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# Promoter, promoter control elements, and combinations, and uses thereof


## Abstract

The present invention is directed to promoter sequences and promoter control elements, polynucleotide constructs comprising the promoters and control elements, and methods of identifying the promoters, control elements, or fragments thereof. The invention further relates to the use of the present promoters or promoter control elements to modulate transcript levels in plants, and plants containing such promoters or promoter control elements.



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## Background/Summary

CROSS-REFERENCE TO RELATED APPLICATIONS (1) This application is a Divisional of application Ser. No. 16/912,222 filed Jun. 25, 2020, now U.S. Pat. No. 11,572,567, which application is a Divisional of application Ser. No. 15/691,458 filed Aug. 30, 2017, now U.S. Pat. No. 10,815,490, which is a Divisional of application Ser. No. 14/476,566 filed on Sep. 3, 2014, now U.S. Pat. No. 9,777,285, which application is a Divisional of application Ser. No. 12/865,719 filed on Apr. 19, 2011 (now abandoned), which is a National Phase of PCT International Application No. PCT/US2009/032485 filed on Jan. 29, 2009, which claims priority to U.S. Provisional Application Ser. No. 61/025,697, filed on Feb. 1, 2008. All of the above applications are hereby expressly incorporated by reference into the present application.

INCORPORATION-BY-REFERENCE OF SEQUENCE LISTING OR TABLE
(1) The material in the accompanying sequence listing is hereby incorporated by reference into this application. The accompanying file, named CRES005USD6_ST26.xml was created on Dec. 6, 2022 and is 153 KB (size as measured in Microsoft Windows® operating system).
FIELD OF THE INVENTION
(2) The present invention relates to promoters and promoter control elements that are useful for modulating transcription of a desired polynucleotide. Such promoters and promoter control elements can be included in polynucleotide constructs, expression cassettes, vectors, or inserted into the chromosome or as an exogenous element, to modulate in vivo and in vitro transcription of a polynucleotide. Host cells, including plant cells, and organisms, such as regenerated plants therefrom, with desired traits or characteristics using polynucleotides comprising the promoters and promoter control elements of the present invention are also a part of the invention.
BACKGROUND OF THE INVENTION
(3) This invention relates to promoter sequences and promoter control element sequences which are useful for the transcription of polynucleotides in a host cell or transformed host organism.
(4) The introduction of genes into plants has resulted in the development of plants having new and useful phenotypes such as pathogen resistance, higher levels of healthier types of oils, novel production of healthful components such as beta-carotene synthesis in rice. An introduced gene is generally a chimeric gene composed of the coding region that confers the desired trait and regulatory sequences. One regulatory sequence is the promoter, which is located 5′ to the coding region. This sequence is involved in regulating the pattern of expression of a coding region 3′ thereof. The promoter sequence binds RNA polymerase complex as well as one or more transcription factors that are involved in producing the RNA transcript of the coding region.
(5) The promoter region of a gene used in plant transformation is most often derived from a different source than is the coding region. It may be from a different gene of the same species of plant, from a different species of plant, from a plant virus, an algae species, a fungal species, or it may be a composite of different natural and/or synthetic sequences. Properties of the promoter sequence generally determine the pattern of expression for the coding region that is operably linked to the promoter. Promoters with different characteristics of expression have been described. The

promoter may confer broad expression as in the case of the widely-used cauliflower mosaic virus (CaMV) 35S promoter. The promoter may confer tissue-specific expression as in the case of the seed-specific phaseolin promoter. The promoter may confer a pattern for developmental changes in expression. The promoter may be induced by an applied chemical compound, or by an environmental condition applied to the plant.

(6) The promoter that is used to regulate a particular coding region is determined by the desired expression pattern for that coding region, which itself is determined by the desired resulting phenotype in the plant. For example, herbicide resistance is desired throughout the plant so the 35S promoter is appropriate for expression of an herbicide-resistance gene. A seed-specific promoter is appropriate for changing the oil content of soybean seed. An endosperm-specific promoter is appropriate for changing the starch composition of corn seed. A root-specific promoter can be important for improving water or nutrient up-take in a plant. Control of expression of an introduced gene by the promoter is important because it is sometimes detrimental to have expression of an introduced gene in non-target tissues. For example, a gene which induces cell death can be expressed in male and/or female gamete cells in connection with bioconfinement.

(7) One of the primary goals of biotechnology is to obtain organisms, such as plants, mammals, yeast, and prokaryotes having particular desired characteristics or traits. Examples of these characteristics or traits abound and may include, for example, in plants, virus resistance, insect resistance, herbicide resistance, enhanced stability or additional nutritional value. Recent advances in genetic engineering have enabled researchers in the field to incorporate polynucleotide sequences into host cells to obtain the desired qualities in the organism of choice. This technology permits one or more polynucleotides from a source different than the organism of choice to be transcribed by the organism of choice. If desired, the transcription and/or translation of these new polynucleotides can be modulated in the organism to exhibit a desired characteristic or trait. Alternatively, new patterns of transcription and/or translation of polynucleotides endogenous to the organism can be produced.

## SUMMARY OF THE INVENTION

(8) The present invention is directed to isolated polynucleotide sequences that comprise promoters and promoter control elements from plants, especially *Arabidopsis thaliana,* and other promoters and promoter control elements functional in plants.

(9) It is an object of the present invention to provide isolated polynucleotides that are promoter or promoter control sequences. These promoter sequences comprise, for example, (1) a polynucleotide having a nucleotide sequence according to any one of SEQ. ID. Nos. 1-26 or residues 601-1000 of SEQ ID NO: 26; (2) a polynucleotide having a nucleotide sequence having at least 80% sequence identity to a sequence according to SEQ. ID. Nos. 1-26 or residues 601-1000 of SEQ ID NO: 26; and (3) a polynucleotide having a nucleotide sequence which hybridizes to a sequence according to SEQ. ID. Nos. 1-26 or nucleic acid residues 601-1000 of SEQ ID NO: 26 under a condition establishing a Tm−5° C.

(10) Promoter or promoter control element sequences of the present invention are capable of modulating preferential transcription.

(11) In another embodiment, the present promoter control elements are capable of serving as or fulfilling the function, for example, as a core promoter, a TATA box, a polymerase binding site, an initiator site, a transcription binding site, an enhancer, an inverted repeat, a locus control region, and/or a scaffold/matrix attachment region.

(12) It is yet another object of the present invention to provide a polynucleotide that includes at least a first and a second promoter control element. The first promoter control element is a promoter control element sequence as discussed above, and the second promoter control element is heterologous to the first control element; wherein, the first and second control elements are operably linked. Such promoters may modulate transcript levels preferentially in a particular tissue or under particular conditions.

(13) In another embodiment, the present isolated polynucleotide comprises a promoter or a promoter control element as described above, wherein the promoter or promoter control element is operably linked to a polynucleotide to be transcribed.
(14) In another embodiment of the present invention, the promoter and promoter control elements of the instant invention are operably linked to a heterologous polynucleotide that is a regulatory sequence.
(15) It is another object of the present invention to provide a host cell comprising an isolated polynucleotide or vector as described above or fragment thereof. Host cells include, for instance, bacterial, yeast, insect, mammalian, fungus, algae, and plant. The host cell can comprise a promoter or promoter control element exogenous to the genome. Such a promoter can modulate transcription in cis- and in trans-.
(16) In yet another embodiment, the host cell is a plant cell capable of regenerating into a plant.
(17) It is yet another embodiment of the present invention to provide a plant comprising an isolated polynucleotide or vector described above.
(18) It is another object of the present invention to provide a method of modulating transcription in a sample that contains either a cell-free system of transcription or host cell. This method comprises providing a polynucleotide or vector according to the present invention as described above, and contacting the sample of the polynucleotide or vector with conditions that permit transcription.
(19) In another embodiment of the present method, the polynucleotide or vector preferentially modulates, depending upon the function of the particular promoter, constitutive transcription, stress induced transcription, light induced transcription, dark induced transcription, leaf transcription, root transcription, stem or shoot transcription, silique or fruit transcription, callus transcription, rhizome transcription, stem node transcription, gamete tissue transcription, flower transcription, immature bud and inflorescence specific transcription, senescing induced transcription, germination transcription and/or drought transcription.
(20) One embodiment of the invention is directed to an isolated nucleic acid molecule having promoter activity comprising a nucleotide sequence selected from the group consisting of: a. a nucleotide sequence according to any one of SEQ ID NOs. 1-26; b. a nucleotide sequence of nucleic acid residues 601-1000 of SEQ ID NO: 26; c. a nucleotide sequence comprising a functional fragment of (a) or (b), wherein said fragment has promoter activity,
and wherein said isolated nucleic acid molecule is not SEQ ID NO: 5.
(21) Another embodiment of the invention is directed to an isolated nucleic acid molecule comprising a nucleotide sequence that shows at least 80 percent sequence identity to any one of SEQ ID NOs: 1-26 or nucleic acid residues 601-1000 of SEQ ID NO: 26, wherein said nucleic acid molecule comprises a regulatory region that directs transcription of an operably linked heterologous polynucleotide, and wherein said isolated nucleic acid molecule is not SEQ ID NO: 5.
(22) In another embodiment of the invention the isolated nucleic acid molecule shows at least 85 percent sequence identity to any one of SEQ ID NOs: 1-26 or nucleic acid residues 601-1000 of SEQ ID NO: 26.
(23) In another embodiment of the invention the isolated nucleic acid molecule has at least 90 percent sequence identity to any one of SEQ ID NOs: 1-26 or nucleic acid residues 601-1000 of SEQ ID NO: 26.
(24) In another embodiment the isolated nucleic acid molecule comprises at least one member selected from the group consisting of a promoter, an enhancer and an intron.
(25) In a further embodiment of the invention, the isolated nucleic acid molecule consists of any one of SEQ ID NOs: 1-4, 6-26 and the nucleic acid residues 61-1000 of SEQ ID NO: 26.
(26) Another embodiment of the invention is directed to a vector construct comprising: a. a first nucleic acid molecule as described above; and b. a transcribable polynucleotide molecule, wherein said first nucleic acid molecule and said transcribable polynucleotide molecule are heterologous to each other and are operably linked.

(27) In another embodiment of the invention, the first nucleic acid molecule consists of the nucleic acid molecule set forth in any one of SEQ ID NOs: 1-26 or nucleic acid residues 601-1000 of SEQ ID NO: 26.
(28) In another embodiment of the invention, the transcribable polynucleotide molecule encodes a polypeptide.
(29) In another embodiment of the invention, the transcribable polynucleotide molecule is operably linked to said first nucleic acid molecule in the sense orientation.
(30) In another embodiment of the invention, the transcribable polynucleotide molecule is transcribed into an RNA molecule that expresses the polypeptide encoded by transcribable polynucleotide molecule.
(31) In another embodiment of the invention, the transcribable polynucleotide molecule is operably linked to said first nucleic acid molecule in the antisense orientation.
(32) In another embodiment of the invention, the transcribable polynucleotide molecule is transcribed into an antisense RNA molecule.
(33) In another embodiment of the invention, the transcribable polynucleotide molecule is transcribed into an interfering RNA against an endogenous gene.
(34) In another embodiment of the invention, the transcribable polynucleotide molecule encodes a polypeptide of agronomic interest.
(35) Another embodiment of the invention is directed to a plant or plant cell comprising: a. the nucleic acid molecule described above that is operably linked to a heterologous polynucleotide, or b. the vector construct described above.
(36) Another embodiment of the invention is directed to a plant or plant cell stably transformed with the vector construct described above.
(37) Another embodiment of the invention is directed to a seed of a plant as described above.
(38) Another embodiment of the invention is directed to a method of directing transcription by combining, in an environment suitable for transcription: a. a first nucleic acid molecule as described above; and b. a transcribable polynucleotide molecule;
wherein said first nucleic acid molecule and said transcribable polynucleotide molecule are heterologous to each other and operably linked.
(39) Another embodiment of the invention is directed to a method of expressing an exogenous coding region in a plant comprising: a. transforming a plant cell with a vector as described above, b. regenerating a stably transformed plant from the transformed plant cell of step (a); and c. selecting plants containing a transformed plant cell,
wherein expression of the transcribable polynucleotide molecule results in production of a polypeptide encoded by said transcribable polynucleotide molecule.
(40) Another embodiment of the invention is directed to a method of altering the expression of a gene in a plant comprising: a. transforming a plant cell with the nucleic acid molecule as described above that is operably linked to a heterologous polynucleotide, and b. regenerating stably transformed plants from said transformed plant cell.
(41) Another embodiment of the invention is directed to a plant prepared according to the method described above.
(42) Another embodiment of the invention is directed to a seed from the plant described above.
(43) Another embodiment of the invention is directed to a method of producing a transgenic plant, said method comprising; a. introducing into a plant cell: (i) an isolated polynucleotide comprising the nucleic acid as described above that is operably linked to a heterologous polynucleotide, or (ii) the vector as described above; and b. growing a plant from said plant cell.
(44) Other and further objects of the present invention will be made clear or become apparent from the following description.
BRIEF DESCRIPTION OF THE TABLES AND FIGURES
(45) The Tables consist of the Expression Reports for some of the promoters of the invention

providing the nucleotide sequence for each promoter and details for expression driven by each of the nucleic acid promoter sequences as observed in transgenic plants. The results are presented as summaries of the spatial expression, which provides information as to gross and/or specific expression in various plant organs and tissues. The observed expression pattern is also presented, which gives details of expression during different generations or different developmental stages within a generation. Additional information is provided regarding the source organism of the promoter, and the vector and marker genes used for the construct. The following symbols are used consistently throughout the Tables: T1: First generation transformant T2: Second generation transformant T3: Third generation transformant (L): low expression level (M): medium expression level (H): high expression level

(46) Each row of the table begins with heading of the data to be found in the section. The following provides a description of the data to be found in each section:

(47) TABLE-US-00001 Heading in Tables Description 1. Promoter Identifies the particular promoter by its Expression construct ID. Report # 2. Promoter Identifies the organism in which the tested in: promoter-marker vector was tested. 3. Spatial Identifies the specific parts of the plant expression where various levels of GFP expression summary: are observed. Expression levels are noted as either low (L), medium (M), or high (H). 4. Observed Provides a general explanation of where expression GFP expression in different generations pattern: of plants was observed. 5. Source Identifies the plant species from which promoter the promoter was derived. organism: 6. Vector: Identifies the vector used into which a promoter was cloned. 7. Marker type: Identifies the type of marker linked to the promoter. The marker is used to determine patterns of gene expression in plant tissue. 8. Generation Identifies the plant generation(s) used in the screened: screening process. T1 plants are those plants T1 Mature subjected to the transformation event while T2 Seedling the T2 generation plants are from the seeds T2 Mature collected from the T1 plants and T3 plants T3 Seedling are from the seeds of T2 plants. 9. Inductions Provides summary of experiment schedule. completed: 10. T1 Mature Identifies plant tissues that were observed Plant for possible expression, and identifies Expression: (H, M or L) level of observed expression. 11. T2 Seedling Identifies plant tissues that were observed Expression: for possible expression, and identifies (H, M or L) level of observed expression. 12. T2 Mature Identifies plant tissues that were observed Plant for possible expression, and identifies Expression: (H, M or L) level of observed expression. 13. Utility Provides a description of the utility of the sequence, including a trait area and, in some instances, a sub-trait area. 14. Construct Identifies the promoter by its construct Promoter ID and internal candidate Candidate I.D. number, and cDNA number cDNA I.D. 15. Lines/Events Identifies the line/event numbers that expressing: expressed under the promoter. Some promoter reports describe additional experiments and results with the particular promoter.

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## Description

BRIEF DESCRIPTION OF THE FIGURES

(1) FIG. **1** is a schematic representation of a vector pNewbin4-HAP1-GFP that is useful to insert promoters of the invention into a plant. The definitions of the abbreviations used in the vector map are as follows:

(2) Ori—the origin of replication used by an *E. coli* host RB—sequence for the right border of the T-DNA from pMOG800 BstXI—restriction enzyme cleavage site used for cloning HAP1VP16—coding sequence for a fusion protein of the HAP1 and VP16 activation domains NOS—terminator region from the nopaline synthase gene HAP1UAS—the upstream activating sequence for HAPI 5ERGFP—the green fluorescent protein gene that has been optimized for localization to the endoplasmic reticulum OCS2—the terminator sequence from the octopine synthase 2 gene OCS—the terminator sequence from the octopine synthase gene p28716 (a.k.a. 28716 short)—promoter

used to drive expression of the PAT (BAR) gene PAT (BAR)—a marker gene conferring herbicide resistance LB—sequence for the left border of the T-DNA from pMOG800 Spec—a marker gene conferring spectinomycin resistance TrfA—transcription repression factor gene RK2-OriV—origin of replication for *Agrobacterium*

DETAILED DESCRIPTION OF THE INVENTION

(3) The following definitions and methods are provided to better define the present invention and to guide those of ordinary skill in the art in the practice of the present invention. Unless otherwise noted, terms are to be understood according to conventional usage by those of ordinary skill in the relevant art.

(4) The invention disclosed herein provides promoters capable of driving the expression of an operably linked transgene. The design, construction, and use of these promoters is one object of this invention. The promoter sequences, SEQ ID NOs: 1-26 and residues 601-1000 of SEQ ID NO: 26, are capable of transcribing operably linked nucleic acid molecules in particular plant tissues/organs or during particular plant growth stages, and therefore can selectively regulate expression of transgenes in these tissues/organs or at these times of plant development.

1. Definitions

(5) Chimeric: The term “chimeric” is used to describe polynucleotides or genes, or constructs wherein at least two of the elements of the polynucleotide or gene or construct, such as the promoter and the polynucleotide to be transcribed and/or other regulatory sequences and/or filler sequences and/or complements thereof, are heterologous to each other.

(6) Broadly Expressing Promoter: Promoters referred to herein as “broadly expressing promoters” actively promote transcription under most, but not necessarily all, environmental conditions and states of development or cell differentiation. Examples of broadly expressing promoters include the cauliflower mosaic virus (CaMV) 35S transcript initiation region and the 1′ or 2′ promoter derived from T-DNA of *Agrobacterium tumefaciens*, and other transcription initiation regions from various plant genes, such as the maize ubiquitin-1 promoter, known to those of skill.

(7) Domain: Domains are fingerprints or signatures that can be used to characterize protein families and/or parts of proteins. Such fingerprints or signatures can comprise conserved (1) primary sequence, (2) secondary structure, and/or (3) three-dimensional conformation. A similar analysis can be applied to polynucleotides. Generally, each domain has been associated with either a conserved primary sequence or a sequence motif. Generally these conserved primary sequence motifs have been correlated with specific in vitro and/or in vivo activities. A domain can be any length, including the entirety of the polynucleotide to be transcribed. Examples of domains include, without limitation, AP2, helicase, homeobox, zinc finger, etc.

(8) Endogenous: The term “endogenous,” within the context of the current invention refers to any polynucleotide, polypeptide or protein sequence which is a natural part of a cell or organism(s) regenerated from said cell. In the context of promoter, the term “endogenous coding region” or “endogenous cDNA” refers to the coding region that is naturally operably linked to the promoter.

(9) Enhancer/Suppressor: An “enhancer” is a DNA regulatory element that can increase the steady state level of a transcript, usually by increasing the rate of transcription initiation. Enhancers usually exert their effect regardless of the distance, upstream or downstream location, or orientation of the enhancer relative to the start site of transcription. In contrast, a “suppressor” is a corresponding DNA regulatory element that decreases the steady state level of a transcript, again usually by affecting the rate of transcription initiation. The essential activity of enhancer and suppressor elements is to bind a protein factor(s). Such binding can be assayed, for example, by methods described below. The binding is typically in a manner that influences the steady state level of a transcript in a cell or in an in vitro transcription extract.

(10) Exogenous: As referred to within, “exogenous” is any polynucleotide, polypeptide or protein sequence, whether chimeric or not, that is introduced into the genome of a host cell or organism regenerated from said host cell by any means other than by a sexual cross. Examples of means by

which this can be accomplished are described below, and include *Agrobacterium*-mediated transformation (of dicots—e.g. Salomon et al. (1984) *EMBO J.* 3:141; Herrera-Estrella et al. (1983) *EMBO J.* 2:987; of monocots, representative papers are those by Escudero et al. (1996) *Plant J.* 10:355), Ishida et al. (1996) *Nature Biotech* 14:745, May et al. (1995) *Bio/Technology* 13:486), biolistic methods (Armaleo et al. (1990) Current Genetics 17:97), electroporation, in planta techniques, and the like. Such a plant containing the exogenous nucleic acid is referred to here as a T.sub.0 for the primary transgenic plant and T.sub.1 for the first generation. The term "exogenous" as used herein is also intended to encompass inserting a naturally found element into a non-naturally found location.

(11) Heterologous sequences: "Heterologous sequences" are those that are not operatively linked or are not contiguous to each other in nature. For example, a promoter from corn is considered heterologous to an *Arabidopsis* coding region sequence. Also, a promoter from a gene encoding a growth factor from corn is considered heterologous to a sequence encoding the corn receptor for the growth factor. Regulatory element sequences, such as UTRs or 3′ end termination sequences that do not originate in nature from the same gene as the coding sequence, are considered heterologous to said coding sequence. Elements operatively linked in nature and contiguous to each other are not heterologous to each other. On the other hand, these same elements remain operatively linked but become heterologous if other filler sequence is placed between them. Thus, the promoter and coding sequences of a corn gene expressing an amino acid transporter are not heterologous to each other, but the promoter and coding sequence of a corn gene operatively linked in a novel manner are heterologous.

(12) Homologous: In the current invention, a "homologous" polynucleotide refers to a polynucleotide that shares sequence similarity with the polynucleotide of interest. This similarity may be in only a fragment of the sequence and often represents a functional domain such as, examples including, without limitation, a DNA binding domain or a domain with tyrosine kinase activity. The functional activities of homologous polynucleotides are not necessarily the same.

(13) Inducible Promoter: An "inducible promoter" in the context of the current invention refers to a promoter, the activity of which is influenced by certain conditions, such as light, temperature, chemical concentration, protein concentration, conditions in an organism, cell, or organelle, etc. A typical example of an inducible promoter, which can be utilized with the polynucleotides of the present invention, is PARSK1, the promoter from an *Arabidopsis* gene encoding a serine-threonine kinase enzyme, and which promoter is induced by dehydration, abscisic acid and sodium chloride (Wang and Goodman (1995) *Plant J.* 8:37). Examples of environmental conditions that may affect transcription by inducible promoters include anaerobic conditions, elevated temperature, the presence or absence of a nutrient or other chemical compound or the presence of light.

(14) Misexpression: The term "misexpression" refers to an increase or a decrease in the transcription of a coding region into a complementary RNA sequence as compared to the wild-type. This term also encompasses expression and/or translation of a gene or coding region or inhibition of such transcription and/or translation for a different time period as compared to the wild-type and/or from a non-natural location within the plant genome, including a gene or coding region from a different plant species or from a non-plant organism.

(15) Modulate Transcription Level: As used herein, the phrase "modulate transcription" describes the biological activity of a promoter sequence or promoter control element. Such modulation includes, without limitation, up- and down-regulation of initiation of transcription, rate of transcription, and/or transcription levels.

