cassettes can be constructed as above, using these plasmids. Many control sequences are known which when coupled to a heterologous coding sequence and transformed into a host organism show fidelity in gene expression with respect to tissue/organ specificity of the original coding sequence. See, e.g., Benfey and Chua, (1989) *Science* 244:174-81. Particularly suitable control sequences for use in these plasmids are promoters for constitutive leaf-specific expression of the gene in the various target plants. Other useful control sequences include a promoter and terminator from the nopaline synthase gene (NOS). The NOS promoter and terminator are present in the plasmid pARC2, available from the American Type Culture Collection and designated ATCC 67238. If such a system is used, the virulence (*vir*) gene

from either the Ti or Ri plasmid must also be present, either along with the T-DNA portion or via a binary system where the *vir* gene is present on a separate vector. Such systems, vectors for use therein, and methods of transforming plant cells are described in US Patent Number 4,658,082; US Patent Application Serial Number 913,914, filed October 1, 1986, as
5 referenced in US Patent Number 5,262,306, issued November 16, 1993 and Simpson, *et al.*, (1986) *Plant Mol. Biol.* 6:403-15 (also referenced in the '306 patent), all incorporated by reference in their entirety.

Once constructed, these plasmids can be placed into *A. rhizogenes* or *A. tumefaciens* and these vectors used to transform cells of plant species, which are ordinarily
10 susceptible to *Fusarium* or *Alternaria* infection. Several other transgenic plants are also contemplated by the present disclosure including but not limited to soybean, corn, sorghum, alfalfa, rice, clover, cabbage, banana, coffee, celery, tobacco, cowpea, cotton, melon and pepper. The selection of either *A. tumefaciens* or *A. rhizogenes* will depend on the plant being transformed thereby. In general *A. tumefaciens* is the preferred organism for
15 transformation. Most dicotyledonous plants, some gymnosperms, and a few monocotyledonous plants (e.g., certain members of the *Liliales* and *Arales*) are susceptible to infection with *A. tumefaciens*. *A. rhizogenes* also has a wide host range, embracing most dicots and some gymnosperms, which includes members of the *Leguminosae*, *Compositae* and *Chenopodiaceae*. Monocot plants can now be transformed with some success. EP
20 Patent Application Number 604 662 A1 discloses a method for transforming monocots using *Agrobacterium*. EP Patent Application Number 672 752 A1 discloses a method for transforming monocots with *Agrobacterium* using the scutellum of immature embryos. Ishida, *et al.*, discuss a method for transforming maize by exposing immature embryos to *A. tumefaciens* (*Nature Biotechnology* 14:745-50 (1996)).

25 Once transformed, these cells can be used to regenerate transgenic plants. For example, whole plants can be infected with these vectors by wounding the plant and then introducing the vector into the wound site. Any part of the plant can be wounded, including leaves, stems and roots. Alternatively, plant tissue, in the form of an explant, such as cotyledonary tissue or leaf disks, can be inoculated with these vectors and cultured under
30 conditions, which promote plant regeneration. Roots or shoots transformed by inoculation of plant tissue with *A. rhizogenes* or *A. tumefaciens*, containing the gene coding for the fumonisin degradation enzyme, can be used as a source of plant tissue to regenerate fumonisin-resistant transgenic plants, either via somatic embryogenesis or organogenesis. Examples of such methods for regenerating plant tissue are disclosed in Shahin, *Theor.*
35 *Appl. Genet.* 69:235-40 (1985); US Patent Number 4,658,082; Simpson, *et al.*, *supra* and US Patent Application Serial Numbers 913,913 and 913,914, both filed October 1, 1986, as

referenced in US Patent Number 5,262,306, issued November 16, 1993, the entire disclosures therein incorporated herein by reference.

Direct Gene Transfer

Despite the fact that the host range for *Agrobacterium*-mediated transformation is broad, some major cereal crop species and gymnosperms have generally been recalcitrant to this mode of gene transfer, even though some success has recently been achieved in rice (Hiei, *et al.*, (1994) *The Plant Journal* 6:271-82). Several methods of plant transformation, collectively referred to as direct gene transfer, have been developed as an alternative to *Agrobacterium*-mediated transformation.

A generally applicable method of plant transformation is microprojectile-mediated transformation, where DNA is carried on the surface of microprojectiles measuring about 1 to 4 μm . The expression vector is introduced into plant tissues with a biolistic device that accelerates the microprojectiles to speeds of 300 to 600 m/s which is sufficient to penetrate the plant cell walls and membranes (Sanford, *et al.*, (1987) *Part. Sci. Technol.* 5:27; Sanford, (1988) *Trends Biotech* 6:299; Sanford, (1990) *Physiol. Plant* 79:206 and Klein, *et al.*, (1992) *Biotechnology* 10:268).

Another method for physical delivery of DNA to plants is sonication of target cells as described in Zang, *et al.*, (1991) *BioTechnology* 9:996. Alternatively, liposome or spheroplast fusions have been used to introduce expression vectors into plants. See, e.g., Deshayes, *et al.*, (1985) *EMBO J.* 4:2731 and Christou, *et al.*, (1987) *Proc. Natl. Acad. Sci. USA* 84:3962. Direct uptake of DNA into protoplasts using CaCl_2 precipitation, polyvinyl alcohol or poly-L-ornithine has also been reported. See, e.g., Hain, *et al.*, (1985) *Mol. Gen. Genet.* 199:161 and Draper, *et al.*, (1982) *Plant Cell Physiol.* 23:451.

Electroporation of protoplasts and whole cells and tissues has also been described. See, e.g., Donn, *et al.*, (1990) in *Abstracts of the VIIIth Int'l. Congress on Plant Cell and Tissue Culture IAPTC*, A2-38, p. 53; D'Halluin, *et al.*, (1992) *Plant Cell* 4:1495-505 and Spencer, *et al.*, (1994) *Plant Mol. Biol.* 24:51-61.

Increasing the Activity and/or Level of a yield improvement Polypeptide

Methods are provided to increase the activity and/or level of the yield improvement polypeptide of the disclosure. An increase in the level and/or activity of the yield improvement polypeptide of the disclosure can be achieved by providing to the plant a yield improvement polypeptide. The yield improvement polypeptide can be provided by introducing the amino acid sequence encoding the yield improvement polypeptide into the plant, introducing into the plant a nucleotide sequence encoding an yield improvement

polypeptide or alternatively by modifying a genomic locus encoding the yield improvement polypeptide of the disclosure.

As discussed elsewhere herein, many methods are known the art for providing a polypeptide to a plant including, but not limited to, direct introduction of the polypeptide into the plant, introducing into the plant (transiently or stably) a polynucleotide construct encoding a polypeptide having cell number regulator activity. It is also recognized that the methods of the disclosure may employ a polynucleotide that is not capable of directing, in the transformed plant, the expression of a protein or an RNA. Thus, the level and/or activity of an yield improvement polypeptide may be increased by altering the gene encoding the yield improvement polypeptide or its promoter. See, e.g., Kmiec, US Patent Number 5,565,350; Zarling, *et al.*, PCT/US93/03868. Therefore mutagenized plants that carry mutations in yield improvement genes, where the mutations increase expression of the yield improvement gene or increase the plant growth and/or organ development activity of the encoded yield improvement polypeptide are provided.

Reducing the Activity and/or Level of a yield improvement Polypeptide

Methods are provided to reduce or eliminate the activity of a yield improvement polypeptide of the disclosure by transforming a plant cell with an expression cassette that expresses a polynucleotide that inhibits the expression of the yield improvement polypeptide. The polynucleotide may inhibit the expression of the yield improvement polypeptide directly, by preventing translation of the yield improvement messenger RNA, or indirectly, by encoding a polypeptide that inhibits the transcription or translation of a yield improvement gene encoding a yield improvement polypeptide. Methods for inhibiting or eliminating the expression of a gene in a plant are well known in the art, and any such method may be used in the present disclosure to inhibit the expression of a yield improvement polypeptide.

In accordance with the present disclosure, the expression of a yield improvement polypeptide is inhibited if the protein level of the yield improvement polypeptide is less than 70% of the protein level of the same yield improvement polypeptide in a plant that has not been genetically modified or mutagenized to inhibit the expression of that yield improvement polypeptide. In particular embodiments of the disclosure, the protein level of the yield improvement polypeptide in a modified plant according to the disclosure is less than 60%, less than 50%, less than 40%, less than 30%, less than 20%, less than 10%, less than 5% or less than 2% of the protein level of the same yield improvement polypeptide in a plant that is not a mutant or that has not been genetically modified to inhibit the expression of that yield improvement polypeptide. The expression level of the yield improvement polypeptide may be measured directly, for example, by assaying for the level of yield improvement polypeptide expressed in the plant cell or plant, or indirectly, for example, by measuring the

plant growth and/or organ development activity of the yield improvement polypeptide in the plant cell or plant or by measuring the biomass in the plant. Methods for performing such assays are described elsewhere herein.

In other embodiments of the disclosure, the activity of the yield improvement polypeptides is reduced or eliminated by transforming a plant cell with an expression cassette comprising a polynucleotide encoding a polypeptide that inhibits the activity of a yield improvement polypeptide. The plant growth and/or organ development activity of a yield improvement polypeptide is inhibited according to the present disclosure if the plant growth and/or organ development activity of the yield improvement polypeptide is less than 70% of the plant growth and/or organ development activity of the same yield improvement polypeptide in a plant that has not been modified to inhibit the plant growth and/or organ development activity of that yield improvement polypeptide. In particular embodiments of the disclosure, the plant growth and/or organ development activity of the yield improvement polypeptide in a modified plant according to the disclosure is less than 60%, less than 50%, less than 40%, less than 30%, less than 20%, less than 10% or less than 5% of the plant growth and/or organ development activity of the same yield improvement polypeptide in a plant that has not been modified to inhibit the expression of that yield improvement polypeptide. The plant growth and/or organ development activity of a yield improvement polypeptide is "eliminated" according to the disclosure when it is not detectable by the assay methods described elsewhere herein. Methods of determining the plant growth and/or organ development activity of a yield improvement polypeptide are described elsewhere herein.

In other embodiments, the activity of a yield improvement polypeptide may be reduced or eliminated by disrupting the gene encoding the yield improvement polypeptide. The disclosure encompasses mutagenized plants that carry mutations in yield improvement genes, where the mutations reduce expression of the yield improvement gene or inhibit the plant growth and/or organ development activity of the encoded yield improvement polypeptide.

Thus, many methods may be used to reduce or eliminate the activity of a yield improvement polypeptide. In addition, more than one method may be used to reduce the activity of a single yield improvement polypeptide. Non-limiting examples of methods of reducing or eliminating the expression of yield improvement polypeptides are given below.

1. Polynucleotide-Based Methods:

In some embodiments of the present disclosure, a plant is transformed with an expression cassette that is capable of expressing a polynucleotide that inhibits the expression of a yield improvement polypeptide of the disclosure. The term "expression" as used herein refers to the biosynthesis of a gene product, including the transcription and/or

translation of said gene product. For example, for the purposes of the present disclosure, an expression cassette capable of expressing a polynucleotide that inhibits the expression of at least one yield improvement polypeptide is an expression cassette capable of producing an RNA molecule that inhibits the transcription and/or translation of at least one yield improvement polypeptide of the disclosure. The "expression" or "production" of a protein or polypeptide from a DNA molecule refers to the transcription and translation of the coding sequence to produce the protein or polypeptide, while the "expression" or "production" of a protein or polypeptide from an RNA molecule refers to the translation of the RNA coding sequence to produce the protein or polypeptide.

Examples of polynucleotides that inhibit the expression of a yield improvement polypeptide are given below.

i. Sense Suppression/Cosuppression

In some embodiments of the disclosure, inhibition of the expression of a yield improvement polypeptide may be obtained by sense suppression or cosuppression. For cosuppression, an expression cassette is designed to express an RNA molecule corresponding to all or part of a messenger RNA encoding a yield improvement polypeptide in the "sense" orientation. Over expression of the RNA molecule can result in reduced expression of the native gene. Accordingly, multiple plant lines transformed with the cosuppression expression cassette are screened to identify those that show the greatest inhibition of yield improvement polypeptide expression.

The polynucleotide used for cosuppression may correspond to all or part of the sequence encoding the yield improvement polypeptide, all or part of the 5' and/or 3' untranslated region of a yield improvement polypeptide transcript or all or part of both the coding sequence and the untranslated regions of a transcript encoding a yield improvement polypeptide. In some embodiments where the polynucleotide comprises all or part of the coding region for the yield improvement polypeptide, the expression cassette is designed to eliminate the start codon of the polynucleotide so that no protein product will be translated.

