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(54) Title: TRANSGENIC PLANTS WITH ENHANCED AGRONOMIC TRAITS

(57) Abstract: This invention provides transgenic plant cells with recombinant DNA for expression of proteins that are useful for imparting enhanced agronomic trait(s) to transgenic crop plants. This invention also provides transgenic plants and progeny seed comprising the transgenic plant cells where the plants are selected for having an enhanced trait selected from the group of traits consisting of enhanced water use efficiency, enhanced cold tolerance, increased yield, enhanced nitrogen use efficiency, enhanced seed protein and enhanced seed oil. Also disclosed are methods for manufacturing transgenic seed and plants with enhanced trait.

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Transgenic Plants with Enhanced Agronomic Traits

Cross Reference To Related Applications

This application claims benefit under 35USC § 119(e) of United States provisional application Serial No. 60/961,192, filed 07/19/2007 herein incorporated by reference in its entirety.

Incorporation of Sequence Listing

Two copies of the sequence listing (Copy 1 and Copy 2) and a computer readable form (CRF) of the sequence listing, all on CD-Rs, each containing the text file named 38-21(54147)A_seqlisting.txt, which is 6,903,808 bytes (measured in MS-WINDOWS), were created on July 16, 2008 and are herein incorporated by reference.

Field Of The Invention

Disclosed herein are recombinant DNA useful for providing enhanced traits to transgenic plants, seeds, pollen, plant cells and plant nuclei of such transgenic plants, methods of making and using such recombinant DNA, plants, seeds, pollen, plant cells and plant nuclei. Also disclosed are methods of producing hybrid seed comprising such recombinant DNA.

Background Of The Invention

This invention employs recombinant DNA for expression of proteins that are useful for imparting enhanced agronomic traits to transgenic plants. Recombinant DNA in this invention is provided in a construct comprising a promoter that is functional in plant cells and that is operably linked to a DNA segment that encodes a protein. In some embodiments of the invention, such protein defined by protein domains e.g. a "Pfam domain module" (as defined herein below) from the group of Pfam domain modules identified in Table 10. In other embodiments of the invention, e.g. where a Pfam domain module is not available, such protein is defined a consensus amino acid sequence of an encoded protein that is targeted for production e.g. a protein having amino acid sequence with at least 90% identity to a consensus amino acid sequence as set forth in SEQ ID NO: 2201. In more specific embodiments of the invention the protein expressed in plant cells is a protein selected from the group of proteins identified in Table 1 and their homologs identified in Table 8.

Other aspects of the invention are specifically directed to plant cell nuclei and transgenic plant cells comprising the recombinant DNA construct of the invention, transgenic plants comprising a plurality of such plant cells, progeny transgenic seed, embryo and transgenic pollen from such transgenic plants. Such transgenic plants are selected from a population of transgenic plants regenerated from plant cells transformed with the recombinant DNA construct provided by the invention and express the protein by screening transgenic plants in the population for an enhanced trait as compared to control plants that do not have the recombinant DNA construct, where the enhanced trait is selected from group of enhanced traits consisting of enhanced water use efficiency, enhanced cold tolerance, increased yield, enhanced nitrogen use efficiency, enhanced seed protein and enhanced seed oil.

In yet another aspect of the invention the plant cell nuclei, plant cells, transgenic plants, seeds, and pollen further comprise recombinant DNA expressing a protein that provides tolerance from exposure to an herbicide applied at levels that are lethal to a wild type plant cell. Such tolerance is especially useful not only as an advantageous trait in such plants but is also useful in a selection step in the methods of the invention. In aspects of the invention such herbicide is a glyphosate, dicamba, or glufosinate compound.

Yet other aspects of the invention provide transgenic plants which are homozygous for the recombinant DNA and transgenic seed of the invention from corn, soybean, cotton, canola, alfalfa, wheat or rice plants.

This invention also provides methods for manufacturing non-natural, transgenic seed that can be used to produce a crop of transgenic plants with an enhanced trait resulting from expression of stably-integrated, recombinant DNA construct provided by herein. More specifically the method comprises (a) providing a population of plants produced from a parental plant having a recombinant DNA construct of the invention; (b) screening this population of plants for at least one enhanced trait and the recombinant DNA construct, where individual plants in the population can exhibit the trait at a level less than, essentially the same as or greater than the level that the trait is exhibited in control plants which do not contain the recombinant DNA construct, where the enhanced trait is selected from the group of enhanced traits consisting of enhanced water use efficiency, enhanced cold tolerance, enhanced heat tolerance, enhanced high salinity tolerance, enhanced shade tolerance, increased yield, enhanced nitrogen use efficiency, enhanced seed protein and enhanced seed oil; (c) selecting from the population one or more plants that exhibit the trait at a level greater than the level that the trait is exhibited in control plants; and (d) collecting seeds from

selected plant selected from step c. The method further comprises (e) verifying that the recombinant DNA construct is stably integrated in said selected plants, and (f) analyzing tissue of a selected plant to determine the production of a protein having the function of a protein selected from SEQ ID NO: 96 through SEQ ID NO: 2166. In one aspect of the invention the plants in the population further comprise DNA expressing a protein that provides tolerance to exposure to a herbicide applied at levels that are lethal to wild type plant cells and the selecting is affected by treating the population with the herbicide, e.g. a glyphosate, dicamba, or glufosinate compound. In another aspect of the invention the plants are selected by identifying plants with the enhanced trait. The methods are especially useful for manufacturing corn, soybean, cotton, canola, alfalfa, wheat or rice seed.

Another aspect of the invention provides a method of producing hybrid corn seed comprising acquiring hybrid corn seed from a herbicide tolerant corn plant which also has stably-integrated, recombinant DNA construct comprising a promoter that is (a) functional in plant cells and (b) is operably linked to DNA that encodes a protein provided by the invention. The methods further comprise producing corn plants from the hybrid corn seed, wherein a fraction of the plants produced from the hybrid corn seed is homozygous for the recombinant DNA, a fraction of the plants produced from the hybrid corn seed is hemizygous for the recombinant DNA construct, and a fraction of the plants produced from the hybrid corn seed has none of the recombinant DNA construct; selecting corn plants which are homozygous and hemizygous for the recombinant DNA construct by treating with an herbicide; collecting seed from herbicide-treated-surviving corn plants and planting the seed to produce further progeny corn plants; repeating the selecting and collecting steps at least once to produce an inbred corn line; and crossing the inbred corn line with a second corn line to produce hybrid seed.

Brief Description Of The Drawings

Figure 1 is a consensus amino acid sequence of SEQ ID NO: 127 and its homologs.

Figures 2-5 are plasmid maps.

Detailed Description Of The Invention

In the attached sequence listing:

SEQ ID NO:1-95 are nucleotide sequences of the coding strand of DNA for “genes” used in the recombinant DNA imparting an enhanced trait in plant cells, i.e. each represents a coding sequence for a protein;

SEQ ID NO: 96-193 are amino acid sequences of the cognate protein of the “genes” with nucleotide coding sequences 1-95;

SEQ ID NO: 194-2166 are amino acid sequences of homologous proteins;

SEQ ID NO: 2167-2200 are nucleotide sequences of the elements in base plasmid

5 vectors

SEQ ID NO: 2201 is a consensus amino acid sequence.

SEQ ID NO: 2202-2203 are nucleotide sequences of two base plasmid vectors useful for corn transformation;

10 SEQ ID NO: 2204 is a nucleotide sequence of a base plasmid vector useful for soybean and canola transformation; and

SEQ ID NO: 2205 is a nucleotide sequence of a base plasmid vector useful for cotton transformation.

15 As used herein a “plant cell” means a plant cell that is transformed with stably-integrated, non-natural, recombinant DNA, e.g. by *Agrobacterium*-mediated transformation or by bombardment using microparticles coated with recombinant DNA or other means. A plant cell of this invention can be an originally-transformed plant cell that exists as a microorganism or as a progeny plant cell that is regenerated into differentiated tissue, e.g. into a transgenic plant with stably-integrated, non-natural recombinant DNA, or seed or
20 pollen derived from a progeny transgenic plant.

As used herein a “transgenic plant” means a plant whose genome has been altered by the stable integration of recombinant DNA. A transgenic plant includes a plant regenerated from an originally-transformed plant cell and progeny transgenic plants from later generations or crosses of a transformed plant.

25 As used herein “recombinant DNA” means DNA which has been genetically engineered and constructed outside of a cell including DNA containing naturally occurring DNA or cDNA or synthetic DNA.

30 As used herein “consensus sequence” means an artificial sequence of amino acids in a conserved region of an alignment of amino acid sequences of homologous proteins, e.g. as determined by a CLUSTALW alignment of amino acid sequence of homolog proteins.

As used herein “homolog” means a protein in a group of proteins that perform the same biological function, e.g. proteins that belong to the same Pfam protein family and that provide a common enhanced trait in transgenic plants of this invention. Homologs are expressed by homologous genes. Homologous genes include naturally occurring alleles and

artificially-created variants. Degeneracy of the genetic code provides the possibility to substitute at least one base of the protein encoding sequence of a gene with a different base without causing the amino acid sequence of the polypeptide produced from the gene to be changed. Hence, a polynucleotide useful in the present invention may have any base
5 sequence that has been changed from SEQ ID NO: 1 through SEQ ID NO: 95 in accordance with degeneracy of the genetic code. Homologs are proteins that, when optimally aligned, have at least 60% identity, more preferably about 70% or higher, more preferably at least 80% and even more preferably at least 90% identity over the full length of a protein identified as being associated with imparting an enhanced trait when expressed in plant cells. Homologs
10 include proteins with an amino acid sequence that has at least 90% identity to a consensus amino acid sequence of proteins and homologs disclosed herein.

Homologs are identified by comparison of amino acid sequence, e.g. manually or by use of a computer-based tool using known homology-based search algorithms such as those commonly known and referred to as BLAST, FASTA, and Smith-Waterman. A local
15 sequence alignment program, e.g. BLAST, can be used to search a database of sequences to find similar sequences, and the summary Expectation value (E-value) used to measure the sequence base similarity. As a protein hit with the best E-value for a particular organism may not necessarily be an ortholog or the only ortholog, a reciprocal query is used in the present invention to filter hit sequences with significant E-values for ortholog identification. The
20 reciprocal query entails search of the significant hits against a database of amino acid sequences from the base organism that are similar to the sequence of the query protein. A hit is a likely ortholog, when the reciprocal query's best hit is the query protein itself or a protein encoded by a duplicated gene after speciation. A further aspect of the invention comprises functional homolog proteins that differ in one or more amino acids from those of disclosed
25 protein as the result of conservative amino acid substitutions, for example substitutions are among: acidic (negatively charged) amino acids such as aspartic acid and glutamic acid; basic (positively charged) amino acids such as arginine, histidine, and lysine; neutral polar amino acids such as glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; neutral nonpolar (hydrophobic) amino acids such as alanine, leucine, isoleucine, valine, proline,
30 phenylalanine, tryptophan, and methionine; amino acids having aliphatic side chains such as glycine, alanine, valine, leucine, and isoleucine; amino acids having aliphatic-hydroxyl side chains such as serine and threonine; amino acids having amide-containing side chains such as asparagine and glutamine; amino acids having aromatic side chains such as phenylalanine, tyrosine, and tryptophan; amino acids having basic side chains such as lysine, arginine, and

histidine; amino acids having sulfur-containing side chains such as cysteine and methionine; naturally conservative amino acids such as valine-leucine, valine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, aspartic acid-glutamic acid, and asparagine-glutamine. A further aspect of the homologs encoded by DNA useful in the transgenic plants of the invention are those proteins that differ from a disclosed protein as the result of deletion or insertion of one or more amino acids in a native sequence.

“Percent identity” describes the extent to which the sequences of DNA or protein segments are invariant throughout a window of alignment of nucleotide or amino acid sequences. An “identity fraction” for a sequence aligned with a reference sequence is the number of identical components which are shared by the sequences, divided by a length of the window of alignment, wherein the length does not include gaps introduced by an alignment algorithm. “Percent identity” (“% identity”) is the identity fraction times 100. The alignment algorithm is preferably a local alignment algorithm, such as BLASTp. As used herein, sequences are “aligned” when the alignment produced by BLASTp has a minimal e-value.

“Pfam” database is a large collection of multiple sequence alignments and hidden Markov models covering many common protein families, e.g. Pfam version 19.0 (December 2005) contains alignments and models for 8183 protein families and is based on the Swissprot 47.0 and SP-TrEMBL 30.0 protein sequence databases. See S.R. Eddy, “Profile Hidden Markov Models”, *Bioinformatics* 14:755-763, 1998. The Pfam database is currently maintained and updated by the Pfam Consortium. The alignments represent some evolutionary conserved structure that has implications for the protein's function. Profile hidden Markov models (profile HMMs) built from the protein family alignments are useful for automatically recognizing that a new protein belongs to an existing protein family even if the homology by alignment appears to be low.

Protein domains are identified by querying the amino acid sequence of a protein against Hidden Markov Models which characterize protein family domains (“Pfam domains”) using HMMER software, a current version of which is publicly available from the Pfam Consortium. A protein domain meeting the gathering cutoff for the alignment of a particular Pfam domain is considered to contain the Pfam domain.

A “Pfam domain module” is a representation of Pfam domains in a protein, in order from N terminus to C terminus. In a Pfam domain module individual Pfam domains are separated by double colons “::”. The order and copy number of the Pfam domains from N to C terminus are attributes of a Pfam domain module. Although the copy number of repetitive

domains is important, varying copy number often enables a similar function. Thus, a Pfam domain module with multiple copies of a domain should define an equivalent Pfam domain module with variance in the number of multiple copies. A Pfam domain module is not specific for distance between adjacent domains, but contemplates natural distances and variations in distance that provide equivalent function. The Pfam database contains both narrowly- and broadly-defined domains, leading to identification of overlapping domains on some proteins. A Pfam domain module is characterized by non-overlapping domains. Where there is overlap, the domain having a function that is more closely associated with the function of the protein (based on the E value of the Pfam match) is selected. One protein is identified as containing a pfam domain when its scores is higher than the gathering cutoff disclosed in Table 12 by Pfam analysis disclosed herein

Once one DNA is identified as encoding a protein which imparts an enhanced trait when expressed in transgenic plants, other DNA encoding proteins with the same Pfam domain module are identified by querying the amino acid sequence of protein encoded by candidate DNA against the Hidden Markov Models which characterizes the Pfam domains using HMMER software. Candidate proteins meeting the same Pfam domain module are in the protein family and have cognate DNA that is useful in constructing recombinant DNA for the use in the plant cells of this invention. Hidden Markov Model databases for use with HMMER software in identifying DNA expressing protein with a common Pfam domain module for recombinant DNA in the plant cells of this invention are also publicly available from the Pfam Consortium.

The HMMER software and Pfam databases were used to identify known domains in the proteins corresponding to amino acid sequence of SEQ ID NO: 96 through SEQ ID NO: 193. All DNA encoding proteins that have at least one of pfam domain modules of this invention can be used in recombinant DNA construct of this invention, e.g. for selecting transgenic plants having enhanced agronomic traits. The relevant Pfams modules for use in this invention, as more specifically disclosed below, are Homeobox, Myb_DNA-binding::Myb_DNA-binding, Myb_DNA-binding, zf-Dof, zf-C2H2::zf-C2H2, AP2, Response_reg::Myb_DNA-binding, B3, B3::Auxin_resp::AUX_IAA, HLH, NAM, B3::B3, AUX_IAA, KNOX1::KNOX2::ELK, GRAS, AT_hook::AT_HOOK::DUF296, TCP, SBP, zf-C2H2, B3::Auxin_resp, EIN3, bZIP_2, zf-B_box::zf-B_box, zf-B_box::CCT, RWP-RK::PB1, F-box::TUB, CBFD_NFYB_HMF, GATA, SRF-TF, K-box, and SRF-TF::K-box.

As used herein "promoter" means regulatory DNA for initializing transcription. A "plant promoter" is a promoter capable of initiating transcription in plant cells whether or not

its origin is a plant cell, e.g. it is well known that *Agrobacterium* promoters are functional in plant cells. Thus, plant promoters include promoter DNA obtained from plants, plant viruses and bacteria such as *Agrobacterium* and *Bradyrhizobium* bacteria. Examples of promoters under developmental control include promoters that preferentially initiate transcription in certain tissues, such as leaves, roots, or seeds. Such promoters are referred to as "tissue preferred". Promoters that initiate transcription only in certain tissues are referred to as "tissue specific". 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 "repressible" promoter is a promoter which is under environmental control. Examples of environmental conditions that may affect transcription by inducible promoters include anaerobic conditions, or certain chemicals, or the presence of light. Tissue specific, tissue preferred, cell type specific, and inducible promoters constitute the class of "non-constitutive" promoters. A "constitutive" promoter is a promoter which is active under most conditions.

As used herein "operably linked" means the association of two or more DNA fragments in a DNA construct so that the function of one, e.g. protein-encoding DNA, is controlled by the other, e.g. a promoter.

As used herein "expressed" means produced, e.g. a protein is expressed in a plant cell when its cognate DNA is transcribed to mRNA that is translated to the protein.

As used herein a "control plant" means a plant that does not contain the recombinant DNA that expresses a protein that imparts an enhanced trait. A control plant is to identify and select a transgenic plant that has an enhance trait. A suitable control plant can be a non-transgenic plant of the parental line used to generate a transgenic plant, i.e. devoid of recombinant DNA. A suitable control plant may in some cases be a progeny of a hemizygous transgenic plant line that is does not contain the recombinant DNA, known as a negative segregant.

As used herein an "enhanced trait" means a characteristic of a transgenic plant that includes, but is not limited to, an enhance agronomic trait characterized by enhanced plant morphology, physiology, growth and development, yield, nutritional enhancement, disease or pest resistance, or environmental or chemical tolerance. In more specific aspects of this invention enhanced trait is selected from group of enhanced traits consisting of enhanced water use efficiency, enhanced cold tolerance, increased yield, enhanced nitrogen use efficiency, enhanced seed protein and enhanced seed oil. In an important aspect of the invention the enhanced trait is enhanced yield including increased yield under non-stress conditions and increased yield under environmental stress conditions. Stress conditions may

include, for example, drought, shade, fungal disease, viral disease, bacterial disease, insect infestation, nematode infestation, cold temperature exposure, heat exposure, osmotic stress, reduced nitrogen nutrient availability, reduced phosphorus nutrient availability and high plant density. "Yield" can be affected by many properties including without limitation, plant height, pod number, pod position on the plant, number of internodes, incidence of pod shatter, grain size, efficiency of nodulation and nitrogen fixation, efficiency of nutrient assimilation, resistance to biotic and abiotic stress, carbon assimilation, plant architecture, resistance to lodging, percent seed germination, seedling vigor, and juvenile traits. Yield can also be affected by efficiency of germination (including germination in stressed conditions), growth rate (including growth rate in stressed conditions), ear number, seed number per ear, seed size, composition of seed (starch, oil, protein) and characteristics of seed fill.

Increased yield of a transgenic plant of the present invention can be measured in a number of ways, including test weight, seed number per plant, seed weight, seed number per unit area (i.e. seeds, or weight of seeds, per acre), bushels per acre, tones per acre, tons per acre, kilo per hectare. For example, maize yield may be measured as production of shelled corn kernels per unit of production area, for example in bushels per acre or metric tons per hectare, often reported on a moisture adjusted basis, for example at 15.5 percent moisture. Increased yield may result from improved utilization of key biochemical compounds, such as nitrogen, phosphorous and carbohydrate, or from improved responses to environmental stresses, such as cold, heat, drought, salt, and attack by pests or pathogens. Recombinant DNA used in this invention can also be used to provide plants having improved growth and development, and ultimately increased yield, as the result of modified expression of plant growth regulators or modification of cell cycle or photosynthesis pathways. Also of interest is the generation of transgenic plants that demonstrate enhanced yield with respect to a seed component that may or may not correspond to an increase in overall plant yield. Such properties include enhancements in seed oil, seed molecules such as tocopherol, protein and starch, or oil particular oil components as may be manifest by alterations in the ratios of seed components.

