Abstract: Water deficit-inducible promoter sequences were identified that may be used to produce transgenic plants that are more tolerant to water deficit and related hyperosmotic stresses than control plants, and yet are wild-type or nearly wild type in appearance. Any of these water deficit-inducible promoters may be incorporated into an expression vector that comprises a polynucleotide regulated by one such promoter and which encodes a polypeptide that, when ectopically expressed, improves water deficit tolerance in plants that are similar to control plants in their morphology and development.
— with sequence listing part of description published separately in electronic form and available upon request from the International Bureau
WATER DEFICIT-INDUCIBLE PROMOTERS

JOINT RESEARCH AGREEMENT

The present invention, in the field of functional genomics and the characterization of plant genes for the improvement of plants, was made by or on behalf of Mendel Biotechnology, Inc. and Monsanto Corporation as a result of activities undertaken within the scope of a joint research agreement in effect on or before the date the present invention was made.

FIELD OF THE INVENTION

The present invention relates to plant genomics and more specifically pertains to water deficit-inducible promoters that mediate gene expression during a plant's response to water deficit.

BACKGROUND OF THE INVENTION

In the natural environment, plants often grow under unfavorable conditions, including water deficit conditions such as drought, a severe form of low water availability generally characterized as a prolonged period of water deficit. Water deficit, or water deprivation, can delay growth and development, reduce productivity, and in extreme cases, cause the plant to die. Low water availability is a major factor in crop yield reduction worldwide. Problems for plants caused by low water availability include mechanical stresses caused by the withdrawal of cellular water. Drought also causes plants to become more susceptible to various diseases (Simpson, ed. (1981) "The Value of Physiological Knowledge of Water Stress in Plants", in Water Stress on Plants, Praeger, NY, pp. 235-265).

A number of polypeptides, including, for example, transcription factors (TFs), have been shown to improve the tolerance of plant species to water deficit conditions (for examples, see publication no. WO2004076638). However, important limitations in the use of various proteins that confer water deficit tolerance to crop species when the proteins are overexpressed may include negative side effects associated with constitutive overexpression of these polypeptides. Possible pleiotropic effects such as small size, delayed growth, increased disease sensitivity, and development and alteration in flowering time are common. It has been proposed that genes conferring tolerance to water deficit impose a cost on overall fitness and development. To overcome these limitations, the present studies were initiated to discover and assess the utility of numerous promoter sequences that respond to water deficit conditions.
These promoter sequences can be used to regulate protein expression during periods of drought or other water deficit conditions, and therefore may be used to induce overexpression of polypeptides that can confer improved water deficit tolerance when they are needed without the adverse developmental or morphological effects that may be associated with their constitutive overexpression. Numerous transgenic plants using these promoter sequences to regulate polypeptides were developed and the plants were analyzed for their tolerance to water deficit conditions. Many of these promoter sequences can be used to produce commercially valuable plants and crops as well as the methods for making them and using them.

The present invention thus relates to methods and compositions for producing transgenic plants, where water deficit-inducible overexpression of transcription factors confers enhanced tolerance to water deficit with reduced or no impact on yield, appearance, quality or fitness, as compared to plants constitutively overexpressing the same transcription factors. Other aspects and embodiments of the invention are described below and can be derived from the teachings of this disclosure as a whole.

**SUMMARY OF THE INVENTION**

The present invention is directed to promoter sequences that may be used to transform a plant. The promoter sequences are able to respond to water deficit conditions and can be used to drive the expression of a polynucleotide sequence that encodes a polypeptide that can confer increased tolerance to water deficit, including drought, desiccation, dehydration, or a related hyperosmotic stress (for example, freezing or high salt concentration). Thus, the polypeptide may be expressed in a water-deficit inducible manner.

The invention also provides an isolated nucleic acid comprising a water deficit-inducible promoter that includes any of the promoter sequences provided by SEQ ID NOs: 1-9. A water deficit-inducible promoter of the invention may comprise a functional part thereof, provided the functional part also includes a water-deficit-inducible promoter function. The functional part of the promoter may have about 50, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 447, 450, 460, 475, 500, 525, 550, 575, 600, 605, 625, 650, 675, 700, 725, 750, 766, 775, 776, 780, 800, 825, 850, 875, 900, 907, 928 or 936 contiguous nucleotides of the nucleic acid sequences of SEQ ID NOs: 1-9, as well as all lengths of contiguous nucleotides within such sizes.
The invention also pertains to expression vectors that can comprise a promoter sequence of the invention. The water deficit-inducible promoter may comprise any of SEQ ID NOs: 1 to 9, or a functional part thereof, provided the functional part also includes a water-deficit-inducible promoter function. The promoter comprises a transcription initiation domain having an RNA polymerase binding site. The promoter is located 5' relative to and is operably linked to a coding sequence encoding a polypeptide that confers to a plant increased tolerance to water deficit conditions. Many of the expression vectors provided as SEQ ID NOs: 10 to 54 (each of which comprises a promoter of any of SEQ ID NOs: 1-9, as well as a nucleic acid sequence encoding a polypeptide that confers increased tolerance to water deficit) have been introduced into plants, and the plants not only have been shown to have greater water deficit tolerance than a control plant, but the transformed plants are often of wild-type or near-wild type morphology and development (many polypeptides that contribute to improved water deficit tolerance can also cause undesirable morphological and/or developmental traits when the polypeptides are constitutively overexpressed).

The invention encompasses a host plant cell comprising a water deficit-inducible promoter of the invention, comprising any of SEQ ID NOs: 1 to 9 or a functional part thereof, wherein the functional part includes a promoter function.

The invention also encompasses a transgenic plant comprising a water deficit-inducible promoter of the invention, comprising any of SEQ ID NOs: 1 to 9 or a functional part thereof, wherein the functional part includes a promoter function, and transgenic seed produced by the transgenic plants of the invention.

Methods for producing a transgenic plant having greater tolerance to water deficit conditions than a control plant, or for increasing the tolerance of a plant to water deficit, are provided. The method steps include the generation of an expression vector that comprises a promoter sequence of any of SEQ ID NOs: 1-9 or a functional part thereof, wherein the functional part includes a promoter function. The promoter sequence is operably linked to a nucleotide sequence that encodes a polypeptide that increases the water deficit tolerance in a plant, and during water deficit conditions the promoter sequence drives expression of the nucleotide sequence that encodes the polypeptide. A target plant is then transformed with the expression vector to produce a transgenic plant. When the polypeptide is overexpressed in the transformed plant (for example, during periods of water deficit), the transformed plant will have greater tolerance to water deficit conditions than the control plant. A transgenic plant that is
produced by this method may be crossed with itself, a plant from the same line as the transgenic plant, a non-transgenic plant, a wild-type plant, or another transgenic plant from a different transgenic line of plants, to produce a transgenic seed that comprises the expression vector.

### Brief Description of the Sequence Listing and Drawings

The Sequence Listing provides exemplary polynucleotide and polypeptide sequences of the invention. The traits associated with the use of the sequences are included in the Examples.

CD-ROMs Copy 1 and Copy 2, and the CRF copy of the Sequence Listing are read-only memory computer-readable compact discs. Each contains a copy of the Sequence Listing in ASCII text format. The Sequence Listing is named "MBI0079P.ST25.txt", the electronic file of the Sequence Listing contained on each of these CD-ROMs was created on February 7, 2007, and is 127 kilobytes in size. The copies of the Sequence Listing on the CD-ROM discs are hereby incorporated by reference in their entirety.

Figure 1 shows a conservative estimate of phylogenetic relationships among the orders of flowering plants (modified from Soltis et al. (1997) *Ann. Missouri Bot. Gard.* 84: 1-49). Those plants with a single cotyledon (monocots) are a monophyletic clade nested within at least two major lineages of dicots; the eudicots are further divided into rosids and asterids. *Arabidopsis* is a rosid eudicot classified within the order Brassicales; rice is a member of the monocot order Poales. Figure 1 was adapted from Daly et al. (2001) *Plant Physiol.* 127: 1328-1333.

Figure 2 shows a phylogenetic dendogram depicting phylogenetic relationships of higher plant taxa, including clades containing tomato and *Arabidopsis*; adapted from Ku et al. (2000) *Proc. Natl. Acad. Sci.* USA 97: 9121-912; and Chase et al. (1993) *Ann. Missouri Bot. Gard.* 80: 528-580.

Figure 3 shows the induction of *Arabidopsis* native genes corresponding to nine drought-promoters in a clay pot drought assay. Drought stressed and well-watered pMEN65 (empty vector) wild-type control plants were used for this experiment. Plants were drought stressed to the wilting point (as is typical for the clay pot assay to the point where they would normally be re-watered), and RT-PCR was performed using gene-specific primers for each of the genes indicated on the x-axis. The cycle threshold counts value was the real-time PCR cycle number at which the RNA transcript of interest was detectable above background, were normalized with
18S RNA. The checkered bars represent the average cycle threshold value for well-watered plants. The solid bars indicate the average cycle threshold value for the drought-stressed plants.

**DETAILED DESCRIPTION**

The present invention relates to polynucleotides and polypeptides for modifying phenotypes of plants, particularly promoter sequences associated with increased tolerance to water deficit such as desiccation, dehydration or drought, with respect to a control plant (for example, a genetically unaltered or non-transgenic plant such as a wild-type plant of the same species, or a transgenic plant line that comprises an empty expression vector). Throughout this disclosure, various information sources are referred to and/or are specifically incorporated. The information sources include scientific journal articles, patent documents, textbooks, and World Wide Web page addresses. While the reference to these information sources clearly indicates that they can be used by one of skill in the art, each and every one of the information sources cited herein are specifically incorporated in their entirety, whether or not a specific mention of "incorporation by reference" is noted. The contents and teachings of each and every one of the information sources can be relied on and used to make and use embodiments of the invention.

As used herein and in the appended claims of the invention, the singular forms "a", "an", and "the" include the plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "a stress" is a reference to one or more stresses and equivalents thereof known to those skilled in the art, and so forth.

**DEFINITIONS**

"Nucleic acid molecule" refers to an oligonucleotide, polynucleotide or any fragment thereof. It may be DNA or RNA of genomic or synthetic origin, double-stranded or single-stranded, and combined with carbohydrate, lipid, protein, or other materials to perform a particular activity such as transformation or form a useful composition such as a peptide nucleic acid (PNA).