(16) Operable Linkage: An "operable linkage" is a linkage in which a promoter sequence or promoter control element is connected to a polynucleotide sequence (or sequences) in such a way as to place transcription of the polynucleotide sequence under the influence or control of the promoter or promoter control element. Two DNA sequences (such as a polynucleotide to be transcribed and a promoter sequence linked to the 5′ end of the polynucleotide to be transcribed)

are said to be operably linked if induction of promoter function results in the transcription of mRNA encoding the polynucleotide and if the nature of the linkage between the two DNA sequences does not (1) result in the introduction of a frame-shift mutation, (2) interfere with the ability of the promoter sequence to direct the expression of the protein, antisense RNA, RNAi or ribozyme, or (3) interfere with the ability of the DNA template to be transcribed. Thus, a promoter sequence would be operably linked to a polynucleotide sequence if the promoter was capable of effecting transcription of that polynucleotide sequence.

(17) Percentage of sequence identity As used herein, the term “percent sequence identity” refers to the degree of identity between any given query sequence, e.g., SEQ ID NOs:1-26, and a subject sequence. A subject sequence typically has a length that is from about 80 percent to 250 percent of the length of the query sequence, e.g., 82, 85, 87, 89, 90, 93, 95, 97, 99, 100, 105, 110, 115, or 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, or 250 percent of the length of the query sequence. A query nucleic acid or amino acid sequence is aligned to one or more subject nucleic acid or amino acid sequences using the computer program ClustalW (version 1.83, default parameters), which allows alignments of nucleic acid or protein sequences to be carried out across their entire length (global alignment). Chenna et al. (2003) *Nucleic Acids Res.* 31(13):3497-500.

(18) ClustalW calculates the best match between a query and one or more subject sequences, and aligns them so that identities, similarities and differences can be determined. Gaps of one or more residues can be inserted into a query sequence, a subject sequence, or both, to maximize sequence alignments. For fast pairwise alignment of nucleic acid sequences, the following default parameters are used: word size: 2; window size: 4; scoring method: percentage; number of top diagonals: 4; and gap penalty: 5. For an alignment of multiple nucleic acid sequences, the following parameters are used: gap opening penalty: 10.0; gap extension penalty: 5.0; and weight transitions: yes. For fast pairwise alignment of protein sequences, the following parameters are used: word size: 1; window size: 5; scoring method: percentage; number of top diagonals: 5; gap penalty: 3. For multiple alignment of protein sequences, the following parameters are used: weight matrix: blosum; gap opening penalty: 10.0; gap extension penalty: 0.05; hydrophilic gaps: on; hydrophilic residues: Gly, Pro, Ser, Asn, Asp, Gln, Glu, Arg, and Lys; residue-specific gap penalties: on. The output is a sequence alignment that reflects the relationship between sequences. ClustalW can be run, for example, at the Baylor College of Medicine Search Launcher website and at the European Bioinformatics Institute website on the World Wide Web.

(19) To determine a percent identity of a subject polypeptide or nucleic acid sequence to a query sequence, the sequences are aligned using Clustal W, the number of identical matches in the alignment is divided by the length of the query sequence, and the result is multiplied by 100. The output is the percent identity of the subject sequence with respect to the query sequence. It is noted that the percent identity value can be rounded to the nearest tenth. For example, 78.11, 78.12, 78.13, and 78.14 are rounded down to 78.1, while 78.15, 78.16, 78.17, 78.18, and 78.19 are rounded up to 78.2.

(20) Plant Promoter: A “plant promoter” is a promoter capable of initiating transcription in plant cells and can modulate transcription of a polynucleotide. Such promoters need not be of plant origin. For example, promoters derived from plant viruses, such as the CaMV35S promoter or from *Agrobacterium tumefaciens* such as the T-DNA promoters, can be plant promoters. A typical example of a plant promoter of plant origin is the maize ubiquitin-1 (ubi-1) promoter known to those of skill in the art.

(21) Plant Tissue: The term “plant tissue” includes differentiated and undifferentiated tissues or plants, including but not limited to roots, stems, shoots, rhizomes, cotyledons, epicotyl, hypocotyl, leaves, pollen, seeds, gall tissue and various forms of cells in culture such as single cells, protoplast, embryos, and callus tissue. The plant tissue may be in plants or in organ, tissue or cell culture.

(22) Preferential Transcription: “Preferential transcription” is defined as transcription that occurs in

a particular pattern of cell types or developmental times or in response to specific stimuli or combination thereof. Non-limitive examples of preferential transcription include: high transcript levels of a desired sequence in root tissues; detectable transcript levels of a desired sequence in certain cell types during embryogenesis; and low transcript levels of a desired sequence under drought conditions. Such preferential transcription can be determined by measuring initiation, rate, and/or levels of transcription.

(23) Promoter: A “promoter” is a DNA sequence that directs the transcription of a polynucleotide. Typically a promoter is located in the 5′ region of a polynucleotide to be transcribed, proximal to the transcriptional start site of such polynucleotide. More typically, promoters are defined as the region upstream of the first exon; more typically, as a region upstream of the first of multiple transcription start sites; more typically, as the region downstream of the preceding gene and upstream of the first of multiple transcription start sites; more typically, the region downstream of the polyA signal and upstream of the first of multiple transcription start sites; even more typically, about 3,000 nucleotides upstream of the ATG of the first exon; even more typically, 2,000 nucleotides upstream of the first of multiple transcription start sites. The promoters of the invention comprise at least a core promoter as defined above. Frequently promoters are capable of directing transcription of genes located on each of the complementary DNA strands that are 3′ to the promoter. Stated differently, many promoters exhibit bidirectionality and can direct transcription of a downstream gene when present in either orientation (i.e. 5′ to 3′ or 3′ to 5′ relative to the coding region of the gene). Additionally, the promoter may also include at least one control element such as an upstream element. Such elements include UARs and optionally, other DNA sequences that affect transcription of a polynucleotide such as a synthetic upstream element.

(24) Promoter Control Element: The term “promoter control element” as used herein describes elements that influence the activity of the promoter. Promoter control elements include transcriptional regulatory sequence determinants such as, but not limited to, enhancers, scaffold/matrix attachment regions, TATA boxes, transcription start locus control regions, UARs, URRs, other transcription factor binding sites and inverted repeats.

(25) Public sequence: The term “public sequence,” as used in the context of the instant application, refers to any sequence that has been deposited in a publicly accessible database prior to the filing date of the present application. This term encompasses both amino acid and nucleotide sequences. Such sequences are publicly accessible, for example, on the BLAST databases on the NCBI FTP web site (accessible via the internet). The database at the NCBI FTP site utilizes “gi” numbers assigned by NCBI as a unique identifier for each sequence in the databases, thereby providing a non-redundant database for sequence from various databases, including GenBank, EMBL, DBBJ (DNA Database of Japan) and PDB (Brookhaven Protein Data Bank).

(26) Regulatory Regions: The term “regulatory region” refers to nucleotide sequences that, when operably linked to a sequence, influence transcription initiation or translation initiation or transcription termination of said sequence and the rate of said processes, and/or stability and/or mobility of a transcription or translation product. As used herein, the term “operably linked” refers to positioning of a regulatory region and said sequence to enable said influence. Regulatory regions include, without limitation, promoter sequences, enhancer sequences, response elements, protein recognition sites, inducible elements, protein binding sequences, 5′ and 3′ untranslated regions (UTRs), transcriptional start sites, termination sequences, polyadenylation sequences, and introns.

(27) The nucleic acid sequence set forth in SEQ ID NOs:1-26 are examples of regulatory regions provided herein. However, a regulatory region can have a nucleotide sequence that deviates from that set forth in SEQ ID NOs:1-26, while retaining the ability to direct expression of an operably linked nucleic acid. For example, a regulatory region having 80% or greater (e.g. 85% or greater, 90% or greater 91% or greater, 92% or greater, 93% or greater, 94% or greater, 95% or greater, 96% or greater, 97% or greater, 98% or greater, or 99% or greater) sequence identity to the nucleotide sequence set forth in SEQ ID NOs:1-25, or 26 can direct expression of an operably

linked nucleic acid.
(28) A regulatory region can also be a fragment of SEQ ID NOs:1-25, or 26, while retaining promoter activity, i.e. the ability to direct expression of an operably linked nucleic acid. Additional examples of regulatory regions are identified in the Sequence Listing.
(29) Regulatory regions can be classified in two categories, promoters and other regulatory regions.
(30) Regulatory Sequence: The term “regulatory sequence,” as used in the current invention, refers to any nucleotide sequence that influences transcription or translation initiation and rate, or stability and/or mobility of a transcript or polypeptide product. Regulatory sequences include, but are not limited to, promoter sequences, enhancer sequences, response elements, protein recognition sites, inducible elements, promoter control elements, protein binding sequences, 5′ and 3′ untranslated regions (UTRs), transcriptional start sites, termination sequences, polyadenylation sequences, introns, certain sequences within amino acid coding sequences such as secretory signals, protease cleavage sites, etc.
(31) A 5′ untranslated region (5′ UTR) of a gene is generally defined as a polynucleotide segment between the transcription start site (TSS) and the coding sequence start site (ATG codon) of a messenger RNA or cDNA. Alternately, 5′ UTR can be synthetically produced or manipulated DNA elements. A “plant 5′UTR” can be a native or non-native 5′UTR that is functional in plant cells. A 5′ UTR can be used as a 5′ regulatory element for modulating expression of an operably linked transcribable polynucleotide molecule. For example, 5′ UTRs derived from heat shock protein genes have been demonstrated to enhance gene expression in plants (see for example, U.S. Pat. Nos. 5,659,122 and 5,362,865, all of which are incorporated herein by reference). Examples of 5′UTRs include those shown in SEQ ID NOs: 1-4, 6-10, 13-26.
(32) Specific Promoters: In the context of the current invention, “specific promoters” refers to a subset of promoters that have a high preference for modulating transcript levels in a specific tissue, or organ or cell and/or at a specific time during development of an organism. By “high preference” is meant at least 3-fold, preferably 5-fold, more preferably at least 10-fold still more preferably at least 20-fold, 50-fold or 100-fold increase in transcript levels under the specific condition over the transcription under any other reference condition considered. Typical examples of temporal and/or tissue or organ specific promoters of plant origin that can be used with the polynucleotides of the present invention, are: PTA29, a promoter which is capable of driving gene transcription specifically in tapetum and only during anther development (Koltonow et al. (1990) *Plant Cell* 2:1201; RCc2 and RCc3, promoters that direct root-specific gene transcription in rice (Xu et al. (1995) *Plant Mol. Biol.* 27:237; TobRB27, a root-specific promoter from tobacco (Yamamoto et al. (1991) *Plant Cell* 3:371). Examples of tissue-specific promoters under developmental control include promoters that initiate transcription only in certain tissues or organs, such as root, ovule, fruit, seeds, or flowers. Other specific promoters include those from genes encoding seed storage proteins or the lipid body membrane protein, oleosin. A few root-specific promoters are noted above. See also “Preferential transcription.”
(33) A regulatory region can contain conserved regulatory motifs. Such a regulatory region can be any one of the sequences set forth in SEQ ID NOs:1-26, or a regulatory region having a nucleotide sequence that deviates from any one of those set forth in SEQ ID NOs:1-26, while retaining the ability to direct expression of an operably linked nucleic acid. For example, a regulatory region can contain a CAAT box or a TATA box. A CAAT box is a conserved nucleotide sequence involved in initiation of transcription. A CAAT box functions as a recognition and binding site for regulatory proteins called transcription factors. A TATA box is another conserved nucleotide sequence involved in transcription initiation. A TATA box seems to be important in determining accurately the position at which transcription is initiated.
(34) Other conserved regulatory motifs can be identified using methods known in the art. For example, a regulatory region can be analyzed using the PLACE (PLAnt Cis-acting regulatory DNA Elements) Web Signal Scan program on the world wide web at

dna.affrc.go.jp/PLACE/signalscan.html. See, Higo et al., *Nucleic Acids Research*, 27(1):297-300 (1999); and Prestridge, *CABIOS*, 7:203-206 (1991). Examples of conserved regulatory motifs can be found in the PLACE database on the world wide web at dna.affrc.go.jp/PLACE/. See, Higo et al., supra.

(35) A regulatory region such as any one of SEQ ID NOs:1-26, or a regulatory region having a nucleotide sequence that deviates from those set forth in SEQ ID NOs:1-26, while retaining the ability to direct expression of an operably linked nucleic acid, can contain one or more conserved regulatory motifs, which can be found in the PLACE database. For example, such a regulatory region can contain a −300CORE motif having the consensus sequence TGTAAAG (SEQ ID NO:27). See, Forde et al., *Nucleic Acids Res* 13:7327-7339 (1985); Colot et al., *EMBO J* 6:3559-3564 (1987); Thomas and Flavell, *Plant Cell* 2:1171-1180 (1990); Thompson et al., *Plant Mol Biol* 15:755-764 (1990); Vicente-Carbajosa et al., *Proc Natl Acad Sci USA* 94:7685-7690 (1997); Mena et al., *Plant J* 16:53-62 (1998); Shing, *Plant Physiol* 118: 1111-1120 (1998). Such a regulatory region can contain an ABREATCONSENSUS motif having the consensus sequence YACGTGGC (SEQ ID NO:28). See, Choi et al., *J Biol Chem* 275: 1723-1730 (2000); Kang et al., *Plant Cell* 14: 343-357 (2002); Oh et al., *Plant Physiology* 138: 341-351 (2005); Choi et al., *Plant Physiol* 139: 1750-1761(2005). Such a regulatory region can contain an ABREATRD22 motif having the consensus sequence RYACGTGGYR (SEQ ID NO:29). See, Iwasaki et al., *Mol Gen Genet* 247:391-398 (1995); Bray, *Trends in Plant Science* 2:48-54 (1997); Busk and Pages, *Plant Mol Biol* 37:425-435 (1998). A regulatory region can contain an ABRELATERD1 motif having the consensus sequence ACGTG (SEQ ID NO:30). See, Simpson et al., *Plant J* 33: 259-270 (2003); Nakashima et al., *Plant Mol Biol* 60:51-68 (2006). A regulatory region can contain an ABREMOTIFAOSOSEM motif having the consensus sequence TACGTGTC (SEQ ID NO:31). See, Hattori et al., *Plant J* 7: 913-925 (1995); Hobo et al., *Proc Natl Acad Sci USA* 96: 15348-15353 (1999). A regulatory region can contain an ABRERATCAL motif having the consensus sequence MACGYGB (SEQ ID NO:32). See, Kaplan et al., *Plant Cell* 18:2733-2748 (2006). A regulatory region can contain an ACGTCBOX motif having the consensus sequence GACGTC (SEQ ID NO:33). See, Foster et al., *FASEB J* 8:192-200 (1994); Izawa et al., *Plant Cell* 6:1277-1287 (1994); Izawa et al., *J Mol Biol* 230:1131-1144 (1993). A regulatory region can contain an ACGTOSGLUB1 motif having the consensus sequence GTACGTG (SEQ ID NO:34). See, Washida et al., *Plant Mol Biol* 40:1-12 (1999); Wu et al., *Plant J* 23: 415-421 (2000). A regulatory region can contain an ACGTTBOX motif having the consensus sequence AACGTT (SEQ ID NO:35). See, Foster et al., *FASEB J* 8:192-200 (1994). A regulatory region can contain an ACIIPVPAL2 motif having the consensus sequence CCACCAACCCCC (SEQ ID NO:36). See, Patzlaff et al., *Plant Mol Biol* 53:597-608 (2003); Hatton et al., *Plant J* 7:859-876 (1995); Gomez-Maldonado et al., *Plant J* 39:513-526 (2004). A regulatory region can contain an AGL2ATCONSENSUS motif having the consensus sequence NNWNCCAWWWWTRGWWAN (SEQ ID NO:37). See, Huang et al., *Plant Cell* 8: 81-94 (1996). A regulatory region can contain an AMYBOX2 motif having the consensus sequence TATCCAT (SEQ ID NO:38). See, Huang et al., *Plant Mol Biol* 14:655-668 (1990); Hwang et al., *Plant Mol Biol* 36:331-341 (1998). A regulatory region can contain an ANAERO1CONSENSUS motif having the consensus sequence AAACAAA (SEQ ID NO:39). See, Mohanty et al., *Ann Bot* (Lond).96: 669-681 (2005). A regulatory region can contain an ARE1 motif having the consensus sequence RGTGACNNNGC (SEQ ID NO:40). See, Rushmore et al., *J Biol Chem* 266:11632-11639 (1991). A regulatory region can contain an ATHB6COREAT motif having the consensus sequence CAATTATTA (SEQ ID NO:41). See, Himmelbach et al., *EMBO J* 21:3029-3038 (2002). A regulatory region can contain an AUXRETGA1GMGH3 motif having the consensus sequence TGACGTAA (SEQ ID NO:42). See, Liu et al., *Plant Cell* 6:645-657 (1994); Liu et al., *Plant Physiol* 115:397-407 (1997); Guilfoyle et al., *Plant Physiol* 118: 341-347 (1998). A regulatory region can contain a BOXIIPCCHS motif having the consensus sequence ACGTGGC (SEQ ID NO:43). See, Block et al., *Proc Natl Acad Sci*

*USA* 87:5387-5391(1990); Terzaghi and Cashmore, *Annu Rev Plant Physiol Plant Mol Biol* 46:445-474 (1995); Nakashima et al., *Plant Mol Biol* 60: 51-68 (2006). A regulatory region can contain a BOXLCOREDCPAL motif having the consensus sequence ACCWWCC (SEQ ID NO:44). See, Meada et al., *Plant Mol Biol* 59: 739-752.(2005). A regulatory region can contain a CACGCAATGMGH3 motif having the consensus sequence CACGCAAT (SEQ ID NO:45). See, Ulmasov et al., *Plant Cell* 7: 1611-1623 (1995). A regulatory region can contain a CARGATCONSENSUS motif having the consensus sequence CCWWWWWWGG (SEQ ID NO:46). See, Hepworth et al., *EMBO J* 21: 4327-4337 (2002); Michaels et al., *Plant J* 33: 867-874 (2003); Hong et al., *Plant Cell* 15:1296-1309 (2003); Folter and Angenent, *Trends Plant Sci* 11:224-231 (2006). A regulatory region can contain a CARGCW8GAT motif having the consensus sequence CWWWWWWWWG (SEQ ID NO:47). See, Tang and Perry, *J Biol Chem* 278:28154-28159 (2003); Folter and Angenent, *Trends Plant Sci* 11:224-231 (2006). A regulatory region can contain a CIACADIANLELHC motif having the consensus sequence CAANNNNATC (SEQ ID NO:48). See, Piechulla et al., *Plant Mol Biol* 38:655-662 (1998). A regulatory region can contain a DPBFCOREDCDC3 motif having the consensus sequence ACACNNG (SEQ ID NO:49). See, Kim et al., *Plant J* 11: 1237-1251 (1997); Finkelstein and Lynch, *Plant Cell* 12: 599-609 (2000); Lopez-Molina and Chua, *Plant Cell Physiol* 41: 541-547 (2000). A regulatory region can contain a DRE2COREZMRAB17 motif having the consensus sequence ACCGAC (SEQ ID NO:50). See, Busk et al., *Plant J* 11: 1285-1295 (1997); Dubouzet et al., *Plant J* 33: 751-763 (2003); Kizis and Pages, *Plant J* 30:679-689 (2002). A regulatory region can contain an E2FCONSENSUS motif having the consensus sequence WTTSSCSS (SEQ ID NO:51). See, Vandepoele et al., *Plant Physiol* 139: 316-328. (2005). A regulatory region can contain an EMHVCHORD motif having the consensus sequence TGTAAAGT (SEQ ID NO:52). See, Muller and Knudsen, *Plant J* 4: 343-355 (1993). A regulatory region can contain an EVENINGAT motif having the consensus sequence AAAATATCT (SEQ ID NO:53). See, Rawat et al., *Plant Mol Biol* 57: 629-643 (2005) and Harmer et al., *Science* 290: 2110-2113 (2000). A regulatory region can contain an GLMHVCHORD motif having the consensus sequence RTGASTCAT (SEQ ID NO:54). See, Albani et al., *Plant Cell* 9: 171-184 (1997); Muller M *Plant J* 4: 343-355 (1993); Onate et al., *J Biol Chem* 274: 9175-9182 (1999). A regulatory region can contain a GT1 Consensus motif having the consensus sequence GRWAAW (SEQ ID NO:55). See, Terzaghi and Cashmore, supra.; Villain et al., *J Biol Chem* 271:32593-32598 (1996); Le Gourrierec et al., *Plant J* 18:663-668 (1999); Buchel et al., *Plant Mol Biol* 40:387-396 (1999); Zhou, *Trends in Plant Science* 4:210-214 (1999). A regulatory region can contain a GT1GMSCAM4 motif having the consensus sequence GAAAAA (SEQ ID NO:56). See, Park et al., *Plant Physiol* 135: 2150-2161 (2004). A regulatory region can contain a HDZIP2ATATHB2 motif having the consensus sequence TAATMATTA (SEQ ID NO:57). See, Ohgishi et al., *Plant J* 25: 389-398 (2001). A regulatory region can contain an IBOXCORENT motif having the consensus sequence GATAAGR (SEQ ID NO:58). See, Martinez-Hernandez et al., *Plant Physiol* 128:1223-1233 (2002). A regulatory region can contain an INRNTPSADB motif having the consensus sequence YTCANTYY (SEQ ID NO:59). See, Nakamura et al., *Plant J* 29: 1-10 (2002). A regulatory region can contain a LEAFYATAG motif having the consensus sequence CCAATGT (SEQ ID NO:60). See, Kamiya et al., *Plant J* 35: 429-441 (2003). A regulatory region can contain a LRENPCABE motif having the consensus sequence ACGTGGCA (SEQ ID NO:61). See, Castresana et al., *EMBO J* 7:1929-1936 (1988). A regulatory region can contain a MARTBOX motif having the consensus sequence TTWTWTTWTT (SEQ ID NO:62). See, Gasser et al., *Intnatl Rev Cyto* 119:57-96 (1989). A regulatory region can contain a MYBGAHV motif having the consensus sequence TAACAAA (SEQ ID NO:63). See, Gubler et al., *Plant Cell* 7:1879-1891 (1995); Morita et al., *FEBS Lett* 423:81-85 (1998); Gubler et al., *Plant J* 17:1-9(1999). A regulatory region can contain a MYBPLANT motif having the consensus sequence MACCWAMC (SEQ ID NO:64). See, Sablowski et al., *EMBO J* 13:128-137 (1994); Tamagnone et al., *Plant Cell* 10: 135-154 (1998). A regulatory region can contain a NRRBNEXTA motif having the consensus sequence