A subset of the DNA molecules of this invention includes fragments of the disclosed recombinant DNA consisting of oligonucleotides of at least 15, preferably at least 16 or 17, more preferably at least 18 or 19, and even more preferably at least 20 or more, consecutive nucleotides. Such oligonucleotides are fragments of the larger molecules having a sequence selected from the group consisting of SEQ ID NO: 1 through SEQ ID NO: 95, and find use,

for example as probes and primers for detection of the polynucleotides of the present invention.

Recombinant DNA constructs are assembled using methods well known to persons of ordinary skill in the art and typically comprise a promoter operably linked to DNA, the expression of which provides the enhanced agronomic trait. Other construct components may include additional regulatory elements, such as 5' leaders and introns for enhancing transcription, 3' untranslated regions (such as polyadenylation signals and sites), DNA for transit or signal peptides.

Numerous promoters that are active in plant cells have been described in the literature. These include promoters present in plant genomes as well as promoters from other sources, including nopaline synthase (NOS) promoter and octopine synthase (OCS) promoters carried on tumor-inducing plasmids of *Agrobacterium tumefaciens* and the CaMV35S promoters from the cauliflower mosaic virus as disclosed in US Patents No. 5,164,316 and 5,322,938. Useful promoters derived from plant genes are found in U.S. Patent 5,641,876, which discloses a rice actin promoter, U.S. Patent No. 7,151,204, which discloses a maize chloroplast aldolase promoter and a maize aldolase (FDA) promoter, and U.S. Patent Application Publication 2003/0131377 A1, which discloses a maize nicotianamine synthase promoter, all of which are incorporated herein by reference. These and numerous other promoters that function in plant cells are known to those skilled in the art and available for use in recombinant polynucleotides of the present invention to provide for expression of desired genes in transgenic plant cells.

In other aspects of the invention, preferential expression in plant green tissues is desired. Promoters of interest for such uses include those from genes such as *Arabidopsis thaliana* ribulose-1,5-bisphosphate carboxylase (Rubisco) small subunit (Fischhoff *et al.* (1992) *Plant Mol Biol.* 20:81-93), aldolase and pyruvate orthophosphate dikinase (PPDK) (Taniguchi *et al.* (2000) *Plant Cell Physiol.* 41(1):42-48).

Furthermore, the promoters may be altered to contain multiple "enhancer sequences" to assist in elevating gene expression. Such enhancers are known in the art. By including an enhancer sequence with such constructs, the expression of the selected protein may be enhanced. These enhancers often are found 5' to the start of transcription in a promoter that functions in eukaryotic cells, but can often be inserted upstream (5') or downstream (3') to the coding sequence. In some instances, these 5' enhancing elements are introns. Particularly useful as enhancers are the 5' introns of the rice actin 1 (see US Patent 5,641,876) and rice

actin 2 genes, the maize alcohol dehydrogenase gene intron, the maize heat shock protein 70 gene intron (U.S. Patent 5,593,874) and the maize shrunken 1 gene.

In other aspects of the invention, sufficient expression in plant seed tissues is desired to affect improvements in seed composition. Exemplary promoters for use for seed

composition modification include promoters from seed genes such as napin (U.S. 5,420,034); maize L3 oleosin (U.S. 6,433,252), zein Z27 (Russell *et al.* (1997) *Transgenic Res.* 6(2):157-166), globulin 1 (Belanger *et al.* (1991) *Genetics* 129:863-872), glutelin 1 (Russell (1997) *supra*), and peroxiredoxin antioxidant (Per1) (Stacy *et al.* (1996) *Plant Mol Biol.* 31(6):1205-1216).

Recombinant DNA constructs prepared in accordance with the invention will also generally include a 3' element that typically contains a polyadenylation signal and site. Well-known 3' elements include those from *Agrobacterium tumefaciens* genes such as *nos* 3', *tml* 3', *tmr* 3', *tms* 3', *ocs* 3', *tr7* 3', for example disclosed in U.S. 6,090,627, incorporated herein by reference; 3' elements from plant genes such as wheat (*Triticum aestivum*) heat shock protein 17 (*Hsp17* 3'), a wheat ubiquitin gene, a wheat fructose-1,6-biphosphatase gene, a rice glutelin gene, a rice lactate dehydrogenase gene and a rice beta-tubulin gene, all of which are disclosed in U.S. published patent application 2002/0192813 A1, incorporated herein by reference; and the pea (*Pisum sativum*) ribulose biphosphate carboxylase gene (*rbs* 3'), and 3' elements from the genes within the host plant.

Constructs and vectors may also include a transit peptide for targeting of a gene to a plant organelle, particularly to a chloroplast, leucoplast or other plastid organelle. For descriptions of the use of chloroplast transit peptides see U.S. Patent 5,188,642 and U.S. Patent No. 5,728,925, incorporated herein by reference. For description of the transit peptide region of an Arabidopsis EPSPS gene useful in the present invention, see Klee, H.J. *et al* (MGG (1987) 210:437-442).

Transgenic plants comprising or derived from plant cells of this invention transformed with recombinant DNA construct can be further enhanced with stacked traits, e.g. a crop plant having an enhanced trait resulting from expression of DNA disclosed herein in combination with herbicide and/or pest resistance traits. For example, genes of the current invention can be stacked with other traits of agronomic interest, such as a trait providing herbicide resistance, or insect resistance, such as using a gene from *Bacillus thuringiensis* to provide resistance against lepidopteran, coliopteran, homopteran, hemiopteran, and other insects. Herbicides for which transgenic plant tolerance has been demonstrated and the method of the present invention can be applied include, but are not limited to, glyphosate,

dicamba, glufosinate, sulfonyleurea, bromoxynil and norflurazon herbicides. Polynucleotide molecules encoding proteins involved in herbicide tolerance are well-known in the art and include, but are not limited to, a polynucleotide molecule encoding 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) disclosed in U.S. Patent 5,094,945; 5,627,061; 5,633,435 and 5 6,040,497 for imparting glyphosate tolerance; polynucleotide molecules encoding a glyphosate oxidoreductase (GOX) disclosed in U.S. Patent 5,463,175 and a glyphosate-N-acetyl transferase (GAT) disclosed in U.S. Patent Application publication 2003/0083480 A1 also for imparting glyphosate tolerance; dicamba monooxygenase disclosed in U.S. Patent Application publication 2003/0135879 A1 for imparting dicamba tolerance; a polynucleotide molecule encoding bromoxynil nitrilase (*Bxn*) disclosed in U.S. Patent 4,810,648 for 10 imparting bromoxynil tolerance; a polynucleotide molecule encoding phytoene desaturase (*crtI*) described in Misawa et al, (1993) *Plant J.* 4:833-840 and in Misawa et al, (1994) *Plant J.* 6:481-489 for norflurazon tolerance; a polynucleotide molecule encoding acetohydroxyacid synthase (AHAS, *aka* ALS) described in Sathasiivan *et al.* (1990) *Nucl. Acids Res.* 18:2188-2193 for imparting tolerance to sulfonyleurea herbicides; polynucleotide molecules known as *bar* genes disclosed in DeBlock, *et al.* (1987) *EMBO J.* 6:2513-2519 for imparting glufosinate and bialaphos tolerance; polynucleotide molecules disclosed in U.S. Patent Application Publication 2003/010609 A1 for imparting N-amino methyl phosphonic acid tolerance; polynucleotide molecules disclosed in U.S. Patent 6,107,549 for imparting 20 pyridine herbicide resistance; molecules and methods for imparting tolerance to multiple herbicides such as glyphosate, atrazine, ALS inhibitors, isoxoflutole and glufosinate herbicides are disclosed in U.S. Patent 6,376,754 and U.S. Patent Application Publication 2002/0112260, all of said U.S. Patents and Patent Application Publications are incorporated herein by reference. Molecules and methods for imparting insect/nematode/virus resistance are disclosed in U.S. Patents 5,250,515; 5,880,275; 6,506,599; 5,986,175 and U.S. Patent 25 Application Publication 2003/0150017 A1, all of which are incorporated herein by reference.

Plant Cell Transformation Methods

Numerous methods for transforming plant cells with recombinant DNA construct are 30 known in the art and may be used in the present invention. Two commonly used methods for plant transformation are *Agrobacterium*-mediated transformation and microprojectile bombardment. Microprojectile bombardment methods are illustrated in U.S. Patents 5,015,580 (soybean); 5,550,318 (corn); 5,538,880 (corn); 5,914,451 (soybean); 6,160,208 (corn); 6,399,861 (corn), 6,153,812 (wheat) and 6,365,807 (rice), and *Agrobacterium*-

mediated transformation is described in U.S. Patents 5,159,135 (cotton); 5,824,877 (soybean); 5,463,174 (canola); 5,591,616 (corn); 6,384,301 (soybean); 7,026,528 (wheat) and 6,329,571 (rice), all of which are incorporated herein by reference. For *Agrobacterium tumefaciens* based plant transformation system, additional elements present on transformation constructs will include T-DNA left and right border sequences to facilitate incorporation of the recombinant polynucleotide into the plant genome.

In general it is useful to introduce recombinant DNA randomly, i.e. at a non-specific location, in the genome of a target plant line. In special cases it may be useful to target recombinant DNA insertion in order to achieve site-specific integration, for example, to replace an existing gene in the genome, to use an existing promoter in the plant genome, or to insert a recombinant polynucleotide at a predetermined site known to be active for gene expression. Several site specific recombination systems exist which are known to function implants include cre-lox as disclosed in U.S. Patent 4,959,317 and FLP-FRT as disclosed in U.S. Patent 5,527,695, both incorporated herein by reference.

Transformation methods of this invention are preferably practiced in tissue culture on media and in a controlled environment. "Media" refers to the numerous nutrient mixtures that are used to grow cells *in vitro*, that is, outside of the intact living organism. Recipient cell targets include, but are not limited to, meristem cells, callus, immature embryos and gametic cells such as microspores, pollen, sperm and egg cells. It is contemplated that any cell from which a fertile plant may be regenerated is useful as a recipient cell. Callus may be initiated from tissue sources including, but not limited to, immature embryos, seedling apical meristems, microspores and the like. Cells capable of proliferating as callus are also recipient cells for genetic transformation. Practical transformation methods and materials for making transgenic plants of this invention, for example various media and recipient target cells, transformation of immature embryo cells and subsequent regeneration of fertile transgenic plants are disclosed in U.S. Patents 6,194,636 and 6,232,526, which are incorporated herein by reference.

The seeds of transgenic plants can be harvested from fertile transgenic plants and be used to grow progeny generations of transformed plants of this invention including hybrid plant lines for selection of plants having an enhanced trait. In addition to direct transformation of a plant with a recombinant DNA, transgenic plants can be prepared by crossing a first plant having a recombinant DNA with a second plant lacking the DNA. For example, recombinant DNA can be introduced into first plant line that is amenable to transformation to produce a transgenic plant which can be crossed with a second plant line to

introgress the recombinant DNA into the second plant line. A transgenic plant with recombinant DNA providing an enhanced trait, e.g. enhanced yield, can be crossed with transgenic plant line having other recombinant DNA that confers another trait, for example herbicide resistance or pest resistance, to produce progeny plants having recombinant DNA that confers both traits. Typically, in such breeding for combining traits the transgenic plant donating the additional trait is a male line and the transgenic plant carrying the base traits is the female line. The progeny of this cross will segregate such that some of the plants will carry the DNA for both parental traits and some will carry DNA for one parental trait; such plants can be identified by markers associated with parental recombinant DNA, e.g. marker identification by analysis for recombinant DNA or, in the case where a selectable marker is linked to the recombinant, by application of the selecting agent such as a herbicide for use with a herbicide tolerance marker, or by selection for the enhanced trait. Progeny plants carrying DNA for both parental traits can be crossed back into the female parent line multiple times, for example usually 6 to 8 generations, to produce a progeny plant with substantially the same genotype as one original transgenic parental line but for the recombinant DNA of the other transgenic parental line

In the practice of transformation DNA is typically introduced into only a small percentage of target plant cells in any one transformation experiment. Marker genes are used to provide an efficient system for identification of those cells that are stably transformed by receiving and integrating a transgenic DNA construct into their genomes. Preferred marker genes provide selective markers which confer resistance to a selective agent, such as an antibiotic or herbicide. Any of the herbicides to which plants of this invention may be resistant are useful agents for selective markers. Potentially transformed cells are exposed to the selective agent. In the population of surviving cells will be those cells where, generally, the resistance-conferring gene is integrated and expressed at sufficient levels to permit cell survival. Cells may be tested further to confirm stable integration of the exogenous DNA. Commonly used selective marker genes include those conferring resistance to antibiotics such as kanamycin and paromomycin (*nptII*), hygromycin B (*aph IV*) and gentamycin (*aac3* and *aacC4*) or resistance to herbicides such as glufosinate (*bar* or *pat*) and glyphosate (*aroA* or EPSPS). Examples of such selectable markers are illustrated in U.S. Patents 5,550,318; 5,633,435; 5,780,708 and 6,118,047, all of which are incorporated herein by reference. Selectable markers which provide an ability to visually identify transformants can also be employed, for example, a gene expressing a colored or fluorescent protein such as a

luciferase or green fluorescent protein (GFP) or a gene expressing a *beta*-glucuronidase or *uidA* gene (GUS) for which various chromogenic substrates are known.

Plant cells that survive exposure to the selective agent, or plant cells that have been scored positive in a screening assay, may be cultured in regeneration media and allowed to mature into plants. Developing plantlets regenerated from transformed plant cells can be transferred to plant growth mix, and hardened off, for example, in an environmentally controlled chamber at about 85% relative humidity, 600 ppm CO₂, and 25-250 microeinsteins m⁻² s⁻¹ of light, prior to transfer to a greenhouse or growth chamber for maturation. Plants are regenerated from about 6 weeks to 10 months after a transformant is identified, depending on the initial tissue. Plants may be pollinated using conventional plant breeding methods known to those of skill in the art and seed produced, for example self-pollination is commonly used with transgenic corn. The regenerated transformed plant or its progeny seed or plants can be tested for expression of the recombinant DNA and selected for the presence of enhanced agronomic trait.

Transgenic Plants and Seeds

Transgenic plants derived from the plant cells of this invention are grown to generate transgenic plants having an enhanced trait as compared to a control plant and produce transgenic seed and pollen of this invention. Such plants with enhanced traits are identified by selection of transformed plants or progeny seed for the enhanced trait. For efficiency a selection method is designed to evaluate multiple transgenic plants (events) comprising the recombinant DNA, for example multiple plants from 2 to 20 or more transgenic events. Transgenic plants grown from transgenic seed provided herein demonstrate improved agronomic traits that contribute to increased yield or other trait that provides increased plant value, including, for example, improved seed quality. Of particular interest are plants having enhanced water use efficiency, enhanced cold tolerance, increased yield, enhanced nitrogen use efficiency, enhanced seed protein and enhanced seed oil.

Table 1 provides a list of protein encoding DNA ("genes") that are useful as recombinant DNA for production of transgenic plants with enhanced agronomic trait, the elements of Table 1 are described by reference to:

"PEP SEQ ID NO" identifies an amino acid sequence from SEQ ID NO: 96 to 193.

“NUC SEQ ID NO” identifies a DNA sequence from SEQ ID NO: 1 to 95.

“BV id” is a reference to the identifying number in Table 4 of base vectors used for construction of the transformation vectors of the recombinant DNA. Construction of plant transformation constructs is illustrated in Example 1.

5 “Gene Name” is a common name for protein encoded by the recombinant DNA.

“Annotation” refers to a description of the top hit protein obtained from an amino acid sequence query of each PEP SEQ ID NO to GenBank database of the National Center for Biotechnology Information (ncbi). More particularly, “gi” is the GenBank ID number for the top BLAST hit;

10 “Description” refers to the description of the top BLAST hit;

“% id” refers to the percentage of identically matched amino acid residues along the length of the portion of the sequences which is aligned by BLAST (-F T) between the sequence of interest provided herein and the hit sequence in GenBank.

15

Table 1.

Nuc seq ID NO	Pep seq ID NO	Gene ID	BV ID	Gene Name	Annotation		
					% ID	GenBank ID	Description
1	96	PHE0004633_5508	4	corn putative transcription factor RAU1	68	50947455	ref XP_483255.1 putative transcription factor RAU1 [Oryza sativa (japonica cultivar-group)]
2	97	PHE0004738_5674	1	rice NAM protein	82	55771315	dbj BAD72224.1 unknown protein [Oryza sativa (japonica cultivar-group)]
3	98	PHE0004814_5801	13	corn KNOX family	75	51535639	dbj BAD37613.1 KNOX family class 2 homeodomain protein [Oryza sativa (japonica cultivar-group)]
4	99	PHE0004817_5809	4	corn PIF3-like family	69	50928761	ref XP_473908.1 OSJNBa0 058K23.6 [Oryza sativa (japonica cultivar-group)]
5	100	PHE0004817_5810	13	corn PIF3-like family gene	69	50928761	ref XP_473908.1 OSJNBa0 058K23.6 [Oryza sativa (japonica cultivar-group)]
6	101	PHE0004821_5819	12	corn PIF3-like family gene	61	55296133	dbj BAD67851.1 basic helix-loop-helix protein SPATULA-like [Oryza sativa (japonica cultivar- group)]

Nuc seq ID NO	Pep seq ID NO	Gene ID	BV ID	Gene Name	Annotation		
					% ID	GenBank ID	Description
7	102	PHE0004828_5826	4	soy PIF3-like family gene	53	92897142	gb ABE93546.1 Helix-loop-helix DNA-binding [Medicago truncatula]
8	103	PHE0004817_5901	12	corn PIF3-like family gene	69	50928761	ref XP_473908.1 OSJNBa0058K23.6 [Oryza sativa (japonica cultivar-group)]
9	104	PHE0004861_5910	4	rice putative SCARECROW protein	87	34899818	ref NP_911255.1 putative SCARECROW protein [Oryza sativa (japonica cultivar-group)]
10	105	PHE0004863_5912	4	corn putative AT-hook protein	61	50916020	ref XP_468474.1 putative AT-hook protein 1 [Oryza sativa (japonica cultivar-group)]
11	106	PHE0002062_5913	4	corn R2R3 Myb protein	59	50946113	ref XP_482584.1 putative typical P-type R2R3 Myb protein [Oryza sativa (japonica cultivar-group)]
12	107	PHE0002531_5926	17	corn DNA-binding protein MNB1a	78	95102176	gb ABF51012.1 DOF1 [Zea mays]
13	108	PHE0004914_5971	4	soy syringolide-induced protein	93	19911577	dbj BAB86892.1 syringolide-induced protein 1-3-1A [Glycine max]
14	109	PHE0004924_5982	4	soy TCP family transcription factor	74	18396089	ref NP_566164.1 PTF1 Plastid Transcription Factor 1 [Arabidopsis thaliana] gb AAM62743.1 unknown [Arabidopsis thaliana]
15	110	PHE0004925_5983	4	Arabidopsis squamosa promoter-binding protein	94	15230904	ref NP_191351.1 DNA binding / transcription factor [Arabidopsis thaliana] sp Q9M2Q6 SPL15_ARATH Squamosa promoter-binding-like protein 15
16	111	PHE0004938_5994	4	Arabidopsis gibberellin-responsive modulator	96	15228553	ref NP_186995.1 RGL2 (RGA-LIKE 2); transcription factor [Arabidopsis thaliana] sp Q8GXW1 RGL2_ARATH DELLA protein RGL2 (RGA-like protein 2) (Scarecrow-like protein 19)
17	112	PHE0004957_6019	4	corn C2H2-type zinc finger protein	68	50933653	ref XP_476354.1 C2H2-type zinc finger protein-like protein [Oryza sativa (japonica cultivar-group)]
18	113	PHE0004958_6020	4	corn putative ascorbate oxidase promoter-binding protein	50	50940107	ref XP_479581.1 putative ascorbate oxidase promoter-binding protein AOBP [Oryza sativa (japonica cultivar-group)] ref XP_506580.1 PREDICTED