"Polynucleotide" is a nucleic acid molecule comprising a plurality of polymerized nucleotides, e.g., at least about 15 consecutive polymerized nucleotides. A polynucleotide may be a nucleic acid, oligonucleotide, nucleotide, or any fragment thereof. In many instances, a polynucleotide comprises a nucleotide sequence
encoding a polypeptide (or protein) or a domain or fragment thereof. Additionally, the
polynucleotide may comprise a promoter, an intron, an enhancer region, a
polyadenylation site, a translation initiation site, 5’ or 3’ untranslated regions, a
reporter gene, a selectable marker, or the like. The polynucleotide can be single-
stranded or double-stranded DNA or RNA. The polynucleotide optionally comprises
modified bases or a modified backbone. The polynucleotide can be, e.g., genomic
DNA or RNA, a transcript (such as an mRNA), a cDNA, a PCR product, a cloned
DNA, a synthetic DNA or RNA, or the like. The polynucleotide can be combined
with carbohydrate, lipids, protein, or other materials to perform a particular activity
such as transformation or form a useful composition such as a peptide nucleic acid
(PNA). The polynucleotide can comprise a sequence in either sense or antisense
orientations. "Oligonucleotide" is substantially equivalent to the terms amplimer,
primer, oligomer, element, target, and probe and is preferably single-stranded.

A "recombinant polynucleotide" is a polynucleotide that is not in its native
state, e.g., the polynucleotide comprises a nucleotide sequence not found in nature, or
the polynucleotide is in a context other than that in which it is naturally found, e.g.,
separated from nucleotide sequences with which it typically is in proximity in nature,
or adjacent (or contiguous with) nucleotide sequences with which it typically is not in
proximity. For example, the sequence at issue can be cloned into a vector, or
otherwise recombined with one or more additional nucleic acid.

An "isolated polynucleotide" is a polynucleotide, whether naturally occurring
or recombinant, that is present outside the cell in which it is typically found in nature,
whether purified or not. Optionally, an isolated polynucleotide is subject to one or
more enrichment or purification procedures, e.g., cell lysis, extraction, centrifugation,
precipitation, or the like.

"Gene" or "gene sequence" refers to the partial or complete coding sequence
of a gene, its complement, and its 5’ or 3’ untranslated regions. A gene is also a
functional unit of inheritance, and in physical terms is a particular segment or
sequence of nucleotides along a molecule of DNA (or RNA, in the case of RNA
viruses) involved in producing a polypeptide chain. The latter may be subjected to
subsequent processing such as chemical modification or folding to obtain a functional
protein or polypeptide. A gene may be isolated, partially isolated, or found with an
organism's genome. By way of example, a transcription factor gene encodes a
transcription factor polypeptide, which may be functional or require processing to function as an initiator of transcription.

Operationally, genes may be defined by the cis-trans test, a genetic test that determines whether two mutations occur in the same gene and that may be used to determine the limits of the genetically active unit (Rieger et al. (1976) Glossary of Genetics and Cytogenetics: Classical and Molecular, 4th ed., Springer Verlag, Berlin). A gene generally includes regions preceding ("leaders"; upstream) and following ("trailers"; downstream) the coding region. A gene may also include intervening, non-coding sequences, referred to as "introns", located between individual coding segments, referred to as "exons". Most genes have an associated promoter region, a regulatory sequence 5' of the transcription initiation codon (there are some genes that do not have an identifiable promoter). The function of a gene may also be regulated by enhancers, operators, and other regulatory elements.

A "promoter" or "promoter region" refers to an RNA polymerase binding site on a segment of DNA, generally found upstream or 5' relative to a coding sequence under the regulatory control of the promoter. The promoter will generally comprise response elements that are recognized by transcription factors. Transcription factors bind to the promoter sequences, recruiting RNA polymerase, which synthesizes RNA from the coding region. Dissimilarities in promoter sequences account for different efficiencies of transcription initiation and hence different relative expression levels of different genes.

"Promoter function" includes regulating expression of the coding sequences under a promoter's control by providing a recognition site for RNA polymerase and/or other factors, such as transcription factors, all of which are necessary for the start of transcription at a transcription initiation site. A "promoter function" may also include the extent to which a gene coding sequence is transcribed to the extent determined by a promoter sequence.

A promoter or promoter region may include variations of promoters found in the present Sequence Listing, which may be derived by ligation to other regulatory sequences, random mutagenesis, controlled mutagenesis, and/or by the addition or duplication of enhancer sequences. Promoters disclosed in the present Sequence Listing and biologically functional equivalents or variations thereof may drive the transcription of operably-linked coding sequences when comprised within an expression vector and introduced into a host plant. Promoters such as those found in
the Sequence Listing (i.e., SEQ ID NOs: 1-9) may be used to generate similarly
functional promoters containing essential promoter elements. Functional promoters of
the invention may also include a functional part of any of SEQ ID NO: 1-9, provided
the functional part also includes a water-deficit-inducible promoter function.

A "polypeptide" is an amino acid sequence comprising a plurality of
consecutive polymerized amino acid residues e.g., at least about 15 consecutive
polymerized amino acid residues. In many of the instances referred to in this
application, a polypeptide comprises a polymerized amino acid residue sequence that
is a transcription factor or a domain or portion or fragment thereof. Additionally, the
polypeptide may comprise: (i) a localization domain; (ii) an activation domain; (iii) a
repression domain; (iv) an oligomerization domain; (v) a DNA-binding domain; or
the like. The polypeptide optionally comprises modified amino acid residues,
naturally occurring amino acid residues not encoded by a codon, non-naturally
occurring amino acid residues.

"Protein" refers to an amino acid sequence, oligopeptide, peptide, polypeptide
or portions thereof whether naturally occurring or synthetic.

A "recombinant polypeptide" is a polypeptide produced by translation of a
recombinant polynucleotide. A "synthetic polypeptide" is a polypeptide created by
consecutive polymerization of isolated amino acid residues using methods well
known in the art. An "isolated polypeptide," whether a naturally occurring or a
recombinant polypeptide, is more enriched in (or out of) a cell than the polypeptide in
its natural state in a wild-type cell, e.g., more than about 5% enriched, more than
about 10% enriched, or more than about 20%, or more than about 50%, or more,
enriched, i.e., alternatively denoted: 105%, 110%, 120%, 150% or more, enriched
relative to wild type standardized at 100%. Such an enrichment is not the result of a
natural response of a wild-type plant. Alternatively, or additionally, the isolated
polypeptide is separated from other cellular components with which it is typically
associated, e.g., by any of the various protein purification methods herein.

"Homology" refers to sequence similarity between a reference sequence and at
least a fragment of a newly sequenced clone insert or its encoded amino acid
sequence.

"Identity" or "similarity" refers to sequence similarity between two
polynucleotide sequences or between two polypeptide sequences, with identity being
a more strict comparison. The phrases "percent identity" and "% identity" refer to the
percentage of sequence similarity found in a comparison of two or more polynucleotide sequences or two or more polypeptide sequences. "Sequence similarity" refers to the percent similarity in base pair sequence (as determined by any suitable method) between two or more polynucleotide sequences. Two or more sequences can be anywhere from 0-100% similar, or any integer value therebetween. Identity or similarity can be determined by comparing a position in each sequence that may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same nucleotide base or amino acid, then the molecules are identical at that position. A degree of similarity or identity between polynucleotide sequences is a function of the number of identical, matching or corresponding nucleotides at positions shared by the polynucleotide sequences. A degree of identity of polypeptide sequences is a function of the number of identical amino acids at corresponding positions shared by the polypeptide sequences. A degree of homology or similarity of polypeptide sequences is a function of the number of amino acids at corresponding positions shared by the polypeptide sequences.

"Complementary" refers to the natural hydrogen bonding by base pairing between purines and pyrimidines. For example, the sequence A-C-G-T (5' -> 3') forms hydrogen bonds with its complements A-C-G-T (5' -> 3') or A-C-G-U (5' -> 3'). Two single-stranded molecules may be considered partially complementary, if only some of the nucleotides bond, or "completely complementary" if all of the nucleotides bond. The degree of complementarity between nucleic acid strands affects the efficiency and strength of hybridization and amplification reactions. "Fully complementary" refers to the case where bonding occurs between every base pair and its complement in a pair of sequences, and the two sequences have the same number of nucleotides.

The terms "paralog" and "ortholog" are defined below in the section entitled "Orthologs and Paralogs". In brief, orthologs and paralogs are evolutionarily related genes that have similar sequences and functions. Orthologs are structurally related genes in different species that are derived by a speciation event. Paralogs are structurally related genes within a single species that are derived by a duplication event.

The term "equivalog" describes members of a set of homologous proteins that are conserved with respect to function since their last common ancestor. Related proteins are grouped into equivalog families, and otherwise into protein families with other
hierarchically defined homology types. This definition is provided at the Institute for Genomic Research (TIGR) World Wide Web (www) website, "tigr.org" under the heading "Terms associated with TIGRFAMs".

In general, the term "variant" refers to molecules with some differences, generated synthetically or naturally, in their base or amino acid sequences as compared to a reference (native) polynucleotide or polypeptide, respectively. These differences include substitutions, insertions, deletions or any desired combinations of such changes in a native polynucleotide of amino acid sequence.

With regard to polynucleotide variants, differences between presently disclosed polynucleotides and polynucleotide variants are limited so that the nucleotide sequences of the former and the latter are closely similar overall and, in many regions, identical. Due to the degeneracy of the genetic code, differences between the former and latter nucleotide sequences may be silent (i.e., the amino acids encoded by the polynucleotide are the same, and the variant polynucleotide sequence encodes the same amino acid sequence as the presently disclosed polynucleotide. Variant nucleotide sequences may encode different amino acid sequences, in which case such nucleotide differences will result in amino acid substitutions, additions, deletions, insertions, truncations or fusions with respect to the similar disclosed polynucleotide sequences. These variations may result in polynucleotide variants encoding polypeptides that share at least one functional characteristic. The degeneracy of the genetic code also dictates that many different variant polynucleotides can encode identical and/or substantially similar polypeptides in addition to those sequences illustrated in the Sequence Listing.

Also within the scope of the invention is a variant of a gene promoter listed in the Sequence Listing, that is, one having a sequence that differs from one of the polynucleotide sequences in the Sequence Listing, or a complementary sequence.