TAGTGGAT (SEQ ID NO:65). See, Elliott and Shirsat, *Plant Mol Biol* 37:675-687 (1998). A regulatory region can contain an O2F3BE2S1 motif having the consensus sequence TCCACGTACT (SEQ ID NO:66). See, Vincentz et al., *Plant Mol Biol* 34:879-889 (1997). A regulatory region can contain a P1BS motif having the consensus sequence GNATATNC (SEQ ID NO:67). See, Rubio et al., *Genes Dev.* 15: 2122-2133.(2001); Shunmann et al., *J Exp Bot.* 55: 855-865. (2004); Shunmann et al., *Plant Physiol* 136: 4205-4214. (2004). A regulatory region can contain a PRECONSCRHSP70A motif having the consensus sequence SCGAYNRNNNNNNNNNNNNHD (SEQ ID NO:68). See, von Gromoff et al., *Nucleic Acids Res* 34:4767-4779 (2006). A regulatory region can contain a PROXBBNNAPA motif having the consensus sequence CAAACACC (SEQ ID NO:69). See, Ezcurra et al., *Plant Mol Biol* 40:699-709 (1999); Busk and Pages, supra.; Ezcurra et al., *Plant J* 24:57-66 (2000). A regulatory region can contain a PYRIMIDINEBOXHVEPB1 motif having the consensus sequence TTTTTTCC (SEQ ID NO:70). See, Cercos et al., *Plant J* 19: 107-118 (1999). A regulatory region can contain a RBCSCONSENSUS motif having the consensus sequence AATCCAA (SEQ ID NO:71). See, Manzara and Gruissem, *Photosynth Res* 16:117-139 (1988); Donald and Cashmore, *EMBO J* 9:1717-1726 (1990). A regulatory region can contain a ROOTMOTIFTAPOX1 motif having the consensus sequence ATATT (SEQ ID NO:72). See, Elmayan and Tepfer, *Transgenic Res* 4:388-396 (1995). A regulatory region can contain a RYREPEATVFLEB4 motif having the consensus sequence CATGCATG (SEQ ID NO:73). See, Curaba et al., *Plant Physiol* 136: 3660-3669. (2004); Nag et al., *Plant Mol Biol* 59: 821-838 (2005). A regulatory region can contain a SEF1MOTIF motif having the consensus sequence ATATTTAWW (SEQ ID NO:74). See, Allen et al., *Plant Cell* 1:623-631 (1989); Lessard et al., *Plant Mol Biol* 16:397-413 (1991). A regulatory region can contain a SORLREP3AT motif having the consensus sequence TGTATATAT (SEQ ID NO:75). See, Hudson and Quail, *Plant Physiol* 133: 1605-1616 (2003). A regulatory region can contain a SURE2STPAT21 motif having the consensus sequence AATACTAAT (SEQ ID NO:76). See, Grierson et al., *Plant J* 5:815-826 (1994). A regulatory region can contain a SV40COREENHAN motif having the consensus sequence GTGGWWHG (SEQ ID NO:77). See, Weiher et al., *Science* 219:626-631 (1983); Green et al., *EMBO J* 6:2543-2549 (1987); Donald and Cashmore, *EMBO J* 9:1717-1726 (1990). A regulatory region can contain a TATABOX2 motif having the consensus sequence TATAAAT (SEQ ID NO:78). See, Shirsat et al., *Mol Gen Genet* 215:326-331 (1989); Grace et al., *Biol Chem* 279:8102-8110 (2004). A regulatory region can contain a TATABOX3 motif having the consensus sequence TATTAAT (SEQ ID NO:79). See, PLACE (PLAnt Cis-acting regulatory DNA Elements) at dna.affrc.go.jp/PLCAE/signalscan.html). A regulatory region can contain a TATABOX4 motif having the consensus sequence TATATAA (SEQ ID NO:80). See, Grace et al., *J Biol Chem* 279:8102-8110 (2004). A regulatory region can contain a TATABOX5 motif having the consensus sequence TTATTT (SEQ ID NO:81). See, Tjaden et al., *Plant Physiol* 108:1109-1117 (1995). A regulatory region can contain a TATABOXOSPAL motif having the consensus sequence TATTTAA (SEQ ID NO:82). See, Zhu et al., *Plant Cell* 14: 795-803 (2002). A regulatory region can contain a TELOBOXATEEF1AA1 motif having the consensus sequence AAACCCTAA (SEQ ID NO:83). See, Tremousayque et al., *Plant J* 20: 553-561 (1999); Axelos et al., *Mol Gen Genet* 219: 106-112 (1989); Welchen and Gonzalez, *Plant Physiol* 139: 88-100 (2005). A regulatory region can contain a TL1ATSAR motif having the consensus sequence CTGAAGAAGAA (SEQ ID NO:84). See, Wang et al., *Science* 308: 1036-1040 (2005). A regulatory region can contain a UP2ATMSD motif having the consensus sequence AAACCCTA (SEQ ID NO:85). See, Tatematsu et al., *Plant Physiology* 138: 757-766 (2005). A regulatory region can contain a WBBOXPCWRKY1 motif having the consensus sequence TTTGACY (SEQ ID NO:86). See, Ishiguro and Nakamura, *Mol Gen Genet* 244:563-571 (1994); Rushton et al., *Plant Mol Biol* 29:691-702 (1995); Rushon et al., *EMBO J* 15:5690-5700 (1996); de Pater et al., *Nucleic Acids Res* 24:4624-4631 (1996); Eulgem et al., *Trends Plant Sci* 5: 199-206 (2000). A regulatory region can contain a XYLAT motif having the consensus sequence ACAAAGAA (SEQ ID NO:87).

See, Ko et al., *Mol Genet Genomics* 276:517-531 (2006).
(36) Stringency: “Stringency,” as used herein is a function of nucleic acid molecule probe length, nucleic acid molecule probe composition (G+C content), salt concentration, organic solvent concentration and temperature of hybridization and/or wash conditions. Stringency is typically measured by the parameter T.sub.m, which is the temperature at which 50% of the complementary nucleic acid molecules in the hybridization assay are hybridized, in terms of a temperature differential from T.sub.m. High stringency conditions are those providing a condition of T.sub.m−5° C. to T.sub.m−10° C. Medium or moderate stringency conditions are those providing T.sub.m−20° C. to T.sub.m−29° C. Low stringency conditions are those providing a condition of T.sub.m−40° C. to T.sub.m−48° C. The relationship between hybridization conditions and T.sub.m (in ° C.) is expressed in the mathematical equation:
*T*.sub.m=81.5−16.6(log.sub.10[Na.sup.+])+0.41(% *G*+*C*)−(600/*N*) (I)
(37) where N is the number of nucleotides of the nucleic acid molecule probe. This equation works well for probes 14 to 70 nucleotides in length that are identical to the target sequence. The equation below, for T.sub.m of DNA-DNA hybrids, is useful for probes having lengths in the range of 50 to greater than 500 nucleotides, and for conditions that include an organic solvent (formamide):
*T*.sub.m=81.5+16.6 log {[Na.sup.+]/(1+0.7[Na.sup.+])}+0.41(% *G*+*C*)−500/*L* 0.63(% formamide) (II)
(38) where L represents the number of nucleotides in the probe in the hybrid (21). The T.sub.m of Equation II is affected by the nature of the hybrid: for DNA-RNA hybrids, T.sub.m is 10-15° C. higher than calculated; for RNA-RNA hybrids, T.sub.m is 20-25° C. higher. Because the T.sub.m decreases about 1° C. for each 1% decrease in homology when a long probe is used (Frischauf et al. (1983) *J. Mol Biol,* 170: 827-842), stringency conditions can be adjusted to favor detection of identical genes or related family members.
(39) Equation II is derived assuming the reaction is at equilibrium. Therefore, hybridizations according to the present invention are most preferably performed under conditions of probe excess and allowing sufficient time to achieve equilibrium. The time required to reach equilibrium can be shortened by using a hybridization buffer that includes a hybridization accelerator such as dextran sulfate or another high volume polymer.
(40) Stringency can be controlled during the hybridization reaction, or after hybridization has occurred, by altering the salt and temperature conditions of the wash solutions. The formulas shown above are equally valid when used to compute the stringency of a wash solution. Preferred wash solution stringencies lie within the ranges stated above; high stringency is 5-8° C. below T.sub.m, medium or moderate stringency is 26-29° C. below T.sub.m and low stringency is 45-48° C. below T.sub.m. T.sub.0: The term “T.sub.0” refers to the whole plant, explant or callus tissue, inoculated with the transformation medium. T.sub.1: The term T.sub.1 refers to either the progeny of the T.sub.0 plant, in the case of whole-plant transformation, or the regenerated seedling in the case of explant or callous tissue transformation. T.sub.2: The term T.sub.2 refers to the progeny of the T.sub.1 plant. T.sub.2 progeny are the result of self-fertilization or cross-pollination of a T.sub.1 plant. T.sub.3: The term T.sub.3 refers to second generation progeny of the plant that is the direct result of a transformation experiment. T.sub.3 progeny are the result of self-fertilization or cross-pollination of a T.sub.2 plant. TATA to start: “TATA to start” shall mean the distance, in number of nucleotides, between the primary TATA motif and the start of transcription. Transgenic plant: A “transgenic plant” is a plant having one or more plant cells that contain at least one exogenous polynucleotide introduced by recombinant nucleic acid methods. Translational start site: In the context of the present invention, a “translational start site” is usually an ATG or AUG in a transcript, often the first ATG or AUG. A single protein encoding transcript, however, may have multiple translational start sites. Transcription start site: “Transcription start site” is used in the current invention to describe the point at which transcription is initiated. This point is typically located about 25 nucleotides downstream from a TFIID binding site, such as a TATA box.

Transcription can initiate at one or more sites within the gene, and a single polynucleotide to be transcribed may have multiple transcriptional start sites, some of which may be specific for transcription in a particular cell-type or tissue or organ. “+1” is stated relative to the transcription start site and indicates the first nucleotide in a transcript. Upstream Activating Region (UAR): An “Upstream Activating Region” or “UAR” is a position or orientation dependent nucleic acid element that primarily directs tissue, organ, cell type, or environmental regulation of transcript level, usually by affecting the rate of transcription initiation. Corresponding DNA elements that have a transcription inhibitory effect are called herein “Upstream Repressor Regions” or “URR”s. The essential activity of these elements is to bind a protein factor. Such binding can be assayed by methods described below. The binding is typically in a manner that influences the steady state level of a transcript in a cell or in vitro transcription extract. Untranslated region (UTR): A “UTR” is any contiguous series of nucleotide bases that is transcribed, but is not translated. A 5′ UTR lies between the start site of the transcript and the translation initiation codon and includes the +1 nucleotide. A 3′ UTR lies between the translation termination codon and the end of the transcript. UTRs can have particular functions such as increasing mRNA message stability or translation attenuation. Examples of 3′ UTRs include, but are not limited to polyadenylation signals and transcription termination sequences.

2. Use of the Promoters of the Invention

(41) The promoters and promoter control elements of this invention are capable of modulating transcription. Such promoters and promoter control elements can be used in combination with native or heterologous promoter fragments, control elements or other regulatory sequences to modulate transcription and/or translation.

(42) Specifically, promoters and control elements of the invention can be used to modulate transcription of a desired polynucleotide, which includes without limitation: (i) antisense; (ii) ribozymes; (iii) coding sequences; or (iv) fragments thereof.

(43) The promoter also can modulate transcription in a host genome in cis- or in trans-.

(44) In an organism, such as a plant, the promoters and promoter control elements of the instant invention are useful to produce preferential transcription which results in a desired pattern of transcript levels in a particular cells, tissues, or organs, or under particular conditions.

4. Identifying and Isolating Promoter Sequences of the Invention

(45) The promoters and promoter control elements of the present invention are presented in the Promoter Reports of the Tables and were identified from *Arabidopsis thaliana* and *Oryza sativa*. Isolation from genomic libraries of polynucleotides comprising the sequences of the promoters and promoter control elements of the present invention is possible using known techniques. For example, polymerase chain reaction (PCR) can amplify the desired polynucleotides utilizing primers designed from SEQ ID NOs: 1-26 or residues 601-1000 of SEQ ID NO: 26. Polynucleotide libraries comprising genomic sequences can be constructed according to Sambrook et al., *Molecular Cloning: A Laboratory Manual,* 2.sup.nd Ed. (1989) Cold Spring Harbor Press, Cold Spring Harbor, NY), for example.

(46) Other procedures for isolating polynucleotides comprising the promoter sequences of the invention include, without limitation, tail-PCR, and 5′ rapid amplification of cDNA ends (RACE). See, for tail-PCR, for example, Liu et al. (1995) *Plant J* 8(3): 457-463; Liu et al. (1995) *Genomics* 25: 674-681; Liu et al. (1993) *Nucl. Acids Res.* 21(14): 3333-3334; and Zoe et al. (1999) *BioTechniques* 27(2): 240-248; for RACE, see, for example, *PCR Protocols: A Guide to Methods and Applications*, (1990) Academic Press, Inc.

(47) In addition, the promoters and promoter control elements described in the Promoter Reports in the Tables (SEQ. ID. Nos. 1-26) can be chemically synthesized according to techniques in common use. See, for example, Beaucage et al. (1981) *Tet. Lett.* 22: 1859 and U.S. Pat. No. 4,668,777. Such chemical oligonucleotide synthesis can be carried out using commercially available devices, such as, Biosearch 4600 or 8600 DNA synthesizer, by Applied Biosystems, a division of Perkin-Elmer

Corp., Foster City, California, USA; and Expedite by Perceptive Biosystems, Framingham, Massachusetts, USA.

(48) Included in the present invention are promoters exhibiting nucleotide sequence identity to SEQ. ID. Nos. 1-26 or nucleic acid residues 601-1000 of SEQ ID NO: 26 namely that exhibits at least 80% sequence identity, at least 85%, at least 90%, and at least 95%, 96%, 97%, 98% or 99% sequence identity compared to SEQ. ID. Nos. 1-26 or residues 601-1000 of SEQ ID NO: 26. Such sequence identity can be calculated by the algorithms and computers programs described above.

(49) The present invention further encompasses “functional variants” or “function fragments” of the disclosed sequences, particularly fragments of SEQ ID NOs: 1-26 and residues 601-1000 of SEQ ID NO: 5 that retain promoter activity. Functional variants include, for example, regulatory sequences of the invention having one or more nucleotide substitutions, deletions or insertions and wherein the variant retains promoter activity. Functional variants can be created by any of a number of methods available to one skilled in the art, such as by site-directed mutagenesis, induced mutation, identified as allelic variants, cleaving through use of restriction enzymes, or the like. Activity can likewise be measured by any variety of techniques, including measurement of reporter activity as is described at U.S. Pat. No. 6,844,484, Northern blot analysis, or similar techniques. The '484 patent describes the identification of functional variants of different promoters.

(50) Functional fragment, that is, a regulatory sequence fragment can be formed by one or more deletions from a larger regulatory element. For example, in some instances, the 5′ portion of a promoter up to the TATA box near the transcription start site can be deleted without abolishing promoter activity, as described by Opsahl-Sorteberg, H-G. et al., “Identification of a 49-bp fragment of the HvLTP2 promoter directing aleruone cell specific expression” Gene 341:49-58 (2004). Such fragments should retain promoter activity, particularly the ability to drive expression of operably linked nucleotide sequences. Activity can be measured by Northern blot analysis, reporter activity measurements when using transcriptional fusions, and the like. See, for example, Sambrook et al., Molecular Cloning, A laboratory Manual (1989). Functional fragments can be obtained by use of restriction enzymes to cleave the naturally occurring regulatory element nucleotide sequences disclosed herein; by synthesizing a nucleotide sequence from the naturally occurring DNA sequence; or can be obtained through the use of PCR technology. See particularly, Mullis et al., Methods Enzymol., 155:335-350 (1987) and Erlich, ed., PCR Technology (Stockton Press, New York), (1989).

(51) For example, a routine way to remove part of a DNA sequence is to use an exonuclease in combination with DNA amplification to produce unidirectional nested deletions of double stranded DNA clones. A commercial kit for this purpose is sold under the trade name Exo-Size™ (New England Biolabs, Beverly, Mass.). Briefly, this procedure entails incubating exonuclease III with DNA to progressively remove nucleotides in the 3′ to 5′ direction at 5′ overhangs, blunt ends or nicks in the DNA template. However, exonuclease III is unable to remove nucleotides at 3′, 4-base overhangs. Timed digests of a clone with this enzyme produces unidirectional nested deletions.

5. Testing of Promoters

(52) Promoters of the invention, including functional fragments, are tested for activity by cloning the sequence into an appropriate vector, transforming plants with the construct and assaying for marker gene expression. Recombinant DNA constructs are prepared which comprise the promoter sequences of the invention inserted into a vector suitable for transformation of plant cells. The construct can be made using standard recombinant DNA techniques (Sambrook et al. 1989) and can be introduced to the species of interest by *Agrobacterium*-mediated transformation or by other means of transformation as referenced below.

(53) The vector backbone can be any of those typical in the art such as plasmids, viruses, artificial chromosomes, BACs, YACs and PACs and vectors of the sort described by (a) BAC: Shizuya et al. (1992) *Proc. Natl. Acad. Sci. USA* 89: 8794-8797; Hamilton et al. (1996) *Proc. Natl. Acad. Sci. USA* 93: 9975-9979; (b) YAC: Burke et al. (1987) *Science* 236:806-812; (c) PAC: Sternberg N. et

al. (1990) Proc Natl Acad Sci USA. 87(l):103-7; (d) Bacteria-Yeast Shuttle Vectors: Bradshaw et al. (1995) *Nucl Acids Res* 23: 4850-4856; (e) Lambda Phage Vectors: Replacement Vector, e.g., Frischauf et al. (1983) *J. Mol Biol* 170: 827-842; or Insertion vector, e.g., Huynh et al. (1985) In: Glover N M (ed) *DNA Cloning: A practical Approach*, Vol. 1 Oxford: IRL Press; T-DNA gene fusion vectors: Walden et al. (1990) *Mol Cell Biol* 1: 175-194; and (g) Plasmid vectors: Sambrook et al., infra.

(54) Typically, the construct comprises a vector containing a promoter sequence of the present invention operationally linked to any marker gene. The promoter was identified as a promoter by the expression of the marker gene. Although many marker genes can be used, Green Fluorescent Protein (GFP) is preferred. The vector may also comprise a marker gene that confers a selectable phenotype on plant cells. The marker may encode biocide resistance, particularly antibiotic resistance, such as resistance to kanamycin, G418, bleomycin, hygromycin, or herbicide resistance, such as resistance to chlorosulfuron or phosphinotricin. Vectors can also include origins of replication, scaffold attachment regions (SARs), markers, homologous sequences, introns, etc.

6. Constructing Promoters with Control Elements

(55) 6.1 Combining Promoters and Promoter Control Elements

(56) The promoter and promoter control elements of the present invention, both naturally occurring and synthetic, can be used alone or combined with each other to produce the desired preferential transcription. Also, the promoters of the invention can be combined with other known sequences to obtain other useful promoters to modulate, for example, tissue transcription specific or transcription specific to certain conditions. Such preferential transcription can be determined using the techniques or assays described above.

(57) Promoters can contain any number of control elements. For example, a promoter can contain multiple transcription binding sites or other control elements. One element may confer tissue or organ specificity; another element may limit transcription to specific time periods, etc. Typically, promoters will contain at least a basal or core promoter as described above. Any additional element can be included as desired. For example, a fragment comprising a basal or “core” promoter can be fused with another fragment with any number of additional control elements.

(58) The following are promoters that are induced under stress conditions and can be combined with those of the present invention: ldh1 (oxygen stress; tomato; see Germain and Ricard (1997) *Plant Mol Biol* 35:949-54), GPx and CAT (oxygen stress; mouse; see Franco et al. (1999) *Free Radic Biol Med* 27:1122-32), ci7 (cold stress; potato; see Kirch et al. (1997) *Plant Mol Biol.* 33:897-909), Bz2 (heavy metals; maize; see Marrs and Walbot (1997) *Plant Physiol* 113:93-102), HSP32 (hyperthermia; rat; see Raju and Maines (1994) *Biochim Biophys Acta* 1217:273-80), and MAPKAPK-2 (heat shock; *Drosophila*; see Larochelle and Suter (1995) *Gene* 163:209-14).

(59) In addition, the following examples of promoters are induced by the presence or absence of light can be used in combination with those of the present invention: Topoisomerase II (pea; see Reddy et al. (1999) *Plant Mol Biol* 41:125-37), chalcone synthase (soybean; see Wingender et al. (1989) *Mol Gen Genet* 218:315-22) mdm2 gene (human tumor; see Saucedo et al. (1998) *Cell Growth Differ* 9:119-30), Clock and BMAL1 (rat; see Namihira et al. (1999) *Neurosci Lett* 271:1-4, PHYA (*Arabidopsis*; see Canton and Quail (1999) *Plant Physiol* 121:1207-16), PRB-1b (tobacco; see Sessa et al. (1995) *Plant Mol Biol* 28:537-47) and Ypr10 (common bean; see Walter et al. (1996) *Eur J Biochem* 239:281-93).

(60) The promoters and control elements of the following genes can be used in combination with the present invention to confer tissue specificity: MipB (iceplant; Yamada et al. (1995) *Plant Cell* 7:1129-42) and SUCS (root nodules; broadbean; Kuster et al. (1993) *Mol Plant Microbe Interact* 6:507-14) for roots, OsSUTI (rice; Hirose et al. (1997) *Plant Cell Physiol* 38:1389-96) for leaves, Msg (soybean; Stomvik et al. (1999) *Plant Mol Biol* 41:217-31) for siliques, cell (*Arabidopsis*; Shani et al. (1997) *Plant Mol Biol* 34(6):837-42) and ACT11 (*Arabidopsis*; Huang et al. (1997) *Plant Mol Biol* 33:125-39) for inflorescence.

(61) Still other promoters are affected by hormones or participate in specific physiological processes, which can be used in combination with those of present invention. Some examples are the ACC synthase gene that is induced differently by ethylene and brassinosteroids (mung bean; Yi et al. (1999) *Plant Mol Biol* 41:443-54), the TAPG1 gene that is active during abscission (tomato; Kalaitzis et al. (1995) *Plant Mol Biol* 28:647-56), and the 1-aminocyclopropane-1-carboxylate synthase gene (carnation; Jones et al. (1995) *Plant Mol Biol* 28:505-12) and the CP-2/cathepsin L gene (rat; Kim and Wright (1997) *Biol Reprod* 57:1467-77), both active during senescence.
(62) Spacing between control elements or the configuration or control elements can be determined or optimized to permit the desired protein-polynucleotide or polynucleotide interactions to occur.
(63) For example, if two transcription factors bind to a promoter simultaneously or relatively close in time, the binding sites are spaced to allow each factor to bind without steric hindrance. The spacing between two such hybridizing control elements can be as small as a profile of a protein bound to a control element. In some cases, two protein binding sites can be adjacent to each other when the proteins bind at different times during the transcription process.
(64) Further, when two control elements hybridize the spacing between such elements will be sufficient to allow the promoter polynucleotide to hairpin or loop to permit the two elements to bind. The spacing between two such hybridizing control elements can be as small as a t-RNA loop, to as large as 10 kb.
(65) Typically, the spacing is no smaller than 5 bases; more typically, no smaller than 8; more typically, no smaller than 15 bases; more typically, no smaller than 20 bases; more typically, no smaller than 25 bases; even more typically, no smaller than 30, 35, 40 or 50 bases.
(66) Usually, the fragment size in no larger than 5 kb bases; more usually, no larger than 2 kb; more usually, no larger than 1 kb; more usually, no larger than 800 bases; more usually, no larger than 500 bases; even more usually, no more than 250, 200, 150 or 100 bases. In some embodiments, the nucleic acid of the invention comprises at least one fragment of YP0286 (SEQ ID NO:5), e.g., YP2219 (SEQ ID NO:4), with the proviso that said nucleic acid does not consist of YP0286 (SEQ ID NO:5).
(67) Such spacing between promoter control elements can be determined using the techniques and assays described above.
(68) 6.2 Vectors Used to Transform Cells/Hosts
(69) A plant transformation construct containing a promoter of the present invention may be introduced into plants by any plant transformation method. Methods and materials for transforming plants by introducing a plant expression construct into a plant genome in the practice of this invention can include any of the well-known and demonstrated methods including electroporation (U.S. Pat. No. 5,384,253); microprojectile bombardment (U.S. Pat. Nos. 5,015,580; 5,550,318; 5,538,880; 6,160,208; 6,399,861; and 6,403,865); *Agrobacterium*-mediated transformation (U.S. Pat. Nos. 5,824,877; 5,591,616; 5,981,840; and 6,384,301); and protoplast transformation (U.S. Pat. No. 5,508,184).
(70) The present promoters and/or promoter control elements may be delivered to a system such as a cell by way of a vector. For the purposes of this invention, such delivery may range from simply introducing the promoter or promoter control element by itself randomly into a cell to integration of a cloning vector containing the present promoter or promoter control element. Thus, a vector need not be limited to a DNA molecule such as a plasmid, cosmid or bacterial phage that has the capability of replicating autonomously in a host cell. All other manner of delivery of the promoters and promoter control elements of the invention are envisioned. The various T-DNA vector types are a preferred vector for use with the present invention. Many useful vectors are commercially available.
(71) It may also be useful to attach a marker sequence to the present promoter and promoter control element in order to determine activity of such sequences. Marker sequences typically include genes that provide antibiotic resistance, such as tetracycline resistance, hygromycin resistance or

ampicillin resistance, or provide herbicide resistance. Specific selectable marker genes may be used to confer resistance to herbicides such as glyphosate, glufosinate or broxynil (Comai et al. (1985) *Nature* 317: 741-744; Gordon-Kamm et al. (1990) *Plant Cell* 2: 603-618; and Stalker et al. (1988) *Science* 242: 419-423). Other marker genes exist which provide hormone responsiveness.