Nuc seq ID NO	Pep seq ID NO	Gene ID	BV ID	Gene Name	Annotation		
					% ID	GenBank ID	Description
							OSJNBa0060017.31 gene product [Oryza sativa (japonica cultivar-group)]
19	114	PHE0004959_6021	4	corn putative EREBP-type transcription factor	41	56567581	gb AAV98700.1 BTH-induced ERF transcriptional factor 1 [Oryza sativa (indica cultivar-group)]
20	115	PHE0004974_6040	4	corn auxin response factor	74	108864436	gb ABG22499.1 Auxin response factor 2, [Oryza sativa (japonica cultivar-group)]
21	116	PHE0004975_6041	4	corn auxin response factor	67	77555450	gb ABA98246.1 Auxin response factor 2, [Oryza sativa (japonica cultivar-group)]
22	117	PHE0004987_6056	4	soy transfactor-like protein	63	77403669	dbj BAE46413.1 MYB-CC type transfactor [Solanum tuberosum]
23	118	PHE0005005_7034	4	corn putative Myb-related protein	52	37536868	ref NP_922736.1 putative Myb-related protein [Oryza sativa (japonica cultivar-group)]
24	119	PHE0004877_7030	12	corn response regulator ARR11	76	56784051	dbj BAD82798.1 putative response regulator 11 [Oryza sativa (japonica cultivar-group)]
25	120	PHE0006057_7048	13	wheat PIF3-like family gene	40	109134123	dbj BAC41905.1 putative bHLH transcription factor bHLH016 [Arabidopsis thaliana]
26	121	PHE0006057_7053	12	wheat PIF3-like family gene	40	109134123	dbj BAC41905.1 putative bHLH transcription factor bHLH016 [Arabidopsis thaliana]
27	122	PHE0006070_7067	4	corn putative transcription factor	64	54291039	dbj BAD61716.1 transcription factor-like [Oryza sativa (japonica cultivar-group)]
28	123	PHE0006073_7072	4	corn putative bZIP transcription factor	64	54291039	dbj BAD61716.1 transcription factor-like [Oryza sativa (japonica cultivar-group)]
29	124	PHE0006004_7082	4	soy NAM like protein	51	15224202	gb AAD22369.1 NAM (no apical meristem)-like protein [Arabidopsis thaliana] sp Q9SK55 NAC42_ARATH Putative NAC domain-containing protein 42 (ANAC042)
30	125	PHE0006022_7105	4	soy transcription factor EIL1	82	18643341	gb AAL76272.1 transcription factor EIL1 [Vigna radiata]

Nuc seq ID NO	Pep seq ID NO	Gene ID	BV ID	Gene Name	Annotation		
					% ID	GenBank ID	Description
31	126	PHE0006023_7240	4	Arabidopsis bHLH family protein	92	15241896	ref NP_201067.1 DNA binding / transcription factor [Arabidopsis thaliana]
32	127	PHE0006191_7251	8	EEM7	60	50936701	ref XP_477878.1 hypothetical protein [Oryza sativa (japonica cultivar-group)]
33	128	PHE0006237_7261	18	Lycopersicon SHN1	86	18650662	gb AAL75809.1 ethylene response factor 1 [Lycopersicon esculentum]
34	129	PHE0006237_7274	17	Lycopersicon SHN1	86	18650662	gb AAL75809.1 ethylene response factor 1 [Lycopersicon esculentum]
35	130	PHE0006237_7268	6	Lycopersicon SHN1	86	18650662	gb AAL75809.1 ethylene response factor 1 [Lycopersicon esculentum]
36	131	PHE0006237_7277	5	Lycopersicon SHN1	86	18650662	gb AAL75809.1 ethylene response factor 1 [Lycopersicon esculentum]
37	132	PHE0006237_7284	11	Lycopersicon SHN1	86	18650662	gb AAL75809.1 ethylene response factor 1 [Lycopersicon esculentum]
38	133	PHE0004816_7303	4	corn PIF3-like family gene	82	50928761	ref XP_473908.1 OSJNBa0058K23.6 [Oryza sativa (japonica cultivar-group)]
39	134	PHE0006291_7319	17	soy putative CONSTANS-like B-box zinc finger protein	42	15227152	ref NP_182310.1 transcription factor/zinc ion binding [Arabidopsis thaliana]
40	135	PHE0004816_7421	12	corn PIF3-like family gene	82	50928761	ref XP_473908.1 OSJNBa0058K23.6 [Oryza sativa (japonica cultivar-group)]
41	136	PHE0004816_7418	4	corn PIF3-like family gene	82	50928761	ref XP_473908.1 OSJNBa0058K23.6 [Oryza sativa (japonica cultivar-group)]
42	137	PHE0003673_7430	4	corn response regulator like	76	56784051	dbj BAD82798.1 putative response regulator 11 [Oryza sativa (japonica cultivar-group)]
43	138	PHE0003664_7436	4	soy AP2/EREBP transcription factor like	48	15227980	gb AAT44934.1 putative AP2/EREBP transcription factor [Arabidopsis thaliana]
44	139	PHE0004816_7445	13	corn PIF3-like family gene	82	50928761	ref XP_473908.1 OSJNBa0058K23.6 [Oryza sativa (japonica cultivar-group)]
45	140	PHE0002149_7487	4	corn DNA-binding protein	66	50941323	ref XP_480189.1 putative LHY protein [Oryza sativa (japonica cultivar-group)]
46	141	PHE0006290_7498	4	corn putative CONSTANS-like B-box zinc finger protein	70	50912285	ref XP_467550.1 zinc-finger protein [Oryza sativa (japonica cultivar-group)]

Nuc seq ID NO	Pep seq ID NO	Gene ID	BV ID	Gene Name	Annotation		
					% ID	GenBank ID	Description
47	142	PHE0006423_7664	4	soy Myb61	56	92873337	gb ABE81808.1 Homeodom ain-related [Medicago truncatula]
48	143	PHE0006384_7737	9	rice R2R3 Myb protein	55	50946113	ref XP_482584.1 putative typical P-type R2R3 Myb protein [Oryza sativa (japonica cultivar-group)]
49	144	PHE0006384_7789	13	rice R2R3 Myb protein	55	50946113	ref XP_482584.1 putative typical P-type R2R3 Myb protein [Oryza sativa (japonica cultivar-group)]
50	145	PHE0006507_7828	17	Corn NFB1_23C	98	50916531	gb ABF96585.1 CCAAT- binding transcription factor subunit A [Oryza sativa (japonica cultivar-group)]
51	146	PHE0006509_7846	19	Arabidopsis Gm2010	60	62856979	gb AAY16440.1 squamosa promoter binding-like protein [Betula platyphylla]
52	147	PHE0006384_7839	19	rice R2R3 Myb protein	55	50946113	ref XP_482584.1 putative typical P-type R2R3 Myb protein [Oryza sativa (japonica cultivar-group)]
53	148	PHE0006448_7859	17	Arabidopsis transcription factor	99	15217662	ref NP_176634.1 transcripti on factor [Arabidopsis thaliana] gb AAN41333.1 unknown protein [Arabidopsis thaliana]
54	149	PHE0006504_7876	17	maize tubby 4	69	55733806	gb AAV59313.1 putative tubby protein [Oryza sativa (japonica cultivar-group)]
55	150	PHE0006057_7929	15	wheat PIF3-like family gene	40	109134123	dbj BAC41905.1 putative bHLH transcription factor bHLH016 [Arabidopsis thaliana]
56	151	PHE0003473_7927	9	soy Zinc finger protein like	50	87162706	gb ABD28501.1 Zinc finger, C2H2-type [Medicago truncatula]
57	152	PHE0002531_7985	45	corn DNA-binding protein MNB1a	78	95102176	gb ABF51012.1 DOF1 [Zea mays]
58	153	PHE0004463_8059	15	soy ethylene response factor	48	15238727	ref NP_197901.1 DNA binding/transcription factor [Arabidopsis thaliana]
59	154	PHE0001067_8154	10	Arabidopsis homeodomain transcription factor	89	15237035	ref NP_195280.1 DNA binding/transcription factor [Arabidopsis thaliana] sp O81788 WOX13_ARAT H WUSCHEL-related homeobox 13
60	155	PHE0006350_8201	15	GIA/RGA-like gibberellin response modulator	46	63054405	gb AAY28970.1 GIA/RGA- like gibberellin response modulator [Gossypium hirsutum]

Nuc seq ID NO	Pep seq ID NO	Gene ID	BV ID	Gene Name	Annotation		
					% ID	GenBank ID	Description
61	156	PHE0006605_8233	17	Arabidopsis Zinc finger (GATA type) family protein	74	15230393	ref NP_190677.1 transcripti on factor [Arabidopsis thaliana]
62	157	PHE0006546_8310	8	Response regulator 9	61	55771374	dbj BAD72541.1 putative response regulator 9 [Oryza sativa (japonica cultivar- group)]
63	158	PHE0006527_8369	17	NFB1-Q185H	97	50916531	gb ABF96585.1 CCAAT- binding transcription factor subunit A [Oryza sativa (japonica cultivar-group)]
64	159	PHE0004938_8370	17	Arabidopsis gibberellin- responsive modulator	96	15228553	sp Q8GXW1 RGL2_ARAT H DELLA protein RGL2 (RGA-like protein 2) (Scarecrow-like protein 19) [Arabidopsis thaliana]
65	160	PHE0006774_8489	15	NFB2_E76R_S83 R	82	115840	sp P25209 NFYB_MaizeNu clear transcription factor Y subunit B (NF-YB) (CAAT- box DNA-binding protein subunit B)
66	161	PHE0006778_8503	15	NFB2_I49R_C73S _C89S	82	115840	sp P25209 NFYB_MaizeNu clear transcription factor Y subunit B (NF-YB) (CAAT- box DNA-binding protein subunit B)
67	162	PHE0006780_8502	15	NFB2_C73S_C89S _L102R	82	115840	sp P25209 NFYB_Maize Nuclear transcription factor Y subunit B (NF-YB) (CAAT-box DNA-binding protein subunit B)
68	163	PHE0006752_8521	16	wheat AP1 (VRN1)	75	30721847	gb AAP33790.1 MADS-box protein TaVRT-1 gb AAW73225.1 VRN-B1 [Triticum aestivum]
69	164	PHE0006779_8565	15	corn NFB2_C73R_C89 S	82	115840	sp P25209 NFYB_MaizeNu clear transcription factor Y subunit B (NF-YB) (CAAT- box DNA-binding protein subunit B)
70	165	PHE0006781_8573	15	corn NFB2_I49R_C73R _C89S_L102R	82	115840	sp P25209 NFYB_MaizeNu clear transcription factor Y subunit B (NF-YB) (CAAT- box DNA-binding protein subunit B)
71	166	PHE0003664_8637	15	soy AP2/EREBP transcription factor	48	15227980	gb AAT44934.1 putative AP2/EREBP transcription factor [Arabidopsis thaliana]
72	167	PHE0006004_8667	15	soy NAM like protein	51	15224202	ref NP_181828.1 ANAC042 ; transcription factor gb AAD22369.1 NAM (no apical meristem)-like protein [Arabidopsis thaliana]

Nuc seq ID NO	Pep seq ID NO	Gene ID	BV ID	Gene Name	Annotation		
					% ID	GenBank ID	Description
73	168	PHE0006022_8690	15	soy transcription factor EIL1	82	18643341	gb AAL76272.1 transcription factor EIL1 [Vigna radiata]
74	169	PHE0006290_8689	15	corn putative CONSTANS-like B-box zinc finger protein	70	50912285	ref XP_467550.1 zinc-finger protein [Oryza sativa (japonica cultivar-group)]
75	170	PHE0006423_8696	15	soy Myb61	56	92873337	gb ABE81808.1 Homeodomain-related [Medicago truncatula]
76	171	PHE0002149_8748	15	corn DNA-binding protein	66	50941323	ref XP_480189.1 putative LHY protein [Oryza sativa (japonica cultivar-group)]
77	172	PHE0006023_8762	15	Arabidopsis bHLH family protein	92	15241896	ref NP_201067.1 DNA binding/transcription factor [Arabidopsis thaliana]
78	173	PHE0004987_8771	15	soy transfactor-like protein	63	77403669	dbj BAE46413.1 MYB-CC type transfactor [Solanum tuberosum]
79	174	PHE0006858_8859	7	corn MADS box protein	97	939781	gb AAB00079.1 MADS box protein
80	175	PHE0006860_8863	7	corn kernel specific MADS	100	939779	gb AAB00078.1 MADS box protein
81	176	PHE0006955_9129	20	Lycopersicon esculentum TAGL12 transcription factor	67	24967140	gb AAM33103.2 TAGL12 transcription factor [Lycopersicon esculentum]
82	177	PHE0006951_9137	20	AT5g52010/MSG15_9	97	15242250	ref NP_200014.1 nucleic acid binding/ transcription factor/ zinc ion binding [Arabidopsis thaliana]
83	178	PHE0006981_9158	15	GRAS family transcription factor	59	92886232	gb ABE88228.1 GRAS family transcription factor [Medicago truncatula]
84	179	PHE0006951_9173	15	AT5g52010/MSG15_9	97	15242250	ref NP_200014.1 nucleic acid binding/ transcription factor/ zinc ion binding [Arabidopsis thaliana]
85	180	PHE0004646_PM ON94356.pep	17	Arabidopsis NAM family protein	86	9758529	dbj BAB08905.1 unnamed protein product [Arabidopsis thaliana]
86	181	PHE0004723_PM ON94660.pep	17	soy auxin-induced protein	92	114733	sp P13088 AUX22_SOYBN Auxin-induced protein AUX22
87	182	PHE0004648_PM ON95051.pep	4	Arabidopsis putative auxin response factor 23	93	20152540	emb CAD29662.1 putative auxin response factor 23 [Arabidopsis thaliana]
88	183	PHE0004357_PM ON94163.pep	2	corn OSJNBa0079A21.14	47	50927517	ref XP_473403.1 OSJNBa0079A21.14 [Oryza sativa (japonica cultivar-group)]

Nuc seq ID NO	Pep seq ID NO	Gene ID	BV ID	Gene Name	Annotation		
					% ID	GenBank ID	Description
89	184	PHE0004646_PM ON94352.pep	2	Arabidopsis NAM family protein	86	9758529	dbj BAB08905.1 unnamed protein product [Arabidopsis thaliana]
90	185	PHE0004624_PM ON94400.pep	2	soy auxin response factor-like protein	65	30027167	gb AAP06759.1 auxin response factor-like protein [Mangifera indica]
91	186	PHE0004463_PM ON94432.pep	2	soy ethylene response factor	48	15238727	ref NP_197901.1 DNA binding/transcription factor [Arabidopsis thaliana]
92	187	PHE0004356_PM ON93862.pep	1	corn LEC2/FUS3	66	56785317	dbj BAD82277.1 regulatory protein Viviparous-1-like [Oryza sativa (japonica cultivar-group)]
93	188	PHE0004332_PM ON95104.pep	14	tomato Pti4	85	3342211	gb AAC50047.1 Pti4 [Lycopersicon esculentum]
94	189	PHE0004644_PM ON95096.pep	3	corn ICE1-like	50	77551194	gb ABA93991.1 Helix-loop- helix DNA-binding domain containing protein [Oryza sativa (japonica cultivar- group)]
95	190	PHE0004723_PM ON95121.pep	4	soy auxin-induced protein	92	114733	sp P13088 AUX22_SOYBN Auxin-induced protein AUX22

Selection Methods for Transgenic Plants with Enhanced Agronomic Trait

Within a population of transgenic plants regenerated from plant cells transformed with the recombinant DNA construct many plants that survive to fertile transgenic plants that produce seeds and progeny plants will not exhibit an enhanced agronomic trait. Selection from the population is necessary to identify one or more transgenic plant cells that can provide plants with the enhanced trait. Transgenic plants having enhanced agronomic traits are selected from populations of plants regenerated or derived from plant cells transformed as described herein by evaluating the plants in a variety of assays to detect an enhanced trait, e.g. enhanced water use efficiency, enhanced cold tolerance, increased yield, enhanced nitrogen use efficiency, enhanced seed protein and enhanced seed oil. These assays also may take many forms including, but not limited to, direct screening for the trait in a greenhouse or field trial or by screening for a surrogate trait. Such analyses can be directed to detecting changes in the chemical composition, biomass, physiological properties, morphology of the plant. Changes in chemical compositions such as nutritional composition of grain can be detected by analysis of the seed composition and content of protein, free amino acids, oil, free fatty acids, starch or tocopherols. Changes in biomass characteristics can be made on

greenhouse or field grown plants and can include plant height, stem diameter, root and shoot dry weights; and, for corn plants, ear length and diameter. Changes in physiological properties can be identified by evaluating responses to stress conditions, for example assays using imposed stress conditions such as water deficit, nitrogen deficiency, cold growing conditions, pathogen or insect attack or light deficiency, or increased plant density. Changes in morphology can be measured by visual observation of tendency of a transformed plant with an enhanced agronomic trait to also appear to be a normal plant as compared to changes toward bushy, taller, thicker, narrower leaves, striped leaves, knotted trait, chlorosis, albino, anthocyanin production, or altered tassels, ears or roots. Other selection properties include days to pollen shed, days to silking, leaf extension rate, chlorophyll content, leaf temperature, stand, seedling vigor, internode length, plant height, leaf number, leaf area, tillering, brace roots, stay green, stalk lodging, root lodging, plant health, barrenness/prolificacy, green snap, and pest resistance. In addition, phenotypic characteristics of harvested grain may be evaluated, including number of kernels per row on the ear, number of rows of kernels on the ear, kernel abortion, kernel weight, kernel size, kernel density and physical grain quality. Although the plant cells and methods of this invention can be applied to any plant cell, plant, seed or pollen, e.g. any fruit, vegetable, grass, tree or ornamental plant, the various aspects of the invention are preferably applied to corn, soybean, cotton, canola, alfalfa, wheat and rice plants. In many cases the invention is applied to corn plants that are inherently resistant to disease from the Mal de Rio Cuarto virus or the *Puccinia sorghi* fungus or both.

The following examples are included to demonstrate aspects of the invention, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific aspects which are disclosed and still obtain a like or similar results without departing from the spirit and scope of the invention.

Example 1. Plant Expression Constructs

This example illustrates the construction of plasmids for transferring recombinant DNA into plant cells which can be regenerated into transgenic plants of this invention.

A. Plant expression constructs for corn transformation

A base corn plant transformation vector pMON93039, as set forth in SEQ ID NO: 2202, illustrated in Table 2 and Figure 2, was fabricated for use in preparing recombinant DNA for *Agrobacterium*-mediated transformation into corn tissue.

Table 2.

