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(57) **Abrégé/Abstract:**

The present invention concerns a method for modifying the growth characteristics of plants by modifying expression in a plant of a nucleic acid sequence encoding a 2xC2H2 zinc finger protein and/or modifying level and/or activity in a plant of a 2xC2H2 zinc finger protein. The invention also relates to transgenic plants having modified growth characteristics, which plants have modified expression of a nucleic acid encoding a 2xC2H2 zinc finger protein. For example yield of crop plants are improved by the methods of the present invention.



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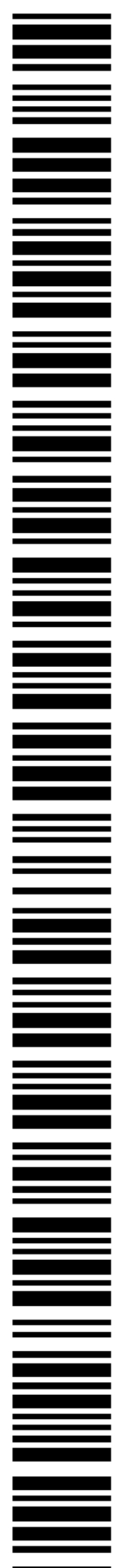
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(54) Title: PLANTS HAVING MODIFIED GROWTH CHARACTERISTICS AND A METHOD FOR MAKING THE SAME

(57) Abstract: The present invention concerns a method for modifying the growth characteristics of plants by modifying expression in a plant of a nucleic acid sequence encoding a 2xC2H2 zinc finger protein and/or modifying level and/or activity in a plant of a 2xC2H2 zinc finger protein. The invention also relates to transgenic plants having modified growth characteristics, which plants have modified expression of a nucleic acid encoding a 2xC2H2 zinc finger protein. For example yield of crop plants are improved by the methods of the present invention.



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## **Plants having modified growth characteristics and a method for making the same**

The present invention concerns a method for modifying plant growth characteristics. More specifically, the present invention concerns a method for modifying the growth characteristics of a plant by modifying expression of a nucleic acid encoding a zinc finger protein and/or by modifying the level and/or activity of a zinc finger protein in a plant, which zinc finger protein has two zinc finger domains of the type C2H2 (2xC2H2). The present invention also concerns plants having modified expression of a nucleic acid encoding a 2xC2H2 zinc finger protein and/or modified levels and/or activity of a 2xC2H2 zinc finger protein, which plants have modified growth characteristics relative to corresponding wild type plants.

Given the ever-increasing world population, it remains a major goal of agricultural research to improve the efficiency of agriculture. Conventional means for crop and horticultural improvements utilise selective breeding techniques to identify plants having desirable characteristics. However, such selective breeding techniques have several drawbacks, namely that these techniques are typically labour intensive and result in plants that often contain heterogeneous genetic components that may not always result in the desirable trait being passed on from parent plants. Advances in molecular biology have allowed mankind to modify the germplasm of animals and plants in a specific and controlled way. Genetic engineering of plants entails the isolation and manipulation of genetic material (typically in the form of DNA or RNA) and the subsequent introduction of that genetic material into a plant. Such technology has led to the development of plants having various improved economic, agronomic or horticultural traits. A trait or growth characteristic of particular economic interest is high yield. Yield is normally defined as the measurable produce of economic value from a crop. This may be defined in terms of quantity and/or quality. Other important growth characteristics include modified architecture, modified growth rate, among others.

The ability to influence one or more of the abovementioned growth characteristics, would have many applications in areas such as crop enhancement, plant breeding, production of ornamental plants, arboriculture, horticulture, forestry, production of algae or plants (for example for use as bioreactors, for the production of substances such as pharmaceuticals, antibodies, or vaccines, or for the bioconversion of organic waste or for use as fuel in the case of high-yielding algae and plants).

The term "zinc finger" describes a nucleic acid-binding domain in a protein that is folded around a tetrahedrally coordinated Zinc ion (Miller et al. 1985. EMBO, 4, 1609-1614). The amino acids that coordinate the zinc ion, are always cysteine or histidine residues, however, diversity occurs in the sequence and length of the zinc finger domain. Zinc finger proteins may contain several zinc finger domains of the same or different type. Further variability is encountered in nature by association of zinc finger domains with other domains. For example, some zinc finger proteins are found in association with ring finger or coil-coil domains, to form a so-called tripartite domain. There are several types of zinc fingers, such as C2H2, C2HC, C2C2. C2H2 is known as the classical zinc finger domain. There are typically two criteria used to classify zinc finger proteins, the first being the type of zinc finger and the second being the number of zinc fingers present in the protein. Zinc finger proteins having a single C2H2 domain have been characterised, for example Superman from *Arabidopsis* and Ramosa I from maize. A well-characterised zinc finger protein having three C2H2 domains is the Indeterminate 1 protein from Maize. Although the first report of this gene (Colasanti et al., Cell. 1998 May 15;93(4):593-603) only mentions the presence of two zinc finger domains, a more sophisticated analysis, using pFAM domain search, revealed the presence of three C2H2 zinc finger domains. Also known are zinc-finger proteins having only two C2H2 domains, for example ZAT10 (STZ) and SCOF-1. This subset of plant zinc finger proteins having two C2H2 domains have been implicated in plant responses to various stresses (Sakamoto et al., Gene 248 (1-2) 23-32 (2000)). Both STZ and SCOF-1 have been used to enhance abiotic stress tolerance. When over-expressed, STZ has been reported to increase salt tolerance in yeast (Lippuner et al., J Biol Chem. 271 (22) 12859-12866 (1996)) and over-expression of the SCOF-1 gene under control of the CaMV 35 S promoter has been reported to enhance cold tolerance in *Arabidopsis thaliana* (Kim et al., Plant J. 25 (3) 247-259 (2001)). Reports of plants having modified expression of a zinc finger encoding gene (whether the zinc finger gene is mutated, over-expressed or otherwise) describe plants having abnormal growth characteristics, none of which (with the exception of cold stress tolerance in transgenic plants expressing SCOF-1) are desirable for crops or describe effects that are only detectable under particular stress conditions.

It has now been found that modifying expression in a plant of a 2xC2H2 zinc finger gene and/or modifying the level and/or activity in a plant of a 2xC2H2 zinc finger protein gives plants having modified growth characteristics. In particular it has been found that introduction into a plant of a 2xC2H2 zinc finger nucleic acid gives plants modified growth characteristics, such as increased yield, modified leaf architecture and altered cycle time, each relative to wild type plants.

Therefore according to one embodiment of the present invention there is provided a method for modifying the growth characteristics of a plant, comprising modifying expression in a plant of a nucleic acid encoding a 2xC2H2 zinc finger protein and/or modifying level and/or activity in a plant of a 2xC2H2 zinc finger protein.

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The term "modifying" as used herein is taken to mean enhancing, decreasing and/or changing in place and/or time. Modifying expression of a nucleic acid encoding a 2xC2H2 zinc finger protein or modifying the level and/or activity of the 2xC2H2 zinc finger protein itself encompasses altered expression of a gene and/or altered level and/or activity of a gene product, namely a polypeptide, in specific cells or tissues, when compared to expression, level and/or activity of a 2xC2H2 zinc finger gene or protein in corresponding wild-type plants. The modified gene expression may result from modified expression of an endogenous 2xC2H2 zinc finger gene and/or may result from modified expression of a 2xC2H2 zinc finger gene previously introduced into a plant. Similarly, modified levels and/or activity of a 2xC2H2 zinc finger protein may be due to modified expression of an endogenous 2xC2H2 zinc finger nucleic acid/gene and/or due to modified expression of a 2xC2H2 zinc finger nucleic acid/gene previously introduced into a plant. Modified expression of a gene/nucleic acid and/or modified level and/or activity of a gene product/protein may be effected, for example, by chemical means and/or recombinant means.

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Therefore there is provided by the present invention, a method for modifying the growth characteristics of a plant, comprising modifying expression, level and/or activity of a 2xC2H2 zinc finger gene or protein by recombinant means and/or by chemical means.

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Advantageously, modifying expression of a nucleic acid encoding a 2xC2H2 zinc finger protein and/or modifying level and/or activity of the 2xC2H2 zinc finger protein itself may be effected by chemical means, i.e. by exogenous application of one or more compounds or elements capable of modifying activity of the 2xC2H2 zinc finger protein and/or capable of modifying expression of a 2xC2H2 zinc finger gene (which may be either an endogenous gene or a transgene introduced into a plant). The term "exogenous application" as defined herein is taken to mean the contacting or administering of a suitable compound or element to a plant. The compound or element may be exogenously applied to a plant in a form suitable for plant uptake (such as through application to the soil for uptake via the roots, or in the case of some plants by applying directly to the leaves, for example by spraying). The exogenous application may take place on wild-type plants or on transgenic plants that have previously been transformed with a 2xC2H2 zinc finger nucleic acid/gene or other transgene.

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Suitable compounds or elements for exogenous application include 2xC2H2 zinc finger proteins or 2xC2H2 zinc finger nucleic acids. Alternatively, exogenous application of compounds or elements capable of modifying levels of factors that directly or indirectly activate or inactivate a 2xC2H2 zinc finger protein will also be suitable in practising the invention. Also  
5 included are antibodies that can recognise or mimic the function of 2xC2H2 zinc finger proteins. Such antibodies may comprise "plantibodies", single chain antibodies, IgG antibodies and heavy chain camel antibodies, as well as fragments thereof.

Additionally or alternatively, the resultant effect may also be achieved by the exogenous  
10 application of an interacting protein or activator or an inhibitor of a 2xC2H2 zinc finger gene/gene product. Additionally or alternatively, the compound or element may be a mutagenic substance, such as a chemical selected from any one or more of: N-nitroso-N-ethylurea, ethylene imine, ethyl methanesulphonate and diethyl sulphate. Mutagenesis may also be achieved by exposure to ionising radiation, such as X-rays or gamma-rays or ultraviolet light.  
15 Methods for introducing mutations and for testing the effect of mutations (such as by monitoring gene expression and/or protein activity) are well known in the art.

Additionally or alternatively, and according to a preferred embodiment of the present invention, modifying expression of a nucleic acid encoding a 2xC2H2 zinc finger protein and/or modifying  
20 level and/or activity of the 2xC2H2 zinc finger protein may be effected by recombinant means. Such recombinant means may comprise a direct and/or indirect approach for modifying expression of a nucleic acid and/or level and/or activity of a protein.

For example, an indirect approach may comprise introducing, into a plant, a nucleic acid  
25 capable of modifying expression of the gene in question (a gene encoding a 2xC2H2 zinc finger protein) and or capable of modifying the level and/or activity of the protein in question (a 2xC2H2 zinc finger protein). Examples of such nucleic acids to be introduced into a plant include nucleic acids encoding transcription factors or activators or inhibitors that bind to the promoter of a 2xC2H2 zinc finger gene or that interact with a 2xC2H2 zinc finger protein.  
30 Methods to test these types of interactions and methods for isolating nucleic acids encoding such interactors include yeast one-hybrid or yeast two-hybrid screens in which the 2xC2H2 zinc finger gene/protein is used as bait. One example of such a transcription regulator is LOS2, described as a transcription regulator for the STZ gene. Therefore, the method of the invention may also be performed using LOS2, wherein expression of a 2xC2H2 zinc finger gene may be  
35 increased or further increased by decreasing expression of LOS2 in plants.

Also encompassed by an indirect approach for modifying expression of a 2xC2H2 zinc finger gene and/or for modifying level and/or activity of a 2xC2H2 zinc finger protein is the provision of, or the inhibition or stimulation of regulatory sequences that drive expression of a native 2xC2H2 zinc finger gene or transgene. Such regulatory sequences may be introduced into a  
5 plant. For example, the regulatory sequence to be introduced into a plant may be a promoter capable of driving expression of an endogenous 2xC2H2 zinc finger gene.

A further indirect approach for modifying expression of a 2xC2H2 zinc finger gene and/or for modifying level and/or activity of a 2xC2H2 zinc finger protein in a plant encompasses  
10 modifying levels in a plant of a factor capable of interacting with a zinc finger protein. Such factors may include ligands of a 2xC2H2 zinc finger protein. Therefore, the present invention also provides a method for modifying growth characteristics of a plant, comprising modifying expression of a gene coding for a protein which is a natural ligand of a 2xC2H2 zinc finger protein. Furthermore, the present invention also provides a method for modifying growth  
15 characteristics of a plant, comprising modifying expression of a gene coding for a protein which is a natural target/substrate of a 2xC2H2 zinc finger protein. Examples of such targets/substrates include stretches of DNA that are bound by the zinc-finger domains.

A direct and preferred approach on the other hand comprises introducing into a plant a nucleic  
20 acid encoding a 2xC2H2 zinc finger protein or a portion thereof or sequences capable of hybridising therewith, which nucleic acid preferably encodes a 2xC2H2 zinc finger protein or a homologue, derivative or active fragment thereof. The nucleic acid may be introduced into a plant by, for example, transformation.

25 Therefore, there is provided a method for modifying growth characteristics of a plant, comprising introducing into a plant a 2xC2H2 zinc finger nucleic acid or a portion thereof.

The 2xC2H2 zinc finger nucleic acid may be derived (either directly or indirectly (if subsequently modified)) from any source provided that the sequence, when expressed in a  
30 plant, leads to modified expression of a 2xC2H2 zinc finger-encoding nucleic acid/gene and/or modified level and/or activity of a 2xC2H2 zinc finger protein. The 2xC2H2 zinc finger gene or protein may be wild type, i.e. the native or endogenous nucleic acid or polypeptide. Alternatively, it may be a protein or nucleic acid derived from the same or another species. The nucleic acid/gene may then be introduced into a plant as a transgene, for example by  
35 transformation.