The term "plant" includes whole plants, shoot vegetative organs/structures (for example, leaves, stems and tubers), roots, flowers and floral organs/structures (for example, bracts, sepals, petals, stamens, carpels, anthers and ovules), seed (including embryo, endosperm, and seed coat) and fruit (the mature ovary), plant tissue (for example, vascular tissue, ground tissue, and the like) and cells (for example, guard cells, egg cells, and the like), and progeny of same. The class of plants that can be used in the method of the invention is generally as broad as the class of higher and lower plants amenable to transformation techniques, including angiosperms.
(monocotyledonous and dicotyledonous plants), gymnosperms, ferns, horsetails, psilophytes, lycophytes, bryophytes, and multicellular algae (see for example, Figure 1, adapted from Daly et al. (2001) supra, Figure 2, adapted from Ku et al. (2000) supra; and see also Tudge (2000) in The Variety of Life, Oxford University Press, New York, NY pp. 547-606.

A "control plant" as used in the present invention refers to a plant cell, seed, plant component, plant tissue, plant organ or whole plant used to compare against transgenic or genetically modified plant for the purpose of identifying an enhanced phenotype in the transgenic or genetically modified plant. A control plant may in some cases be a transgenic plant line that comprises an empty vector or marker gene, but does not contain the recombinant polynucleotide of the present invention that is expressed in the transgenic or genetically modified plant being evaluated. In general, a control plant is a plant of the same line or variety as the transgenic or genetically modified plant being tested. A suitable control plant would include a genetically unaltered or non-transgenic plant of the parental line used to generate a transgenic plant herein.

A "transgenic plant" refers to a plant that contains genetic material not found in a wild-type plant of the same species, variety or cultivar. The genetic material may include a transgene, an insertional mutagenesis event (such as by transposon or T-DNA insertional mutagenesis), an activation tagging sequence, a mutated sequence, a homologous recombination event or a sequence modified by chimeraplasty. Typically, the foreign genetic material has been introduced into the plant by human manipulation, but any method can be used as one of skill in the art recognizes.

A transgenic plant may contain an expression vector or cassette. The expression cassette typically comprises a polypeptide-encoding sequence operably linked (i.e., under regulatory control of) to an inducible regulatory sequence, such as a promoter of the invention, that allows for the controlled expression of polypeptide. The expression cassette can be introduced into a plant by transformation or by breeding after transformation of a parent plant. A plant refers to a whole plant as well as to a plant part, such as seed, fruit, leaf, or root, plant tissue, plant cells or any other plant material, e.g., a plant explant, as well as to progeny thereof, and to in vitro systems that mimic biochemical or cellular components or processes in a cell.

"Wild type" or "wild-type", as used herein, refers to a plant cell, seed, plant component, plant tissue, plant organ or whole plant that has not been genetically
modified or treated in an experimental sense. Wild-type cells, seed, components, tissue, organs or whole plants may be used as controls to compare levels of expression and the extent and nature of trait modification with cells, tissue or plants of the same species in which expression of a polypeptide, such as a transcription factor polypeptide, is altered, e.g., in that it has been overexpressed or ectopically expressed.

A "trait" refers to a physiological, morphological, biochemical, or physical characteristic of a plant or particular plant material or cell. In some instances, this characteristic is visible to the human eye, such as seed or plant size, or can be measured by biochemical techniques, such as detecting the protein, starch, or oil content of seed or leaves, or by observation of a metabolic or physiological process, e.g. by measuring tolerance to a form of water deficit such as drought, or particular salt or sugar concentrations, or by the observation of the expression level of a gene or genes, e.g., by employing Northern analysis, RT-PCR, microarray gene expression assays, or reporter gene expression systems, or by agricultural observations such as extent of wilting, turgor, hyperosmotic stress tolerance or yield. Any technique can be used to measure the amount of, comparative level of, or difference in any selected chemical compound or macromolecule in the transgenic plants, however.

"Trait modification" refers to a detectable difference in a characteristic in a plant ectopically expressing a polynucleotide or polypeptide of the present invention relative to a plant not doing so, such as a wild-type plant. In some cases, the trait modification can be evaluated quantitatively. For example, the trait modification can entail at least about a 2% increase or decrease, or an even greater difference, in an observed trait as compared with a control or wild-type plant. It is known that there can be a natural variation in the modified trait. Therefore, the trait modification observed entails a change of the normal distribution and magnitude of the trait in the plants as compared to control or wild-type plants.

When two or more plants are "morphologically similar" they have comparable forms or appearances, including analogous features such as dimension, height, width, mass, root mass, shape, glossiness, color, stem diameter, leaf size, leaf dimension, leaf density, internode distance, branching, root branching, number and form of inflorescences, and other macroscopic characteristics at a particular stage of growth. If the plants are morphologically similar at all stages of growth, they are also "developmentally similar". It may be difficult to distinguish two plants that are
genotypically distinct but morphologically similar based on morphological characteristics alone.

The term "transcript profile" refers to the expression levels of a set of genes in a cell in a particular state, particularly by comparison with the expression levels of that same set of genes in a cell of the same type in a reference state. For example, the transcript profile of a particular transcription factor protein in a suspension cell is the expression levels of a set of genes in a cell knocking out or overexpressing that transcription factor protein compared with the expression levels of that same set of genes in a suspension cell that has normal levels of that transcription factor protein.

The transcript profile can be presented as a list of those genes whose expression level is significantly different between the two treatments, and the difference ratios. Differences and similarities between expression levels may also be evaluated and calculated using statistical and clustering methods.

"Ectopic expression or altered expression" in reference to a polynucleotide indicates that the pattern of expression in, e.g., a transgenic plant or plant tissue, is different from the expression pattern in a wild-type plant or a reference plant of the same species. The pattern of expression may also be compared with a reference expression pattern in a wild-type plant of the same species. For example, the polynucleotide or polypeptide is expressed in a cell or tissue type other than a cell or tissue type in which the sequence is expressed in the wild-type plant, or by expression at a time other than at the time the sequence is expressed in the wild-type plant, or by a response to different inducible agents, such as hormones or environmental signals, or at different expression levels (either higher or lower) compared with those found in a wild-type plant. The term also refers to altered expression patterns that are produced by lowering the levels of expression to below the detection level or completely abolishing expression. The resulting expression pattern can be transient or stable, constitutive or inducible. In reference to a polypeptide, the term "ectopic expression or altered expression" further may relate to altered activity levels resulting from the interactions of the polypeptides with exogenous or endogenous modulators or from interactions with factors or as a result of the chemical modification of the polypeptides.

The term "overexpression" as used herein refers to a greater expression level of a gene in a plant, plant cell or plant tissue, compared to expression in a wild-type plant, cell or tissue, at any developmental or temporal stage for the gene.
Overexpression can occur when, for example, the genes encoding one or more transcription factor proteins are under the control of a strong promoter (e.g., the cauliflower mosaic virus 35S transcription initiation region). Overexpression may also occur under the control of an inducible promoter such as a water deficit-inducible promoter. Thus, overexpression may occur throughout a plant or in the presence of particular environmental signals, depending on the promoter used.

Overexpression may take place in plant cells normally lacking expression of polypeptides functionally equivalent or identical to a polypeptide that can confer increased water deficit tolerance. Overexpression may also occur in plant cells where endogenous expression of the present proteins that confer enhanced water deficit tolerance, or functionally equivalent molecules, normally occurs, but such normal expression is at a lower level. Overexpression thus results in a greater than normal production, or "overproduction" of the protein that confers improved water deficit tolerance in the plant, cell or tissue.

The term "transcription regulating region" refers to a DNA regulatory sequence that regulates expression of one or more genes in a plant when a transcription factor having one or more specific binding domains binds to the DNA regulatory sequence. Transcription factors of the present invention possess an conserved domain. Transcription factors of the invention also comprise an amino acid subsequence that forms a transcription activation domain that regulates expression of one or more biotic stress resistance genes in a plant when the transcription factor binds to the regulating region.

**DESCRIPTION OF THE SPECIFIC EMBODIMENTS**

A number of polypeptides produced by plants are involved in numerous pathways that can confer enhanced tolerance to water deprivation. We have shown that overexpression of transcription factors can lead to enhanced water deficit tolerance in *Arabidopsis* plants. However, overexpression of these transcription factors may come at a price; the overexpressing plant may be small, may have increased disease susceptibility, or may have other undesirable developmental effects such as delayed development, low yield or fertility. This raises an obvious question: can regulation of transcription factor pathways be controlled in a manner that confers water deficit tolerance and yet avoids much or all of the growth and developmental penalty? Overexpression and associated water deficit tolerance without significant adverse morphological effects would make these transcription factors available as
effective commercial tools for enhancing water deficit tolerance. One such means is the use of drought-inducible promoters that can confer water deficit tolerance while mitigating the undesirable effects of constitutive overexpression of transcription factors responsible for that tolerance.

The development of effective water deficit tolerance in these plants is likely to require a promoter(s) that responds rapidly to low water availability, as well as sustained expression throughout the period of low water availability to maximize effectiveness. The selection strategy for identifying commercially valuable drought-inducible promoters thus considered the following criteria. Promoters of interest would be:

- expressed at a low basal level, that is, in the absence of water deficit;
- induced strongly and at a sustained induction level early in the course of reduced water availability; and
- specific to the response to water deficit (the ability to be induced by other environmental factors increases frequency of expression and the likelihood that the plant would have reduced size or yield).

Transcript profiling (TxP) is a powerful tool for promoter discovery, providing a global insight into gene expression, regulation and induction levels in the plant's response to water deficit. As outlined below, water deficit-inducible promoters have been identified in microarrays by transcript profiling of plants exposed to water deficit-related challenges. When a polynucleotide sequence that encodes a polypeptide (for example, a transcription factor) known to confer water deficit tolerance but which also causes significant adverse morphological consequences was overexpressed, and the polynucleotide expression was under the regulatory control of water deficit-inducible promoters, the result was often the production of water deficit tolerance plants of normal (i.e., wild type) or near-normal stature and development.

