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(54) Title: MODULATION OF ABSCISIC ACID

(57) Abstract: Compositions and methods for modulating abscisic acid (ABA) perception and signal transduction in developing seed are provided. The methods and compositions find use in increasing yield in plants. Compositions comprise genetic constructs known to affect ABA sensitivity, particularly ABA biosynthetic mutants and fragments and variants thereof. Such compositions can be expressed with seed-preferred promoters.

MODULATION OF ABSCISIC ACID

FIELD OF THE INVENTION

The present invention is drawn to methods for the genetic modification of plants, particularly for modulating plant response to abscisic acid.

BACKGROUND OF THE INVENTION

Abscisic acid (ABA) is a phytohormone that plays an essential regulatory role for a variety of physiological processes. The phytohormone is involved in embryo development, seed dormancy, transpiration, and adaptation to environmental stresses. ABA regulates many agronomically important aspects of plant development including synthesis of seed storage proteins and lipids as well as regulating stomatal closure. The analysis of ABA-responsive promoters has revealed a diversity of potential *cis*-acting regulatory elements.

Mutations in ABA biosynthesis are known in a variety of plant species. See, for example, Leung and Giraudat (1998) *Annu. Rev. Plant Physiol. Plant Mol. Biol. 49*:199-222, and the references cited therein. In *Arabidopsis*, a number of genetically distinct *Arabidopsis* acid-insensitive loci have been identified. These mutants were selected based on the ability of seeds to germinate in the presence of inhibitory concentrations of ABA. The mutations have also been shown to affect several additional aspects of seed development, including accumulation of storage proteins and lipids, chlorophyll breakdown, and desiccation tolerance.

To date, numerous mutants and genes have been characterized in plants. Five mutationally identified ABA response loci have been cloned. These represent three classes of proteins. The classes include two orthologous transcriptional regulators (Viviparous1-Vp1) of maize and ABA-insensitive 3 of *Arabidopsis* (ABI3), two highly homologous members of the protein phosphotase 2C family, and a farnesyl transferase of *Arabidopsis*. See, for example, McCarty *et al.* (1991) *Cell 66*:895-905; Giraudat *et al.* (1992) *Plant Cell 4*:1251-1261; Leung *et al.* (1994) *Science 264*:1448-1452; Leung *et al.* (1997) *Plant Cell 9*:759-771; and Cuither *et al.* (1996) *Science 273*:1239-1241.

During the maturation phase of seed development, the embryo becomes quiescent in tissues that are destined to remain viable and the dry seed acquire tolerance to desiccation. In maize and other grasses, this includes cells in the aleurone layer of the seed endosperm. The viviparous mutants of maize are blocked in the maturation program. Thus, the mutant embryo proceeds precociously into seedling development while attached to the mother plant. The nine characterized vivipary loci affect early steps in the biosynthesis of carotenoids and abscisic acid. vp1 embryos exhibit reduced sensitivity to ABA in culture. It has been suggested that the initial Vp1 may encode a factor involved in ABA perception.

At the molecular level, embryonic maturation is associated with a broad range of gene activation. Many of the genes expressed are regulated by the hormone ABA. However, the molecular mechanisms of ABA action are largely unknown.

ABA mediated growth control is a fundamental response of plants to adverse environmental conditions. Because little is known about the molecular mechanism of ABA-mediated growth control, methods are needed to modulate the response of plants to ABA, particularly to increase yield.

SUMMARY OF THE INVENTION

Compositions and methods for increasing yield in plants, particularly seed plants, are provided. The methods involve modulating abscisic acid (ABA) perception and signal transduction in developing seed. Methods are useful for protecting plants against the harmful/detrimental effects of stress and adverse environmental conditions. Compositions comprise genetic constructs known to affect ABA sensitivity in a plant or plant cell. Of particular interest are ABA-associated sequences. Such sequences include mutants, fragments and variants thereof, as well as antisense nucleotide sequences, for genes and mutants involved in the perception and signal transduction of ABA. The DNA sequences may be provided in constructs for temporal, developmental, and tissue specificity.

Compositions are useful in methods for increasing yield in plants under stress, particularly abiotic stress. In this manner, detrimental effects of ABA on ear and kernel growth are ablated.

Transformed plants, plant cells, tissues, and seeds are additionally provided.

DETAILED DESCRIPTION OF THE INVENTION

Methods for modulating early plant response to abscisic acid (ABA) are provided, particularly to insulate crop yield by ablating the detrimental effects of ABA on seed development. In particular, the invention provides compositions and methods for disrupting ABA signaling or function. The compositions and methods are useful for disrupting ABA function in a tissue and developmental-preferred manner to insulate female reproductive tissue growth from stress and adverse environmental conditions.

For purposes of the invention "early plant response" is intended the development of reproductive tissue, seed development, endosperm development, and seed maturation. ABA is involved in many other physiological and developmental processes throughout the life cycle of plants, including seed dormancy, adaptation to abiotic environmental stresses, such as cold, drought, salinity, etc., accumulation of nutritive reserves, acquisition of desiccation tolerance, stomatal closure, and the like. In the early phases, the phytohormone ABA regulates seed maturation and the maintenance of embryo dormancy. Later, at the onset of ontogenesis, ABA mediates several adaptational responses towards environmental cues such as desiccation, cold, salt stress, and other stresses, and acts as a negative growth regulator. Generally, ABA imposes a bimodal growth control by regulating the potential of the cell to enlarge, possibly by turgor control, and by inducing mitotic growth arrest in plants in accordance with its role as a negative growth regulator.

The invention involves controlling or modulating the early response of the plant to the signaling of ABA. By "modulating" is intended the up-regulation or down-regulation of the plant response to ABA. For purposes of the invention, modulation is generally down-regulation by the disruption of ABA synthesis or the disruption of the perception and signal transduction of ABA. It is recognized that total disruption of ABA function in plants is not practical as ABA performs many useful roles in plant development. For purposes of the invention, it is generally preferable to disrupt the effects of ABA at the site of the eventual effect, *i.e.* ears and kernels for cereal crops. In this manner, disruption of ABA perception or its signal transduction provides an effective strategy in insulating cereal female reproductive tissue growth from stress.

Environmental stresses following fertilization inhibit early events in establishment of sink capacity and can decrease yield. In cereals, for example, the endosperm is the major source of stored reserves within the mature seed. Storage capacity is established during an early stage of seed development. Recognizing ABA involvement in early plant responses to stress, the present invention is drawn to ablating the detrimental effects of ABA on the developing seed and improve the nature and quantity of seed and seed products, particularly cereals and grains. See, Mambelli and Setter (1998) *Physiologia Plantarum 104*:266-72 and Tuberosa *et al.* (1998) *Theor. Appl. Genet* 744-55.

As indicated, the invention comprises introducing sequences that modulate ABA perception and signal transduction into a target plant. By "sequences that modulate ABA perception and signal transduction" and "sequences involved in the perception and signal transduction of ABA" are intended mutants and genes that disrupt ABA synthesis or its perception and signal transduction. These mutants, genes, and sequences that disrupt ABA synthesis or its perception or signal transduction, are also called "ABA-associated sequences" herein. Such sequences include, but are not limited to, ABA-insensitive and hypersensitive mutants or antisense sequences corresponding to the mutant or wild-type genes. ABA mutants are known in the art and include *abi*1-5, *era*1-3 (Cutler *et al.* (1996) *Science 273*:1239-41), *gca*1/8 (Benning *et al.* (1996) *Proc. Workshop Abscisic Acid Signal Transduction in Arabidopsis,* Madrid, p. 34), *axr*2 (Wilson *et al.* (1990) *Mol. Gen. Genet. 222*:377-83), *jar*1 (Staswick *et al.* (1992) *Proc. Natl. Acad. Sci. USA 89*:6837-40), *jin*4 (Berger *et al.* (1996) *Plant Physiol. 111*:525-31), *bri*1 (Clouse *et al.* (1996) *Plant Physiol. 111*:671-78), *sax* (*Arabidopsis thaliana*); *vp*1 (McCarty *et al.* (1991) *Cell 66*:895-905 and Robichaud *et al.* (1986) *J. Plant Physiol. 126:*235-42), and *rea*1 (Sturaro *et al.* (1996) *J. Exp. Bot. 47*:755-62) (*Zea mays*); *cool* (Raskin *et al.* (1988) *Planta 173*:73-78) (*Hordeum vulgare*); *aba*1 (Bitoun et al. (1990) Mol. Gen. Genet. 220:234-39 and Leydecker *et al.* (1995) *Plant Physiol. 107:*1427-31) (*Nicotiana plumbaginifolia*); and the like. These and other ABA-associated mutants can be used in the practice of the invention.

By "corresponding" to a gene or sequence is intended that the sequence is capable of hybridizing to the gene or sequence to the extent necessary to disrupt transcription. It is recognized that depending on the ABA-associated sequence

utilized in the invention, the coding sequence or the antisense sequence may be preferred. However, the coding sequence may also be used to co-suppress expression of the target gene. For example, one strategy includes expression of mutant genes, such as *abi1* or *abi2* with an early kernel/embryo promoter to dominantly disrupt ABA action in tissues at early stages. Such an approach would not disrupt the later required ABA function in seed maturation. Alternatively, wild-type alleles of genes such as Vp1 may be down-regulated by co-suppression or antisense strategies to disrupt ABA action. In this latter example, an early kernel/embryo promoter may be used to drive expression of a coding sequence for Vp1 (to co-suppress) or to drive expression of an antisense sequence for Vp1. A third example includes the transformation of a plant with a promoter for late period kernel development driving a wild-type Vp1 sequence. This transformed plant can then be crossed to a *vp1* mutant plant. In this example, the inability of the *vp1* mutant to be induced by ABA works to insulate early kernels from deleterious effects. At the same time, the DNA construct supplies kernels with the ability to mature normally. Thus, as described more fully below, several candidate gene targets are available to be coupled with promoters with limited expression patterns to provide increased yield stability in the face of abiotic stress.

The viviparous-1 (Vp1) gene of maize is required for expression of the maturation program in seed development. VP1 is a novel transcription factor possibly involved in potentiation of a seed-specific hormone response. The nucleic acid and amino acid sequence of Vp1 is in SEQ ID NOS: 1 and 2. The viviparous mutants of maize are blocked in the maturation program. As a result, the mutant embryo proceeds precociously into seedling development while attached to the mother plant. Several vivipary mutants have been identified. Further characteristics of a loss of function *vp1* mutant include, for example, an ABA insensitive phenotype (*i.e.*, a reduced sensitivity to germination inhibition by exogenous ABA in culture) and/or a decrease in *Em* promoter activation. It is well within skill in the art to identify loss of function mutations in Vp1 that are useful in the methods of the present invention. For example, Hill *et al.* ((1996) *Journal Biological Chemistry 7*:3366) have identified a role for the NH^2-terminal acidic region and the highly conserved BR1 domain of VP1 as being essential for VP1 function. Other *vp1* mutants are known. See, for example, Neill *et al.* (1986) *Planta 169*:87-96; McCarthy *et al.* (1991) *Cell 66*:895-905;

Robichaud *et al.* (1980) *Dev. Genet. 1*:325-330; Robichaud and Sussex (1987) *Plant Physiol. 130*:181-188; Robichaud *et al.* (1986) *J. Plant Physiol. 126*:235-42; McCarthy *et al.* (1990) *Physiol. Plant 81*:267-72; and, Eyster *et al.* (1931) *Genetics 16*:457-590; all of which are herein incorporated by reference.

Arabidopsis ABA-insensitive, *ABI*, mutants are also available. Such mutants have pleiotropic defects in seed development, including decreased sensitivity to ABA inhibition of germination in altered seed-specific gene expression. See, Finkelstein *et al.* (1998) *The Plant Cell 10*:1043-1045; Leung *et al.* (1994) *Science 264*:1448-1452; Leung (1997) *Plant Cell 9*:759-771; Giraudat *et al.* (1992) *Plant Cell 4*:1251-1261; Myer *et al.* (1994) *Science 264*:1452-1455; Koornneef *et al.* (1989) *Plant Physiol. 90*:463-469; Nambara *et al.* (1992) *Plant J. 2*:435-441; Finkelstein and Somerville (1990) *Plant Physiol. 94*:1172-1179; Leung and Giraudat (1998) *Annu. Rev. Plant Physiol. Plant Mol. Biol. 49*:199-222; Robinson and Hill (1999) *Plant, Cell and Environment 22*:117-123; and Rodriguez *et al.* (1998) *FEBS Letters 421:*185-190, and the references cited therein, all of which are herein incorporated by reference. In addition, the nucleic acid and amino acid sequences of wild type ABI1, ABI2, ABI3, and ABI4 are set forth in SEQ ID NOS:3-10. Other ABA-associated mutants include *bri1* from *Arabidopsis thaliana* the sequence of which can be found in Genbank Accession No. AF017056 and Li e*t al.* (1997) Cell 90:929-938, both of which are herein incorporated by reference.

An *abi* mutant of interest includes, for example, *abi1*. *abi1* is a dominant mutation in the structural part of the ABI1 gene. The mutation has been characterized and comprises a nucleotide base transition from guanine to adenine and changes the DNA sequence GGC to GAC, thus causing the wild type glycine residue at amino acid position 180 of SEQ ID NO:3 to be replaced with aspartic acid (Meyer *et al.* (1994) *Science 264*:1452-1455). *abi2* is another dominant mutation of interest in the methods of the invention. *abi2* is characterized by a GGC to GAC transition leading to the replacement the Gly residue at amino acid position 168 of SEQ ID NO:6 to Asp (Rodriquez *et al.* (1998) *FEBS Letters 421*:18-190). It is well within skill in the art to identify other mutations (both dominant and recessive) in other ABA-associated sequences that will be useful in the methods of the present invention.

Such mutants listed above are designated "ABA-associated mutants." By "ABA-associated mutants" is intended genes and sequences which disrupt ABA

signaling and/or perception in a plant. Utilizing the sequences above, related sequences from other plants, including cereals, can be isolated. In some instances, it may be beneficial to use the ABA-associated sequence that corresponds with a sequence from the target plant of interest. For example, for use in maize, the maize homolog of the ABA-associated sequence, or a sequence corresponding to the maize homolog, may be preferred.

The invention utilizes the ABA-associated sequences to control the plant response to ABA. Generally, it will be beneficial to block ABA signaling or perception to prevent a loss of yield. Utilizing the ABA-associated sequences, coding sequences, and antisense sequences, the expression and perception of ABA in a plant can be controlled. As described in more detail below, such sequences can be introduced into plants of interest by recombinant methods as well as by traditional breeding methods.

The nucleotide sequences of the invention can be used to isolate corresponding sequences from other organisms, particularly other plants more particularly cereals. In this manner, methods such as PCR, hybridization, and the like can be used to identify such sequences based on their sequence homology to the ABA-associated sequences known in the art. Sequences may be isolated based on their sequence identity to the entire ABA-associated sequence or to fragments thereof.