Cosuppression may be used to inhibit the expression of plant genes to produce plants having undetectable protein levels for the proteins encoded by these genes. See, for example, Broin, *et al.*, (2002) *Plant Cell* 14:1417-1432. Cosuppression may also be used to inhibit the expression of multiple proteins in the same plant. See, for example, US Patent Number 5,942,657. Methods for using cosuppression to inhibit the expression of endogenous genes in plants are described in Flavell, *et al.*, (1994) *Proc. Natl. Acad. Sci. USA* 91:3490-3496; Jorgensen, *et al.*, (1996) *Plant Mol. Biol.* 31:957-973; Johansen and Carrington, (2001) *Plant Physiol.* 126:930-938; Broin, *et al.*, (2002) *Plant Cell* 14:1417-1432; Stoutjesdijk, *et al.*, (2002) *Plant Physiol.* 129:1723-1731; Yu, *et al.*, (2003) *Phytochemistry*

63:753-763 and US Patent Numbers 5,034,323, 5,283,184 and 5,942,657, each of which is herein incorporated by reference. The efficiency of cosuppression may be increased by including a poly-dT region in the expression cassette at a position 3' to the sense sequence and 5' of the polyadenylation signal. See, US Patent Application Publication Number 5 2002/0048814, herein incorporated by reference. Typically, such a nucleotide sequence has substantial sequence identity to the sequence of the transcript of the endogenous gene, optimally greater than about 65% sequence identity, more optimally greater than about 85% sequence identity, most optimally greater than about 95% sequence identity. See US Patent Numbers 5,283,184 and 5,034,323, herein incorporated by reference.

10 ii. *Antisense Suppression*

In some embodiments of the disclosure, inhibition of the expression of the yield improvement polypeptide may be obtained by antisense suppression. For antisense suppression, the expression cassette is designed to express an RNA molecule 15 complementary to all or part of a messenger RNA encoding the yield improvement polypeptide. Over expression of the antisense RNA molecule can result in reduced expression of the native gene. Accordingly, multiple plant lines transformed with the antisense suppression expression cassette are screened to identify those that show the greatest inhibition of yield improvement polypeptide expression.

20 The polynucleotide for use in antisense suppression may correspond to all or part of the complement of the sequence encoding the yield improvement polypeptide, all or part of the complement of the 5' and/or 3' untranslated region of the yield improvement transcript or all or part of the complement of both the coding sequence and the untranslated regions of a transcript encoding the yield improvement polypeptide. In addition, the antisense 25 polynucleotide may be fully complementary (i.e., 100% identical to the complement of the target sequence) or partially complementary (i.e., less than 100% identical to the complement of the target sequence) to the target sequence. Antisense suppression may be used to inhibit the expression of multiple proteins in the same plant. See, for example, US Patent Number 5,942,657. Furthermore, portions of the antisense nucleotides may be used 30 to disrupt the expression of the target gene. Generally, sequences of at least 50 nucleotides, 100 nucleotides, 200 nucleotides, 300, 400, 450, 500, 550 or greater may be used. Methods for using antisense suppression to inhibit the expression of endogenous genes in plants are described, for example, in Liu, *et al.*, (2002) *Plant Physiol.* 129:1732-1743 and US Patent Numbers 5,759,829 and 5,942,657, each of which is herein 35 incorporated by reference. Efficiency of antisense suppression may be increased by including a poly-dT region in the expression cassette at a position 3' to the antisense

sequence and 5' of the polyadenylation signal. See, US Patent Application Publication Number 2002/0048814, herein incorporated by reference.

iii. Double-Stranded RNA Interference

In some embodiments of the disclosure, inhibition of the expression of a yield improvement polypeptide may be obtained by double-stranded RNA (dsRNA) interference. For dsRNA interference, a sense RNA molecule like that described above for cosuppression and an antisense RNA molecule that is fully or partially complementary to the sense RNA molecule are expressed in the same cell, resulting in inhibition of the expression of the corresponding endogenous messenger RNA.

Expression of the sense and antisense molecules can be accomplished by designing the expression cassette to comprise both a sense sequence and an antisense sequence. Alternatively, separate expression cassettes may be used for the sense and antisense sequences. Multiple plant lines transformed with the dsRNA interference expression cassette or expression cassettes are then screened to identify plant lines that show the greatest inhibition of yield improvement polypeptide expression. Methods for using dsRNA interference to inhibit the expression of endogenous plant genes are described in Waterhouse, *et al.*, (1998) *Proc. Natl. Acad. Sci. USA* 95:13959-13964, Liu, *et al.*, (2002) *Plant Physiol.* 129:1732-1743 and WO 1999/49029, WO 1999/53050, WO 1999/61631 and WO 2000/49035, each of which is herein incorporated by reference.

iv. Hairpin RNA Interference and Intron-Containing Hairpin RNA Interference

In some embodiments of the disclosure, inhibition of the expression of one or a yield improvement polypeptide may be obtained by hairpin RNA (hpRNA) interference or intron-containing hairpin RNA (ihpRNA) interference. These methods are highly efficient at inhibiting the expression of endogenous genes. See, Waterhouse and Helliwell, (2003) *Nat. Rev. Genet.* 4:29-38 and the references cited therein.

For hpRNA interference, the expression cassette is designed to express an RNA molecule that hybridizes with itself to form a hairpin structure that comprises a single-stranded loop region and a base-paired stem. The base-paired stem region comprises a sense sequence corresponding to all or part of the endogenous messenger RNA encoding the gene whose expression is to be inhibited, and an antisense sequence that is fully or partially complementary to the sense sequence. Thus, the base-paired stem region of the molecule generally determines the specificity of the RNA interference. hpRNA molecules are highly efficient at inhibiting the expression of endogenous genes, and the RNA interference they induce is inherited by subsequent generations of plants. See, for example,

Chuang and Meyerowitz, (2000) *Proc. Natl. Acad. Sci. USA* 97:4985-4990; Stoutjesdijk, *et al.*, (2002) *Plant Physiol.* 129:1723-1731 and Waterhouse and Helliwell, (2003) *Nat. Rev. Genet.* 4:29-38. Methods for using hpRNA interference to inhibit or silence the expression of genes are described, for example, in Chuang and Meyerowitz, (2000) *Proc. Natl. Acad. Sci. USA* 97:4985-4990; Stoutjesdijk, *et al.*, (2002) *Plant Physiol.* 129:1723-1731; Waterhouse and Helliwell, (2003) *Nat. Rev. Genet.* 4:29-38; Pandolfini, *et al.*, *BMC Biotechnology* 3:7 and US Patent Application Publication Number 2003/0175965, each of which is herein incorporated by reference. A transient assay for the efficiency of hpRNA constructs to silence gene expression *in vivo* has been described by Panstruga, *et al.*, (2003) *Mol. Biol. Rep.* 30:135-140, herein incorporated by reference.

For ihpRNA, the interfering molecules have the same general structure as for hpRNA, but the RNA molecule additionally comprises an intron that is capable of being spliced in the cell in which the ihpRNA is expressed. The use of an intron minimizes the size of the loop in the hairpin RNA molecule following splicing, and this increases the efficiency of interference. See, for example, Smith, *et al.*, (2000) *Nature* 407:319-320. In fact, Smith, *et al.*, show 100% suppression of endogenous gene expression using ihpRNA-mediated interference. Methods for using ihpRNA interference to inhibit the expression of endogenous plant genes are described, for example, in Smith, *et al.*, (2000) *Nature* 407:319-320; Wesley, *et al.*, (2001) *Plant J.* 27:581-590; Wang and Waterhouse, (2001) *Curr. Opin. Plant Biol.* 5:146-150; Waterhouse and Helliwell, (2003) *Nat. Rev. Genet.* 4:29-38; Helliwell and Waterhouse, (2003) *Methods* 30:289-295 and US Patent Application Publication Number 2003/0180945, each of which is herein incorporated by reference.

The expression cassette for hpRNA interference may also be designed such that the sense sequence and the antisense sequence do not correspond to an endogenous RNA. In this embodiment, the sense and antisense sequence flank a loop sequence that comprises a nucleotide sequence corresponding to all or part of the endogenous messenger RNA of the target gene. Thus, it is the loop region that determines the specificity of the RNA interference. See, for example, WO 2002/00904, herein incorporated by reference.

v. *Amplicon-Mediated Interference*

Amplicon expression cassettes comprise a plant virus-derived sequence that contains all or part of the target gene but generally not all of the genes of the native virus. The viral sequences present in the transcription product of the expression cassette allow the transcription product to direct its own replication. The transcripts produced by the amplicon may be either sense or antisense relative to the target sequence (i.e., the messenger RNA for the yield improvement polypeptide). Methods of using amplicons to inhibit the expression of endogenous plant genes are described, for example, in Angell and Baulcombe, (1997)

EMBO J. 16:3675-3684, Angell and Baulcombe, (1999) *Plant J.* 20:357-362 and US Patent Number 6,646,805, each of which is herein incorporated by reference.

vi. *Ribozymes*

5 In some embodiments, the polynucleotide expressed by the expression cassette of the disclosure is catalytic RNA or has ribozyme activity specific for the messenger RNA of the yield improvement polypeptide. Thus, the polynucleotide causes the degradation of the endogenous messenger RNA, resulting in reduced expression of the yield improvement polypeptide. This method is described, for example, in US Patent Number 4,987,071, herein
10 incorporated by reference.

vii. *Small Interfering RNA or Micro RNA*

In some embodiments of the disclosure, inhibition of the expression of a yield improvement polypeptide may be obtained by RNA interference by expression of a gene
15 encoding a micro RNA (miRNA). miRNAs are regulatory agents consisting of about 22 ribonucleotides. miRNA are highly efficient at inhibiting the expression of endogenous genes. See, for example, Javier, *et al.*, (2003) *Nature* 425:257-263, herein incorporated by reference.

For miRNA interference, the expression cassette is designed to express an RNA
20 molecule that is modeled on an endogenous miRNA gene. The miRNA gene encodes an RNA that forms a hairpin structure containing a circa 22-nucleotide sequence that is complementary to another endogenous gene (target sequence). For suppression of yield improvement expression, the 22-nucleotide sequence is selected from a yield improvement transcript sequence and contains 22 nucleotides of said yield improvement sequence in
25 sense orientation and 21 nucleotides of a corresponding antisense sequence that is complementary to the sense sequence. miRNA molecules are highly efficient at inhibiting the expression of endogenous genes and the RNA interference they induce is inherited by subsequent generations of plants.

30 2. *Polypeptide-Based Inhibition of Gene Expression*

In one embodiment, the polynucleotide encodes a zinc finger protein that binds to a gene encoding a yield improvement polypeptide, resulting in reduced expression of the gene. In particular embodiments, the zinc finger protein binds to a regulatory region of a yield improvement gene. In other embodiments, the zinc finger protein binds to a
35 messenger RNA encoding a yield improvement polypeptide and prevents its translation. Methods of selecting sites for targeting by zinc finger proteins have been described, for example, in US Patent Number 6,453,242, and methods for using zinc finger proteins to

inhibit the expression of genes in plants are described, for example, in US Patent Application Publication Number 2003/0037355, each of which is herein incorporated by reference.

3. *Polypeptide-Based Inhibition of Protein Activity*

5 In some embodiments of the disclosure, the polynucleotide encodes an antibody that binds to at least one yield improvement polypeptide and reduces the cell number regulator activity of the yield improvement polypeptide. In another embodiment, the binding of the antibody results in increased turnover of the antibody-yield improvement complex by cellular quality control mechanisms. The expression of antibodies in plant cells and the inhibition of
10 molecular pathways by expression and binding of antibodies to proteins in plant cells are well known in the art. See, for example, Conrad and Sonnewald, (2003) *Nature Biotech.* 21:35-36, incorporated herein by reference.

4. *Gene Disruption*

15 In some embodiments of the present disclosure, the activity of an yield improvement polypeptide is reduced or eliminated by disrupting the gene encoding the yield improvement polypeptide. The gene encoding the yield improvement polypeptide may be disrupted by any method known in the art. For example, in one embodiment, the gene is disrupted by transposon tagging. In another embodiment, the gene is disrupted by mutagenizing plants
20 using random or targeted mutagenesis and selecting for plants that have reduced cell number regulator activity.

i. *Transposon Tagging*

In one embodiment of the disclosure, transposon tagging is used to reduce or
25 eliminate the yield improvement activity of one or more yield improvement polypeptide. Transposon tagging comprises inserting a transposon within an endogenous yield improvement gene to reduce or eliminate expression of the yield improvement polypeptide. "yield improvement gene" is intended to mean the gene that encodes a yield improvement polypeptide according to the disclosure.

30 In this embodiment, the expression of one or more yield improvement polypeptide is reduced or eliminated by inserting a transposon within a regulatory region or coding region of the gene encoding the yield improvement polypeptide. A transposon that is within an exon, intron, 5' or 3' untranslated sequence, a promoter or any other regulatory sequence of a yield improvement gene may be used to reduce or eliminate the expression and/or activity
35 of the encoded yield improvement polypeptide.