Promoters showing early induction in a water deficit-related stress and little or no background expression could be used to drive expression of transcription factors to provide enhanced water deficit tolerance with little yield loss ("drag"). Promoters of genes that are induced relatively late in responding to water deficit are less likely to be effective, since they reflect a late induction of response factors. Therefore, we concentrated on early time points and early events following recognition of water deficit-derived stress response proteins.
Promoters of the invention are provided as SEQ ID NO: 1-9, and examples of expression vectors that have been or may be constructed using these promoters that may be able to confer improved water deficit tolerance include SEQ ID NOs: 10-54. The invention also encompasses a water deficit-inducible promoter that comprises a functional part of any of SEQ ID NOs: 1-9, provided that the functional part of the promoter also includes a water-deficit-inducible promoter function. The functional part of the promoter may have about 100, 150, 200, 250, 300, 350, 400, 447, 450, 460, 500, 550, 600, 605, 650, 700, 750, 766, 776, 780, 800, 850, 900, 907, 928 or 936 contiguous nucleotides of the nucleic acid sequences of SEQ ID NOs: 1-9, as well as all lengths of contiguous nucleotides within such sizes, provided that the functional part of the promoter includes a water-deficit-inducible promoter function.

Promoters that are similar to those listed in the Sequence Listing may be made that have some alterations in the nucleotide sequence and yet retain the function of the listed sequences. At the nucleotide level, the promoter sequences of the invention will typically share at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% nucleotide sequence identity with any of SEQ ID NOs: 1-9, or with constructs SEQ ID NOs: 10-54.

Percent identity can be determined electronically, e.g., by using the MEGALIGN program (DNASTAR, Inc. Madison, Wis.). The MEGALIGN program can create alignments between two or more sequences according to different methods, for example, the clustal method (see, for example, Higgins and Sharp (1988). The clustal algorithm groups sequences into clusters by examining the distances between all pairs. The clusters are aligned pairwise and then in groups. Other alignment algorithms or programs may be used, including FASTA, BLAST, or ENTREZ, FASTA and BLAST, and which may be used to calculate percent similarity. These are available as a part of the GCG sequence analysis package (University of Wisconsin, Madison, WI), and can be used with or without default settings. ENTREZ is available through the National Center for Biotechnology Information. In one embodiment, the percent identity of two sequences can be determined by the GCG program with a gap weight of 1 (see USPN 6,262,333).

Software for performing BLAST analyses is publicly available, e.g., through the National Center for Biotechnology Information (see internet website at http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence,
which either match or satisfy some positive-valued threshold score $T$ when aligned
with a word of the same length in a database sequence. $T$ is referred to as the
neighborhood word score threshold (Altschul (1990); Altschul et al. (1993)). These
initial neighborhood word hits act as seeds for initiating searches to find longer HSPs
containing them. The word hits are then extended in both directions along each
sequence for as far as the cumulative alignment score can be increased. Cumulative
scores are calculated using, for nucleotide sequences, the parameters $M$ (reward score
for a pair of matching residues; always $> 0$) and $N$ (penalty score for mismatching
residues; always $< 0$). For amino acid sequences, a scoring matrix is used to calculate
the cumulative score. Extension of the word hits in each direction are halted when: the
cumulative alignment score falls off by the quantity $X$ from its maximum achieved
value; the cumulative score goes to zero or below, due to the accumulation of one or
more negative-scoring residue alignments; or the end of either sequence is reached.
The BLAST algorithm parameters $W$, $T$, and $X$ determine the sensitivity and speed of
the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a
wordlength ($W$) of 11, an expectation ($E$) of 10, a cutoff of 100, $M=5$, $N=-4$, and a
comparison of both strands. For amino acid sequences, the BLASTP program uses as
defaults a wordlength ($W$) of 3, an expectation ($E$) of 10, and the BLOSUM62 scoring
matrix (see Henikoff & Henikoff (1989)). Unless otherwise indicated for comparisons
of predicted polynucleotides, "sequence identity" refers to the % sequence identity
generated from a tblastx using the NCBI version of the algorithm at the default
settings using gapped alignments with the filter "off" (see, for example, internet

EXAMPLES

Example 1. Microarray time course experiments and selection criteria

Candidate drought-inducible promoters were primarily selected based on a
drought time course TxP experiment performed. In this experiment, clay pots of well-
watered late-rosette stage Arabidopsis plants grown under short day conditions were
transferred to absorbent paper, and further watering was withheld during the
subsequent drought period. Data were generated for five physiologically determined
drought stages: mild stress, moderate stress, severe stress, and two stages after re-
watering. The stress state of each plant was determined by measuring physiological
indicators of drought, including relative water content, photosynthetic carbon
assimilation, as well as ABA and proline levels. Leaf tissue samples during a two
week drought period were collected daily, and sample from plants that had similar physiology for each pre-defined state were pooled for microarray analysis. Well-watered controls were also sampled each day. For each of the five physiological states analyzed, one microarray replicate consisted of leaf tissue pooled from six plants (18 leaves). Two replicates of each drought stage were analyzed on microarrays, and the resulting data were averaged. Expression ratios were generated by comparing each drought stage sample to an appropriate (age-matched) well-watered control.

Promoter candidates were selected based on the following criteria: 1) strong expression during at least moderate and severe stages of water deficit, and a return to well-watered baseline levels within 24 hr of re-watering, 2) low basal expression level in most non-stressed Arabidopsis tissues, and 3) similar drought inducibility for orthologous soybean genes, if these data were available.

**Example II. Drought-induction of Native Candidate Promoter Genes**

Drought-inducible promoter candidates were initially identified based on a wild-type baseline drought transcription profile (TxP) experiment. In assessing the effectiveness of drought-inducible-transcription factor combinations, it was essential to ensure that the drought imposed on plants during the water deficit treatment (we used a clay pot, soil-based drought assay) was sufficient to drive inducible gene expression via the promoters in a similar manner as observed in the original drought TxP experiment. The nine endogenous genes in Figure 3 showed strong induction upon drought treatment. These results confirmed that the clay pot soil-based drought assay was sufficient for water deficit-based induction of promoter candidate genes. Most of these genes are expressed at undetectable or extremely low levels in non-stressed plants. In most cases, however, there is considerably high expression in maturing seeds, due to the drought-like drying process inherent during this developmental stage. Additionally, several of the genes showed a response to ABA, osmotic, or cold treatment. Only one gene (AT3G46230) showed a response to heat treatment. This gene encodes a 17.4 kDa heat shock protein, so it is not surprising that in addition to drought induction, this gene also shows temperature regulation.

Drought stressed and well-watered pMen65 empty vector control plants were examined for induction of the candidate promoter genes on the x-axis of Figure 3. Plants were drought stressed to the wilting point and RT-PCR was performed using gene-specific primers for each of the genes indicated on the x-axis of Figure 3. Cycle threshold counts were normalized with 18S RNA.
Example III. Preparation of transgenic plants

Promoter cloning. For genes showing appropriate patterns of regulation, approximately 1.2 kb of upstream sequence were cloned by PCR (unless this region contained another gene, in which case the upstream sequence up to the next gene was cloned). Each promoter was cloned into an expression vector (vectors used in this study may include SEQ ID NOs: 10-54, and SEQ ID NO: 10-27, 33-36, 43-45, and 51-54 have been tested in plants) in front of either green fluorescent protein (GFP) or a polynucleotide encoding a transcription factor, such as SEQ ID NOs: 55, 57, 59, 61, or 59, that has been shown to provide increased tolerance to water deficit. In some of these cases, the transcription factors also produce deleterious morphological effects in the plants when they are constitutively overexpressed.

Transformation. Transformation of Arabidopsis was performed by an Agrobacterium-mediated protocol based on the method of Bechtold and Pelletier (1998) Methods Mol. Biol. 82: 259-266. Unless otherwise specified, all experimental work was performed using the Columbia ecotype.

Plant preparation. Arabidopsis seeds were sown on mesh covered pots. The seedlings were thinned so that 6-10 evenly spaced plants remained on each pot 10 days after planting. The primary bolts were cut off a week before transformation to break apical dominance and encourage auxiliary shoots to form. Transformation was typically performed at 4-5 weeks after sowing.

Bacterial culture preparation. Agrobacterium stocks were inoculated from single colony plates or from glycerol stocks and grown with the appropriate antibiotics and grown until saturation. On the morning of transformation, the saturated cultures were centrifuged and bacterial pellets are re-suspended in Infiltration Media (0.5X MS, 1X B5 Vitamins, 5% sucrose, 1 mg/ml benzylaminopurine riboside , 200 µl/L Silwet L77) until an A600 reading of 0.8 was reached.

Transformation and seed harvest. The Agrobacterium solution was poured into dipping containers. All flower buds and rosette leaves of the plants were immersed in this solution for 30 seconds. The plants were laid on their side and wrapped to keep the humidity high. The plants were kept this way overnight at 4 °C and then the pots were turned upright, unwrapped, and moved to the growth racks.

The plants were maintained on the growth rack under 24-hour light until seeds were ready to be harvested. Seeds were harvested when 80% of the siliques of the transformed plants were ripe (approximately 5 weeks after the initial transformation).
This seed was deemed T0 seed, since it was obtained from the T0 generation, and was later plated on selection plates (either kanamycin or sulfonamide). Resistant plants that were identified on such selection plates comprise the T1 generation.

For polynucleotides encoding transcription factors used in these experiments (SEQ ID NOs: 55, 57, 59, 61 or 63, encoding SEQ ID NOs: 56, 58, 60, 62 or 64, respectively), RT-PCR may be performed to confirm the ability of cloned promoter fragments to drive expression of the transcription factor transgene in plants transformed with the vectors.

T1 plants transformed with promoter-TF combinations listed in the Sequence Listing (those designated with a construct designation (SEQ ID NO: 10-27, 33-36, 43-45, and 51-54), were subjected to morphological analysis. Promoters that produced a substantial amelioration of the negative effects of TF overexpression were subjected to further analysis by propagation into the T2 generation, where the plants were analyzed for water deficit tolerance.

**Example IV. GFP fusion expression patterns**

While the majority of cloned promoter fragments were shown to have the necessary sequences to drive drought-inducible expression of RNA, it was unknown if the elements required for efficient protein translation during stress were also included in these constructs. To assess this, promoter-GFP fusions were used to measure visually the accumulation of GFP protein during and after water deficit treatment. All nine promoters were examined, and three promoters in particular, prATIG52690 (from a LEA protein), prAT5G52300 (from RD29B), and prAt5G43840 (from heat shock TF G1947), were found to drive high levels of detectable protein during water deficit stress.

The promoter from prATIG52690 (SEQ ID NO: 4) was reliably and strongly induced upon drought in multiple events. The expression level, as measured by GFP fluorescence, was stronger than that of either the constitutively expressed cauliflower mosaic (CaMV) 35S promoter or the RD29A stress-inducible promoter. Upon re-watering, there was a slight decrease in the expression level over time, but levels were still well-above background expression. In terms of tissue-specific expression, in addition to leaves, this promoter was found to drive expression in flowers, especially floral guard cells.