In a PCR approach, oligonucleotide primers can be designed for use in PCR reactions to amplify corresponding DNA sequences from cDNA or genomic DNA extracted from any plant of interest. Methods for designing PCR primers and PCR cloning are generally known in the art and are disclosed in Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York). See also Innis *et al.*, eds. (1990) *PCR Protocols: A Guide to Methods and Applications* (Academic Press, New York); Innis and Gelfand, eds. (1995) *PCR Strategies* (Academic Press, New York); and Innis and Gelfand, eds. (1999) *PCR Methods Manual* (Academic Press, New York). Known methods of PCR include, but are not limited to, methods using paired primers, nested primers, single specific primers, degenerate primers, gene-specific primers, vector-specific primers, partially-mismatched primers, and the like.

In hybridization techniques, all or part of a known nucleotide sequence is used as a probe that selectively hybridizes to other corresponding nucleotide sequences

present in a population of cloned genomic DNA fragments or cDNA fragments (*i.e.*, genomic or cDNA libraries) from a chosen plant. The hybridization probes may be genomic DNA fragments, cDNA fragments, RNA fragments, or other oligonucleotides, and may be labeled with a detectable group such as ^{32}P, or any other detectable marker. Thus, for example, probes for hybridization can be made by labeling synthetic oligonucleotides based on the ABA-associated sequences of the invention. Methods for preparation of probes for hybridization and for construction of cDNA and genomic libraries are generally known in the art and are disclosed in Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

For example, an entire ABA-associated sequence, or one or more portions thereof, may be used as a probe capable of specifically hybridizing to corresponding sequences and messenger RNAs. To achieve specific hybridization under a variety of conditions, such probes include sequences that are unique among the sequences of interest and are preferably at least about 10 nucleotides in length, and most preferably at least about 20 nucleotides in length. Such probes may be used to amplify corresponding sequences from a chosen plant by PCR. This technique may be used to isolate additional coding sequences from a desired plant or as a diagnostic assay to determine the presence of coding sequences in a plant. Hybridization techniques include hybridization screening of plated DNA libraries (either plaques or colonies; see, for example, Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

Hybridization of such sequences may be carried out under stringent conditions. By "stringent conditions" or "stringent hybridization conditions" is intended conditions under which a probe will hybridize to its target sequence to a detectably greater degree than to other sequences (*e.g.*, at least 2-fold over background). Stringent conditions are sequence-dependent and will be different in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences that are 100% complementary to the probe can be identified (homologous probing). Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing). Generally, a probe is less than about 1000 nucleotides in length, preferably less than 500 nucleotides in length.

Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (*e.g.*, 10 to 50 nucleotides) and at least about 60°C for long probes (*e.g.*, greater than 50 nucleotides). Duration of hybridization is generally less than about 24 hours, usually about 4 to 12. Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1.0 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C.

Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m can be approximated from the equation of Meinkoth and Wahl (1984) *Anal. Biochem. 138*:267-284: $T_m = 81.5°C + 16.6 (\log M) + 0.41 (\%GC) - 0.61 (\%\ form) - 500/L$; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The T_m is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1°C for each 1% of mismatching; thus, T_m, hybridization, and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with $\geq$90% identity are sought, the T_m can be decreased 10°C. Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3, or 4°C lower than the thermal melting point (T_m); moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9, or 10°C lower than the thermal melting point (T_m); low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20°C lower than the thermal

melting point (T_m). Using the equation, hybridization and wash compositions, and desired T_m, those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a T_m of less than 45°C (aqueous solution) or 32°C (formamide solution), it is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology—Hybridization with Nucleic Acid Probes,* Part I, Chapter 2 (Elsevier, New York); and Ausubel *et al.*, eds. (1995) *Current Protocols in Molecular Biology*, Chapter 2 (Greene Publishing and Wiley-Interscience, New York). See Sambrook *et al.* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Plainview, New York).

Thus, isolated "corresponding ABA-associated sequences" that modulate the plant response to ABA and which hybridize under stringent conditions to the ABA-associated sequences disclosed herein, or to fragments thereof, are encompassed by the present invention. Such sequences will be at least about 40% to 50% homologous, about 60%, 65%, or 70% homologous, and even at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more homologous with the disclosed sequences. That is, the sequence identity of sequences may range, sharing at least about 40% to 50%, about 60%, 65%, or 70%, and even at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more sequence identity.

The ABA-associated sequences of the invention can be utilized with tissue or developmental-specific promoters to disrupt ABA function in a tissue or a developmentally specific manner. Promoters of particular interest include seed-preferred promoters, particularly early kernel/embryo promoters and late kernel/embryo promoters.

Kernel development post-pollination is divided into approximately three primary phases. The lag phase of kernel growth occurs from about 0 to 10-12 days after pollination. During this phase the kernel is not growing significantly in mass, but rather important events are being carried out that will determine kernel vitality (*i.e.*, number of cells). The linear grain fill stage occurs from about 10-12 to about 40 DAP. During this stage of kernel development, the kernel attains almost all of its

final mass and various storage products (*i.e.,* starch, protein, oil) are produced. Finally, the maturation phase occurs from about 40 DAP to harvest. During this phase of kernel development the kernel becomes quiescent and begins to dry down in preparation for a long period of dormancy prior to germination. As defined herein "Early kernel/embryo promoters" are promoters that are on during the first phase of development (i.e., from about 0 to about 12 DAP). "Late kernel/embryo promoters", as defined herein, are on from about 12 DAP through maturation. The choice of the promoter will depend on the ABA associated sequence utilized.

Early kernel/embryo promoters include, for example, cim1, a pollen and whole kernel specific promoter that is active 5 DAP. See, for example, WO 00/11177, which is herein incorporated by reference. Other early kernel/embryo promoters include the seed-preferred promoters end1, which is active 7-10 DAP, and end2, which is active 9-14 DAP in the whole kernel and active 10 DAP in the endosperm and pericarp. See, for example, WO 00/12733, herein incorporated by reference. Additional early kernel/embryo promoters that find use in the methods of the present invention include the seed-preferred promoter lpt2 (SEQ ID NO:13) which is active 6 to 24 DAP (U.S. Patent No. 5,525,716, herein incorporated by reference).

Such early kernel/embryo promoters can be used with genes or mutants in the perception/signal transduction pathway for ABA. In this manner, mutant genes such as *abi1* or *abi2* operably linked to an early kernel/embryo promoter would dominantly disrupt ABA action in tissues prior to the later required ABA function in seed maturation. Alternatively, an early kernel/embryo promoter can be operably linked to a wild type (co suppression) or antisence nucleotide sequence of an ABA associated sequence. The early kernel/embryo promoter would be utilized to disrupt ABA action in tissues prior to seed maturation.

Late kernel/embryo promoters include, for example, promoters from oleosin genes. See, for example, Plant *et al.* (1994) *Plant Mol. Biol. 25*:193-205; Keddie *et al.* (1994) *Plant Mol. Biol. 24*:327-40; Keddie *et al.* (1992) *Plant Mol. Biol. 19*:443-53; and Hong *et al.* (1997) *34*:549-55; herein incorporated by reference. See also, Genbank Accession Nos. U71381 (SEQ ID NO:11), AF134411 (SEQ ID NO:12), and U.S. Patent No. 5,977,436, which contain oleosin promoter sequences from *Glycine max*, *Brassica juncea,* and *Arabidopsis thaliana*, respectively. All of these references are herein incorporated by reference. Additional late kernel/embryo promoters

include, smi1ps, an embryo specific promoter that is active 13-14 DAP and cz19B1 a whole kernel specific promoter that is active 13-40 DAP. See, for example, WO 00/11177, which is herein incorporated by reference. The seed-preferred promoter al3 is active 24-40 days after flowering and may also be used in the methods of the invention. See, for example, WO 00/40710, which is herein incorporated by reference.

Late kernel/promoters, such as those from oleosin genes, can be used to drive expression of a wild-type Vp1 allele. Such plants can then be crossed to a plant having a *vp1* mutant. In this example, the inability of the *vp1* mutant allele to be complemented by ABA would insulate early kernels from deleterious effects. The Vp1 gene product is on very early in kernel development. In the presence of ABA, the VP1 becomes effective. The engineered gene supplied by the transgenic parent would supply the kernels with the ability to mature normally. As used herein, an "endogenous ABA associated sequence" is defined as any ABA associated sequence not introduced into the plant via a transformation event.

Such ABA-associated genes can be utilized to control the effects of stress on the plant. Since the accumulation of nutritive reserves in the acquisition of desiccation tolerance are associated with the expression of specific sets of mRNAs. Transcripts encoding either storage proteins or late-embryogenesis-abundant (LEA) proteins thought to participate in desiccation tolerance can be precociously induced by exogenous ABA in cultured embryos. Thus, late expression of ABA genes can be coupled with transgenic seed storage proteins to increase nutritive reserves in seeds.

By "introducing" sequences that modulate ABA perception and signal transduction into a target plant encompasses any means for incorporating the sequence of interest into the target plant. Such means includes conventional breeding methods, genetic transformation methods, or other such means as may be available. The methods of the invention do not depend on a particular method for introducing a nucleotide construct to a plant, only that the nucleotide construct gains access to the interior of at least one cell of the plant. By "stable transformation" is intended that the nucleotide construct introduced into a plant integrates into the genome of the plant and is capable of being inherited by progeny thereof.

Wild-type alleles of genes such as Vp1 may be down-regulated with early promoters via either cosuppression or antisense strategies. It is recognized that with

these nucleotide sequences, antisense constructions complementary to at least a portion of the messenger RNA (mRNA) for the ABA-associated sequences can be constructed. Antisense nucleotides are constructed to hybridize with the corresponding mRNA. Modifications of the antisense sequences may be made as long as the sequences hybridize to and interfere with expression of the corresponding mRNA. In this manner, antisense constructions having 70%, preferably 80%, more preferably 85% sequence similarity to the corresponding antisensed sequences may be used. Furthermore, portions of the antisense nucleotides may be used to disrupt the expression of the target gene. Generally, sequences of at least 50 nucleotides, 100 nucleotides, 200 nucleotides, or greater may be used.

Methods for suppressing gene expression in plants using nucleotide sequences in the sense orientation are known in the art. The methods generally involve transforming plants with a DNA construct comprising a promoter that drives expression in a plant operably linked to at least a portion of a nucleotide sequence that corresponds to the transcript of the endogenous gene (i.e, an ABA-associated sequence). Typically, such a nucleotide sequence has substantial sequence identity to the sequence of the transcript of the endogenous gene, preferably greater than about 65% sequence identity, more preferably greater than about 85% sequence identity, most preferably greater than about 95% sequence identity. See, U.S. Patent Nos. 5,283,184 and 5,034,323; herein incorporated by reference.

It is recognized that fragments and/or variants of the ABA-associated genes can be utilized in the invention. Fragments and variants of the ABA-associated nucleotide sequences and proteins encoded thereby are thus encompassed by the present invention. By "fragment" is intended a portion of the nucleotide sequence or a portion of the amino acid sequence and hence protein encoded thereby. Fragments of a nucleotide sequence may encode protein fragments that retain the biological activity of the native protein and hence act to modulate responses to ABA. Alternatively, fragments of a nucleotide sequence that are useful as hybridization probes generally do not encode fragment proteins retaining biological activity. Thus, fragments of a nucleotide sequence may range from at least about 20 nucleotides, about 50 nucleotides, about 100 nucleotides, and up to the full-length nucleotide sequence of the invention.

By "variants" is intended substantially similar sequences. For nucleotide sequences naturally occurring variants such as these can be identified with the use of well-known molecular biology techniques, as, for example, with polymerase chain reaction (PCR) and hybridization techniques as outlined below. Variant nucleotide sequences also include synthetically derived nucleotide sequences, such as those generated, for example, by using site-directed mutagenesis. Generally, variants of a particular nucleotide sequence of the invention will have at least about 40%, 50%, 60%, 65%, 70%, generally at least about 75%, 80%, 85%, preferably at least about 90%, 92%, 94%, 95%, 96%, 97%, and more preferably at least about 98%, 99% or more sequence identity to that particular nucleotide sequence as determined by sequence alignment programs described elsewhere herein using default parameters.

Methods of alignment of sequences for comparison are well known in the art. Thus, the determination of percent identity between any two sequences can be accomplished using a mathematical algorithm. Non-limiting examples of such mathematical algorithms are the algorithm of Myers and Miller (1988) *CABIOS 4*:11-17; the local homology algorithm of Smith *et al.* (1981) *Adv. Appl. Math. 2*:482; the homology alignment algorithm of Needleman *and Wunsch* (1970) *J. Mol. Biol. 48*:443-453; the search-for-similarity-method of Pearson *and Lipman* (1988) *Proc. Natl. Acad. Sci. 85*:2444-2448; the algorithm of Karlin and Altschul (1990) *Proc. Natl. Acad. Sci. USA 872*264, modified as in Karlin and Altschul (1993) *Proc. Natl. Acad. Sci. USA 90*:5873-5877.

Computer implementations of these mathematical algorithms can be utilized for comparison of sequences to determine sequence identity. Such implementations include, but are not limited to: CLUSTAL in the PC/Gene program (available from Intelligenetics, Mountain View, California); the ALIGN program (Version 2.0) and GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Version 8 (available from Genetics Computer Group (GCG), 575 Science Drive, Madison, Wisconsin, USA). Alignments using these programs can be performed using the default parameters. The CLUSTAL program is well described by Higgins *et al.* (1988) *Gene 73*:237-244 (1988); Higgins *et al.* (1989) *CABIOS 5*:151-153; Corpet *et al.* (1988) *Nucleic Acids Res. 16*:10881-90; Huang *et al.* (1992) *CABIOS 8*:155-65; and Pearson *et al.* (1994) *Meth. Mol. Biol. 24*:307-331. The ALIGN program is based on the algorithm of Myers and Miller (1988) *supra*. A

PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used with the ALIGN program when comparing amino acid sequences. The BLAST programs of Altschul *et al* (1990) *J. Mol. Biol. 215*:403 are based on the algorithm of Karlin and Altschul (1990) *supra*. BLAST nucleotide searches can be performed with the BLASTN program, score = 100, wordlength = 12, to obtain nucleotide sequences homologous to a nucleotide sequence encoding a protein of the invention. BLAST protein searches can be performed with the BLASTX program, score = 50, wordlength = 3, to obtain amino acid sequences homologous to a protein or polypeptide of the invention. To obtain gapped alignments for comparison purposes, Gapped BLAST (in BLAST 2.0) can be utilized as described in Altschul *et al.* (1997) *Nucleic Acids Res. 25*:3389. Alternatively, PSI-BLAST (in BLAST 2.0) can be used to perform an iterated search that detects distant relationships between molecules. See Altschul *et al.* (1997) *supra*. When utilizing BLAST, Gapped BLAST, PSI-BLAST, the default parameters of the respective programs (*e.g.*, BLASTN for nucleotide sequences, BLASTX for proteins) can be used. See http://www.ncbi.hlm.nih.gov. Alignment may also be performed manually by inspection.