The nucleic acid may be isolated from a bacteria, yeast or fungi, or from a plant, algae, insect or animal (including human) source. This nucleic acid may be substantially modified from its native form in composition and/or genomic environment through deliberate human manipulation. The nucleic acid is preferably obtained from a plant, whether from the same plant species in which it is to be introduced or whether from a different plant species. Further preferably, the nucleic acid is from a dicot, preferably from the family *Brassicaceae*, further preferably from *Arabidopsis thaliana*. More preferably, the nucleic acid is essentially similar to a nucleic acid as represented by SEQ ID NO 1, or a portion of SEQ ID NO 1, or a nucleic acid capable of hybridising therewith or is a nucleic acid encoding an amino acid sequence essentially similar to an amino acid as represented by SEQ ID NO 2, or a homologue, derivative or active fragment thereof.

Advantageously, the methods according to the invention may also be practised using variant 2xC2H2 zinc finger nucleic acids and variant 2xC2H2 zinc finger amino acids, preferably wherein the variant nucleic acids are variants of SEQ ID NO 1 and wherein the variant amino acids are variants of SEQ ID NO 2. Examples of variant sequences suitable in performing the methods of the invention include:

- (i) Functional portions of a 2xC2H2 zinc finger nucleic acid/gene;
- (ii) Sequences capable of hybridising with a 2xC2H2 zinc finger nucleic acid/gene;
- (iii) Alternative splice variants of a 2xC2H2 zinc finger nucleic acid/gene;
- (iv) Allelic variants of a 2xC2H2 zinc finger nucleic acid/gene;
- (v) Homologues, derivatives and active fragments of a 2xC2H2 zinc finger protein.

The abovementioned variants may also be described as being "essentially similar" to a 2xC2H2 zinc finger nucleic acid/gene, particularly to the 2xC2H2 zinc finger encoding nucleic acid of SEQ ID NO 1, or essentially similar to a 2xC2H2 zinc finger amino acid/protein, particularly that of SEQ ID NO 2. The term "essentially similar to" also includes variants of SEQ ID NO 1 in the form of a complement, DNA, RNA, cDNA or genomic DNA. The variant nucleic acid encoding a 2xC2H2 zinc finger protein or the variant of a 2xC2H2 zinc finger protein may be synthesized in whole or in part, it may be a double-stranded nucleic acid or a single-stranded nucleic acid. Also, the term encompasses a variant due to the degeneracy of the genetic code; a family member of the gene or protein; and variants that are interrupted by one or more intervening sequences.

An example of a variant 2xC2H2 zinc finger nucleic acid is a functional portion of a 2xC2H2 zinc-finger gene. Advantageously, the method according to the present invention may also be practised using portions of a DNA or nucleic acid encoding a 2xC2H2 zinc finger protein. A

functional portion refers to a piece of DNA derived or prepared from an original (larger) DNA molecule, which DNA portion, when expressed in a plant, gives plants having modified growth characteristics. The portion may comprise many genes, with or without additional control elements or may contain spacer sequences. The portion may be made by making one or more deletions and/or truncations to the nucleic acid. Techniques for introducing truncations and deletions into a nucleic acid are well known in the art. Portions suitable for use in the methods according to the invention may readily be determined by following the methods described in the Examples section by simply substituting the sequence used in the actual Example with the portion to be tested for functionality.

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An example of a further variant 2xC2H2 zinc finger nucleic acid is a sequence that is capable of hybridising to a 2xC2H2 zinc finger nucleic acid, for example to any of SEQ ID NO 1, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 41, 43, 45, 47 or 49. Advantageously, the methods according to the present invention may also be practised using these variants.

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Hybridising sequences suitable for use in the methods according to the invention may readily be determined for example by following the methods described in the Examples section by simply substituting the sequence used in the actual Example with the hybridising sequence.

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The term "hybridisation" as defined herein is a process wherein substantially homologous complementary nucleotide sequences anneal to each other. The hybridisation process can occur entirely in solution, i.e. both complementary nucleic acids are in solution. Tools in molecular biology relying on such a process include the polymerase chain reaction (PCR; and all methods based thereon), subtractive hybridisation, random primer extension, nuclease S1 mapping, primer extension, reverse transcription, cDNA synthesis, differential display of RNAs, and DNA sequence determination. The hybridisation process can also occur with one of the complementary nucleic acids immobilised to a matrix such as magnetic beads, Sepharose beads or any other resin. Tools in molecular biology relying on such a process include the isolation of poly (A+) mRNA. The hybridisation process can furthermore occur with one of the complementary nucleic acids immobilised to a solid support such as a nitro-cellulose or nylon membrane or immobilised by e.g. photolithography to, for example, a siliceous glass support (the latter known as nucleic acid arrays or microarrays or as nucleic acid chips). Tools in molecular biology relying on such a process include RNA and DNA gel blot analysis, colony hybridisation, plaque hybridisation, *in situ* hybridisation and microarray hybridisation. In order to allow hybridisation to occur, the nucleic acid molecules are generally thermally or chemically denatured to melt a double strand into two single strands and/or to remove hairpins or other secondary structures from single stranded nucleic acids. The stringency of hybridisation is influenced by conditions such as temperature, salt concentration and hybridisation buffer

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composition. High stringency conditions for hybridisation include high temperature and/or low salt concentration (salts include NaCl and Na<sub>3</sub>-citrate) and/or the inclusion of formamide in the hybridisation buffer and/or lowering the concentration of compounds such as SDS (detergent) in the hybridisation buffer and/or exclusion of compounds such as dextran sulphate or polyethylene glycol (promoting molecular crowding) from the hybridisation buffer. Conventional hybridisation conditions are described in, for example, Sambrook (2001) *Molecular Cloning: a laboratory manual*, 3rd Edition Cold Spring Harbor Laboratory Press, CSH, New York, but the skilled craftsman will appreciate that numerous different hybridisation conditions may be designed in function of the known or the expected homology and/or length of the nucleic acid sequence. Sufficiently low stringency hybridisation conditions are particularly preferred (at least in the first instance) to isolate nucleic acids heterologous to the DNA sequences of the invention defined supra. An example of low stringency conditions is 4-6x SSC / 0.1-0.5% w/v SDS at 37-45°C for 2-3 hours. Depending on the source and concentration of the nucleic acid involved in the hybridisation, alternative conditions of stringency may be employed, such as medium stringency conditions. Examples of medium stringency conditions include 1-4x SSC / 0.25% w/v SDS at  $\geq 45^{\circ}\text{C}$  for 2-3 hours. An example of high stringency conditions includes 0.1 to 2x SSC / 0.1% w/v SDS at 60°C for 1-3 hours. The skilled man will be aware of various parameters which may be altered during hybridisation and washing and which will either maintain or change the stringency conditions. The stringency conditions may start low and be progressively increased until there is provided a hybridising nucleic acid, as defined hereinabove. Elements contributing to heterology include allelism, degeneration of the genetic code and differences in preferred codon usage.

Another variant 2xC2H2 zinc finger nucleic acid useful in practising the methods according to the present invention is an alternative splice variant of a nucleic acid sequence encoding a 2xC2H2 zinc finger protein. The term "alternative splice variant" as used herein encompasses variants of a nucleic acid sequence in which selected introns and/or exons have been excised, replaced or added. Such splice variants may be found in nature or may be manmade. Methods for making such splice variants are well known in the art. Splice variants suitable for use in the methods according to the invention may readily be determined for example by following the methods described in the Examples section by simply substituting the sequence used in the actual Example with the splice variant.

Another variant 2xC2H2 zinc finger nucleic acid useful in practising the methods according to the present invention is an allelic variant of a nucleic acid encoding a 2xC2H2 zinc finger protein. Allelic variants exist in nature and encompassed within the methods of the present invention is the use of these natural alleles. Allelic variants also encompass Single Nucleotide

Polymorphisms (SNPs), as well as Small Insertion/Deletion Polymorphisms (INDELs). The size of INDELs is usually less than 100 bp. SNPs and INDELs form the largest set of sequence variants in naturally occurring polymorphic strains of most organisms. Allelic variants suitable for use in the methods according to the invention may readily be determined for example by following the methods described in the Examples section by simply substituting the sequence used in the actual Example with the allelic variant.

The present invention provides a method for modifying plant growth characteristics, comprising modifying expression in a plant of an alternative splice variant or expression in a plant of an allelic variant of a nucleic acid encoding a 2xC2H2 zinc finger protein and/or by modifying level and/or activity in a plant of a 2xC2H2 zinc finger protein encoded by the alternative splice variant or allelic variant.

Examples of variant 2xC2H2 zinc finger proteins useful in practicing the methods of the present invention are homologues, derivatives or functional fragments of a 2xC2H2 zinc finger protein.

"Homologues" of a 2xC2H2 zinc finger protein encompass peptides, oligopeptides, polypeptides, proteins and enzymes having amino acid substitutions, deletions and/or insertions relative to the unmodified protein in question and having similar biological and functional activity as the unmodified protein from which they are derived. To produce such homologues, amino acids of the protein may be replaced by other amino acids having similar properties (such as similar hydrophobicity, hydrophilicity, antigenicity, propensity to form or break  $\alpha$ -helical structures or  $\beta$ -sheet structures). Conservative substitution tables are well known in the art (see for example Creighton (1984) Proteins. W.H. Freeman and Company). The homologues useful in the method according to the invention have at least in increasing order of preference 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 52%, 54%, 56%, 58%, 60%, 62%, 64%, 66%, 68%, 70%, 72%, 74%, 76%, 78%, 80%, 82%, 84%, 86%, 88%, 90%, 92%, 94%, 96%, 98% sequence identity or similarity to an unmodified protein.

The percentage of identity may be calculated by using an alignment program well known in the art. For example, the percentage of identity may be calculated using the program GAP, or needle (EMBOSS package) or stretcher (EMBOSS package) or the program align X, as a module of the vector NTI suite 5.5 software package, using the standard parameters (for example GAP penalty 5, GAP opening penalty 15, GAP extension penalty 6.6).

According to another embodiment of the present invention, the nucleic acid sequence useful in the methods of the present invention is a nucleic acid encoding a protein homologous to SEQ ID NO 2.

5 Methods for the search and identification of 2xC2H2 zinc finger protein homologues, for example STZ zinc finger homologues, would be well within the realm of a person skilled in the art. Such methods, involve screening sequence databases with the sequences provided by the present invention, for example SEQ ID NO 2 (or SEQ ID NO 1), preferably in a computer readable format. This sequence information may be available in public databases, that include but are not limited to Genbank (<http://www.ncbi.nlm.nih.gov/web/Genbank>), the European  
10 Molecular Biology Laboratory Nucleic acid Database (EMBL) (<http://w.ebi.ac.uk/ebi-docs/embl-db.html>) or versions thereof or the MIPS database (<http://mips.gsf.de/>). Different search algorithms and software for the alignment and comparison of sequences are well known in the art. Such methods include GAP, BESTFIT, BLAST, FASTA and TFASTA. GAP uses the algorithm of Needleman and Wunsch (J. Mol. Biol. 48: 443-453, 1970) to find the alignment of  
15 two complete sequences that maximises the number of matches and minimises the number of gaps. The BLAST algorithm calculates percent sequence identity and performs a statistical analysis of the similarity between the two sequences. The suite of programs referred to as BLAST programs has 5 different implementations: three designed for nucleotide sequence queries (BLASTN, BLASTX, and TBLASTX) and two designed for protein sequence queries  
20 (BLASTP and TBLASTN) (Coulson, Trends in Biotechnology: 76-80, 1994; Birren et al., GenomeAnalysis, 1: 543, 1997). The software for performing BLAST analysis is publicly available through the National Centre for Biotechnology Information.

Default blast parameters to find useful homologues of any of SEQ ID NO 1, SEQ ID NO 2 or  
25 any of SEQ ID NO 10 to SEQ ID NO 50, are, when comparing nucleotide sequence G (Cost to open a gap) 5, E (Cost to extend a gap default) 2, q (Penalty for a mismatch) -3, r (Reward for a match) 1, e (Expectation value (E)) 10.0, W (Word size) 11, V (Number of one-line descriptions) 100 and B (Number of alignments to show) 100. When comparing protein sequences, the default parameters are preferably G 11, E 1, e value 10.0, W 3, V 100 and B  
30 100.

The above-mentioned analyses for comparing sequences, for the calculation of sequence identity and for the search for homologues, is preferentially done with full-length sequences or within a conserved region of the sequence. Therefore, these analyses may be based on a  
35 comparison of certain regions such as conserved domains, motifs or boxes.

The identification of such domains or motifs for examples the motif and boxes as represented by SEQ ID NO 5, 6, 7, 8 and 9, would also be well within the realm of a person skilled in the art and involves for example, a computer readable format of proteins of the present invention, the use of alignment software programs and the use of publicly available information on protein domains, conserved motifs and boxes. This protein domain information is available in the PRODOM (<http://www.biochem.ucl.ac.uk/bsm/dbbrowser/jj/prodomsrchjj.html>), PIR (<http://pir.georgetown.edu/>) or pFAM (<http://pfam.wustl.edu/>) database. For the identification of Zinc finger domains, such as the 2xC2H2 zinc finger domain, pFAM is preferred. Sequence analysis programs designed for motif searching may be used for identification of fragments, regions and conserved domains as mentioned above. Preferred computer programs would include but are not limited to MEME, SIGNALSCAN, and GENESCAN. A MEME algorithm (Version 3.0) may be found in the GCG package; or on the Internet site <http://www.sdsc.edu/MEME/meme>. SIGNALSCAN version 4.0 information is available on the Internet site <http://biosci.cbs.umn.edu/software/sigscan.html>. GENESCAN may be found on the Internet site <http://gnomic.stanford.edu/GENESCANW.html>.

At present, zinc finger motifs are subdivided in more than 40 different classes as can be found in the Pfam database of protein families present at the Sanger institute (<http://www.sanger.ac.uk/Software/Pfam/browse/Z.shtml>).