Methods for the transposon tagging of specific genes in plants are well known in the art. See, for example, Maes, *et al.*, (1999) *Trends Plant Sci.* 4:90-96; Dharmapuri and Sonti,

(1999) *FEMS Microbiol. Lett.* 179:53-59; Meissner, *et al.*, (2000) *Plant J.* 22:265-274; Phogat, *et al.*, (2000) *J. Biosci.* 25:57-63; Walbot, (2000) *Curr. Opin. Plant Biol.* 2:103-107; Gai, *et al.*, (2000) *Nucleic Acids Res.* 28:94-96; Fitzmaurice, *et al.*, (1999) *Genetics* 153:1919-1928). In addition, the TUSC process for selecting Mu insertions in selected genes has been described in Bensen, *et al.*, (1995) *Plant Cell* 7:75-84; Mena, *et al.*, (1996) *Science* 274:1537-1540 and US Patent Number 5,962,764, each of which is herein incorporated by reference.

ii. *Mutant Plants with Reduced Activity*

Additional methods for decreasing or eliminating the expression of endogenous genes in plants are also known in the art and can be similarly applied to the instant disclosure. These methods include other forms of mutagenesis, such as ethyl methanesulfonate-induced mutagenesis, deletion mutagenesis and fast neutron deletion mutagenesis used in a reverse genetics sense (with PCR) to identify plant lines in which the endogenous gene has been deleted. For examples of these methods see, Ohshima, *et al.*, (1998) *Virology* 243:472-481; Okubara, *et al.*, (1994) *Genetics* 137:867-874 and Quesada, *et al.*, (2000) *Genetics* 154:421-436, each of which is herein incorporated by reference. In addition, a fast and automatable method for screening for chemically induced mutations, TILLING (Targeting Induced Local Lesions In Genomes), using denaturing HPLC or selective endonuclease digestion of selected PCR products is also applicable to the instant disclosure. See, McCallum, *et al.*, (2000) *Nat. Biotechnol.* 18:455-457, herein incorporated by reference.

Mutations that impact gene expression or that interfere with the function (cell number regulator activity) of the encoded protein are well known in the art. Insertional mutations in gene exons usually result in null-mutants. Mutations in conserved residues are particularly effective in inhibiting the cell number regulator activity of the encoded protein. Conserved residues of nutrient uptake improvement polypeptides suitable for mutagenesis with the goal to eliminate cell number regulator activity have been described. Such mutants can be isolated according to well-known procedures, and mutations in different yield improvement loci can be stacked by genetic crossing. See, for example, Gruis, *et al.*, (2002) *Plant Cell* 14:2863-2882.

In another embodiment of this disclosure, dominant mutants can be used to trigger RNA silencing due to gene inversion and recombination of a duplicated gene locus. See, for example, Kusaba, *et al.*, (2003) *Plant Cell* 15:1455-1467.

The disclosure encompasses additional methods for reducing or eliminating the activity of one or more yield improvement polypeptide. Examples of other methods for altering or mutating a genomic nucleotide sequence in a plant are known in the art and

include, but are not limited to, the use of RNA:DNA vectors, RNA:DNA mutational vectors, RNA:DNA repair vectors, mixed-duplex oligonucleotides, self-complementary RNA:DNA oligonucleotides and recombinogenic oligonucleobases. Such vectors and methods of use are known in the art. See, for example, US Patent Numbers 5,565,350; 5,731,181; 5,756,325; 5,760,012; 5,795,972 and 5,871,984, each of which are herein incorporated by reference. See also, WO 1998/49350, WO 1999/07865, WO 1999/25821 and Beetham, *et al.*, (1999) *Proc. Natl. Acad. Sci. USA* 96:8774-8778, each of which is herein incorporated by reference.

iii. *Modulating plant growth and/or organ development activity*

In specific methods, the level and/or activity of a cell number regulator in a plant is increased by increasing the level or activity of the yield improvement polypeptide in the plant. Methods for increasing the level and/or activity of yield improvement polypeptides in a plant are discussed elsewhere herein. Briefly, such methods comprise providing a yield improvement polypeptide of the disclosure to a plant and thereby increasing the level and/or activity of the yield improvement polypeptide. In other embodiments, an yield improvement nucleotide sequence encoding an yield improvement polypeptide can be provided by introducing into the plant a polynucleotide comprising an yield improvement nucleotide sequence of the disclosure, expressing the yield improvement sequence, increasing the activity of the yield improvement polypeptide and thereby increasing the number of tissue cells in the plant or plant part. In other embodiments, the yield improvement nucleotide construct introduced into the plant is stably incorporated into the genome of the plant.

In other methods, the number of cells and biomass of a plant tissue is increased by increasing the level and/or activity of the yield improvement polypeptide in the plant. Such methods are disclosed in detail elsewhere herein. In one such method, a yield improvement nucleotide sequence is introduced into the plant and expression of said yield improvement nucleotide sequence decreases the activity of the yield improvement polypeptide and thereby increasing the plant growth and/or organ development in the plant or plant part. In other embodiments, the yield improvement nucleotide construct introduced into the plant is stably incorporated into the genome of the plant.

As discussed above, one of skill will recognize the appropriate promoter to use to modulate the level/activity of a plant growth and/or organ development polynucleotide and polypeptide in the plant. Exemplary promoters for this embodiment have been disclosed elsewhere herein.

Accordingly, the present disclosure further provides plants having a modified plant growth and/or organ development when compared to the plant growth and/or organ development of a control plant tissue. In one embodiment, the plant of the disclosure has an

increased level/activity of the yield improvement polypeptide of the disclosure and thus has increased plant growth and/or organ development in the plant tissue. In other embodiments, the plant of the disclosure has a reduced or eliminated level of the yield improvement polypeptide of the disclosure and thus has decreased plant growth and/or organ development in the plant tissue. In other embodiments, such plants have stably incorporated into their genome a nucleic acid molecule comprising a yield improvement nucleotide sequence of the disclosure operably linked to a promoter that drives expression in the plant cell.

iv. Modulating Root Development

Methods for modulating root development in a plant are provided. By "modulating root development" is intended any alteration in the development of the plant root when compared to a control plant. Such alterations in root development include, but are not limited to, alterations in the growth rate of the primary root, the fresh root weight, the extent of lateral and adventitious root formation, the vasculature system, meristem development or radial expansion.

Methods for modulating root development in a plant are provided. The methods comprise modulating the level and/or activity of the yield improvement polypeptide in the plant. In one method, a yield improvement sequence of the disclosure is provided to the plant. In another method, the yield improvement nucleotide sequence is provided by introducing into the plant a polynucleotide comprising a yield improvement nucleotide sequence of the disclosure, expressing the yield improvement sequence and thereby modifying root development. In still other methods, the yield improvement nucleotide construct introduced into the plant is stably incorporated into the genome of the plant.

In other methods, root development is modulated by altering the level or activity of the yield improvement polypeptide in the plant. An increase in yield improvement activity can result in at least one or more of the following alterations to root development, including, but not limited to, larger root meristems, increased in root growth, enhanced radial expansion, an enhanced vasculature system, increased root branching, more adventitious roots and/or an increase in fresh root weight when compared to a control plant.

As used herein, "root growth" encompasses all aspects of growth of the different parts that make up the root system at different stages of its development in both monocotyledonous and dicotyledonous plants. It is to be understood that enhanced root growth can result from enhanced growth of one or more of its parts including the primary root, lateral roots, adventitious roots, etc.

Methods of measuring such developmental alterations in the root system are known in the art. See, for example, US Patent Application Publication Number 2003/0074698 and

Werner, *et al.*, (2001) *PNAS* 18:10487-10492, both of which are herein incorporated by reference.

As discussed above, one of skill will recognize the appropriate promoter to use to modulate root development in the plant. Exemplary promoters for this embodiment include constitutive promoters and root-preferred promoters. Exemplary root-preferred promoters have been disclosed elsewhere herein.

Stimulating root growth and increasing root mass by increasing the activity and/or level of the yield improvement polypeptide also finds use in improving the standability of a plant. The term "resistance to lodging" or "standability" refers to the ability of a plant to fix itself to the soil. For plants with an erect or semi-erect growth habit, this term also refers to the ability to maintain an upright position under adverse (environmental) conditions. This trait relates to the size, depth and morphology of the root system. In addition, stimulating root growth and increasing root mass by increasing the level and/or activity of the yield improvement polypeptide also finds use in promoting *in vitro* propagation of explants.

Furthermore, higher root biomass production due to an increased level and/or activity of yield improvement activity has a direct effect on the yield and an indirect effect of production of compounds produced by root cells or transgenic root cells or cell cultures of said transgenic root cells. One example of an interesting compound produced in root cultures is shikonin, the yield of which can be advantageously enhanced by said methods.

Accordingly, the present disclosure further provides plants having modulated root development when compared to the root development of a control plant. In some embodiments, the plant of the disclosure has an increased level/activity of the yield improvement polypeptide of the disclosure and has enhanced root growth and/or root biomass. In other embodiments, such plants have stably incorporated into their genome a nucleic acid molecule comprising a yield improvement nucleotide sequence of the disclosure operably linked to a promoter that drives expression in the plant cell.

v. *Modulating Shoot and Leaf Development*

Methods are also provided for modulating shoot and leaf development in a plant. By "modulating shoot and/or leaf development" is intended any alteration in the development of the plant shoot and/or leaf. Such alterations in shoot and/or leaf development include, but are not limited to, alterations in shoot meristem development, in leaf number, leaf size, leaf and stem vasculature, internode length and leaf senescence. As used herein, "leaf development" and "shoot development" encompasses all aspects of growth of the different parts that make up the leaf system and the shoot system, respectively, at different stages of their development, both in monocotyledonous and dicotyledonous plants. Methods for measuring such developmental alterations in the shoot and leaf system are known in the art.

See, for example, Werner, *et al.*, (2001) PNAS 98:10487-10492 and US Patent Application Publication Number 2003/0074698, each of which is herein incorporated by reference.

The method for modulating shoot and/or leaf development in a plant comprises modulating the activity and/or level of a yield improvement polypeptide of the disclosure. In one embodiment, a yield improvement sequence of the disclosure is provided. In other embodiments, the yield improvement nucleotide sequence can be provided by introducing into the plant a polynucleotide comprising a yield improvement nucleotide sequence of the disclosure, expressing the yield improvement sequence, and thereby modifying shoot and/or leaf development. In other embodiments, the yield improvement nucleotide construct introduced into the plant is stably incorporated into the genome of the plant.

In specific embodiments, shoot or leaf development is modulated by decreasing the level and/or activity of the yield improvement polypeptide in the plant. A decrease in yield improvement activity can result in at least one or more of the following alterations in shoot and/or leaf development, including, but not limited to, reduced leaf number, reduced leaf surface, reduced vascular, shorter internodes and stunted growth and retarded leaf senescence, when compared to a control plant.

As discussed above, one of skill will recognize the appropriate promoter to use to modulate shoot and leaf development of the plant. Exemplary promoters for this embodiment include constitutive promoters, shoot-preferred promoters, shoot meristem-preferred promoters and leaf-preferred promoters. Exemplary promoters have been disclosed elsewhere herein.

Decreasing yield improvement activity and/or level in a plant results in shorter internodes and stunted growth. Thus, the methods of the disclosure find use in producing dwarf plants. In addition, as discussed above, modulations of yield improvement activity in the plant modulates both root and shoot growth. Thus, the present disclosure further provides methods for altering the root/shoot ratio. Shoot or leaf development can further be modulated by decreasing the level and/or activity of the yield improvement polypeptide in the plant.

Accordingly, the present disclosure further provides plants having modulated shoot and/or leaf development when compared to a control plant. In some embodiments, the plant of the disclosure has an increased level/activity of the yield improvement polypeptide of the disclosure, altering the shoot and/or leaf development. Such alterations include, but are not limited to, increased leaf number, increased leaf surface, increased vascularity, longer internodes and increased plant stature, as well as alterations in leaf senescence, as compared to a control plant. In other embodiments, the plant of the disclosure has a decreased level/activity of the yield improvement polypeptide of the disclosure.

vi Modulating Reproductive Tissue Development

Methods for modulating reproductive tissue development are provided. In one embodiment, methods are provided to modulate floral development in a plant. By "modulating floral development" is intended any alteration in a structure of a plant's reproductive tissue as compared to a control plant in which the activity or level of the yield improvement polypeptide has not been modulated. "Modulating floral development" further includes any alteration in the timing of the development of a plant's reproductive tissue (i.e., a delayed or an accelerated timing of floral development) when compared to a control plant in which the activity or level of the yield improvement polypeptide has not been modulated. Macroscopic alterations may include changes in size, shape, number or location of reproductive organs, the developmental time period that these structures form or the ability to maintain or proceed through the flowering process in times of environmental stress. Microscopic alterations may include changes to the types or shapes of cells that make up the reproductive organs.