Unless otherwise stated, sequence identity/similarity values provided herein refer to the value obtained using GAP Version 10 using the following parameters: % identity using GAP Weight of 50 and Length Weight of 3; % similarity using Gap Weight of 12 and Length Weight of 4, or any equivalent program. By "equivalent program" is intended any sequence comparison program that, for any two sequences in question, generates an alignment having identical nucleotide or amino acid residue matches and an identical percent sequence identity when compared to the corresponding alignment generated by the preferred program.

GAP uses the algorithm of Needleman and Wunsch (1970) *J. Mol. Biol.* 48: 443-453, to find the alignment of two complete sequences that maximizes the number of matches and minimizes the number of gaps. GAP considers all possible alignments and gap positions and creates the alignment with the largest number of matched bases and the fewest gaps. It allows for the provision of a gap creation penalty and a gap extension penalty in units of matched bases. GAP must make a profit of gap creation penalty number of matches for each gap it inserts. If a gap extension penalty greater than zero is chosen, GAP must, in addition, make a profit for each gap inserted of the

length of the gap times the gap extension penalty. Default gap creation penalty values and gap extension penalty values in Version 10 of the Wisconsin Genetics Software Package for protein sequences are 8 and 2, respectively. For nucleotide sequences the default gap creation penalty is 50 while the default gap extension penalty is 3. The gap creation and gap extension penalties can be expressed as an integer selected from the group of integers consisting of from 0 to 200. Thus, for example, the gap creation and gap extension penalties can be 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65 or greater.

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

By "variant" protein is intended a protein derived from the native protein by deletion (so-called truncation) or addition of one or more amino acids to the N-terminal and/or C-terminal end of the native protein; deletion or addition of one or more amino acids at one or more sites in the native protein; or substitution of one or more amino acids at one or more sites in the native protein. Such variants may result from, for example, genetic polymorphism or from human manipulation.

The proteins of the invention may be altered in various ways including amino acid substitutions, deletions, truncations, and insertions. Methods for such manipulations are generally known in the art. Methods for mutagenesis and nucleotide sequence alterations are well known in the art. See, for example, Kunkel (1985) *Proc. Natl. Acad. Sci. USA 82*:488-492; Kunkel *et al.* (1987) *Methods in Enzymol. 154*:367-382; US Patent No. 4,873,192; Walker and Gaastra, eds. (1983) *Techniques in Molecular Biology* (MacMillan Publishing Company, New York) and the references cited therein. Guidance as to appropriate amino acid substitutions that

do not affect biological activity of the protein of interest may be found in the model of Dayhoff *et al.* (1978) *Atlas of Protein Sequence and Structure* (Natl. Biomed. Res. Found., Washington, D.C.), herein incorporated by reference. Conservative substitutions, such as exchanging one amino acid with another having similar properties, may be preferred.

Thus, the genes and nucleotide sequences of the invention include both the naturally occurring sequences as well as mutant forms. Likewise, the proteins of the invention encompass both naturally occurring proteins as well as variations and modified forms thereof. Such variants will continue to possess the desired activity. Obviously, the mutations that will be made in the DNA encoding the variant must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. See, EP Patent Application Publication No. 75,444.

The deletions, insertions, and substitutions of the protein sequences encompassed herein are not expected to produce radical changes in the characteristics of the protein. However, when it is difficult to predict the exact effect of the substitution, deletion, or insertion in advance of doing so, one skilled in the art will appreciate that the effect will be evaluated by routine screening assays.

Variant nucleotide sequences and proteins also encompass sequences and proteins derived from a mutagenic and recombinogenic procedure such as DNA shuffling. With such a procedure, one or more different ABA-associated coding sequences can be manipulated to create a new ABA-associated protein possessing the desired properties. In this manner, libraries of recombinant polynucleotides are generated from a population of related sequence polynucleotides comprising sequence regions that have substantial sequence identity and can be homologously recombined *in vitro* or *in vivo*. Strategies for such DNA shuffling are known in the art. See, for example, Stemmer (1994) *Proc. Natl. Acad. Sci. USA 91*:10747-10751; Stemmer (1994) *Nature 370*:389-391; Crameri *et al.* (1997) *Nature Biotech. 15*:436-438; Moore *et al.* (1997) *J. Mol. Biol. 272*:336-347; Zhang *et al. (1997) Proc. Natl. Acad. Sci. USA 94*:4504-4509; Crameri *et al.* (1998) *Nature 391*:288-291; and U.S. Patent Nos. 5,605,793 and 5,837,458.

The ABA-associated sequences of the invention are provided in expression cassettes for expression in the plant of interest. The cassette will include 5' and 3'

regulatory sequences operably linked to an ABA-associated sequence of the invention. By "operably linked" is intended a functional linkage between a promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. Generally, operably linked means that the nucleic acid sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame. The cassette may additionally contain at least one additional gene to be cotransformed into the organism. Alternatively, the additional gene(s) can be provided on multiple expression cassettes.

Such an expression cassette is provided with a plurality of restriction sites for insertion of the sequence of interest to be under the transcriptional regulation of the regulatory regions. The expression cassette may additionally contain selectable marker genes.

The expression cassette will include in the 5'-3' direction of transcription, a transcriptional and translational initiation region, a DNA sequence of the invention, and a transcriptional and translational termination region functional in plants. The transcriptional initiation region, the promoter, may be native or analogous or foreign or heterologous to the plant host. Additionally, the promoter may be the natural sequence or alternatively a synthetic sequence. By "foreign" is intended that the transcriptional initiation region is not found in the native plant into which the transcriptional initiation region is introduced. As used herein, a chimeric gene comprises a coding sequence operably linked to a transcription initiation region that is heterologous to the coding sequence. While it may be preferable to express the sequences using heterologous promoters, the native promoter sequences may be used. Thus, the phenotype of the plant or plant cell is altered.

The termination region may be native with the transcriptional initiation region, may be native with the operably linked DNA sequence of interest, or may be derived from another source. Convenient termination regions are available from the Ti-plasmid of *A. tumefaciens*, such as the octopine synthase and nopaline synthase termination regions. See also Guerineau *et al.* (1991) *Mol. Gen. Genet. 262*:141-144; Proudfoot (1991) *Cell 64*:671-674; Sanfacon *et al.* (1991) *Genes Dev. 5*:141-149; Mogen *et al.* (1990) *Plant Cell 2*:1261-1272; Munroe *et al.* (1990) *Gene 91*:151-158;

Ballas *et al.* (1989) *Nucleic Acids Res. 17*:7891-7903; and Joshi *et al.* (1987) *Nucleic Acid Res. 15*:9627-9639.

Where appropriate, the gene(s) may be optimized for increased expression in the transformed plant. That is, the genes can be synthesized using plant-preferred codons for improved expression. Methods are available in the art for synthesizing plant-preferred genes. See, for example, U.S. Patent Nos. 5,380,831, and 5,436,391, and Murray *et al.* (1989) *Nucleic Acids Res. 17*:477-498, herein incorporated by reference.

Additional sequence modifications are known to enhance gene expression in a cellular host. These include elimination of sequences encoding spurious polyadenylation signals, exon-intron splice site signals, transposon-like repeats, and other such well-characterized sequences that may be deleterious to gene expression. The G-C content of the sequence may be adjusted to levels average for a given cellular host, as calculated by reference to known genes expressed in the host cell. When possible, the sequence is modified to avoid predicted hairpin secondary mRNA structures.

The expression cassettes may additionally contain 5' leader sequences in the expression cassette construct. Such leader sequences can act to enhance translation. Translation leaders are known in the art and include: picornavirus leaders, for example, EMCV leader (Encephalomyocarditis 5' noncoding region) (Elroy-Stein *et al.* (1989) *PNAS USA 86*:6126-6130); potyvirus leaders, for example, TEV leader (Tobacco Etch Virus) (Allison *et al.* (1986); MDMV leader (Maize Dwarf Mosaic Virus); *Virology 154*:9-20), and human immunoglobulin heavy-chain binding protein (BiP), (Macejak *et al.* (1991) *Nature 353*:90-94); untranslated leader from the coat protein mRNA of alfalfa mosaic virus (AMV RNA 4) (Jobling *et al.* (1987) *Nature 325*:622-625); tobacco mosaic virus leader (TMV) (Gallie *et al.* (1989) in *Molecular Biology of RNA*, ed. Cech (Liss, New York), pp. 237-256); and maize chlorotic mottle virus leader (MCMV) (Lommel *et al.* (1991) *Virology 81*:382-385). See also, Della-Cioppa *et al.* (1987) *Plant Physiol. 84*:965-968. Other methods known to enhance translation can also be utilized, for example, introns, and the like.

In preparing the expression cassette, the various DNA fragments may be manipulated, so as to provide for the DNA sequences in the proper orientation and, as appropriate, in the proper reading frame. Toward this end, adapters or linkers may be

employed to join the DNA fragments or other manipulations may be involved to provide for convenient restriction sites, removal of superfluous DNA, removal of restriction sites, or the like. For this purpose, *in vitro* mutagenesis, primer repair, restriction, annealing, resubstitutions, *e.g.*, transitions and transversions, may be involved.

Transformation protocols as well as protocols for introducing nucleotide sequences into plants may vary depending on the type of plant or plant cell, *i.e.*, monocot or dicot, targeted for transformation. Suitable methods of introducing nucleotide sequences into plant cells and subsequent insertion into the plant genome include microinjection (Crossway *et al.* (1986) *Biotechniques 4*:320-334), electroporation (Riggs *et al.* (1986) *Proc. Natl. Acad. Sci. USA 83*:5602-5606, *Agrobacterium*-mediated transformation (Townsend *et al.*, U.S. Pat No. 5,563,055; U.S. Patent No. 5,981,840), direct gene transfer (Paszkowski *et al.* (1984) *EMBO J. 3*:2717-2722), and ballistic particle acceleration (see, for example, Sanford *et al.*, U.S. Patent No. 4,945,050; Tomes *et al.* (1995) "Direct DNA Transfer into Intact Plant Cells via Microprojectile Bombardment," in *Plant Cell, Tissue, and Organ Culture: Fundamental Methods*, ed. Gamborg and Phillips (Springer-Verlag, Berlin); and McCabe *et al.* (1988) *Biotechnology 6*:923-926). Also see Weissinger *et al.* (1988) *Ann. Rev. Genet. 22*:421-477; Sanford *et al.* (1987) *Particulate Science and Technology 5*:27-37 (onion); Christou *et al.* (1988) *Plant Physiol. 87*:671-674 (soybean); McCabe *et al.* (1988) *Bio/Technology 6*:923-926 (soybean); Finer and McMullen (1991) *In Vitro Cell Dev. Biol.* 27P:175-182 (soybean); Singh *et al.* (1998) *Theor. Appl. Genet. 96*:319-324 (soybean); Datta *et al.* (1990) *Biotechnology 8*:736-740 (rice); Klein *et al.* (1988) *Proc. Natl. Acad. Sci. USA 85*:4305-4309 (maize); Klein *et al.* (1988) *Biotechnology 6*:559-563 (maize); Tomes, U.S. Patent No. 5,240,855; Buising *et al.*, U.S. Patent Nos. 5,322,783 and 5,324,646; Tomes *et al. (1995)* "Direct DNA Transfer into Intact Plant Cells via Microprojectile Bombardment," in *Plant Cell, Tissue, and Organ Culture: Fundamental Methods*, ed. Gamborg (Springer-Verlag, Berlin) (maize); Klein *et al.* (1988) *Plant Physiol. 91*:440-444 (maize); Fromm *et al.* (1990) *Biotechnology 8*:833-839 (maize); Hooykaas-Van Slogteren *et al.* (1984) *Nature (London) 311*:763-764; Bowen *et al.*, U.S. Patent No. 5,736,369 (cereals); Bytebier *et al.* (1987) *Proc. Natl. Acad. Sci. USA 84*:5345-5349 (Liliaceae); De Wet *et al.* (1985) in *The Experimental Manipulation of*

Ovule Tissues, ed. Chapman *et al.* (Longman, New York), pp. 197-209 (pollen); Kaeppler *et al.* (1990) *Plant Cell Reports 9*:415-418 and Kaeppler *et al.* (1992) *Theor. Appl. Genet. 84*:560-566 (whisker-mediated transformation); D'Halluin *et al.* (1992) *Plant Cell 4*:1495-1505 (electroporation); Li *et al.* (1993) *Plant Cell Reports 12*:250-255 and Christou and Ford (1995) *Annals of Botany 75*:407-413 (rice); Osjoda *et al.* (1996) *Nature Biotechnology 14*:745-750 (maize via *Agrobacterium tumefaciens*); all of which are herein incorporated by reference.

The cells that have been transformed may be grown into plants in accordance with conventional ways. See, for example, McCormick *et al.* (1986) *Plant Cell Reports 5*:81-84. These plants may then be grown, and either pollinated with the same transformed strain or different strains, and the resulting hybrid having constitutive expression of the desired phenotypic characteristic identified. Two or more generations may be grown to ensure that constitutive expression of the desired phenotypic characteristic is stably maintained and inherited and then seeds harvested to ensure constitutive expression of the desired phenotypic characteristic has been achieved.

The present invention may be used for transformation of any plant species, including, but not limited to, monocots and dicots. Examples of plants of interest include, but are not limited to, corn (*Zea mays*), *Brassica* sp. (*e.g.*, *B. napus, B. rapa, B. juncea*), particularly those *Brassica* species useful as sources of seed oil, alfalfa (*Medicago sativa*), rice (*Oryza sativa*), rye (*Secale cereale*), sorghum (*Sorghum bicolor, Sorghum vulgare*), millet (*e.g.*, pearl millet (*Pennisetum glaucum*), proso millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*), finger millet (*Eleusine coracana*)), sunflower (*Helianthus annuus*), safflower (*Carthamus tinctorius*), wheat (*Triticum aestivum*), soybean (*Glycine max*), tobacco (*Nicotiana tabacum*), potato (*Solanum tuberosum*), peanuts (*Arachis hypogaea*), cotton (*Gossypium barbadense, Gossypium hirsutum*), sweet potato (*Ipomoea batatus*), cassava (*Manihot esculenta*), coffee (*Coffea* spp.), coconut (*Cocos nucifera*), pineapple (*Ananas comosus*), citrus trees (*Citrus* spp.), cocoa (*Theobroma cacao*), tea (*Camellia sinensis*), banana (*Musa* spp.), avocado (*Persea americana*), fig (*Ficus casica*), guava (*Psidium guajava*), mango (*Mangifera indica*), olive (*Olea europaea*), papaya (*Carica papaya*), cashew (*Anacardium occidentale*), macadamia (*Macadamia integrifolia*), almond (*Prunus amygdalus*), sugar

beets (*Beta vulgaris*), sugarcane (*Saccharum* spp.), oats, barley, vegetables, ornamentals, and conifers.