The C2H2 zinc finger (Zf-C2H2) motif is the classical zinc finger domain. It was first recognized in the transcription factor IIIA (TFIIIA) of *Xenopus* (Miller et al. 1985). The domain is typically 25 to 30 amino-acid residues in length. The following pattern describes the zinc finger \*X-C-X(1-5)-C-X3-\*X5-\*X2-H-X(3-6)-[H/C] where X can be any amino acid, and numbers in brackets indicate the number of residues. The positions marked \* are those that are important for the stable folding of the zinc finger. The final position can be either his or cys, while still being a C2H2 zinc finger domain. In view of recent publications on the design of zinc finger domains it becomes feasible also to replace one or more of the Cys or His amino acids, whilst still retaining the original functionality of the C2H2 domain. The residues separating the second Cys and the first His are mainly polar and basic. The canonical C2H2 zinc finger is composed of two short beta strands followed by an alpha helix. DNA binding of the zinc finger motif is mediated by amino terminal part of the alpha helix which binds the major groove in DNA binding zinc fingers. C2H2 domains have been shown to interact with RNA, DNA and proteins. The tetracoordination of a Zinc ion by the conserved cysteine and histidine residues determines the conserved tertiary structure of the motif. Conserved hydrophobic residues are commonly found at positions -2 and also at 4 amino acids after the second cysteine (that participates in zinc binding) and at position three before the first histidine (that participates in zinc binding). In

plant multi zinc finger proteins, spacing between the C2H2 domains is generally about 15 to about 65 amino acids.

- Thus, plant zinc finger proteins are characterized by long spacers of diverse lengths between adjacent fingers. Moreover, they are characterised by a highly conserved sequence of six amino acids, located within a putative DNA-contacting surface of each finger. Two forms of such conserved sequence are most commonly found in plant C2H2 zinc fingers, the QALGGH (SEQ ID NO 5) and the NNM/WQMH (SEQ ID NO 6). Despite the high sequence conservation of the QALGGH, some variants or the so-called 'modified type' occur in nature where one or two amino acids can have a different form, most typically the +1 "Q" can be a "G", "K" or "R" (these amino acids share the same turn-like characteristic), the +2 "A" can be "S" (both of which share the characteristic of being small amino acids) or the +3 "L" can be "F" (these two amino acids are both hydrophobic). The QALGGH-motif as used herein comprises all these variants. In the NNM/WQMH motif at position 3 there is mostly an "M" or a "W".
- Therefore, the present invention provides a method as described hereinabove, wherein said 2xC2H2 zinc finger protein comprises a QALGGH motif. Further, The present invention provides a s described hereinabove, wherein said 2xC2H2 zinc finger protein comprises a NNM/WQMH motif.
- According to one embodiment of the invention, both C2H2 domains are of the same type. More preferably, both C2H2 zinc finger domains have the same conserved GALGGH or NNM/WQMH motif. According to another embodiment, each C2H2 zinc finger domain has a different conserved motif.
- According to one embodiment, the 2xC2H2 protein useful in the methods of the present invention is characterized by an EAR motif, which is an ERF-Associated amphiphilic repression motif. This motif has been recognized in two unrelated types of transcription factors, namely the ERF transcription factors of the AP2 type and in the zinc finger transcription factors. In the latter class, the EAR motif is generally located at the C-terminus of the protein.
- The pattern for the EAR motif has the conserved sequence hDLNh(X)P (SEQ ID NO 7), where "h" is a hydrophobic residue (any one of A,C,F,G,H,I,K,L,M,R,T,V,W,Y) most typically L/F/I and where "X" can be one (any amino acid) or no amino acid. A characteristic feature of the EAR motif is the alternation of hydrophilic and hydrophobic residues with the aspartic acid (D) residue being amphiphilic. Ohta et al. (The plant cell, 2001, 13, p1959-1968), which reference is cited herein by reference, previously characterized EAR motifs present in 2xC2H2 zinc finger proteins.

Therefore, the present invention provides a method as described hereinabove, wherein the 2xC2H2 zinc finger protein comprises an EAR motif. According to one embodiment, the EAR motif is located in the C-terminal region of the protein, preferably between the second zinc finger domain and the C-terminus.

5

According to a further embodiment, the zinc finger proteins used in the methods of the present invention have two zinc finger domains and a nuclear localization signal (B-box). A cluster of basic amino acids that resembles the B-box (Basic box) were described by Chua et al. (EMBO 1992- 11, 241-9) and were hypothesized to be a nuclear localization signal for the protein.

10 These have been recognized in 2xC2H2 proteins (Sakamoto et al., Gene 248 (2000) 23-32). The cluster is rich in Lysine (K) and Arginine (R) residues. A consensus sequence defining the most frequent form of the B-box in 2xC2H2 genes is KR(S)KRXR (SEQ ID NO 8) where "S" at the 3rd position may be absent or present. However other variants may occur in nature that still retain the characteristic of being a charged region rich in basic amino acids. The location of  
15 the basic box is most frequently at the N-terminus of the protein, but can also occur in other locations. It has been speculated that due to its basic nature the B-box could also participate in DNA binding.

Accordingly, the present invention provides a method as described hereinabove, wherein the  
20 2xC2H2 zinc finger protein further comprises a B-box. According to one embodiment the B-box is located in the N-terminal region of the zinc finger protein. Preferably the proteins useful in the methods of the present invention have a B-box located between the N-terminus and the first zinc finger domain.

25 According to a further embodiment, the zinc finger proteins useful in the methods of the present invention have two C2H2 zinc finger domains and an L-box. A conserved motif, named L-box, of yet unknown function has been identified in 2xC2H2 proteins and has been described previously by Sakamoto et al. (Gene 248 (2000) 23-32). The L-box is typically located at the N-terminus, between the B-box and the first C2H2 zinc finger. The L-box is represented by the  
30 sequence EXEXXAXCLXXL (SEQ ID NO 9). This region may be involved in protein-protein interactions. Zinc finger proteins lacking the L-box, may for example have serine rich regions at a similar position, which regions are putative sites for protein-protein interactions.

Therefore, the present invention provides a method as described hereinabove, wherein the  
35 2xC2H2 protein comprises an L-box.

Particular zinc finger homologues useful in the methods of the present invention have one or more of the conserved motifs as depicted in SEQ ID NO 5, 6, 7, 8 and 9, or motifs that are 80% identical to these motifs or motifs that have conserved substitutions of amino acids. The 2xC2H2 protein as set forth in SEQ ID NO 2 comprises all the boxes as set forth in SEQ ID NO 5, 7, 8 and 9. All its paralogues and orthologues also comprise all of these boxes.

Homologues of a 2xC2H2 protein as presented in SEQ ID NO 2 and isolated from *Arabidopsis thaliana*, that are useful in the constructs and the methods of the present invention are also identified in other plant species.

Two special forms of homologue, orthologues and paralogues, are evolutionary concepts used to describe ancestral relationships of genes. The term "paralogue" relates to a gene-duplication within the genome of a species leading to paralogous genes. The term "orthologue" relates to a homologous gene in different organisms due to ancestral relationship. The term "homologue" as used herein also encompasses paralogues and orthologues of the proteins useful in the methods according to the invention.

Orthologues in other plant species may easily be found by performing a so-called reciprocal blast search. Orthologous genes can be identified by querying one or more gene databases with a query gene or protein of interest (SEQ ID NO 1 or 2), using for example BLAST program. The highest-ranking subject genes that result from the search are then again subjected to a BLAST analysis, and only those subject genes that match again with the query sequence (SEQ ID NO 1 or 2) are retained as true orthologous genes. For example, to find a rice orthologue of an *Arabidopsis thaliana* gene, one may perform a BLASTN or TBLASTX analysis on a rice database such as (but not limited to) the *Oryza sativa Nipponbare* database available at the NCBI website (<http://www.ncbi.nlm.nih.gov>) or the genomic sequences of rice (cultivars *indica* or *japonica*). In a next step, the obtained rice sequences are used in a reverse BLAST analysis using an *Arabidopsis* database. The results may be further refined when the resulting sequences are analysed with ClustalW and visualised in a neighbour joining tree. The method can be used to identify orthologues from many different species.

The closest homologues in other species (orthologues of the protein of SEQ ID NO 2), include those from a variety of dicot and monocot plants, for example from *Datisca glomerata* (AF119050\_1, AAD26942, SEQ ID NO 10 and 11), from soybean (T09602, SCOF-1, SEQ ID NO 12 and 13), *Medicago sativa* (CAB77055.1, SEQ ID NO 14 and 15), from tobacco (T01985, SEQ ID NO 16 and 17) from rice, (AF332876\_1, AAK01713.1, SEQ ID NO 18 and 19), from petunia (BAA05079.1, SEQ ID NO 20 and 21), from wheat (S39045 and BAA03901,

WZF1, SEQ ID NO 22 and 23), from *Capsicum annum* (SEQ ID NO 24 and 25), from turnip (T14408, T14409) and from sugarcane (CA279020).

Close homologues of the same species (paralogues of the protein of SEQ ID NO 2 from  
5 *Arabidopsis thaliana*) are described below.

The MIPS database contains the sequence of the *Arabidopsis thaliana* genome with prediction and functional annotation of the proteins encoded. Searching this database with the STZ gene of SEQ ID NO 1 (MIPS accession number At1g27730), showed that in the *Arabidopsis* genome there are 2 genes encoding very close homologues of SEQ ID NO 2, At5g43170  
10 (NM\_123683, SEQ ID NO 32 and 33) and At5g04340 (NM\_120516 SEQ ID NO 28 and 29), and 3 others with high similarity: At3g19580 (NM\_112848, SEQ ID NO 26 and 27), At5g67450 (NM\_126145, SEQ ID NO 34 and 35) and At3g49930 (NM\_114853, SEQ ID NO 30-31). These genes are spread over 3 chromosomes, 1, 3 and 5. Similarly, a number of paralogues of the orthologue in *Petunia* have been isolated and sequenced. Advantageously, paralogues from  
15 the same species may be used in the methods of the present invention.

Furthermore, a number of family members of the STZ protein of SEQ ID NO 2 have been found in *Arabidopsis*. The STZ gene and protein of SEQ ID NO 1 and 2 have been previously published in the database under the MIPS accession number At1g27730 or in Genbank under  
20 the accession numbers NP\_174094.1, X95573 or CAA64820. Additionally, several other cDNA's, isolated from other tissues or at different developmental stages of *Arabidopsis* have been reported and encode the same protein as that of SEQ ID NO 2. Such sequences sequences deposited under the Genbank accession number AY034998, NM\_102538, AC12375, X95573, AY063006, X98671, X98670, or AF250336. These isolates illustrate the  
25 differential expression of the STZ gene in different plant tissues at different developmental stages. The differential regulation of these different cDNA's is reflected by the differences at their 5'UTR and the 3'UTR regions, while the encoded protein remains the same. Advantageously, the members of the same gene family as SEQ ID NO 1 or the members of the same family of any of the orthologues of SEQ ID NO 1, may be used in the methods of the  
30 present invention.

Other close homologues useful in the methods of the present invention are the sequences as deposited in the public database under the following accession numbers, which sequences are herein incorporated by reference: homologues isolated from *Petunia*: BAA21923.1,  
35 BAA21922.1, BAA21926.1, BAA21925.1, BAA19110.1, BAA19926.1, BAA21924.1, BAA19111.1, BAA21921.1, BAA19114.1, BAA05076.1, BAA05079.1, CAA43111.1, BAA21920.1, BAA21919.1, BAA05077.1, BAA05078.1, BAA20137.1; homologues isolated

from *Arabidopsis*: CAA67229.1, BAC43454.1, NP\_196054.1, AAM67193.1, NP\_199131.1, NP\_188592.1, NP\_201546.1, NP\_190562.1, NP\_182037.1, BAC43008.1, Q8VWG3, CAC86393.1, CAC86168.1, CAC86167.1, CAC86166.1, CAB67667.1, CAC01747.1, CAB90936.1, CAB90935.1, CAB80245.1, CAB41188.1, CAA18741.1, CAA67234.1, CAA67236.1, CAA67231.1, CAA67230.1, CAA67228.1, CAA67235.1, CAA67233.1, CAA67232.1, CAA67229.1, CAA64820.1 and homologues isolated from rice: BAB16855.1, AAO06972.1, CAC09475.1, BAB63718.1, P0683F02.21, BAB67885.1, P0031D11.19, BAB64114.1, AAK01713.1, AF332876\_1, AAL76091.1, BAB67879.1, P0031D11.12 and BAC15513.1.

10

A phylogenetic tree may be constructed with all the homologues, paralogues and orthologues are defined herein above. Multiple alignment may be made using clustal W present in the VNTi (version 5.0) program with for example Gap opening penalty 10 and Gap extension 5. For making a phylogenetic tree the Phylip software package available at <http://evolution.genetics.washington.edu/phylip.html> may be used. Sequences clustering around SEQ ID NO 1 or SEQ ID NO 2, identify genes or proteins suitable for use in the methods of the present invention.

15

The sequence of SEQ ID NO 2 and its rice orthologue AF332876 (SEQ ID NO 19) have 36% sequence identity when using the program Needle with the parameters Gap penalty 5 and Gap extension penalty 6. Therefore, homologues particularly useful in the methods of the present invention are homologues having 36% or more sequence identity with the 2xC2H2 zinc finger protein as presented in SEQ ID NO 2 or having 36% or more sequence identity to the closest orthologue of SEQ ID NO 2 from another species.

20

Preferred homologues useful in practicing the methods of the present invention are plant homologues, i.e. proteins obtained from a plant nucleic acid. More preferably, the nucleic acid sequence is from a dicot, more preferably from the family *Brassicaceae*, further preferably from *Arabidopsis thaliana*.

25

Preferably the 2xC2H2 zinc finger protein useful in the methods of the present invention belongs to the same gene family as the salt tolerant zinc finger protein (STZ) of *Arabidopsis thaliana*, or is a homologue thereof. The name ZAT10 can also be used to identify the STZ zinc finger protein of *Arabidopsis thaliana*.

30

Another variant of a zinc finger protein useful in the methods of the present invention is a substitutional variant. The term "Substitutional variants" of a protein refers to those variants in

35

which at least one residue in an amino acid sequence has been removed and a different residue inserted in its place. Amino acid substitutions are typically of single residues, but may be clustered depending upon functional constraints placed upon the polypeptide; insertions will usually be of the order of about 1-10 amino acid residues, and deletions will range from about  
5 1-20 residues. Preferably, amino acid substitutions comprise conservative amino acid substitutions. Particular substitutional variants of the C2H2 zinc finger protein are substitutional variants in which one or more of the conserved Cys and/or His residues is replaced, whilst retaining the same zinc finger functionality. To retain the same functionality, the residues around these conserved Cys or His residues may also be substituted.