The method for modulating floral development in a plant comprises modulating yield improvement activity in a plant. In one method, a yield improvement sequence of the disclosure is provided. A yield improvement nucleotide sequence can be provided by introducing into the plant a polynucleotide comprising a yield improvement nucleotide sequence of the disclosure, expressing the yield improvement sequence and thereby modifying floral development. In other embodiments, the yield improvement nucleotide construct introduced into the plant is stably incorporated into the genome of the plant.

In specific methods, floral development is modulated by decreasing the level or activity of the yield improvement polypeptide in the plant. A decrease in yield improvement activity can result in at least one or more of the following alterations in floral development, including, but not limited to, retarded flowering, reduced number of flowers, partial male sterility and reduced seed set, when compared to a control plant. Inducing delayed flowering or inhibiting flowering can be used to enhance yield in forage crops such as alfalfa. Methods for measuring such developmental alterations in floral development are known in the art. See, for example, Mouradov, *et al.*, (2002) *The Plant Cell* S111-S130, herein incorporated by reference.

As discussed above, one of skill will recognize the appropriate promoter to use to modulate floral development of the plant. Exemplary promoters for this embodiment include constitutive promoters, inducible promoters, shoot-preferred promoters and inflorescence-preferred promoters.

In other methods, floral development is modulated by increasing the level and/or activity of the yield improvement sequence of the disclosure. Such methods can comprise introducing a yield improvement nucleotide sequence into the plant and increasing the

activity of the yield improvement polypeptide. In other methods, the yield improvement nucleotide construct introduced into the plant is stably incorporated into the genome of the plant. Increasing expression of the yield improvement sequence of the disclosure can modulate floral development during periods of stress. Such methods are described
5 elsewhere herein. Accordingly, the present disclosure further provides plants having modulated floral development when compared to the floral development of a control plant. Compositions include plants having an increased level/activity of the yield improvement polypeptide of the disclosure and having an altered floral development. Compositions also include plants having an increased level/activity of the yield improvement polypeptide of the
10 disclosure wherein the plant maintains or proceeds through the flowering process in times of stress.

Methods are also provided for the use of the yield improvement sequences of the disclosure to increase seed size and/or weight. The method comprises increasing the activity of the yield improvement sequences in a plant or plant part, such as the seed. An
15 increase in seed size and/or weight comprises an increased size or weight of the seed and/or an increase in the size or weight of one or more seed parts including, for example, the embryo, endosperm, seed coat, aleurone or cotyledon.

As discussed above, one of skill will recognize the appropriate promoter to use to increase seed size and/or seed weight. Exemplary promoters of this embodiment include
20 constitutive promoters, inducible promoters, seed-preferred promoters, embryo-preferred promoters and endosperm-preferred promoters.

The method for decreasing seed size and/or seed weight in a plant comprises decreasing yield improvement activity in the plant. In one embodiment, the yield improvement nucleotide sequence can be provided by introducing into the plant a
25 polynucleotide comprising a yield improvement nucleotide sequence of the disclosure, expressing the yield improvement sequence, and thereby decreasing seed weight and/or size. In other embodiments, the yield improvement nucleotide construct introduced into the plant is stably incorporated into the genome of the plant.

It is further recognized that increasing seed size and/or weight can also be
30 accompanied by an increase in the speed of growth of seedlings or an increase in early vigor. As used herein, the term "early vigor" refers to the ability of a plant to grow rapidly during early development, and relates to the successful establishment, after germination, of a well-developed root system and a well-developed photosynthetic apparatus. In addition, an increase in seed size and/or weight can also result in an increase in nutrient uptake when
35 compared to a control.

Accordingly, the present disclosure further provides plants having an increased seed weight and/or seed size when compared to a control plant. In other embodiments, plants

having an increased vigor and nutrient uptake are also provided. In some embodiments, the plant of the disclosure has an increased level/activity of the yield improvement polypeptide of the disclosure and has an increased seed weight and/or seed size. In other embodiments, such plants have stably incorporated into their genome a nucleic acid molecule comprising a yield improvement nucleotide sequence of the disclosure operably linked to a promoter that drives expression in the plant cell.

vii. Method of Use for yield improvement promoter polynucleotides

The polynucleotides comprising the yield improvement promoters disclosed in the present disclosure, as well as variants and fragments thereof, are useful in the genetic manipulation of any host cell, preferably plant cell, when assembled with a DNA construct such that the promoter sequence is operably linked to a nucleotide sequence comprising a polynucleotide of interest. In this manner, the yield improvement promoter polynucleotides of the disclosure are provided in expression cassettes along with a polynucleotide sequence of interest for expression in the host cell of interest. As discussed in Example 2 below, the yield improvement promoter sequences of the disclosure are expressed in a variety of tissues and thus the promoter sequences can find use in regulating the temporal and/or the spatial expression of polynucleotides of interest.

Synthetic hybrid promoter regions are known in the art. Such regions comprise upstream promoter elements of one polynucleotide operably linked to the promoter element of another polynucleotide. In an embodiment of the disclosure, heterologous sequence expression is controlled by a synthetic hybrid promoter comprising the yield improvement promoter sequences of the disclosure, or a variant or fragment thereof, operably linked to upstream promoter element(s) from a heterologous promoter. Upstream promoter elements that are involved in the plant defense system have been identified and may be used to generate a synthetic promoter. See, for example, Rushton, *et al.*, (1998) *Curr. Opin. Plant Biol.* 1:311-315. Alternatively, a synthetic yield improvement promoter sequence may comprise duplications of the upstream promoter elements found within the yield improvement promoter sequences.

It is recognized that the promoter sequence of the disclosure may be used with its native yield improvement coding sequences. A DNA construct comprising the yield improvement promoter operably linked with its native yield improvement gene may be used to transform any plant of interest to bring about a desired phenotypic change, such as modulating cell number, modulating root, shoot, leaf, floral and embryo development, stress tolerance and any other phenotype described elsewhere herein.

The promoter nucleotide sequences and methods disclosed herein are useful in regulating expression of any heterologous nucleotide sequence in a host plant in order to

vary the phenotype of a plant. Various changes in phenotype are of interest including modifying the fatty acid composition in a plant, altering the amino acid content of a plant, altering a plant's pathogen defense mechanism, and the like. These results can be achieved by providing expression of heterologous products or increased expression of endogenous products in plants. Alternatively, the results can be achieved by providing for a reduction of expression of one or more endogenous products, particularly enzymes or cofactors in the plant. These changes result in a change in phenotype of the transformed plant.

Genes of interest are reflective of the commercial markets and interests of those involved in the development of the crop. Crops and markets of interest change, and as developing nations open up world markets, new crops and technologies will emerge also. In addition, as our understanding of agronomic traits and characteristics such as yield and heterosis increase, the choice of genes for transformation will change accordingly. General categories of genes of interest include, for example, those genes involved in information, such as zinc fingers, those involved in communication, such as kinases and those involved in housekeeping, such as heat shock proteins. More specific categories of transgenes, for example, include genes encoding important traits for agronomics, insect resistance, disease resistance, herbicide resistance, sterility, grain characteristics and commercial products. Genes of interest include, generally, those involved in oil, starch, carbohydrate or nutrient metabolism as well as those affecting kernel size, sucrose loading, and the like.

In certain embodiments the nucleic acid sequences of the present disclosure can be used in combination ("stacked") with other polynucleotide sequences of interest in order to create plants with a desired phenotype. The combinations generated can include multiple copies of any one or more of the polynucleotides of interest. The polynucleotides of the present disclosure may be stacked with any gene or combination of genes to produce plants with a variety of desired trait combinations, including but not limited to traits desirable for animal feed such as high oil genes (e.g., US Patent Number 6,232,529); balanced amino acids (e.g., hordothionins (US Patent Numbers 5,990,389; 5,885,801; 5,885,802 and 5,703,409); barley high lysine (Williamson, *et al.*, (1987) *Eur. J. Biochem.* 165:99-106 and WO 1998/20122) and high methionine proteins (Pedersen, *et al.*, (1986) *J. Biol. Chem.* 261:6279; Kirihaara, *et al.*, (1988) *Gene* 71:359 and Musumura, *et al.*, (1989) *Plant Mol. Biol.* 12:123)); increased digestibility (e.g., modified storage proteins (US Patent Application Serial Number 10/053,410, filed November 7, 2001) and thioredoxins (US Patent Application Serial Number 10/005,429, filed December 3, 2001)), the disclosures of which are herein incorporated by reference. The polynucleotides of the present disclosure can also be stacked with traits desirable for insect, disease or herbicide resistance (e.g., *Bacillus thuringiensis* toxic proteins (US Patent Numbers 5,366,892; 5,747,450; 5,737,514; 5,723,756; 5,593,881; Geiser, *et al.*, (1986) *Gene* 48:109); lectins (Van Damme, *et al.*, (1994) *Plant Mol.*

Biol. 24:825); fumonisin detoxification genes (US Patent Number 5,792,931); avirulence and disease resistance genes (Jones, *et al.*, (1994) *Science* 266:789; Martin, *et al.*, (1993) *Science* 262:1432; Mindrinos, *et al.*, (1994) *Cell* 78:1089); acetolactate synthase (ALS) mutants that lead to herbicide resistance such as the S4 and/or Hra mutations; inhibitors of glutamine synthase such as phosphinothricin or basta (e.g., bar gene) and glyphosate resistance (EPSPS gene)) and traits desirable for processing or process products such as high oil (e.g., US Patent Number 6,232,529); modified oils (e.g., fatty acid desaturase genes (US Patent Number 5,952,544; WO 94/11516)); modified starches (e.g., ADPG pyrophosphorylases (AGPase), starch synthases (SS), starch branching enzymes (SBE) and starch debranching enzymes (SDBE)) and polymers or bioplastics (e.g., US Patent Number 5,602,321; beta-ketothiolase, polyhydroxybutyrate synthase and acetoacetyl-CoA reductase (Schubert, *et al.*, (1988) *J. Bacteriol.* 170:5837-5847) facilitate expression of polyhydroxyalkanoates (PHAs)), the disclosures of which are herein incorporated by reference. One could also combine the polynucleotides of the present disclosure with polynucleotides affecting agronomic traits such as male sterility (e.g., see, US Patent Number 5,583,210), stalk strength, flowering time or transformation technology traits such as cell cycle regulation or gene targeting (e.g., WO 1999/61619; WO 2000/17364; WO 1999/25821), the disclosures of which are herein incorporated by reference.

In one embodiment, sequences of interest improve plant growth and/or crop yields. For example, sequences of interest include agronomically important genes that result in improved primary or lateral root systems. Such genes include, but are not limited to, nutrient/water transporters and growth inducers. Examples of such genes, include but are not limited to, maize plasma membrane H⁺-ATPase (MHA2) (Frias, *et al.*, (1996) *Plant Cell* 8:1533-44); AKT1, a component of the potassium uptake apparatus in *Arabidopsis*, (Spalding, *et al.*, (1999) *J Gen Physiol* 113:909-18); RML genes which activate cell division cycle in the root apical cells (Cheng, *et al.*, (1995) *Plant Physiol* 108:881); maize glutamine synthetase genes (Sukanya, *et al.*, (1994) *Plant Mol Biol* 26:1935-46) and hemoglobin (Duff, *et al.*, (1997) *J. Biol. Chem* 27:16749-16752, Arredondo-Peter, *et al.*, (1997) *Plant Physiol.* 115:1259-1266; Arredondo-Peter, *et al.*, (1997) *Plant Physiol* 114:493-500, and references cited therein). The sequence of interest may also be useful in expressing antisense nucleotide sequences of genes that negatively affects root development.

Additional, agronomically important traits such as oil, starch and protein content can be genetically altered in addition to using traditional breeding methods. Modifications include increasing content of oleic acid, saturated and unsaturated oils, increasing levels of lysine and sulfur, providing essential amino acids and also modification of starch. Hordothionin protein modifications are described in US Patent Numbers 5,703,049, 5,885,801, 5,885,802 and 5,990,389, herein incorporated by reference. Another example is

lysine and/or sulfur rich seed protein encoded by the soybean 2S albumin described in US Patent Number 5,850,016 and the chymotrypsin inhibitor from barley, described in Williamson, *et al.*, (1987) *Eur. J. Biochem.* 165:99-106, the disclosures of which are herein incorporated by reference.

5 Derivatives of the coding sequences can be made by site-directed mutagenesis to increase the level of preselected amino acids in the encoded polypeptide. For example, the gene encoding the barley high lysine polypeptide (BHL) is derived from barley chymotrypsin inhibitor, US Patent Application Serial Number 08/740,682, filed November 1, 1996 and WO 1998/20133, the disclosures of which are herein incorporated by reference. Other proteins
10 include methionine-rich plant proteins such as from sunflower seed (Lilley, *et al.*, (1989) *Proceedings of the World Congress on Vegetable Protein Utilization in Human Foods and Animal Feedstuffs*, ed. Applewhite, (American Oil Chemists Society, Champaign, Illinois), pp. 497-502, herein incorporated by reference); corn (Pedersen, *et al.*, (1986) *J. Biol. Chem.* 261:6279; Kirihaara, *et al.*, (1988) *Gene* 71:359, both of which are herein incorporated by
15 reference) and rice (Musumura, *et al.*, (1989) *Plant Mol. Biol.* 12:123, herein incorporated by reference). Other agronomically important genes encode latex, Flourey 2, growth factors, seed storage factors and transcription factors.