Vegetables include tomatoes (*Lycopersicon esculentum*), lettuce (*e.g., Lactuca sativa*), green beans (*Phaseolus vulgaris*), lima beans (*Phaseolus limensis*), peas (*Lathyrus* spp.), and members of the genus *Cucumis* such as cucumber (*C. sativus*), cantaloupe (*C. cantalupensis*), and musk melon (*C. melo*). Ornamentals include azalea (*Rhododendron* spp.), hydrangea (*Macrophylla hydrangea*), hibiscus (*Hibiscus rosasanensis*), roses (*Rosa* spp.), tulips (*Tulipa* spp.), daffodils (*Narcissus* spp.), petunias (*Petunia hybrida*), carnation (*Dianthus caryophyllus*), poinsettia (*Euphorbia pulcherrima*), and chrysanthemum. Conifers that may be employed in practicing the present invention include, for example, pines such as loblolly pine (*Pinus taeda*), slash pine (*Pinus elliotii*), ponderosa pine (*Pinus ponderosa*), lodgepole pine (*Pinus contorta*), and Monterey pine (*Pinus radiata*); Douglas-fir (*Pseudotsuga menziesii*); Western hemlock (*Tsuga canadensis*); Sitka spruce (*Picea glauca*); redwood (*Sequoia sempervirens*); true firs such as silver fir (*Abies amabilis*) and balsam fir (*Abies balsamea*); and cedars such as Western red cedar (*Thuja plicata*) and Alaska yellow-cedar (*Chamaecyparis nootkatensis*). Preferably, plants of the present invention are crop plants (for example, corn, alfalfa, sunflower, *Brassica*, soybean, cotton, safflower, peanut, sorghum, wheat, millet, tobacco, etc.), more preferably corn and soybean plants, yet more preferably corn plants.

Plants of particular interest include grain plants that provide seeds of interest, oil-seed plants, and leguminous plants. Seeds of interest include grain seeds, such as corn, wheat, barley, rice, sorghum, rye, etc. Oil seed plants include cotton, soybean, safflower, sunflower, *Brassica*, maize, alfalfa, palm, coconut, etc. Leguminous plants include beans and peas. Beans include guar, locust bean, fenugreek, soybean, garden beans, cowpea, mungbean, lima bean, fava bean, lentils, chickpea, etc.

The following examples are offered by way of illustration and not by way of limitation.

EXPERIMENTAL

Transformation and Regeneration of Transgenic Plants

Example 1: Transformation and Regeneration of Transgenic Maize Plants

Immature maize embryos from greenhouse donor plants are bombarded with a plasmid containing the ABI3 sequence operably linked to an early kernel/embryo promoter plus a plasmid containing the selectable marker gene PAT (Wohlleben *et al.* (1988) *Gene 70*:25-37) that confers resistance to the herbicide Bialaphos. Transformation is performed as follows. All media recipes are below.

Preparation of Target Tissue

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

Preparation of DNA

A plasmid vector comprising the ABI3 sequence operably linked to an early kernel/embryo promoter is made. This plasmid DNA plus plasmid DNA containing a PAT selectable marker is precipitated onto 1.1 μm (average diameter) tungsten pellets using a $CaCl_2$ precipitation procedure as follows:

100 μl prepared tungsten particles in water

10 μl (1 μg) DNA in TrisEDTA buffer (1 μg total)

100 μl 2.5 M $CaCl_2$

10 μl 0.1 M spermidine

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

Particle Gun Treatment

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

Subsequent Treatment

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

Bombardment and Culture Media

Bombardment medium (560Y) comprises 4.0 g/l N6 basal salts (SIGMA C-1416), 1.0 ml/l Eriksson's Vitamin Mix (1000X SIGMA-1511), 0.5 mg/l thiamine HCl, 120.0 g/l sucrose, 1.0 mg/l 2,4-D, and 2.88 g/l L-proline (brought to volume with D-I H_20 following adjustment to pH 5.8 with KOH); 2.0 g/l Gelrite (added after bringing to volume with D-I H_20); and 8.5 mg/l silver nitrate (added after sterilizing the medium and cooling to room temperature). Selection medium (560R) comprises 4.0 g/l N6 basal salts (SIGMA C-1416), 1.0 ml/l Eriksson's Vitamin Mix (1000X SIGMA-1511), 0.5 mg/l thiamine HCl, 30.0 g/l sucrose, and 2.0 mg/l 2,4-D (brought to volume with D-I H_20 following adjustment to pH 5.8 with KOH); 3.0 g/l Gelrite (added after bringing to volume with D-I H_20); and 0.85 mg/l silver nitrate and 3.0 mg/l bialaphos(both added after sterilizing the medium and cooling to room temperature).

Plant regeneration medium (288J) comprises 4.3 g/l MS salts (GIBCO 11117-074), 5.0 ml/l MS vitamins stock solution (0.100 g nicotinic acid, 0.02 g/l thiamine HCL, 0.10 g/l pyridoxine HCL, and 0.40 g/l glycine brought to volume with polished D-I H_20) (Murashige and Skoog (1962) *Physiol. Plant. 15*:473), 100 mg/l myo-inositol, 0.5 mg/l zeatin, 60 g/l sucrose, and 1.0 ml/l of 0.1 mM abscisic acid (brought to volume with polished D-I H_20 after adjusting to pH 5.6); 3.0 g/l Gelrite (added after bringing to volume with D-I H_20); and 1.0 mg/l indoleacetic acid and 3.0 mg/l bialaphos (added after sterilizing the medium and cooling to 60°C). Hormone-free medium (272V) comprises 4.3 g/l MS salts (GIBCO 11117-074), 5.0 ml/l MS vitamins stock solution (0.100 g/l nicotinic acid, 0.02 g/l thiamine HCL, 0.10 g/l pyridoxine HCL, and 0.40 g/l glycine brought to volume with polished D-I H_20), 0.1 g/l myo-inositol, and 40.0 g/l sucrose (brought to volume with polished D-I H_20 after adjusting pH to 5.6); and 6 g/l bacto-agar (added after bringing to volume with polished D-I H_20), sterilized and cooled to 60° C.

Example 2: *Agrobacterium*-mediated Transformation

For *Agrobacterium*-mediated transformation of maize with an ABI3 sequence operably linked to an early kernel/embryo promoter, preferably the method of Zhao is employed (U.S. Patent No. 5,981,840, and PCT patent publication WO98/32326; the contents of which are hereby incorporated by reference). Briefly, immature embryos are isolated from maize and the embryos contacted with a suspension of *Agrobacterium*, where the bacteria are capable of transferring the ABI3 sequence operably linked to an early kernel/embryo promoter to at least one cell of at least one of the immature embryos (step 1: the infection step). In this step the immature embryos are preferably immersed in an *Agrobacterium* suspension for the initiation of inoculation. The embryos are co-cultured for a time with the *Agrobacterium* (step 2: the co-cultivation step). Preferably the immature embryos are cultured on solid medium following the infection step. Following this co-cultivation period an optional "resting" step is contemplated. In this resting step, the embryos are incubated in the presence of at least one antibiotic known to inhibit the growth of *Agrobacterium* without the addition of a selective agent for plant transformants (step 3: resting step). Preferably the immature embryos are cultured on solid medium with antibiotic, but without a selecting agent, for elimination of *Agrobacterium* and for a resting phase for

the infected cells. Next, inoculated embryos are cultured on medium containing a selective agent and growing transformed callus is recovered (step 4: the selection step). Preferably, the immature embryos are cultured on solid medium with a selective agent resulting in the selective growth of transformed cells. The callus is then regenerated into plants (step 5: the regeneration step), and preferably calli grown on selective medium are cultured on solid medium to regenerate the plants.

Example 3: Soybean Embryo Transformation

Soybean embryos are bombarded with a plasmid containing the ABI3 nucleotide sequence operably linked to an early embryo/kernel promoter as follows. To induce somatic embryos, cotyledons, 3-5 mm in length dissected from surface-sterilized, immature seeds of the soybean cultivar A2872, are cultured in the light or dark at 26°C on an appropriate agar medium for six to ten weeks. Somatic embryos producing secondary embryos are then excised and placed into a suitable liquid medium. After repeated selection for clusters of somatic embryos that multiplied as early, globular-staged embryos, the suspensions are maintained as described below.

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

Soybean embryogenic suspension cultures may then be transformed by the method of particle gun bombardment (Klein *et al.* (1987) *Nature* (London) *327*:70-73, U.S. Patent No. 4,945,050). A Du Pont Biolistic PDS1000/HE instrument (helium retrofit) can be used for these transformations.

A selectable marker gene that can be used to facilitate soybean transformation is a transgene composed of the 35S promoter from Cauliflower Mosaic Virus (Odell *et al.* (1985) *Nature 313*:810-812), the hygromycin phosphotransferase gene from plasmid pJR225 (from *E. coli*; Gritz *et al.* (1983) *Gene 25*:179-188), and the 3' region of the nopaline synthase gene from the T-DNA of the Ti plasmid of *Agrobacterium tumefaciens*. The expression cassette comprising the ABI3 nucleotide sequence operably linked to an early kernel/embryo promoter can be isolated as a restriction fragment. This fragment can then be inserted into a unique restriction site of the vector carrying the marker gene.

To 50 µl of a 60 mg/ml 1 µm gold particle suspension is added (in order): 5 µl DNA (1 µg/µl), 20 µl spermidine (0.1 M), and 50 µl $CaCl_2$ (2.5 M). The particle preparation is then agitated for three minutes, spun in a microfuge for 10 seconds and the supernatant removed. The DNA-coated particles are then washed once in 400 µl 70% ethanol and resuspended in 40 µl of anhydrous ethanol. The DNA/particle suspension can be sonicated three times for one second each. Five microliters of the DNA-coated gold particles are then loaded on each macro carrier disk.

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

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

Example 4: Sunflower Meristem Tissue Transformation

Sunflower meristem tissues are transformed with an expression cassette containing the ABI3 sequence operably linked to an early kernel embryo promoter as follows (see also European Patent Number EP 0 486233, herein incorporated by reference, and Malone-Schoneberg *et al.* (1994) *Plant Science 103*:199-207). Mature sunflower seed (*Helianthus annuus* L.) are dehulled using a single wheat-head thresher. Seeds are surface sterilized for 30 minutes in a 20% Clorox bleach solution

with the addition of two drops of Tween 20 per 50 ml of solution. The seeds are rinsed twice with sterile distilled water.

Split embryonic axis explants are prepared by a modification of procedures described by Schrammeijer *et al.* (Schrammeijer *et al.*(1990) *Plant Cell Rep. 9*: 55-60). Seeds are imbibed in distilled water for 60 minutes following the surface sterilization procedure. The cotyledons of each seed are then broken off, producing a clean fracture at the plane of the embryonic axis. Following excision of the root tip, the explants are bisected longitudinally between the primordial leaves. The two halves are placed, cut surface up, on GBA medium consisting of Murashige and Skoog mineral elements (Murashige *et al.* (1962) *Physiol. Plant., 15*: 473-497), Shepard's vitamin additions (Shepard (1980) in *Emergent Techniques for the Genetic Improvement of Crops* (University of Minnesota Press, St. Paul, Minnesota), 40 mg/l adenine sulfate, 30 g/l sucrose, 0.5 mg/l 6-benzyl-aminopurine (BAP), 0.25 mg/l indole-3-acetic acid (IAA), 0.1 mg/l gibberellic acid (GA_3), pH 5.6, and 8 g/l Phytagar.

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

Disarmed *Agrobacterium tumefaciens* strain EHA105 is used in all transformation experiments. A binary plasmid vector comprising the expression cassette that contains the ABI3 gene operably linked to an early kernel/embryo promoter is introduced into *Agrobacterium* strain EHA105 via freeze-thawing as described by Holsters *et al.* (1978) *Mol. Gen. Genet. 163*:181-187. This plasmid further comprises a kanamycin selectable marker gene (i.e, *nptII*). Bacteria for plant transformation experiments are grown overnight (28°C and 100 RPM continuous agitation) in liquid YEP medium (10 gm/l yeast extract, 10 gm/l Bactopeptone, and 5 gm/l NaCl, pH 7.0) with the appropriate antibiotics required for bacterial strain and binary plasmid maintenance. The suspension is used when it reaches an OD_{600} of

about 0.4 to 0.8. The *Agrobacterium* cells are pelleted and resuspended at a final OD_{600} of 0.5 in an inoculation medium comprised of 12.5 mM MES pH 5.7, 1 gm/l NH_4Cl, and 0.3 gm/l $MgSO_4$.

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

NPTII-positive shoots are grafted to Pioneer® hybrid 6440 *in vitro*-grown sunflower seedling rootstock. Surface sterilized seeds are germinated in 48-0 medium (half-strength Murashige and Skoog salts, 0.5% sucrose, 0.3% gelrite, pH 5.6) and grown under conditions described for explant culture. The upper portion of the seedling is removed, a 1 cm vertical slice is made in the hypocotyl, and the transformed shoot inserted into the cut. The entire area is wrapped with parafilm to secure the shoot. Grafted plants can be transferred to soil following one week of *in vitro* culture. Grafts in soil are maintained under high humidity conditions followed by a slow acclimatization to the greenhouse environment. Transformed sectors of T_0 plants (parental generation) maturing in the greenhouse are identified by, for example, NPTII ELISA of leaf extracts while transgenic seeds harvested from NPTII-positive T_0 plants are identified by assaying for a modulation in the plant response to ABA.

Example 5

Transgenic maize plants are generated by the methods of example 1 using a DNA construction comprising a wild type Vp1 sequence (SEQ ID NO:1) operably

linked to the oleosin promoter. The plasmid further contains the selectable marker PAT (Wohlleben *et al.* (1998) *Gene 70*:25-37). As described in Example 1, plants having stably incorporated the oleosin:Vp1 DNA construct are isolated.

Maize plants having a loss of function mutation in *vp1* are isolated as described in Eyster *et al.* (1931) *Genetics 16*:574-590, herein incorporated by reference. Such plants are characterized as having a reduced sensitivity to ABA. Transgenic maize plants having stably incorporated the oleosin:Vp1 DNA construct are crossed to the maize plant having the *vp1* loss of function mutation. The resulting progeny are backcrossed to produce a plant having the following genotype: *vp1/vp1*; oleosin:Vp1/oleosin:Vp1. This plant will be insulted from the deleterious effects of ABA in the early embryo and will be supplied with VP1 in late kernel/embryo development, allowing kernels to mature normally.

All publications and patent applications mentioned in the specification are indicative of the level of those skilled in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications may be practiced within the scope of the appended claims.