10

"Insertional variants" of a protein are those in which one or more amino acid residues are introduced into a predetermined site in said protein. Insertions can comprise amino-terminal and/or carboxy-terminal fusions as well as intra-sequence insertions of single or multiple amino acids. Generally, insertions within the amino acid sequence will be smaller than amino- or  
15 carboxy-terminal fusions, of the order of about 1 to 10 residues. Examples of amino- or carboxy-terminal fusion proteins or peptides include the binding domain or activation domain of a transcriptional activator as used in the yeast two-hybrid system, phage coat proteins, (histidine)<sub>6</sub>-tag, glutathione S-transferase-tag, protein A, maltose-binding protein, dihydrofolate reductase, Tag•100 epitope, c-myc epitope, FLAG<sup>®</sup>-epitope, lacZ, CMP (calmodulin-binding  
20 peptide), HA epitope, protein C epitope and VSV epitope.

"Deletion variants" of a protein are characterised by the removal of one or more amino acids from the protein. Amino acid variants of a protein may readily be made using peptide synthetic techniques well known in the art, such as solid phase peptide synthesis and the like, or by  
25 recombinant DNA manipulations. Methods for the manipulation of DNA sequences to produce substitution, insertion or deletion variants of a protein are well known in the art. For example, techniques for making substitution mutations at predetermined sites in DNA are well known to those skilled in the art and include M13 mutagenesis, T7-Gen *in vitro* mutagenesis (USB, Cleveland, OH), QuickChange Site Directed mutagenesis (Stratagene, San Diego, CA), PCR-mediated site-directed mutagenesis or other site-directed mutagenesis protocols.

30

The term "derivatives" refers to peptides, oligopeptides, polypeptides, proteins and enzymes which may comprise substitutions, deletions or additions of naturally and non-naturally occurring amino acid residues compared to the amino acid sequence of a naturally-occurring  
35 form of the 2xC2H2 protein such as for example the 2xC2H2 zinc finger protein as presented in SEQ ID NO 2. "Derivatives" of a 2xC2H2 zinc finger protein encompass peptides, oligopeptides, polypeptides, proteins and enzymes which may comprise naturally occurring

altered, glycosylated, acylated or non-naturally occurring amino acid residues compared to the amino acid sequence of a naturally-occurring form of the polypeptide. A derivative may also comprise one or more non-amino acid substituents compared to the amino acid sequence from which it is derived, for example a reporter molecule or other ligand, covalently or non-covalently bound to the amino acid sequence such as, for example, a reporter molecule which is bound to facilitate its detection, and non-naturally occurring amino acid residues relative to the amino acid sequence of a naturally-occurring protein.

Another variant of a 2xC2H2 zinc finger protein useful in the methods of the present invention is an active fragment of a zinc finger protein. "Active fragments" of a 2xC2H2 zinc finger protein encompasses at least five contiguous amino acid residues of a protein, which residues retain similar biological and/or functional activity to the naturally occurring protein. For example, useful fragments comprise at least 10 contiguous amino acid residues of a 2xC2H2 zinc finger protein. Other preferred fragments are fragments of a 2xC2H2 zinc finger protein starting at the second or third or further internal methionin residues. These fragments originate from protein translation, starting at internal ATG codons. Functional fragments of a 2xC2H2 zinc finger protein useful in practising the methods of the present invention may have one, two or no C2H2 domains, without affecting its functionality in the methods of the present invention.

According to a preferred feature of the present invention, enhanced or increased expression of a nucleic acid encoding a 2xC2H2 zinc finger protein is envisaged. Methods for obtaining enhanced or increased expression of genes or gene products are well documented in the art and include, for example, over-expression driven by a strong promoter, the use of transcription enhancers or translation enhancers. The term over-expression as used herein means any form of expression that is additional to the original wild-type expression level. Preferably the nucleic acid to be introduced into the plant and/or the nucleic acid that is to be overexpressed in the plant is in the sense direction with respect to the promoter to which it is operably linked. Preferably, the nucleic acid sequence represented by SED ID NO 1 is over-expressed in a plant. However, it should be clear that the applicability of the invention is not limited to use of the nucleic acid represented by SEQ ID NO 1 nor to the nucleic acid sequence encoding the amino acid sequence of SEQ ID NO 2, but that other nucleic acid sequences encoding homologues, derivatives or active fragments of SED ID NO 1 or SED ID NO 2 may be useful in the methods of the present invention. Examples of nucleic acids or proteins are provided in SEQ ID NO 10 to SEQ ID NO 50.

Alternatively and/or additionally, increased expression of a 2xC2H2 encoding gene or increased level and/or activity of a 2xC2H2 protein in a plant cell, is achieved by mutagenesis.

For example these mutations may be responsible for altered control of the 2xC2H2 gene, resulting in more expression of the gene, relative to the wild-type gene. Mutations can also cause conformational changes in a protein, resulting in more activity and/or higher levels of the 2xC2H2 protein.

5

Modifying gene expression (whether by a direct or indirect approach) encompasses altered transcript levels of a gene. Altered transcript levels may be sufficient to induce certain phenotypic effects, for example via the mechanism of cosuppression. Here the overall effect of introduction of a transgene is that there is less activity in the cell of the protein encoded by a native gene having homology to the introduced transgene. Therefore, according to another embodiment of the present invention, there is provided a method for modifying growth characteristics in a plant, comprising decreasing expression of a gene encoding a 2xC2H2 zinc finger protein or decreasing level and/or activity of a 2xC2H2 zinc finger protein. Examples of decreasing expression, level and/or activity of a protein in a cell are well documented in the art and include, for example, downregulation of expression by anti-sense techniques, RNAi techniques, small interference RNAs (siRNAs) and microRNA (miRNA) .

10

15

20

Another method for downregulation of gene expression or gene silencing comprises use of ribozymes, for example as described in Atkins et al. 1994 (WO 94/00012), Lenée et al. 1995 (WO 95/03404), Lutziger et al. 2000 (WO 00/00619), Prinsen et al. 1997 (WO 97/3865) and Scott et al. 1997 (WO 97/38116).

25

Gene silencing may also be achieved by insertion mutagenesis (for example, T-DNA insertion or transposon insertion) or by gene silencing strategies as described by, among others, Angell and Baulcombe 1998 (WO 98/36083), Lowe et al. 1989 (WO 98/53083), Lederer et al. 1999 (WO 99/15682) or Wang et al. 1999 (WO 99/53050).

30

Expression of an endogenous gene may also be reduced if it contains a mutation. Such a mutation or such a mutant gene may be isolated and introduced into the same or different plant species in order to obtain plants having modified growth characteristics. Examples of such mutants are dominant negative mutants of a 2xC2H2 zinc finger gene.

35

Genetic constructs aimed at silencing gene expression may comprise the 2xC2H2 zinc finger nucleic acid, for example as represented by SEQ ID NO 1 (or one or more portions thereof or a sequence capable of hybridising therewith), in a sense and/or antisense orientation relative to the promoter sequence. The sense or antisense copies of at least part of the endogenous gene in the form of direct or inverted repeats may also be utilised in the methods according to

the invention. The growth characteristics of plants may also be modified by introducing into a plant at least part of an antisense version of the nucleotide sequence represented by SEQ ID NO 1.

5 According to a further embodiment of the present invention, genetic constructs and vectors to facilitate introduction and/or to facilitate expression of the 2xC2H2 zinc finger nucleotide sequences useful in the methods according to the invention are provided. Therefore, according to the present invention, there is provided a construct comprising:

- 10 (i) a nucleic acid capable of modifying expression of a nucleic acid encoding a 2xC2H2 zinc finger protein and/or modifying level and/or activity of a 2xC2H2 zinc finger protein;
- (ii) one or more control sequence capable of driving expression of the nucleic acid sequence of (i); and optionally
- 15 (iii) a transcription termination sequence.

Constructs useful in the methods according to the present invention may be constructed using recombinant DNA technology well known to persons skilled in the art. The gene constructs may be inserted into vectors, which may be commercially available, suitable for transforming into plants and suitable for expression of the gene of interest in the transformed cells.  
20 Preferably the genetic construct is a plant expression vector.

The nucleic acid according to (i) is advantageously any of the nucleic acids described hereinbefore. A preferred nucleic acid is the nucleic acid represented by SEQ ID NO 1 or a variant thereof as hereinbefore defined, or is a nucleic acid sequence encoding a sequence  
25 represented by SEQ ID NO 2 or a variant as hereinbefore defined. For example such variants encode a protein as presented in any of SEQ ID NO 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 42, 44, 46, 48 and 50.

The terms "regulatory element" and "control sequence" are used herein interchangeably and  
30 are to be taken in a broad context to refer to regulatory nucleic acids capable of effecting expression of the sequences to which they are operably linked. Encompassed by the aforementioned terms are promoters. A "promoter" encompasses transcriptional regulatory sequences derived from a classical eukaryotic genomic gene (including the TATA box which is required for accurate transcription initiation, with or without a CCAAT box sequence) and  
35 additional regulatory elements (i.e. upstream activating sequences, enhancers and silencers) which alter gene expression in response to developmental and/or external stimuli, or in a tissue-specific manner. Also included within the term is a transcriptional regulatory sequence

of a classical prokaryotic gene, in which case it may include a -35 box sequence and/or -10 box transcriptional regulatory sequences. The term "regulatory element" also encompasses a synthetic fusion molecule or derivative which confers, activates or enhances expression of a nucleic acid molecule in a cell, tissue or organ. The term "operably linked" as used herein  
5 refers to a functional linkage between the promoter sequence and the gene of interest, such that the promoter sequence is able to initiate transcription of the gene of interest. Preferably, the gene of interest is operably linked to a promoter in a sense direction.

Advantageously, any type of promoter may be used to drive expression of the nucleic acid  
10 sequence depending on the desired outcome.

Promoters useful for the present invention are described in EP 03075331.3, which promoters and sequences are incorporated herein by reference.

Other examples of preferred promoters are presented in Table I (a) to (c), which promoters or  
15 derivatives thereof are useful in the methods and/or in making the constructs of the present invention. Accordingly, genetic constructs comprising of the nucleic acids of (i), for example a 2xC2H2 nucleic acid, and at least part of a promoter from Table I (a) to (c) or from EP 03075331.3, preferably, wherein said parts are operably linked, are also provided by the present invention.

20 According to another embodiment, the nucleic acid of (i) is operably linked to a constitutive promoter. The term "constitutive" as defined herein refers to a promoter that is expressed substantially continuously. Furthermore, preferably the constitutive promoter is a ubiquitous promoter, which is expressed in more than one, preferably in most or substantially all tissues  
25 of the plant. Preferably, the constitutive promoter to be used in the methods of the present invention, or cloned in the genetic constructs of the present invention, is a plant promoter, preferably a constitutive promoter, such as a GOS2 promoter or a promoter with similar strength and/or similar expression pattern. Preferably plant promoters derived from a plant nucleic acid are used. Alternatively, promoters operable in plant, such as promoters derived  
30 from plant pathogens are used.

According to another embodiment of the invention, the nucleic acid of (i) is operably linked to a plant promoter, preferably a tissue-preferred promoter. The term "tissue-preferred" as used herein refers to a promoter that is expressed predominantly in at least one tissue or organ. For  
35 example, the tissue-preferred promoter is a seed-preferred promoter, such as a pWS18 (Joshee et al. Plant Cell Physiol. 1998 Jan;39(1):64-72. ) or a promoter of similar strength and/or similar expression pattern.

Promoters with similar strength and/or similar expression pattern may be found by coupling the promoter to a reporter gene and checking the function of the reporter gene in different tissues of a plant. One suitable reporter gene is beta-glucuronidase and the colorimetric GUS staining to visualize the beta-glucuronidase activity in a plant tissue is well known to a person skilled in the art.