Promoter prAT5G52300 from AT5G52300 (RD29B; SEQ ID NO: 2), produced some variable penetrance, but lines could be obtained that produced
relatively stronger expression upon induction than either constitutive 35S or stress-inducible RD29A reference promoters. This indicates that the promoter from prAT5G52300 may be easily influenced by the insertion point in the genome. Expression from this promoter was persistent after re-watering, and, except for expression in leaves, no tissue-specific expression was noted.

Promoter prAt5G43840 from At5G43840 (heat shock TF G1947; SEQ ID NO: 6) also produced variably penetrant lines in terms of strong drought-inducibility, but again, strong lines could be isolated. The expression from this promoter was very slightly weakened after re-watering, and except for leaf tissue no tissue-specific expression was noted.

**Example V. Promoter-G481 fusion expression patterns**

These experiments, conducted with the G481 protein (SEQ ID NO: 56) illustrate the types of experiments that can be used to identify promoters that effectively induce water deficit-related protein expression.

In addition to characterizing the ability of these promoters to drive GFP protein expression, the drought-inducible accumulation of the G481 protein was also examined in lines transformed with three different promoter-G481 combinations, the promoters being prATIG52690 (SEQ ID NO: 4), prAT5G52300 (SEQ ID NO: 2), and prAT2G37870 (SEQ ID NO: 5). The first two of these promoters, prATIG52690 and prAT5G52300, had shown strong GFP induction when Arabidopsis plants were subjected to drought treatment. Protein from well-watered or drought-treated plants was subjected to gel electrophoresis and the gels were then probed with an antibody to the G481 protein. Coomassie blue staining was used to verify equivalent protein loading. G481 was found to accumulate to high levels upon water deficit treatment when expressed under the regulatory control of either prATIG52690 or prAT5G52300, similar to what was expected from GFP experiments in which it was shown that either of these promoters effectively induced G481 expression. The third promoter tested (G481 under the regulatory control of prAT2G37870), did not provide significant drought-inducible G481 protein expression. Again, this result is similar to what was observed with GFP experiments.

**Example VI. Soil-based water deficit assays**

The soil-based water deficit assays (also referred to as "drought assays") were performed in clay pots and were based on the procedure described by Haake et al. (2002) *Plant Physiol.* 130: 639-648.
Seeds were sterilized by a 2 minute ethanol treatment followed by 20 minutes in 30% bleach / 0.01% Tween and five washes in distilled water. Seeds were sown on MS agar in 0.1% agarose and stratified for 3 days at 4°C, before transfer to growth cabinets with a temperature of 22°C. After 7 days of growth on selection plates, seedlings were transplanted to 3.5 inch diameter clay pots containing 80g of a 50:50 mix of vermiculite:perlite topped with 80g of ProMix. Typically, each pot contained 14 seedlings, and plants of the transgenic line being tested were in separate pots to the wild-type controls. Pots containing the transgenic line versus control pots were interspersed in the growth room, maintained under 24-hour light conditions (18 - 23°C, and 90 - 100 µE m⁻² s⁻¹) and watered for a period of 14 days. Water was then withheld and pots were placed on absorbent paper for a period of 8-10 days. After this period, a visual qualitative "drought score" from 0-6 was assigned to record the extent of visible drought stress symptoms. A score of "6" corresponded to no visible symptoms whereas a score of "0" corresponded to extreme wilting and the leaves having a "crispy" texture. At the end of the drought period, pots were re-watered and scored after 5-6 days; the number of surviving plants in each pot was counted, and the proportion of the total plants in the pot that survived was calculated.

Analysis of results. Typically, 6 or more pots of a transgenic line were compared with 6 or more pots of the appropriate control plants. The mean drought score and mean proportion of plants surviving (survival rate) were calculated for both the transgenic line and the wild-type pots. In each case, a p-value that indicated the significance of the difference between the two mean values was calculated. The results for each transgenic line across each planting for a particular project were then presented in a results table.

Calculation of p-values. Survival was analyzed with a logistic regression to account for the fact that the random variable was a proportion between 0 and 1. The reported p-value was the significance of the experimental proportion contrasted to the control, based upon regressing the logit-transformed data.

Drought score, being an ordered factor with no real numeric meaning, was analyzed with a non-parametric test between the experimental and control groups. The p-value was calculated with a Mann-Whitney rank-sum test. Significance was indicated if the experimental line performed better or worse than controls at p < 0.11.
Example VII. Water deficit tolerance of transgenic plants transformed with transcription factors under the regulatory control of drought-inducible promoters

Generally, for the water deficit assays described in this example, three lines of overexpressors were tested, and the results are presented if they were determined to be statistically significant (p < 0.11). In a typical set of experiments, lines that were wild-type in appearance were chosen for soil drought assays, except as noted below.

G481 (SEQ ID NOS: polynucleotide 55 and polypeptide 56)

G481 is a CAAT family transcription factor sequence that has been shown to confer improved drought tolerance when constitutively expressed. However, unwelcome morphological or physical characteristics may be associated with constitutive overexpression of G481 (for example, late flowering). It is believed that a drought-inducible promoter regulating G481 expression may provide drought tolerance in plants, as well as a normal morphology and development.

Lines for each promoter-G481 combination generally appeared wild-type, although some lines showed changes in flowering time, possibly indicating low level background expression of G481.

In general, many of the drought inducible promoter-G481 combinations performed no better or worse than controls in water deficit experiments. However, some lines for five of the constructs tested (prAT5G15240, prG1947, prAT5G66780, prAT3G46230, and prAT2G37870) were shown to be more tolerant to water deficit than controls on at least one of two plant dates, as noted below.

One line of prAT5G15240::G481 overexpressors recovered from water deficit treatment better than controls in one of two runs of the assay. No obvious induction of the G481 transgene was evident in an RT-PCR experiment.

One line of prG1947::G481 overexpressors was more tolerant to water deficit treatment in one of two runs of the assay. Drought induction of the G481 transgene was seen in the RT-PCR experiment.

One line of prAT3G46230::G481 overexpressors recovered from water deficit treatment better than controls in one of two runs of the assay. Very mild drought induction of the G481 transgene was seen in the RT-PCR experiment.

One line of prAT5G66780::G481 overexpressors was statistically more tolerant to water deficit treatment in one of two runs and a separate planting of this
line recovered from water deficit treatment better than controls. Drought induction of the G481 transgene was seen in the RT-PCR experiment

Two lines of prAT2G37870::G481 overexpressors demonstrated a statistically better performance in drought assays than controls on separate planting dates. For the first line, plants from one of the planting dates were both more tolerant to water deficit than controls and recovered better than controls. For the second line, plants in two separate experiments were more tolerant to water deficit than controls and/or recovered better from the treatment than controls. Drought induction of the G481 transgene has not been examined by RT-PCR.

G1073 (SEQ ID NOs: polynucleotide 57 and polypeptide 58)

G1073 is a member of the AT-hook family transcription factors and has been shown to confer improved drought tolerance when constitutively expressed. However, unwelcome morphological or physical characteristics such as large size (which may be a disadvantage under some circumstances) may be associated with constitutive overexpression of G1073. It is believed that a drought-inducible promoter regulating G1073 expression may provide drought tolerance in plants, as well as a more normal morphology and development.

T2 lines tested in clay pot drought assays for the different promoter-G1073 combinations generally appeared wild-type. However, in the T1 generation, modest alterations in flowering time and size were observed for some of the constructs, possibly indicating low level background expression of G1073.

In general, many of the drought inducible promoter-G1073 combinations performed no better or worse than controls in water deficit experiments. However, a number of individual lines for five of the constructs did show a better performance than controls on at least one of two plant dates (prAT5G15240, prAT5G66780, prG1947, prAT3G17520 and prAT5G52300).

One line of prAT5G15240::G1073 overexpressors recovered better from a water deficit treatment in one of two runs of the assay. No induction of the G1073 transgene was observed in RT-PCR experiments.

One line of prAT5G66780::G1073 overexpressors recovered better from a water deficit treatment in one of two runs of the assay. Induction of the G1073 transgene by the drought treatment was confirmed by RT-PCR.

One line of prG1947::G1073 overexpressors performed better than controls in the water deficit tolerance and in their recovery from the treatment in one of two runs.
of the assay. Induction of the G1073 transgene by the drought treatment was confirmed by RT-PCR.

One line of prAT3G17520::G1073 overexpressors recovered from water deficit better than controls in two separate experiments. Induction of the G1073 transgene by the drought treatment has been confirmed.

One line of prAT5G52300::G1073 overexpressors recovered from water deficit better than controls in one of two runs of the assay. Induction of the G1073 transgene by the drought treatment has been confirmed.

**G1274 (SEQ ID NOs: polynucleotide 59 and polypeptide 60)**

G1274 is a member of the WRKY family of transcription factors and has been shown to confer improved drought tolerance when constitutively expressed. However, unwelcome morphological or physical characteristics such as effects on size (both small and large plants have been observed, both of which may be disadvantageous under some circumstances) may be associated with constitutive overexpression of G1274. It is believed that a drought-inducible promoter regulating G1274 expression may provide drought tolerance in plants with more normal morphology and development.

Overall, the drought-inducible promoter-G1274 lines did not show any consistent differences from wild-type morphology, with the exception of some inconsistent variation in flowering time. Besides prAT5G15240, all other promoters were confirmed to produce drought-induced G1274 transcript, as measured by RT-PCR.

In general, many of the lines transformed with G1274 under the regulatory control of a drought-inducible promoter performed no better or even worse than controls in drought assays. However, a number of individual lines for three of the five constructs tested, prAT5G66780, prAT3G17520 and prAT4G09600 did show a better performance than controls in water deficit experiments on one of two plant dates, as noted below.

One line of prAT5G66780::G1274 overexpressors recovered from water deficit treatment better than controls in one of two runs of the assay. Induction of the G1274 transgene by the drought treatment was confirmed by RT-PCR.

In one of two runs of a water deficit assay, one of the prAT3G17520::G1274 lines of overexpressors recovered from water deficit better than controls. Induction of the G1274 transgene by the drought treatment was confirmed by RT-PCR.
One line of prAT4G09600::G1274 overexpressors also recovered from water deficit treatment better than controls in one of two runs of the assay. Induction of the G1274 transgene by the drought treatment was confirmed by RT-PCR.