Function	Name	Annotation	Coordinates of SEQ ID NO: 2202
Agro T-DNA transfer	B-AGRtu.right border	Agro right border sequence, essential for transfer of T-DNA.	11364-11720
Gene of interest expression cassette	E-Os.Act1	Upstream promoter region of the rice actin 1 gene	19-775
	E-CaMV.35S.2xA1-B3	Duplicated 35S A1-B3 domain without TATA box	788-1120
	P-Os.Act1	Promoter region of the rice actin 1 gene	1125-1204
	L-Ta.Lhcb1	5' untranslated leader of wheat major chlorophyll a/b binding protein	1210-1270
	I-Os.Act1	First intron and flanking UTR exon sequences from the rice actin 1 gene	1287-1766
	T-St.Pis4	3' non-translated region of the potato proteinase inhibitor II gene which functions to direct polyadenylation of the mRNA	1838-2780
Plant selectable marker expression cassette	P-Os.Act1	Promoter from the rice actin 1 gene	2830-3670
	L-Os.Act1	First exon of the rice actin 1 gene	3671-3750
	I-Os.Act1	First intron and flanking UTR exon sequences from the rice actin 1 gene	3751-4228
	TS-At.ShkG-CTP2	Transit peptide region of Arabidopsis EPSPS	4238-4465
	CR-AGRtu.aroA-CP4.nat	Coding region for bacterial strain CP4 native <i>aroA</i> gene	4466-5833

Function	Name	Annotation	Coordinates of SEQ ID NO: 2202
	T-AGRtu.nos	A 3' non-translated region of the nopaline synthase gene of <i>Agrobacterium tumefaciens</i> Ti plasmid which functions to direct polyadenylation of the mRNA.	5849-6101
Agro T-DNA transfer	B-AGRtu.left border	Agro left border sequence, essential for transfer of T-DNA.	6168-6609
Maintenance in <i>E. coli</i>	OR-Ec.oriV-RK2	The vegetative origin of replication from plasmid RK2.	6696-7092
	CR-Ec.rop	Coding region for repressor of primer from the ColE1 plasmid. Expression of this gene product interferes with primer binding at the origin of replication, keeping plasmid copy number low.	8601-8792
	OR-Ec.ori-ColE1	The minimal origin of replication from the <i>E. coli</i> plasmid ColE1.	9220-9808
	P-Ec.aadA-SPC/STR	Promoter for Tn7 adenyltransferase (AAD (3"))	10339-10380
	CR-Ec.aadA-SPC/STR	Coding region for Tn7 adenyltransferase (AAD (3")) conferring spectinomycin and streptomycin resistance.	10381-11169
	T-Ec.aadA-SPC/STR	3' UTR from the Tn7 adenyltransferase (AAD (3")) gene of <i>E. coli</i> .	11170-11227

Another embodiment of corn plant transformation base vector is pMON92705, as set forth in SEQ ID NO: 2203, illustrated in Table 3 and Figure 3, which was fabricated for use in preparing recombinant DNA for *Agrobacterium*-mediated transformation into corn tissue.

Other base vectors similar to those described above were also constructed as listed in Table 4. See Table 4 for a summary of base vector plasmids and base vector ID's which are referenced in Table 1. Also see Table 5 for a summary of regulatory elements used in the gene expression cassette for these base vectors and SEQ ID NOs for elements.

5

Table 3.

Function	Name	Annotation	Coordinates of SEQ ID NO: 2203
Agro T-DNA transfer	B-AGRtu. right border	Agro right border sequence, essential for transfer of T-DNA.	5206-5562
Gene of interest expression cassette	P-Os.Act1	Promoter from rice actin 1 gene	5580-6423
	L-Os.Act1	5' UTR of rice Act7 (or 1) gene	6424-6503
	I-Os.Act1	Intron from the rice actin7 gene	6504-6980
	T-St.Pis4	3' non-translated region of the potato proteinase inhibitor II gene which functions to direct polyadenylation of the mRNA	7055-7997
Plant selectable marker expression cassette	P-Os.Act1	Promoter from the rice actin 1 gene	8047-8887
	L-Os.Act1	First exon of the rice actin 1 gene	8888-8967
	I-Os.Act1	First intron and flanking UTR exon sequences from the rice actin 1 gene	8968-9445
	TS-At.ShkG-CTP2	Transit peptide region of Arabidopsis EPSPS	9455-9682
	CR-AGRtu.aroA-CP4.nat	Coding region for bacterial strain CP4 native <i>aroA</i> gene	9683-11050
	T-AGRtu.nos	A 3' non-translated region of the nopaline synthase gene of <i>Agrobacterium tumefaciens</i> Ti plasmid which functions to direct polyadenylation of the mRNA.	11066-11318
Agro T-DNA transfer	B-AGRtu.left border	Agro left border sequence, essential for transfer of T-DNA.	10-451
Maintenance in <i>E. coli</i>	OR-Ec.oriV-RK2	The vegetative origin of replication from plasmid RK2.	538-934

Function	Name	Annotation	Coordinates of SEQ ID NO: 2203
	CR-Ec.rop	Coding region for repressor of primer from the ColE1 plasmid. Expression of this gene product interferes with primer binding at the origin of replication, keeping plasmid copy number low.	2443-2634
	OR-Ec.ori-ColE1	The minimal origin of replication from the E. coli plasmid ColE1.	3062-3650
	P-Ec.aadA-SPC/STR	Promoter for Tn7 adenylyltransferase (AAD (3"))	4181-4222
	CR-Ec.aadA-SPC/STR	Coding region for Tn7 adenylyltransferase (AAD (3")) conferring spectinomycin and streptomycin resistance.	4223-5011
	T-Ec.aadA-SPC/STR	3' UTR from the Tn7 adenylyltransferase (AAD (3")) gene of E. coli.	5012-5069

Primers for PCR amplification of protein coding nucleotides of recombinant DNA are designed at or near the start and stop codons of the coding sequence, in order to eliminate most of the 5' and 3' untranslated regions. Each recombinant DNA coding for a protein identified in Table 1 is amplified by PCR prior to insertion into the insertion site within the gene of interest expression cassette of one of the base vectors as referenced in Table 1.

Table 4

Base Vector ID	Base Vector for Corn
1	pMON74577
2	pMON82060
3	pMON82921
4	pMON92705
5	pMON92708
6	pMON92712
7	pMON92713
8	pMON92715
9	pMON92716
10	pMON92718

Base Vector ID	Base Vector for Corn
11	pMON92719
12	pMON92722
13	pMON92724
14	pMON92725
15	pMON93039
16	pMON93043
Base Vector ID	Base Vector for Soybean And Canola
17	pMON82053
18	pMON92669
19	pMON92671
20	pMON99006

Table 5

Vector	Promoter	SEQ ID NO	Leader	SEQ ID NO	Intron	SEQ ID NO
pMON74577	P-Hv.Per1	2167	L-Hv.Per1	2182	I-Zm.DnaK	2197
pMON82053	P-CaMV.35S-enh	2168	NONE	/	NONE	/
pMON82060	P-Os.Act1	2169	L-Os.Act1	2183	I-Os.Act1	2198
pMON82921	P-Zm.Cik 1	2170	L-Zm.Cik 1	2184	I-Zm.Cik 1	2199
pMON92705	P-Os.Act1	2169	L-Os.Act1	2183	I-Os.Act1	2198
pMON92708	P-Zm.CA4H	2171	L-Zm.CA4H	2185	NONE	/
pMON92712	P-Os.Cut1	2172	L-Os.Cut1	2186	I-Zm.DnaK	2197
pMON92713	P-Zm.P39486	2173	L-Zm.39486	2187	I-Zm.DnaK	2197
pMON92715	P-Hv.Per1	2167	L-Hv.Per1	2182	I-Zm.DnaK	2197
pMON92716	P-Zm.FDA	2174	L-Zm.FDA	2188	I-Zm.DnaK	2197
pMON92718	P-Zm.Cik 1	2170	L-Zm.Cik1	2184	I-Zm.Cik1	2199
pMON92719	P-Zm.RAB17	2175	L-Zm.RAB17	2189	I-Zm.DnaK	2197
pMON92722	P-CaMV.35S-enh	2168	L-CaMV.35S	2190	I-Zm.DnaK	2197
pMON92724	P-Zm.-636aldolase-0:1:2 + P-Zm.PPDK	2176	L-Zm.PPDK	2191	I-Zm.DnaK	2197
pMON93039	E-Os.Act1 + E-CaMV.35S.2xA1-B3 + P-Os.Act1	2177	L-Ta.Lhcb1	2192	I-Os.Act1	2198
pMON93043	P-Zm.EM	2178	L-Zm.EM	2193	I-Zm.DnaK	2197
pMON92669	P-At.Rca	2179	L-At.Rca	2194	NONE	/
pMON92671	P-At.SAMS3	2180	L-At.SAMS3	2195	I-At.SAMS3	2200
pMON99006	P-CaMV.35S-enh	2168	NONE	/	NONE	/
pMON92725	P-Zm.HRGP	2181	L-Zm.HRGP	2196	I-Zm.DnaK	2197

B. Plasmids for use in transformation of soybean and canola are also prepared.

Elements of an exemplary common expression vector plasmid pMON82053 are shown in Table 6 below and Figure 4.

Table 6

Function	Name	Annotation	Coordinates of SEQ ID NO: 2204
Agro T-DNA transfer	B-AGRtu.left border	Agro left border sequence, essential for transfer of T-DNA.	6144-6585
Plant selectable marker expression cassette	P-At.Act7	Promoter from the Arabidopsis actin 7 gene	6624-7861
	L-At.Act7	5' UTR of Arabidopsis Act7 gene	
	I-At.Act7	Intron from the Arabidopsis actin7 gene	
	TS-At.ShkG-CTP2	Transit peptide region of Arabidopsis EPSPS	7864-8091
	CR-AGRtu.aroA-CP4.nno_At	Synthetic CP4 coding region with dicot preferred codon usage.	8092-9459
	T-AGRtu.nos	A 3' non-translated region of the nopaline synthase gene of Agrobacterium tumefaciens Ti plasmid which functions to direct polyadenylation of the mRNA.	9466-9718
Gene of interest expression cassette	P-CaMV.35S-enh	Promoter for 35S RNA from CaMV containing a duplication of the -90 to -350 region.	1-613
	T-Gb.E6-3b	3' untranslated region from the fiber protein E6 gene of sea-island cotton;	688-1002
Agro T-DNA transfer	B-AGRtu.right border	Agro right border sequence, essential for transfer of T-DNA.	1033-1389
Maintenance in <i>E. coli</i>	OR-Ec.oriV-RK2	The vegetative origin of replication from plasmid RK2.	5661-6057
	CR-Ec.rop	Coding region for repressor of primer from the ColE1 plasmid. Expression of this gene product interferes with primer binding at the origin of replication, keeping plasmid copy number low.	3961-4152
	OR-Ec.ori-ColE1	The minimal origin of replication from the E. coli plasmid ColE1.	2945-3533
	P-Ec.aadA-SPC/STR	Promoter for Tn7 adenyltransferase (AAD (3"))	2373-2414
	CR-Ec.aadA-SPC/STR	Coding region for Tn7 adenyltransferase (AAD (3")) conferring spectinomycin and streptomycin resistance.	1584-2372
	T-Ec.aadA-SPC/STR	3' UTR from the Tn7 adenyltransferase (AAD (3")) gene of E. coli.	1526-1583

Primers for PCR amplification of protein coding nucleotides of recombinant DNA are designed at or near the start and stop codons of the coding sequence, in order to eliminate most of the 5' and 3' untranslated regions. Each recombinant DNA coding for a protein identified in Table 1 is amplified by PCR prior to insertion into the insertion site within the gene of interest expression cassette of one of the base vectors as referenced in Table 1.

C. Cotton transformation vector

Recombinant DNA constructs for use in transformation of cotton are also prepared. Elements of an exemplary common expression vector plasmid pMON99053 are shown in Table 7 below and Figure 5. Primers for PCR amplification of protein coding nucleotides of recombinant DNA are designed at or near the start and stop codons of the coding sequence, in order to eliminate most of the 5' and 3' untranslated regions. Each recombinant DNA coding for a protein identified in Table 1 is amplified by PCR prior to insertion into the insertion site within the gene of interest expression cassette of the base vector in Table 7.

Table 7

Function	Name	Annotation	Coordinates of SEQ ID NO: 2205
Agrobacterium T-DNA transfer	B-AGRtu.right border	Agro right border sequence, essential for transfer of T-DNA.	1-357
Gene of interest expression cassette	Exp-CaMV.35S-enh+Ph.DnaK	Enhanced version of the 35S RNA promoter from CaMV plus the petunia hsp70 5' untranslated region	388-1091
	T-Ps.RbcS2-E9	The 3' non-translated region of the pea RbcS2 gene which functions to direct polyadenylation of the mRNA.	1165- 1797
Plant selectable marker expression cassette	Exp-CaMV.35S	Promoter and 5' untranslated region from the 35S RNA of CaMV	1828-2151
	CR-Ec.nptII-Tn5	Coding region for neomycin phosphotransferase gene from transposon Tn5 which confers resistance to neomycin and kanamycin.	2185-2979
	T-AGRtu.nos	A 3' non-translated region of the nopaline synthase gene of Agrobacterium tumefaciens Ti plasmid which functions to direct polyadenylation of the mRNA.	3011-3263
Agrobacterium T-DNA transfer	B-AGRtu.left border	Agro left border sequence, essential for transfer of T-DNA.	3309-3750

Function	Name	Annotation	Coordinates of SEQ ID NO: 2205
Maintenance in E. coli	OR-Ec.oriV-RK2	The vegetative origin of replication from plasmid RK2.	3837-4233
	CR-Ec.rop	Coding region for repressor of primer from the ColE1 plasmid. Expression of this gene product interferes with primer binding at the origin of replication, keeping plasmid copy number low.	5742-5933
	OR-Ec.ori-ColE1	The minimal origin of replication from the E. coli plasmid ColE1.	6361-6949
	P-Ec.aadA-SPC/STR	Promoter for Tn7 adenyltransferase (AAD(3"))	7480-7521
	CR-Ec.aadA-SPC/STR	Coding region for Tn7 adenyltransferase (AAD(3")) conferring spectinomycin and streptomycin resistance.	7522-8310
	T-Ec.aadA-SPC/STR	3' UTR from the Tn7 adenyltransferase (AAD(3")) gene of E. coli.	8311-8368

Example 2. Corn Transformation

This example illustrates the production and identification of transgenic corn cells in seed of transgenic corn plants having an enhanced agronomic trait, i.e. enhanced nitrogen use efficiency, increased yield, enhanced water use efficiency, enhanced tolerance to cold and/or improved seed compositions as compared to control plants. Transgenic corn cells are prepared with recombinant DNA construct expressing each of the protein encoding DNAs listed in Table 1 by *Agrobacterium*-mediated transformation using the corn transformation vectors as disclosed in Example 1. Corn transformation is effected using methods disclosed in U.S. Patent Application Publication 2004/0344075 A1 where corn embryos are inoculated and co-cultured with the *Agrobacterium tumefaciens* strain ABI and the corn transformation vector. To regenerate transgenic corn plants the transgenic callus resulting from transformation is placed on media to initiate shoot development in plantlets which are transferred to potting soil for initial growth in a growth chamber followed by a mist bench before transplanting to pots where plants are grown to maturity. The plants are self fertilized and seed is harvested for screening as seed, seedlings or progeny R2 plants or hybrids, e.g., for yield trials in the screens indicated above.

Many transgenic events which survive to fertile transgenic plants that produce seeds and progeny plants do not exhibit an enhanced agronomic trait. The transgenic plants and seeds having the transgenic cells of this invention which have recombinant DNA imparting the enhanced agronomic traits are identified by screening for nitrogen use efficiency, yield, water use efficiency, cold tolerance and improved seed composition as reported in Example 7.

Example 3. Soybean transformation

This example illustrates the production and identification of transgenic soybean cells in seed of transgenic soybean plants having an enhanced agronomic trait, i.e. enhanced nitrogen use efficiency, increased yield, enhanced water use efficiency, enhanced tolerance to cold and/or improved seed compositions as compared to control plants. Transgenic soybean cells are prepared with recombinant DNA expressing each of the protein encoding DNAs listed in Table 1 by *Agrobacterium*-mediated transformation using the soybean transformation vectors disclosed in Example 1. Soybean transformation is effected using methods disclosed in U.S. Patent 6,384,301 where soybean meristem explants are wounded then inoculated and co-cultured with the soybean transformation vector, then transferred to selection media for 6-8 weeks to allow selection and growth of transgenic shoots.

Transgenic shoots producing roots are transferred to the greenhouse and potted in soil. Many transgenic events which survive to fertile transgenic plants that produce seeds and progeny plants do not exhibit an enhanced agronomic trait. The transgenic plants and seeds having the transgenic cells of this invention which have recombinant DNA imparting the enhanced agronomic traits are identified by screening for nitrogen use efficiency, yield, water use efficiency, cold tolerance and improved seed composition as reported in Example 7.

Example 4. Cotton transgenic plants with enhanced agronomic traits

Cotton transformation is performed as generally described in WO0036911 and in U.S. Pat. No. 5,846,797. Transgenic cotton plants containing each of the recombinant DNA construct having a sequence of SEQ ID NO: 1 through SEQ ID NO: 95 are obtained by transforming with recombinant DNA from each of the genes identified in Table 1. Progeny transgenic plants are selected from a population of transgenic cotton events under specified growing conditions and are compared with control cotton plants. Control cotton plants are substantially the same cotton genotype but without the recombinant DNA, for example, either a parental cotton plant of the same genotype that was not transformed with the identical

recombinant DNA or a negative isolate of the transformed plant. Additionally, a commercial cotton cultivar adapted to the geographical region and cultivation conditions, i.e. cotton variety ST474, cotton variety FM 958, and cotton variety Siokra L-23, are used to compare the relative performance of the transgenic cotton plants containing the recombinant DNA.

5 The specified culture conditions are growing a first set of transgenic and control plants under “wet” conditions, i.e. irrigated in the range of 85 to 100 percent of evapotranspiration to provide leaf water potential of -14 to -18 bars, and growing a second set of transgenic and control plants under “dry” conditions, i.e. irrigated in the range of 40 to 60 percent of evapotranspiration to provide a leaf water potential of -21 to -25 bars. Pest control, such as
10 weed and insect control is applied equally to both wet and dry treatments as needed. Data gathered during the trial includes weather records throughout the growing season including detailed records of rainfall; soil characterization information; any herbicide or insecticide applications; any gross agronomic differences observed such as leaf morphology, branching habit, leaf color, time to flowering, and fruiting pattern; plant height at various points during
15 the trial; stand density; node and fruit number including node above white flower and node above crack boll measurements; and visual wilt scoring. Cotton boll samples are taken and analyzed for lint fraction and fiber quality. The cotton is harvested at the normal harvest timeframe for the trial area. Enhanced water use efficiency is indicated by increased yield, improved relative water content, enhanced leaf water potential, increased biomass, enhanced
20 leaf extension rates, and improved fiber parameters.

The transgenic cotton plants of this invention are identified from among the transgenic cotton plants by agronomic trait screening as having increased yield and enhanced water use efficiency.

25 **Example 5. Canola transformation**

This example illustrates plant transformation useful in producing the transgenic canola plants of this invention and the production and identification of transgenic seed for transgenic canola having enhanced water use efficiency, enhanced cold tolerance, increased yield, enhanced nitrogen use efficiency, enhanced seed protein and enhanced seed oil.