Table I (a): flower preferred promoters useful in the present invention. Sequences of these promoters are described in the cited reference, which sequences are herein incorporated by reference.

| Gene                     | Expression | Reference                                                                                       |
|--------------------------|------------|-------------------------------------------------------------------------------------------------|
| AtPRP4                   | flowers    | <a href="http://salus.medium.edu/mmg/tierney/html">http://salus.medium.edu/mmg/tierney/html</a> |
| chalcone synthase (chsA) | flowers    | Van der Meer, <i>et al.</i> , <i>Plant Mol. Biol.</i> 15, 95-109, 1990.                         |
| LAT52                    | anther     | Twell <i>et al</i> <i>Mol. Gen Genet.</i> 217:240-245 (1989)                                    |
| <i>apetala-3</i>         | flowers    |                                                                                                 |

Table I (b): seed-preferred promoters useful in the present invention. Sequences of these promoters are described in the cited reference, which sequences are herein incorporated by reference.

| Gene                | Expression | Reference                                                                                                                                                                                           |
|---------------------|------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| seed-specific genes | seed       | Simon, <i>et al.</i> , <i>Plant Mol. Biol.</i> 5: 191, 1985; Scofield, <i>et al.</i> , <i>J. Biol. Chem.</i> 262: 12202, 1987.; Baszczyński, <i>et al.</i> , <i>Plant Mol. Biol.</i> 14: 633, 1990. |
| Brazil Nut albumin  | seed       | Pearson, <i>et al.</i> , <i>Plant Mol. Biol.</i> 18: 235-245, 1992.                                                                                                                                 |
| legumin             | seed       | Ellis, <i>et al.</i> , <i>Plant Mol. Biol.</i> 10: 203-214, 1988.                                                                                                                                   |
| glutelin (rice)     | seed       | Takaiwa, <i>et al.</i> , <i>Mol. Gen. Genet.</i> 208: 15-22, 1986; Takaiwa, <i>et al.</i> , <i>FEBS Letts.</i> 221: 43-47, 1987.                                                                    |
| zein                | seed       | Matzke <i>et al</i> <i>Plant Mol Biol</i> , 14(3):323-32 1990                                                                                                                                       |
| napA                | seed       | Stalberg, <i>et al</i> , <i>Planta</i> 199: 515-519, 1996.                                                                                                                                          |

|                                               |                            |                                                                                         |
|-----------------------------------------------|----------------------------|-----------------------------------------------------------------------------------------|
| wheat LMW and HMW glutenin-1                  | endosperm                  | Mol Gen Genet 216:81-90, 1989; NAR 17:461-2, 1989                                       |
| wheat SPA                                     | seed                       | Albani <i>et al</i> , Plant Cell, 9: 171-184, 1997                                      |
| wheat $\alpha$ , $\beta$ , $\gamma$ -gliadins | endosperm                  | EMBO 3:1409-15, 1984                                                                    |
| barley <i>ltr1</i> promoter                   | endosperm                  |                                                                                         |
| barley B1, C, D, hordein                      | endosperm                  | Theor Appl Gen 98:1253-62, 1999; Plant J 4:343-55, 1993; Mol Gen Genet 250:750-60, 1996 |
| barley DOF                                    | endosperm                  | Mena <i>et al</i> , The Plant Journal, 116(1): 53-62, 1998                              |
| <i>blz2</i>                                   | endosperm                  | EP99106056.7                                                                            |
| synthetic promoter                            | endosperm                  | Vicente-Carbajosa <i>et al</i> , Plant J. 13: 629 - 640, 1998.                          |
| rice prolamin NRP33                           | endosperm                  | Wu <i>et al</i> , Plant Cell Physiology 39(8) 885-889, 1998                             |
| rice $\alpha$ -globulin Glb-1                 | endosperm                  | Wu <i>et al</i> , Plant Cell Physiology 39(8) 885-889, 1998                             |
| rice OSH1                                     | embryo                     | Sato <i>et al</i> , Proc. Natl. Acad. Sci. USA, 93: 8117-8122, 1996                     |
| rice $\alpha$ -globulin REB/OHP-1             | endosperm                  | Nakase <i>et al</i> . Plant Mol. Biol. 33: 513-522, 1997                                |
| rice ADP-glucose PP                           | endosperm                  | Trans Res 6:157-68, 1997                                                                |
| maize ESR gene family                         | endosperm                  | Plant J 12:235-46, 1997                                                                 |
| sorgum $\gamma$ -kafirin                      | endosperm                  | PMB 32:1029-35, 1996                                                                    |
| KNOX                                          | embryo                     | Postma-Haarsma <i>et al</i> , Plant Mol. Biol. 39:257-71, 1999                          |
| rice oleosin                                  | embryo and aleuron         | Wu <i>et al</i> , J. Biochem., 123:386, 1998                                            |
| sunflower oleosin                             | seed (embryo and dry seed) | Cummins, <i>et al</i> , Plant Mol. Biol. 19: 873-876, 1992                              |

Table I (c): constitutive promoters useful in the present invention. Sequences of these promoters are described in the cited reference, which sequences are herein incorporated by reference.

5

| Gene     | Expression   | Reference                                           |
|----------|--------------|-----------------------------------------------------|
| Actin    | constitutive | McElroy <i>et al</i> , Plant Cell, 2: 163-171, 1990 |
| CAMV 35S | constitutive | Odell <i>et al</i> , Nature, 313: 810-812,          |

|                  |              |                                                                       |
|------------------|--------------|-----------------------------------------------------------------------|
|                  |              | 1985                                                                  |
| CaMV 19S         | constitutive | Nilsson <i>et al.</i> , <i>Physiol. Plant.</i> 100:456-462, 1997      |
| GOS2             | constitutive | de Pater <i>et al.</i> , <i>Plant J</i> Nov;2(6):837-44, 1992         |
| ubiquitin        | constitutive | Christensen <i>et al.</i> , <i>Plant Mol. Biol.</i> 18: 675-689, 1992 |
| rice cyclophilin | constitutive | Buchholz <i>et al.</i> , <i>Plant Mol Biol.</i> 25(5): 837-43, 1994   |
| maize H3 histone | constitutive | Lepetit <i>et al.</i> , <i>Mol. Gen. Genet.</i> 231:276-285, 1992     |
| actin 2          | constitutive | An <i>et al.</i> , <i>Plant J.</i> 10(1); 107-121, 1996               |

Optionally, one or more terminator sequences may also be used in the construct introduced into a plant. The term "terminator" encompasses a control sequence which is a DNA sequence at the end of a transcriptional unit which signals 3' processing and polyadenylation of a primary transcript and termination of transcription. Additional regulatory elements may include transcriptional as well as translational enhancers. Those skilled in the art will be aware of terminator and enhancer sequences which may be suitable for use in the invention. Such sequences would be known or may readily be obtained by a person skilled in the art.

- 5 The genetic constructs of the invention may further include an origin of replication sequence which is required for maintenance and/or replication in a specific cell type. One example is when a genetic construct is required to be maintained in a bacterial cell as an episomal genetic element (e.g. plasmid or cosmid molecule). Preferred origins of replication include, but are not limited to, the f1-ori and colE1.

15

The genetic construct may optionally comprise a selectable marker gene. As used herein, the term "selectable marker gene" includes any gene which confers a phenotype on a cell in which it is expressed to facilitate the identification and/or selection of cells which are transfected or transformed with a genetic construct of the invention. Suitable markers may be selected from markers that confer antibiotic or herbicide resistance. Cells containing the recombinant DNA will thus be able to survive in the presence of antibiotic or herbicide concentrations that kill untransformed cells. Examples of selectable marker genes include genes conferring resistance to antibiotics (such as *nptII* encoding neomycin phosphotransferase capable of phosphorylating neomycin and kanamycin, or *hpt* encoding hygromycin phosphotransferase

20

capable of phosphorylating hygromycin), to herbicides (for example bar which provides resistance to Basta; aroA or gox providing resistance against glyphosate), or genes that provide a metabolic trait (such as manA that allows plants to use mannose as sole carbon source). Visual marker genes result in the formation of colour (for example beta-glucuronidase, GUS), luminescence (such as luciferase) or fluorescence (Green Fluorescent Protein, GFP, and derivatives thereof). Further examples of suitable selectable marker genes include the ampicillin resistance (Ampr), tetracycline resistance gene (Tcr), bacterial kanamycin resistance gene (Kanr), phosphinothricin resistance gene, and the chloramphenicol acetyltransferase (CAT) gene, amongst others

10

The present invention also encompasses plants obtainable by the methods according to the present invention. The present invention therefore provides plants obtainable by the method according to the present invention, which plants have modified growth characteristics, which plants have altered 2xC2H2 zinc finger protein level and/or activity and/or altered expression of a nucleic acid sequence encoding a 2xC2H2 zinc finger protein.

15

Therefore, according to one aspect of the present invention, there is provided a method for the production of plants, having modified growth characteristics, comprising introducing, into a plant, a nucleic acid capable of modifying activity of a 2xC2H2 zinc finger protein and/or capable of modifying expression of a 2xC2H2 zinc-finger gene. According to a further embodiment of the present invention, there is provided a method for the production of transgenic plants having modified growth characteristics, comprising introduction and expression in a plant of a 2xC2H2 nucleic acid.

20

More specifically, the present invention provides a method for the production of transgenic plants having modified growth characteristics, which method comprises:

25

- (i) introducing into a plant or plant cell a 2xC2H2 zinc finger nucleic acid;
- (iii) cultivating the plant cell under conditions promoting plant growth.

30

The growth characteristic may be any of the characteristics defined hereinunder.

35

The 2xC2H2 zinc finger nucleic acid includes all variant nucleic acids as described herein before and includes all nucleic acids encoding all variant proteins as described herein before. Cultivating the plant cell under conditions promoting plant growth, may or may not include regeneration and or growth to maturity.

The protein itself and/or the nucleic acid itself may be introduced directly into a plant cell or into the plant itself (including introduction into a tissue, organ or any other part of the plant). According to a preferred feature of the present invention, the nucleic acid is preferably introduced into a plant by transformation.

5

The term "transformation" as referred to herein encompasses the transfer of an exogenous polynucleotide into a host cell, irrespective of the method used for transfer. Plant tissue capable of subsequent clonal propagation, whether by organogenesis or embryogenesis, may be transformed with a genetic construct of the present invention and a whole plant regenerated therefrom. The particular tissue chosen will vary depending on the clonal propagation systems available for, and best suited to, the particular species being transformed. Exemplary tissue targets include leaf disks, pollen, embryos, cotyledons, hypocotyls, megagametophytes, callus tissue, existing meristematic tissue (e.g., apical meristem, axillary buds, and root meristems), and induced meristem tissue (e.g., cotyledon meristem and hypocotyl meristem). The polynucleotide may be transiently or stably introduced into a host cell and may be maintained non-integrated, for example, as a plasmid. Alternatively, it may be integrated into the host genome. The resulting transformed plant cell can then be used to regenerate a transformed plant in a manner known to persons skilled in the art.

20 Transformation of a plant species is now a fairly routine technique. Advantageously, any of several transformation methods may be used to introduce the nucleic acid of interest (e.g. the 2xC2H2 nucleic acid) into a suitable ancestor cell. Transformation methods include the use of liposomes, electroporation, chemicals that increase free DNA uptake, injection of the DNA directly into the plant, particle gun bombardment, transformation using viruses or pollen and microprojection. Methods may be selected from the calcium/polyethylene glycol method for protoplasts (Krens, F.A. et al., 1982, Nature 296, 72-74; Negrutiu I. et al., June 1987, Plant Mol. Biol. 8, 363-373); electroporation of protoplasts (Shillito R.D. et al., 1985 Bio/Technol 3, 1099-1102); microinjection into plant material (Crossway A. et al., 1986, Mol. Gen Genet 202, 179-185); DNA or RNA-coated particle bombardment (Klein T.M. et al., 1987, Nature 327, 70) infection with (non-integrative) viruses and the like. A preferred transformation method is an *Agrobacterium* mediated transformation method.

35 Transgenic rice plants expressing a 2xC2H2 gene are preferably produced via *Agrobacterium*-mediated transformation using any of the well-known methods for rice transformation, such as the ones described in any of the following: published European patent application EP 1198985 A1, Aldemita and Hodges (Planta, 199, 612-617, 1996); Chan et al. (Plant Mol. Biol. 22 (3) 491-506, 1993); Hiei et al. (Plant J. 6 (2) 271-282, 1994); which disclosures are incorporated

by reference herein as if fully set forth. In the case of corn transformation, the preferred method is as described in either Ishida et al. (Nat. Biotechnol. 1996 Jun; 14(6): 745-50) or Frame et al. (Plant Physiol. 2002 May; 129(1): 13-22), which disclosures are incorporated by reference herein as if fully set forth.

5

Generally after transformation, plant cells or cell groupings are selected for the presence of one or more markers which are encoded by plant-expressible genes co-transferred with the gene of interest, following which the transformed material is regenerated into a whole plant.

10 Following DNA transfer and regeneration, putatively transformed plants may be evaluated, for instance using Southern analysis, for the presence of the gene of interest, copy number and/or genomic organisation. Alternatively or additionally, expression levels of the newly introduced DNA may be undertaken using Northern and/or Western analysis, both techniques being well known to persons having ordinary skill in the art.

15

The generated transformed plants may be propagated by a variety of means, such as by clonal propagation or classical breeding techniques. For example, a first generation (or T1) transformed plant may be selfed to give homozygous second generation (or T2) transformants, and the T2 plants further propagated through classical breeding techniques.

20

The generated transformed organisms may take a variety of forms. For example, they may be chimeras of transformed cells and non-transformed cells; clonal transformants (e.g., all cells transformed to contain the expression cassette); grafts of transformed and untransformed tissues (e.g., in plants, a transformed rootstock grafted to an untransformed scion).

25

The present invention clearly extends to any plant cell or plant produced by any of the methods described herein, and to all plant parts and propagules thereof. The present invention extends further to encompass the progeny of a primary transformed or transfected cell, tissue, organ or whole plant that has been produced by any of the aforementioned methods, the only requirement being that progeny exhibit the same genotypic and/or phenotypic characteristic(s) as those produced in the parent by the methods according to the invention. The invention also includes host cells having modified expression and/or level and/or activity of a 2xC2H2 zinc finger protein. Such host cells for example comprise genetic constructs as mentioned above. Preferred host cells according to the invention are derived from a plant, algae, bacterium, 30 fungus, yeast, insect or animal. The invention also extends to harvestable parts of a plant such as but not limited to seeds, leaves, fruits, flowers, petals, stamen, stem cultures, stem, 35 rhizomes, roots, tubers, bulbs or cotton fibers.