(72) The promoter or promoter control element of the present invention may be operably linked to a polynucleotide to be transcribed. In this manner, the promoter or promoter control element may modify transcription by modulating transcript levels of that polynucleotide when inserted into a genome.

(73) However, prior to insertion into a genome, the promoter or promoter control element need not be linked, operably or otherwise, to a polynucleotide to be transcribed. For example, the promoter or promoter control element may be inserted alone into the genome in front of a polynucleotide already present in the genome. In this manner, the promoter or promoter control element may modulate the transcription of a polynucleotide that was already present in the genome. This polynucleotide may be native to the genome or inserted at an earlier time.

(74) Alternatively, the promoter or promoter control element may be inserted into a genome alone to modulate transcription. See, for example, Vaucheret, H et al. (1998) *Plant J* 16: 651-659. Rather, the promoter or promoter control element may be simply inserted into a genome or maintained extrachromosomally as a way to divert transcription resources of the system to itself. This approach may be used to downregulate the transcript levels of a group of polynucleotide(s).

(75) The nature of the polynucleotide to be transcribed is not limited. Specifically, the polynucleotide may include sequences that will have activity as RNA as well as sequences that result in a polypeptide product. These sequences may include, but are not limited to antisense sequences, RNAi sequences, ribozyme sequences, spliceosomes, amino acid coding sequences, and fragments thereof. Specific coding sequences may include, but are not limited to endogenous proteins or fragments thereof, or heterologous proteins including marker genes or fragments thereof.

(76) Constructs of the present invention would typically contain a promoter operably linked to a transcribable nucleic acid molecule operably linked to a 3′ transcription termination nucleic acid molecule. In addition, constructs may include but are not limited to additional regulatory nucleic acid molecules from the 3′-untranslated region (3′ UTR) of plant genes (e.g., a 3′ UTR to increase mRNA stability of the mRNA, such as the PI-II termination region of potato or the octopine or nopaline synthase 3′ termination regions). Constructs may include but are not limited to the 5′ untranslated regions (5′ UTR) of an mRNA nucleic acid molecule which can play an important role in translation initiation and can also be a genetic component in a plant expression construct. For example, non-translated 5′ leader nucleic acid molecules derived from heat shock protein genes have been demonstrated to enhance gene expression in plants (see for example, U.S. Pat. Nos. 5,659,122 and 5,362,865, all of which are hereby incorporated by reference). These additional upstream and downstream regulatory nucleic acid molecules may be derived from a source that is native or heterologous with respect to the other elements present on the promoter construct.

(77) Thus, one embodiment of the invention is a promoter such as provided in SEQ ID NOs: 1-26 or residues 601-1000 of SEQ ID NO: 26, operably linked to a transcribable nucleic acid molecule so as to direct transcription of said transcribable nucleic acid molecule at a desired level or in a desired tissue or developmental pattern upon introduction of said construct into a plant cell. In some cases, the transcribable nucleic acid molecule comprises a protein-coding region of a gene, and the promoter provides for transcription of a functional mRNA molecule that is translated and expressed as a protein product. Constructs may also be constructed for transcription of antisense RNA molecules or other similar inhibitory RNA in order to inhibit expression of a specific RNA molecule of interest in a target host cell.

(78) Exemplary transcribable nucleic acid molecules for incorporation into constructs of the present invention include, for example, nucleic acid molecules or genes from a species other than the target

gene species, or even genes that originate with or are present in the same species, but are incorporated into recipient cells by genetic engineering methods rather than classical reproduction or breeding techniques. Exogenous gene or genetic element is intended to refer to any gene or nucleic acid molecule that is introduced into a recipient cell. The type of nucleic acid molecule included in the exogenous nucleic acid molecule can include a nucleic acid molecule that is already present in the plant cell, a nucleic acid molecule from another plant, a nucleic acid molecule from a different organism, or a nucleic acid molecule generated externally, such as a nucleic acid molecule containing an antisense message of a gene, or a nucleic acid molecule encoding an artificial or modified version of a gene.

(79) The promoters of the present invention can be incorporated into a construct using marker genes as described, and tested in transient analyses that provide an indication of gene expression in stable plant systems. As used herein the term “marker gene” refers to any transcribable nucleic acid molecule whose expression can be screened for or scored in some way. Methods of testing for marker gene expression in transient assays are known to those of skill in the art. Transient expression of marker genes has been reported using a variety of plants, tissues, plant cell(s), and DNA delivery systems. For example, types of transient analyses can include but are not limited to direct gene delivery via electroporation or particle bombardment of tissues in any transient plant assay using any plant species of interest. Such transient systems would include, but are not limited to, electroporation of protoplasts from a variety of tissue sources or particle bombardment of specific tissues of interest. The present invention encompasses the use of any transient expression system to evaluate promoters or promoter fragments operably linked to any transcribable nucleic acid molecules, including but not limited to selected reporter genes, marker genes, or genes of agronomic interest. Examples of plant tissues envisioned to test in transients via an appropriate delivery system would include, but are not limited to, leaf base tissues, callus, cotyledons, roots, endosperm, embryos, floral tissue, pollen, and epidermal tissue.

(80) Promoters and control elements of the present invention are useful for modulating metabolic or catabolic processes. Such processes include, but are not limited to, secondary product metabolism, amino acid synthesis, seed protein storage, increased biomass, oil development, pest defense and nitrogen usage. Some examples of genes, transcripts and peptides or polypeptides participating in these processes, which can be modulated by the present invention: are tryptophan decarboxylase (tdc) and strictosidine synthase (str1), dihydrodipicolinate synthase (DHDPS) and aspartate kinase (AK), 2S albumin and alpha-, beta-, and gamma-zeins, ricinoleate and 3-ketoacyl-ACP synthase (KAS), *Bacillus thuringiensis* (Bt) insecticidal protein, cowpea trypsin inhibitor (CpTI), asparagine synthetase and nitrite reductase. Alternatively, expression constructs can be used to inhibit expression of these peptides and polypeptides by incorporating the promoters in constructs for antisense use, co-suppression use or for the production of dominant negative mutations.

(81) As explained above, several types of regulatory elements exist concerning transcription regulation. Each of these regulatory elements may be combined with the present vector if desired. Translation of eukaryotic mRNA is often initiated at the codon that encodes the first methionine. Thus, when constructing a recombinant polynucleotide according to the present invention for expressing a protein product, it is preferable to ensure that the linkage between the 3′ portion, preferably including the TATA box, of the promoter and the polynucleotide to be transcribed, or a functional derivative thereof, does not contain any intervening codons which are capable of encoding a methionine.

(82) The vector of the present invention may contain additional components. For example, an origin of replication allows for replication of the vector in a host cell. Additionally, homologous sequences flanking a specific sequence allow for specific recombination of the specific sequence at a desired location in the target genome. T-DNA sequences also allow for insertion of a specific sequence randomly into a target genome.

(83) The vector may also be provided with a plurality of restriction sites for insertion of a polynucleotide to be transcribed as well as the promoter and/or promoter control elements of the present invention. The vector may additionally contain selectable marker genes. The vector may also contain a transcriptional and translational initiation region, and a transcriptional and translational termination region functional in the host cell. The termination region may be native with the transcriptional initiation region, may be native with the polynucleotide to be transcribed, or may be derived from another source. Convenient termination regions are available from the Ti-plasmid of *A. tumefaciens*, such as the octopine synthase and nopaline synthase termination regions. See also, Guerineau et al. (1991) *Mol. Gen. Genet.* 262:141-144; Proudfoot (1991) *Cell* 64:671-674; Sanfacon et al. (1991) *Genes Dev.* 5:141-149; Mogen et al. (1990) *Plant Cell* 2:1261-1272; Munroe et al. (1990) *Gene* 91:151-158; Ballas et al. (1989) *Nucleic Acids Res.* 17:7891-7903; Joshi et al. (1987) *Nucleic Acid Res.* 15:9627-9639.
(84) Where appropriate, the polynucleotide to be transcribed may be optimized for increased expression in a certain host cell. For example, the polynucleotide can be synthesized using preferred codons for improved transcription and translation. See U.S. Pat. Nos. 5,380,831, 5,436,391; see also and Murray et al. (1989) *Nucleic Acids Res.* 17:477-498.
(85) Additional sequence modifications include elimination of sequences encoding spurious polyadenylation signals, exon intron splice site signals, transposon-like repeats, and other such sequences well characterized as deleterious to expression. The G-C content of the polynucleotide may be adjusted to levels average for a given cellular host, as calculated by reference to known genes expressed in the host cell. The polynucleotide sequence may be modified to avoid hairpin secondary mRNA structures.
(86) A general description of expression vectors and reporter genes can be found in Gruber, et al. (1993) “Vectors for Plant Transformation” In *Methods in Plant Molecular Biology* & *Biotechnology*, Glich et al. Eds. pp. 89-119, CRC Press. Moreover GUS expression vectors and GUS gene cassettes are available from Clonetech Laboratories, Inc., Palo Alto, California while luciferase expression vectors and luciferase gene cassettes are available from Promega Corp. (Madison, Wisconsin). GFP vectors are available from Aurora Biosciences.
(87) 6.3 Polynucleotide Insertion into a Host Cell
(88) The promoters according to the present invention can be inserted into a host cell. A host cell includes but is not limited to a plant, mammalian, insect, yeast, and prokaryotic cell, preferably a plant cell.
(89) The method of insertion into the host cell genome is chosen based on convenience. For example, the insertion into the host cell genome may either be accomplished by vectors that integrate into the host cell genome or by vectors which exist independent of the host cell genome.
(90) The promoters of the present invention can exist autonomously or independent of the host cell genome. Vectors of these types are known in the art and include, for example, certain type of non-integrating viral vectors, autonomously replicating plasmids, artificial chromosomes, and the like.
(91) Additionally, in some cases transient expression of a promoter may be desired.
(92) The promoter sequences, promoter control elements or vectors of the present invention may be transformed into host cells. These transformations may be into protoplasts or intact tissues or isolated cells. Preferably expression vectors are introduced into intact tissue. General methods of culturing plant tissues are provided for example by Maki et al. (1993) “Procedures for Introducing Foreign DNA into Plants” In *Methods in Plant Molecular Biology* & *Biotechnology*, Glich et al. Eds. pp. 67-88 CRC Press; and by Phillips et al. (1988) “Cell-Tissue Culture and In-Vitro Manipulation” In *Corn* & *Corn Improvement,* 3*rd Edition* Sprague et al. eds., pp. 345-387, American Society of Agronomy Inc. et al.
(93) Methods of introducing polynucleotides into plant tissue include the direct infection or co-cultivation of plant cell with *Agrobacterium tumefaciens,* Horsch et al. (1985) *Science,* 227:1229. Descriptions of *Agrobacterium* vector systems and methods for *Agrobacterium*-mediated gene

transfer provided by Gruber et al. supra.
(94) Alternatively, polynucleotides are introduced into plant cells or other plant tissues using a direct gene transfer method such as microprojectile-mediated delivery, DNA injection, electroporation and the like. More preferably polynucleotides are introduced into plant tissues using the microprojectile media delivery with the biolistic device. See, for example, Tomes et al., "Direct DNA transfer into intact plant cells via microprojectile bombardment" In: Gamborg and Phillips (Eds.) Plant Cell, Tissue and Organ Culture: Fundamental Methods, Springer Verlag, Berlin (1995).
(95) Methods for specifically transforming dicots are well known to those skilled in the art. Transformation and plant regeneration using these methods have been described for a number of crops including, but not limited to, cotton (*Gossypium hirsutum*), soybean (*Glycine max*), peanut (*Arachis hypogaea*), and members of the genus *Brassica.*
(96) Methods for transforming monocots are well known to those skilled in the art. Transformation and plant regeneration using these methods have been described for a number of crops including, but not limited to, barley (*Hordeum vulgarae*); maize (*Zea mays*); oats (*Avena sativa*); orchard grass (*Dactylis glomerata*); rice (*Oryza sativa*, including indica and *japonica* varieties); sorghum (*Sorghum bicolor*); sugar cane (*Saccharum* sp); tall fescue (*Festuca arundinacea*); turfgrass species (e.g. species: *Agrostis stolonifera, Poa pratensis, Stenotaphrum secundatum*); wheat (*Triticum aestivum*), switchgrass (*Panicum vigatum*) and alfalfa (*Medicago sativa*). It is apparent to those of skill in the art that a number of transformation methodologies can be used and modified for production of stable transgenic plants from any number of target plants of interest.
(97) The polynucleotides and vectors described herein can be used to transform a number of monocotyledonous and dicotyledonous plants and plant cell systems, including species from one of the following families: Acanthaceae, Alliaceae, Alstroemeriaceae, Amaryllidaceae, Apocynaceae, Arecaceae, Asteraceae, Berberidaceae, Bixaceae, Brassicaceae, Bromeliaceae, Cannabaceae, Caryophyllaceae, Cephalotaxaceae, Chenopodiaceae, Colchicaceae, Cucurbitaceae, Dioscoreaceae, Ephedraceae, Erythroxylaceae, Euphorbiaceae, Fabaceae, Lamiaceae, Linaceae, Lycopodiaceae, Malvaceae, Melanthiaceae, Musaceae, Myrtaceae, Nyssaceae, Papaveraceae, Pinaceae, Plantaginaceae, Poaceae, Rosaceae, Rubiaceae, Salicaceae, Sapindaceae, Solanaceae, Taxaceae, Theaceae, or Vitaceae.
(98) Suitable species may include members of the genus *Abelmoschus, Abies, Acer, Agrostis, Allium, Alstroemeria, Ananas, Andrographis, Andropogon, Artemisia, Arundo, Atropa, Berberis, Beta, Bixa, Brassica, Calendula, Camellia, Camptotheca, Cannabis, Capsicum, Carthamus, Catharanthus, Cephalotaxus, Chrysanthemum, Cinchona, Citrullus, Coffea, Colchicum, Coleus, Cucumis, Cucurbita, Cynodon, Datura, Dianthus, Digitalis, Dioscorea, Elaeis, Ephedra, Erianthus, Erythroxylum, Eucalyptus, Festuca, Fragaria, Galanthus, Glycine, Gossypium, Helianthus, Hevea, Hordeum, Hyoscyamus, Jatropha, Lactuca, Linum, Lolium, Lupinus, Lycopersicon, Lycopodium, Manihot, Medicago, Mentha, Miscanthus, Musa, Nicotiana, Oryza, Panicum, Papaver, Parthenium, Pennisetum, Petunia, Phalaris, Phleum, Pinus, Poa, Poinsettia, Populus, Rauwolfia, Ricinus, Rosa, Saccharum, Salix, Sanguinaria, Scopolia, Secale, Solanum, Sorghum, Spartina, Spinacea, Tanacetum, Taxus, Theobroma, Triticosecale, Triticum, Uniola, Veratrum, Vinca, Vitis*, and *Zea.*
(99) Suitable species include *Panicum* spp. or hybrids thereof, Sorghum spp. or hybrids thereof, sudangrass, *Miscanthus* spp. or hybrids thereof, *Saccharum* spp. or hybrids thereof, *Erianthus* spp., *Populus* spp., *Andropogon gerardii* (big bluestem), *Pennisetum purpureum* (elephant grass) or hybrids thereof (e.g., *Pennisetum purpureum*×*Pennisetum typhoidum*), *Phalaris arundinacea* (reed canarygrass), *Cynodon dactylon* (bermudagrass), *Festuca arundinacea* (tall fescue), *Spartina pectinata* (prairie cord-grass), *Medicago sativa* (alfalfa), *Arundo donax* (giant reed) or hybrids thereof, *Secale cereale* (rye), Salix spp. (willow), *Eucalyptus* spp. (*eucalyptus*), Triticosecale (*Triticum*—wheat×rye), *Tripsicum dactyloides* (Eastern gammagrass), *Leymus cinereus* (basin

wildrye), *Leymus condensatus* (giant wildrye), and bamboo.
(100) In some embodiments, a suitable species can be a wild, weedy, or cultivated sorghum species such as, but not limited to, *Sorghum almum, Sorghum amplum, Sorghum angustum, Sorghum arundinaceum, Sorghum bicolor* (such as bicolor, guinea, caudatum, kafir, and durra), *Sorghum brachypodum, Sorghum bulbosum, Sorghum burmahicum, Sorghum controversum, Sorghum drummondii, Sorghum ecarinatum, Sorghum exstans, Sorghum grande, Sorghum halepense, Sorghum interjectum, Sorghum intrans, Sorghum laxiflorum, Sorghum leiocladum, Sorghum macrospermum, Sorghum matarankense, Sorghum miliaceum, Sorghum nigrum, Sorghum nitidum, Sorghum plumosum, Sorghum propinquum, Sorghum purpureosericeum, Sorghum stipoideum, Sorghum sudanensese, Sorghum timorense, Sorghum trichocladum, Sorghum versicolor, Sorghum virgatum, Sorghum vulgare,* or hybrids such as *Sorghum*×*almum, Sorghum*×sudangrass or *Sorghum*×*drummondii.*
(101) Suitable species also include *Helianthus annuus* (sunflower), *Carthamus tinctorius* (safflower), *Jatropha curcas* (jatropha), *Ricinus communis* (castor), *Elaeis guineensis* (palm), *Linum usitatissimum* (flax), and *Brassica juncea.*
(102) Suitable species also include *Beta vulgaris* (sugarbeet), and *Manihot esculenta* (cassava).
(103) Suitable species also include *Lycopersicon esculentum* (tomato), *Lactuca sativa* (lettuce), *Musa paradisiaca* (banana), *Solanum tuberosum* (potato), *Brassica oleracea* (broccoli, cauliflower, brusselsprouts), *Camellia sinensis* (tea), *Fragaria ananassa* (strawberry), *Theobroma cacao* (cocoa), *Coffea arabica* (coffee), *Vitis vinifera* (grape), *Ananas comosus* (pineapple), *Capsicum annum* (hot & sweet pepper), *Allium cepa* (onion), *Cucumis melo* (melon), *Cucumis sativus* (cucumber), *Cucurbita maxima* (squash), *Cucurbita moschata* (squash), *Spinacea oleracea* (spinach), *Citrullus lanatus* (watermelon), *Abelmoschus esculentus* (okra), and *Solanum melongena* (eggplant).
(104) Suitable species also include *Papaver somniferum* (opium poppy), *Papaver orientale, Taxus baccata, Taxus brevifolia, Artemisia annua, Cannabis sativa, Camptotheca acuminate, Catharanthus roseus, Vinca rosea, Cinchona officinalis, Colchicum autumnale, Veratrum californica, Digitalis lanata, Digitalis purpurea, Dioscorea* spp., *Andrographis paniculata, Atropa belladonna, Datura stomonium, Berberis* spp., *Cephalotaxus* spp., *Ephedra sinica, Ephedra* spp., *Erythroxylum coca, Galanthus wornorii, Scopolia* spp., *Lycopodium serratum* (*=Huperzia serrata*), *Lycopodium* spp., *Rauwolfia serpentina, Rauwolfia* spp., *Sanguinaria canadensis, Hyoscyamus* spp., *Calendula officinalis, Chrysanthemum parthenium, Coleus forskohlii,* and *Tanacetum parthenium.*
(105) Suitable species also include *Parthenium argentatum* (guayule), *Hevea* spp. (rubber), *Mentha spicata* (mint), *Mentha piperita* (mint), *Bixa orellana*, and *Alstroemeria* spp.
(106) Suitable species also include *Rosa* spp. (rose), *Dianthus caryophyllus* (carnation), *Petunia* spp. (*petunia*) and *Poinsettia pulcherrima* (poinsettia).
(107) Suitable species also include *Nicotiana tabacum* (tobacco), *Lupinus albus* (lupin), *Uniola paniculata* (oats), bentgrass (*Agrostis* spp.), *Populus tremuloides* (aspen), *Pinus* spp. (pine), *Abies* spp. (fir), Acer spp. (maple, *Hordeum vulgare* (barley), *Poa pratensis* (bluegrass), *Lolium* spp. (ryegrass) and *Phleum pratense* (timothy).
(108) Thus, the methods and compositions can be used over a broad range of plant species, including species from the dicot genera *Brassica, Carthamus, Glycine, Gossypium, Helianthus, Jatropha, Parthenium, Populus,* and *Ricinus*; and the monocot genera *Elaeis, Festuca, Hordeum, Lolium, Oryza, Panicum, Pennisetum, Phleum, Poa, Saccharum, Secale, Sorghum, Triticosecale, Triticum,* and *Zea*. In some embodiments, a plant is a member of the species *Panicum virgatum* (switchgrass), *Sorghum bicolor* (sorghum, sudangrass), *Miscanthus giganteus* (*miscanthus*), *Saccharum* sp. (energycane), *Populus balsamifera* (poplar), *Zea mays* (corn), *Glycine max* (soybean), *Brassica napus* (canola), *Triticum aestivum* (wheat), *Gossypium hirsutum* (cotton), *Oryza sativa* rice), *Heianthus annuus* (sunflower), *Medicago sativa* (alfalfa), *Beta vulgaris*