30 Tissues from *in vitro* grown canola seedlings are prepared and inoculated with overnight-grown *Agrobacterium* cells containing the recombinant DNA construct containing the DNA segment for the gene of interest cassette and a plant selectable marker cassette. Following co-cultivation with *Agrobacterium*, the infected tissues are allowed to grow on selection to promote growth of transgenic shoots, followed by growth of roots from the

transgenic shoots. The selected plantlets are then transferred to the greenhouse and potted in soil. Molecular characterization are performed to confirm the presence of the gene of interest, and its expression in transgenic plants and progenies. Progeny transgenic plants are selected from a population of transgenic canola events under specified growing conditions and are compared with control canola plants. Control canola plants are substantially the same canola genotype but without the recombinant DNA, for example, either a parental canola plant of the same genotype that is not transformed with the identical recombinant DNA or a negative isolate of the transformed plant

Transgenic canola plant cells are transformed with recombinant DNA construct from each of the genes identified in Table 1. Transgenic progeny plants and seed of the transformed plant cells are screened for enhanced water use efficiency, enhanced cold tolerance, increased yield, enhanced nitrogen use efficiency, enhanced seed protein and enhanced seed oil as reported in Example 7.

Example 6. Homolog Identification

This example illustrates the identification of homologs of proteins encoded by the DNA identified in Table 1 which is used to provide transgenic seed and plants having enhanced agronomic traits. From the sequence of the homologs, homologous DNA sequence can be identified for preparing additional transgenic seeds and plants of this invention with enhanced agronomic traits.

An "All Protein Database" was constructed of known protein sequences using a proprietary sequence database and the National Center for Biotechnology Information (NCBI) non-redundant amino acid database (nr.aa). For each organism from which a polynucleotide sequence provided herein was obtained, an "Organism Protein Database" was constructed of known protein sequences of the organism; it is a subset of the All Protein Database based on the NCBI taxonomy ID for the organism.

The All Protein Database was queried using amino acid sequences provided herein as SEQ ID NO: 96 through SEQ ID NO: 193 using NCBI "blastp" program with E-value cutoff of $1e-8$. Up to 1000 top hits were kept, and separated by organism names. For each organism other than that of the query sequence, a list was kept for hits from the query organism itself with a more significant E-value than the best hit of the organism. The list contains likely duplicated genes of the polynucleotides provided herein, and is referred to as the Core List. Another list was kept for all the hits from each organism, sorted by E-value, and referred to as the Hit List.

The Organism Protein Database was queried using polypeptide sequences provided herein as SEQ ID NO: 96 through SEQ ID NO: 193 using NCBI “blastp” program with E-value cutoff of 1e-4. Up to 1000 top hits were kept. A BLAST searchable database was constructed based on these hits, and is referred to as “SubDB”. SubDB was queried with each sequence in the Hit List using NCBI “blastp” program with E-value cutoff of 1e-8. The hit with the best E-value was compared with the Core List from the corresponding organism. The hit is deemed a likely ortholog if it belongs to the Core List, otherwise it is deemed not a likely ortholog and there is no further search of sequences in the Hit List for the same organism. Homologs from a large number of distinct organisms were identified and are reported by amino acid sequences of SEQ ID NO: 194 through SEQ ID NO: 2166. The relationship of proteins of SEQ ID NO: 96 through 193 and homologs of SEQ ID NO: 194 through 2166 is identified in Table 8. The source organism for each homolog is found in the Sequence Listing.

Table 8.

15 PEP SEQ ID NO: homolog SEQ ID Nos

	96:	1993	479	2151	697	1028	1041	1751	979	522	1247		
	97:	747	592	1014	1740	1904	1641	1354	1207	2134	1569	1635	407
		602	635	397	2029	975							
	98:	1246	1099	1100	2152	1152	1301	1474	1482	1994	1196	737	736
20		324	1078	1076	615	339	322	335	775	608	611	613	1745
		1744	1742	1697	486	2049	1971	454	1472	789	1829	1058	1941
		368	574	1555	1416	1228	1020	456	205	1081	2047	1746	489
		821	1409	2081	534	330	1884	2138	1748	1621	1361	1505	1199
		1985	1756	371	693	1702	1287	1766	1571	1573	550	552	1726
25		1891	1727	1633	634	253	457	288	290	398	405	401	1516
		1356	2038	551	417	1553	2055	863	1284	1282	1917	1102	735
		768	531	720	616	466	772	1708					
	99:	1041	1906	491	927	1788	1116	404	1636	1231	1676	1684	139
		136	135	133	1132	1026							
30	100:	1041	1906	491	927	1788	1116	404	1636	1231	1676	1684	139
		136	135	133	1132	1026							
	101:	894	956	458	1085	809	1448	102	1855	797	1620	714	505
		1339	927	715	979	1805	639	384	578				
	102:	956	1085	458	809	1145	1448	505	927	715	570	459	1018
35		1104	477	810	455	654	101	578	1704				
	103:	1041	1906	491	927	1788	1116	404	1636	1231	1676	1684	139
		136	135	133	1132	1026							
	104:	781	1325	2061	1348	1350	1346	467	256	903	915	1759	1882
		1432	1433	734	669	1639	575	1203	544	525	1547	970	1403
40		1665	1366	652	1566	920	1984	470	319	1288	1290	1375	1296
		1395	1328	1330	1311	292	661	2131	2146	2085	542	916	1519
		1367	1364	1313	1314	1324	1355	1352	1293	1294	1378	1401	1399
		1310	2068	2082	1160	1773							
	105:	1497	1872	730	361	732	2066	1954	690	201	1359	473	1879
45		745	1129	411	415	1715	2144	1074	1476	1417			
	106:	806	869	1412	264	265	248	246	1096	286	312	238	1864
		1860	1243	1261	744	428	930	496	629	851	852	2148	497
		516	933	1814	1478	1463	573	377	641	867	665	221	1455
		991	653	601	1524	1418	526	883	2110	1185	1368	1630	1441
50		2004	1771	919	1098	1138	424	1731	1379	413	1186	778	833
		1172	2034	1607	1791	147	144	143	887	504	988	228	936
		499	1931	403	382	609	680	733	982	1725	1713	1711	1710

		414	1044									
	107:	1369	1400	831	344	218	588	1863	1489	2031	1450	375 1709
		798	1837	1681	543	1500	1005	1159	1900			
5	108:	1936	853	1935	2105	350	594	893	2101	1268	1233	866 1422
	109:	1151	1167	1166	1178	1358	630	500	538	535	1413	1564 1721
		198	1898	723	1411	1019	1316	1331	1318	1999	2007	1822 519
		709	329	840	1511	1502	766	671	1951	1436	501	610 612
		539	1414	648	1263	997						
10	110:	769	1861	1156	1828	1093	568	677	1659	252	250	370 276
		197	1133	275	262	261	259	257	241	239	236	889 1012
		1209										
	111:	781	1325	2061	1348	1350	1346	1332	196	903	915	1759 1882
		1639	2006	667	2035	652	1566	1169	830	1220	2051	920 470
15		1105	1103	1290	1288	1375	1370	1296	1395	1328	1330	1311 509
		579	1488	628	292	1875	952	1504	1803	779	1430	285 1857
		1598	1718	796	1101	1739	423	542	916	1519	945	1313 1364
		1367	1324	1314	1352	1355	1293	1294	1378	1401	1399	1310 2082
		2068	1597	1160	1773	1599	1586	1582	1976	1580	1722	1545
20	112:	823	1253	1750	247	1859	1753	2147	1752	1544	688	1631 1347
		1769	875	510	503	1663	374	722	752	942	2128	512 987
		1298	1066									
	113:	1396	1221	433	326	406	2093	1273	1670	1655	1672	2031 1147
		338	1712	553	619	986	1479	792	1837	1681	543	741
25	114:	1144	1143	1813	386	995	502	708	707	1886	206	1007 1279
		1809	1389	1657	1644	882	1075	1069	911	1867	1023	223 1934
		1541	356	1960	2062	914	1051	2057	672	476	394	438 1637
		605	1079	1513	1795	1862	2028	1970	1983	646	304	971 1577
		1539	755	1624	1895	2098	913					
30	115:	800	2016	213	1056	254	799	1217	600	334	848	1134 444
		185	1950	2046	1468	1888	1124	1923	953	985	1587	1000 999
		1001	716	1877	828	1887	1937	621				
	116:	800	2016	213	1056	799	1217	848	600	334	1134	444 1139
		185	1950	904	985	1587	1000	999	372	1162	1887	1937 1768
		1091										
35	117:	203	711	1833	1765	1956	1295	410	1050	822	1496	1781 762
		1095	981	1006	1097	1827	1611	1632	559	527	1512	445 1473
	118:	853	1935	2105	393	1499	993	1595	1514	478	299	1873 1148
		893	1552	803	2090	1646	1533	1615	1260	866		
40	119:	1517	1030	1583	359	655	1521	327	1276	815	1323	245 318
		962	2078	549	888	296	2084	513	422	378	1398	1501 1785
		679	2037	864	1986	390	463	1525	1576	306	1674	1245 380
		1142	367	1821	1437	767	923	1878	2043	1381	1678	465 1550
		1618	902	137								
45	120:	150	121	1491	1380							
	121:	120	150	1491	1380							
	122:	659	1724	1835	507	1242	836	738	895	1572	1625	1218 879
		1990	365	1299	1425	597	790	1338	1945	1691	1737	2129 1690
		924	791	855	123	2154	1852	837	1981	2112		
50	123:	659	1724	1835	507	1242	836	738	895	1572	1625	1218 879
		1990	365	1299	1425	597	790	1338	1945	1691	1737	2129 1690
		924	791	855	122	211	2154	1852	837	1981	2112	
	124:	1177	1754	521	1254	2067	471	2012	1673	834	1280	1464 400
		807	622	1027	1939	1556	426	565	1360	1452	493	1569 1200
		1892	321	2071	427	1831	1557	1701	1890	1889	785	1334 1149
55		1255	1438	1554	1838							
	125:	1475	1302	388	2165	2070	2073	2065	865	1077	484	589 487
		604	1067	1068	760	994	585	495	890	1885	876	1285 2033
		222	1762	1015	801	839	842	1871	844	435	857	859 650
		1237	1238	861	1111	2111	432	694	1929	314	1170	1942 1908
60		1560	1216	1964	537	311	825	841	1530	1002	1909	443 1682
		664	572	2083	1070	1153						
	126:	894	956	2013	430	1041	1448	713	1021	591	325	786 928
		1034	1522	1506	1743	1905	522					
65	127:	1503	1108	1543	1315	1881	1922	1239	2030	266	873	
	128:	1528	387	206	1007	271	607	1692	1991	1848	1345	963 1651
		1213	249	1903	402	186	153	773	1901	1874	412	1190 480
		1297	1003	1122	898	1540	660	564	1616	1051	555	683 1987
		273	244	672	438	1384	1520	698	793	2052	1173	756 336

		1269	1094	2145	1234	1807	943	1949	776	704	1486	1961	1776
		482	625	1775	483	1526							
5	129:	1528	387	206	1007	271	607	1692	1991	1848	1345	963	1651
		1213	249	1903	402	186	153	773	1901	1874	412	1190	480
		1297	1003	1122	898	1540	660	564	1616	1051	555	683	1987
		273	244	672	438	1384	1520	698	793	2052	1173	756	336
		1269	1094	2145	1234	1807	943	1949	776	704	1486	1961	1776
		482	625	1775	483	1526							
10	130:	1528	387	206	1007	271	607	1692	1991	1848	1345	963	1651
		1213	249	1903	402	186	153	773	1901	1874	412	1190	480
		1297	1003	1122	898	1540	660	564	1616	1051	555	683	1987
		273	244	672	438	1384	1520	698	793	2052	1173	756	336
		1269	1094	2145	1234	1807	943	1949	776	704	1486	1961	1776
		482	625	1775	483	1526							
15	131:	1528	387	206	1007	271	607	1692	1991	1848	1345	963	1651
		1213	249	1903	402	186	153	773	1901	1874	412	1190	480
		1297	1003	1122	898	1540	660	564	1616	1051	555	683	1987
		273	244	672	438	1384	1520	698	793	2052	1173	756	336
		1269	1094	2145	1234	1807	943	1949	776	704	1486	1961	1776
		482	625	1775	483	1526							
20	132:	1528	387	206	1007	271	607	1692	1991	1848	1345	963	1651
		1213	249	1903	402	186	153	773	1901	1874	412	1190	480
		1297	1003	1122	898	1540	660	564	1616	1051	555	683	1987
		273	244	672	438	1384	1520	698	793	2052	1173	756	336
25		1269	1094	2145	1234	1807	943	1949	776	704	1486	1961	1776
		482	625	1775	483	1526							
	133:	956	1906	927	979	1788	1116	404	1636	1231	1026	103	100
		99	1834										
30	134:	1157	758	451	548	1281	832	1136	1816	1804	710	858	289
		618	1049	1933	532	1567	1256	878	620	1031	2118		
	135:	956	1906	927	979	1788	1116	404	1636	1231	1026	103	100
		99	1834										
	136:	956	1906	927	979	1788	1116	404	1636	1231	1026	103	100
		99	1834										
35	137:	1517	1030	1583	359	655	1521	327	1276	815	1323	245	318
		962	2078	549	888	296	2084	513	422	378	1398	1501	1785
		679	2037	864	1986	390	463	1525	1576	306	1674	1245	380
		1142	367	1821	1437	767	923	1878	2043	1381	1678	465	1550
		1618	902	119									
40	138:	1144	708	202	364	1321	219	216	1541	356	603	488	587
		581	2126	394	438	1272	1666	225	651	1604	1602	989	1060
		1594	243	1082	1054	1970	1865						
	139:	956	1906	927	979	1788	1116	404	1636	1231	1026	103	100
		99	1834										
45	140:	416	1799	662	298	1278	1126	1694	1176	1187	1819	2119	1397
		468	1664	1493	932	1393	1498	2088	1811	1259	1184	369	1008
		1009	1966	395	1036	1037	1039	1033	1995	1992	1040	1071	1065
		1155	1141	352	354	1926	1826	1661	1053	301	1642	233	1627
		2048	1593	1613	1483	1667	1010	437	1125	1080	1092	529	331
50		347	1982	1165	1568	1542	1451						
	141:	1320	2158	541	295	1150	984	284	743	1714	1728	2008	1136
		1823	847	1508	2115	2130	1121	1977	1802	481	1466	1244	1140
		717	1996	1979	461								
55	142:	806	1210	871	1412	248	265	264	246	2075	286	312	238
		1243	744	428	629	1131	2148	1110	1426	497	933	1814	1927
		1924	641	1918	1578	926	958	1847	1197	2096	939	1368	1630
		1441	886	490	1731	1729	782	557	1227	1419	1179	2045	1792
		1774	1186	1219	1423	977	1607	1791	1763	1761	1789	1777	682
		644	268	226	623	829	1793	1836	1265	1790	263	258	260
60		731	566	1376	1720	447	1579	850	1953	676	849	1308	1869
		2092	567	967	2009	499	936	1931	403	382	609	680	733
		982	1725	1713	1711	1710	1693	1214					
	143:	806	1191	1194	869	868	1412	1303	264	265	248	246	1096
		286	312	315	238	1864	1860	1243	1261	744	428	930	496
65		629	851	852	1127	1128	2148	497	516	933	1017	1814	1478
		1463	1212	573	377	641	653	601	867	991	1455	665	1524
		221	1418	526	883	2110	1185	1630	1368	1441	2004	1771	919
		1098	316	881	1138	424	816	1731	1792	1774	1186	464	778

		1916	1955	1219	827	826	724	1423	725	727	833	1172	2034
		977	1607	1791	228	106	1608	1044	1154	936	499	1931	403
		382	609	680	733	982	1725	1713	1711	1710	414	887	
5	144:	806	1191	1194	869	868	1412	1303	264	265	248	246	1096
		286	312	315	238	1864	1860	1243	1261	744	428	930	496
		629	851	852	1127	1128	2148	497	516	933	1017	1814	1478
		1463	1212	573	377	641	653	601	867	991	1455	665	1524
		221	1418	526	883	2110	1185	1630	1368	1441	2004	1771	919
10		1098	316	881	1138	424	816	1731	1792	1774	1186	464	778
		1916	1955	1219	827	826	724	1423	725	727	833	1172	2034
		977	1607	1791	228	106	1608	1044	1154	936	499	1931	403
		382	609	680	733	982	1725	1713	1711	1710	414	887	
	145:	1570	294	1640	2053	1248	1703	1252	547	1201	1362	1386	2132
		712	1327	453	363	1841	379	212	921	2060	1340	446	998
15		891	961	627	1928	910	805	1064	1899	1469	2108	1716	237
		1972	1038	949	1732	1289	1808	1824	2010	695	560	1029	1946
		1089	657	1088	158	1730	328						
	146:	769	281	279	282	267	1861	2102	978	1106	569	421	1638
		360	2143	370	2032	1283	276	272	270	261	241	275	262
20		257	236	259	239	889	1012						
	147:	806	1191	1194	869	868	1412	1303	264	265	248	246	1096
		286	312	315	238	1864	1860	1243	1261	744	428	930	496
		629	851	852	1127	1128	2148	497	516	933	1017	1814	1478
		1463	1212	573	377	641	653	601	867	991	1455	665	1524
25		221	1418	526	883	2110	1185	1630	1368	1441	2004	1771	919
		1098	316	881	1138	424	816	1731	1792	1774	1186	464	778
		1916	1955	1219	827	826	724	1423	725	727	833	1172	2034
		977	1607	1791	228	106	1608	1044	1154	936	499	1931	403
		382	609	680	733	982	1725	1713	1711	1710	414	887	
30	148:	210	1183	1548	1291	1471	2140	436	1120	1119	1115	1118	2141
		687	1656	1534	1286	905	1251	1372	313	1603	1300	638	1181
		1198	220										
	149:	1277	524	955	658	1609	317	1180	1536	355	353	357	2005
		399	229	645	425	1561	1958	1894	1312	1940	1783	1250	1390
35		1391	1507	917	227	640	996	2106	1446	518	1649	606	794
		520	1610	1492	1208	1223	1800	1851	1706	1914	2039	941	1870
		1698	434	2040	293	1778	1733	1322	1059	449	376	1846	2103
		1025	1107										
	150:	120	121	1491	1380								
40	151:	696	333	1013	1336	1257	1606	804	392	506	835	1193	593
		1114	637	974	556	1734	906	701	1371	1086	1683	2095	1612
		862	1896	362	419	1974	813	765					
	152:	1369	1400	831	344	218	588	1863	1489	2031	1450	375	1709
		798	1837	1681	543	1500	1005	1159	1900				
45	153:	1144	1143	1813	386	995	502	1668	1007	1279	271	1651	1024
		1541	356	1540	660	1689	1051	488	2121	555	683	1987	581
		132	131	130	129	128	973	672	438	1272	1666	1384	1520
		698	1173	1513	336	1269	756	943	1949	776	243	1241	1970
		646	304	402									
50	154:	1405	1794	1087	2097	1304	1035	681	969	1628	2163	2100	452
		1055	636	899	1083	885	2077	1549	582	1700	1947	1932	1084
	155:	781	1325	2061	1348	1350	1346	467	256	903	915	1432	1433
		1685	1688	1699	1669	1806	159	111	1224	1722	1645	748	1545
		1853	1707	345	1798	429	787	278	1639	901	2006	614	1236
55		544	525	1547	667	1566	1169	1220	830	2051	920	1984	2036
		470	319	1105	1288	1290	1375	1296	1395	1328	1330	1311	579
		292	1875	1718	796	542	916	1519	945	1313	1367	1364	1314
		1324	1352	1355	1293	1294	1378	1401	1399	1310	2068	2082	1597
		1160	1773	1586	1599	1976	1582	1580	1817	673			
60	156:	586	1434	2042	280	1523	2079	1647	705	817	1820	1915	617
		1830	1135	2125	2149	1858	492	1757	819	918	818	759	692
	157:	1517	1030	1583	359	655	1705	740	439	2020	1973	908	1090
		1796	1696	962	318	2084	2017	1057	1117	422	378	684	554
		1501	474	679	1910	864	880	624	1584	1307	234	1204	306
65		546	1674	1782	1245	396	1189	1211	678	367	1779	1821	1437
		870	1878	2043	2058	1686	445	1381	1678	558	465	1550	1618
		892	1680										
	158:	1570	294	1640	2053	1248	1252	1703	547	1201	1362	1386	2132