The term "plant" as used herein encompasses whole plants, ancestors and progeny of the plants and plant parts, including seeds, shoots, stems, roots (including tubers), and plant cells, tissues and organs. The term "plant" also encompasses suspension cultures, embryos, meristematic regions, callus tissue, leaves, gametophytes, sporophytes, pollen, and microspores. Plants that are particularly useful in the methods of the invention include all plants which belong to the superfamily *Viridiplantae*, in particular monocotyledonous and dicotyledonous plants including, fodder or forage legumes, ornamental plants, food crop, tree, or shrub selected from the list comprising *Acacia* spp., *Acer* spp., *Actinidia* spp., *Aesculus* spp., *Agathis australis*, *Albizia amara*, *Alsophila tricolor*, *Andropogon* spp., *Arachis* spp, *Areca catechu*, *Astelia fragrans*, *Astragalus cicer*, *Baikiaea plurijuga*, *Betula* spp., *Brassica* spp., *Bruguiera gymnorhiza*, *Burkea africana*, *Butea frondosa*, *Cadaba farinosa*, *Calliandra* spp, *Camellia sinensis*, *Canna indica*, *Capsicum* spp., *Cassia* spp., *Centroema pubescens*, *Chaenomeles* spp., *Cinnamomum cassia*, *Coffea arabica*, *Colophospermum mopane*, *Coronillia varia*, *Cotoneaster serotina*, *Crataegus* spp., *Cucumis* spp., *Cupressus* spp., *Cyathea dealbata*, *Cydonia oblonga*, *Cryptomeria japonica*, *Cymbopogon* spp., *Cynthea dealbata*, *Cydonia oblonga*, *Dalbergia monetaria*, *Davallia divaricata*, *Desmodium* spp., *Dicksonia squarosa*, *Diheteropogon amplexans*, *Dioclea* spp, *Dolichos* spp., *Dorycnium rectum*, *Echinochloa pyramidalis*, *Ehrartia* spp., *Eleusine coracana*, *Eragrestis* spp., *Erythrina* spp., *Eucalyptus* spp., *Euclea schimperi*, *Eulalia villosa*, *Fagopyrum* spp., *Feijoa sellowiana*, *Fragaria* spp., *Flemingia* spp, *Freycinetia banksii*, *Geranium thunbergii*, *Ginkgo biloba*, *Glycine javanica*, *Gliricidia* spp, *Gossypium hirsutum*, *Grevillea* spp., *Guibourtia coleosperma*, *Hedysarum* spp., *Hemarthia altissima*, *Heteropogon contortus*, *Hordeum vulgare*, *Hyparrhenia rufa*, *Hypericum erectum*, *Hyperthelia dissoluta*, *Indigo incarnata*, *Iris* spp., *Leptarrhena pyrolifolia*, *Lespediza* spp., *Lettuca* spp., *Leucaena leucocephala*, *Loudetia simplex*, *Lotonus bainesii*, *Lotus* spp., *Macrotyloma axillare*, *Malus* spp., *Manihot esculenta*, *Medicago sativa*, *Metasequoia glyptostroboides*, *Musa sapientum*, *Nicotianum* spp., *Onobrychis* spp., *Ornithopus* spp., *Oryza* spp., *Peltophorum africanum*, *Pennisetum* spp., *Persea gratissima*, *Petunia* spp., *Phaseolus* spp., *Phoenix canariensis*, *Phormium cookianum*, *Photinia* spp., *Picea glauca*, *Pinus* spp., *Pisum sativum*, *Podocarpus totara*, *Pogonarthria fleckii*, *Pogonarthria squarrosa*, *Populus* spp., *Prosopis cineraria*, *Pseudotsuga menziesii*, *Pterolobium stellatum*, *Pyrus communis*, *Quercus* spp., *Rhaphiolepis umbellata*, *Rhopalostylis sapida*, *Rhus natalensis*, *Ribes grossularia*, *Ribes* spp., *Robinia pseudoacacia*, *Rosa* spp., *Rubus* spp., *Salix* spp., *Schyzachyrium sanguineum*, *Sciadopitys verticillata*, *Sequoia sempervirens*, *Sequoiadendron giganteum*, *Sorghum bicolor*, *Spinacia* spp., *Sporobolus fimbriatus*, *Stiburus alopecuroides*, *Stylosanthos humilis*, *Tadehagi* spp, *Taxodium distichum*, *Themeda triandra*, *Trifolium* spp., *Triticum* spp., *Tsuga heterophylla*, *Vaccinium*

*spp.*, *Vicia spp.*, *Vitis vinifera*, *Watsonia pyramidata*, *Zantedeschia aethiopica*, *Zea mays*, amaranth, artichoke, asparagus, broccoli, brussel sprout, cabbage, canola, carrot, cauliflower, celery, collard greens, flax, kale, lentil, oilseed rape, okra, onion, potato, rice, soybean, straw, sugarbeet, sugar cane, sunflower, tomato, squash, and tea, trees and algae amongst others.

5 According to a preferred embodiment of the present invention, the plant is a crop plant such soybean, sunflower, canola, alfalfa, rapeseed, cotton, tomato, potato or tobacco. According to another preferred embodiment of the present invention, the plant is a monocotyledonous plant, such as sugar cane, further preferably a cereal, most preferably the plant is selected from the group consisting of rice, maize, wheat, barley, millet, rye or oats.

10

In a particular embodiment of the present invention, proteins of one plant species (for example *Arabidopsis*) are introduced in another plant species (for example rice). It has been shown in the present invention that plant growth characteristics are improved by introduction of a 2xC2H2 zinc finger gene or protein from a dicot into a monocot.

15

According to a particular embodiment of the invention, there are provided methods as described above, wherein the plant is a monocot. More preferably the plant is rice or corn.

Advantageously, performance of the methods according to the present invention leads to  
20 plants having modified growth characteristics.

The term "growth characteristic" as used herein, preferably refers to anyone or more of, but is not limited to, yield, architecture and cycle time.

The term "yield" means the amount of harvested material. For crop plants yield also means the  
25 amount of harvested material per acre of production. Depending on the crop the harvested part of the plant may be a different part or tissue of the plant, such as seed (e.g. rice, sorghum or corn when grown for seed); total above-ground biomass (e.g. for corn, when used as silage), root (e.g. sugarbeet), fruit (e.g. tomato), cotton fibers, or any other part of the plant which is of economic value. "Yield" also encompasses yield stability of the plants, meaning that year after  
30 year, one can obtain the same yield from the progeny of the plants, without too much interference of external factors, such as weather conditions. "Yield" also encompasses yield potential, which as the maximum obtainable yield.

Yield maybe dependent on a number of yield components. The parameters for these components are well known by a person skilled in the art. For example breeders are well  
35 aware of the specific yield components and the corresponding parameters for the crop they are aiming to improve.

For example key yield components for corn include number of plants per hectare or acre, number of ears per plant, number of rows (of seeds) per ear, number of kernels per row, and thousand kernel weight. For silage corn typical parameters are the above ground biomass and energy content.

- 5 Key yield components for rice include number of plants per hectare or acre, number of panicles per plant, number of spikelets per panicle, seed filling rate (number of filled seeds) and thousand kernel weight. Preferentially methods for increasing yield of rice encompass increased number of flowers per panicle and an increased number of filled seeds. The parameter of increased total number of seeds may be linked to increased number of flowers.
- 10 "Yield" further encompasses typical biomass components, such as above ground parts of a plant and the root system. General biomass parameters are area and dry weight. Specific parameters for above ground biomass further encompass above ground area and plant height. Specific parameters for the root system encompass root ratio, root length and penetration depth, root branching, root hair density, root pulling resistance and aerenchyma formation.

- 15 The plants of the present invention are characterized by increased number of filled seeds, increased total seed weight, increased total number of seeds and increased harvest index. Therefore the methods of the present invention are particularly favorable to be applied in cereals such as rice and corn (maize). Accordingly, a particular embodiment of the present invention relates to a method to increase yield of corn, comprising modifying expression of a
- 20 nucleic acid encoding a 2xC2H2 zinc finger protein.

- The plants of the present invention are characterized by an increase in thousand kernel weight and therefore the seed size or seed volume and/or the seed content and/or seed composition
- 25 are altered by the methods of the present invention. The seeds provided by the methods of the present invention may have more nutritional value, more starch and/or more oil, possibly due to their bigger size.

- The plants of the present invention are characterized by more above ground area. Therefore,
- 30 the methods of the present invention are particularly favorable for crops grown for their green tissue and/or grown for their above ground biomass. The methods of the present invention are particularly useful for grasses, forage crops (such as forage corn (maize), clover, medicago etc.), trees, sugar cane etc.

- 35 The improvement in yield as obtained by the methods of the invention, may be obtained as a result of improvement of one or more of the above mentioned yield components and/or parameters.

The term "architecture" as used herein encompasses the appearance or morphology of a plant, including any one or more structural features or combination of structural features thereof. Such structural features include the shape, size, number, position, texture, arrangement, and pattern of any cell, tissue or organ or groups of cells, tissues or organs of a plant, including the root, leaf, shoot, stem, petiole, trichome, flower, petal, stigma, style, stamen, pollen, ovule, seed, embryo, endosperm, seed coat, aleurone, fibre, cambium, wood, heartwood, parenchyma, aerenchyma, sieve element, phloem or vascular tissue, amongst others. Particular architectural characteristics that may be modified by the methods of the present invention are increased plant height, increased number or size of stems or stalks or tillers or panicles or pedicles, increased number or size of inflorescences, increased branching of for example of tassels and ears or altered flowering characteristics. A preferred architectural characteristic that may be modified by the methods of the present invention is leaf architecture. The term "leaf architecture" as used herein comprises typical leaf characteristics such as length, width, thickness, cell number, cell size and greenness.

Typically, the plants of the present invention display increased leaf surface area and /or increased leaf blade width. This trait is particularly important as it allows the plant to optimize the shape of its leaf to maximize the area used for photosynthesis. For that purpose, preferably the leaf blade is widened, but alternatively, the leaves are longer or smaller or rounder. These effects may lead to more healthy plants. Alternatively, this trait attributes aesthetic properties to the plant such as greenness and stronger leaves.

"cycle time" of the plant as used herein means the time wherein a plant reaches 90% of its maximum total area. This parameter is an indication of the duration of the vegetative growth. Prolonged vegetative growth was only displayed in some of the plants according to the present invention and may be controlled by choice of the transformation event and/or by choice of the promoter driving the 2xC2H2 nucleic acid. For example this characteristic was not displayed when a seed-preferred promoter was used.

Other "growth characteristics" that may be improved by the methods of the present invention are growth rate, early vigour, modified Tmid, T90 or A42 or altered growth curve.

It is clear from the data as presented in the examples that one or more of the growth characteristics as defined herein above, may be combined in one plant. Alternatively, depending on the chosen transformation event and/or depending on the promoter used, one

or more of these growth characteristics may be present or absent or more or less pronounced in the plant.

5 The methods of the present invention may also be used to confer stress tolerance to plants. In particular, a 2xC2H2 of the STZ type may be used to confer to a plant salt stress tolerance and/or drought stress tolerance. According to a specific embodiment, a tissue preferred promoter, such as a seed-preferred promoter" is used in these methods.

10 The present invention also relates to use of a nucleic acid sequence encoding a zinc finger protein and homologues, derivatives and active fragments thereof in modifying the growth characteristics of plants, preferably in increasing yield, further preferably increasing seed yield. The present invention also relates to use of a nucleic acid sequence encoding a 2xC2H2 zinc finger protein and homologues, derivatives and active fragments thereof and to the 2xC2H2 zinc finger protein itself and to homologues, derivatives and active fragments thereof as a  
15 growth regulator. The sequences represented by SEQ ID NO 1, and portions thereof and SEQ ID NO 2, and homologues, derivatives and active fragments thereof are useful in modifying the growth characteristics of plants, as hereinbefore described. The sequences would therefore find use as growth regulators, such as herbicides or growth stimulators. The present invention also provides a composition comprising a protein represented by SEQ ID NO 2, or a  
20 homologue, derivative or active fragment thereof for the use as a growth regulator. A growth regulator is used herein as meaning a regulator that increased yield and is therefore also referred to as yield regulator.

In particular, the present invention provides a yield regulating composition comprising a nucleic acid encoding a 2xC2H2 protein, and/ or comprising a 2xC2H2 protein, and/or comprising a  
25 construct as defined herein above. Such a yield regulating composition further comprises additives normally use in yield regulating compositions, such as a solvent or carrier.

Conversely, the sequences according to the present invention may also be interesting targets for agrochemical compounds, such as herbicides or growth stimulators. Accordingly, the  
30 present invention encompasses use of a nucleic acid encoding a 2xC2H2 protein, of a 2xC2H2 protein and/or of a construct as defined in any of claims 20 to 22 as target for an agrochemical, such as a herbicide or a growth stimulator.

The methods according to the present invention may also be practised by co-expression of a gene encoding a 2xC2H2 zinc finger protein in a plant with at least one other gene that  
35 cooperates with the gene encoding a 2xC2H2 zinc finger protein. Such a gene may be a gene encoding a target protein of the 2xC2H2 zinc finger protein. Co-expression may be effected by cloning the genes under the control of a plant expressible promoter in a plant expressible

vector and introducing the expression vector(s) into a plant cell using *Agrobacterium*-mediated plant transformation. Therefore, the methods according to the present invention may result in plants having modified growth characteristics, particularly increased yield, as described hereinbefore in combination with other economically advantageous traits, such as further yield-enhancing traits, tolerance to various stresses, traits modifying various architectural features and/or biochemical and/or physiological features.

Since the plants of the present invention have excellent growth characteristics and have high yield, they are suitable for the production of enzymes, pharmaceuticals or agrochemicals. Also, there are suitable to produce food or feed products.

The invention clearly extends to enzymes, pharmaceuticals or agrochemicals as well as food or feed products isolated from these plants.

Further a nucleic acid encoding a 2xC2H2 protein, a 2xC2H2 protein and/or the constructs of the present invention may be used breeding programs aiming at the development of plants with increased yield.

Particularly, the use of allelic variants as defined above in particular conventional breeding programmes, such as in marker-assisted breeding is also encompassed by the present invention; this may be in addition to their use in the methods according to the present invention. Such breeding programmes sometimes require the introduction of allelic variations in the plants by mutagenic treatment of a plant. One suitable mutagenic method is EMS mutagenesis. Identification of allelic variants then takes place by, for example, PCR. This is followed by a selection step for selection of superior allelic variants of the sequence in question and which give rise to altered growth characteristics in a plant. Selection is typically carried out by monitoring growth performance of plants containing different allelic variants of the sequence in question, for example, SEQ ID NO 1. Monitoring growth performance may be done in a greenhouse or in the field. Further optional steps include crossing plants in which the superior allelic variant was identified with another plant. This could be used, for example, to make a combination of interesting phenotypic features

According to another type of breeding programme a DNA marker is identified which may be genetically linked to a gene capable of modifying expression of a nucleic acid encoding a 2xC2H2 zinc finger protein in a plant, which gene may be a gene encoding the 2xC2H2 zinc finger protein itself or any other gene which may directly or indirectly influence expression of the gene encoding a 2xC2H2 zinc finger protein and/or activity of the 2xC2H2 zinc finger protein itself. This DNA marker may then be used in breeding programs to select plants having altered growth characteristics.