(sugarbeet), or *Pennisetum glaucum* (pearl millet).
(109) In certain embodiments, the polynucleotides and vectors described herein can be used to transform a number of monocotyledonous and dicotyledonous plants and plant cell systems, wherein such plants are hybrids of different species or varieties of a specific species (e.g., *Saccharum* sp.×*Miscanthus* sp., *Panicum virgatum*×*Panicum amarum, Panicum virgatum*×*Panicum amarulum*, and *Pennisetum purpureum*×*Pennisetum typhoidum*).
(110) In another embodiment of the current invention, expression constructs can be used for gene expression in callus culture for the purpose of expressing marker genes encoding peptides or polypeptides that allow identification of transformed plants. Here, a promoter that is operatively linked to a polynucleotide to be transcribed is transformed into plant cells and the transformed tissue is then placed on callus-inducing media. If the transformation is conducted with leaf discs, for example, callus will initiate along the cut edges. Once callus growth has initiated, callus cells can be transferred to callus shoot-inducing or callus root-inducing media. Gene expression will occur in the callus cells developing on the appropriate media: callus root-inducing promoters will be activated on callus root-inducing media, etc. Examples of such peptides or polypeptides useful as transformation markers include, but are not limited to barstar, glyphosate, chloramphenicol acetyltransferase (CAT), kanamycin, spectinomycin, streptomycin or other antibiotic resistance enzymes, green fluorescent protein (GFP), and β-glucuronidase (GUS), etc. Some of the promoters provided in SEQ ID NOs: 1-26 or nucleic acid residues 601-1000 of SEQ ID NO: 26 will also be capable of sustaining expression in some tissues or organs after the initiation or completion of regeneration. Examples of these tissues or organs are somatic embryos, cotyledon, hypocotyl, epicotyl, leaf, stems, roots, flowers and seed.
(111) Integration into the host cell genome also can be accomplished by methods known in the art, for example, by the homologous sequences or T-DNA discussed above or using the cre-lox system (A. C. Vergunst et al. (1998) *Plant Mol. Biol.* 38:393).
7. Uses of the Promoters of the Invention
(112) 7.1 Use of the Promoters to Study and Screen for Expression
(113) The promoters of the present invention can be used to further understand developmental mechanisms. For example, promoters that are specifically induced during callus formation, somatic embryo formation, shoot formation or root formation can be used to explore the effects of overexpression, repression or ectopic expression of target genes, or for isolation of trans-acting factors.
(114) The vectors of the invention can be used not only for expression of coding regions but may also be used in exon-trap cloning, or promoter trap procedures to detect differential gene expression in various tissues (see Lindsey et al. (1993) *Transgenic Research* 2:3347. Auch and Reth (1990) *Nucleic Acids Research* 18: 6743).
(115) Entrapment vectors, first described for use in bacteria (Casadaban and Cohen (1979) *Proc. Nat. Aca. Sci. U.S.A.* 76: 4530; Casadaban et al. (1980) *J. Bacteriol.* 143: 971) permit selection of insertional events that lie within coding sequences. Entrapment vectors can be introduced into pluripotent ES cells in culture and then passed into the germline via chimeras (Gossler et al. aaa91989) *Science* 244: 463; Skarnes (1990) *Biotechnology* 8: 827). Promoter or gene trap vectors often contain a reporter gene, e.g., lacZ, lacking its own promoter and/or splice acceptor sequence upstream. That is, promoter gene traps contain a reporter gene with a splice site but no promoter. If the vector lands in a gene and is spliced into the gene product, then the reporter gene is expressed.
(116) Recently, the isolation of preferentially-induced genes has been made possible with the use of sophisticated promoter traps (e.g. IVET) that are based on conditional auxotrophy complementation or drug resistance. In one IVET approach, various bacterial genome fragments are placed in front of a necessary metabolic gene coupled to a reporter gene. The DNA constructs are inserted into a bacterial strain otherwise lacking the metabolic gene, and the resulting bacteria are used to infect the host organism. Only bacteria expressing the metabolic gene survive in the

host organism; consequently, inactive constructs can be eliminated by harvesting only bacteria that survive for some minimum period in the host. At the same time, broadly active constructs can be eliminated by screening only bacteria that do not express the reporter gene under laboratory conditions. The bacteria selected by such a method contain constructs that are selectively induced only during infection of the host. The IVET approach can be modified for use in plants to identify genes induced in either the bacteria or the plant cells upon pathogen infection or root colonization. For information on IVET see the articles by Mahan et al. (1993) *Science* 259:686-688, Mahan et al. (1995) *Proc. Natl. Acad. Sci. USA* 92:669-673, Heithoff et al. (1997) *Proc. Natl. Acad. Sci USA* 94:934-939, and Wang et al. (1996) *Proc. Natl. Acad. Sci USA* 93:10434.

(117) 7.2 Use of the Promoters to Transcribe Genes of Interest

(118) In one embodiment of the invention, a nucleic acid molecule as shown in SEQ ID NOs: 1-26 or nucleic acid residues 601-1000 of SEQ ID NO: 26 is incorporated into a construct such that a promoter of the present invention is operably linked to a transcribable nucleic acid molecule that is a gene of agronomic interest. As used herein, the term “gene of agronomic interest” refers to a transcribable nucleic acid molecule that includes but is not limited to a gene that provides a desirable characteristic associated with plant morphology, physiology, growth and development, yield, nutritional enhancement, disease or pest resistance, or environmental or chemical tolerance. The expression of a gene of agronomic interest is desirable in order to confer an agronomically important trait. A gene of agronomic interest that provides a beneficial agronomic trait to crop plants may be, for example, including, but not limited to genetic elements comprising herbicide resistance, increased yield, increased biomass, insect control, fungal disease resistance, virus resistance, nematode resistance, bacterial disease resistance, starch production, modified oils production, high oil production, modified fatty acid content, high protein production, fruit ripening, enhanced animal and human nutrition, biopolymers, environmental stress resistance, pharmaceutical peptides, improved processing traits, improved digestibility, industrial enzyme production, improved flavor, nitrogen fixation, hybrid seed production, and biofuel production. The genetic elements, methods, and transgenes described in the patents listed above are hereby incorporated by reference.

(119) Alternatively, a transcribable nucleic acid molecule can effect the above mentioned phenotypes by encoding a RNA molecule that causes the targeted inhibition of expression of an endogenous gene, for example via antisense, inhibitory RNA (RNAi), or cosuppression-mediated mechanisms. The RNA could also be a catalytic RNA molecule (i.e., a ribozyme) engineered to cleave a desired endogenous mRNA product. Thus, any nucleic acid molecule that encodes a protein or mRNA that expresses a phenotype or morphology change of interest may be useful for the practice of the present invention.

(120) 7.3. Stress Induced Preferential Transcription

(121) Promoters and control elements providing modulation of transcription under oxidative, drought, oxygen, wound, and methyl jasmonate stress are particularly useful for producing host cells or organisms that are more resistant to biotic and abiotic stresses. In a plant, for example, modulation of genes, transcripts, and/or polypeptides in response to oxidative stress can protect cells against damage caused by oxidative agents, such as hydrogen peroxide and other free radicals.

(122) Drought induction of genes, transcripts, and/or polypeptides are useful to increase the viability of a plant, for example, when water is a limiting factor. In contrast, genes, transcripts, and/or polypeptides induced during oxygen stress can help the flood tolerance of a plant.

(123) The promoters and control elements of the present invention can modulate stresses similar to those described in, for example, stress conditions are VuPLD1 (drought stress; Cowpea; see Pham-Thi et al. (1999) *Plant Mol Biol* 39:1257-65), pyruvate decarboxylase (oxygen stress; rice; see Rivosal et al. (1997) *Plant Physiol* 114(3): 1021-29), chromoplast specific carotenoid gene (oxidative stress; *Capsicum*; see Bouvier et al. (1998) *J Biol Chem* 273: 30651-59).

(124) Promoters and control elements providing preferential transcription during wounding or

induced by methyl jasmonate can produce a defense response in host cells or organisms. In a plant, for example, preferential modulation of genes, transcripts, and/or polypeptides under such conditions is useful to induce a defense response to mechanical wounding, pest or pathogen attack or treatment with certain chemicals.
(125) Promoters and control elements of the present invention also can trigger a response similar to those described for cf9 (viral pathogen; tomato; see O'Donnell et al. (1998) Plant J 14(1): 137-42), hepatocyte growth factor activator inhibitor type 1 (HAI-1), which enhances tissue regeneration (tissue injury; human; Koono et al. (1999) *J Histochem Cytochem* 47: 673-82), copper amine oxidase (CuAO), induced during ontogenesis and wound healing (wounding; chick-pea; Rea et al. (1998) *FEBS Lett* 437: 177-82), proteinase inhibitor II (wounding; potato; see Pena-Cortes et al. (1988) *Planta* 174: 84-89), protease inhibitor II (methyl jasmonate; tomato; see Farmer and Ryan (1990) *Proc Natl Acad Sci USA* 87: 7713-7716), two vegetative storage protein genes VspA and VspB (wounding, jasmonic acid, and water deficit; soybean; see Mason and Mullet (1990) *Plant Cell* 2: 569-579).
(126) Up-regulation and transcription down-regulation are useful for these applications. For instance, genes, transcripts, and/or polypeptides that increase oxidative, flood, or drought tolerance may require up-regulation of transcription.
(127) Typically, promoter or control elements, which provide preferential transcription in wounding or under methyl jasmonate induction, produce transcript levels that are statistically significant as compared to cell types, organs or tissues under other conditions.
(128) For preferential up-regulation of transcription, promoter and control elements produce transcript levels that are above background of the assay.
(129) 7.4. Light Induced Preferential Transcription
(130) Promoters and control elements providing preferential transcription when induced by light exposure can be utilized to modulate growth, metabolism, and development; to increase drought tolerance; and decrease damage from light stress for host cells or organisms. In a plant, for example, modulation of genes, transcripts, and/or polypeptides in response to light is useful (1) to increase the photosynthetic rate; (2) to increase storage of certain molecules in leaves or green parts only, e.g. silage with high protein or starch content; (3) to modulate production of exogenous compositions in green tissue, e.g. certain feed enzymes; (4) to induce growth or development, such as fruit development and maturity, during extended exposure to light; (5) to modulate guard cells to control the size of stomata in leaves to prevent water loss, or (6) to induce accumulation of beta-carotene to help plants cope with light induced stress.
(131) The promoters and control elements of the present invention also can trigger responses similar to those described in: abscisic acid insensitive3 (ABI3) (dark-grown *Arabidopsis* seedlings, see Rohde et al. (2000) *Plant Cell* 12: 35-52), asparagine synthetase (pea root nodules, see Tsai and Coruzzi (1990) *EMBO J* 9: 323-32), mdm2 gene (human tumor, see Saucedo et al. (1998) *Cell Growth Differ* 9: 119-30).
(132) Up-regulation and transcription down-regulation are useful for these applications. For instance, genes, transcripts, and/or polypeptides that increase drought or light tolerance may require up-regulation of transcription.
(133) Typically, promoter or control elements, which provide preferential transcription in cells, tissues or organs exposed to light, produce transcript levels that are statistically significant as compared to cells, tissues, or organs under decreased light exposure (intensity or length of time).
(134) For preferential up-regulation of transcription, promoter and control elements produce transcript levels that are above background of the assay.
(135) 7.5. Dark Induced Preferential Transcription
(136) Promoters and control elements providing preferential transcription when induced by dark or decreased light intensity or decreased light exposure time can be utilized to time growth, metabolism, and development, to modulate photosynthesis capabilities for host cells or organisms.

In a plant, for example, modulation of genes, transcripts, and/or polypeptides in response to dark is useful, for example, (1) to induce growth or development, such as fruit development and maturity, despite lack of light; (2) to modulate genes, transcripts, and/or polypeptide active at night or on cloudy days; or (3) to preserve the plastid ultra structure present at the onset of darkness.
(137) The present promoters and control elements can also trigger response similar to those described in the section above.
(138) Up-regulation and transcription down-regulation is useful for these applications. For instance, genes, transcripts, and/or polypeptides that increase or decrease growth and development may require up-regulation of transcription.
(139) Typically, promoter or control elements, which provide preferential transcription under exposure to dark or decrease light intensity or decrease exposure time, produce transcript levels that are statistically significant.
(140) For preferential up-regulation of transcription, promoter and control elements produce transcript levels that are above background of the assay.
(141) 7.6. Leaf Preferential Transcription
(142) Promoters and control elements providing preferential transcription in a leaf can modulate growth, metabolism, and development or modulate energy and nutrient utilization in host cells or organisms. In a plant, for example, preferential modulation of genes, transcripts, and/or polypeptide in a leaf, is useful, for example, (1) to modulate leaf size, shape, and development; (2) to modulate the number of leaves; or (3) to modulate energy or nutrient usage in relation to other organs and tissues
(143) Up-regulation and transcription down-regulation is useful for these applications. For instance, genes, transcripts, and/or polypeptides that increase growth, for example, may require up-regulation of transcription.
(144) Typically, promoter or control elements, which provide preferential transcription in the cells, tissues, or organs of a leaf, produce transcript levels that are statistically significant as compared to other cells, organs or tissues.
(145) For preferential up-regulation of transcription, promoter and control elements produce transcript levels that are above background of the assay.
(146) 7.7. Root Preferential Transcription
(147) Promoters and control elements providing preferential transcription in a root can modulate growth, metabolism, development, nutrient uptake, nitrogen fixation, or modulate energy and nutrient utilization in host cells or organisms. In a plant, for example, preferential modulation of genes, transcripts, and/or polypeptide in a root, is useful, (1) to modulate root size, shape, and development; (2) to modulate the number of roots, or root hairs; (3) to modulate mineral, fertilizer, or water uptake; (4) to modulate transport of nutrients; or (4) to modulate energy or nutrient usage in relation to other cells, organs and tissues.
(148) Up-regulation and transcription down-regulation is useful for these applications. For instance, genes, transcripts, and/or polypeptides that increase or decrease growth, for example, may require up-regulation of transcription.
(149) Typically, promoter or control elements, which provide preferential transcription in cells, tissues, or organs of a root, produce transcript levels that are statistically significant as compared to other cells, organs or tissues.
(150) For preferential up-regulation of transcription, promoter and control elements produce transcript levels that are above background of the assay.
(151) 7.8. Stem/Shoot Preferential Transcription
(152) Promoters and control elements providing preferential transcription in a stem or shoot can modulate growth, metabolism, and development or modulate energy and nutrient utilization in host cells or organisms. In a plant, for example, preferential modulation of genes, transcripts, and/or polypeptide in a stem or shoot, is useful, for example, (1) to modulate stem/shoot size, shape, and

development; or (2) to modulate energy or nutrient usage in relation to other organs and tissues
(153) Up-regulation and transcription down-regulation is useful for these applications. For instance, genes, transcripts, and/or polypeptides that increase growth, for example, may require up-regulation of transcription.
(154) Typically, promoter or control elements, which provide preferential transcription in the cells, tissues, or organs of a stem or shoot, produce transcript levels that are statistically significant as compared to other cells, organs or tissues.
(155) For preferential up-regulation of transcription, promoter and control elements produce transcript levels that are above background of the assay.
(156) 7.9. Fruit and Seed Preferential Transcription
(157) Promoters and control elements providing preferential transcription in a silique or fruit can time growth, development, or maturity; or modulate fertility; or modulate energy and nutrient utilization in host cells or organisms. In a plant, for example, preferential modulation of genes, transcripts, and/or polypeptides in a fruit, is useful (1) to modulate fruit size, shape, development, and maturity; (2) to modulate the number of fruit or seeds; (3) to modulate seed shattering; (4) to modulate components of seeds, such as, storage molecules, starch, protein, oil, vitamins, anti-nutritional components, such as phytic acid; (5) to modulate seed and/or seedling vigor or viability; (6) to incorporate exogenous compositions into a seed, such as lysine rich proteins; (7) to permit similar fruit maturity timing for early and late blooming flowers; or (8) to modulate energy or nutrient usage in relation to other organs and tissues.
(158) Up-regulation and transcription down-regulation is useful for these applications. For instance, genes, transcripts, and/or polypeptides that increase or decrease growth, for example, may require up-regulation of transcription.
(159) Typically, promoter or control elements, which provide preferential transcription in the cells, tissues, or organs of siliques or fruits, produce transcript levels that are statistically significant as compared to other cells, organs or tissues.
(160) For preferential up-regulation of transcription, promoter and control elements produce transcript levels that are above background of the assay.
(161) 7.10. Callus Preferential Transcription
(162) Promoters and control elements providing preferential transcription in a callus can be useful to modulating transcription in dedifferentiated host cells. In a plant transformation, for example, preferential modulation of genes, transcripts, in callus is useful to modulate transcription of a marker gene, which can facilitate selection of cells that are transformed with exogenous polynucleotides.
(163) Up-regulation and transcription down-regulation is useful for these applications. For instance, genes, transcripts, and/or polypeptides that increase marker gene detectability, for example, may require up-regulation of transcription.
(164) For preferential up-regulation of transcription, promoter and control elements produce transcript levels that are above background of the assay.
(165) 7.11. Flower Specific Transcription
(166) Promoters and control elements providing preferential transcription in flowers can modulate pigmentation; or modulate fertility in host cells or organisms. In a plant, for example, preferential modulation of genes, transcripts, and/or polypeptides in a flower, is useful, (1) to modulate petal color; or (2) to modulate the fertility of pistil and/or stamen.
(167) Up-regulation and transcription down-regulation is useful for these applications. For instance, genes, transcripts, and/or polypeptides that increase or decrease pigmentation, for example, may require up-regulation of transcription
(168) Typically, promoter or control elements, which provide preferential transcription in flowers, produce transcript levels that are statistically significant as compared to other cells, organs or tissues.

(169) For preferential up-regulation of transcription, promoter and control elements produce transcript levels that are above background of the assay.
(170) 7.12. Immature Bud and Inflorescence Preferential Transcription
(171) Promoters and control elements providing preferential transcription in a immature bud or inflorescence can time growth, development, or maturity; or modulate fertility or viability in host cells or organisms. In a plant, for example, preferential modulation of genes, transcripts, and/or polypeptide in a immature bud and/or inflorescence, is useful, (1) to modulate embryo development, size, and maturity; (2) to modulate endosperm development, size, and composition; (3) to modulate the number of seeds and fruits; or (4) to modulate seed development and viability.
(172) Up-regulation and transcription down-regulation is useful for these applications. For instance, genes, transcripts, and/or polypeptides that increase or decrease growth, for example, may require up-regulation of transcription.
(173) Typically, promoter or control elements, which provide preferential transcription in immature buds and inflorescences, produce transcript levels that are statistically significant as compared to other cell types, organs or tissues.
(174) For preferential up-regulation of transcription, promoter and control elements produce transcript levels that are above background of the assay.
(175) 7.13. Senescence Preferential Transcription
(176) Promoters and control elements providing preferential transcription during senescence can be used to modulate cell degeneration, nutrient mobilization, and scavenging of free radicals in host cells or organisms. Other types of responses that can be modulated include, for example, senescence associated genes (SAG) that encode enzymes thought to be involved in cell degeneration and nutrient mobilization (*Arabidopsis*; see Hensel et al. (1993) *Plant Cell* 5: 553-64), and the CP-2/cathepsin L gene (rat; Kim and Wright (1997) *Biol Reprod* 57: 1467-77), both induced during senescence.
(177) In a plant, for example, preferential modulation of genes, transcripts, and/or polypeptides during senescence is useful to modulate fruit ripening.
(178) Up-regulation and transcription down-regulation is useful for these applications. For instance, genes, transcripts, and/or polypeptides that increase or decrease scavenging of free radicals, for example, may require up-regulation of transcription.
(179) Typically, promoter or control elements, which provide preferential transcription in cells, tissues, or organs during senescence, produce transcript levels that are statistically significant as compared to other conditions.
(180) For preferential up-regulation of transcription, promoter and control elements produce transcript levels that are above background of the assay.
(181) 7.14. Germination Preferential Transcription
(182) Promoters and control elements providing preferential transcription in a germinating seed can time growth, development, or maturity; or modulate viability in host cells or organisms. In a plant, for example, preferential modulation of genes, transcripts, and/or polypeptide in a germinating seed, is useful, (1) to modulate the emergence of the hypocotyls, cotyledons and radical; or (2) to modulate shoot and primary root growth and development;
(183) Up-regulation and transcription down-regulation is useful for these applications. For instance, genes, transcripts, and/or polypeptides that increase or decrease growth, for example, may require up-regulation of transcription.
(184) Typically, promoter or control elements, which provide preferential transcription in a germinating seed, produce transcript levels that are statistically significant as compared to other cell types, organs or tissues.
(185) For preferential up-regulation of transcription, promoter and control elements produce transcript levels that are above background of the assay.
8. GFP Experimental Procedures and Results

(186) Procedures
(187) The polynucleotide sequences of the present invention were tested for promoter activity using Green Fluorescent Protein (GFP) assays in the following manner.
(188) Approximately 1-3 kb of genomic sequence occurring immediately upstream of the ATG translational start site of the gene of interest was isolated using appropriate primers tailed with BstXI restriction sites. Standard PCR reactions using these primers and genomic DNA were conducted. The resulting product was isolated, cleaved with BstXI and cloned into the BstXI site of an appropriate vector, such as pNewBin4-HAP1-GFP (see FIG. **1**).
(189) *Agrobacterium*-Mediated Transformation of *Arabidopsis*
(190) Host Plants and Transgenes: Wild-type *Arabidopsis thaliana* Wassilewskja (WS) plants are transformed with Ti plasmids containing nucleic acid sequences to be expressed, as noted in the respective examples, in the sense orientation relative to the 35S promoter in a Ti plasmid. A Ti plasmid vector useful for these constructs, CRS 338, contains the Ceres-constructed, plant selectable marker gene phosphinothricin acetyltransferase (PAT), which confers herbicide resistance to transformed plants.
(191) Ten independently transformed events are typically selected and evaluated for their qualitative phenotype in the T.sub.1 generation.
(192) Preparation of Soil Mixture: 24 L Sunshine Mix #5 soil (Sun Gro Horticulture, Ltd., Bellevue, WA) is mixed with 16 L Therm-O-Rock vermiculite (Therm-O-Rock West, Inc., Chandler, AZ) in a cement mixer to make a 60:40 soil mixture. To the soil mixture is added 2 Tbsp Marathon 1% granules (Hummert, Earth City, MO), 3 Tbsp OSMOCOTE® 14-14-14 (Hummert, Earth City, MO) and 1 Tbsp Peters fertilizer 20-20-20 (J. R. Peters, Inc., Allentown, PA), which are first added to 3 gallons of water and then added to the soil and mixed thoroughly. Generally, 4-inch diameter pots are filled with soil mixture. Pots are then covered with 8-inch squares of nylon netting.
(193) Planting: Using a 60 mL syringe, 35 mL of the seed mixture is aspirated. 25 drops are added to each pot. Clear propagation domes are placed on top of the pots that are then placed under 55% shade cloth and subirrigated by adding 1 inch of water.
(194) Plant Maintenance: 3 to 4 days after planting, lids and shade cloth are removed. Plants are watered as needed. After 7-10 days, pots are thinned to 20 plants per pot using forceps. After 2 weeks, all plants are subirrigated with Peters fertilizer at a rate of 1 Tsp per gallon of water. When bolts are about 5-10 cm long, they are clipped between the first node and the base of stem to induce secondary bolts. Dipping infiltration is performed 6 to 7 days after clipping.
(195) Preparation of *Agrobacterium*: To 150 mL fresh YEB is added 0.1 mL each of carbenicillin, spectinomycin and rifampicin (each at 100 mg/ml stock concentration). *Agrobacterium* starter blocks are obtained (96-well block with *Agrobacterium* cultures grown to an OD.sub.600 of approximately 1.0) and inoculated one culture vessel per construct by transferring 1 mL from appropriate well in the starter block. Cultures are then incubated with shaking at 27° C. Cultures are spun down after attaining an OD.sub.600 of approximately 1.0 (about 24 hours). 200 mL infiltration media is added to resuspend *Agrobacterium* pellets. Infiltration media is prepared by adding 2.2 g MS salts, 50 g sucrose, and 5 μL 2 mg/ml benzylaminopurine to 900 ml water.
(196) Dipping Infiltration: The pots are inverted and submerged for 5 minutes so that the aerial portion of the plant is in the *Agrobacterium* suspension. Plants are allowed to grow normally and seed is collected.
(197) High-throughput Screening of T.sub.1 Transgenic Plants: Seed is evenly dispersed into water-saturated soil in pots and placed into a dark 4° C. cooler for two nights to promote uniform germination. Pots are then removed from the cooler and covered with 55% shade cloth for 4-5 days. Cotyledons are fully expanded at this stage. FINALE® (Sanofi Aventis, Paris, France) is sprayed on plants (3 ml FINALE® diluted into 48 oz. water) and repeated every 3-4 days until only transformants remain.