		712	1327	453	363	212	1841	379	2060	446	998	1340	961
		627	910	805	1928	1899	1469	1052	2108	1716	237	1972	1038
		949	1289	1808	1824	2010	695	560	1029	1946	1089	657	1088
		1730	328										
5	159:	781	1325	2061	1348	1350	1346	1332	196	903	915	1759	1882
		1639	2006	667	2035	652	1566	1169	830	1220	2051	920	470
		1105	1103	1290	1288	1375	1370	1296	1395	1328	1330	1311	509
		579	1488	628	292	1875	952	1504	1803	779	1430	285	1857
		1598	1718	796	1101	1739	423	542	916	1519	945	1313	1364
10		1367	1324	1314	1352	1355	1293	1294	1378	1401	1399	1310	2082
		2068	1597	1160	1773	1599	1586	1582	1976	1580	1722	1545	
	160:	1570	294	1640	2053	1248	1252	1703	547	1201	1362	1386	2132
		712	1327	453	363	1559	2091	1735	1596	1402	385	1842	1695
		703	1484	1373	961	1658	563	2104	702	1736	1662	1899	1469
15		1052	1767	699	1801	237	1716	240	242	1972	1038	528	2021
		1249	300	1824	2010	695	560	1029	1946	1089	657	1088	1854
		164	162	161	165	420	1113	1738	685	1843	1415		
	161:	1570	294	1640	2053	1248	1252	1703	547	1201	1362	1386	2132
		712	1327	453	363	1559	2091	1735	1596	1402	385	1842	1695
20		703	1484	1373	961	1658	563	2104	702	1736	1662	1899	1469
		1052	1767	699	1801	237	1716	240	242	1972	1038	528	2021
		1249	300	1824	2010	695	560	1029	1946	1089	657	1088	165
		164	162	1854	160	420	1113	1738	685	1843	1415		
	162:	1570	294	1640	2053	1248	1252	1703	547	1201	1362	1386	2132
25		712	1327	453	363	1559	2091	1735	1596	1402	385	1842	1695
		703	1484	1373	961	1658	563	2104	702	1736	1662	1899	1469
		1052	1767	699	1801	237	1716	240	242	1972	1038	528	2021
		1249	300	1824	2010	695	560	1029	1946	1089	657	1088	165
		164	161	1854	160	420	1113	1738	685	1843	1415		
30	163:	1605											
	164:	1570	294	1640	2053	1248	1252	1703	547	1201	1362	1386	2132
		712	1327	453	363	1559	2091	1735	1596	1402	385	1842	1695
		703	1484	1373	961	1658	563	2104	702	1736	1662	1899	1469
		1052	1767	699	1801	237	1716	240	242	1972	1038	528	2021
35		1249	300	1824	2010	695	560	1029	1946	1089	657	1088	162
		165	161	1854	160	420	1113	1738	685	1843	1415		
	165:	1570	294	1640	2053	1248	1252	1703	547	1201	1362	1386	2132
		712	1327	453	363	1559	2091	1735	1596	1402	385	1842	1695
		703	1484	1373	961	1658	563	2104	702	1736	1662	1899	1469
40		1052	1767	699	1801	237	1716	240	242	1972	1038	528	2021
		1249	300	1824	2010	695	560	1029	1946	1089	657	1088	161
		162	164	1854	160	420	1113	1738	685	1843	1415		
	166:	1144	708	202	364	1321	219	216	1541	356	603	488	587
		581	2126	394	438	1272	1666	225	651	1604	1602	989	1060
45		1594	243	1082	1054	1970	1865						
	167:	1177	1754	521	1254	2067	471	2012	1673	834	1280	1464	400
		807	622	1027	1939	1556	426	565	1360	1452	493	1569	1200
		1892	321	2071	427	1831	1557	1701	1890	1889	785	1334	1149
		1255	1438	1554	1838								
50	168:	1475	1302	388	2165	2070	2073	2065	865	1077	484	589	487
		604	1067	1068	760	994	585	495	890	1885	876	1285	2033
		222	1762	1015	801	839	842	1871	844	435	857	859	650
		1237	1238	861	1111	2111	432	694	1929	314	1170	1942	1908
		1560	1216	1964	537	311	825	841	1530	1002	1909	443	1682
55		664	572	2083	1070	1153							
	169:	1320	2158	541	295	1150	984	284	743	1714	1728	2008	1136
		1823	847	1508	2115	2130	1121	1977	1802	481	1466	1244	1140
		717	1996	1979	461								
60	170:	806	1210	871	1412	248	265	264	246	2075	286	312	238
		1243	744	428	629	1131	2148	1110	1426	497	933	1814	1927
		1924	641	1918	1578	926	958	1847	1197	2096	939	1368	1630
		1441	886	490	1731	1729	782	557	1227	1419	1179	2045	1792
		1774	1186	1219	1423	977	1607	1791	1763	1761	1789	1777	682
		644	268	226	623	829	1793	1836	1265	1790	263	258	260
65		731	566	1376	1720	447	1579	850	1953	676	849	1308	1869
		2092	567	967	2009	499	936	1931	403	382	609	680	733
		982	1725	1713	1711	1710	1693	1214					
	171:	416	1799	662	298	1278	1126	1694	1176	1187	1819	2119	1397

		468	1664	1493	932	1393	1498	2088	1811	1259	1184	369	1008
		1009	1966	395	1036	1037	1039	1033	1995	1992	1040	1071	1065
		1155	1141	352	354	1926	1826	1661	1053	301	1642	233	1627
		2048	1593	1613	1483	1667	1010	437	1125	1080	1092	529	331
5		347	1982	1165	1568	1542	1451						
	172:	894	956	2013	430	1041	1448	713	1021	591	325	786	928
		1034	1522	1506	1743	1905	522						
	173:	203	711	1833	1765	1956	1295	410	1050	822	1496	1781	762
		1095	981	1006	1097	1827	1611	1632	559	527	1512	445	1473
10	174:	2086	2089	2087	1061	1588	1980	757	1601	1581	2120	2107	1679
		1818	1998	1997	1959	1309	1270	2056	2001	2002	2003	2018	1978
		761	700	1271	2041	1975	283	291	2026	1206	1363	1495	1565
		1048	1537	1016	2011	1989	1652	561	929	753	1531	1515	754
		631	912	1687	1264	200	1952	2123	2135	656	960	381	966
15		2063	937	545	1222	1072	1392	590	2150	1856	1262	959	925
		1589	940	1591	1614	811	343	1797	1205	571	1832	877	1510
		1011	944	846	938	948	649	726	897	1292	1047	1944	1962
		964	965	1592	968	1509	277	1893	909	1435	1648	1866	1357
		1377	643	2074	2072	1623	1527	1957	1342	1344	763	1319	472
20		632	647	633	856	1353	1158	1410	1046	195	2166	2069	2064
		907	2139	1677	2159	2160	2136	214	232	224	1845	2022	2023
		1485	1487	383	440	1913	675	1600	946	274	1192	1229	175
		323	1335										
	175:	2086	2089	1061	1588	1349	1351	1921	235	1980	757	1601	1581
25		2120	2107	1535	1538	1679	1818	1998	1997	1959	1309	1270	2056
		1168	2001	2002	2003	2018	1978	700	1271	2041	1975	283	291
		2026	2024	1206	1363	1495	1565	1048	1537	1016	2011	1989	1652
		561	929	753	1634	1531	1515	754	1383	631	912	1687	1264
		200	1952	2123	656	960	381	966	2063	1222	1072	1392	590
30		2150	1856	1262	959	925	1589	940	1591	1614	811	343	1797
		1205	571	1832	877	1510	1011	944	846	938	948	649	726
		897	1292	1047	1944	1962	964	1741	1592	965	968	1509	1590
		1341	277	1893	909	1435	1648	1866	1377	1357	643	2074	2072
		1623	1527	1957	1342	1344	763	1319	472	632	647	856	1353
35		1158	1046	2069	2064	907	2139	1677	2159	2160	214	224	232
		1845	750	721	2022	2023	1485	1487	383	440	1913	441	1600
		946	1335	1229	174	1192	274						
	176:	2086	1911	1456	1447	1449	751	1581	2120	1780	1431	208	1998
		1997	1959	1306	2056	1168	2001	2003	2018	1978	700	1465	1444
40		1461	1429	1428	1967	1912	1271	2041	1215	1439	1457	1442	1016
		1634	1585	596	631	215	217	200	1952	2123	656	950	960
		207	209	381	2027	1671	749	1258	2063	1222	1195	1551	1902
		462	194	1392	450	1424	1407	1408	1558	1109	959	925	1591
		940	1385	1382	1614	494	2116	1815	877	1510	944	1460	771
45		783	770	1274	938	1387	1275	1988	580	1626	2153	964	1592
		965	968	1787	418	1719	1230	460	1619	1232	1305	297	1494
		2059	1377	1333	1948	517	643	691	2074	1844	1410	2166	1643
		907	2139	954	2022	2023	1490	1406	1427	946	2137		
	177:	303	408	860	2124	373	1812	795	729	814	2015		
50	178:	781	1325	2061	1350	1348	1346	467	903	915	1759	1882	1685
		1688	808	2117	2094	544	1547	1770	1566	1174	920	1984	2036
		470	319	1288	1290	1375	1395	1296	1330	1328	1311	342	1758
		1723	292	1532	2044	990	1112	764	668	1063	1123	1969	542
		916	945	1367	1364	1313	1314	1324	1355	1352	1293	1294	1378
55		1401	1399	1310	2082	2068	1597	1160	1773	1529	2025		
	179:	303	408	860	2124	373	1812	795	729	814	2015		
	180:	1754	508	469	1907	1965	595	1464	1458	400	514	1617	1810
		1839	565	348	366	389	391	431	1825	1004			
	181:	1919	972	351	349	1188	2164	1749	305	287	1329	983	935
60		1876	780	1747	1883	1175	1454	1326	2161	1042	1920	1654	843
		1717	2014	900	824	820	530	1653					
	182:	1225	666	2099	1453	1467	774	1337	1137	562	2080	1235	1574
		1963	1518	598	670	1462	719	442	523	1443	947	2000	1445
		2054	1675	931	706	584	1240	718	838	1764	2114	2109	1480
65		1772	1022	1171	332								
	183:	1528	1786	1660	387	502	1562	204	1007	992	1880	1073	788
		686	1388	1267	1840	2019	2050	1938	1943	1546	872	1024	485
		1934	1622	1629	1541	340	674	1477	742	511	922	231	1960

		2062	1440	1689	1051	2122	1755	583	2156	2162	188	2127	1481
		1563	1897	672	394	225	784	1526	448	1795	302	1862	2028
		646	304	310	739	1459							1983
	184:	1754	508	469	1907	1965	595	1464	1458	400	514	1617	1810
5		1839	565	348	366	389	391	431	1825	1004			
	185:	2016	213	1056	254	799	1217	600	848	334	1134	444	1343
		1124	1923	904	812	953	985	1587	1000	999	716	1877	828
		372	1162	854	934	599	884	1887	115	621	251	1091	1768
		116	2155										
10	186:	1144	1143	1813	386	995	502	1668	1007	1279	271	1651	1024
		1541	356	1540	660	1689	1051	488	2121	555	683	1987	581
		132	131	130	129	128	973	672	438	1272	1666	1384	1520
		698	1173	1513	336	1269	756	943	1949	776	243	1241	1970
		646	304	402									
15	187:	269	1470	1317	255	976	320	957	1849	1365	1760	577	1968
		1161	576	777	308								
	188:	502	1562	1007	992	1073	1880	788	686	1840	2019	1938	1267
		1388	2050	1943	1546	872	663	1024	485	1629	1622	674	1477
		340	511	742	922	231	1130	1202	1226	1440	1689	1051	2122
20		1755	583	1266	1563	672	1868	784	346	802	309	2076	358
		1146	475	1575	199	536	1420	1421	1394	310	739	183	1062
		1459	2133	2157	409	625	689	646	304	2162			
	189:	1163	896	1164	1850	337	341	1032	874	1182	230	307	540
		626	1374	1045	728	746	2113	2142	533	951	980	1043	1784
25		845	1650	1925	498	515	1404	1930	642				
	190:	1919	972	351	349	1188	2164	1749	305	287	1329	983	935
		1876	780	1747	1883	1175	1454	1326	2161	1042	1920	1654	843
		1717	2014	900	824	820	530	1653					
30													

Example 7. Selection of Transgenic Plants with Enhanced Agronomic Trait(s)

This example illustrates identification of transgenic plant cells of the invention by screening derived plants and seeds for enhanced trait. Transgenic seed and plants in corn, soybean, cotton or canola with recombinant DNA constructs identified in Table 1 are prepared by plant cells transformed with DNA that is stably integrated into a chromosome of the plant cell. Progeny transgenic plants and seed of the transformed plant cells are screened for enhanced water use efficiency, enhanced cold tolerance, increased yield, enhanced nitrogen use efficiency, enhanced seed protein and enhanced seed oil as compared to control plants

A. Selection for enhanced Nitrogen Use Efficiency (NUE)

Transgenic corn seeds provided by the present invention are planted in fields with three levels of nitrogen (N) fertilizer being applied, i.e. low level (0 N), medium level (80 lb/ac) and high level (180 lb/ac). A variety of physiological traits are monitored. Plants with enhanced NUE provide higher yield as compared to control plants.

B. Selection for increased yield

Effective selection of enhanced yielding transgenic plants uses hybrid progeny of the transgenic plants for corn, cotton, and canola, or inbred progeny of transgenic plants for soybean, canola and cotton over multiple locations with plants grown under optimal

production management practices, and maximum pest control. A useful target for improved yield is a 5% to 10% increase as compared to yield produced by plants grown from seed for a control plant. Selection methods may be applied in multiple and diverse geographic locations, for example up to 16 or more locations, over one or more planting seasons, for example at least two planting seasons, to statistically distinguish yield improvement from natural environmental effects.

C. Selection for enhanced water use efficiency (WUE)

The selection process imposes a water withholding period to induce drought stress followed by watering. For example, for corn, a useful selection process imposes 3 drought/re-water cycles on plants over a total period of 15 days after an initial stress free growth period of 11 days. Each cycle consists of 5 days, with no water being applied for the first four days and a water quenching on the 5th day of the cycle. The primary phenotypes analyzed by the selection method are the changes in plant growth rate as determined by height and biomass during a vegetative drought treatment.

D. Selection for Growth Under Cold Stress

(1) Cold germination assay - Trays of transgenic and control seeds are placed in a growth chamber at 9.7°C for 24 days (no light). Seeds having higher germination rates as compared to the control are identified.

(2) Cold field efficacy trial - A cold field efficacy trial is used to identify recombinant DNA constructs that confer enhanced cold vigor at germination and early seedling growth under early spring planting field conditions in conventional-till and simulated no-till environments. Seeds are planted into the ground around two weeks before local farmers begin to plant corn so that a significant cold stress is exerted onto the crop, named as cold treatment. Seeds also are planted under local optimal planting conditions such that the crop has little or no exposure to cold condition, named as normal treatment. At each location, seeds are planted under both cold and normal conditions with 3 repetitions per treatment. Two temperature monitors are set up at each location to monitor both air and soil temperature daily.

Seed emergence is defined as the point when the growing shoot breaks the soil surface. The number of emerged seedlings in each plot is counted everyday from the day the earliest plot begins to emerge until no significant changes in emergence occur. In addition, for each planting date, the latest date when emergence is 0 in all plots is also recorded. Seedling vigor is also rated at V3-V4 stage before the average of corn plant height reaches 10

inches, with 1=excellent early growth, 5=Average growth and 9=poor growth. Days to 50% emergence, maximum percent emergence and seedling vigor are used to determine plants with enhanced cold tolerance.

E. Screens for transgenic plant seeds with increased protein and/or oil levels

5 This example sets forth a high-throughput selection for identifying plant seeds with improvement in seed composition using the Infratec 1200 series Grain Analyzer, which is a near-infrared transmittance spectrometer used to determine the composition of a bulk seed sample (Table 9). Near infrared analysis is a non-destructive, high-throughput method that can analyze multiple traits in a single sample scan. An NIR calibration for the analytes of
10 interest is used to predict the values of an unknown sample. The NIR spectrum is obtained for the sample and compared to the calibration using a complex chemometric software package that provides predicted values as well as information on how well the sample fits in the calibration.

Infratec Model 1221, 1225, or 1227 with transport module by Foss North America is
15 used with cuvette, item # 1000-4033, Foss North America or for small samples with small cell cuvette, Foss standard cuvette modified by Leon Girard Co. Corn and soy check samples of varying composition maintained in check cell cuvettes are supplied by Leon Girard Co. NIT collection software is provided by Maximum Consulting Inc. Software. Calculations are performed automatically by the software. Seed samples are received in packets or containers
20 with barcode labels from the customer. The seed is poured into the cuvettes and analyzed as received.

Table 9.

Typical sample(s):	Whole grain corn and soybean seeds
Analytical time to run method:	Less than 0.75 min per sample
Total elapsed time per run:	1.5 minute per sample
Typical and minimum sample size:	Corn typical: 50cc; minimum 30cc Soybean typical: 50cc; minimum 5cc
Typical analytical range:	Determined in part by the specific calibration. Corn – moisture 5-15%, oil 5-20%, protein 5-30%, starch 50-75%, and density 1.0-1.3%. Soybean – moisture 5-15%, oil 15-25%,

	and protein 35-50%.
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Example 8. Consensus sequence

This example illustrates the identification of consensus amino acid sequence for the proteins and homologs encoded by DNA that is used to prepare the transgenic seed and plants of this invention having enhanced agronomic traits.

ClustalW program was selected for multiple sequence alignments of the amino acid sequence of SEQ ID NO: 127 and its 10 homologs. Three major factors affecting the sequence alignments dramatically are (1) protein weight matrices; (2) gap open penalty; (3) gap extension penalty. Protein weight matrices available for ClustalW program include Blosum, Pam and Gonnet series. Those parameters with gap open penalty and gap extension penalty were extensively tested. On the basis of the test results, Blosum weight matrix, gap open penalty of 10 and gap extension penalty of 1 were chosen for multiple sequence alignment. Figure 1 shows the sequences of SEQ ID NO: 127, its homologs and the consensus sequence (SEQ ID NO: 2201) at the end. The symbols for consensus sequence are (1) uppercase letters for 100% identity in all positions of multiple sequence alignment output; (2) lowercase letters for $\geq 70\%$ identity; symbol; (3) "X" indicated $< 70\%$ identity; (4) dashes "-" meaning that gaps were in $\geq 70\%$ sequences.

The consensus amino acid sequence can be used to identify DNA corresponding to the full scope of this invention that is useful in providing transgenic plants, for example corn and soybean plants with enhanced agronomic traits, for example improved nitrogen use efficiency, improved yield, improved water use efficiency and/or improved growth under cold stress, due to the expression in the plants of DNA encoding a protein with amino acid sequence identical to the consensus amino acid sequence.

Example 9. Identification of amino acid domain by Pfam analysis

This example illustrates the identification of protein domain and domain module by Pfam analysis.