The methods according to the present invention may also be practised by introducing into a plant at least a part of a (natural or artificial) chromosome (such as a Bacterial Artificial Chromosome (BAC)), which chromosome contains at least a gene encoding a 2xC2H2 zinc  
 5 finger protein, optionally together with one or more related gene family members. Therefore, according to a further aspect of the present invention, there is provided a method for modifying growth characteristics of plants by expressing in a plant at least a part of a chromosome comprising at least a gene encoding a 2xC2H2 zinc finger protein.

10 The present invention will now be described with reference to the following figures in which:

**Fig. 1** is a map of an expression vector for the expression in plants of a 2xC2H2zinc finger protein under the control of a GOS2 promoter. CDS1536 is the internal code for the *Arabidopsis thaliana* salt tolerant zinc finger (STZ) protein cDNA. The zinc finger protein  
 15 expression cassette has a GOS2 promoter and a double terminator sequence (T-zein and T-rbcS-deltaGA) located within the left border (LB repeat) and the right border (RB repeat) of the Ti plasmid. Cloned within these T-borders are also a screenable marker and a selectable marker, each under the control of a constitutive promoter (Prom), followed by a terminator sequence (poly a and t-NOS). Furthermore, this vector also contains an origin of replication  
 20 (pBR322 (ori + bom)) for bacterial replication and a selectable marker (Sm/SpR) for bacterial selection.

**Fig. 2A** shows digital images from a T1 positive line transformed with an STZ zinc finger transgene under control of a GOS2 promoter and **Fig. 2B** shows digital images of  
 25 corresponding nullizygotes plants.

**Fig. 3** lists sequences useful in the methods of the present invention. SEQ ID NO 1 is an STZ encoding nucleic acid isolated from *Arabidopsis thaliana*; the start and the stop codon are highlighted in bold. SEQ ID NO 2 is the STZ protein sequence encoded by SEQ ID NO 1. In  
 30 the STZ protein the nuclear localization signal also called the KRS motif or B-box is annotated (bold, italics, underlined), as well as the L-box (bold, underlined), the EAR motif (bold, italics), and the two C2H2 zinc finger domains with QALGGH motif (bold and boxed). SEQ ID NO 10 to SEQ ID NO 25 provides the sequences of various orthologs of the *Arabidopsis thaliana* STZ protein from other plant species. SEQ ID NO 26 to SEQ ID NO 35 provides the sequences of  
 35 various paralogs (from *Arabidopsis*) of the STZ protein. SEQ ID NO 36 to SEQ ID NO 50 provides the sequences of related 2xC2H2 genes and proteins useful in the methods of the present invention.

**Fig. 4** is a photograph of T3 plants grown in a greenhouse (A) or in a field (B). The photograph shows yield increase (especially in aboveground biomass and plant height) in subsequent generations of STZ transformed plants.

5

**Fig. 5** shows the binary vector for expression in *Oryza sativa* of the *Arabidopsis thaliana* STZ gene (CDS1536) under the control of a seed preferred WSI18 promoter (PRO0151). This vector contains a T-DNA derived from the Ti Plasmid, limited by a left border (LB repeat, LB Ti C58) and a right border (RB repeat, RB Ti C58)).

- 10 The zinc finger protein expression cassette has a WSI18 (PRO0151) promoter and a double terminator sequence (T-zein and T-rbcS-deltaGA) located within the left border (LB repeat) and the right border (RB repeat) of the Ti plasmid. Cloned within these T-borders are also a screenable marker and a selectable marker, each under the control of a constitutive promoter (Prom), followed by a terminator sequence (poly a and t-NOS). Furthermore, this vector also
- 15 contains an origin of replication (pBR322 (ori + bom)) for bacterial replication and a selectable marker (Sm/SpR) for bacterial selection.

### Examples

- The present invention will now be described with reference to the following examples, which
- 20 are by way of illustration alone.

### DNA Manipulation

- Unless otherwise stated, recombinant DNA techniques are performed according to standard protocols described in Sambrook (2001) Molecular Cloning: a laboratory manual, 3rd Edition
- 25 Cold Spring Harbor Laboratory Press, CSH, New York or in Volumes 1 and 2 of Ausubel *et al.* (1988), Current Protocols in Molecular Biology, Current Protocols. Standard materials and methods for plant molecular work are described in Plant Molecular Biology Labfase (1993) by R.D.D. Croy, published by BIOS Scientific Publications Ltd (UK) and Blackwell Scientific Publications (UK).

30

### Example 1: Gene Cloning

- A gene encoding an STZ protein was amplified by PCR from an *Arabidopsis thaliana* seedling cDNA library (Invitrogen, Paisley, UK). After reverse transcription of RNA extracted from seedlings, the cDNAs were cloned into pCMV Sport 6.0. Average insert size of the bank was
- 35 1.5 kb, and original number of clones was of  $1.59 \times 10^7$  cfu. Original titer was determined to be  $9.6 \times 10^5$  cfu/ml, after first amplification of  $6 \times 10^{11}$  cfu/ml. After plasmid extraction, 200 ng of template was used in a 50 µl PCR mix. Sequences of the primers used for PCR amplification

were, including the attB sites for Gateway recombination (in bold) were PRM3204 (sense, start codon in italics) 5' **GGGGACAAGTTTGTACAAAAAAGCAGGCTTCACAATGGCG** CTCGAGGCTC 3' (SEQ ID NO 3) and PRM3205 (reverse, complementary stop codon in italics) 5' **GGGGACCACTTTGTACAAGAAAGCTGGGTAAATTCCTTAAAGTTGAAGTTTGA** 3' (SEQ ID NO 4).

PCR was performed using Hifi Taq DNA polymerase in standard conditions. The PCR fragment (CDS1536) was amplified and purified using standard methods. The first step of the Gateway procedure, the BP reaction, was then performed, during which the PCR fragment was recombined *in vivo* with the pDONR plasmid to produce, according to Gateway terminology, an "entry clone", p3359. PDONR was purchased from Invitrogen, as part of the Gateway technology.

#### **Example 2: Vector construction for rice transformation with pGOS2::AtSTZ**

The entry clone p3359 was subsequently used in an LR reaction with p0640, a destination vector used for rice transformation. This vector contains as functional elements within the T-DNA borders a plant selectable marker and a Gateway cassette intended for LR *in vivo* recombination with the sequence of interest already cloned in the donor vector. Upstream of this Gateway cassette lies the rice GOS2 promoter for constitutive expression of the zinc finger gene (De Pater *et al.*, Plant J. 2 (6) 837-844, 1992). After the recombination step, the resulting expression vector with the expression cassette CD4398 (Figure 1) was transformed into *Agrobacterium* strain LBA4404 and subsequently into plants. Transformed rice plants were allowed to grow and then examined for various parameters as described in Example 3.

#### **Example 3: Evaluation of T0, T1 and T2 transgenic rice plants transformed with pGOS2::AtSTZ (CD4398)**

Approximately 15 to 20 independent T0 transformants were generated. The primary T0 transformants were transferred from tissue culture chambers to a greenhouse for growing and harvest of T1 seed. Six events of which the T1 progeny segregated 3:1 for presence/absence of the transgene were retained. For each of these events, approximately 10 T1 seedlings containing the transgene (hetero- and homo-zygotes), and approximately 10 T1 seedlings lacking the transgene (nullizygotes), were selected by PCR. Based on the results of the T1 evaluation three events were chosen, for further characterisation in the T2 generation, one event being very positive for a number of parameters, a second event being positive for a number of parameters, but less pronounced, and a third event being neutral. Seed batches from the positive plants (both hetero- and homozygotes) in T1, were screened by monitoring marker expression. For each chosen event, the heterozygote seed batches were then selected

for T2 evaluation. An equal number of positives and negatives within each seed batch were transplanted for evaluation in the greenhouse (i.e., for each event 40 plants were grown of which there were about 20 positives for the transgene and about 20 negative). Therefore, the total number for the three events amounted to 120 plants for evaluation in the T2 generation.

5

T1 and T2 plants were transferred to the greenhouse and evaluated for vegetative growth parameters and seed parameters, as described hereunder.

#### **(I) Statistical analysis of phenotypic characteristics**

10 A two factor ANOVA (analyses of variance) was used as statistical model for the overall evaluation of plant phenotypic characteristics. An F-test was carried out on all the parameters measured, for all the plants of all the events transformed with the gene of interest. The F-test was carried out to check for an effect of the gene over all the transformation events and to verify an overall effect of the gene or "global gene effect". Significant data, as determined by  
15 the value of the f-test, indicates a "gene" effect, meaning that the phenotype observed is caused by more than the presence or position of the gene. In case of the F-test, the threshold for significance for a global gene effect is set at 5% probability level.

To check for an effect of the genes within an event, i.e., for a line-specific effect, a t-test was  
20 performed within each event using data sets from the transgenic plants and the corresponding null plants. "Null plants" or "Null segregants" are the plants treated in the same way as the transgenic plant, but from which the transgene has segregated. Null plants can also be described as homozygous negative transformant plants. The threshold for significance for the t-test is set at 10% probability level. Within one population of transformation events, some  
25 events may be under or above this t-test threshold. This is based on the hypothesis that a gene might only have an effect in certain positions in the genome, and that the occurrence of this position-dependent effect is not uncommon. This kind of gene effect may also be referred to as a "line effect of a gene". The p- value is obtained by comparing the t-value to the t-distribution or alternatively, by comparing the F-value to the f-distribution. The p- value stand  
30 for the probability that the null hypothesis (null hypothesis being "there is no effect of the transgene") is correct.

#### **(II) Vegetative growth measurements**

The selected plants were grown in a greenhouse. Each plant received a unique barcode label  
35 to link unambiguously the phenotyping data to the corresponding plant. The selected plants were grown on soil in 10 cm diameter pots under the following environmental settings: photoperiod= 11.5 h, daylight intensity= 30,000 lux or more, daytime temperature= 28°C or

higher, night time temperature= 22°C, relative humidity= 60-70%. Transgenic plants and the corresponding nullizygotes were grown side-by-side at random positions. From the stage of sowing until the stage of maturity (which is the stage where there is no more increase in biomass) the plants were passed weekly through a digital imaging cabinet (examples of pictures are shown in Figures 2A and 2B). At each time point digital images (2048x1536 pixels, 16 million colours) were taken of each plant from at least 6 different angles. The parameters described below were derived in an automated way from the digital images using image analysis software.

#### 10 **(a) Aboveground area**

Plant above ground area was determined by counting the total number of pixels from aboveground plant parts discriminated from the background. This value was averaged for the pictures taken on the same time point from the different angles and was converted to a physical surface value expressed in square mm by calibration. Experiments show that the aboveground plant area measured this way correlates with the biomass of plant parts above ground.

Results of the maximum above ground area values of the lines selected for T2 evaluation are summarized in Table 1. The plants of the best performing line showed an increase in biomass of 34 % , compared to the nullizygotes.

When an F-test was carried out on all the plants of all the T2 events it became clear that the transgenic plants show a significant increase in above ground area, in average an increase of approximately 18%. A significant increase in above ground biomass is also displayed by STZ transformed plants grown under field conditions (see figure 4).

25

Table 1: *Aboveground area of STZ transgenic T2 plants. Each row corresponds to one event, for which the average maximum aboveground area (expressed in mm<sup>2</sup>) was determined for the transgenics (TR) and the null plants (null). The difference in absolute values between the transgenic population and the nullizygotes of each event are presented (dif.) as well as the percentage of difference between the two populations (% dif). P stands for the probability produced by the t-test for each event. The last row presents the average numbers calculated from all the events. Here the p-value is produced by the F-test.*

30

| Total above ground Area Max (mm <sup>2</sup> ) |       |       |       |       |         |
|------------------------------------------------|-------|-------|-------|-------|---------|
| Line                                           | TR    | null  | dif   | % dif | p-value |
| CD4396 L1                                      | 63947 | 47606 | 16341 | 34    | 0.0021  |
| CD4396 L2                                      | 42509 | 41342 | 1167  | 3     | 0.8063  |
| CD4396 L3                                      | 41116 | 33687 | 7429  | 22    | 0.1107  |
| Overall                                        | 49178 | 41657 | 7522  | 18    | 0.0047  |

**(b) Plant height measurements**

Plant height was determined by the distance between the horizontal lines going through the upper pot edge and the uppermost pixel corresponding to a plant part above ground. This value was averaged for the pictures taken on the same time point from the different angles and was converted, by calibration, to a physical distance expressed in mm. Experiments showed that plant height measured this way correlate with plant height measured manually with a ruler.

The increase in plant height was displayed very clearly in STZ transformed plants when measured at the end of the vegetative growth (see figure 4A). Also, this parameter, was displayed by STZ transformed plants when grown in the field (see figure 4B) at the time of harvest.

**(c) Total area cycle time measurements**

Plants were imaged weekly along the complete cell cycle and the maximum total area of the plants was determined as mentioned above. Total Area Cycle Time is the time when a plant reaches 90% of its maximum total area. This parameter is an indication of the duration of the vegetative growth.

Only in some transgenic lines there was an effect on cycle time. These few lines showed a prolonged vegetative growth.

**(III) Measurement of seed-related parameters**

The mature primary panicles were harvested, bagged, barcode-labelled and then dried for three days in the oven at 37°C. The panicles were then threshed and all the seeds collected. The filled husks were separated from the empty ones using an air-blowing device. After separation, both seed lots were then counted using a commercially available counting machine. The empty husks were discarded. The filled husks were weighed on an analytical balance and the cross-sectional area of the seeds was measured using digital imaging. This procedure resulted in the set of seed-related parameters described below.

**(a) Total number of filled seeds per plant**

The number of filled seeds was determined by counting the number of filled husks that remained after the separation step.