(198) GFP Assay
(199) Tissues are dissected by eye or under magnification using INOX 5 grade forceps and placed on a slide with water and coversliped. An attempt is made to record images of observed expression patterns at earliest and latest stages of development of tissues listed below. Specific tissues will be preceded with High (H), Medium (M), Low (L) designations.
(200) TABLE-US-00002 Flower Pedicel, receptacle, nectary, sepal, petal, filament, anther, pollen, carpel, style, papillae, vascular, epidermis, stomata, trichome Silique Stigma, style, carpel, septum, placentae, transmitting tissue, vascular, epidermis, stomata, abscission zone, ovule Ovule Pre-fertilization: inner integument, outer integument, embryo sac, funiculus, chalaza, micropyle, gametophyte Post-fertilization: zygote, inner integument, outer integument, seed coat, primordia, chalaza, micropyle, early endosperm, mature endosperm, embryo Embryo Suspensor, preglobular, globular, heart, torpedo, late mature, provascular, hypophysis, radicle, cotyledons, hypocotyl Stem Epidermis, cortex, vascular, xylem, phloem, pith, stomata, trichome Leaf Petiole, mesophyll, vascular, epidermis, trichorne, primordia, stomata, stipule, margin
(201) T1 Mature: These are the T1 plants resulting from independent transformation events. These are screened between stage 6.50-6.90 (i.e. the plant is flowering and 50-90% of the flowers that the plant will make have developed), which is 4-6 weeks of age. At this stage the mature plant possesses flowers, siliques at all stages of development, and fully expanded leaves. The plants are initially imaged under UV with a Leica Confocal microscope to allow examination of the plants on a global level. If expression is present, they are re-imaged using scanning laser confocal microscopy.
(202) T2 Seedling: Progeny are collected from the T1 plants giving the same expression pattern and the progeny (T2) are sterilized and plated on agar-solidified medium containing M&S salts. In the event that there is no expression in the T1 plants, T2 seeds are planted from all lines. The seedlings are grown in Percival incubators under continuous light at 22° C. for 10-12 days. Cotyledons, roots, hypocotyls, petioles, leaves, and the shoot meristem region of individual seedlings were screened until two seedlings were observed to have the same pattern. In general, the same expression pattern was found in the first two seedlings. However, up to 6 seedlings were screened before “no expression pattern” was recorded. All constructs are screened as T2 seedlings even if they did not have an expression pattern in the T1 generation.
(203) T2 Mature: The T2 mature plants were screened in a similar manner to the T1 plants. The T2 seeds were planted in the greenhouse, exposed to selection and at least one plant screened to confirm the T1 expression pattern. In instances where there were any subtle changes in expression, multiple plants were examined and the changes noted in the tables.
(204) T3 Seedling: This was done similar to the T2 seedlings except that only the plants for which we are trying to confirm the pattern are planted.
(205) Image Data:
(206) Images are collected by scanning laser confocal microscopy. Scanned images are taken as 2-D optical sections or 3-D images generated by stacking the 2-D optical sections collected in series. All scanned images are saved as TIFF files by imaging software, edited in Adobe Photoshop, and labeled in Powerpoint specifying organ and specific expressing tissues.
Results
(207) The Promoter Expression Reports of the Tables present the results of the GFP assays as reported by their corresponding construct number and line number.
(208) TABLE-US-00003 Promoter Expression Report For PT0960 (SEQ ID NO: 1) Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Spatial expression summary: Hypocotyl H vascular Cotyledon H vascular Rosette Leaf H vascular Primary Root L epidermis L cortex H endodermis H vascular Observed expression pattern: T1 Mature expression: None observed. T2 Seedling expression: High GFP expression throughout vasculature of seedlings. Source Promoter *Arabidopsis thaliana*, Organism: Columbia (Col) ecotype Vector: pNewbin4-

HAP1-GFP Marker Type: GFP-ER Generation Screened: XT1 Mature XT2 Seedling Inductions completed. Events Time Screened/ Treatment: Age: Gen: points: Response Response: 1. 14.3 mM KNO.sub.3 4 wks T2 72 hrs 2/0 No to 28.6 Mannitol post transfer TABLE 1-1. T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 3 Events Expressing: n = 0 No GFP Expression Detected TABLE 2-1. T2 Seedling Expression Tissues Screened Events Screened: n = 3 Events Expressing: n = 3 Expression Detected Hypocotyl H vascular Cotyledon H vascular Rosette Leaf H vascular Primary Root L epidermis L cortex H endodermis H vascular Construct: PT0960 Promoter candidate 22254785 I.D: cDNA I.D: 23518786 Events expressing: 01-03

(209) TABLE-US-00004 Promoter Expression Report YP2585 (SEQ ID NO: 2) deletion of PT0743 Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Spatial expression summary: Primary Root H epidermis H cortex H root hairs Mature Root H epidermis H vascular H pericycle H stele Observed expression pattern: T1 Mature expression: No expression detected T2 Seedling expression: Expression in root epidermis and cortex T2 Mature expression: No expression detected Source Promoter Organism: *Arabidopsis thaliana*, Columbia (Col) ecotype Vector: pNewbin4-HAP1-GFP Marker Type: GFP-ER Generation Screened: X T1 Mature X T2 Seedling X T2 Mature TABLE 1-2. T1 Mature Plant Expression Organs/Tissues screened No GFP Expression Detected TABLE 2-2. T2 Seedling Expression Tissues Screened Events Screened: n = 6 Events Expressing: n = 5 (02-06) Expression Detected Primary Root H epidermis H cortex H root hairs TABLE 3-2. T2 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 3 (03, 04, 06 Expression Detected Mature Root H epidermis H vascular H pericycle H stele TABLE 4-2. Promoter utility Trait Area: Water Use Efficiency, Nutrient Use Efficiency Sub-trait Area: Drought Tolerance Utility: Among other uses this promoter sequence is useful to improve the uptake of water and nutrients from the soil. Construct: YP2585 Promoter candidate I.D: 40983033 cDNA I.D: 23509083 Events expressing: 02-06 One or more fragments of the above described promoter are identified in the miscellaneous feature section of the relevant SEQ ID in the Sequence Listing. Those fragments were tested for promoter activity by the same procedures as described above, and the results are summarized below. No expression is observed for Ceres Promoter YP2581.

(210) TABLE-US-00005 Promoter Expression Report For PT0998 (SEQ ID NO: 3) Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Spatial expression summary: Flower H anther H tapetum H silique Silique L vascular L epidermis Mature root H mature root Primary Root H cortex H endodermis H vascular L xylem H phloem H pericycle Observed expression pattern: T1 Mature expression: High GFP expression in roots, tapetum cells of developing anthers and siliques. T2 Seedling expression: High GFP expression in cortex, endodermis, and surrounding vascular bundle. T2 Mature expression: High GFP expression in roots (vasculature) of mature plants. Source Promoter *Arabidopsis thaliana*, Organism: Columbia (Col) ecotype Vector: pNewbin4-HAP1-GFP Marker Type: GFP-ER Generation Screened: X T1 Mature X T2 Seedling X T2 Mature TABLE 1-3. T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 4 Events Expressing: n = 4 Expression Detected Flower H anther H tapetum H silique H Silique L vascular L epidermis Root H Yes TABLE 2-3. T2 Seedling Expression Tissues Screened Events Screened: n = 4 Events Expressing: n = 4 GFP Expression Detected X Primary Root H cortex H endodermis H vascular L xylem H phloem H pericycle TABLE 3-3. T2 Mature Plant Expression Organs/Tissues screened Events Screened: n = 4 Events Expressing: n = 4 Expression Detected Mature Root H mature root TABLE 4-3. RT-PCR Results: Plants were grown hydroponically for 4 weeks until tissue collection. Roots and aerial tissues (Aerials) were harvested separately in liquid nitrogen. For each event, two sets of samples are collected for qRT-PCR analysis. The data presented is the average ratio of roots and aerials between two replicates. PT0998-2 PT0998-3 PT0998-4 Ratio Ratio Ratio (Roots/ (Roots/ (Roots/ Aerials) Aerials) Aerials) HAP 6.33 23.16 33.01 GFP 253.98 575.31 555.41 TABLE 5-3. Promoter utility Trait Area: Stress, Nutrients Sub-trait Area: nitrogen utilization, drought tolerance, UV-B tolerance Utility: Among other uses this

promoter sequence is useful to improve: Modulate nutrients, water uptake from soil and transportation within plants. Provide drought or UV protection. Construct: PT0998 Promoter candidate I.D: 24469840 cDNA I.D: 23506262 + 23545255 Events expressing: 01, 02, 03, 04 One or more fragments of the above described promoter are identified in the miscellaneous feature section of the relevant SEQ ID in the Sequence Listing. Those fragments were tested for promoter activity by the same procedures as described above, and the results are summarized below. No expression is observed for Ceres Promoter PD3457.

(211) TABLE-US-00006 Promoter Expression Report YP2219 (SEQ ID NO: 4) Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Spatial expression summary: Hypocotyl L epidermis L cortex L vascular Cotyledon L hydathode L petiole L epidermis Rosette Leaf L epidermis H petiole Primary Root L epidermis H cortex H vascular Lateral root H lateral root cap Observed expression pattern: T1 Mature expression: No GFP expression observed T2 Seedling expression: Hypocotyl, cotyledon, rosette leaf, primary, and lateral root T2 Mature expression: Source Promoter Organism: *Arabidopsis thaliana*, Columbia (Col) ecotype Vector: pNewbin4-HAP1-GFP Marker Type: GFP-ER Generation Screened: X T1 Mature X T2 Seedling Inductions completed. Events Time Screened/ Re- Treatment: Age: Gen: points: Response sponse: 1. Drought 4 wks T2 1.0% 6/4 Yes moisture Inducible expression summary: Time point Organs Treatment: induced: induced: Tissues induced: 1. Drought 1.0% Stem, leaf, moisture leaf TABLE 1-4. T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 0 No GFP Expression Detected TABLE 2-4. T2 Seedling Expression Tissues Screened Events Screened: n = 6 Events Expressing: n = 4 Expression Detected Hypocotyl L epidermis L cortex L vascular Cotyledon L epidermisd L petiole L hydathode Rosette Leaf L epidermis H petiole Primary Root L epidermis H cortex H vascular Lateral root H lateral root cap TABLE 4-4. Promoter utility Trait Area: Water Use Efficiency Sub-trait Area: Drought Tolerance Utility: Among other uses this promoter sequence is useful to engineer drought tolerance in plants. Construct: YP2219 Promoter candidate I.D: 37172464 cDNA I.D: 23494283 Events expressing: 01, 02, 04, 06 One or more fragments of the above described promoter are identified in the miscellaneous feature section of the relevant SEQ ID in the Sequence Listing. Those fragments were tested for promoter activity by the same procedures as described above, and the results are summarized below. No induction is observed for Ceres Promoter YP2229.

(212) TABLE-US-00007 Promoter Expression Report For YP0286 (SEQ ID NO: 5) Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Spatial expression summary: Flower L pedicel L epidermis Stem L epidermis Hypocotyl H epidermis Cotyledon H mesophyll H vascular H epidermis H petiole Rosette Leaf H epidermis H petiole Primary Root H epidermis Lateral root H lateral root cap Observed expression pattern: T1 mature: Low epidermal expression in stem and pedicles near inflorescence apical meristem. T2 seedling: High epidermal expression in cotyledons, petioles of emerging rosette leaves, hypocotyl, and root. Expression observed in vascular and mesophyll cells of cotyledons. Source Promoter Organism: *Arabidopsis thaliana*, WS ecotype Vector: pNewbin4.HAP1-GFP Marker Type: GFP-ER Generation Screened: XT1 Mature XT2 Seedling Inductions completed: Events Time Screened/ Re- Treatment: Age: Gen: points: Response: sponse: 1. Drought 7 days T2 3 Hrs Air dry 2/0 No 2. Drought 4 weeks T2 10-12 day 2/2 Yes No H20 Inducible expression summary: Time point Treatment: induced: Organs induced: Tissues induced: 2. Drought 10-12 day Flowers Pedicel, Epidermis No H20 Siliques Epidermis Leaf Epidermis, Vascular Stem Epidermis TABLE 1-5. T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 2 GFP Expression Detected Flower L pedicel L epidermis Stem L epidermis TABLE 2-5. T2 Seedling Expression Tissues Screened Events Screened: n = 2 Events Expressing: n = 2 Seedlings expressing/Seedlings screened Event-04: 6/6 Event-06: 4/6 Expression Detected Hypocotyl H epidermis Cotyledon H mesophyll H vascular H epidermis H petiole Rosette Leaf H epidermis H petiole Primary Root H epidermis Lateral root H lateral root cap TABLE 3-5. Promoter utility Trait Area: Water use efficiency Sub-

trait Area: Drought Utility: Among other uses this promoter sequence is useful to improve: Modulation growth and development. Modulation of nutrient uptake and loading. Expression of nitrate transports and water pumps. Modulation of drought responses, including modulation of water uptake and transport under drought conditions. Notes: Candidate to drive genes involved in osmotic stresses such as NCED. Endogenous promoter induced under drought. Construct: YP0286 Promoter candidate I.D: 11768589 cDNA I.D: 12669548 (OCKHAM3-C) Lines expressing: YP0286 −04, −06; 7/3/03

(213) TABLE-US-00008 Promoter Expression Report YP1692 (SEQ ID NO: 6) Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Spatial expression summary: Flower L petal L vascular Hypocotyl L epidermis L vascular Cotyledon L vascular Primary Root H vascular Lateral root H vascular Root H mature root Observed expression pattern: T1 mature expression: GFP expressed in vasculature of petals in flowers. T2 seedling expression: High GFP expression in vasculature of root. Low GFP expression in hypocotyl and cotyledons. GFP expressed in epidermis of hypocotyl near root transition zone. T1 mature expression: GFP expressed in vasculature of root. Source Promoter *Arabidopsis thaliana*, Columbia Organism: (Col) ecotype Vector: pNewbin4-HAP1-GFP Marker Type: GFP-ER Generation Screened: X T1 Mature X T2 Seedling X T2 Mature Inductions completed. Events Time Screened/ Re- Treatment: Age: Gen: points: Response sponse: 1. Drought 4 wks T2 1.0% 6/2 Yes moisture Inducible expression summary: Time point Tissues Treatment: induced: Organs induced: induced: TABLE 1-6. T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 3 Expression Detected Flower L petal L vascular TABLE 2-6. T2 Seedling Expression Tissues Screened Events Screened: n = 6 Events Expressing: n = 1 Expression Detected Hypocotyl L epidermis L vascular Cotyledon L vascular Primary Root H vascular Lateral root H vascular TABLE 3-6. T2 Mature Plant Expression Organs/Tissues screened Events Screened: n = 5 Events Expressing: n = 2 Expression Detected X Root H mature root Construct: YP1692 Promoter candidate I.D: 15371608 cDNA I.D: 36534367 Events expressing: 01-06

(214) TABLE-US-00009 Promoter Expression Report YP1894 (SEQ ID NO: 7) Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Spatial expression summary: Cotyledon L epidermis Primary Root H epidermis H cortex Root H mature root Observed expression pattern: Root Specific. T1 Mature expression: No expression detected. T2 Seedling expression: High GFP expression in epidermis and cortex cells of root. Low GFP expression in cotyledons. T2 Mature expression: High GFP expression in roots. Inductions: Expression enhanced by high-nitrogen conditions (see Induction table below). Source Promoter *Arabidopsis thaliana*, Organism: Columbia (Col) ecotype Vector: pNewbin4-HAP1-GFP Marker Type: GFP-ER Generation Screened: X T1 Mature X T2 Seedling X T2 Mature Inductions Table. Upregulation of gene expression was expected in low-nitrogen conditions. This was not observed. However, enhanced GFP was detected in response to the high-nitrogen condition (control test) (see Table 4 below). Events Time Screened/ Re- Treatment: Age: Gen: points: Response sponse: 1. Nitrogen (High 4 wks T2 90 hrs 6/0 No to Low)-14.3 mM KNO.sub.3 to 28.6 mM Mannitol Inducible expression summary: Time point Organs Treatment: induced: induced: Tissues induced: High nitrogen 90 hrs Leaves and stems TABLE 1-7. T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 0 No GFP Expression Detected TABLE 2-7. T2 Seedling Expression Tissues Screened Events Screened: n = 6 Events Expressing: n = 6 (01-06) Expression Detected Cotyledon L epidermis Primary Root H epidermis H cortex TABLE 3-7. T2 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 6 (01-06) Expression Detected Root H mature root TABLE 5-7. Promoter utility Trait Area: Water Use Efficiency, Nutrient Sub-trait Area: Drought, Nitrogen use efficiency, Utility: Among other uses this promoter sequence is useful to improve: Expression in the roots is useful for improving water and nutrient uptake into the root mass without undesirable affects on the above ground tissues. The high-nitrogen induction is useful in reducing the toxicity associated with very high levels of

nitrogen and/or bypassing the feedback loop that limits nitrogen uptake when nitrogen levels are not limiting, thus accelerating nitrogen uptake for either storage for later use or for enhancing growth. Construct: YP1894 Promoter candidate I.D: 25518825 cDNA I.D: 23499704

(215) TABLE-US-00010 Promoter Expression Report For YP1976 (SEQ ID NO: 8) Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Spatial expression summary: Hypocotyl L epidermis Primary Root H cortex Lateral root H cortex Root H mature root Observed expression pattern: T1 mature expression: No GFP expression observed in aerial organs. T2 seedling expression: High GFP expressed in epidermal cells at root transition zone. GFP is expressed in cortex cells of lower main root and lateral root. T2 mature expression: High GFP expression in roots of mature plant. No GFP expression observed in aerial tissues. Source Promoter Organism: *Arabidopsis thaliana*, Columbia (Col) Vector: ecotype pNewbin4-HAP1-GFP Marker Type: GFP-ER Generation Screened: X T1 Mature X T2 Seedling X T2 Mature TABLE 1-8. T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 0 No GFP Expression Detected TABLE 2-8. T2 Seedling Expression Tissues Screened Events Screened: n = 6 Events Expressing: n = 6 Expression Detected X Hypocotyl L epidermis X Primary Root H cortex X Lateral rant H cortex TABLE 3-8. T2 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 6 Expression Detected X Root H mature root TABLE 4-8 Promoter utility Trait Area: Nutrient and water economy. Sub-trait Area: Nitrogen use efficiency Water use efficiency. Utility: Among other uses this promoter sequence is useful to improve: Nitrogen use efficiency in lower/non-limiting nitrogen environments, enhanced water uptake in drought and non-limiting water environments, and protection against soil-borne nematodes, root worms, fungal and bacterial pathogens. Construct: YP1976 Promoter candidate I.D: 15371806 cDNA I.D: 23523729 Events expressing: 01-06

(216) TABLE-US-00011 Promoter Expression Report YP2016 (SEQ ID NO: 9) Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Spatial expression summary: Flower H pedicel H receptacle H nectary H sepal H petal H filament H tapetum H carpel H style H papillae H vascular H epidermis H stomata H silique Silique H stigma H style H carpel H septum H vascular H epidermis H abscission zone H ovule Ovule Post-fertilization: H inner integument H outer integument H seed coat H embryo Embryo H torpedo H late H mature Stem H vascular H xylem H phloem H pith H epidermis H cortex Leaf H petiole H mesophyll H epidermis H stomata Hypocotyl H epidermis H cortex H vascular H xylem H phloem Cotyledon H mesophyll H vascular H epidermis H petiole Rosette Leaf H mesophyll H vascular H epidermis Primary Root H cortex H vascular Lateral root H root cap Observed expression pattern: T1 Mature expression: GFP broadly expressed throughout mature plant with highest expression at inflorescence meristems. High GFP expression throughout organs of flowers. Not expressed in anther walls of mature stamen. High GFP expression in silique, developing ovules, seed and embryos. GFP expression in epidermis, vasculature and mesophyll in leaves. GFP expressed in epidermis, cortex, pith, and vascular bundles throughout stem, highest near apex decreasing toward rosette leaves. GFP expression in mature root. T2 Seedling expression: GFP expression throughout epidermis, cortex, vascular and mesophyll cells in aerial tissues of seedling. In root, GFP expressed in cortex and vasculature. T2 Mature expression: GFP broadly expressed throughout mature plant with highest expression at inflorescence meristems and root. Source Promoter *Arabidopsis thaliana*, Organism: Columbia (Col) ecotype Vector: pNewbin4-HAP1-GFP Marker Type: GFP-ER Generation Screened: X T1 Mature X T2 Seedling X T2 Mature TABLE 1-9. T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 6 Expression Detected Flower H pedicel H receptacle H nectary H sepal H petal H filament H tapetum H carpel H style H papillae H vascular H epidermis H stomata H silique Silique H stigma H style H carpel H septum H vascular H epidermis H abscission zone H ovule Ovule Post- fertilization:H inner integument H outer integument H seed coat H embryo Embryo H torpedo H late H mature Stem H epidermis H cortex H vascular H xylem H phloem H pith Leaf H petiole H mesophyll H epidermis H stomata

TABLE 2-9. T2 Seedling Expression Tissues Screened Events Screened: n = 4 Events Expressing: n = 4 Expression Detected Hypocotyl H epidermis H cortex H vascular H xylem H phloem Cotyledon H mesophyll H vascular H epidermis H petiole Rosette Leaf H mesophyll H vascular H epidermis Primary Root H cortex H vascular Lateral root H root cap TABLE 3-9. T2 Mature Plant Expression Organs/Tissues screened Events Screened: n = 4 Events Expressing: n = 4 TABLE 4-9. Promoter utility Trait Area: Plant growth and development Sub-trait Area: Size and source capacity Utility: Among other uses this promoter sequence is useful to improve: Plant size and architecture, growth rate, seedling establishment, responses to shade and low light, responses to drought and cold, source capacity and sucrose loading, seed filling, seed size and plant yield. Construct: YP2016 Promoter candidate I.D: 25087074 cDNA I.D: 23495629 Events expressing: 01-04

(217) TABLE-US-00012 Promoter Expression Report YP2097 (SEQ ID NO: 10) Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Spatial expression summary: Flower H pollen Primary Root L epidermis Root L mature root Observed expression pattern: T1 mature expression: High GFP expression specific to pollen. T2 seedling expression: GFP expression in epidermal cells at root transition zone decreasing toward root tip. T2 mature expression: High GFP expression in pollen. GFP expression in epidermis and vascular cells of root. Source Promoter Organism: *Arabidopsis thaliana*, Columbia (Col) ecotype Vector: pNewbin4-HAP1-GFP Marker Type: GFP-ER Generation Screened: X T1 Mature X T2 Seedling X T2 Mature Inductions completed. Events Time Screened/ Treatment: Age: Gen: points: Response Response: 1. Drought 4 wks T2 1.0% 6/2 Yes moisture Inducible expression summary: Time point Organs Tissues Treatment: induced: induced: induced: 1. Drought 1.0% Flower Pollen, moisture Leaf Epidermis Stem Guard cells, Root Epidermis Guard cells Vascular TABLE 1-10. T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 4 Expression Detected Flower H pollen TABLE 2-10. T2 Seedling Expression Tissues Screened Events Screened: n = 6 Events Expressing: n = 2 Expression Detected Primary Root L epidermis TABLE 3-10. T2 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 3 Expression Detected Root L mature root TABLE 5-10. Promoter utility Trait Area: PG&D, water use efficiency Sub-trait Area: Drought tolerance, Utility: Among other uses this promoter sequence is useful to improve: Desiccation tolerance, recovery from drought, drought tolerance, improve water use efficiency, seed size and nutrient use efficiency and nitrogen use efficiency. Construct: YP2097 Promoter candidate I.D: 29223804 cDNA I.D: 36541759 Events expressing: 02, 04