**G1792 (SEQ ID NOs: polynucleotide 61 and polypeptide 62)**

G1792 is a member of the AP2 family transcription factors and has been shown to confer improved drought tolerance when constitutively expressed. However, unwelcome morphological or physical characteristics such as small size and reduced fertility may be associated with constitutive overexpression of G1792. It is believed that a drought-inducible promoter regulating G1792 expression may provide drought tolerance in plants with more normal morphology and development.

Lines for each promoter-G1792 combination generally appeared wild-type, although a minority of the T1 plants for each promoter showed dark green and/or late flowering phenotypes, indicating leaky expression of G1792 in these lines.

Lines for five different drought inducible promoters directly fused to G1792 have been through two runs of the soil drought clay pot assay. These drought inducible promoter combinations did not produce compelling results with G1792, and many lines performed the same, or worse, than controls in this water deficit assay. However, one line for one of the five constructs did show a better performance than controls on one of two plant dates, as noted below.

One line of prAT4G09600::G1792 overexpressors recovered from the water deficit treatment better than controls in one of two runs of the assay. Drought induction of the G1792 transgene was seen in the RT-PCR experiment.

**G47 (SEQ ID NOs: polynucleotide 63 and polypeptide 64)**

G47 is a member of the AP2 family transcription factors and has been shown to confer improved drought tolerance when constitutively expressed. However, unwelcome morphological or physical characteristics such as small size and reduced fertility may be associated with constitutive overexpression of G47. It is believed that a drought-inducible promoter regulating G47 expression may provide drought tolerance in plants with more normal morphology and development.

Two of the promoter-G47 combinations tested, prAT5G66780, prG1947, resulted in plants that were somewhat late developing, possible indicating leaky expression of G47 in these lines.

Lines for five different drought inducible promoters directly fused to G47 were tested in two runs of the soil drought clay pot assay. A number of individual
lines for four of the five constructs, comprising prAT5G15240, prAT3G17520, prAT4G09600 and prG1947, showed a better performance than controls on at least one of two plant dates, as noted below.

One line of prAT5G15240::G47 overexpressors recovered better from a water deficit treatment, and another line was visibly more tolerant and recovered better from the water deficit treatment than control plants in one of two runs of the assay. Drought-based induction of the G47 transgene was not confirmed by RT-PCR.

One line of prAT3G17520::G47 overexpressors recovered better from a water deficit treatment than controls in one of two runs of the assay. Induction of the G47 transgene by the drought treatment was confirmed by RT-PCR.

One line of prAT4G09600::G47 overexpressors recovered better from a water deficit treatment than controls in one of two runs of the assay. A second line was visibly more tolerant to water deficit (observed in one run of the assay) and recovered better from the treatment than control plants (observed in both runs of the assay).

Induction of the G47 transgene by the drought treatment was confirmed by RT-PCR.

One line of prG1947::G47 overexpressors recovered better from a water deficit treatment than controls in one of two runs of the assay. A second line was visibly more tolerant to water deficit and recovered better from the treatment than control plants in one of the two runs of the assay. Induction of the G47 transgene by the drought treatment was confirmed by RT-PCR in the former of these lines but did not confirm induction in the latter.

**Example VIII. Regulating expression of polynucleotides encoding RNA species which act at a non-protein level**

In addition to use of the water deficit inducible promoters to regulate the expression of a polynucleotide encoding a polypeptide, these promoters can also be used to regulate the expression of a polynucleotide encoding a non-coding RNA species (that is, one which acts at a non-protein level), such as a microRNA, a microRNA precursor, or a sequence designed to act through RNA interference (RNAi). For example, a substantial number of microRNA (miRNA) species have been implicated in stress responses and these molecules have been shown to be involved in the control of many aspects of plant growth and development (Bartel and Bartel (2003) *Plant Physiol.* 132: 709-717; Aukerman and Sakai (2003). *Plant Cell* 15:; 2730-2741; Bartel (2004) *Cell* 116: 281-297; Juarez et al. (2004) *Nature* 428: 84-
It should be noted that, for particular families of highly related plant transcription factors, overexpression of one or more of the family members produces a comparable phenotype to that obtained from reducing expression (for example, by mutation or knockdown approaches such as antisense or RNA interference) of one or more of the family members. For instance, overexpression of the CBF family proteins has been widely demonstrated to confer tolerance to drought and low temperature stress (e.g., Jaglo et al. (2001) *Plant Physiol.* 127: 910-917). Nonetheless, Novillo et al. (2004) *Proc. Natl. Acad. ScL USA* 101:, 3985-3990, showed that homozygous *cbf2* mutant *Arabidopsis* plants carrying a disruption in the CBF2 gene also exhibit enhanced freezing tolerance. Such results can be accounted for by cross regulation between the genes encoding different transcription factor family members. In the study by Novillo et al, (2004) supra, CBF2 was shown to be a negative transcriptional regulator of the *CBFl* and *CBF3* genes. Comparable mechanisms likely account for the fact that we have observed stress tolerance from both overexpression and from knockdown approaches with certain NF-Y family genes.

We have shown using a 35S promoter that overexpression of precursors for miRNA169 (SEQ ID NOs: 71, 72, 73 or 74), which targets NF-YA (HAP2 class transcription factor genes; Bartel and Bartel, supra; Jones-Rhoades and Bartel (2004) *Mol. Cell* 14: 787-799) produces tolerance to dehydration and osmotic stress, but this is often accompanied by developmental changes such as alterations in flowering time or reduced size. Expression of miRNA169 using a drought inducible promoter is therefore expected to produce water deficit tolerance without undesirable effects on development. We have obtained similar results from overexpression of a polynucleotide (P21305, SEQ ID NO: 66) designed to effect RNA interference on Non-LECl-like NF-YB proteins; The RNAi construct P21305 (carrying KanR) targets the G481 clade (this clade is comprised of sequences that are closely and evolutionarily related to G481, SEQ ID NO: 55 encoding polypeptide SEQ ID NO: 56). It contains two fragments each comprised of G481 clade sequences from G2345, SEQ ID NO: 67 (base pairs 185-315 from the start codon; this fragment is represented by SEQ ID NO: 69) and G485 SEQ ID NO: 68 (base pairs 61-170 from the start codon, this fragment is represented by SEQ ID NO: 70). A number of bases were
mutated in order to increase the percentage homology with the other clade members. The bases that appear as capital letters in the two sequence fragments listed below indicate positions where the point mutations were introduced in the cloning primers to increase the percentage homology with other clade members.

G2345 fragment (bases 185-315), SEQ ID NO: 69
aggaatgTgtctctgaAttcatcagcttcacctgactagtgataactgctccaaagagagaAaggaagaccat
caatggagatgatttgttgtgcatgtgccaactttaggttCgaAgattac

G485 fragment (bases 61-170), SEQ ID NO: 70
gagcaagataggcctAcggatcgtaacggactgcatgaagaaacacttcttgcaacgcaaatctctaa
GgatgcTaaagaAacggttcaagagtgtgt

The fragments are expected to form a hairpin structure as follows:

35S::sense_fragment(G2345-G485)::pdkintron::antisense_fragment(G2345-G485). Transgenic Arabidopsis lines overexpressing P21305 showed improved tolerance to water deficit and heat stress, but exhibited developmental abnormalities and changes in flowering time. Expression of such a polynucleotide using a drought inducible promoter is therefore expected to produce stress tolerance without undesirable effects on development and/or morphology.

**Example IX. Transformation of dicots to produce increased water deficit stress tolerance**

Crop species including tomato and soybean plants that overexpress transcription factor polypeptides that confer increased tolerance to water deficit, including dehydration, desiccation, drought or another hyperosmotic stress such as high salt or sugar concentrations, may produce plants with increased water deficit tolerance when placed under the regulatory control of water deficit-inducible promoters of the invention. These observations indicate that these genes, when overexpressed, will result in improved quality and larger yields than non-transformed plants in stressed conditions, which may occur in the field to even a low, imperceptible degree at any time in the growing season.

Thus, promoter sequences listed in the Sequence Listing recombined into, for example, one of the expression vectors of the invention, or another suitable expression vector, may be transformed into a plant for the purpose of regulating water response
sequences and modifying plant traits for the purpose of improving yield and/or quality. The cloning vector may be introduced into a variety of plants by means well known in the art such as, for example, direct DNA transfer or Agrobacterium *tumefaciens-mediated* transformation. It is now routine to produce transgenic plants using most dicot plants (see Weissbach and Weissbach, (1989) *Methods for Plant Molecular Biology*, Academic Press; Gelvin et al. (1990) *Plant Molecular Biology Manual*, Kluwer Academic Publishers; Herrera-Estrella et al. (1983) *Nature* 303: 209; Bevan (1984) *Nucleic Acids Res.* 12: 8711-8721; and Klee (1985) *Bio/Technology* 3: 637-642). Methods for analysis of traits are routine in the art and examples are disclosed above.

Numerous protocols for the transformation of tomato and soy plants have been previously described, and are well known in the art. Gruber et al. (1993) in *Methods in Plant Molecular Biology and Biotechnology*, p. 89-119, and Glick and Thompson ((1993) *Methods in Plant Molecular Biology and Biotechnology*, CRC Press., Boca Raton, FL) describe several expression vectors and culture methods that may be used for cell or tissue transformation and subsequent regeneration. For soybean transformation, methods are described by Miki et al. (1993) in *Methods in Plant Molecular Biology and Biotechnology*, p. 67-88, Glick and Thompson, eds., CRC Press, Inc., Boca Raton; and U.S. Pat. No. 5,563,055, (Townsend and Thomas), issued Oct. 8, 1996.


Alternatively, sonication methods (see, for example, Zhang et al. (1991) *Bio/Technology* 9: 996-997); direct uptake of DNA into protoplasts using CaCl2 precipitation, polyvinyl alcohol or poly-L-ornithine (Hain et al. (1985) *Mol. Gen. Genet.* 199: 161-168; Draper et al. (1982) *Plant Cell Physiol.* 23: 451-458); liposome or spheroplast fusion (see, for example, Deshayes et al. (1985) *EMBO J.* 4: 2731-
2737; Christou et al. (1987) *Proc. Natl. Acad. Sci. USA* 84: 3962-3966; and
electroporation of protoplasts and whole cells and tissues (see, for example, Donn et
al.(1990) in Abstracts of VIIth International Congress on Plant Cell and Tissue
Culture IAPTC, A2-38: 53; D'Halluin et al. (1992) *Plant Cell* 4: 1495-1505; and
Spencer et al. (1994) *Plant Mol. Biol.* 24: 51-61) have been used to introduce foreign
DNA and expression vectors into plants.