THAT WHICH IS CLAIMED

1. A method for modulating a response in a target plant to abscisic acid (ABA) said method comprising:

introducing a DNA construct comprising an ABA-associated sequence operably linked to an early kernel/embryo promoter into said plant.

2. The method of claim 1, wherein said modulating comprises a decreasing response to ABA.

3. The method of claim 2, wherein said ABA-associated sequence is selected from the group consisting of:

a) a nucleic acid molecule comprising a nucleotide sequence set forth in any one of SEQ ID NO: 1, 3, 5, 7, or 9;

b) a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide set forth in any one of SEQ ID NO: 2, 4, 6, 8, or 10;

c) a nucleic acid molecule comprising a nucleotide sequence having at least 70% sequence identity to the sequence set forth in any one of SEQ ID NO: 1, 3, 5, 7 or 9; and, wherein expression of said sequence decreases the response to ABA;

d) a nucleic acid molecule comprising a nucleotide sequence hybridizing under stringent conditions to the nucleotide sequence of any one of SEQ ID NO: 1, 3, 5, 7, or 9, wherein expression of said sequence decreases the response to ABA; and,

e) a nucleic acid molecule comprising an antisense sequence of the nucleotide sequence of a), b), c), or d).

4. The method of claim 2, wherein said ABA-associated sequence is an antisense sequence for an ABA-associated sequence.

5. The method of claim 2, wherein said ABA-associated sequence is an ABA-associated mutant sequence and comprises a sequence selected from the group consisting of *abi1* and *abi2*.

6. The method of claim 1, wherein said ABA-associated sequence corresponds to a sequence from the target plant.

7. The method of claim 1, wherein said plant is a cereal.

8. The method of claim 7, wherein said cereal is maize.

9. The method of claim 2, wherein said introducing is by breeding.

10. The method of claim 2, wherein said introducing is by transformation.

11. A method for preventing detrimental effects of stress on developing plant seed, said method comprising:

introducing a DNA construct comprising an ABA-associated sequence operably linked to an early kernel/embryo promoter into said plant.

12. The method of claim 11, wherein expression of said ABA-associated sequence modulates a response to ABA.

13. The method of claim 12, wherein said modulation is a decrease response to ABA.

14. The method of claim 13, wherein said ABA-associated sequence is selected from the group consisting of:

a) a nucleic acid molecule comprising a nucleotide sequence set forth in any one of SEQ ID NO: 1, 3, 5, 7, or 9;

b) a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide set forth in any one of SEQ ID NO: 2, 4, 6, 8 or 10;

c) a nucleic acid molecule comprising a nucleotide sequence having at least 70% sequence identity to the sequence set forth in any one of SEQ ID NO: 1, 3, 5, 7 or 9; and, wherein expression of said sequence decreases the response to ABA;

d) a nucleic acid molecule comprising a nucleotide sequence hybridizing under stringent conditions to the nucleotide sequence of any one of SEQ ID NO: 1, 3, 5, 7, or 9, wherein expression of said sequence decreases the response to ABA; and,

e) a nucleic acid molecule comprising an antisense sequence of the nucleotide sequence of a), b), c), or d).

15. A plant having stably introduced a DNA construct comprising an ABA-associated sequence operably linked to an early kernel/embryo promoter.

16. The plant of claim 15, wherein expression of said ABA-associated sequence modulates a response to ABA.

17. The plant of claim 16, wherein said modulation decreases a response to ABA.

18. The plant of claim 16, wherein said sequence is involved in the perception and signal transduction of ABA and is a coding sequence.

19. The plant of claim 16, wherein said ABA-associated sequence is selected from the group consisting of:

a) a nucleic acid molecule comprising a nucleotide sequence set forth in any one of SEQ ID NO: 1, 3, 5, 7, or 9;

b) a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide set forth in any one of SEQ ID NO: 2, 4, 6, 8 or 10;

c) a nucleic acid molecule comprising a nucleotide sequence having at least 70% sequence identity to the sequence set forth in any one of SEQ ID NO: 1, 3, 5, 7 or 9; and, wherein expression of said fragment decreases the response to ABA;

d) a nucleic acid molecule comprising a nucleotide sequence hybridizing under stringent conditions to the nucleotide sequence of any one of SEQ ID NO: 1, 3, 5, 7, or 9, wherein expression of said sequence decreases the response to ABA; and,

e) a nucleic acid molecule comprising an antisense sequence of the nucleotide sequence of a), b), c), or d).

20. The plant of claim 15, wherein said DNA construct is stably integrated into the genome of the plant.

21. The transformed seed of the plant of claim 15.

22. Progeny of the plant of claim 15.

23. A plant cell having introduced into its genome a DNA construct comprising an ABA-associated sequence operably linked to an early kernel/embryo promoter.

24. A method for preventing detrimental effects of stress on developing plant seed, said method comprising:

introducing a DNA construct comprising an ABA-associated sequence operably linked to a late kernel/embryo promoter into a plant, wherein the plant has a loss of function mutation for the ABA-associated sequence.

25. The method of claim 24, wherein said introducing comprises breeding a first plant having stably incorporated into its genome said DNA construct to a second plant having a loss of function mutation for the ABA-associated sequence and isolating the progeny.

26. The method of claim 24, wherein said modulating comprises a decreasing response to ABA.

27. The method of claim 24, wherein said ABA-associated sequence is selected from the group consisting of:

a) a nucleic acid molecule comprising a nucleotide sequence set forth in any one of SEQ ID NO: 1 or 7;

b) a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide set forth in any one of SEQ ID NO: 2 or 8;

c) a nucleic acid molecule comprising a nucleotide sequence having at least 70% sequence identity to the sequence set forth in any one of SEQ ID NO: 1 or 7; and, wherein said sequence encodes a polypeptide having VP1 activity; and,

d) a nucleic acid molecule comprising a nucleotide sequence hybridizing under stringent conditions to the nucleotide sequence of any one of SEQ ID NO: 1 or 7, wherein said sequence encodes a polypeptide having VP1 activity.

28. The method of claim 24, wherein said promoter is an oleosin promoter.

29. The method of claim 24, wherein said DNA construct is introduced by transformation.

30. A plant having stably introduced a DNA construct comprising an ABA-associated sequence operably linked to a late kernel/embryo promoter.

31. The plant of claim 30, further comprising a loss of function mutation in an endogenous ABA-associated sequence.

32. The plant of claim 30, wherein said ABA-associated sequence is selected from the group consisting of:

a) a nucleic acid molecule comprising a nucleotide sequence set forth in any one of SEQ ID NO: 1 or 7;

b) a nucleic acid molecule comprising a nucleotide sequence encoding a polypeptide set forth in any one of SEQ ID NO: 2 or 8;

c) a nucleic acid molecule comprising a nucleotide sequence having at least 70% sequence identity to the sequence set forth in any one of SEQ ID NO: 1 or 7; and, wherein said sequence encodes a polypeptide having VP1 activity; and,

d) a nucleic acid molecule comprising a nucleotide sequence hybridizing under stringent conditions to the nucleotide sequence of any one of SEQ ID NO: 1 or 7, wherein said sequence encodes a polypeptide having VP1 activity.

33. The transformed seed of the plant of claim 31.

34. Progeny of the plant of claim 31.

SEQUENCE LISTING

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Arg Gln Gly Thr Met Gln Leu Leu Lys Leu Ile Leu Thr Trp Val Gln
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Asn His His Leu Gln Arg Lys Arg Pro Arg Asp Val Met Glu Glu Glu
225                 230                 235                 240
Ala Gly Leu His Val Gln Leu Pro Ser Pro Val Ala Asn Pro Pro Gly
                245                 250                 255
Tyr Glu Phe Pro Ala Gly Gly Gln Asp Met Ala Ala Gly Gly Gly Thr
            260                 265                 270
Ser Trp Met Pro His Gln Gln Ala Phe Thr Pro Pro Ala Ala Tyr Gly
        275                 280                 285
Gly Asp Ala Val Tyr Pro Ser Ala Ala Gly Gln Gln Tyr Ser Phe His
    290                 295                 300
Gln Gly Pro Ser Thr Ser Ser Val Val Val Asn Ser Gln Pro Phe Ser
305                 310                 315                 320
Pro Pro Pro Val Gly Asp Met His Gly Ala Asn Met Ala Trp Pro Gln
                325                 330                 335
Gln Tyr Val Pro Phe Pro Pro Pro Gly Ala Ser Thr Gly Ser Tyr Pro
            340                 345                 350
Met Pro Gln Pro Phe Ser Pro Gly Phe Gly Gly Gln Tyr Ala Gly Ala
        355                 360                 365
Gly Ala Gly His Leu Ser Val Ala Pro Gln Arg Met Ala Gly Val Glu
    370                 375                 380
Ala Ser Ala Thr Lys Glu Ala Arg Lys Lys Arg Met Ala Arg Gln Arg
385                 390                 395                 400
Arg Leu Ser Cys Leu Gln Gln Gln Arg Ser Gln Gln Leu Ser Leu Gly
                405                 410                 415
Gln Ile Gln Thr Ser Val His Leu Gln Glu Pro Ser Pro Arg Ser Thr
            420                 425                 430
His Ser Gly Pro Val Thr Pro Ser Ala Gly Gly Trp Gly Phe Trp Ser
        435                 440                 445
Pro Ser Ser Gln Gln Gln Val Gln Asn Pro Leu Ser Lys Ser Asn Ser
    450                 455                 460
Ser Arg Ala Pro Pro Ser Ser Leu Glu Ala Ala Ala Ala Ala Pro Gln
465                 470                 475                 480
Thr Lys Pro Ala Pro Ala Gly Ala Arg Gln Asp Asp Ile His His Arg
                485                 490                 495
Leu Ala Ala Ala Ser Asp Lys Arg Gln Gly Ala Lys Ala Asp Lys Asn
            500                 505                 510
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Leu Arg Phe Leu Leu Gln Lys Val Leu Lys Gln Ser Asp Val Gly Ser
        515                 520                 525
Leu Gly Arg Ile Val Leu Pro Lys Lys Glu Ala Glu Val His Leu Pro
    530                 535                 540
Glu Leu Lys Thr Arg Asp Gly Ile Ser Ile Pro Met Glu Asp Ile Gly
545                 550                 555                 560
Thr Ser Arg Val Trp Asn Met Arg Tyr Arg Phe Trp Pro Asn Asn Lys
                565                 570                 575
Ser Arg Met Tyr Leu Leu Glu Asn Thr Gly Glu Phe Val Arg Ser Asn
            580                 585                 590
Glu Leu Gln Glu Gly Asp Phe Ile Val Ile Tyr Ser Asp Val Lys Ser
        595                 600                 605
Gly Lys Tyr Leu Ile Arg Gly Val Lys Val Arg Pro Pro Pro Ala Gln
    610                 615                 620
Glu Gln Gly Ser Gly Ser Ser Gly Gly Gly Lys His Arg Pro Leu Cys
625                 630                 635                 640
Pro Ala Gly Pro Glu Arg Ala Ala Ala Ala Gly Ala Pro Glu Asp Ala
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Val Val Asp Gly Val Ser Gly Ala Cys Lys Gly Arg Ser Pro Glu Gly
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180
caggaaaccc ttattgaaat tcaattcatt tctttctttc tgtgtttgtg attttcccgg
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470
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518
Pro Phe Ser Glu Thr Gln Met Asp Phe Thr Gly Ile Arg Leu Gly Lys
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ggt tac tgc aat aac caa tac tca aat caa gat tcc gag aac gga gat
566
Gly Tyr Cys Asn Asn Gln Tyr Ser Asn Gln Asp Ser Glu Asn Gly Asp
 30                  35                  40                  45
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614
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                 50                  55                  60

cat ggt tct gaa tct agg aaa gtt ttg att tct cgg atc aat tct cct
662
His Gly Ser Glu Ser Arg Lys Val Leu Ile Ser Arg Ile Asn Ser Pro
             65                  70                  75

aat tta aac atg aag gaa tca gca gct gct gat ata gtc gtc gtt gat
710
Asn Leu Asn Met Lys Glu Ser Ala Ala Ala Asp Ile Val Val Val Asp
         80                  85                  90

atc tcc gcc gga gat gag atc aac ggc tca gat att act agc gag aag
758
Ile Ser Ala Gly Asp Glu Ile Asn Gly Ser Asp Ile Thr Ser Glu Lys
     95                  100                 105

aag atg atc agc aga aca gag agt agg agt ttg ttt gaa ttc aag agt
806
Lys Met Ile Ser Arg Thr Glu Ser Arg Ser Leu Phe Glu Phe Lys Ser
110                 115                 120                 125

gtg cct ttg tat ggt ttt act tcg att tgt gga aga aga cct gag atg
854
Val Pro Leu Tyr Gly Phe Thr Ser Ile Cys Gly Arg Arg Pro Glu Met
                130                 135                 140

gaa gat gct gtt tcg act ata cca aga ttc ctt caa tct tcc tct ggt
902
Glu Asp Ala Val Ser Thr Ile Pro Arg Phe Leu Gln Ser Ser Ser Gly
            145                 150                 155

tcg atg tta gat ggt cgg ttt gat cct caa tcc gcc gct cat ttc ttc
950
Ser Met Leu Asp Gly Arg Phe Asp Pro Gln Ser Ala Ala His Phe Phe
        160                 165                 170

ggt gtt tac gac ggc cat ggc ggt tct cag gta gcg aac tat tgt aga
998
Gly Val Tyr Asp Gly His Gly Gly Ser Gln Val Ala Asn Tyr Cys Arg
    175                 180                 185

gag agg atg cat ttg gct ttg gcg gag gag ata gct aag gag aaa ccg
1046
Glu Arg Met His Leu Ala Leu Ala Glu Glu Ile Ala Lys Glu Lys Pro
190                 195                 200                 205

atg ctc tgc gat ggt gat acg tgg ctg gag aag tgg aag aaa gct ctt
1094
Met Leu Cys Asp Gly Asp Thr Trp Leu Glu Lys Trp Lys Lys Ala Leu
                210                 215                 220

ttc aac tcg ttc ctg aga gtt gac tcg gag att gag tca gtt gcg ccg
1142
Phe Asn Ser Phe Leu Arg Val Asp Ser Glu Ile Glu Ser Val Ala Pro
            225                 230                 235

gag acg gtt ggg tca acg tcg gtg gtt gcc gtt gtt ttc ccg tct cac
1190
Glu Thr Val Gly Ser Thr Ser Val Val Ala Val Val Phe Pro Ser His
        240                 245                 250
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atc ttc gtc gct aac tgc ggt gac tct aga gcc gtt ctt tgc cgc ggc
1238
Ile Phe Val Ala Asn Cys Gly Asp Ser Arg Ala Val Leu Cys Arg Gly
    255                 260                 265

aaa act gca ctt cca tta tcc gtt gac cat aaa ccg gat aga gaa gat
1286
Lys Thr Ala Leu Pro Leu Ser Val Asp His Lys Pro Asp Arg Glu Asp
270                 275                 280                 285