(218) TABLE-US-00013 Promoter Expression Report YP2538 (SEQ ID NO: 11) Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Spatial expression summary: Flower H pedicel H anther H silique Silique H carpel H epidermis Ovule Post-fertilization: H embryo Embryo H mature Hypocotyl H epidermis H vascular Cotyledon H mesophyll H vascular H epidermis Rosette Leaf H mesophyll H epidermis Primary Root H vascular H root cap Aerial organs H inflorescence H flowers H silique H stem Root H mature root Observed expression pattern: T1 Mature expression: High GFP expression at inflorescence. GFP expressed in stem and pedicels near apex. High GFP expression in anthers of developing flowers and in carpels and mature embryos in mature siliques. T2 Seedling expression: High GFP expression in vasculature of root, hypocotyl and cotyledons. High GFP expression in epidermis, mesophyll and vasculature of cotyledons. High GFP expression in emerging rosette leaves. T2 Mature expression: High GFP expression at inflorescence, flowers, stem and root. Source Promoter *Arabidopsis thaliana*, Organism: Columbia (Col) ecotype Vector: pNewbin4-HAP1-GFP Marker Type: GFP-ER Generation Screened: X T1 Mature X T2 Seedling X T2 Mature Inductions completed. Events Screen- Time ed/Re- Re- Treatment: Age: Gen: points: sponse sponse: 1. Drought 4 wks T2 1.0% 4/3 Yes moisture Inducible expression summary: Time point Organs Tissues Treatment: induced: induced: induced: 1. Drought 1.0% Inflorescence Pedicels, moisture Flower Silique, Silique Stem Silique, Stem Stamens Root Epidermis, Vascular Vascular Epidermis TABLE 1-11. T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 5 Events Expressing: n = 2 (02,

05) Expression Detected Flower H pedicel H anther H silique Silique H carpel H epidermis Ovule Post-fertilization: H embryo Embryo H mature TABLE 2-11. T2 Seedling Expression Tissues Screened Events Screened: n = 3 Events Expressing: n = 2 (03, 05) Expression Detected Hypocotyl H epidermis H vascular Cotyledon H mesophyll H vascular H epidermis Rosette Leaf H mesophyll H epidermis Primary Root H vascular H root cap TABLE 3-11. T2 Mature Plant Expression Organs/Tissues screened Events Screened: n = 4 Events Expressing: n = 2 (03, 05) Expression Detected Aerial organs H inflorescence H flowers H silique H stem Root H mature root TABLE 5-11. Promoter utility Trait Area: Water Use Efficiency, Nutrient Use Efficiency, Yield Sub-trait Area: Drought Tolerance, Low Nitrogen Tolerance, Low Phosphorous Tolerance, Seed Size and Yield Utility: Among other uses this promoter sequence is useful to improve: response to drought conditions and low soil nutrient levels. Expression in the embryo could be valuable for engineering of seed size and yield. Construct: YP2538 Promoter candidate I.D: 25087125 cDNA I.D: 23519856 Events expressing: 03-05

(219) TABLE-US-00014 Promoter Expression Report YP2552 (SEQ ID NO: 12) Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Spatial expression summary: Primary Root H epidermis H vascular H root hairs Mature Root H epidermis H vasculature H xylem H phloem H root hairs lateral root L stele Observed expression pattern: T1 Mature expression: No GFP expression detected. T2 Seedling expression: Root specific GFP expression. High GFP expression in epidermal and vascular cells. T2 Mature expression: Root specific GFP expression. High GFP expression in epidermal and vascular cells. Source Promoter Organism: *Arabidopsis thaliana*, Columbia (Col) ecotype Vector: pNewbin4-HAP1-GFP Marker Type: GFP-ER Generation Screened: X T1 Mature X T2 Seedling X T2 Mature Inductions completed: Events Time Screened/ Treatment: Age: Gen: points: Response Response: 1. Cold 10 T2 4 hr 6/0 None day 2. Nitrogen - high N 4 T2 4 hr 6/0 None to low N [14.3 mM weeks KNO.sub.3 to 28.6 mM Mannitol] 3. Far Red Far Red.sub.730 = 10 T2 4 hr 6/0 None 525 μW/cm day Inducible expression summary: Time point Organs Tissues Treatment: induced: induced: induced: Table 1-12. T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 0 No GFP Expression Detected Table 2-12. T2 Seedling Expression Tissues Screened Events Screened: n = 6 Events Expressing: n = 4 (01-03, 06) Expression Detected X Primary Root H epidermis H vasculature H root hairs Table 3-12. T2 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 5 (01, 02, 03, 05, 06) Expression Detected Mature Root H epidermis H vasculature H xylem H phloem H root hairs lateral root L stele Table 4-12. Promoter utility Trait Area: Water Use Efficiency, Nutrient Use Efficiency Sub-trait Area: Drought Tolerance, Nitrogen and Phosphorous Use Efficiency Utility: Among other uses this promoter sequence is useful to improve the uptake of water and nutrients. Construct: YP2552 Promoter candidate I.D: 25659462 cDNA I.D: 23504306 Events expressing: 01, 02, 03, 05, 06

(220) TABLE-US-00015 Promoter Expression Report YP2563 (SEQ ID NO: 13) Promoter Tested In: *Ambidopsis thaliana*, Wassilewskija (WS) ecotype Spatial expression summary: Flower H carpel H silique Silique H carpel Ovule Post-fertilization: H funiculus H seed coat Hypocotyl H epidermis L vascular Cotyledon L epidermis H mesophyll H epidermis Primary Root L epidermis L cortex H vascular H root cap H root hairs Lateral root L epidermis Observed expression pattern: T1 Mature expression: High GFP expression in carpels and in seed coats and funiculus of mature siliques. T2 Seedling expression: High GFP expression in vasculature throughout root and in epidermis and cortex cells near root tip at elongation zone. GFP expressed in root hair and root cap. T2 Mature expression: High GFP expression in roots and lateral inflorescences. Source Promoter *Arabidopsis thaliana*, Columbia (Col) ecotype Organism: Vector: pNewbin4-HAP1-GFP Marker Type: GFP-ER Generation Screened: X T1 Mature X T2 Seedling X T2 Mature Inductions completed. Events Time Screened/ Treatment: Age: Gen: points Response Response: 1. Nitrogen-high 4 wks T2 72 Hr 12/0 None N to low N [14.3 mM KNO.sub.3 to 28.6 mM Mannitol] Inducible expression summary: Time point Organs Tissues Treatment: induced: induced: induced: Table 1-13.

T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 12 Events Expressing: n = 12 (01-12) Expression Detected Flower H carpel H silique Silique H carpel Ovule Post-fertilization: H funiculus H seed coat Table 2-13. T2 Seedling Expression Tissues Screened Events Screened: n = 11 Events Expressing: n = 9 (02-04, 06, 09-11, 13, 14) Expression Detected Hypocotyl H epidermis L vascular Cotyledon L epidermis Rosette Leaf H mesophyll H epidermis Primary Root L epidermis L cortex H vascular H root cap H root hairs Lateral root L epidermis Table 3-13. T2 Mature Plant Expression Organs/Tissues screened Events Screened: n = 5 Events Expressing: n = 4 (11-15) Expression Detected Aerial organs H inflorescence L stem Root H mature root Table 4-13. Promoter utility Trait Area: Nutrient Use Efficiency, Water Use Efficiency, Yield, PG&D, Confinement Sub-trait Area: Nitrogen and Phosphorous Utilization, Drought Tolerance, Seed Size, Plant Establishment, Seed Confinement Utility: Among other uses this promoter sequence could be useful to improve enhance the uptake of nutrients and water from the soil (root), and seed size (seed coat). Expression in the seed could also be used to engineer seed ablation and seed confinement. Construct: YP2563 Promoter candidate I.D: 25518834 cDNA I.D: 36516796 Events expressing: 01-12

(221) TABLE-US-00016 Promoter Expression Report YP2571 (SEQ ID NO: 14) Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Spatial expression summary: Flower H sepal H petal H anther H vascular H stomata H silique Silique H style H carpel H funiculus H vascular Leaf L vascular Aerial organs H inflorescence Root H mature root Hypocotyl H vascular Cotyledon H mesophyll H vascular H epidermis Primary Root H vascular Observed expression pattern: T1 Mature expression: High GFP expression detected in inflorescences. T2 Seedling expression: High GFP expression in vasculature of hypocotyls, cotyledons and root. GFP expression in epidermis and mesophyll cells of cotyledons. T2 Mature expression: High GFP expression in vasculature and guard cells of sepals and in anthers, petals and silique of flowers. GFP expressed in style, carpels and vasculature of silique. Not expressed in ovules or seed. Low GFP expression in leaf vasculature. High GFP expression in root. Source Promoter Organism: *Arabidopsis thaliana*, Columbia (Col) ecotype Vector: pNewbin4-HAP1-GFP Marker Type: GFP-ER Generation Screened: X T1 Mature X T2 Seedling X T2 Mature Inductions completed. Events Time Screened/ Re- Treatment: Age: Gen: points: Response sponse: 1. ABA—[uM] 14 T2 4 hrs 6/0 No days 2. Heat—28 C. 15 T2 24 hrs 3/3 Low days 3. Heat—36 C. 9 T2 >24 hrs 6/3 High days 4. Heat—41 C. 14 T2 4 hrs 3/3 Medium days 5. Heat—41 C. 15 T2 24 hrs 3/0 No days 6. Heat—46 C. 14 T2 4 hrs 3/0 No days 7. Heat—40 C. 4 wks T2 24 hrs 6/6 Low 8. Drought 4 wks T2 1.0% 6/6 High moisture Inducible expression summary: Time point Organs Tissues Treatment: induced: induced: induced: 2. Heat—28 C. 24 hrs Cotyledons Rosette leaf 3. Heat—36 C. >24hrs Cotyledons Ep, Me, Vs Rosette leaf Ep, Me, Vs Root Vs 4. Heat—41 C. 4 hrs Cotyledons Rosette leaf 7. Heat—40 C. 24 hrs Flower Se, Pe, Vs Stem Ph, Vs Leaf Vs 8. Drought 1.0% Flower Se, Pe, Ca moisture Stem Xy, Ph, Vs Leaf Ep, Me Root Ep TABLE 1-14. T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 1 (04) Expression Detected Inflorescence H flowers TABLE 2-14. T2 Seedling Expression Tissues Screened Events Screened: n = 6 Events Expressing: n = 2 (04, 05) Expression Detected Hypocotyl H vascular Cotyledon H mesophyll H vascular H epidermis Primary Root H vascular TABLE 3-14. T2 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 6 (01-06) Expression Detected Flower H sepal H petal H anther H vascular H stomata H silique Silique H style H carpel H funiculus H vascular Leaf L vascular Aerial organs H inflorescence Root H mature root TABLE 5-14. Promoter utility Trait Area: Water Use Efficiency Sub-trait Area: Drought Tolerance, Heat Stress Tolerance Utility: Among other uses this promoter sequence is useful to improve plant tolerance to heat and drought stress. Construct: YP2571 Promoter candidate I.D: 29223786 cDNA I.D: 23618816 Events expressing: 01-06

(222) TABLE-US-00017 Promoter Expression Report YP2590 (SEQ ID NO: 15) Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Spatial expression summary: *Arabidopsis*

Flower H pedicel Silique H ovule Ovule Pre-fertilization: H funiculus Post-fertilization: H funiculus Aerial organs H flowers Root H mature root Rice Root H not-specific Meristem H not-specific Observed expression pattern: *Arabidopsis*: T1 Mature expression: High GFP expression at the distal end of the funiculus in developing ovules and seed. GFP highly localized to adaxial side at the base of the pedicel, in structures resembling stipules of leaves. T2 Seedling expression: No GFP expression detected. T2 Mature expression: GFP detected at the base of pedicles and roots. Rice: T0 Seedling expression: GFP expression was detected strongly throughout the root and in meristematic tissues corresponding to the stem nodes. Source Promoter Organism: *Arabidopsis thaliana*, Columbia (Col) ecotype Vector: pNewbin4-HAP1-GFP Marker Type: GFP-ER Generation Screened: X T1 Mature X T2 Seedling X T2 Mature Table 1-14. T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 3 Expression Detected Flower H pedicel Silique H ovule Ovule Pre-fertilization: H funiculus Post-fertilization: H funiculus Table 2-14. T2 Seedling Expression Tissues Screened Events Screened: n = 6 Events Expressing: n = 0 No GFP Expression Detected Table 3-14. T2 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 2 (01, 03, 04, 06) Expression Detected Aerial organs H flowers Root H mature root Table 4-14, T0 Rice Seedlings Organs/Tissues screened Events Screened: n = 13 Events Expressing: n = 4 (01, 02, 06, 09) Expression Detected Root H not-specific Meristem L not-specific Table 5-14. Promoter utility Trait Area: Yield, PG&D, Confinement, Water Use Efficiency Sub-trait Area: Seed Number, Seed Growth, Plant Size, Growth Rate Utility: Among other uses this promoter sequence is useful to improve plant architecture and increase the number of secondary floral branches. Expression in the root could be valuable for improved uptake and transport of water and nutrients. *Arabidopsis* Construct: YP2590-*Arabidopsis* Promoter candidate I.D: 25659363 cDNA I.D: 23523207 Events expressing: 02, 03, 04 Rice Construct: PD3146 Promoter candidate I.D: 55210297 cDNA I.D: 23523207 Events expressing: 01, 02, 06, 09

(223) TABLE-US-00018 Promoter Expression Report YP2606 (SEQ ID NO: 16) Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Spatial expression summary: Primary Root H epidermis H cortex Mature Root H epidermis L cortex H stele H vasculature Observed expression pattern: T1 Mature expression: No GFP expression detected. T2 Seedling expression: Root specific GFP expression. GFP expressed in root epidermis and cortex cells. T2 Mature expression: Root specific GFP expression. GFP expressed in root epidermis and the stele, non-ground cell vascular region of root. Source Promoter Organism: *Arabidopsis thaliana*, Columbia (Col) ecotype Vector: pNewbin4-HAP1-GFP Marker Type: GFP-ER Generation Screened: X T1 Mature X T2 Seedling X T2 Mature Table 1-15. T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 0 No Expression Detected Table 2-15. T2 Seedling Expression Tissues Screened Events Screened: n = 6 Events Expressing: n = 3 (04-06) Expression Detected Primary Root H epidermisH cortex Table 3-15. T2 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 2 (04, 06) Expression Detected Mature Root H epidermis H cortex H stele H vasculature Table 4-15. Promoter utility Trait Area: Water Use Efficiency, Nutrient Use Efficiency Sub-trait Area: Drought Tolerance, Nitrogen and Phosphorous Utilization Utility: Among other uses this promoter sequence is useful to improve the uptake of water and nutrients. Construct: YP2606 Promoter candidate I.D: 25086987 cDNA I.D: 36511228 Events expressing: 04-06 One or more fragments of the above described promoter are identified in the miscellaneous feature section of the relevant SEQ ID in the Sequence Listing. Those fragments were tested for promoter activity by the same procedures as described above, and the results are summarized below. Ceres Promoter PD3464 expresses weakly in roots in rice.

(224) TABLE-US-00019 Promoter Expression Report YP2608 (SEQ ID NO: 17) Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Spatial expression summary: Primary Root H epidermis H cortex Aerial organs H flowers Flower H stamen H filament H anther Mature Root

H epidermis L cortex H stele Observed expression pattern: T1 Mature expression: No GFP expression detected. T2 Seedling expression: GFP expressed in root epidermis. T2 Mature expression: High stamen specific GFP expression corresponding to third organ whorl in *Arabidopsis* flower. GFP expressed in sub-epidermal cells in root. Source Promoter Organism: *Arabidopsis thaliana*, Columbia (Col) ecotype Vector: pNewbin4-HAP1-GFP Marker Type: GFP-ER Generation Screened: X T1 Mature X T2 Seedling X T2 Mature T3 Seedling Table 1-16. T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 4 Events Expressing: n = 0 No GFP Expression Detected Table 2-16. T2 Seedling Expression Tissues Screened Events Screened: n = 5 Events Expressing: n = 5 (01-05) Expression Detected Primary Root H epidermis H cortex Table 3-16. T2 Mature Plant Expression Organs/Tissues screened Events Screened: n = 4 Events Expressing: n = 3 (02, 03, 04) Expression Detected Flower H stamen H filament H anther Aerial organs H flowers Mature Root H epidermis L cortex H stele Table 4-16. Promoter utility Trait Area: Water Use Efficiency, Nutrient Use Efficiency, Sterility Sub-trait Area: Drought Tolerance, Nitrogen and Phosphorous Utilization, Male Sterility Utility: Among other uses this promoter sequence is useful to improve the uptake of water and nutrients from the soil. It could also be used to engineer male sterility. Construct: YP2608 Promoter candidate I.D: 25656951 cDNA I.D: 5680676 Events expressing: 02, 03 ,04 One or more fragments of the above described promoter are identified in the miscellaneous feature section of the relevant SEQ ID in the Sequence Listing. Those fragments were tested for promoter activity by the same procedures as described above, and the results are summarized below. For Ceres Promoter PD3739, no expression is observed.

(225) TABLE-US-00020 Promoter Expression Report YP2683 (SEQ ID NO: 18) Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Spatial expression summary: Flower L petal L anther H pollen Stem L cortex L vascular H xylem H procambium Leaf H vascular L epidermis L stomata Cotyledon L epidermis L hydathode Primary Root L cortex L epidermis H vascular Aerial Organs L flower Mature Root L epidermis H cortex H vascular H quiescent center Observed expression pattern: T1 Mature expression: Expression is primarily in pollen and in vascular tissues in leaves and stems. Expression appears to be strongest in the meristematic vascular cells (procambium), which gives rise to the xylem and phloem. T2 Seedling expression: Expression is observed primarily in the root vasculature. Weak expression was also observed in the epidermis of the seedling. T2 Mature: Weak expression was observed in the floral tissue. Strong expression was detected in the root, primarily in the cortex and vascular tissues as well as the quiescent center. Source Promoter Organism: *Arabidopsis thaliana*, Columbia (Col) ecotype Vector: pNewbin4-HAP1-GFP Marker Type: GFP-ER Generation Screened: X T1 Mature X T2 Seedling X T2 Mature Table 1-17. T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 8 Events Expressing: n = 3 (02, 03, 06) Expression Detected Flower L petal L anther H pollen Stem L cortex L vascular H xylem H procambium Leaf H vascular L epidermis L stomata Table 2-17. T2 Seedling Expression Tissues Screened Events Screened: n = 6 Events Expressing: n = 3 (02, 05, 06) Expression Detected Cotyledon L epidermis L hydathode Primary Root L epidermis L cortex H vascular Table 3-17. T2 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 4 (02, 03, 05, 06) Expression Detected Aerial organs L flower Mature Root L epidermis H cortex H vascular H quiescent center Table 4-17. Promoter utility Trait Area: Sterility, Water Use Efficiency, Nutrient Use Efficiency Sub-trait Area: Male-Sterility, Drought Tolerance, Nitrogen and Phosphorous Utilization Utility: Among other uses this promoter sequence is useful to improve the uptake of water and nutrients from the soil. The pollen expression could be used to engineer male sterility for gene-confinement. Construct: YP2683 Promoter candidate I.D: 41958148 cDNA I.D: 36558613 Events expressing: 02, 03, 05, 06

(226) TABLE-US-00021 Promoter Expression Report YP2816 (SEQ ID NO: 19) Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Spatial expression summary: Flower H vascular H ovule Silique H septum Ovule Pre-fertilization: H outer integument Post-fertilization: H

seed coat H embryo Embryo H radicle H late mature Stem H epidermis H vascular H xylem H phloem Leaf L vascular H trichome Hypocotyl L epidermis Cotyledon L epidermis Rosette Leaf H epidermis H trichome H primordia Primary Root H epidermis H cortex H vascular L quiescent center L columella Mature Root H epidermis H cortex H vascular H endodermis H parenchyma H stele Observed expression pattern: All cells in root. T1 Mature expression: Promoter expression is observed in the pre-fertilized ovule integuments as well as the seed coat and the developing radicle of the embryo. Expression is also observed weakly in the vasculature of the stem and leaf trichomes. T2 Seedling expression: Seedling expression is primarily in all cells of the root. T2 Mature: Expression is strong in all cells of the root analyzed. Source Promoter Organism: *Arabidopsis thaliana*, Columbia (Col) ecotype Vector: pNewbin4-HAP1-GFP Marker Type: GFP-ER Generation Screened: X T1 Mature X T2 Seedling X T2 Mature Table 1-18. T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 8 Events Expressing: n = 5 Expression Detected (02, 03, 05, 07, 08) Flower H vascular H ovule Silique H septum Ovule Pre-fertilization: H outer integument Post-fertilization: H seed coat H embryo Embryo H late H radicle Stem H epidermis H vascular H xylem H phloem Leaf L vascular H trichome Table 2-18. T2 Seedling Expression Tissues Screened Events Screened: n = 6 Events Expressing: n = 3 (02, 03, 05) Expression Detected Hypocotyl L epidermis Cotyledon L epidermis Rosette Leaf H epidermis H trichome H primordia Primary Root H epidermis H cortex H vascular L quiescent center L columella Table 3-18. T2 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 5 (01-05) Expression Detected Leaf H trichome Aerial organs L stem Mature Root H epidermis H cortex H vascular H endodermis H parenchyma H stele Table 4-18. Promoter utility Trait Area: Water Use Efficiency, Nutrient Use Efficiency Sub-trait Area: Drought Tolerance, Nitrogen and Phosphorous Use Efficiency Utility: Among other uses this promoter sequence is useful to improve the uptake and utilization of water and nutrients from the soil and the transport of water throughout the vasculature. Construct: YP2816 Promoter candidate I.D: 15372106 cDNA I.D: 36540030 Events expressing: 01-05, 07, 08 One or more fragments of the above described promoter are identified in the miscellaneous feature section of the relevant SEQ ID in the Sequence Listing. Those fragments were tested for promoter activity by the same procedures as described above, and the results are summarized below. Ceres Promoter PD3238 expresses weakly in roots in rice. No expression is observed for Ceres Promoter PD3229 and Ceres Promoter PD3243.