After a plant or plant cell is transformed (and the latter regenerated into a
plant), the transformed plant may be crossed with itself or a plant from the same line,
a non-transformed or wild-type plant, or another transformed plant from a different
transgenic line of plants. Crossing provides the advantages of producing new and
often stable transgenic varieties. Genes and the traits they confer that have been
introduced into a tomato or soybean line may be moved into distinct line of plants
using traditional backcrossing techniques well known in the art. Transformation of
tomato plants may be conducted using the protocols of Koornneef et al (1986) in

*Tomato Biotechnology*; Alan R. Liss, Inc., 169-178, and in U.S. Patent 6,613,962, the
latter method described in brief here. Eight day old cotyledon explants are precultured
for 24 hours in Petri dishes containing a feeder layer of Petunia hybrida suspension
cells plated on MS medium with 2% (w/v) sucrose and 0.8% agar supplemented with
10 µM α-naphthalene acetic acid and 4.4 µM 6-benzylaminopurine. The explants are
then infected with a diluted overnight culture of Agrobacterium tumefaciens
containing an expression vector comprising a polynucleotide of the invention for 5-10
minutes, blotted dry on sterile filter paper and cocultured for 48 hours on the original
feeder layer plates. Culture conditions are as described above. Overnight cultures of
*Agrobacterium tumefaciens* are diluted in liquid MS medium with 2% (w/v) sucrose,
pH 5.7) to an OD600 of 0.8.

Following cocultivation, the cotyledon explants are transferred to Petri dishes
with selective medium comprising MS medium with 4.56 µM zeatin, 67.3 µM
vancomycin, 418.9 µM cefotaxime and 171.6 µM kanamycin sulfate, and cultured
under the culture conditions described above. The explants are subcultured every
three weeks onto fresh medium. Emerging shoots are dissected from the underlying
callus and transferred to glass jars with selective medium without zeatin to form roots.
The formation of roots in a kanamycin sulfate-containing medium is a positive
indication of a successful transformation.
Transformation of soybean plants may be conducted using the methods found in, for example, U.S. Patent 5,563,055 (Townsend et al., issued October 8, 1996), described in brief here. In this method soybean seed is surface sterilized by exposure to chlorine gas evolved in a glass bell jar. Seeds are germinated by plating on 1/10 strength agar solidified medium without plant growth regulators and culturing at 28° C. with a 16 hour day length. After three or four days, seed may be prepared for cocultivation. The seedcoat is removed and the elongating radicle removed 3-4 mm below the cotyledons.

Overnight cultures of Agrobacterium tumefaciens harboring the expression vector comprising a polynucleotide of the invention are grown to log phase, pooled, and concentrated by centrifugation. Inoculations are conducted in batches such that each plate of seed was treated with a newly resuspended pellet of Agrobacterium. The pellets are resuspended in 20 ml inoculation medium. The inoculum is poured into a Petri dish containing prepared seed and the cotyledonary nodes are macerated with a surgical blade. After 30 minutes the explants are transferred to plates of the same medium that has been solidified. Explants are embedded with the adaxial side up and level with the surface of the medium and cultured at 22° C. for three days under white fluorescent light. These plants may then be regenerated according to methods well established in the art, such as by moving the explants after three days to a liquid counter-selection medium (see U.S. Patent 5,563,055).

The explants may then be picked, embedded and cultured in solidified selection medium. After one month on selective media transformed tissue becomes visible as green sectors of regenerating tissue against a background of bleached, less healthy tissue. Explants with green sectors are transferred to an elongation medium. Culture is continued on this medium with transfers to fresh plates every two weeks. When shoots are 0.5 cm in length they may be excised at the base and placed in a rooting medium.

**Example X: Transformation of monocots to produce increased water deficit stress tolerance**

Cereal plants and other grasses such as, but not limited to, corn, wheat, rice, sorghum, barley, Miscanthus, and switchgrass may be transformed with the present promoter sequences such as those presented in the present Sequence Listing, cloned into a vector such as pGA643 and containing a kanamycin-resistance marker, and inducibly express, for example, a transcription factor that confers improved water
deficit tolerance. The expression vectors may be one found in the Sequence Listing, or any other suitable expression vector that incorporates a promoter sequence of the invention, may be similarly used. For example, pMEN020 may be modified to replace the NptII coding region with the BAR gene of Streptomyces hygroscopicus that
5 confers resistance to phosphinothricin. The KpnI and BglII sites of the Bar gene are removed by site-directed mutagenesis with silent codon changes.

The cloning vector may be introduced into a variety of cereal plants by means well known in the art including direct DNA transfer or Agrobacterium tumefaciens-mediated transformation. The latter approach may be accomplished by a variety of means, including, for example, that of U.S. Patent No. 5,591,616, in which
10 monocotyledon callus is transformed by contacting dedifferentiating tissue with the Agrobacterium containing the cloning vector.

The sample tissues are immersed in a suspension of 3x10-9 cells of Agrobacterium containing the cloning vector for 3-10 minutes. The callus material is cultured on solid medium at 25°C in the dark for several days. The calli grown on this medium are transferred to Regeneration medium. Transfers are continued every 2-3 weeks (2 or 3 times) until shoots develop. Shoots are then transferred to Shoot-
Elongation medium every 2-3 weeks. Healthy looking shoots are transferred to rooting medium and after roots have developed, the plants are placed into moist potting soil.

The transformed plants are then analyzed for the presence of the NPTII gene/kanamycin resistance by ELISA, using the ELISA NPTII kit from 5Prime-3Prime Inc. (Boulder, CO).

cells derived from immature scutellum tissues are the preferred cellular targets for
transformation (Hiei et al. (1997) supra; Vasil (1994) supra). For transforming corn
embryogenic cells derived from immature scutellar tissue using microprojectile
bombardment, the A188XB73 genotype is the preferred genotype (Fromm et al.
(1990) supra; Gordon-Kamm et al. (1990) supra). After microprojectile bombardment
the tissues are selected on phosphinothricin to identify the transgenic embryogenic
cells (Gordon-Kamm et al. (1990) supra). Transgenic plants are regenerated by
standard corn regeneration techniques (Fromm et al. (1990) supra; Gordon-Kamm et
al. (1990) supra). Agrobacterium-mediated transformation of switchgrass has also

Example XI: Transcription factor expression and analysis of water deficit stress
tolerance

Northern blot analysis, RT-PCR or microarray analysis of the regenerated,
transformed plants may be used to show expression of a transcription factor
polypeptide of the invention and related genes that are capable of inducing improved
water deficit stress tolerance as compared to a control plant.

To verify the ability to confer increased water deficit tolerance, mature plants
overexpressing a transcription factor under the regulatory control of a water deficit-
inducible promoter of the invention, or alternatively, seedling progeny of these plants,
may be challenged by a stress such as a dehydration, drought, desiccation, or a related
hyperosmotic stress tolerance such as salt or mannitol. Alternatively, these plants may
challenged in a hyperosmotic stress condition that may also measure altered sugar
sensing, such as a high sugar (e.g., sucrose) condition. By comparing control plants
(for example, wild type or parental line untransformed plants, or plants transformed
with an empty vector or one lacking the transcription factor) and transgenic plants
similarly treated, the transgenic plants may be shown to have greater tolerance to the
particular water deficit-related stress.

After a dicot plant, monocot plant or plant cell has been transformed (and the
latter regenerated into a plant) and shown to have greater size or tolerance to water
deficit-related stress, or produce greater yield or quality relative to a control plant
under the stress conditions, the transformed monocot plant may be crossed with itself
or a plant from the same line, a non-transformed or wild-type monocot plant, or
another transformed monocot plant from a different transgenic line of plants.
These experiments would demonstrate that transcription factor polypeptides of the invention can be identified and shown to confer greater water deficit-related stress tolerance, greater yield, or greater quality in dicots or monocots, including tolerance to more than one water deficit-related stresses.

5 Example XII: Sequences that Confer Significant Improvements to non-
Arabidomis species

The function of promoter sequences of the invention has been analyzed and may be further characterized and the sequences may be incorporated into crop plants. The ectopic overexpression of transcription factor sequences, or any other sequence that may confer increased tolerance to water-deficit related stress (e.g., to drought, desiccation, dehydration and/or other hyperosmotic stress) may be regulated using regulatory elements of the invention. In addition to these sequences, it is expected that newly discovered polynucleotide sequences from, for example, other species having similar sequences, may be closely related to polynucleotide sequences found in the

Sequence Listing can also confer improved water deficit tolerance in a similar manner to the sequences found in the Sequence Listing, when transformed into a any of a considerable variety of plants of different species, and including dicots and monocots. The polynucleotide and polypeptide sequences derived from monocots (e.g., the rice sequences) may be used to transform both monocot and dicot plants, and those derived from dicots (e.g., the Arabidopsis and soy genes) may be used to transform either group, although some of these sequences may function best if the gene is transformed into a plant from the same class as that from which the sequence is derived.

The results presented in the Examples above indicate that proteins such as transcription factors that confer improved water deficit tolerance may do so when they are overexpressed under the regulatory control of a promoter sequence of the invention, without having a significant adverse impact on plant morphology and/or development. The lines that display useful traits may be selected for further study or commercial development.

Monocotyledonous plants, including rice, corn, wheat, rye, sorghum, barley and others, may be transformed with a plasmid containing a transcription factor polynucleotide. The transcription factor gene sequence may include dicot or monocot-derived sequences such as those presented herein. These transcription factor genes may be cloned into an expression vector containing a kanamycin-resistance marker,
and then expressed in an inducible manner under the regulatory control of a promoter sequence of the invention.

The cloning vector may be introduced into monocots by, for example, means described in the previous Example, including direct DNA transfer or Agrobacterium tumefaciens-mediated transformation. The latter approach may be accomplished by a variety of means, including, for example, that of U.S. Patent No. 5,591,616, in which monocotyledon callus is transformed by contacting dedifferentiating tissue with the Agrobacterium containing the cloning vector.

The sample tissues are immersed in a suspension of 3x10-9 cells of Agrobacterium containing the cloning vector for 3-10 minutes. The callus material is cultured on solid medium at 25° C in the dark for several days. The calli grown on this medium are transferred to Regeneration medium. Transfers are continued every 2-3 weeks (2 or 3 times) until shoots develop. Shoots are then transferred to Shoot-Elongation medium every 2-3 weeks. Healthy looking shoots are transferred to rooting medium and after roots have developed, the plants are placed into moist potting soil.