gaa gct gcg agg att gaa gcc gca gga ggg aaa gtg att cag tgg aat
1334
Glu Ala Ala Arg Ile Glu Ala Ala Gly Gly Lys Val Ile Gln Trp Asn
                290                 295                 300

gga gct cgt gtt ttc ggt gtt ctc gcc atg tcg aga tcc att ggc gat
1382
Gly Ala Arg Val Phe Gly Val Leu Ala Met Ser Arg Ser Ile Gly Asp
            305                 310                 315

aga tac ttg aaa cca tcc atc att cct gat ccg gaa gtg acg gct gtg
1430
Arg Tyr Leu Lys Pro Ser Ile Ile Pro Asp Pro Glu Val Thr Ala Val
        320                 325                 330

aag aga gta aaa gaa gat gat tgt ctg att ttg gcg agt gac ggg gtt
1478
Lys Arg Val Lys Glu Asp Asp Cys Leu Ile Leu Ala Ser Asp Gly Val
    335                 340                 345

tgg gat gta atg acg gat gaa gaa gcg tgt gag atg gca agg aag cgg
1526
Trp Asp Val Met Thr Asp Glu Glu Ala Cys Glu Met Ala Arg Lys Arg
350                 355                 360                 365

att ctc ttg tgg cac aag aaa aac gcg gtg gct ggg gat gca tcg ttg
1574
Ile Leu Leu Trp His Lys Lys Asn Ala Val Ala Gly Asp Ala Ser Leu
                370                 375                 380

ctc gcg gat gag cgg aga aag gaa ggg aaa gat cct gcg gcg atg tcc
1622
Leu Ala Asp Glu Arg Arg Lys Glu Gly Lys Asp Pro Ala Ala Met Ser
            385                 390                 395

gcg gct gag tat ttg tca aag ctg gcg ata cag aga gga agc aaa gac
1670
Ala Ala Glu Tyr Leu Ser Lys Leu Ala Ile Gln Arg Gly Ser Lys Asp
        400                 405                 410

aac ata agt gtg gtg gtg gtt gat ttg aag cct cgg agg aaa ctc aag
1718
Asn Ile Ser Val Val Val Val Asp Leu Lys Pro Arg Arg Lys Leu Lys
    415                 420                 425

agc aaa ccc ttg aac tga ggcagagagg gtccttttc ttaatttta
1766
Ser Lys Pro Leu Asn  *
430

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1826
ttaactaaca agttataacc atatggagat aatgaagctt aatgtgttaa gctcttttgt
1886
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1946
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35 40 45
Ser Leu Pro Glu Thr Ser Ser Cys Ser Val Ser Gly Ser His Gly Ser
50 55 60
Glu Ser Arg Lys Val Leu Ile Ser Arg Ile Asn Ser Pro Asn Leu Asn
65 70 75 80
Met Lys Glu Ser Ala Ala Ala Asp Ile Val Val Val Asp Ile Ser Ala
85 90 95
Gly Asp Glu Ile Asn Gly Ser Asp Ile Thr Ser Glu Lys Lys Met Ile
100 105 110
Ser Arg Thr Glu Ser Arg Ser Leu Phe Glu Phe Lys Ser Val Pro Leu
115 120 125
Tyr Gly Phe Thr Ser Ile Cys Gly Arg Arg Pro Glu Met Glu Asp Ala
130 135 140
Val Ser Thr Ile Pro Arg Phe Leu Gln Ser Ser Ser Gly Ser Met Leu
145 150 155 160
Asp Gly Arg Phe Asp Pro Gln Ser Ala Ala His Phe Phe Gly Val Tyr
165 170 175
Asp Gly His Gly Gly Ser Gln Val Ala Asn Tyr Cys Arg Glu Arg Met
180 185 190
His Leu Ala Leu Ala Glu Glu Ile Ala Lys Glu Lys Pro Met Leu Cys
195 200 205
Asp Gly Asp Thr Trp Leu Glu Lys Trp Lys Lys Ala Leu Phe Asn Ser
210 215 220
Phe Leu Arg Val Asp Ser Glu Ile Glu Ser Val Ala Pro Glu Thr Val
225 230 235 240
Gly Ser Thr Ser Val Val Ala Val Val Phe Pro Ser His Ile Phe Val
245 250 255
Ala Asn Cys Gly Asp Ser Arg Ala Val Leu Cys Arg Gly Lys Thr Ala
260 265 270
Leu Pro Leu Ser Val Asp His Lys Pro Asp Arg Glu Asp Glu Ala Ala
275 280 285
Arg Ile Glu Ala Ala Gly Gly Lys Val Ile Gln Trp Asn Gly Ala Arg
290 295 300
Val Phe Gly Val Leu Ala Met Ser Arg Ser Ile Gly Asp Arg Tyr Leu
305 310 315 320
Lys Pro Ser Ile Ile Pro Asp Pro Glu Val Thr Ala Val Lys Arg Val
325 330 335
Lys Glu Asp Asp Cys Leu Ile Leu Ala Ser Asp Gly Val Trp Asp Val
340 345 350
Met Thr Asp Glu Glu Ala Cys Glu Met Ala Arg Lys Arg Ile Leu Leu
355 360 365
Trp His Lys Lys Asn Ala Val Ala Gly Asp Ala Ser Leu Leu Ala Asp
370 375 380
Glu Arg Arg Lys Glu Gly Lys Asp Pro Ala Ala Met Ser Ala Ala Glu
385 390 395 400
Tyr Leu Ser Lys Leu Ala Ile Gln Arg Gly Ser Lys Asp Asn Ile Ser
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104
Glu Val Ser Pro Ala Val Ala Val Pro Phe Arg Pro Phe Thr Asp Pro
5 10 15

cac gcc gga ctt aga ggc tat tgc aac ggt gaa tct agg gtt act tta
152
His Ala Gly Leu Arg Gly Tyr Cys Asn Gly Glu Ser Arg Val Thr Leu
20 25 30

ccg gaa agt tct tgt tct ggc gac gga gct atg aaa gat tct tcc ttt
200
Pro Glu Ser Ser Cys Ser Gly Asp Gly Ala Met Lys Asp Ser Ser Phe
35 40 45 50

gag atc aat aca aga caa gat tca ttg aca tca tca tca tct gct atg
248
Glu Ile Asn Thr Arg Gln Asp Ser Leu Thr Ser Ser Ser Ser Ala Met
55 60 65

gca ggt gtg gat atc tcc gcc gga gat gaa atc aac ggt tca gat gag
296
Ala Gly Val Asp Ile Ser Ala Gly Asp Glu Ile Asn Gly Ser Asp Glu
70 75 80

ttt gat ccg aga tcg atg aat cag agt gag aag aaa gta ctt agt aga
344
Phe Asp Pro Arg Ser Met Asn Gln Ser Glu Lys Lys Val Leu Ser Arg
85 90 95

aca gag agt aga agt ctg ttt gag ttc aag tgt gtt cct tta tat gga
392
Thr Glu Ser Arg Ser Leu Phe Glu Phe Lys Cys Val Pro Leu Tyr Gly
100 105 110

gtg act tcg att tgt ggt aga cga cca gag atg gaa gat tct gtc tca
440
Val Thr Ser Ile Cys Gly Arg Arg Pro Glu Met Glu Asp Ser Val Ser
115 120 125 130

acg att cct aga ttc ctt caa gtt tct tct agt tcg ttg ctt gat ggt
488
Thr Ile Pro Arg Phe Leu Gln Val Ser Ser Ser Ser Leu Leu Asp Gly
135 140 145

cga gtc act aat gga ttt aat cct cac ttg agt gct cat ttc ttt ggt
536
Arg Val Thr Asn Gly Phe Asn Pro His Leu Ser Ala His Phe Phe Gly
150 155 160

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gtt tac gat ggc cat ggc ggt tct cag gta gcg aat tat tgt cgt gag
584
Val Tyr Asp Gly His Gly Gly Ser Gln Val Ala Asn Tyr Cys Arg Glu
        165                 170                 175

agg atg cat ctg gct ttg acg gag gag ata gtg aag gag aaa ccg gag
632
Arg Met His Leu Ala Leu Thr Glu Glu Ile Val Lys Glu Lys Pro Glu
    180                 185                 190

ttt tgt gac ggt gac acg tgg caa gag aag tgg aag aag gct ttg ttc
680
Phe Cys Asp Gly Asp Thr Trp Gln Glu Lys Trp Lys Lys Ala Leu Phe
195                 200                 205                 210

aac tct ttt atg aga gtt gac tcg gag att gaa act gtg gct cat gct
728
Asn Ser Phe Met Arg Val Asp Ser Glu Ile Glu Thr Val Ala His Ala
                215                 220                 225

ccg gaa act gtt ggg tct acc tcg gtg gtt gcg gtt gtc ttt ccg act
776
Pro Glu Thr Val Gly Ser Thr Ser Val Val Ala Val Val Phe Pro Thr
            230                 235                 240

cac atc ttt gtc gcg aat tgc ggc gac tct agg gcg gtt ttg tgt cgc
824
His Ile Phe Val Ala Asn Cys Gly Asp Ser Arg Ala Val Leu Cys Arg
        245                 250                 255

ggc aaa acg cca ctc gcg ttg tcg gtt gat cac aaa ccg gat agg gat
872
Gly Lys Thr Pro Leu Ala Leu Ser Val Asp His Lys Pro Asp Arg Asp
    260                 265                 270

gat gaa gcg gcg agg ata gaa gct gcc ggt ggg aaa gta atc cgg tgg
920
Asp Glu Ala Ala Arg Ile Glu Ala Ala Gly Gly Lys Val Ile Arg Trp
275                 280                 285                 290

aac ggg gct cgt gta ttt ggt gtt ctc gca atg tca aga tcc att ggc
968
Asn Gly Ala Arg Val Phe Gly Val Leu Ala Met Ser Arg Ser Ile Gly
                295                 300                 305

gat aga tac ctt aaa ccg tca gta att ccg gat cca gaa gtg act tca
1016
Asp Arg Tyr Leu Lys Pro Ser Val Ile Pro Asp Pro Glu Val Thr Ser
            310                 315                 320

gtg cgg cga gta aaa gaa gat gat tgt ctc atc tta gca agt gat ggt
1064
Val Arg Arg Val Lys Glu Asp Asp Cys Leu Ile Leu Ala Ser Asp Gly
        325                 330                 335

ctt tgg gat gta atg aca aac gaa gaa gtg tgc gat ttg gct cgg aaa
1112
Leu Trp Asp Val Met Thr Asn Glu Glu Val Cys Asp Leu Ala Arg Lys
    340                 345                 350

cgg att tta cta tgg cat aag aag aac gcg atg gcc gga gag gct ttg
1160
Arg Ile Leu Leu Trp His Lys Lys Asn Ala Met Ala Gly Glu Ala Leu
355                 360                 365                 370
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ctt ccg gcg gag aaa aga gga gaa gga aaa gat cct gca gca atg tcc
1208
Leu Pro Ala Glu Lys Arg Gly Glu Gly Lys Asp Pro Ala Ala Met Ser
                375                 380                 385

gcg gca gag tat ttg tcg aag atg gct ttg caa aaa gga agc aaa gac
1256
Ala Ala Glu Tyr Leu Ser Lys Met Ala Leu Gln Lys Gly Ser Lys Asp
            390                 395                 400

aat ata agt gtg gta gtg gtt gat ttg aag gga ata agg aaa ttc aag
1304
Asn Ile Ser Val Val Val Val Asp Leu Lys Gly Ile Arg Lys Phe Lys
        405                 410                 415

agc aaa tcc ttg aat tga aaaagaaggt ttggaagaaa agtgaaaaaa
1352
Ser Lys Ser Leu Asn  *
    420

aaagttttga tggtgggtaa aaattctctt tagtgaaaaa agaaagataa aacaacaggt
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        35                  40                  45
Ser Phe Glu Ile Asn Thr Arg Gln Asp Ser Leu Thr Ser Ser Ser Ser
    50                  55                  60
Ala Met Ala Gly Val Asp Ile Ser Ala Gly Asp Glu Ile Asn Gly Ser
65                  70                  75                  80
Asp Glu Phe Asp Pro Arg Ser Met Asn Gln Ser Glu Lys Lys Val Leu
                85                  90                  95
Ser Arg Thr Glu Ser Arg Ser Leu Phe Glu Phe Lys Cys Val Pro Leu
            100                 105                 110
Tyr Gly Val Thr Ser Ile Cys Gly Arg Arg Pro Glu Met Glu Asp Ser
        115                 120                 125
Val Ser Thr Ile Pro Arg Phe Leu Gln Val Ser Ser Ser Ser Leu Leu
    130                 135                 140
Asp Gly Arg Val Thr Asn Gly Phe Asn Pro His Leu Ser Ala His Phe
145                 150                 155                 160
Phe Gly Val Tyr Asp Gly His Gly Gly Ser Gln Val Ala Asn Tyr Cys
                165                 170                 175
Arg Glu Arg Met His Leu Ala Leu Thr Glu Glu Ile Val Lys Glu Lys
            180                 185                 190
Pro Glu Phe Cys Asp Gly Asp Thr Trp Gln Glu Lys Trp Lys Lys Ala
        195                 200                 205
Leu Phe Asn Ser Phe Met Arg Val Asp Ser Glu Ile Glu Thr Val Ala
    210                 215                 220
His Ala Pro Glu Thr Val Gly Ser Thr Ser Val Val Ala Val Val Phe
225                 230                 235                 240
Pro Thr His Ile Phe Val Ala Asn Cys Gly Asp Ser Arg Ala Val Leu
                245                 250                 255
Cys Arg Gly Lys Thr Pro Leu Ala Leu Ser Val Asp His Lys Pro Asp
            260                 265                 270
Arg Asp Asp Glu Ala Ala Arg Ile Glu Ala Ala Gly Gly Lys Val Ile
        275                 280                 285
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Arg Trp Asn Gly Ala Arg Val Phe Gly Val Leu Ala Met Ser Arg Ser
    290                 295                 300
Ile Gly Asp Arg Tyr Leu Lys Pro Ser Val Ile Pro Asp Pro Glu Val
305                 310                 315                 320
Thr Ser Val Arg Arg Val Lys Glu Asp Asp Cys Leu Ile Leu Ala Ser
                325                 330                 335
Asp Gly Leu Trp Asp Val Met Thr Asn Glu Glu Val Cys Asp Leu Ala
            340                 345                 350
Arg Lys Arg Ile Leu Leu Trp His Lys Lys Asn Ala Met Ala Gly Glu
        355                 360                 365
Ala Leu Leu Pro Ala Glu Lys Arg Gly Glu Gly Lys Asp Pro Ala Ala
    370                 375                 380
Met Ser Ala Ala Glu Tyr Leu Ser Lys Met Ala Leu Gln Lys Gly Ser
385                 390                 395                 400
Lys Asp Asn Ile Ser Val Val Val Val Asp Leu Lys Gly Ile Arg Lys
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Phe Lys Ser Lys Ser Leu Asn
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417
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465
His Val Ala Ala Asn Ala Gly Asp Leu Ala Glu Asp Cys Gly Ile Leu
 5                  10                  15                  20