Insect resistance genes may encode resistance to pests that have great yield drag such as rootworm, cutworm, European Corn Borer, and the like. Such genes include, for
20 example, *Bacillus thuringiensis* toxic protein genes (US Patent Numbers 5,366,892; 5,747,450; 5,736,514; 5,723,756; 5,593,881 and Geiser, *et al.*, (1986) *Gene* 48:109), and the like.

Genes encoding disease resistance traits include detoxification genes, such as against fumonisin (US Patent Number 5,792,931); avirulence (avr) and disease resistance
25 (R) genes (Jones, *et al.*, (1994) *Science* 266:789; Martin, *et al.*, (1993) *Science* 262:1432 and Mindrinos, *et al.*, (1994) *Cell* 78:1089), and the like.

Herbicide resistance traits may include genes coding for resistance to herbicides that act to inhibit the action of acetolactate synthase (ALS), in particular the sulfonylurea-type herbicides (e.g., the acetolactate synthase (ALS) gene containing mutations leading to such
30 resistance, in particular the S4 and/or Hra mutations), genes coding for resistance to herbicides that act to inhibit action of glutamine synthase, such as phosphinothricin or basta (e.g., the bar gene) or other such genes known in the art. The bar gene encodes resistance to the herbicide basta, the *nptII* gene encodes resistance to the antibiotics kanamycin and geneticin and the ALS-gene mutants encode resistance to the herbicide chlorsulfuron.

35 Sterility genes can also be encoded in an expression cassette and provide an alternative to physical detasseling. Examples of genes used in such ways include male tissue-preferred genes and genes with male sterility phenotypes such as QM, described in

US Patent Number 5,583,210. Other genes include kinases and those encoding compounds toxic to either male or female gametophytic development.

The quality of grain is reflected in traits such as levels and types of oils, saturated and unsaturated, quality and quantity of essential amino acids and levels of cellulose. In corn, modified hordothionin proteins are described in US Patent Numbers 5,703,049, 5,885,801, 5,885,802 and 5,990,389.

Commercial traits can also be encoded on a gene or genes that could increase for example, starch for ethanol production, or provide expression of proteins. Another important commercial use of transformed plants is the production of polymers and bioplastics such as described in US Patent Number 5,602,321. Genes such as β -Ketothiolase, PHBase (polyhydroxybutyrate synthase) and acetoacetyl-CoA reductase (see, Schubert, *et al.*, (1988) *J. Bacteriol.* 170:5837-5847) facilitate expression of polyhydroxyalkanoates (PHAs).

Exogenous products include plant enzymes and products as well as those from other sources including procaryotes and other eukaryotes. Such products include enzymes, cofactors, hormones and the like. The level of proteins, particularly modified proteins having improved amino acid distribution to improve the nutrient value of the plant, can be increased. This is achieved by the expression of such proteins having enhanced amino acid content.

When referring to the relationship between two genetic elements, such as a genetic element contributing to tolerance and a proximal marker, "coupling" phase linkage indicates the state where the "favorable" allele at the tolerance locus is physically associated on the same chromosome strand as the "favorable" allele of the respective linked marker locus. In coupling phase, both favorable alleles are inherited together by progeny that inherit that chromosome strand. In "repulsion" phase linkage, the "favorable" allele at the locus of interest (e.g., a QTL for tolerance) is physically linked with an "unfavorable" allele at the proximal marker locus, and the two "favorable" alleles are not inherited together (i.e., the two loci are "out of phase" with each other).

"Linkage disequilibrium" generally refers to a phenomenon wherein alleles tend to remain together in linkage groups when segregating from parents to offspring, with a greater frequency than expected from their individual frequencies.

"Linkage group" generally refers to traits or markers that generally co-segregate. A linkage group generally corresponds to a chromosomal region containing genetic material that encodes the traits or markers. "Locus" refers to a segment of DNA.

A "map location," "map position" or "relative map position" is an assigned location on a genetic map relative to linked genetic markers where a specified marker can be found within a given species. Map positions are generally provided in centimorgans. A "physical position" or "physical location" is the position, typically in nucleotide bases, of a particular nucleotide, such as a SNP nucleotide, on the chromosome.

"Mapping" is the process of defining the linkage relationships of loci through the use of genetic markers, populations segregating for the markers and standard genetic principles of recombination frequency.

"Marker" or "molecular marker" is a term used to denote a nucleic acid or amino acid sequence that is sufficiently unique to characterize a specific locus on the genome. Any detectable polymorphic trait can be used as a marker so long as it is inherited differentially and exhibits linkage disequilibrium with a phenotypic trait of interest. Each marker is an indicator of a specific segment of DNA, having a unique nucleotide sequence. The map positions provide a measure of the relative positions of particular markers with respect to one another. When a trait is stated to be linked to a given marker, it will be understood that the actual DNA segment whose sequence affects the trait generally co-segregates with the marker. More precise and definite localization of a trait can be obtained if markers are identified on both sides of the trait. By measuring the appearance of the marker(s) in progeny of crosses, the existence of the trait can be detected by relatively simple molecular tests without actually evaluating the appearance of the trait itself, which can be difficult and time-consuming because the actual evaluation of the trait requires growing plants to a stage and/or under environmental conditions where the trait can be expressed. Molecular markers have been widely used to determine genetic composition in crop plants. "Marker assisted selection" refers to the process of selecting a desired trait or traits in a plant or plants by detecting one or more nucleic acids from the plant, where the nucleic acid is linked to the desired trait, and then selecting the plant or germplasm possessing those one or more nucleic acids.

"Haplotype" generally refers to a combination of particular alleles present within a particular plant's genome at two or more linked marker loci, for instance at two or more loci on a particular linkage group.

"Polymorphism" means a change or difference between two related nucleic acids. A "nucleotide polymorphism" refers to a nucleotide that is different in one sequence when compared to a related sequence when the two nucleic acids are aligned for maximal correspondence.

"Quantitative trait loci" or "QTL" refer to the genetic elements controlling a quantitative trait.

Provided are markers and haplotypes associated with tolerance of abiotic to root-knot nematode, as well as related primers and/or probes and methods for the use of any of the foregoing for identifying and/or selecting soybean plants with improved tolerance to root-knot nematode. A method for determining the presence or absence of at least one allele of a particular marker or haplotype associated with tolerance to root-knot nematode comprises analyzing genomic DNA from a soybean plant or germplasm to determine if at least one, or a

plurality, of such markers is present or absent and if present, determining the allelic form of the marker(s). If a plurality of markers on a single linkage group are investigated, this information regarding the markers present in the particular plant or germplasm can be used to determine a haplotype for that plant/germplasm.

This disclosure can be better understood by reference to the following non-limiting examples. It will be appreciated by those skilled in the art that other embodiments of the disclosure may be practiced without departing from the spirit and the scope of the disclosure as herein disclosed and claimed.

EXAMPLES

Example 1: Identification of sequences of interest

A multi-faceted computational analysis was done to identify a set of genes that can improve crop yield. The yield enhancement may occur through various physiological avenues, but especially via drought tolerance or WUE efficiency. These genes comprised a set of 1703 genes. These genes were identified by analyses relying on multiple sets of profiling data, pathway-network curation and literature interpretation. Most of the genes hail from sorghum, which is known to be a drought tolerant crop and many have root or root-preferred expression. This work consisted of several substeps, including: Part_1. Generate sorghum orthologs for genes already in the testing pipeline as well as newly nominated genes slated for that pipeline. Part_2. Literature and Nominations. A set of genes from literature were identified, and also a complex search of proprietary software that intersects various genomic and genetic information was used to generate a subset of genes of interest. Part_3. Sorghum Profiling Analyses, especially emphasizing sorghum genes that are stress/drought responsive where the maize orthologs are not. Part_4. Sorghum orthologs to maize mRNA profiling results of a proprietary set of elite germplasm tested under well-watered and drought conditions where this set of genes correlated to yield performance. These were dubbed yield stability genes, with the stability being under drought. Part_5. Root Hair Specific Set. As set of sorghum orthologs were identified to the Arabidopsis root hair formation genes. All the genes from parts 1-5 were gathered, sequence redundancy removed and they were further filtered by whether the ORF was complete and the degree of sorghum root preference in expression.

Example 2: Transgenic FAST Corn

Transgenic FAST Corn plants transformed with three sorghum genes expressed from the constitutive ubiquitin promoter from maize were subjected to a reproductive drought screen at the T1 generation. The three constructs, Sb09g004150, Sb03g011680 and Sb06g033870, were selected for the T1 reproductive drought evaluation based on phenomic

data from T0 FAST Corn plants. T0 phenotyping involves measurement of overall growth of the plant as well as measurement of yield components. T1 reproductive drought assay involves imposition of a chronic drought stress starting at the vegetative stage and continuing through to the flowering stage. The experiment is terminated prior to grain filling, at 8 days after silking and the reproductive parameters including ear area, ear length, ear width and silk count are determined.

Evaluation of T0 plants of Sb09g004150 indicated that 3 out of 10 tested events had statistically significant increase in ear area and maximum total plant area. At the construct level, several traits were statistically significant on the positive side, and these traits include ear area, ear length, ear width, maximum total plant area and seed number. In the T1 reproductive drought assay, 6 events were evaluated, and some parameters were positive and some negative amongst these events. T0 plants of Sb03g011680 showed significantly positive maximum total plant area for 2 of 10 events. T1 assay under drought for 6 events of this construct revealed two events with significantly improved ear area of which one had significantly increased ear length as well. In the case of Sb06g033870, 4 of 10 events evaluated at the T0 stage had significantly positive ear area and three had significantly positive seed number as well. At the construct level, ear area, ear length, maximum total plant area and seed number were all significantly positive. This construct, when tested in the T1 reproductive assay, showed one of six events with significantly positive ear area, ear length and silk count. The anthesis silking interval was significantly high for this event as well.

Example 3: Transformation and Regeneration of Transgenic Plants

Immature maize embryos from greenhouse donor plants are bombarded with a plasmid containing the sorghum uptake or stress tolerance sequence operably linked to the drought-inducible promoter RAB17 promoter (Vilardell, *et al.*, (1990) *Plant Mol Biol* 14:423-432) and the selectable marker gene PAT, which confers resistance to the herbicide Bialaphos. Alternatively, the selectable marker gene is provided on a separate plasmid. Transformation is performed as follows. Media recipes follow below.

Preparation of Target Tissue:

The ears are husked and surface sterilized in 30% Clorox® bleach plus 0.5% Micro detergent for 20 minutes and rinsed two times with sterile water. The immature embryos are excised and placed embryo axis side down (scutellum side up), 25 embryos per plate, on 560Y medium for 4 hours and then aligned within the 2.5-cm target zone in preparation for bombardment.

Preparation of DNA:

A plasmid vector comprising the nutrient uptake/stress tolerance sequence operably linked to an ubiquitin promoter is made. This plasmid DNA plus plasmid DNA containing a PAT selectable marker is precipitated onto 1.1 μm (average diameter) tungsten pellets using a CaCl_2 precipitation procedure as follows:

- 100 μl prepared tungsten particles in water
- 10 μl (1 μg) DNA in Tris EDTA buffer (1 μg total DNA)
- 100 μl 2.5 M CaCl_2
- 10 μl 0.1 M spermidine

Each reagent is added sequentially to the tungsten particle suspension, while maintained on the multitube vortexer. The final mixture is sonicated briefly and allowed to incubate under constant vortexing for 10 minutes. After the precipitation period, the tubes are centrifuged briefly, liquid removed, washed with 500 ml 100% ethanol and centrifuged for 30 seconds. Again the liquid is removed and 105 μl 100% ethanol is added to the final tungsten particle pellet. For particle gun bombardment, the tungsten/DNA particles are briefly sonicated and 10 μl spotted onto the center of each macrocarrier and allowed to dry about 2 minutes before bombardment.

Particle Gun Treatment:

The sample plates are bombarded at level #4 in particle gun #HE34-1 or #HE34-2. All samples receive a single shot at 650 PSI, with a total of ten aliquots taken from each tube of prepared particles/DNA.