The transformed plants are then analyzed for the presence of the NPTII gene/kanamycin resistance by ELISA, using the ELISA NPTII kit from 5Prime-3Prime Inc. (Boulder, CO).

Northern blot analysis, RT-PCR or microarray analysis of the regenerated, transformed plants may be used to show expression of a transcription factor polypeptide of the invention that is capable of conferring improved water deficit-related stress tolerance, or increased yield or quality, in the transformed plants.

To verify the ability to confer improved water deficit-related stress tolerance, mature plants or seedling progeny of these plants expressing a monocot-derived equivalog gene may be challenged using methods described herein. By comparing control plants and the transgenic plants, the latter are shown to be more tolerant to one or more water deficit-related stresses such as drought, dehydration, desiccation, or other hyperosmotic stress, as compared to control plants similarly treated. As an example of a first step to determine water deficit-related tolerance, seeds of transgenic plants may be subjected to germination assays to measure sucrose sensing. For example, sterile dicot seeds including, but not limited to soybean and alfalfa, are sown on 80% MS medium plus vitamins with 9.4% sucrose; control media lack sucrose. All assay plates are then incubated at 22° C under 24-hour light, 120-130 µEin/m2/s, in a growth
chamber. Evaluation of germination and seedling vigor is then conducted three days after planting. Plants overexpressing proteins that confer improved tolerance to water deficit, where the proteins are under the regulatory control of promoters of the invention, may be found to be more tolerant to high sucrose by having better germination, longer radicles, and more cotyledon expansion, than control plants in the presence of the sugar concentration. It is expected that closely related and structurally similar promoter sequences, may also confer altered sugar sensing or improved hyperosmotic stress tolerance.

Plants overexpressing proteins that confer increased tolerance to water deficit, where the proteins are under the regulatory control of the promoter sequences of the invention, may also be subjected to soil-based drought assays to identify those lines that are more tolerant to water deprivation than control plants. For example, drought experiments in a greenhouse may be conducted. Pre-germinated seedlings of transgenic plants (progeny of a heterozygous transgenic plant that inherit the exogenous transcription factor DNA construct) and wild type plants (progeny of a heterozygous transgenic plant that inherit the exogenous transcription factor DNA construct) are planted in soil. The plants are well watered for one week and then allowed to dry for 4 days. An equal number of transgenic and wild type plants are selected based on matched height. A drought assay is then started by measuring plant heights and resuming daily watering for "wet" pots. "Dry" pots are generated by maintaining the average "dry" pot weight (e.g., about 400 g) well below that of the "wet" pots (e.g., about 500 g or more); water is added to the "dry" pots when necessary to maintain the "dry" pots at around the average "dry" pot weight. The height of transgenic plants and controls are measured for nine days, after which full watering is resumed for the "dry" flat pots for three days, after which heights are again measured. Recovered plants may be subjected to a second round of drought as described above. A number of the lines of plants transformed with sequences of the invention will be significantly larger and greener, with less wilting or desiccation, than control plants, particularly after a period of water deficit is followed by rewatering and a subsequent incubation period. Unlike plants constitutively overexpressing the proteins that confer increased tolerance to water deficit, transgenic plants overexpressing these proteins under the regulatory control of the water deficit-inducible promoters described herein will be morphologically and developmentally
similar to control plants such as wild type or plants transformed with an "empty" vector.

It is expected that the same methods may be applied to identify other useful and valuable promoter sequences, and the sequences may be derived from a diverse range of species.
We claim:

1. An expression vector comprising a water deficit-inducible promoter comprising any of SEQ ID NOs: 1 to 9 or a functional part thereof, wherein the functional part includes a promoter function.

2. The expression vector of Claim 1, wherein the expression vector comprises any of SEQ ID NOs: 10 to 54.

3. The expression vector of Claim 1, wherein the expression vector comprises an RNA polymerase binding site located 5' relative to and operably linked to a coding sequence encoding a polypeptide that confers increased tolerance to water deficit conditions.

4. The expression vector of Claim 1, wherein the water deficit-inducible promoter regulates expression of a polynucleotide encoding a polypeptide that confers increased tolerance to water deficit conditions.

5. The expression vector of Claim 4, wherein the polypeptide is a transcription factor.

6. The expression vector of Claim 5, wherein the transcription factor is selected from the group consisting of SEQ ID NOs: 56, 58, 60, 62 and 64.

7. A host plant cell comprising a water deficit-inducible promoter comprising any of SEQ ID NOs: 1 to 9 or a functional part thereof, wherein the functional part includes a promoter function.

8. The host plant cell of Claim 7, wherein the expression vector comprises any of SEQ ID NOs: 10 to 54.

9. The host plant cell of Claim 7, wherein the expression vector comprises an RNA polymerase binding site located 5' relative to and operably linked to a coding sequence encoding a polypeptide that confers increased tolerance to water deficit conditions.

10. The host plant cell of Claim 7, wherein the water deficit-inducible promoter regulates expression of a polynucleotide encoding a polypeptide that confers increased tolerance to water deficit conditions.

11. The host plant cell of Claim 10, wherein the polypeptide is a transcription factor.

12. The host plant cell of Claim 11, wherein the transcription factor is selected from the group consisting of SEQ ID NOs: 56, 58, 60, 62 and 64.
13. A transgenic plant comprising an expression vector comprising a water deficit-inducible promoter comprising any of SEQ ID NOs: 1-9 or a functional part thereof, wherein the functional part includes a promoter function.

14. The transgenic plant of Claim 13, wherein the expression vector comprises a recombinant polynucleotide that comprises an RNA polymerase binding site located 5' relative to and operably linked to a coding sequence encoding a polypeptide that confers increased tolerance to water deficit conditions.

15. The transgenic plant of Claim 13, wherein the expression vector comprises a water deficit-inducible promoter that regulates expression of a polynucleotide encoding a polypeptide that confers increased tolerance to water deficit conditions.

16. The transgenic plant of Claim 15, wherein the polypeptide is a transcription factor.

17. The transgenic plant of Claim 16, wherein the transcription factor is selected from the group consisting of SEQ ID NOs: 56, 58, 60, 62 and 64.

18. The transgenic plant of Claim 13, wherein the expression vector comprises any of SEQ ID NOs: 8 to 26.

19. The transgenic plant of Claim 13, wherein the transgenic plant has greater tolerance to water deficit conditions than a control plant.


21. A method for producing a transgenic plant having greater tolerance to water deficit conditions than a control plant, the method steps including:

(a) generating an expression vector comprising:

(i) a promoter sequence comprising any of SEQ ID NOs: 1-9 or a functional part thereof, wherein the functional part includes a promoter function; and

(ii) a nucleotide sequence that encodes a polypeptide that increases the water deficit tolerance in the transgenic plant;

wherein the promoter sequence is operably linked to the nucleotide sequence that encodes the polypeptide, and during water deficit conditions the promoter sequence drives the expression of the nucleotide sequence that encodes the polypeptide; and

(b) transforming a target plant with the expression vector to produce the transgenic plant;
wherein when the polypeptide is overexpressed in the transgenic plant, the transgenic plant has greater tolerance to water deficit conditions than the control plant.

22. The method of Claim 21, wherein the transgenic plant comprises any of SEQ ID NOs: 10-54.

23. The method of Claim 21, wherein the promoter sequence comprises an RNA polymerase binding site located 5' relative to and operably linked to a coding sequence encoding the polypeptide that regulates tolerance to water deficit in a plant.

24. The method of Claim 21, wherein the polypeptide is a transcription factor.

25. The method of Claim 24, wherein the transcription factor is selected from the group consisting of SEQ ID NOs: 56, 58, 60, 62 and 64.

26. The method of Claim 21, the method steps further including:
   (c) crossing the transgenic plant with itself, a plant from the same line as the transgenic plant, a non-transgenic plant, a wild-type plant, or another transgenic plant from a different transgenic line of plants, to produce a transgenic seed.

27. A method for increasing a plant's tolerance to water deficit conditions in relation to the tolerance to water deficit conditions of a control plant, the method steps including:
   (a) generating an expression vector comprising:
      (i) a promoter sequence comprising any of SEQ ID NOs: 1-9 or a functional part thereof, wherein the functional part includes a promoter function; and
      (ii) a nucleotide sequence that encodes a polypeptide that increases the water deficit tolerance in the transgenic plant;
      wherein the promoter sequence is operably linked to the nucleotide sequence that encodes the polypeptide, and during water deficit conditions the promoter sequence drives the expression of the nucleotide sequence that encodes the polypeptide; and
   (b) transforming a target plant with the expression vector to produce the transgenic plant;
wherein when the polypeptide is overexpressed in the transgenic plant, 
the transgenic plant has greater tolerance to water deficit conditions than 
the control plant.

28. An expression vector comprising a water deficit-inducible promoter 
comprising any of SEQ ID NOs: 1 to 9 or a functional part thereof, wherein 
the functional part includes a promoter function;

wherein the water deficit-inducible promoter controls the expression of a non-coding RNA species that regulates transcription in a plant;

wherein said regulation of transcription by said non-coding RNA species results in said plant being more tolerant to water deficit than a control plant; 
and

said plant is morphologically similar and/or developmentally similar to the control plant.

29. The expression vector of Claim 28, wherein the non-coding RNA species is an interference RNA (RNAi) or a microRNA (miRNA).

30. A transgenic plant comprising the expression vector of Claim 28.
Fig. 2

- Asterida I
  - Solanaceae (tomato, pepper, eggplant)
  - Cucurbitaceae (melon)
  - Leguminosae (alfalfa, bean)
  - Rosaceae (peach, cherry, apple)
  - Salicaceae (poplar)
  - Malvaceae (cotton, Sterculiaceae (cocoa))
  - Brassicaceae (Arabidopsis)
  - Chenopodiaceae (sugarbeet, spinach)
  - Rosidae
  - Hamamelida II
  - Hamamelid II
  - Rosidae
  - Carophyllidae

- Rosidae
  - Ranunculidae
  - Magnoliidae
  - Monocots
  - Grasses (maize, wheat, rice)
  - Musaceae (banana)

- Coniferidae
  - Pinaceae (pine)
  - Ginkgo
  - Cycads
  - Ferns, mosses and other non-seed plants
  - Chlorophyta "algae"