ggt gga gac gct gat gat act gtt ttg atg gat gga att gat gaa gtt
513
Gly Gly Asp Ala Asp Asp Thr Val Leu Met Asp Gly Ile Asp Glu Val
                25                  30                  35

ggt aga gag atc tgg tta gat gac cat gga gga gat aat aat cat gtt
561
Gly Arg Glu Ile Trp Leu Asp Asp His Gly Gly Asp Asn Asn His Val
            40                  45                  50

cat ggt cat caa gat gat gat ttg att gtt cat cat gac cct tca atc
609
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His Gly His Gln Asp Asp Asp Leu Ile Val His His Asp Pro Ser Ile
         55                  60                  65

ttc tat gga gat ctc cca acg ctt cct gat ttc cca tgc atg tcg tct
657
Phe Tyr Gly Asp Leu Pro Thr Leu Pro Asp Phe Pro Cys Met Ser Ser
     70                  75                  80

tca tca tcg tct tca aca tct cca gct cct gtc aac gca atc gtc tcc
705
Ser Ser Ser Ser Ser Thr Ser Pro Ala Pro Val Asn Ala Ile Val Ser
 85                  90                  95                 100

tca gcc tct tct tct tcg gca gct tct tcc tcc act tcc tca gct gct
753
Ser Ala Ser Ser Ser Ser Ala Ala Ser Ser Ser Thr Ser Ser Ala Ala
                105                 110                 115

tct tgg gct ata ttg aga tca gat gga gaa gat ccg act cca aac caa
801
Ser Trp Ala Ile Leu Arg Ser Asp Gly Glu Asp Pro Thr Pro Asn Gln
            120                 125                 130

aac caa tac gca tca gga aac tgt gac gac tct tct ggt gca ttg caa
849
Asn Gln Tyr Ala Ser Gly Asn Cys Asp Asp Ser Ser Gly Ala Leu Gln
        135                 140                 145

tcc aca gct tcc atg gag att cca tta gac agc agt caa ggt ttt ggt
897
Ser Thr Ala Ser Met Glu Ile Pro Leu Asp Ser Ser Gln Gly Phe Gly
    150                 155                 160

tgc ggc gaa ggc ggt ggt gat tgc att gat atg atg gag act ttc ggg
945
Cys Gly Glu Gly Gly Gly Asp Cys Ile Asp Met Met Glu Thr Phe Gly
165                 170                 175                 180

tac atg gat cta ctt gat agc aac gag ttc ttt gac acc tca gct ata
993
Tyr Met Asp Leu Leu Asp Ser Asn Glu Phe Phe Asp Thr Ser Ala Ile
                185                 190                 195

ttt agc caa gac gac gac acg caa aac cct aac ttg atg gac caa acc
1041
Phe Ser Gln Asp Asp Asp Thr Gln Asn Pro Asn Leu Met Asp Gln Thr
            200                 205                 210

ctt gag aga caa gaa gac cag gtc gtt gtt ccg atg atg gag aat aac
1089
Leu Glu Arg Gln Glu Asp Gln Val Val Val Pro Met Met Glu Asn Asn
        215                 220                 225

agt ggt gga gac atg caa atg atg aat tct tcc ttg gaa cag gac gat
1137
Ser Gly Gly Asp Met Gln Met Met Asn Ser Ser Leu Glu Gln Asp Asp
    230                 235                 240

gat ctc gct gct gtg ttt ttg gag tgg cta aag aac aac aag gag act
1185
Asp Leu Ala Ala Val Phe Leu Glu Trp Leu Lys Asn Asn Lys Glu Thr
245                 250                 255                 260

gtg tcg gct gag gat ttg agg aaa gta aag ata aag aaa gct acg att
1233
Val Ser Ala Glu Asp Leu Arg Lys Val Lys Ile Lys Lys Ala Thr Ile
                265                 270                 275
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gaa tca gcg gca aga aga cta ggc ggt ggt aaa gaa gcg atg aag cag
1281
Glu Ser Ala Ala Arg Arg Leu Gly Gly Gly Lys Glu Ala Met Lys Gln
            280                 285                 290

ctt tta aag ctg att ctt gaa tgg gtc caa act aat cac tta caa aga
1329
Leu Leu Lys Leu Ile Leu Glu Trp Val Gln Thr Asn His Leu Gln Arg
        295                 300                 305

aga cgc acc acc acc acc acc acc aac ctc tct tat caa caa tca ttc
1377
Arg Arg Thr Thr Thr Thr Thr Thr Asn Leu Ser Tyr Gln Gln Ser Phe
    310                 315                 320

caa caa gat cca ttt caa aac cct aac cct aat aac aac aac cta atc
1425
Gln Gln Asp Pro Phe Gln Asn Pro Asn Pro Asn Asn Asn Asn Leu Ile
325                 330                 335                 340

cca ccg tcc gac caa acc tgt ttc tca cct tca aca tgg gtt cct cca
1473
Pro Pro Ser Asp Gln Thr Cys Phe Ser Pro Ser Thr Trp Val Pro Pro
                345                 350                 355

cca cca caa caa caa gct ttt gtc tcg gac ccg ggt ttt gga tac atg
1521
Pro Pro Gln Gln Gln Ala Phe Val Ser Asp Pro Gly Phe Gly Tyr Met
            360                 365                 370

cct gct cca aac tat ccg cca cag cca gag ttc ctt cct tta ctt gaa
1569
Pro Ala Pro Asn Tyr Pro Pro Gln Pro Glu Phe Leu Pro Leu Leu Glu
        375                 380                 385

tct cca ccg tca tgg cca cca cca cca cag tct ggt ccc atg cca cat
1617
Ser Pro Pro Ser Trp Pro Pro Pro Pro Gln Ser Gly Pro Met Pro His
    390                 395                 400

caa caa ttc ccc atg ccg cca acc tcg cag tat aat caa ttt gga gat
1665
Gln Gln Phe Pro Met Pro Pro Thr Ser Gln Tyr Asn Gln Phe Gly Asp
405                 410                 415                 420

cca aca ggt ttc aat gga tac aac atg aat ccg tac caa tat cct tat
1713
Pro Thr Gly Phe Asn Gly Tyr Asn Met Asn Pro Tyr Gln Tyr Pro Tyr
                425                 430                 435

gtt cct gca gga caa atg aga gat cag aga tta ctc cgt ttg tgt tcc
1761
Val Pro Ala Gly Gln Met Arg Asp Gln Arg Leu Leu Arg Leu Cys Ser
            440                 445                 450

tca gca act aaa gag gca aga aag aaa cgg atg gcg aga cag agg agg
1809
Ser Ala Thr Lys Glu Ala Arg Lys Lys Arg Met Ala Arg Gln Arg Arg
        455                 460                 465

ttc ttg tct cat cac cac aga cat aac aac aac aac aac aac aac aac
1857
Phe Leu Ser His His His Arg His Asn Asn Asn Asn Asn Asn Asn Asn
    470                 475                 480
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aat aat cag cag aac caa acc caa atc gga gaa acc tgt gcc gcg gtg
1905
Asn Asn Gln Gln Asn Gln Thr Gln Ile Gly Glu Thr Cys Ala Ala Val
485 490 495 500

gct cca caa ctt aac ccc gtg gcc aca acc gcc acg gga ggg acc tgg
1953
Ala Pro Gln Leu Asn Pro Val Ala Thr Thr Ala Thr Gly Gly Thr Trp
505 510 515

atg tat tgg cct aat gtc ccg gca gtg ccg cct caa tta ccg cca gtg
2001
Met Tyr Trp Pro Asn Val Pro Ala Val Pro Pro Gln Leu Pro Pro Val
520 525 530

atg gag act cag tta cct acc atg gac cga gct ggc tca gct tct gct
2049
Met Glu Thr Gln Leu Pro Thr Met Asp Arg Ala Gly Ser Ala Ser Ala
535 540 545

atg cca cgt cag cag gtg gta cca gat cgc cgg cag gga tgg aaa cca
2097
Met Pro Arg Gln Gln Val Val Pro Asp Arg Arg Gln Gly Trp Lys Pro
550 555 560

gaa aag aat ttg cgg ttt ctc ttg cag aaa gtc ttg aag caa agc gac
2145
Glu Lys Asn Leu Arg Phe Leu Leu Gln Lys Val Leu Lys Gln Ser Asp
565 570 575 580

gtg ggt aac ctc gga agg atc gtt ttg cca aaa aaa gaa gct gag aca
2193
Val Gly Asn Leu Gly Arg Ile Val Leu Pro Lys Lys Glu Ala Glu Thr
585 590 595

cac ttg ccg gag cta gag gca aga gac ggc atc tct ctg gcc atg gaa
2241
His Leu Pro Glu Leu Glu Ala Arg Asp Gly Ile Ser Leu Ala Met Glu
600 605 610

gac atc gga acc tct cgt gtt tgg aac atg cgc tac agg ttt tgg cct
2289
Asp Ile Gly Thr Ser Arg Val Trp Asn Met Arg Tyr Arg Phe Trp Pro
615 620 625

aac aac aaa agc agg atg tat ctc ctc gag aac acc ggc gat ttt gtg
2337
Asn Asn Lys Ser Arg Met Tyr Leu Leu Glu Asn Thr Gly Asp Phe Val
630 635 640

aaa acc aat ggg ctc caa gaa ggt gat ttc ata gtc ata tac tcc gac
2385
Lys Thr Asn Gly Leu Gln Glu Gly Asp Phe Ile Val Ile Tyr Ser Asp
645 650 655 660

gtc aaa tgt ggc aaa tat ttg ata cga ggg gtt aaa gta aga caa ccg
2433
Val Lys Cys Gly Lys Tyr Leu Ile Arg Gly Val Lys Val Arg Gln Pro
665 670 675

agc gga caa aag ccg gag gcc cca ccg tcg tca gca gct acg aag aga
2481
Ser Gly Gln Lys Pro Glu Ala Pro Pro Ser Ser Ala Ala Thr Lys Arg
680 685 690

caa aac aag tcg caa agg aac ata aac aat aac tct ccg tcg gcg aat
2529

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Gln Asn Lys Ser Gln Arg Asn Ile Asn Asn Asn Ser Pro Ser Ala Asn
        695                 700                 705

gtg gtg gtc gct tca cca act tct caa act gtt aaa tga aaaacagaga
2578
Val Val Val Ala Ser Pro Thr Ser Gln Thr Val Lys  *
    710                 715                 720

caaaaagaaa caatataaat attattatgt accaaataag aaagagggca aaaggaaaaa
2638
atggcagcgt acccgagtgt gccacttctc gtgcatgcat gggatcttga agacaaatgg
2698
agggtcatga ttaaagctgt ttggtcaggg tccgggtttt tactccattt tttgcctttt
2758
cttgtcgagt cggttctttt ataactcttt actcttttta ccttcaggat attgtagaga
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2868
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<213> Arabidopsis thaliana

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            20                  25                  30
Ile Asp Glu Val Gly Arg Glu Ile Trp Leu Asp Asp His Gly Gly Asp
        35                  40                  45
Asn Asn His Val His Gly His Gln Asp Asp Asp Leu Ile Val His His
    50                  55                  60
Asp Pro Ser Ile Phe Tyr Gly Asp Leu Pro Thr Leu Pro Asp Phe Pro
65                  70                  75                  80
Cys Met Ser Ser Ser Ser Ser Ser Ser Thr Ser Pro Ala Pro Val Asn
                85                  90                  95
Ala Ile Val Ser Ser Ala Ser Ser Ser Ser Ala Ala Ser Ser Ser Thr
            100                 105                 110
Ser Ser Ala Ala Ser Trp Ala Ile Leu Arg Ser Asp Gly Glu Asp Pro
        115                 120                 125
Thr Pro Asn Gln Asn Gln Tyr Ala Ser Gly Asn Cys Asp Asp Ser Ser
    130                 135                 140
Gly Ala Leu Gln Ser Thr Ala Ser Met Glu Ile Pro Leu Asp Ser Ser
145                 150                 155                 160
Gln Gly Phe Gly Cys Gly Glu Gly Gly Gly Asp Cys Ile Asp Met Met
                165                 170                 175
Glu Thr Phe Gly Tyr Met Asp Leu Leu Asp Ser Asn Glu Phe Phe Asp
            180                 185                 190
Thr Ser Ala Ile Phe Ser Gln Asp Asp Asp Thr Gln Asn Pro Asn Leu
        195                 200                 205
Met Asp Gln Thr Leu Glu Arg Gln Glu Asp Gln Val Val Val Pro Met
    210                 215                 220
Met Glu Asn Asn Ser Gly Gly Asp Met Gln Met Met Asn Ser Ser Leu
225                 230                 235                 240
Glu Gln Asp Asp Asp Leu Ala Ala Val Phe Leu Glu Trp Leu Lys Asn
                245                 250                 255
Asn Lys Glu Thr Val Ser Ala Glu Asp Leu Arg Lys Val Lys Ile Lys
            260                 265                 270
Lys Ala Thr Ile Glu Ser Ala Ala Arg Arg Leu Gly Gly Gly Lys Glu
        275                 280                 285
Ala Met Lys Gln Leu Leu Lys Leu Ile Leu Glu Trp Val Gln Thr Asn
    290                 295                 300
His Leu Gln Arg Arg Arg Thr Thr Thr Thr Thr Thr Asn Leu Ser Tyr
305                 310                 315                 320
Gln Gln Ser Phe Gln Gln Asp Pro Phe Gln Asn Pro Asn Pro Asn Asn
                325                 330                 335
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Asn Asn Leu Ile Pro Pro Ser Asp Gln Thr Cys Phe Ser Pro Ser Thr
            340                 345                 350
Trp Val Pro Pro Pro Pro Gln Gln Gln Ala Phe Val Ser Asp Pro Gly
        355                 360                 365
Phe Gly Tyr Met Pro Ala Pro Asn Tyr Pro Pro Gln Pro Glu Phe Leu
    370                 375                 380
Pro Leu Leu Glu Ser Pro Pro Ser Trp Pro Pro Pro Pro Gln Ser Gly
385                 390                 395                 400
Pro Met Pro His Gln Gln Phe Pro Met Pro Pro Thr Ser Gln Tyr Asn
                405                 410                 415
Gln Phe Gly Asp Pro Thr Gly Phe Asn Gly Tyr Asn Met Asn Pro Tyr
            420                 425                 430
Gln Tyr Pro Tyr Val Pro Ala Gly Gln Met Arg Asp Gln Arg Leu Leu
        435                 440                 445
Arg Leu Cys Ser Ser Ala Thr Lys Glu Ala Arg Lys Lys Arg Met Ala
    450                 455                 460
Arg Gln Arg Arg Phe Leu Ser His His His Arg His Asn Asn Asn Asn
465                 470                 475                 480
Asn Asn Asn Asn Asn Asn Gln Gln Asn Gln Thr Gln Ile Gly Glu Thr
                485                 490                 495
Cys Ala Ala Val Ala Pro Gln Leu Asn Pro Val Ala Thr Thr Ala Thr
            500                 505                 510
Gly Gly Thr Trp Met Tyr Trp Pro Asn Val Pro Ala Val Pro Pro Gln
        515                 520                 525
Leu Pro Pro Val Met Glu Thr Gln Leu Pro Thr Met Asp Arg Ala Gly
    530                 535                 540
Ser Ala Ser Ala Met Pro Arg Gln Gln Val Val Pro Asp Arg Arg Gln
545                 550                 555                 560
Gly Trp Lys Pro Glu Lys Asn Leu Arg Phe Leu Leu Gln Lys Val Leu
                565                 570                 575
Lys Gln Ser Asp Val Gly Asn Leu Gly Arg Ile Val Leu Pro Lys Lys
            580                 585                 590
Glu Ala Glu Thr His Leu Pro Glu Leu Glu Ala Arg Asp Gly Ile Ser
        595                 600                 605
Leu Ala Met Glu Asp Ile Gly Thr Ser Arg Val Trp Asn Met Arg Tyr
    610                 615                 620
Arg Phe Trp Pro Asn Asn Lys Ser Arg Met Tyr Leu Leu Glu Asn Thr
625                 630                 635                 640
Gly Asp Phe Val Lys Thr Asn Gly Leu Gln Glu Gly Asp Phe Ile Val
                645                 650                 655
Ile Tyr Ser Asp Val Lys Cys Gly Lys Tyr Leu Ile Arg Gly Val Lys
            660                 665                 670
Val Arg Gln Pro Ser Gly Gln Lys Pro Glu Ala Pro Pro Ser Ser Ala
        675                 680                 685
Ala Thr Lys Arg Gln Asn Lys Ser Gln Arg Asn Ile Asn Asn Asn Ser
    690                 695                 700
Pro Ser Ala Asn Val Val Val Ala Ser Pro Thr Ser Gln Thr Val Lys
705                 710                 715                 720