(227) TABLE-US-00022 Promoter Expression Report YP2832 (SEQ ID NO: 20) Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Spatial expression summary: Flower H pedicel H receptacle H style Silique H style H septum H abscission zone Stem H epidermis H cortex Leaf L stomata Primary Root L epidermis H cortex H endodermis H phloem Lateral Root H primordia Aerial Organs H stem Mature Root L epidermis H cortex H endodermis H phloem H lateral roots Observed expression pattern: T1 Mature expression: Expression is primarily observed in the epidermis and cortical layers of the stem tissue. T2 Seedling expression: The promoter is expressed in the most mature parts of the primary root, and is not detected in the root tips. The root expression is also observed in the buds giving rise to the secondary root branches. No expression is observed in the hypocotyl or cotyledons. T2 Mature expression: Expression observed in non-leaf tissues. Source Promoter Organism: *Arabidopsis thaliana*, Columbia (Col) ecotype Vector: pNewbin4-HAP1-GFP Marker Type: GFP-ER Generation Screened: X T1 Mature X T2 Seedling XT2 Mature Table 1-19. T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 5 (01-05) Expression Detected Flower H pedicel H receptacle H style Silique H style H septum H abscission zone Stem H epidermis H cortex Leaf L stomata Table 2-19. T2 Seedling Expression Tissues Screened Events Screened: n = 6 Events Expressing: n = 4 (01, 02, 04, 05) Expression Detected Primary Root L epidermis H cortex H endodermis H phloem Lateral root H primordia Table 3-19. T2 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 6 (01-06) Expression Detected Aerial organs H stem

Mature Root H lateral root L epidermis H cortex H endodermis H phloem Table 4-19. Promoter utility Trait Area: Source, Water Use Efficiency, Nutrient Use Efficiency Sub-trait Area: Carbon/Nitrogen Partitioning, Drought Tolerance, Nitrogen and Phosphorous Use Efficiency Utility: Among other uses this promoter sequence is useful to engineer the storage of carbon into stem and root material, to improve the uptake of water and nutrients into the roots from the soil, and to protect the plants from pests. Construct: YP2832 Promoter candidate I.D: 32258957 cDNA I.D: 36551046 Events expressing: 01-06

(228) TABLE-US-00023 Promoter Expression Report PD2995 (aka PD2263) (SEQ ID NO: 21) Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Spatial expression summary: Flower H epidermis L petal H carpel H style H silique H pedicel Silique H epidermis H style H carpel H septum H ovule Ovule Pre-fertilization: H outer integument Post-fertilization: H seed coat H outer integument Embryo H mature endosperm H mature Stem H epidermis H pith Leaf L epidermis H mesophyll Inflorescence H floral meristem H floral primordia Hypocotyl H epidermis H cortex H vascular Cotyledon H epidermis H mesophyll Rosette Leaf H epidermis H mesophyll H stipule Primary Root H epidermis H cortex H vascular H quiescent center H root meristem H root cap Lateral root H primordia H flanking cells H lateral root cap Aerial organs H inflorescence H floral meristem H floral primordia H flowers H silique H stem H leaf Root H mature root Observed expression pattern: T1 Mature expression: High broad GFP expression throughout aerial organs. High GFP expression in flowers, stems, and leaves. GFP expressed in floral meristems and primordia. High GFP expression in carpels, ovules and developing seed in silique. GFP expressed in outer integuments, endosperm, seed coat and embryos in ovules. GFP expressed in pith and epidermis in stem and epidermis and mesophyll of leaf. T2 Seedling expression: High GFP expression in aerial organs and root. High GFP expression in epidermis and mesophyll cells of cotyledon and rosette leaf. High GFP expression in epidermis, cortex and vasculature of hypocotyl and root. GFP expressed in root meristem cells and root cap. T2 Mature Expression: High boad GFP expression throughout aerial organs and roots. Source Promoter Organism: *Arabidopsis thaliana*, Columbia (Col) ecotype Vector: CRS338-GFP Marker Type: GFP-ER Generation Screened: X T1 Mature X T2 Seedling X T2 Mature Table 1-20. T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 6 (01-06) Expression Detected X Flower H pedicel L petal H carpel H style H epidermis H silique X Silique H style H carpel H septum H epidermis H ovule X Ovule Pre-fertilization: H outer integument Post-fertilization: H outer integument H seed coat H mature endosperm X Embryo H mature X Stem H epidermis H pith X Leaf H mesophyll L epidermis X Inflorescence H floral meristem H floral primordia Table 2-20. T2 Seedling Expression Tissues Screened Events Screened: n = 6 Events Expressing: n = 6 (01-06) GFP Expression Detected X Hypocotyl H epidermis H cortex H vascular X Cotyledon H mesophyll H epidermis X Rosette Leaf H mesophyll H epidermis H stipule X Primary Root H epidermis H cortex H vascular H quiescent center H root meristem H root cap X Lateral root H primordia H flanking cells □ H lateral root cap Table 3-20. T2 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 6 (01-06) GFP Expression Detected X Aerial organs H inflorescence H floral meristem H floral primordia H flowers H silique H stem H leaf X Root H mature root Table 4-20. Promoter utility Trait Area: Water Use Efficiency, PG&D, Seed, Nutrient, and Yield Sub-trait Area: Water use efficiency, growth rate, seed yield, and nutrient utilization Utility: Among other uses this promoter sequence is useful to modulate plant growth and architecture and the utilization and water and nutrients. Construct: PD2263 Promoter candidate I.D: 38960222 cDNA I.D: 36549595 Events expressing: 01-06 One or more fragments of the above described promoter are identified in the miscellaneous feature section of the relevant SEQ ID in the Sequence Listing. Those fragments were tested for promoter activity by the same procedures as described above, and the results are summarized below. For Ceres Promoter PD2926, expression is weak compared to the full-length promoter PD2995; the promoter remains active in all tissues except the embryo. For Ceres Promoter

PD3048, expression is weak compared to the full-length promoter PD2995; the promoter remains active in all tissues. For Ceres Promoter PD3182, no expression is observed. For Ceres Promoter PD3345, expression is very weak. For Ceres Promoter PD3503, no expression is observed. For Ceres Promoter PD3676, expression is weak compared to the full-length promoter PD2995; the promoter expresses at higher levels in vegetative tissues than in reproductive tissues.

(229) TABLE-US-00024 Promoter Expression Report PD2999 (aka PD2258) (SEQ ID NO: 22) Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Flower H pedicel H sepal H carpel H style H stigma H silique Silique H stigma H style H carpel H placentae H funiculus H epidermis H ovule Ovule Pre-fertilization: H primordia H inner integument H outer integument H funiculus Post-fertilization: H outer integument H seed coat H early endosperm H mature endosperm H embryo Embryo H suspensor H heart H mature H radicle H cotyledons Stem H epidermis H cortex H interfascicular region H vascular H xylem H phloem H pith Leaf H mesophyll H epidermis Inflorescence H floral meristem H floral primordia Hypocotyl H epidermis H cortex H vascular Cotyledon H epidermis H vascular Rosette Leaf H epidermis Primary Root H vascular H root cap Observed expression pattern: T1 Mature expression: Broad high GFP expression throughout aerial organs. High GFP expression in inflorescence, floral meristem, flowers, siliques. High GFP expression throughout tissues of silique. GFP expressed in carpels, placenta, ovule primordia, developing ovules, embryo, and endosperm. High GFP expression in outer integuments and seed coats of developing ovules and seed. High GFP expression in vascular and ground tissues of stem. High GFP expression in epidermis and mesophyll cells of leaf. T2 Seedling expression: Broad expression throughout aerial organs and vasculature of root. Source Promoter Organism: *Arabidopsis thaliana*, Columbia (Col) ecotype Vector: CRS338-GFP Marker Type: GFP-ER Generation Screened: X T1 Mature X 12 Seedling Table 1-21. T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 4 (01, 02, 04, 06) GFP Expression Detected X Flower H pedicel H sepal H carpel H style H stigma H silique X Silique H stigma H style H carpel H placentae H funiculus H epidermis H ovule X Ovule Pre-fertilization: H primordia H inner integument H outer integument H funiculus Post-fertilization: H outer integument H seed coat H early endosperm H mature endosperm H embryo X Embryo H suspensor H heart H mature H radicle H cotyledons X Stem H epidermis H cortex H interfascicular region H vascular H xylem H phloem H pith X Leaf H mesophyll H epidermis X Inflorescence H floral meristem H floral primordia Table 2-21. T2 Seedling Expression Tissues Screened Events Screened: n = 6 Events Expressing: n = 5 (01-04, 06) □ No GFP Expression Detected X Hypocotyl H epidermis H cortex H vascular X Cotyledon H epidermis H vascular X Rosette Leaf H epidermis X Primary Root H vascular H root cap Table 3-21. Promoter utility Trait Area: Water use efficiency, PG&D, Seed, Nutrient, Yield Sub-trait Area: Water use efficiency, growth rate, seed size and yield, and nutrient use Utility: Among other uses this promoter sequence is useful to improve: Water use efficiency, PG&D, Seed, Nutrient, Yield Construct: PD2258 Promoter candidate I.D: 38960200 cDNA I.D: 23478038 Events expressing: 01, 02, 04, 06 One or more fragments of the above described promoter are identified in the miscellaneous feature section of the relevant SEQ ID in the Sequence Listing. Those fragments were tested for promoter activity by the same procedures as described above, and the results are summarized below. For Ceres Promoter PD2929, expression is very weak compared to the full-length promoter PD2999; the promoter remains active in all tissues. For Ceres Promoter PD3183, expression is only detected in anthers and stigma. For Ceres Promoter PD3240, expression is very weak compared to the full-length promoter PD2999; the promoter remains active in all tissues. For Ceres Promoter PD3266, no expression is detected in rice.

(230) TABLE-US-00025 Promoter Expression Report PD3141 (SEQ TD NO: 23) Promoter Tested In: *Oryza sativa* Spatial expression summary: Tiller: not-specific Main culm: bundle sheath, endodermis, epidermis, internode, ligule, node, pericycle, phloem, sclerenchyma layer, vasculature, xylem Root: cortex, epidermis, vascular Leaf: epidermis, leaf blade, leaf sheath, mesophyll, petiole,

stipule, stomata, vasculature Meristem: floral meristem, shoot apical meristem, vegetative meristem Panicle: flag leaf, ovary, peduncle, primary branch, rachilla, rachis, spiklet Spikelet: floral meristem, shoot apical meristem, vegetative meristem Observed expression pattern: Broad Source Promoter Organism: *Oryza sativa* Vector: Binary DF EGFP Marker Type: EGFP Generation Screened: T0 Seedling and T0 Mature Induction Data Table 1-22. T0 Seedling Expression Organs/Tissues screened Events Screened: n = 12 Events Expressing: n = 9 (01, 03-04, 06-11) Organs Tiller: not-specific Main culm: not-specific Root: not-specific Leaf: not-specific Meristem: not-specific Table 2-22. T0 Mature Plant Expression Tissues Screened Events Screened: n = 6 Events Expressing: n = 6 (01, 04, 07-08, 11, 14) Organs Main culm: bundle sheath, endodermis, epidermis, internode, ligule, node, pericycle, phloem, sclerenchyma layer, vasculature, xylem Root: cortex, vascular Panicle: flag leaf, ovary, peduncle, primary branch, rachilla, rachis, spiklet Spiklet: flag leaf, floret(palea), lemma, ovule, pedicle, pollen, seed, stigma Leaf: epidermis, leaf blade, leaf sheath, mesophyll Meristem: floral meristem, shoot apical meristem, vegetative meristem Promoter utility Trait Area: Yield, Composition, Disease, Stress Tolerance, Nutrient Use Efficiency, Nutrient Utilization Sub-trait Area: Biomass, Lignin composition, Disease resistance, Salt tolerance, Drought tolerance, Phosphate and Nitrate Use Efficiency, Phosphate and Nitrate Utilization Utility: Among other uses, this promoter sequence is useful to improve: the biomass of the plants under normal and stressful conditions through the overexpression of trait-specific transgenes. Construct: PD3141 SR/OS Line: OS00486 Promoter candidate I.D: 54507599 cDNA I.D: NA Events expressing: 01, 03-04, 06-11,14

(231) TABLE-US-00026 Promoter Expression Report YP2680 (SEQ ID NO: 24) Promoter Tested In: *Arabidopsis thaliana* Spatial expression summary: Leaf: epidermis, vasculature Flower: vascular Silique: abscission zone, vascular Primary root: cortex, epidermis, root hairs, vascular Lateral root: cortex, epidermis, vascular Mature root: not-specific Observed expression pattern: Primarily root expression Source Promoter Organism: *Arabidopsis thaliana* Vector: Binary TC(815) Marker Type: erGFP Generation Screened: T1 Mature, T2 Seedling, T2 Mature Table 1-23. T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 8 Events Expressing: n = 7 (01, 03-08) Organs Leaf: epidermis, vasculature Flower: vascular Silique: abscission zone, vascular Table 2-23. T1 Seedling Expression Tissues Screened Events Screened: n = 5 Events Expressing: n = 5 (01-05) Primary root: cortex, epidermis, root hairs, vascular Lateral root: cortex, epidermis, vascular Table 3-23. T2 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 5 (01-03, 06, 08) Organs Mature root: not-specific Primary root: cortex, epidermis, vascular Lateral root: cortex, epidermis, vascular Promoter utility: Trait Area: Drought Tolerance, Nutrient Utilization Sub-trait Area: Water Utilization, Phosphate and Nitrate Utilization Utility: Among other uses, this promoter sequence is useful to improve: the uptake and transport of water and nutrients from the soil to support vegetative growth. Notes: 1000 nt upstream of atg Construct: YP2664 SR/OS Line: SR04406 Promoter candidate I.D: 41958160 cDNA I.D: 36511557 Events expressing: 01-08 One or more fragments of the above described promoter are identified in the miscellaneous feature section of the relevant SEQ ID in the Sequence Listing. Those fragments were tested for promoter activity by the same procedures as described above, and the results are summarized below. Ceres Promoter PD3584 is a strong, broadly expressing promoter; comparable to the full-length promoter PD3141.

(232) TABLE-US-00027 Promoter Expression Report PD3147 (aka YP2663) (SEQ ID NO: 25) Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Spatial expression summary: Flower H pedicel H sepal H petal H filament H anther L carpel L silique Silique L carpel L funiculus Stem H epidermis H cortex H vascular H xylem H phloem L pith Leaf H epidermis H mesophyll H vascular Hypocotyl H epidermis H cortex Cotyledon H epidermis H mesophyll Rosette Leaf H epidermis H mesophyll Aerial organs H inflorescence H flowers L silique H stem H leaf Observed expression pattern: T1 Mature expression: High GFP expressed in aerial tissues. High GFP expression in leaf, stem and inflorescence. High GFP expressed in pedicels, sepals,

petals and stamens. Low GFP expression in epidermis of silique. No GFP expression observed in embryo. High GFP expression in epidermis, cortex, phloem and xylem cells of vasculature. Low GFP expression in pith sells of stem. High GFP expression in epidermis, mesophyll and vasculature of leaves. T2 Seedling expression: High GFP expressed in aerial tissues. High GFP expression in epidermis and mesophyll cells of cotyledons and rosette leaves and in epidermis and cortex of hypocotyls. No GFP expression observed in roots. T2 Mature expression: GFP expressed in aerial tissues. High GFP expression in leaf, stem and inflorescence. No GFP expression observed in roots. Source Promoter Organism: *Arabidopsis thaliana*, Columbia (Col) ecotype Vector: pNewbin4-HAP1-GFP Marker Type: GFP-ER Generation Screened: X T1 Mature X T2 Seedling X T2 Mature Inductions completed. Events Screened/ Treatment: Age: Gen: Time points: Response Response: 1. Nitrogen-high N to low N 4 wks T2 72 hrs 6/0 No [14.3 mM KNO.sub.3 to 28.6 mM Mannitol] Inducible expression summary: Treatment: Time point induced: Organs induced: Tissues induced: Table 1-24. T1 Mature Plant Expression Organs/Tissues screened Events Screened: n = 6 Events Expressing: n = 4 (02, 04-06) GFP Expression Detected X Flower H pedicel H sepal H petal H filament H anther L carpel L silique X Silique L carpel L fimiculus X Stem H epidermis H cortex H vascular H xylem H phloem L pith X Leaf H mesophyll H vascular H epidermis Table 2-24. T2 Seedling Expression Tissues Screened Events Screened: n = 6 Events Expressing: n = 4 (02, 04-06) GFP Expression Detected X Hypocotyl H epidermis H cortex X Cotyledon H mesophyll H epidermis X Rosette Leaf H mesophyll H epidermis Table 3-24. T2 Mature Plant Expression Organs/Tissues screened Events Screened: n = 5 Events Expressing: n = 4 (02, 04-05) GFP Expression Detected X Aerial organs H inflorescence H flowers L silique H stem H leaf Table 5-24. Promoter utility Trait Area: PG&D, Confinement, Hybrid Production Sub-trait Area: Plant Size, Growth Rate, Carbon Fixation Utility: Among other uses this promoter sequence is useful to improve carbon fixation in above ground tissues to increase biomass and seed yield. Expression is useful for engineering of carbon and nitrogen ratios. Expression in flowers is deployed for sterility and confinement. Construct: Y132663 Promoter candidate LD: 25087104 cDNA I.D: 36567145 Events expressing: 02, 04-06 One or more fragments of the above described promoter are identified in the miscellaneous feature section of the relevant SEQ ID in the Sequence Listing. Those fragments were tested for promoter activity by the same procedures as described above, and the results are summarized below. Ceres Promoter PD3721 is a strong, vegetatively expressing promoter; comparable to the full-length promoter PD3147.

(233) TABLE-US-00028 Promoter Expression Report For PT0822 (SEQ ID NO: 26) Promoter Tested In: *Arabidopsis thaliana*, Wassilewskija (WS) ecotype Observed expression pattern: T1 Mature expression: Expression observed in mature root only. T2 Seedling expression: Expression specific to epidermis and cortex cells of root. Source Promoter Organism: *Arabidopsis thaliana*, Columbia (Col) ecotype Vector: pNewbin4-HAP1-GFP Marker Type: Generation Screened: T1 Mature T2 Seedling T2 Mature Table 1-25. T1 Mature Plant Expression Organs/Tissues screened Root H epidermis Table 2-25. T2 Seedling Expression Tissues Screened Expression Detected Primary Root H epidermis H cortex Promoter Expression Report for PD3389 (nucleotides 601-1000 of SEQ ID NO: 26) Promoter Tested In: *Oryza sativa* Spatial expression summary: T0 Seedling X Root expression in: CORTEX EPIDERMIS ROOT CAP T0 Mature X Root expression: non-specific. Observed expression pattern: T0 Seedling: Expression observed in the root only. T2 Seedling expression: Expression specific to epidermis and cortex cells of root. Deletion of root-specific promoter PT0822. 600 nucleotides were deleted from the 5'end of the 1000 nucleotide PT0822 promoter (SEQ ID NO: 26). Source Promoter Organism: *Arabidopsis thaliana*, Columbia (Col) ecotype Generation Screened: T0 Seedling and T0 Mature Table 1-26. T0 Seedling Expression Organs/Tissues screened Events Screened: n = 16 Events Expressing: n = 3 (01, 05,13) Organs X Root expression in: CORTEX EPIDERMIS ROOT CAP Table 2-26. T0 Mature Plant Expression Organs/Tissues screened Events Screened: n = 4 Events Expressing: n = 2 (05,13) Organs X Root expression: nonspecific Promoter utility Trait Area: Salt tolerance, Drought

Tolerance, Nutrient Use Efficiency, Nutrient Utilization Sub-trait Area: Salt tolerance, Drought tolerance, Phosphate and Nitrate Use Efficiency, Phosphate and Nitrate Utilization Utility: Among other uses, this promoter sequence is useful to improve: the plants biomass of the under normal and stressful conditions through the overexpression of trait-specific transgenes. One or more fragments of the above described promoter are identified in the miscellaneous feature section of the relevant SEQ ID in the Sequence Listing. Those fragments were tested for promoter activity by the same procedures as described above, and the results are summarized below. For Ceres Promoter PD3389, expression is observed in the roots of rice seedlings.

(234) The invention being thus described, it will be apparent to one of ordinary skill in the art that various modifications of the materials and methods for practicing the invention can be made. Such modifications are to be considered within the scope of the invention as defined by the following claims.

(235) Each of the references from the patent and periodical literature cited herein is hereby expressly incorporated in its entirety by such citation.

## Claims

1. A vector construct comprising: a) a first nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:16 or a nucleotide sequence having at least 95% identity to the nucleotide sequence of SEQ ID NO:16, wherein said first nucleic acid molecule functions as a promoter; and b) a second nucleic acid molecule to be expressed, wherein said first nucleic acid molecule and said second nucleic acid molecule are heterologous to each other and are operably linked.

2. The vector construct according to claim 1, wherein said first nucleic acid molecule comprises the nucleic acid sequence of SEQ ID NO:16.

3. The vector construct according to claim 1, wherein said second nucleic acid molecule encodes a polypeptide.

4. The vector construct according to claim 3, wherein said second nucleic acid molecule is operably linked to said first nucleic acid molecule in the sense orientation.

5. The vector construct according to claim 4, wherein said second nucleic acid molecule is transcribed into an RNA molecule that expresses the polypeptide encoded by said second nucleic acid molecule.

6. The vector construct according to claim 1, wherein said second nucleic acid molecule is operably linked to said first nucleic acid molecule in the antisense orientation.

7. The vector construct according to claim 6, wherein transcription of said second nucleic acid molecule produces an antisense molecule.

8. The vector construct according to claim 1, wherein transcription of said second nucleic acid molecule produces an RNAi molecule against an endogenous gene.

9. The vector construct according to claim 3, wherein said second nucleic acid molecule encodes a polypeptide of agronomic interest.

10. A plant or plant cell comprising the vector construct according to claim 1.

11. A plant or plant cell stably transformed with the vector construct according to claim 1.

12. A transgenic seed of the plant according to claim 10.

13. A method of directing transcription comprising combining, in an environment suitable for transcription: a) a first nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 16 or a nucleotide sequence having at least 95% identity to the nucleotide sequence of SEQ ID NO: 16, wherein said first nucleic acid molecule functions as a promoter; and b) a second nucleic acid molecule; wherein said first nucleic acid molecule and said second nucleic acid molecule are heterologous to each other and operably linked, and transcribing said second nucleic acid molecule.

14. A method of expressing an exogenous coding region in a plant comprising: (a) transforming a plant cell with the vector of claim 1; (b) regenerating a stably transformed plant from the

transformed plant cell of step (a); and (c) selecting at least one plant containing the transformed plant cell, wherein expression of the second nucleic acid molecule results in production of a polypeptide encoded by said second transcribable nucleic acid molecule.

15. A method of altering the expression of an endogenous gene in a plant comprising: a) transforming a plant cell with the vector construct according to claim 1, and b) regenerating at least one stably transformed plant from said transformed plant cell, wherein transcription of said second nucleic acid molecule produces an RNAi molecule that suppresses expression of the endogenous gene.

16. A plant prepared according to the method of claim 14.

17. A transgenic seed from the plant according to claim 16.

18. A method of producing a transgenic plant, said method comprising: (a) transforming a plant cell comprising the vector according to claim 1; and (b) growing a plant from said plant cell.

19. The method of claim 18, wherein said second nucleic acid molecule comprises a nucleic acid sequence encoding a polypeptide.

20. The method of claim 18, wherein said second nucleic acid molecule is operably linked to said first nucleic acid molecule in an antisense orientation.

21. The method of claim 18, wherein transcription of said second nucleic acid molecule produces an RNAi molecule.

22. A plant or plant cell comprising the vector construct according to claim 2.

23. A plant or plant cell stably transformed with the vector construct according to claim 2.

24. A transgenic seed of the plant according to claim 22, wherein the transgenic seed comprises the vector construct.

25. A method of producing a transgenic plant, said method comprising: (a) introducing into a plant cell the vector according to claim 2; and (b) growing a plant from said plant cell.