Subsequent Treatment:

Following bombardment, the embryos are kept on 560Y medium for 2 days, then transferred to 560R selection medium containing 3 mg/liter Bialaphos and subcultured every 2 weeks. After approximately 10 weeks of selection, selection-resistant callus clones are transferred to 288J medium to initiate plant regeneration. Following somatic embryo maturation (2-4 weeks), well-developed somatic embryos are transferred to medium for germination and transferred to the lighted culture room. Approximately 7-10 days later, developing plantlets are transferred to 272V hormone-free medium in tubes for 7-10 days until plantlets are well established. Plants are then transferred to inserts in flats (equivalent to 2.5" pot) containing potting soil and grown for 1 week in a growth chamber, subsequently grown an additional 1-2 weeks in the greenhouse, then transferred to classic 600 pots (1.6 gallon) and grown to maturity. Plants are monitored and scored for increased abiotic stress. Assays to measure improved abiotic stress are routine in the art and include, for example, increased kernel-earring capacity yields under drought conditions when compared to control

maize plants under identical environmental conditions. Alternatively, the transformed plants can be monitored for a modulation in meristem development (i.e., a decrease in spikelet formation on the ear). See, for example, Bruce, *et al.*, (2002) *Journal of Experimental Botany* 53:1-13.

5

Bombardment and Culture Media:

Bombardment medium (560Y) comprises 4.0 g/l N6 basal salts (SIGMA C-1416), 1.0 ml/l Eriksson's Vitamin Mix (1000X SIGMA-1511), 0.5 mg/l thiamine HCl, 120.0 g/l sucrose, 1.0 mg/l 2,4-D and 2.88 g/l L-proline (brought to volume with D-I H₂O following adjustment to pH 5.8 with KOH); 2.0 g/l Gelrite® (added after bringing to volume with D-I H₂O) and 8.5 mg/l silver nitrate (added after sterilizing the medium and cooling to room temperature). Selection medium (560R) comprises 4.0 g/l N6 basal salts (SIGMA C-1416), 1.0 ml/l Eriksson's Vitamin Mix (1000X SIGMA-1511), 0.5 mg/l thiamine HCl, 30.0 g/l sucrose and 2.0 mg/l 2,4-D (brought to volume with D-I H₂O following adjustment to pH 5.8 with KOH); 3.0 g/l Gelrite® (added after bringing to volume with D-I H₂O) and 0.85 mg/l silver nitrate and 3.0 mg/l bialaphos (both added after sterilizing the medium and cooling to room temperature).

Plant regeneration medium (288J) comprises 4.3 g/l MS salts (GIBCO 11117-074), 5.0 ml/l MS vitamins stock solution (0.100 g nicotinic acid, 0.02 g/l thiamine HCL, 0.10 g/l pyridoxine HCL and 0.40 g/l glycine brought to volume with polished D-I H₂O) (Murashige and Skoog, (1962) *Physiol. Plant.* 15:473), 100 mg/l myo-inositol, 0.5 mg/l zeatin, 60 g/l sucrose and 1.0 ml/l of 0.1 mM abscisic acid (brought to volume with polished D-I H₂O after adjusting to pH 5.6); 3.0 g/l Gelrite® (added after bringing to volume with D-I H₂O) and 1.0 mg/l indoleacetic acid and 3.0 mg/l bialaphos (added after sterilizing the medium and cooling to 60°C). Hormone-free medium (272V) comprises 4.3 g/l MS salts (GIBCO 11117-074), 5.0 ml/l MS vitamins stock solution (0.100 g/l nicotinic acid, 0.02 g/l thiamine HCL, 0.10 g/l pyridoxine HCL and 0.40 g/l glycine brought to volume with polished D-I H₂O), 0.1 g/l myo-inositol and 40.0 g/l sucrose (brought to volume with polished D-I H₂O after adjusting pH to 5.6) and 6 g/l bacto™-agar (added after bringing to volume with polished D-I H₂O), sterilized and cooled to 60°C.

Example 4: *Agrobacterium*-mediated Transformation

For *Agrobacterium*-mediated transformation of maize with an antisense sequence of the nutrient uptake/stress tolerance sequence of the present disclosure, preferably the method of Zhao is employed (US Patent Number 5,981,840 and PCT Patent Publication WO 1998/32326, the contents of which are hereby incorporated by reference). Briefly, immature embryos are isolated from maize and the embryos contacted with a suspension of *Agrobacterium*, where

the bacteria are capable of transferring the sequence to at least one cell of at least one of the immature embryos (step 1: the infection step). In this step the immature embryos are preferably immersed in an *Agrobacterium* suspension for the initiation of inoculation. The embryos are co-cultured for a time with the *Agrobacterium* (step 2: the co-cultivation step).
5 Preferably the immature embryos are cultured on solid medium following the infection step. Following this co-cultivation period an optional "resting" step is contemplated. In this resting step, the embryos are incubated in the presence of at least one antibiotic known to inhibit the growth of *Agrobacterium* without the addition of a selective agent for plant transformants (step 3: resting step). Preferably the immature embryos are cultured on solid medium with
10 antibiotic, but without a selecting agent, for elimination of *Agrobacterium* and for a resting phase for the infected cells. Next, inoculated embryos are cultured on medium containing a selective agent and growing transformed callus is recovered (step 4: the selection step). Preferably, the immature embryos are cultured on solid medium with a selective agent resulting in the selective growth of transformed cells. The callus is then regenerated into
15 plants (step 5: the regeneration step) and preferably calli grown on selective medium are cultured on solid medium to regenerate the plants. Plants are monitored and scored for a modulation in meristem development, for instance, alterations of size and appearance of the shoot and floral meristems and/or increased yields of leaves, flowers and/or fruits.

20 **Example 5: Transgenic maize plants overexpressing sorghum genes showed improved ear traits and yield components**

Sorghum genomic clones (SEQ ID NOS: 3553, 3563, 3564, 3589, 3680, 4042, 4548, 4202, 4306, 4345, 4530, 4724, 4887, 4910) containing the corresponding 13 genes were isolated and each individual gene was transformed into maize plants. In the designed
25 vector, transgene expression was driven by a constitutive maize ubiquitin promoter. T0 plants overexpressing the transgenes were generated. Transgenic plants from multiple events were subjected to T1 reproductive assay under low nitrogen stress treatment (4mM concentration). Multiple ear traits were collected from multiple events of the transgenic plants corresponding to these 13 genes, respectively. Compared to non-transgenic controls,
30 the transgenic plants showed significant improvement in plant growth especially ear traits, such as ear length, ear width, ear area and silk number, which reflects the seed number potential per ear (Table 2, below). These data demonstrate the efficacy of these sorghum genes in improving yield components and potential yield of maize and under stressed condition of low nitrogen.

Table 2

Gene Loc	Seq ID NO	Ear area (cm ²)	Ear length (cm)	Ear width (cm)	Silk count (no.)	Stress type
Sb03g011680	3553	2/6 events sig increase up to 31%	2/6 events sig increase up to 22%	2/6 events sig increase up to 8%	2/6 events sig increase up to 23%	NUE and Drought
Sb06g033870	3563	1/6 events sig increase up to 24%	1/6 events sig increase up to 18%	4/6 events; NS	1/6 events sig increase up to 16%	NUE and Drought
Sb03g034260	3564	1/6 events sig increase up to 15%	3/6 events; NS	3/6 events; NS	6/6 events; NS	NUE
Sb09g029110	3589	2/6 events sig increase up to 32%	2/6 events sig increase up to 18%	2/6 events sig increase up to 11%	2/6 events sig increase up to 32%	NUE
Sb03g029150	3680	1/6 events sig increase up to 16%	4/6 events; NS	1/6 events sig increase up to 5%	3/6 events; NS	NUE
Sb02g000230	4042	4/5 events; NS	4/5 events; NS	4/5 events; NS	4/5 events; NS	NUE
Sb04g034130	4548	1/6 events sig increase up to 18%	5/6 events; NS	1/6 events sig increase up to 6%	1/6 events sig increase up to 18%	NUE
Sb02g041830	4202	2/3 events; NS	2/3 events; NS	2/3 events; NS	2/3 events sig increase up to 26%	NUE
Sb03g027470	4306	6/6 events; NS	1/6 events sig increase up to 14%	1/6 events sig increase up to 6%	1/6 events sig increase up to 16%	NUE
Sb03g034500	4345	2/3 events; NS	2/3 events; NS	2/3 events; NS	3/3 events; NS	NUE
Sb04g030895	4530	1/6 events sig increase up to 21%	1/6 events sig increase up to 16%	1/6 events sig increase up to 6%	3/6 events; NS	NUE
Sb06g029070	4724	1/6 events sig increase up to 31%	2/6 events sig increase up to 26%	1/6 events sig increase up to 10%	1/6 events; NS	NUE
Sb08g021630	4887	4/6 events; NS	3/6 events; NS	3/6 events; NS	3/6 events; NS	NUE
Sb09g004150	4910	4/6 events; NS	4/6 events; NS	2/6 events; NS	1/6 events sig increase up to 25%	Drought

NS - increase not significant

P<0.10

5

Example 6: Soybean Embryo Transformation

Soybean embryos are bombarded with a plasmid containing nutrient uptake/stress tolerance sequence operably linked to an ubiquitin promoter as follows. To induce somatic

embryos, cotyledons, 3-5 mm in length dissected from surface-sterilized, immature seeds of the soybean cultivar A2872, are cultured in the light or dark at 26°C on an appropriate agar medium for six to ten weeks. Somatic embryos producing secondary embryos are then excised and placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos that multiplied as early, globular-staged embryos, the suspensions are maintained as described below.

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

Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Klein, *et al.*, (1987) *Nature* (London) 327:70-73, US Patent Number 4,945,050). A Du Pont Biolistic PDS1000/HE instrument (helium retrofit) can be used for these transformations.

A selectable marker gene that can be used to facilitate soybean transformation is a transgene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell, *et al.*, (1985) *Nature* 313:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from *E. coli*; Gritz, *et al.*, (1983) *Gene* 25:179-188) and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The expression cassette comprising nutrient uptake/stress tolerance sense sequence operably linked to the ubiquitin promoter can be isolated as a restriction fragment. This fragment can then be inserted into a unique restriction site of the vector carrying the marker gene.

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

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

Five to seven days post bombardment, the liquid media may be exchanged with fresh media, and eleven to twelve days post-bombardment with fresh media containing 50 mg/ml hygromycin. This selective media can be refreshed weekly. Seven to eight weeks post-bombardment, green, transformed tissue may be observed growing from untransformed, necrotic embryogenic clusters. Isolated green tissue is removed and inoculated into individual flasks to generate new, clonally propagated, transformed embryogenic suspension cultures. Each new line may be treated as an independent transformation event. These suspensions can then be subcultured and maintained as clusters of immature embryos or regenerated into whole plants by maturation and germination of individual somatic embryos.

Example 7: Sunflower Meristem Tissue Transformation

Sunflower meristem tissues are transformed with an expression cassette containing the nutrient uptake/stress tolerance sequence operably linked to a ubiquitin promoter as follows (see also, EP Patent Number 0 486233, herein incorporated by reference and Malone-Schoneberg, *et al.*, (1994) *Plant Science* 103:199-207). Mature sunflower seed (*Helianthus annuus* L.) are dehulled using a single wheat-head thresher. Seeds are surface sterilized for 30 minutes in a 20% Clorox® bleach solution with the addition of two drops of Tween® 20 per 50 ml of solution. The seeds are rinsed twice with sterile distilled water.

Split embryonic axis explants are prepared by a modification of procedures described by Schrammeijer, *et al.*, (Schrammeijer, *et al.*, (1990) *Plant Cell Rep.* 9:55-60). Seeds are imbibed in distilled water for 60 minutes following the surface sterilization procedure. The cotyledons of each seed are then broken off, producing a clean fracture at the plane of the embryonic axis. Following excision of the root tip, the explants are bisected longitudinally between the primordial leaves. The two halves are placed, cut surface up, on GBA medium consisting of Murashige and Skoog mineral elements (Murashige, *et al.*, (1962) *Physiol. Plant.*, 15:473-497), Shepard's vitamin additions (Shepard, (1980) in *Emergent Techniques for the Genetic Improvement of Crops* (University of Minnesota Press, St. Paul, Minnesota), 40 mg/l adenine sulfate, 30 g/l sucrose, 0.5 mg/l 6-benzyl-aminopurine (BAP), 0.25 mg/l indole-3-acetic acid (IAA), 0.1 mg/l gibberellic acid (GA₃), pH 5.6 and 8 g/l Phytagar.

The explants are subjected to microprojectile bombardment prior to *Agrobacterium* treatment (Bidney, *et al.*, (1992) *Plant Mol. Biol.* 18:301-313). Thirty to forty explants are placed in a circle at the center of a 60 X 20 mm plate for this treatment. Approximately 4.7 mg of 1.8 mm tungsten microprojectiles are resuspended in 25 ml of sterile TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0) and 1.5 ml aliquots are used per bombardment. Each plate is bombarded twice through a 150 mm nytex screen placed 2 cm above the samples in a PDS 1000® particle acceleration device.