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<211> 1500
<212> DNA
<213> Arabidopsis thaliana

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<223> ABI4 cDNA (Genbank Accession No. AF040959)

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gtcctctcca ctatttttct caatttcttt aatctctctc tttctctctc tacttcctct
120
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tcctcttctt cttcttcttc ttcttcatct atg gac cct tta gct tcc caa cat
174
                                  Met Asp Pro Leu Ala Ser Gln His
                                   1               5

caa cac aac cat ctg gaa gat aat aac caa acc cta acc cat aat aat
222
Gln His Asn His Leu Glu Asp Asn Asn Gln Thr Leu Thr His Asn Asn
     10                  15                  20

cct caa tcc gat tcc acc acc gac tca tca act tcc tcc gct caa cgc
270
Pro Gln Ser Asp Ser Thr Thr Asp Ser Ser Thr Ser Ser Ala Gln Arg
 25                  30                  35                  40

aaa cgc aaa ggc aaa ggt ggt ccg gac aac tcc aag ttc cgt tac cgt
318
Lys Arg Lys Gly Lys Gly Gly Pro Asp Asn Ser Lys Phe Arg Tyr Arg
                 45                  50                  55

ggc gtt cga caa aga agc tgg ggc aaa tgg gtc gcc gag atc cga gag
366
Gly Val Arg Gln Arg Ser Trp Gly Lys Trp Val Ala Glu Ile Arg Glu
             60                  65                  70

cca cgt aag cgc act cgc aag tgg ctt ggt act ttc gca acc gcc gaa
414
Pro Arg Lys Arg Thr Arg Lys Trp Leu Gly Thr Phe Ala Thr Ala Glu
         75                  80                  85

gac gcc gca cgt gcc tac gac cgg gct gcc gtt tac cta tac ggg tca
462
Asp Ala Ala Arg Ala Tyr Asp Arg Ala Ala Val Tyr Leu Tyr Gly Ser
     90                  95                  100

cgt gct cag ctc aac tta acc cct tcg tct cct tcc tcc gtc tct tcc
510
Arg Ala Gln Leu Asn Leu Thr Pro Ser Ser Pro Ser Ser Val Ser Ser
105                 110                 115                 120

tct tcc tcc tcc gtc tcc gcc gct tct tct cct tcc acc tcc tct tcc
558
Ser Ser Ser Ser Val Ser Ala Ala Ser Ser Pro Ser Thr Ser Ser Ser
                125                 130                 135

tcc act caa acc cta aga cct ctc ctc cct cgc ccc gcc gcc gcc acc
606
Ser Thr Gln Thr Leu Arg Pro Leu Leu Pro Arg Pro Ala Ala Ala Thr
            140                 145                 150

gta gga gga gga gcc aac ttt ggt ccg tac ggt atc cct ttt aac aac
654
Val Gly Gly Gly Ala Asn Phe Gly Pro Tyr Gly Ile Pro Phe Asn Asn
        155                 160                 165

aac atc ttc ctt aat ggt ggg acc tct atg tta tgc cct agt tat ggt
702
Asn Ile Phe Leu Asn Gly Gly Thr Ser Met Leu Cys Pro Ser Tyr Gly
    170                 175                 180

ttt ttc cct caa caa caa caa caa caa aat cag atg gtc cag atg gga
750
Phe Phe Pro Gln Gln Gln Gln Gln Gln Asn Gln Met Val Gln Met Gly
185                 190                 195                 200

caa ttc caa cac caa cag tat cag aat ctt cat tct aat act aac aat
798
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Gln Phe Gln His Gln Gln Tyr Gln Asn Leu His Ser Asn Thr Asn Asn
                205                 210                 215

aac aag att tct gac atc gag ctc act gat gtt ccg gta act aat tcg
846
Asn Lys Ile Ser Asp Ile Glu Leu Thr Asp Val Pro Val Thr Asn Ser
            220                 225                 230

act tcg ttt cat cat gag gtg gcg tta ggg cag gaa caa gga gga agt
894
Thr Ser Phe His His Glu Val Ala Leu Gly Gln Glu Gln Gly Gly Ser
        235                 240                 245

ggg tgt aat aat aat agt tcg atg gag gat ttg aac tct cta gct ggt
942
Gly Cys Asn Asn Asn Ser Ser Met Glu Asp Leu Asn Ser Leu Ala Gly
    250                 255                 260

tcg gtg ggt tcg agt cta tca ata act cat cca ccg ccg ttg gtt gat
990
Ser Val Gly Ser Ser Leu Ser Ile Thr His Pro Pro Pro Leu Val Asp
265                 270                 275                 280

ccg gta tgt tct atg ggt ctg gat ccg ggt tat atg gtt gga gat gga
1038
Pro Val Cys Ser Met Gly Leu Asp Pro Gly Tyr Met Val Gly Asp Gly
                285                 290                 295

tct tcg acc att tgg cct ttt gga gga gaa gaa gaa tat agt cat aat
1086
Ser Ser Thr Ile Trp Pro Phe Gly Gly Glu Glu Glu Tyr Ser His Asn
            300                 305                 310

tgg ggg agt att tgg gat ttt att gat ccc atc ttg ggg gaa ttc tat
1134
Trp Gly Ser Ile Trp Asp Phe Ile Asp Pro Ile Leu Gly Glu Phe Tyr
        315                 320                 325

taa tttgtttttg tggaagatca tattatatac gatgagcatc cctaaggtcg
1187
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gtcaagagca ttggagattc attgttgaga ggaatcaaag agattgcatt ctatgaggag
1247
ctctgcatgc aaaattttgg aggatttttt tactacctat agagataaat aagagggtat
1307
ttttattatt tttttgaaga tttttatttt caaggaattc gtaaaagaga ttacggttcc
1367
aataaagtat gtatatgtgg aagagaatcg gaggagatgg tggaaagttg tatgggaatt
1427
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1487
atgataattt ctg
1500

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<211> 328
<212> PRT
<213> Arabidopsis thaliana

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Asn Gln Thr Leu Thr His Asn Asn Pro Gln Ser Asp Ser Thr Thr Asp
            20                  25                  30
Ser Ser Thr Ser Ser Ala Gln Arg Lys Arg Lys Gly Lys Gly Gly Pro
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        35                  40                  45
Asp Asn Ser Lys Phe Arg Tyr Arg Gly Val Arg Gln Arg Ser Trp Gly
    50                  55                  60
Lys Trp Val Ala Glu Ile Arg Glu Pro Arg Lys Arg Thr Arg Lys Trp
65                  70                  75                  80
Leu Gly Thr Phe Ala Thr Ala Glu Asp Ala Ala Arg Ala Tyr Asp Arg
                85                  90                  95
Ala Ala Val Tyr Leu Tyr Gly Ser Arg Ala Gln Leu Asn Leu Thr Pro
            100                 105                 110
Ser Ser Pro Ser Ser Val Ser Ser Ser Ser Ser Ser Val Ser Ala Ala
        115                 120                 125
Ser Ser Pro Ser Thr Ser Ser Ser Ser Thr Gln Thr Leu Arg Pro Leu
    130                 135                 140
Leu Pro Arg Pro Ala Ala Ala Thr Val Gly Gly Gly Ala Asn Phe Gly
145                 150                 155                 160
Pro Tyr Gly Ile Pro Phe Asn Asn Asn Ile Phe Leu Asn Gly Gly Thr
                165                 170                 175
Ser Met Leu Cys Pro Ser Tyr Gly Phe Phe Pro Gln Gln Gln Gln Gln
            180                 185                 190
Gln Asn Gln Met Val Gln Met Gly Gln Phe Gln His Gln Gln Tyr Gln
        195                 200                 205
Asn Leu His Ser Asn Thr Asn Asn Asn Lys Ile Ser Asp Ile Glu Leu
    210                 215                 220
Thr Asp Val Pro Val Thr Asn Ser Thr Ser Phe His His Glu Val Ala
225                 230                 235                 240
Leu Gly Gln Glu Gln Gly Gly Ser Gly Cys Asn Asn Asn Ser Ser Met
                245                 250                 255
Glu Asp Leu Asn Ser Leu Ala Gly Ser Val Gly Ser Ser Leu Ser Ile
            260                 265                 270
Thr His Pro Pro Pro Leu Val Asp Pro Val Cys Ser Met Gly Leu Asp
        275                 280                 285
Pro Gly Tyr Met Val Gly Asp Gly Ser Ser Thr Ile Trp Pro Phe Gly
    290                 295                 300
Gly Glu Glu Glu Tyr Ser His Asn Trp Gly Ser Ile Trp Asp Phe Ile
305                 310                 315                 320
Asp Pro Ile Leu Gly Glu Phe Tyr
                325
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<210> 11
<211> 286
<212> DNA
<213> Glycine max

<220>
<221> promoter
<222> (0)...(0)
<223> Oleosin promoter (Genbank Accession No. U71381)

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ccaacaccaa caccaccacc acctgcgaaa ctgtacgtat ctcaattgtc cttaataaaa
120
atgtaaatag tacattattc tccttgcctg tcattattta tgtgccccca gcttaatttt
180
tctgatgtac ttaacccagg gcaaaactga aacaagttcc tcatgcaaag ccccaactca
240
tcatgcatca tgtaccgtgt catcatccag caactccact tttgca
286

<210> 12
<211> 940
<212> DNA
<213> Brassica juncea

<220>
<221> promoter

<222> (0)...(0)
<223> Oleosin promoter (Genbank Accession No. AF134411)

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cccagaagac atcgagtaag ctctcgaagc tgacctcttg cggatcgtac tgaacccgaa
120
caatctcgtt atgtcccgtc gtctccgaac agacatcctc gtatctcgga ttatcgacta
180
atccatggct atacccaacc tccgtcttcg tcacgcctgg aaccctctgg tacgccaatt
240
ccgctcccca gaaacaaccg gcgccgaatt gcgcgaattg ctgacctggg agacggaaca
300
tcgtcgtcgg gtccttgcgc gattgcggcg gaagccgggt cgggttgggg acgaaaccga
360
atccgagcct ggtgaatagg ttgttcatcg gagatttata gacggagatg gatctagcgt
420
tttgggaaag ggaagtggtt tggctctttt ggatagagag agtgcagctt tggagagaga
480
ctggagaggt ttagagagag acgcggcgga gattaccgga ggagaggcga cgagagatag
540
cattatcgaa gggaagggag aaagagtgac gtggagaaat aagaaaccgt taagagtcgg
600
atatttatta tattaaaagc ccaatgggcc taaacccatt taaacaagac aagataaatg
660
ggccgtgtgg taacagagtg ttacgttcgg cttcaaatgc caacgccata ggaacaaaac
720
aaacgtgtcc tcaagtaaac ccctgccgtt tacacctcaa tgactgcatg gtgaagccat
780
taacacgtgg cgtaggatgc atgacgacgc cattgacacc tgactttctt cccttctctt
840
catatatctc taatcaattc aactactcac agtcatagct attcggaaaa tacatacaca
900
tccttttctc ttcgatctct ctcaattcac aagaagcaaa
940

<210> 13
<211> 807
<212> DNA
<213> Hordeum vulgare

<220>
<221> promoter
<222> (0)...(0)
<223> lpt2 promoter

<400> 13
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taaaaataag ccgatgagga taaataaaat gtggtggtac agtacttcaa gaggtttact
120
catcaagagg atgcttttcc gatgagctct agtagtacat cggacctcac atacctccat
180
tgtggtgaaa tattttgtgc tcatttagtg atgggtaaat tttgtttatg tcactctagg
240
ttttgacatt tcagttttgc cactcttagg ttttgacaaa taatttccat tccgcggcaa
300
aagcaaaaca attttatttt acttttacca ctcttagctt tcacaatgta tcacaaatgc
360
cactctagaa attctgttta tgccacagaa tgtgaaaaaa aacactcact tatttgaagc
420
caaggtgttc atggcatgga aatgtgacat aaagtaacgt tcgtgtataa gaaaaaattg
480
tactcctcgt aacaagagac ggaaacatca tgagacaatc gcgtttggaa ggctttgcat
540

caccttttgga tgatgcgcat gaatggagtc gtctgcttgc tagccttcgc ctaccgccca
600
ctgagtccgg gcggcaacta ccatcggcga acgacccagc tgacctctac cgaccggact
660
tgaatgcgct accttcgtca gcgacgatgg ccgcgtacgc tggcgacgtg cccccgcatg
720
catggcggca catggcgagc tcagaccgtg cgtggctggc tacaaatacg taccccgtga
780
gtgccctagc tagaaactta cacctgc
807