The amino acid sequence of the expressed proteins that are shown to be associated with an enhanced trait were analyzed for Pfam protein family against the current Pfam collection of multiple sequence alignments and Hidden Markov models using the HMMER software. The Pfam protein domains and modules for the proteins for the proteins of SEQ ID NO: 96

- 5 through 193 are shown in Tables 11 and 10 respectively. The Hidden Markov model databases for the identified pfam domains are allowing identification of other homologous proteins and their cognate encoding DNA to enable the full breadth of the invention for a person of ordinary skill in the art. Certain proteins are identified by a single Pfam domain and others by multiple Pfam domains. For instance, the protein with amino acids of SEQ ID
- 10 NO: 98 is characterized by three Pfam domains, i.e. KNOX1, KNOX2 and ELK.

Table 10.. Pfam domain module annotation

PEP SEQ ID NO	Gene ID	Pfam domain module	Position
154	PHE0001067_8154.pep	Homeobox	97-158
106	PHE0002062_5913.pep	Myb_DNA-binding::Myb_DNA-binding	14-61::67-112
140	PHE0002149_7487.pep	Myb_DNA-binding	24-69
171	PHE0002149_8748.pep	Myb_DNA-binding	24-69
107	PHE0002531_5926.pep	zf-Dof	39-101
152	PHE0002531_7985.pep	zf-Dof	39-101
151	PHE0003473_7927.pep	zf-C2H2::zf-C2H2	72-94::149-171
138	PHE0003664_7436.pep	AP2	21-84
166	PHE0003664_8637.pep	AP2	21-84
137	PHE0003673_7430.pep	Response_reg::Myb_DNA-binding	13-126::197-247
188	PHE0004332_PMON95104.pep	AP2	104-168
187	PHE0004356_PMON93862.pep	B3	23-128
183	PHE0004357_PMON94163.pep	AP2	135-199
153	PHE0004463_8059.pep	AP2	6-69
186	PHE0004463_PMON94432.pep	AP2	6-69
185	PHE0004624_PMON94400.pep	B3::Auxin_resp::AUX_IAA	141-246::268-350::640-805
96	PHE0004633_5508.pep	HLH	104-152
189	PHE0004644_PMON95096.pep	HLH	327-374
184	PHE0004646_PMON94352.pep	NAM	17-139
180	PHE0004646_PMON94356.pep	NAM	17-139
182	PHE0004648_PMON95051.pep	B3::B3	13-105::148-244
181	PHE0004723_PMON94660.pep	AUX_IAA	6
190	PHE0004723_PMON95121.pep	AUX_IAA	6
97	PHE0004738_5674.pep	NAM	96
98	PHE0004814_5801.pep	KNOX1::KNOX2::ELK	38-82::92-

PEP SEQ ID NO	Gene ID	Pfam domain module	Position
			147::203-244
133	PHE0004816_7303.pep	HLH	19-68
136	PHE0004816_7418.pep	HLH	19-68
135	PHE0004816_7421.pep	HLH	19-68
139	PHE0004816_7445.pep	HLH	19-68
99	PHE0004817_5809.pep	HLH	20-69
100	PHE0004817_5810.pep	HLH	20-69
103	PHE0004817_5901.pep	HLH	20-69
101	PHE0004821_5819.pep	HLH	105-154
102	PHE0004828_5826.pep	HLH	107-156
104	PHE0004861_5910.pep	GRAS	26-325
105	PHE0004863_5912.pep	AT_hook::AT_HOOK::DUF296	121-133
119	PHE0004877_7030.pep	Response_reg::Myb_DNA-binding	13-126::197-247
108	PHE0004914_5971.pep	Myb_DNA-binding::Myb_DNA-binding	11-60::117-164
109	PHE0004924_5982.pep	TCP	38-253
110	PHE0004925_5983.pep	SBP	58-136
111	PHE0004938_5994.pep	GRAS	154-454
159	PHE0004938_8370.pep	GRAS	154-454
112	PHE0004957_6019.pep	zf-C2H2	68-90
113	PHE0004958_6020.pep	zf-Dof	104-166
114	PHE0004959_6021.pep	AP2	128-191
115	PHE0004974_6040.pep	B3::Auxin_resp	148-253
116	PHE0004975_6041.pep	B3::Auxin_resp	136-241::263-345
117	PHE0004987_6056.pep	Myb_DNA-binding	21-72
173	PHE0004987_8771.pep	Myb_DNA-binding	21-72
118	PHE0005005_7034.pep	Myb_DNA-binding	96-143
124	PHE0006004_7082.pep	NAM	18-147
167	PHE0006004_8667.pep	NAM	18-147
125	PHE0006022_7105.pep	EIN3	30-426
168	PHE0006022_8690.pep	EIN3	30-426
126	PHE0006023_7240.pep	HLH	160-210
172	PHE0006023_8762.pep	HLH	160-210
120	PHE0006057_7048.pep	HLH	12-61
121	PHE0006057_7053.pep	HLH	12-61
150	PHE0006057_7929.pep	HLH	12-61
122	PHE0006070_7067.pep	bZIP_2	96-153
123	PHE0006073_7072.pep	bZIP_2	96-153
128	PHE0006237_7261.pep	AP2	6-69
130	PHE0006237_7268.pep	AP2	6-69

PEP SEQ ID NO	Gene ID	Pfam domain module	Position
129	PHE0006237_7274.pep	AP2	6-69
131	PHE0006237_7277.pep	AP2	6-69
132	PHE0006237_7284.pep	AP2	6-69
141	PHE0006290_7498.pep	zf-B_box::zf-B_box	1-47::48-90
169	PHE0006290_8689.pep	zf-B_box::zf-B_box	1-47::48-90::355-393
134	PHE0006291_7319.pep	zf-B_box::CCT	3-50::309-347
155	PHE0006350_8201.pep	GRAS	98-403
143	PHE0006384_7737.pep	Myb_DNA-binding::Myb_DNA-binding	14-61::67-112
144	PHE0006384_7789.pep	Myb_DNA-binding::Myb_DNA-binding	14-61::67-112
147	PHE0006384_7839.pep	Myb_DNA-binding::Myb_DNA-binding	14-61::67-112
142	PHE0006423_7664.pep	Myb_DNA-binding::Myb_DNA-binding	14-61::67-112
170	PHE0006423_8696.pep	Myb_DNA-binding::Myb_DNA-binding	14-61::67-112
148	PHE0006448_7859.pep	RWP-RK::PB1	553-604::741-823
149	PHE0006504_7876.pep	F-box::TUB	49-104::115-424
145	PHE0006507_7828.pep	CBFD_NFYB_HMF	1-40
146	PHE0006509_7846.pep	SBP	64-142
158	PHE0006527_8369.pep	CBFD_NFYB_HMF	26-91
157	PHE0006546_8310.pep	Response_reg::Myb_DNA-binding	28-141::225-275
156	PHE0006605_8233.pep	GATA	223-258
163	PHE0006752_8521.pep	SRF-TF	9-59
163	PHE0006752_8521.pep	K-box	75-174
160	PHE0006774_8489.pep	CBFD_NFYB_HMF	34-106
161	PHE0006778_8503.pep	CBFD_NFYB_HMF	34-106
164	PHE0006779_8565.pep	CBFD_NFYB_HMF	34-106
162	PHE0006780_8502.pep	CBFD_NFYB_HMF	34-106
165	PHE0006781_8573.pep	CBFD_NFYB_HMF	34-106
174	PHE0006858_8859.pep	SRF-TF::K-box	9-59::75-174
175	PHE0006860_8863.pep	SRF-TF::K-box	9-59::75-174
177	PHE0006951_9137.pep	zf-C2H2	152-175
179	PHE0006951_9173.pep	zf-C2H2	152-175
176	PHE0006955_9129.pep	SRF-TF::K-box	9-59::85-175
178	PHE0006981_9158.pep	GRAS	149-456

Table 11. Pfam domain annotation

PEP SEQ ID NO	GENE ID	Pfam domain name	Begin	Stop	Score	E-value
154	PHE0001067_8154.pep	Homeobox	97	158	68	2.70E-17
106	PHE0002062_5913.pep	Myb_DNA-binding	14	61	44.5	3.20E-10
106	PHE0002062_5913.pep	Myb_DNA-binding	67	112	47.8	3.20E-11
140	PHE0002149_7487.pep	Myb_DNA-binding	24	69	54.4	3.40E-13
171	PHE0002149_8748.pep	Myb_DNA-binding	24	69	54.4	3.40E-13
107	PHE0002531_5926.pep	zf-Dof	39	101	133.7	4.60E-37
152	PHE0002531_7985.pep	zf-Dof	39	101	133.7	4.60E-37
151	PHE0003473_7927.pep	zf-C2H2	72	94	25.6	0.00016
151	PHE0003473_7927.pep	zf-C2H2	149	171	20.5	0.0055
138	PHE0003664_7436.pep	AP2	21	84	135.2	1.60E-37
166	PHE0003664_8637.pep	AP2	21	84	135.2	1.60E-37
137	PHE0003673_7430.pep	Response_reg	13	126	104.9	2.20E-28
137	PHE0003673_7430.pep	Myb_DNA-binding	197	247	46.4	8.90E-11
188	PHE0004332_PMON95104.pep	AP2	104	168	156.7	5.40E-44
187	PHE0004356_PMON93862.pep	B3	23	128	64.1	4.20E-16
183	PHE0004357_PMON94163.pep	AP2	135	199	150.2	5.10E-42
153	PHE0004463_8059.pep	AP2	6	69	116.5	7.10E-32
186	PHE0004463_PMON94432.pep	AP2	6	69	116.5	7.10E-32
185	PHE0004624_PMON94400.pep	B3	141	246	110.7	4.00E-30
185	PHE0004624_PMON94400.pep	Auxin_resp	268	350	198.6	1.30E-56
185	PHE0004624_PMON94400.pep	AUX_IAA	640	805	-72.2	0.00025
96	PHE0004633_5508.pep	HLH	104	152	39.4	1.10E-08
189	PHE0004644_PMON95096.pep	HLH	327	374	36.7	7.20E-08
184	PHE0004646_PMON94352.pep	NAM	17	139	58.4	2.20E-14
180	PHE0004646_PMON94356.pep	NAM	17	139	58.4	2.20E-14
182	PHE0004648_PMON95051.pep	B3	13	105	117	4.90E-32
182	PHE0004648_PMON95051.pep	B3	148	244	110.3	5.20E-30
181	PHE0004723_PMON94660.pep	AUX_IAA	6	173	339.7	4.50E-99
190	PHE0004723_PMON95121.pep	AUX_IAA	6	173	339.7	4.50E-99
97	PHE0004738_5674.pep	NAM	96	239	184.9	1.80E-52
98	PHE0004814_5801.pep	KNOX1	38	82	63.3	7.00E-16
98	PHE0004814_5801.pep	KNOX2	92	147	83.5	6.00E-22
98	PHE0004814_5801.pep	ELK	203	224	34.1	4.50E-07
133	PHE0004816_7303.pep	HLH	19	68	62.5	1.30E-15
136	PHE0004816_7418.pep	HLH	19	68	62.5	1.30E-15
135	PHE0004816_7421.pep	HLH	19	68	62.5	1.30E-15
139	PHE0004816_7445.pep	HLH	19	68	62.5	1.30E-15
99	PHE0004817_5809.pep	HLH	20	69	57.6	3.70E-14
100	PHE0004817_5810.pep	HLH	20	69	57.6	3.70E-14
103	PHE0004817_5901.pep	HLH	20	69	57.6	3.70E-14
101	PHE0004821_5819.pep	HLH	105	154	61.6	2.40E-15
102	PHE0004828_5826.pep	HLH	107	156	60	7.00E-15
104	PHE0004861_5910.pep	GRAS	26	325	369.5	4.70E-108
105	PHE0004863_5912.pep	AT_hook	121	133	17.5	0.02
105	PHE0004863_5912.pep	AT_hook	182	194	12.6	0.14
105	PHE0004863_5912.pep	DUF296	212	332	177.3	3.40E-50

PEP SEQ ID NO	GENE ID	Pfam domain name	Begin	Stop	Score	E-value
119	PHE0004877_7030.pep	Response_reg	13	126	104.9	2.20E-28
119	PHE0004877_7030.pep	Myb_DNA-binding	197	247	46.4	8.90E-11
108	PHE0004914_5971.pep	Myb_DNA-binding	11	60	22.4	0.0014
108	PHE0004914_5971.pep	Myb_DNA-binding	117	164	49.3	1.20E-11
109	PHE0004924_5982.pep	TCP	38	253	139.2	1.00E-38
110	PHE0004925_5983.pep	SBP	58	136	173.4	5.30E-49
111	PHE0004938_5994.pep	GRAS	154	454	524.3	1.20E-154
159	PHE0004938_8370.pep	GRAS	154	454	524.3	1.20E-154
112	PHE0004957_6019.pep	zf-C2H2	68	90	21.6	0.0026
113	PHE0004958_6020.pep	zf-Dof	104	166	140.5	4.20E-39
114	PHE0004959_6021.pep	AP2	128	191	141.1	2.70E-39
115	PHE0004974_6040.pep	B3	148	253	114.1	3.60E-31
115	PHE0004974_6040.pep	Auxin_resp	275	357	156.9	4.80E-44
115	PHE0004974_6040.pep	AUX_IAA	623	809	-63.7	6.00E-05
116	PHE0004975_6041.pep	B3	136	241	113.4	6.10E-31
116	PHE0004975_6041.pep	Auxin_resp	263	345	170	5.40E-48
117	PHE0004987_6056.pep	Myb_DNA-binding	21	72	48.3	2.30E-11
173	PHE0004987_8771.pep	Myb_DNA-binding	21	72	48.3	2.30E-11
118	PHE0005005_7034.pep	Myb_DNA-binding	96	143	54	4.60E-13
124	PHE0006004_7082.pep	NAM	18	147	257.9	1.90E-74
167	PHE0006004_8667.pep	NAM	18	147	257.9	1.90E-74
125	PHE0006022_7105.pep	EIN3	30	426	983.5	7.20E-293
168	PHE0006022_8690.pep	EIN3	30	426	983.5	7.20E-293
126	PHE0006023_7240.pep	HLH	160	210	36.8	6.80E-08
172	PHE0006023_8762.pep	HLH	160	210	36.8	6.80E-08
120	PHE0006057_7048.pep	HLH	12	61	60.5	5.10E-15
121	PHE0006057_7053.pep	HLH	12	61	59.8	8.10E-15
150	PHE0006057_7929.pep	HLH	12	61	60.5	5.10E-15
122	PHE0006070_7067.pep	bZIP_2	96	153	65.7	1.30E-16
122	PHE0006070_7067.pep	bZIP_1	96	156	18.3	0.0014
123	PHE0006073_7072.pep	bZIP_1	96	156	18.3	0.0014
123	PHE0006073_7072.pep	bZIP_2	96	153	65.7	1.30E-16
128	PHE0006237_7261.pep	AP2	6	69	121.7	1.90E-33
130	PHE0006237_7268.pep	AP2	6	69	121.7	1.90E-33
129	PHE0006237_7274.pep	AP2	6	69	121.7	1.90E-33
131	PHE0006237_7277.pep	AP2	6	69	121.7	1.90E-33
132	PHE0006237_7284.pep	AP2	6	69	121.7	1.90E-33
141	PHE0006290_7498.pep	zf-B_box	1	47	44.6	3.00E-10
141	PHE0006290_7498.pep	zf-B_box	48	90	23.5	0.00039
141	PHE0006290_7498.pep	CCT	355	393	72.3	1.40E-18
169	PHE0006290_8689.pep	zf-B_box	1	47	44.6	3.00E-10
169	PHE0006290_8689.pep	zf-B_box	48	90	23.5	0.00039
169	PHE0006290_8689.pep	CCT	355	393	72.3	1.40E-18
134	PHE0006291_7319.pep	zf-B_box	3	50	56.9	6.10E-14
134	PHE0006291_7319.pep	CCT	309	347	69.6	9.10E-18
155	PHE0006350_8201.pep	GRAS	98	403	400	3.20E-117
143	PHE0006384_7737.pep	Myb_DNA-binding	14	61	43.1	8.70E-10

PEP SEQ ID NO	GENE ID	Pfam domain name	Begin	Stop	Score	E-value
143	PHE0006384_7737.pep	Myb_DNA-binding	67	112	50	7.30E-12
144	PHE0006384_7789.pep	Myb_DNA-binding	14	61	43.1	8.70E-10
144	PHE0006384_7789.pep	Myb_DNA-binding	67	112	50	7.30E-12
147	PHE0006384_7839.pep	Myb_DNA-binding	14	61	43.1	8.70E-10
147	PHE0006384_7839.pep	Myb_DNA-binding	67	112	50	7.30E-12
142	PHE0006423_7664.pep	Myb_DNA-binding	14	61	51.6	2.50E-12
142	PHE0006423_7664.pep	Myb_DNA-binding	67	112	35.1	2.20E-07
170	PHE0006423_8696.pep	Myb_DNA-binding	14	61	51.6	2.50E-12
170	PHE0006423_8696.pep	Myb_DNA-binding	67	112	35.1	2.20E-07
148	PHE0006448_7859.pep	RWP-RK	553	604	110.7	3.80E-30
148	PHE0006448_7859.pep	PB1	741	823	92.6	1.10E-24
149	PHE0006504_7876.pep	F-box	49	104	29.5	1.10E-05
149	PHE0006504_7876.pep	Tub	115	424	691.2	7.00E-205
145	PHE0006507_7828.pep	CBFD_NFYB_HMF	1	40	31.5	2.80E-06
146	PHE0006509_7846.pep	SBP	64	142	188.1	1.90E-53
158	PHE0006527_8369.pep	CBFD_NFYB_HMF	26	91	130.9	3.20E-36
157	PHE0006546_8310.pep	Response_reg	28	141	92.2	1.40E-24
157	PHE0006546_8310.pep	Myb_DNA-binding	225	275	45.6	1.50E-10
156	PHE0006605_8233.pep	GATA	223	258	67.8	3.20E-17
163	PHE0006752_8521.pep	SRF-TF	9	59	117.1	4.70E-32
163	PHE0006752_8521.pep	K-box	75	174	163.9	3.80E-46
160	PHE0006774_8489.pep	CBFD_NFYB_HMF	34	106	112	1.60E-30
161	PHE0006778_8503.pep	CBFD_NFYB_HMF	34	106	106.2	8.90E-29
164	PHE0006779_8565.pep	CBFD_NFYB_HMF	34	106	106.5	7.30E-29
162	PHE0006780_8502.pep	CBFD_NFYB_HMF	34	106	102.1	1.50E-27
165	PHE0006781_8573.pep	CBFD_NFYB_HMF	34	106	95.6	1.30E-25
174	PHE0006858_8859.pep	SRF-TF	9	59	115.3	1.60E-31
174	PHE0006858_8859.pep	K-box	74	173	148.4	1.70E-41
175	PHE0006860_8863.pep	SRF-TF	9	59	121.5	2.20E-33
175	PHE0006860_8863.pep	K-box	74	172	152.7	8.70E-43
177	PHE0006951_9137.pep	zf-C2H2	152	175	20.1	0.0071
179	PHE0006951_9173.pep	zf-C2H2	152	175	20.1	0.0071
176	PHE0006955_9129.pep	SRF-TF	9	59	95.2	1.80E-25
176	PHE0006955_9129.pep	K-box	85	175	22.5	5.10E-06
178	PHE0006981_9158.pep	GRAS	149	456	481	1.30E-141