Disarmed *Agrobacterium tumefaciens* strain EHA105 is used in all transformation experiments. A binary plasmid vector comprising the expression cassette that contains the nutrient uptake/stress tolerance gene operably linked to the ubiquitin promoter is introduced into *Agrobacterium* strain EHA105 via freeze-thawing as described by Holsters, *et al.*, (1978) *Mol. Gen. Genet.* 163:181-187. This plasmid further comprises a kanamycin selectable marker gene (i.e., *nptII*). Bacteria for plant transformation experiments are grown overnight (28°C and 100 RPM continuous agitation) in liquid YEP medium (10 gm/l yeast extract, 10 gm/l Bacto®peptone and 5 gm/l NaCl, pH 7.0) with the appropriate antibiotics required for bacterial strain and binary plasmid maintenance. The suspension is used when it reaches an OD₆₀₀ of about 0.4 to 0.8. The *Agrobacterium* cells are pelleted and resuspended at a final OD₆₀₀ of 0.5 in an inoculation medium comprised of 12.5 mM MES pH 5.7, 1 gm/l NH₄Cl and 0.3 gm/l MgSO₄.

Freshly bombarded explants are placed in an *Agrobacterium* suspension, mixed, and left undisturbed for 30 minutes. The explants are then transferred to GBA medium and co-cultivated, cut surface down, at 26°C and 18-hour days. After three days of co-cultivation, the explants are transferred to 374B (GBA medium lacking growth regulators and a reduced sucrose level of 1%) supplemented with 250 mg/l cefotaxime and 50 mg/l kanamycin sulfate. The explants are cultured for two to five weeks on selection and then transferred to fresh 374B medium lacking kanamycin for one to two weeks of continued development. Explants with differentiating, antibiotic-resistant areas of growth that have not produced shoots suitable for excision are transferred to GBA medium containing 250 mg/l cefotaxime for a second 3-day phytohormone treatment. Leaf samples from green, kanamycin-resistant shoots are assayed for the presence of NPTII by ELISA and for the presence of transgene expression by assaying for a modulation in meristem development (i.e., an alteration of size and appearance of shoot and floral meristems).

NPTII-positive shoots are grafted to Pioneer® hybrid 6440 *in vitro*-grown sunflower seedling rootstock. Surface sterilized seeds are germinated in 48-0 medium (half-strength Murashige and Skoog salts, 0.5% sucrose, 0.3% gelrite®, pH 5.6) and grown under conditions described for explant culture. The upper portion of the seedling is removed, a 1 cm vertical slice is made in the hypocotyl, and the transformed shoot inserted into the cut. The entire area is wrapped with parafilm® to secure the shoot. Grafted plants can be transferred to soil following one week of *in vitro* culture. Grafts in soil are maintained under high humidity conditions followed by a slow acclimatization to the greenhouse environment. Transformed sectors of T₀ plants (parental generation) maturing in the greenhouse are identified by NPTII ELISA and/or by nutrient uptake/stress tolerance activity analysis of leaf extracts while transgenic seeds harvested from NPTII-positive T₀ plants are identified by nutrient uptake/stress tolerance activity analysis of small portions of dry seed cotyledon.

An alternative sunflower transformation protocol allows the recovery of transgenic progeny without the use of chemical selection pressure. Seeds are dehulled and surface-sterilized for 20 minutes in a 20% Clorox® bleach solution with the addition of two to three drops of Tween® 20 per 100 ml of solution, then rinsed three times with distilled water. Sterilized seeds are imbibed in the dark at 26°C for 20 hours on filter paper moistened with water. The cotyledons and root radical are removed, and the meristem explants are cultured on 374E (GBA medium consisting of MS salts, Shepard vitamins, 40 mg/l adenine sulfate, 3% sucrose, 0.5 mg/l 6-BAP, 0.25 mg/l IAA, 0.1 mg/l GA, and 0.8% Phytagar at pH 5.6) for 24 hours under the dark. The primary leaves are removed to expose the apical meristem, around 40 explants are placed with the apical dome facing upward in a 2 cm circle in the center of 374M (GBA medium with 1.2% Phytagar) and then cultured on the medium for 24 hours in the dark.

Approximately 18.8 mg of 1.8 µm tungsten particles are resuspended in 150 µl absolute ethanol. After sonication, 8 µl of it is dropped on the center of the surface of macrocarrier. Each plate is bombarded twice with 650 psi rupture discs in the first shelf at 26 mm of Hg helium gun vacuum.

The plasmid of interest is introduced into *Agrobacterium tumefaciens* strain EHA105 via freeze thawing as described previously. The pellet of overnight-grown bacteria at 28°C in a liquid YEP medium (10 g/l yeast extract, 10 g/l Bacto®peptone and 5 g/l NaCl, pH 7.0) in the presence of 50 µg/l kanamycin is resuspended in an inoculation medium (12.5 mM 2-mM 2-(N-morpholino) ethanesulfonic acid, MES, 1 g/l NH₄Cl and 0.3 g/l MgSO₄ at pH 5.7) to reach a final concentration of 4.0 at OD 600. Particle-bombarded explants are transferred to GBA medium (374E) and a droplet of bacteria suspension is placed directly onto the top of the meristem. The explants are co-cultivated on the medium for 4 days, after which the explants are transferred to 374C medium (GBA with 1% sucrose and no BAP, IAA, GA3 and supplemented with 250 µg/ml cefotaxime). The plantlets are cultured on the medium for about two weeks under 16-hour day and 26°C incubation conditions.

Explants (around 2 cm long) from two weeks of culture in 374C medium are screened for a modulation in meristem development (i.e., an alteration of size and appearance of shoot and floral meristems). After positive (i.e., a change in nutrient uptake/stress tolerance expression) explants are identified, those shoots that fail to exhibit an alteration in nutrient uptake/stress tolerance activity are discarded and every positive explant is subdivided into nodal explants. One nodal explant contains at least one potential node. The nodal segments are cultured on GBA medium for three to four days to promote the formation of auxiliary buds from each node. Then they are transferred to 374C medium and allowed to develop for an additional four weeks. Developing buds are separated and cultured for an additional four weeks on 374C medium. Pooled leaf samples from each

newly recovered shoot are screened again by the appropriate protein activity assay. At this time, the positive shoots recovered from a single node will generally have been enriched in the transgenic sector detected in the initial assay prior to nodal culture.

Recovered shoots positive for altered nutrient uptake/stress tolerance expression are grafted to Pioneer hybrid 6440 *in vitro*-grown sunflower seedling rootstock. The rootstocks are prepared in the following manner. Seeds are dehulled and surface-sterilized for 20 minutes in a 20% Clorox® bleach solution with the addition of two to three drops of Tween® 20 per 100 ml of solution, and are rinsed three times with distilled water. The sterilized seeds are germinated on the filter moistened with water for three days, then they are transferred into 48 medium (half-strength MS salt, 0.5% sucrose, 0.3% gelrite® pH 5.0) and grown at 26°C under the dark for three days, then incubated at 16-hour-day culture conditions. The upper portion of selected seedling is removed, a vertical slice is made in each hypocotyl, and a transformed shoot is inserted into a V-cut. The cut area is wrapped with parafilm®. After one week of culture on the medium, grafted plants are transferred to soil. In the first two weeks, they are maintained under high humidity conditions to acclimatize to a greenhouse environment.

Example 8. Abiotic stress screening of transgenic plants expressing sorghum stress tolerance proteins

A qualitative drought screen was performed with plants over-expressing different sorghum stress tolerance genes under the control of different promoters. The soil is watered to saturation and then plants are grown under standard conditions (i.e., 16 hour light, 8 hour dark cycle; 22°C; ~60% relative humidity). No additional water is given.

Digital images of the plants are taken at the onset of visible drought stress symptoms. Images are taken once a day (at the same time of day), until the plants appear dessicated. Typically, four consecutive days of data is captured.

Color analysis is employed for identifying potential drought tolerant lines. Color analysis can be used to measure the increase in the percentage of leaf area that falls into a yellow color bin. Using hue, saturation and intensity data ("HSI"), the yellow color bin consists of hues 35 to 45.

Maintenance of leaf area is also used as another criterion for identifying potential drought tolerant lines, since *Arabidopsis* leaves wilt during drought stress. Maintenance of leaf area can be measured as reduction of rosette leaf area over time. Leaf area is measured in terms of the number of green pixels obtained using the LemnaTec imaging system. Transgenic and non-transgenic control plants are grown side by side in flats.

When wilting begins, images are taken for a number of days to monitor the wilting process. From these data wilting profiles are determined based on the green pixel counts

obtained over four consecutive days for transgenic and accompanying control plants. The profile is selected from a series of measurements over the four day period that gives the largest degree of wilting.

The ability to withstand drought is measured by the tendency of transgenic plants to resist wilting compared to control plants.

Estimates of the leaf area of the *Arabidopsis* plants are obtained in terms of the number of green pixels. The data for each image is averaged to obtain estimates of mean and standard deviation for the green pixel counts for transgenic and non-transgenic control plants. Parameters for a noise function are obtained by straight line regression of the squared deviation versus the mean pixel count using data for all images in a batch. Error estimates for the mean pixel count data are calculated using the fit parameters for the noise function. The mean pixel counts for transgenic and control plants are summed to obtain an assessment of the overall leaf area for each image. The four-day interval with maximal wilting is obtained by selecting the interval that corresponds to the maximum difference in plant growth. The individual wilting responses of the transgenic and control plants are obtained by normalization of the data using the value of the green pixel count of the first day in the interval. The drought tolerance of the transgenic plant compared to the control plant is scored by summing the weighted difference between the wilting response of transgenic plants and control plants over day two to day four; the weights are estimated by propagating the error in the data. A positive drought tolerance score corresponds to a transgenic plant with slower wilting compared to the control plant. Significance of the difference in wilting response between transgenic and control plants is obtained from the weighted sum of the squared deviations.

Transgenic events with a significant delay in yellow color accumulation and/or with significant maintenance of rosette leaf area, when compared to the control are considered drought tolerant

Example 9: Variants of Sequences

A. *Variant Nucleotide Sequences That Do Not Alter the Encoded Amino Acid Sequence*

The nucleotide sequences are used to generate variant nucleotide sequences having the nucleotide sequence of the open reading frame with about 70%, 75%, 80%, 85%, 90% and 95% nucleotide sequence identity when compared to the starting unaltered ORF nucleotide sequence of the corresponding SEQ ID NO. These functional variants are generated using a standard codon table. While the nucleotide sequences of the variants are altered, the amino acid sequence encoded by the open reading frames does not change.

B. Variant Amino Acid Sequences of Polypeptides

Variant amino acid sequences of the polypeptides are generated. In this example, one amino acid is altered. Specifically, the open reading frames are reviewed to determine the appropriate amino acid alteration. The selection of the amino acid to change is made by consulting the protein alignment (with the other orthologs and other gene family members from various species). An amino acid is selected that is deemed not to be under high selection pressure (not highly conserved) and which is rather easily substituted by an amino acid with similar chemical characteristics (i.e., similar functional side-chain). Using a protein alignment, an appropriate amino acid can be changed. Once the targeted amino acid is identified, the procedure outlined in the following section C is followed. Variants having about 70%, 75%, 80%, 85%, 90% and 95% nucleic acid sequence identity are generated using this method.

C. Additional Variant Amino Acid Sequences of Polypeptides

In this example, artificial protein sequences are created having 80%, 85%, 90% and 95% identity relative to the reference protein sequence. This latter effort requires identifying conserved and variable regions and then the judicious application of an amino acid substitutions table. These parts will be discussed in more detail below.

Largely, the determination of which amino acid sequences are altered is made based on the conserved regions among protein or among the other polypeptides. Based on the sequence alignment, the various regions of the polypeptide that can likely be altered are represented in lower case letters, while the conserved regions are represented by capital letters. It is recognized that conservative substitutions can be made in the conserved regions below without altering function. In addition, one of skill will understand that functional variants of the sequence of the disclosure can have minor non-conserved amino acid alterations in the conserved domain.

Artificial protein sequences are then created that are different from the original in the intervals of 80-85%, 85-90%, 90-95% and 95-100% identity. Midpoints of these intervals are targeted, with liberal latitude of plus or minus 1%, for example. The amino acids substitutions will be effected by a custom Perl script. The substitution table is provided below in Table 3.

Table 3. Substitution Table

Amino Acid	Strongly Similar and Optimal Substitution	Rank of Order to Change	Comment
I	L,V	1	50:50 substitution
L	I,V	2	50:50 substitution
V	I,L	3	50:50 substitution
A	G	4	
G	A	5	
D	E	6	
E	D	7	
W	Y	8	
Y	W	9	
S	T	10	
T	S	11	
K	R	12	
R	K	13	
N	Q	14	
Q	N	15	
F	Y	16	
M	L	17	First methionine cannot change
H		Na	No good substitutes
C		Na	No good substitutes
P		Na	No good substitutes

First, any conserved amino acids in the protein that should not be changed is identified and "marked off" for insulation from the substitution. The start methionine will of course be added to this list automatically. Next, the changes are made.

H, C and P are not changed in any circumstance. The changes will occur with isoleucine first, sweeping N-terminal to C-terminal. Then leucine and so on down the list until the desired target is reached. Interim number substitutions can be made so as not to cause reversal of changes. The list is ordered 1-17, so start with as many isoleucine changes as needed before leucine and so on down to methionine. Clearly many amino acids will in this manner not need to be changed. L, I and V will involve a 50:50 substitution of the two alternate optimal substitutions.

The variant amino acid sequences are written as output. Perl script is used to calculate the percent identities. Using this procedure, variants of the polypeptides are generating having about 80%, 85%, 90% and 95% amino acid identity to the disclosed sequences.

All publications and patent applications in this specification are indicative of the level of ordinary skill in the art to which this disclosure pertains. All publications and patent

applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated by reference.

The disclosure has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and
5 modifications may be made while remaining within the spirit and scope of the disclosure.

WHAT IS CLAIMED IS:

1. An isolated polynucleotide selected from the group consisting of:
 - a. a polynucleotide having at least 70% sequence identity, as determined by the
5 GAP algorithm under default parameters, to the full length sequence of a
polynucleotide selected from the group consisting of SEQ ID NOS: 1, 3, 5, 7,
9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49,
51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89,
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 a polypeptide that functions as a modifier of nitrogen utilization efficiency;
 30 b. a polynucleotide encoding a polypeptide that is at least 90% identical to the
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d. A polynucleotide which is complementary to the polynucleotide of (a), (b) or (c).

- 5 2. A recombinant expression cassette, comprising the polynucleotide of claim 1, wherein the polynucleotide is operably linked, in sense or anti-sense orientation, to a promoter.
3. A host cell comprising the expression cassette of claim 2.
4. A transgenic plant comprising the recombinant expression cassette of claim 2.
- 10 5. The transgenic plant of claim 4, wherein said plant is a monocot.
6. The transgenic plant of claim 4, wherein said plant is a dicot.
7. The transgenic plant of claim 4, wherein said plant is selected from the group consisting of: maize, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, millet, peanut and cocoa.
- 15 8. A transgenic seed from the transgenic plant of claim 4.
9. A method of modulating nitrogen utilization efficiency in plants, comprising:
 - a. introducing into a plant cell a recombinant expression cassette comprising the polynucleotide of claim 1 operably linked to a promoter; and
 - b. culturing the plant under plant cell growing conditions; wherein the nitrogen
 - 20 utilization in said plant cell is modulated.
10. The method of claim 9, wherein the plant cell is from a plant selected from the group consisting of: maize, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, millet, peanut and cocoa.
11. A method of modulating the nitrogen utilization efficiency in a plant, comprising:
 - 25 a. introducing into a plant cell a recombinant expression cassette comprising the polynucleotide of claim 1 operably linked to a promoter;
 - b. culturing the plant cell under plant cell growing conditions; and
 - c. regenerating a plant from said plant cell; wherein the nitrogen utilization efficiency in said plant is modulated.
- 30 12. The method of claim 11, wherein the plant is selected from the group consisting of: maize, soybean, sorghum, canola, wheat, alfalfa, cotton, rice, barley, millet, peanut and cocoa.
13. A method of decreasing the NUE polypeptide activity in a plant cell, comprising:
 - 35 a. providing a nucleotide sequence comprising at least 18 consecutive nucleotides of the complement of SEQ ID NOS: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97,

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- 5 c. introducing the nucleotide sequence of step (a) into the plant cell of step (b), wherein the nucleotide sequence inhibits expression of the mRNA in the plant cell.
- 10 14. The method of claim 13, wherein said plant cell is from a monocot.
15. The method of claim 14, wherein said monocot is maize, wheat, rice, barley, sorghum or rye.
16. The method of claim 13, wherein said plant cell is from a dicot.
17. The transgenic plant of claim 4, wherein the nitrogen utilization efficiency activity in
- 15 said plant is increased.
18. The transgenic plant of claim 17, wherein the plant has enhanced root growth.
19. The transgenic plant of claim 17, wherein the plant has increased seed size.
20. The transgenic plant of claim 17, wherein the plant has increased seed weight.
21. The transgenic plant of claim 17, wherein the plant has seed with increased embryo
- 20 size.
22. The transgenic plant of claim 17, wherein the plant has increased leaf size.
23. The transgenic plant of claim 17, wherein the plant has increased seedling vigor.
24. The transgenic plant of claim 17, wherein the plant has enhanced silk emergence.
25. The transgenic plant of claim 17, wherein the plant has increased ear size.
- 25 26. A method of improving an agronomic parameter of a maize plant, the method comprising expressing a polynucleotide that encodes a polypeptide of at least 90% identical to the amino acid sequence selected from the group consisting of SEQ ID NOS: 298, 318, 320, 370, 552, 1276, 2288, 1596, 1804, 1882, 2252, 2640 and 2966.
27. The method of claim 26, wherein the agronomic parameter is selected from the group
- 30 consisting of increased grain filling, increased silking, increased ear area, increased ear length, increased ear width and increased silk count.
28. A method for increasing abiotic stress tolerance in a plant, said method comprising:
- a. expressing a recombinant nucleotide sequence encoding a polypeptide
- 35 having at least 90% sequence identity to the amino acid sequence selected from the group consisting of SEQ ID NOS: 298, 318, 320, 370, 552, 1276, 2288, 1596, 1804, 1882, 2252, 2640 and 2966, wherein said nucleotide sequence is operably linked to a heterologous promoter selected from the

group consisting of a weak constitutive promoter, an organ- or tissue-preferred promoter a stress-inducible promoter, a chemical-induced promoter, a light-responsive promoter, and a diurnally-regulated promoter; and

- b. expressing said nucleotide sequence in said plant; whereby abiotic stress tolerance of said plant is increased relative to a control plant.

29. The method of claim 28, wherein said organ- or tissue-preferred promoter is a leaf-preferred promoter, a root-preferred promoter, a vasculature-specific promoter or a promoter without expression in developing or mature ears.

30. The method of claim 28, wherein said stress-inducible promoter is a *Rab17* promoter or an *Rd29a* promoter.

31. A method for increasing yield of a seed crop plant exposed to drought stress, said method comprising increasing expression of a polypeptide having at least 90% sequence identity to one of SEQ ID NOS: 298, 318, 320, 370, 552, 1276, 2288, 1596, 1804, 1882, 2252, 2640 and 2966 in said plant.

32. The method of claim 31, wherein the polynucleotide sequence is selected from the group consisting of SEQ ID NOS: 297, 317, 319, 369, 552, 1275, 2287, 1595, 1803, 1881, 2251, 2639 and 2965 or a sequence that is at least 90% identical to one of SEQ ID NOS: 297, 317, 319, 369, 552, 1275, 2287, 1595, 1803, 1881, 2251, 2639 and 2965.

33. The method of claim 28, wherein the plant further comprises a gene conferring tolerance to a herbicide or an insect.

34. The method of claim 28, wherein the plant is maize.

35. The method of claim 28, wherein the plant is wheat.

36. The method of claim 28, wherein the plant is rice.

37. The method of claim 28, wherein the plant is sorghum.

38. The method of claim 28, wherein the plant is soybean.

39. The method of claim 28, wherein the plant is a vegetable.

40. The method of claim 28, wherein the plant is brassica.

41. A method for increasing abiotic stress tolerance or yield in a plant, said method comprising:

- a. expressing a genomic nucleotide sequence encoding a polypeptide having at least 90% sequence identity to the amino acid sequence selected from the group consisting of SEQ ID NOS: 298, 318, 320, 370, 552, 1276, 2288, 1596, 1804, 1882, 2252, 2640 and 2966, wherein said nucleotide sequence is operably linked to a heterologous promoter selected from the group consisting of a weak constitutive promoter, an organ- or tissue-preferred

promoter a stress-inducible promoter, a chemical-induced promoter, a light-responsive promoter and a diurnally-regulated promoter; and

- b. expressing said nucleotide sequence in said plant; whereby abiotic stress tolerance of said plant is increased relative to a control plant.

42. A method of developing a marker for marker-assisted breeding of sorghum, the method comprising identifying a marker within a polynucleotide sequence selected from the group consisting nucleotide sequences listed in Table 1 or in linkage disequilibrium with the nucleotide sequences and identifying a sorghum plant that comprises the marker.

43. A method of identifying an allelic variant of a polynucleotide in a sorghum plant that is associated with increased tolerance to an abiotic stress or increased yield, the method comprising the steps of:

- a. crossing two sorghum plants with differing levels of abiotic stress tolerance;
- b. evaluating allelic variations in the progeny plants with respect to a polynucleotide sequence encoding a protein comprising selected from the group consisting of polypeptide sequences listed in Table 1 or in the genomic region that regulates the expression of the polynucleotide encoding the protein;
- c. phenotyping the progeny plants for abiotic stress tolerance;
- d. associating allelic variations with said tolerance; and
- e. identifying the alleles that are associated with increased tolerance to said abiotic stress.

44. A method of identifying a sorghum plant that exhibits an improved agronomic parameter, the method comprising screening a population of sorghum plants for enhanced nutrient utilization efficiency or drought tolerance and analyzing the sequence of a polynucleotide encoding a protein comprising a polypeptide selected from the group listed in Table 1 or a regulatory sequence thereof and identifying the sorghum plant with enhanced nutrient utilization efficiency or drought tolerance.

45. A method of identifying alleles in sorghum plants or germplasm that are associated with tolerance to abiotic stress, the method comprising:

- a. obtaining a population of sorghum plants, wherein one or more plants exhibit differing levels of enhanced tolerance to abiotic stress;
- b. evaluating allelic variations with respect to the polynucleotide sequence encoding a protein comprising a polypeptide selected from the group listed in Table 1 or in the genomic region that regulates the expression of the polynucleotide encoding the protein;

- 5
- c. obtaining phenotypic values of abiotic stress tolerance for a plurality of maize plants in the population;
 - d. associating the allelic variations in the genomic region with a polynucleotide selected from the group listed in Table 1; and
 - e. identifying the alleles that are associated with increased tolerance to abiotic stress.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/019905

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N15/82 C07K14/415 C12Q1/68
ADD.

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

B. FIELDS SEARCHED

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

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

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/094717 A1 (TROUKHAN MAXIM [US] ET AL) 9 April 2009 (2009-04-09) claims 1-22; sequences 7275, 7276	1-9
X	-& DATABASE UniProt [Online] 1 September 2009 (2009-09-01), "SubName: Full=Putative uncharacterized protein Sb01g042400;", XP002725315, retrieved from EBI accession no. UNIPROT:C5WSM4 Database accession no. C5WSM4 sequence	1
A	----- WO 2011/025840 A1 (TARGETED GROWTH INC [US]; NGUYEN THU [US]; DEROCHER JAY [US]) 3 March 2011 (2011-03-03) paragraph [0011] ----- -/-	1-25, 42-45



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

4 June 2014

Date of mailing of the international search report

28/08/2014

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
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Authorized officer

Bilang, Jürg

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/019905

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2010/075143 A1 (MONSANTO TECHNOLOGY LLC [US]; COFFIN MARIE [US]) 1 July 2010 (2010-07-01) page 51 -----	1-25, 42-45
A	WO 2009/056566 A2 (BASF PLANT SCIENCE GMBH [DE]; REUZEAU CHRISTOPHE [FR]; SANZ MOLINERO A) 7 May 2009 (2009-05-07) abstract -----	1-25, 42-45
A	QING N HAO ET AL: "Identification of genes associated with nitrogen-use efficiency by genome-wide transcriptional analysis of two soybean genotypes", BMC GENOMICS, BIOMED CENTRAL LTD, LONDON, UK, vol. 12, no. 1, 26 October 2011 (2011-10-26), page 525, XP021112872, ISSN: 1471-2164, DOI: 10.1186/1471-2164-12-525 the whole document -----	1-25, 42-45

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/019905

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-25, 42-45(all partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-25, 42-45(all partially)

Nucleic acid sequence having at least 70% sequence identity to SEQ ID NO: 1 or encoding a protein having at least 90% sequence identity to SEQ ID NO: 2, and uses thereof

2-1689. claims: 1-25, 42-45(all partially)

As invention 1, but the reference nucleic acid sequence being one of SEQ ID NO: 3 to 3403 (odd numbers) or SEQ ID NO: 3404, with the exception of SEQ ID NO: 297, 317, 319, 369, 552, 1275, 2287, 1595, 1803, 1881, 2251, 2639, and 2965

1690-1703. claims: 1-45(partially)

As invention 1, but the reference nucleic acid sequence being one of SEQ ID NO: 297, 317, 319, 369, 552, 1275, 2287, 1595, 1803, 1881, 2251, 2639, 2965

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2014/019905

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