Table 12. Description of Pfam domain

Pfam domain name	Accession number	Gathering cutoff	Domain description
AP2	PF00847.9	0	AP2 domain
AT_hook	PF02178.8	3.6	AT hook motif
AUX_IAA	PF02309.6	-83	AUX/IAA family
Auxin_resp	PF06507.3	25	Auxin response factor
B3	PF02362.12	26.5	B3 DNA binding domain
CBFD_NFYB_HMF	PF00808.12	18.4	Histone-like transcription factor (CBF/NF-Y) and archaeal histone

Pfam domain name	Accession number	Gathering cutoff	Domain description
CCT	PF06203.4	25	CCT motif
DUF296	PF03479.4	-11	Domain of unknown function (DUF296)
EIN3	PF04873.3	-137.6	Ethylene insensitive 3
ELK	PF03789.3	25	ELK domain
F-box	PF00646.21	13.6	F-box domain
GATA	PF00320.16	28.5	GATA zinc finger
GRAS	PF03514.4	-78	GRAS family transcription factor
HLH	PF00010.15	8.2	Helix-loop-helix DNA-binding domain
Homeobox	PF00046.18	-4.1	Homeobox domain
K-box	PF01486.7	0	K-box region
KNOX1	PF03790.3	25	KNOX1 domain
KNOX2	PF03791.3	25	KNOX2 domain
Myb_DNA-binding	PF00249.19	2.8	Myb-like DNA-binding domain
NAM	PF02365.5	-19	No apical meristem (NAM) protein
PB1	PF00564.13	12.3	PB1 domain
RWP-RK	PF02042.5	25	RWP-RK domain
Response_reg	PF00072.12	4	Response regulator receiver domain
SBP	PF03110.5	25	SBP domain
SRF-TF	PF00319.8	11	SRF-type transcription factor (DNA-binding and dimerisation domain)
TCP	PF03634.3	-38	TCP family transcription factor
Tub	PF01167.7	-98	Tub family
bZIP_1	PF00170.10	16.5	bZIP transcription factor
bZIP_2	PF07716.4	15	Basic region leucine zipper
zf-B_box	PF00643.14	15.3	B-box zinc finger
zf-C2H2	PF00096.15	16.8	Zinc finger, C2H2 type
zf-Dof	PF02701.5	25	Dof domain, zinc finger

Example 10. Selection of transgenic plants with enhanced agronomic trait(s)

This example illustrates the preparation and identification by selection of transgenic seeds and plants derived from transgenic plant cells of this invention where the plants and seed are identified by screening for an enhanced agronomic trait imparted by expression of a protein selected from the group including the homologous proteins identified in Example 6. Transgenic plant cells of corn, soybean, cotton, canola, wheat and rice are transformed with recombinant DNA for expressing each of the homologs identified in Example 6. Plants are regenerated from the transformed plant cells and used to produce progeny plants and seed that are screened for enhanced water use efficiency, enhanced cold tolerance, increased yield, enhanced nitrogen use efficiency, enhanced seed protein and enhanced seed oil. Plants are identified exhibiting enhanced traits imparted by expression of the homologous proteins.

What is claimed is:

1. A plant cell nucleus with stably integrated, recombinant DNA construct, wherein said
5 recombinant DNA construct comprises a promoter that is functional in a plant cell and that is operably linked to a DNA segment encoding a protein comprising an amino acid sequence of SEQ ID NO: 177; and
wherein said recombinant DNA construct is stably integrated into a chromosome in said plant cell nucleus which is selected by screening a population of transgenic plants that have said
10 recombinant DNA construct and an enhanced trait as compared to control plants that do not have said recombinant DNA construct in their nuclei; and
wherein said enhanced trait is selected from group of enhanced traits consisting of enhanced water use efficiency, enhanced cold tolerance, enhanced heat tolerance, enhanced high salinity tolerance, enhanced shade tolerance, increased yield, enhanced nitrogen use
15 efficiency, enhanced seed protein and enhanced seed oil.

2. A recombinant DNA construct comprising a promoter that is functional in a plant cell and that is operably linked to a DNA segment that encodes:
 - a. at least one protein having an amino acid sequence comprising a Pfam domain module
20 selected from the group consisting of Homeobox, Myb_DNA-binding::Myb_DNA-binding, Myb_DNA-binding, zf-Dof, zf-C2H2::zf-C2H2, AP2, Response_reg::Myb_DNA-binding, B3, B3::Auxin_resp::AUX_IAA, HLH, NAM, B3::B3, AUX_IAA, KNOX1::KNOX2::ELK, GRAS, AT_hook::AT_HOOK::DUF296, TCP, SBP, zf-C2H2, B3::Auxin_resp, EIN3, bZIP_2, zf-B_box::zf-B_box, zf-B_box::CCT, RWP-RK::PB1, F-box::TUB,
25 CBFD_NFYB_HMF, GATA, SRF-TF, K-box, and SRF-TF::K-box;
 - b. a protein comprising an amino acid sequence with at least 90% identity to a consensus amino acid sequence as set forth in SEQ ID NO: 2201;
 - c. a protein having an amino acid sequence having at least 70% identity to an amino acid sequence selected from the group consisting of SEQ ID NOs 96 through SEQ ID NO: 193;
30 or
 - d. a protein having an amino acid sequence selected from the group consisting of SEQ ID NO: 96 through SEQ ID NO: 193;
and wherein said recombinant DNA construct is stably integrated into a chromosome in a plant cell nucleus which is selected by screening a population of transgenic plants that have

said recombinant DNA construct and an enhanced trait as compared to control plants that do not have said recombinant DNA construct in their nuclei; and wherein said enhanced trait is selected from group of enhanced traits consisting of enhanced water use efficiency, enhanced cold tolerance, enhanced heat tolerance, enhanced high salinity tolerance, enhanced shade
5 tolerance, increased yield, enhanced nitrogen use efficiency, enhanced seed protein and enhanced seed oil.

3. A transgenic plant cell nucleus comprising a recombinant DNA construct of claim 2.

10 4. A transgenic plant cell having a plant cell nucleus of claim 3.

5. The transgenic plant cell of claim 4 wherein said transgenic plant cell is homozygous for said recombinant DNA construct.

15 6. The transgenic plant cell of claim 4 further comprising DNA expressing a protein that provides tolerance from exposure to an herbicide applied at levels that are lethal to a wild type of said plant cell.

20 7. The transgenic plant cell of claim 5 wherein said herbicide is a glyphosate, dicamba, or glufosinate compound.

8. A transgenic plant comprising a plurality of plant cells of claim 4.

25 9. The transgenic plant of claim 8 wherein said transgenic plant is homozygous for said recombinant DNA construct.

10. A transgenic seed comprising a recombinant DNA construct of claim 2.

30 11. The transgenic seed of claim 10 from a corn, soybean, cotton, canola, alfalfa, wheat or rice plant.

12. A transgenic pollen grain comprising a recombinant DNA construct of claim 2.

13. A method for manufacturing transgenic seeds that can be used to produce a crop of transgenic plants with an enhanced trait resulting from expression of a DNA segment in a plant cell nucleus comprising a recombinant DNA construct of claim 2, wherein said method comprises:

- 5 (a) providing a population of plants produced from a parental plant having a recombinant DNA construct of claim 2;
- (b) screening said population of plants for at least one of said enhanced trait and said recombinant DNA construct, wherein individual plants in said population can exhibit said trait at a level less than, essentially the same as or greater than the level that said trait is
- 10 exhibited in control plants which do not contain said recombinant DNA construct, wherein said enhanced trait is selected from the group of enhanced traits consisting of enhanced water use efficiency, enhanced cold tolerance, enhanced heat tolerance, enhanced high salinity tolerance, enhanced shade tolerance, increased yield, enhanced nitrogen use efficiency, enhanced seed protein and enhanced seed oil;
- 15 (c) selecting from said population one or more plants that exhibit said trait at a level greater than the level that said trait is exhibited in control plants; and
- (d) collecting seeds from selected plant selected from step c.

14. The method of claim 13, wherein said method further comprises:

- 20 (e) verifying that said recombinant DNA construct is stably integrated in said selected plants; and
- (f) analyzing tissue of said selected plant to determine the expression of a protein having the function of a protein having an amino acid sequence selected from the group consisting of one of SEQ ID NO: 96 through SEQ ID NO: 193.

25

15. The method of claim 14 wherein said seed is corn, soybean, cotton, alfalfa, canola wheat or rice seed and said recombinant DNA construct is homozygous in said plant.

16. A method of producing hybrid corn seed comprising:

- 30 (a) acquiring hybrid corn seed from a herbicide tolerant corn plant which also has a stably-integrated, recombinant DNA construct of claim 2;
- (b) producing corn plants from said hybrid corn seed, wherein a fraction of the plants produced from said hybrid corn seed is homozygous for said recombinant DNA construct, a fraction of the plants produced from said hybrid corn seed is hemizygous for said

recombinant DNA construct, and a fraction of the plants produced from said hybrid corn seed has none of said recombinant DNA construct;

- (c) selecting corn plants which are homozygous or hemizygous for said recombinant DNA construct by treating with an herbicide;
- 5 (d) collecting seeds from herbicide-treated-surviving corn plants and planting said seed to produce further progeny corn plants;
- (e) repeating steps (c) and (d) at least once to produce an inbred corn line; and
- (f) crossing said inbred corn line with a second corn line to produce hybrid corn seed.

Figure 1 of 5

SEQ ID NO: homolog SEQ ID NOs

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873      ---MSA---SRSSSPNSMSQWSQKENKMFEEALAYYGEGTPNRWDKVSSAMGGIKSAEEV
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266      ---MSSGSSSRSNSPSSDSEWSKKENKMFEEALAYYGVSAPNLCEKVASAMGGTKSTEEV
10 1239    ---MSS-----PSSDSEWSKKENKMFEEALAYYGMGAPNLWEKVASAMGGTKSAEEV
1108     MSSRSSSSSRNSNSVNMDSSEWSKKENKLFEEALAYYGEGAPDLFHKVSRAMGGTKTADEV
1543     -----MDSEWSKKENKLFEEALAYYGEGAPDLFHKVSRAMGGTKTADEV
1503     -----MASEWSKEENKLFEEALAYYGEGAPDLFHKVSRAMGGTKTADEV
consensus ---msxxxxxsxspxsxSeWskkENKmFEEAlAYYGxxapnlwxKVxxAMGGtKsaxEv
15 2201

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RCHYEDLTDDVKTIESGRVQFPKYKTQGYWT-----
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RRHFQFLVDDVKIEHGRIFFPKYKTRGFWT-----
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RLHFEILVDDIKLIEARRVPFPKYNTQGAWN-----
RxHxxxLxdDvxxIExgrxpfPkyktxgxwt-----

30 -----
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35 -----
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-----
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40 PAADSPLPGRLLSRPTTERPRPDGLISPNGPYTFTGRPASLDHRMGHVAGPAKLGDGDKS
-----
-----

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45 -----*
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-----*
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Figure 2 of 5

Plasmid map of pMON93039

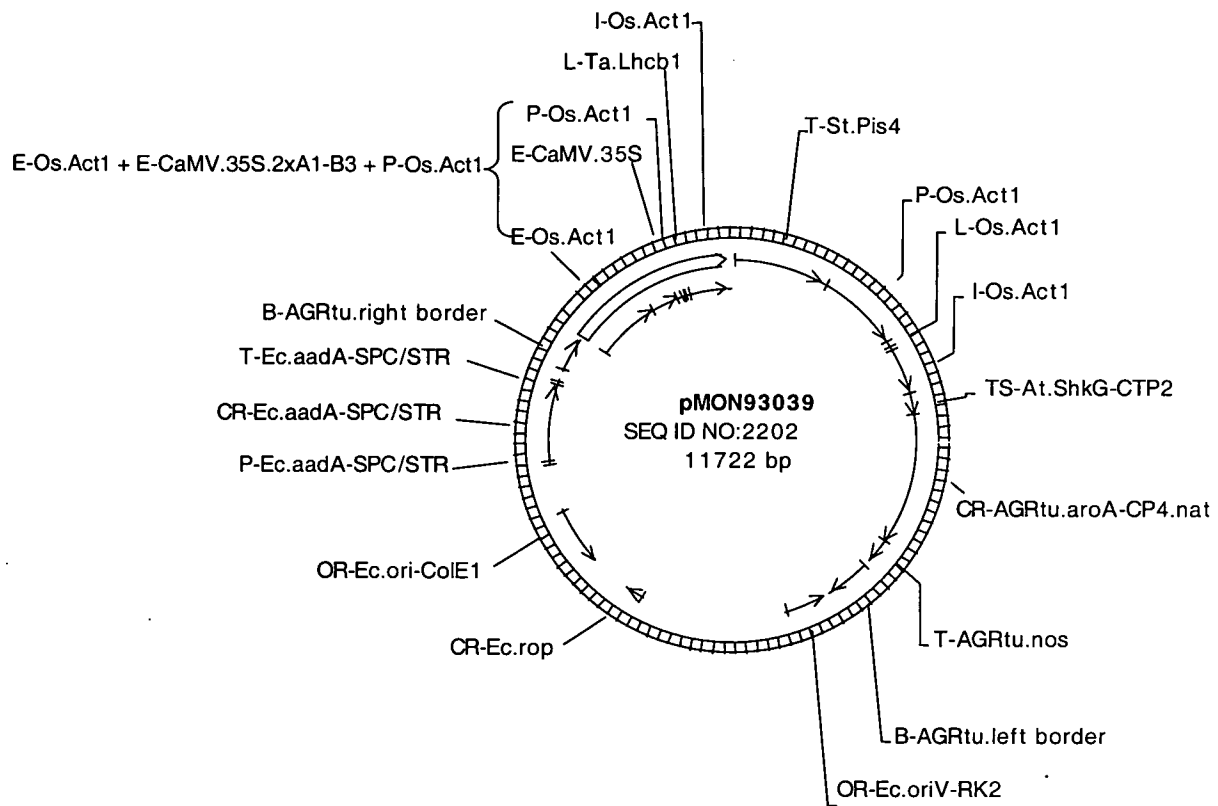


Figure 3 of 5

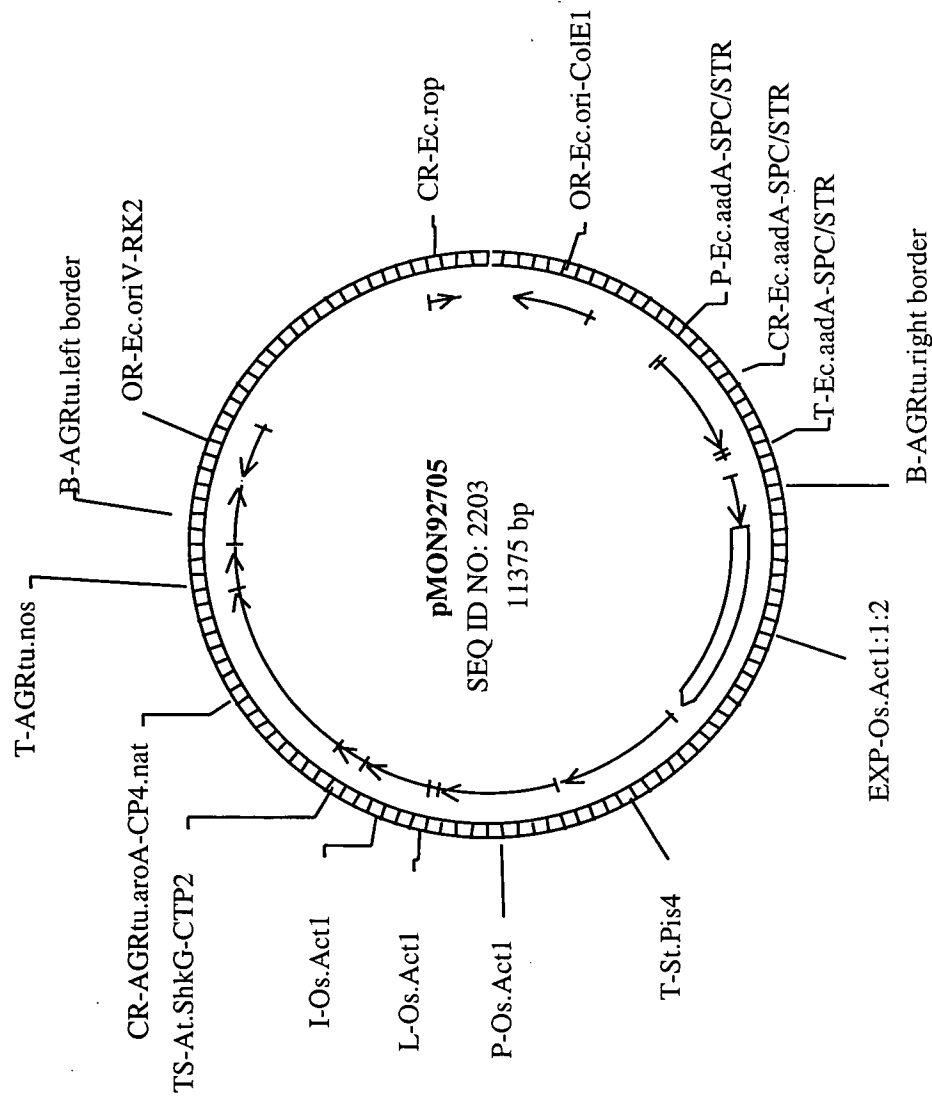


Figure 4 of 5

Plasmid map of pMON82053

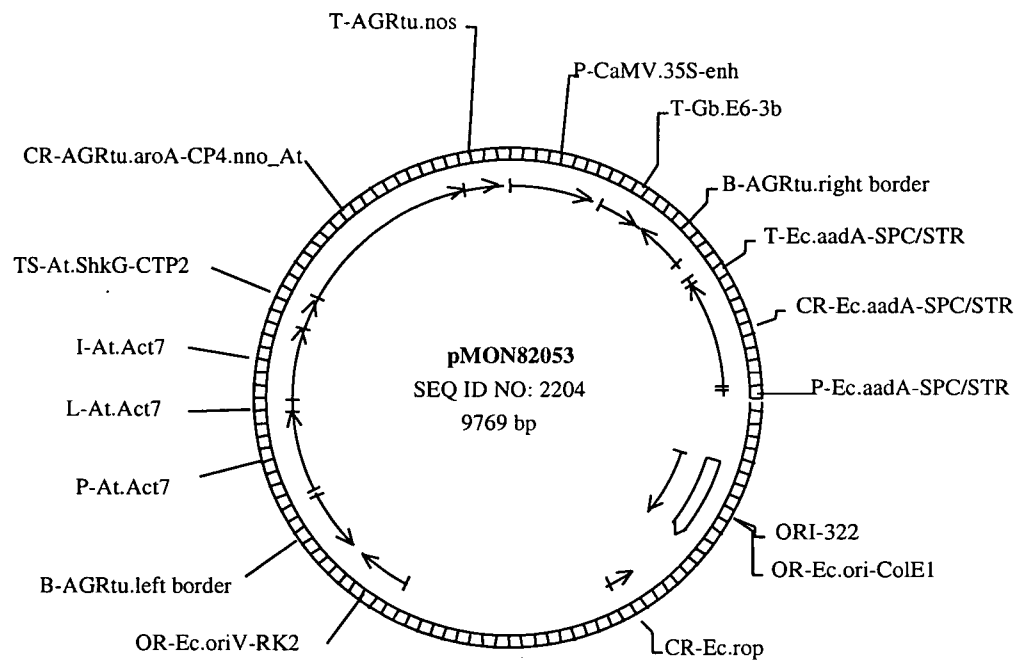


Figure 5 of 5

Plasmid map of pMON99053.