- 5 **Total numbers of filled seeds per plant are summarized in Table 2. The t-test shows that for two events, transgenic plants produce 106% and 130% more filled seeds than the nullizygotes.**

10 Table 2: *Number of filled seeds of STZ transgenic T2 plants. Each row corresponds to one event, for which the average number of filled seeds was determined for the transgenics (TR) and the null plants (null). The difference in absolute values between the transgenic population and the nullizygotes of each event are presented (dif.) as well as the percentage of difference between the two populations (% dif). P stands for the probability produced by the t-test for each event. The last row presents the average numbers calculated from all the events. Here*

15 *the p-value is produced by the F-test.*

| Number of filled seeds |       |       |        |       |         |
|------------------------|-------|-------|--------|-------|---------|
| Line                   | TR    | null  | dif    | % dif | p-value |
| CD4396 L1              | 387.9 | 188.7 | 199.19 | 106   | <0.0001 |
| CD4396 L2              | 163.8 | 156.5 | 7.22   | 5     | 0.8382  |
| CD4396 L3              | 236.9 | 102.9 | 133.98 | 130   | 0.0004  |
| Overall                | 264.9 | 159.7 | 105.25 | 66    | <0.0001 |

**(b) Total seed weight per plant**

The total seed weight was measured by weighing all filled husks harvested from a plant.

- 20 The total seed weight values of STZ transformed plants are summarized in Table 3. STZ transgenic plants produce significantly more seed weight than the corresponding nullizygotes. The difference in seed weight of the transgenics may be as high as 138% or higher.

25 Table 3: *Total seed weight per plant of STZ transgenic T2 plants. Each row corresponds to one event, for which the average total seed weigh (in gram) was determined for the transgenics (TR) and the null plants (null). The difference in absolute values between the transgenic population and the nullizygotes of each event are presented (dif.) as well as the percentage of difference between the two populations (% dif). P stands for the probability produced by the t-test for each event. The last row presents the average numbers calculated*

30 *from all the events. Here the p-value is produced by the F-test.*

| Total weight of seeds |     |      |      |       |         |
|-----------------------|-----|------|------|-------|---------|
| Line                  | TR  | null | dif  | % dif | p-value |
| CD4396 L1             | 9.8 | 4.5  | 5.25 | 116   | <0.0001 |
| CD4396 L2             | 3.4 | 3.3  | 0.1  | 3     | 0.908   |
| CD4396 L3             | 6.1 | 2.6  | 3.56 | 138   | 0.0001  |
| Overall               | 6.5 | 3.7  | 2.75 | 74    | <0.0001 |

### (c) Harvest index

The harvest index in the present invention is defined as the ratio between the total seed yield and the above ground area (mm<sup>2</sup>), multiplied by a factor 10<sup>6</sup>.

5

The harvest index values of the STZ-transgenic plants are summarized in Table 4. STZ transgenic plants have a significant increase in harvest index. The increase in harvest index of the transgenic plants may be as high as 66%, when compared to the corresponding nullizygotes.

10

Table 4: *Harvest index of STZ transgenic T2 plants. Each row corresponds to one event, for which the average harvest index was determined for the transgenics (TR) and the null plants (null). The difference in absolute values between the transgenic population and the nullizygotes of each event are presented (dif.) as well as the percentage of difference between the two populations (% dif). P stands for the probability produced by the t-test for each event. The last row presents the average numbers calculated from all the events. Here the p-value is produced by the F-test.*

15

| Harvest index |       |      |       |       |         |
|---------------|-------|------|-------|-------|---------|
| Line          | TR    | null | dif   | % dif | p-value |
| CD4396 L1     | 149.1 | 90   | 59.11 | 66    | <0.0001 |
| CD4396 L2     | 74    | 73.4 | 0.55  | 1     | 0.9574  |
| CD4396 L3     | 121.3 | 75.9 | 45.32 | 60    | <0.0001 |
| Overall       | 114.8 | 82.6 | 32.16 | 39    | <0.0001 |

### (d) Thousand kernel weight (TKW) of plants

20

Thousand Kernel Weight (TKW) is a parameter extrapolated from the number of filled seeds counted, and their total weight.

The weight values of thousand kernels of STZ transgenic plants are presented in Table 5. STZ transgenic plants have increased thousand kernel weight. The increase of TKW of transgenic plants may be as high as 6% when compared to the corresponding nullizygotes.

Table 5: Thousand kernel weight of STZ transgenic T2 plants. Each row corresponds to one event, for which the average TKW was determined for the transgenics (TR) and the null plants (null). The difference in absolute values between the transgenic population and the nullizygotes of each event are presented (dif.) as well as the percentage of difference between the two populations (% dif). P stands for the probability produced by the t-test for each event. The last row presents the average numbers calculated from all the events. Here the p-value is produced by the F-test.

| TKW       |      |      |       |       |         |
|-----------|------|------|-------|-------|---------|
| Line      | TR   | null | dif   | % dif | p-value |
| CD4396 L1 | 25.2 | 23.8 | 1.46  | 6     | 0.0128  |
| CD4396 L2 | 20.6 | 20.7 | -0.14 | -1    | 0.7963  |
| CD4396 L3 | 25.5 | 24.5 | 0.99  | 4     | 0.0812  |
| Overall   | 23.7 | 23   | 0.71  | 3     | 0.0213  |

#### 10 (e) Total number of seeds

The total number of seeds per plant was measured by counting the number of husks harvested from a plant.

15 The total numbers of seeds per plant are summarized in Table 6. STZ transformed plants have an increase in total number of seeds. The increase of total number of seeds may be as high as 68%, when compared to the corresponding nullizygotes.

20 Table 6: Total number of seeds of STZ transgenic T2 plants. Each row corresponds to one event, for which the average total number of seeds was determined for the transgenics (TR) and the null plants (null). The difference in absolute values between the transgenic population and the nullizygotes of each event are presented (dif.) as well as the percentage of difference between the two populations (% dif). P stands for the probability produced by the t-test for each event. The last row presents the average numbers calculated from all the events. Here the p-value is produced by the F-test.

| Total number of seeds |       |       |        |       |         |
|-----------------------|-------|-------|--------|-------|---------|
| Line                  | TR    | null  | dif    | % dif | p-value |
| CD4396 L1             | 483.5 | 367.4 | 116.03 | 32    | 0.0146  |
| CD4396 L2             | 353.9 | 327.5 | 26.42  | 8     | 0.5473  |
| CD4396 L3             | 383.6 | 228.2 | 155.48 | 68    | 0.0009  |
| Overall               | 406   | 312.5 | 93.52  | 30    | 0.0002  |

**Conclusion**

It may be concluded that vegetative growth is increased in the STZ transgenic plants when compared to the control non-transgenic plants, as reflected by parameters such as above ground area, where the increase is above 20 %. This effect may be attributed to the expression of the STZ gene in the transgenic plants. Additionally, in some transformation events, the length of the vegetative growth is altered in the STZ transgenic plants. For those transformation events in which this effect occurs, in average the vegetative growth was prolonged with about 4 to 6 days, under the conditions tested.

Furthermore, yield was increased in STZ transgenic plants. Several seed parameters reflect this yield increase. The total number of seeds harvested was at least 100% higher in the transgenics than in the control plants, for those events showing a differential. For these events, there was also an increase in the total number of seeds of the transgenics, which increase was higher than 30 %. Seed filling in those transgenics was greatly improved, reaching differences above 100% in the number of filled seeds.

Seed of the transgenic plants were also heavier, and probably bigger, as suggested by the higher values obtained for the thousand kernel weigh. The TKW parameter is a very stable parameter in rice cultivars, such as nipponbare, and in the growth conditions here used. This means that this parameter is not easily influenced and makes it an important yield parameter. Therefore a TKW increase of 6 % represents a significantly increase in yield.

Harvest index, another important yield parameter, was increased in the transgenic plants with more than 50 %.

In summary, based on the evaluation of STZ transgenic plants in the T1, T2 and further generations, it may be concluded that the presence of an STZ transgene, has a positive effect on the size of the plant and/or its organs, as well as a positive effect on the final yield harvested.

**(iii) Root growth measurements**

Transgenic plants are grown next to their corresponding non-transgenic null segregant in transparent pots. In average, for each construct comprising a particular promoter-2xC2H2 combination, a minimum of 5 independent transformation events are evaluated for root growth, root development and root architecture. Typically, per transformation event, 10 transgenics are compared to 10 nullizygotes. Root pictures are taken weekly during plant growth. The pictures are processed and analyzed to extract the values for the root parameters as detailed below. Statistical analysis as described above are applied to these data.

**a) Root Area**

Total root area is calculated from the summed number of pixels of each root images. A positive linear correlation between root area and dry weight and root biomass of the root has been previously established by similar experiments. Therefore, root area is a good approximation for root biomass.

**b) Root Length**

The total perimeter of the roots of a plant is calculated as the sum of the perimeter of all roots in the images. A linear correlation between this measurement and root length has been previously established. Thus, root length is extrapolated from the total root perimeter.

**c) Root Width**

Average root width of a plant is expressed as the ratio between the Root Area and the Root Length.

STZ transgenic plants of the invention show a superior performance when compared to control plants. Transgenic plants are altered in one or more the root parameters detailed above. In particular the transgenic have increased root biomass, for example due to increased root dry weight or area, and/or increased root length and/or increased root width.

**Example 4: Leaf Blade Width Measurement.**

Leaves of STZ transgenic plants appeared bigger and wider when compared to the corresponding control non-transgenic plants. To quantify the increase in leaf width, leaf blade width (length of transversal axe) of the flag leaf was measured with a ruler at the widest point of the leaf, which is approximately at half of the length, in plants that have reached the end of the vegetative growth phase. The results shown in the Table 7, indicate that the increase in the leaf blade width in at least the event here measured was around 15 % when compared to the corresponding nullizygote.

Table 7: Leaf blade width of STZ transgenic T2 plants. The average leaf blade width was determined for the transgenics (TR) and the null plants (null) of the selected event. The difference in absolute values between the transgenic population and the nullizygotes of the event is presented (dif.) as well as the percentage of difference between the two populations (% dif). P stands for the probability produced by the t-test .

| Leaf blade width |      |      |      |       |         |
|------------------|------|------|------|-------|---------|
| Line             | TR   | null | dif  | % dif | p-value |
| CD4396 L1        | 1.56 | 1.35 | 0.21 | 15    | 0.098   |

#### Example 5: Vector construction for rice transformation with pWSI18::AtSTZ

Vector construction for transformation with the pWSI18 (PRO0151) - AtSTZ (CDS1536) cassette was carried out essentially as in example 2. The entry clone p3359, described earlier, was subsequently used in an LR reaction with p05653, a destination vector used for rice transformation. This destination vector contains as functional elements within the T-DNA borders a plant selectable marker and a Gateway cassette intended for LR in vivo recombination with the sequence of interest already cloned in the donor vector. A WSI18 promoter for seed preferred expression (PRO0151) is located upstream of this Gateway cassette. After the recombination step, the resulting expression vector with the expression cassette CD4398 (Figure 5) was transformed into *Agrobacterium* strain LBA4404 and subsequently this vector was transformed to *Oryza sativa* plants. Transformed rice plants were allowed to grow and then examined for various parameters as described in example 3.

15

#### Example 6: Evaluation of T0 and T1 transgenic rice plants transformed with the seed preferred expression cassette pWSI18::AtSTZ (CD4398)

Preparations of calli and of the *Agrobacterium tumefaciens* strain containing the expression vector with the CD4398 expression cassette, were carried out as described in example 3, as were the calli transformation and plant regeneration.

20

Approximately 15 to 20 independent T0 rice transformants were generated. The primary transformants were transferred from tissue culture chambers to a greenhouse for growing and harvest of T1 seed. Events, of which the T1 progeny segregated 3:1 for presence/absence of the transgene, were retained. For each of these events, approximately 10 T1 seedlings containing the transgene (hetero- and homo-zygotes), and approximately 10 T1 seedlings lacking the transgene (nullizygotes), were selected by monitoring marker expression.

25

Transgenic plantlets were grown next to control nullizygotes, seeds were harvested and thousand kernel weight determined as previously described.

30

Transformed plants comprising the expression cassette CD8490 (seed preferred pWSI18::STZ), had a normal and healthy appearance and were harvested at the same time as the control plants. The seeds harvested from the transgenic plants had an increase in

thousand kernel weight when compared to the control plants. As shown in Table 8 increase in thousand kernel weight was above 10%.

Table 8: *Thousand kernel weight of STZ transgenic T1 plants. The average 1 thousand kernel weight was determined for the transgenics (TR) and the null plants (null) of the selected event. The difference in absolute values between the transgenic population and the nullizygotes of the event is presented (dif.) as well as the percentage of difference between the two populations (% dif). P stands for the probability produced by the t-test .*

| Thousand kernel weight |      |      |      |       |         |
|------------------------|------|------|------|-------|---------|
| Line                   | TR   | null | dif  | % dif | p-value |
| CD8490 L1              | 29.6 | 26.8 | 2.82 | 11    | 0.001   |

#### Example 7: Cloning, transformation and evaluation of other 2xC2H2 encoding genes.

In Table 9 an overview is given of constructs with STZ or other 2xC2H2 zinc finger proteins, under control of various promoters, which constructs are made for use in the methods of the present invention. The coding regions of the 2xC2H2 genes to be cloned (GOI, Gene of Interest) are amplified by PCR from cDNA , following the protocol as in Example 1. Specific primers for each 2xC2H2 gene were designed at the start and stop codons of the gene sequence as present in the public database under the accession number as indicated in Table 9. These cloned sequences are also herein incorporated under the SEQ ID NO number as mentioned in the table. Moreover, the isolated PCR fragments were also given a unique CDS number.

The PCR fragment with a 2xC2H2 gene is then cloned under the control of a particular promoter. Different combinations for different genes are made (see Table 9). Chimeric constructs are made and CD numbers represent bacterial strains carrying the chimeric construct. Corresponding transgenic plants are obtained by transforming the plants with the chimeric constructs, following the protocols as mentioned herein before. Evaluation of the transgenic events reveals an increase in yield, and increase in leaf surface area and/or an increase in duration of vegetative growth in the transgenic plants when compared to the control non-transgenic plants.

**CD-070-PCT****Table 9:** examples of 2xC2H2 chimeric constructs useful for the methods of the present invention. \*see Table 1

| CDS                                    | Accession number<br>(cDNA on which primers<br>were designed to amplify<br>the CDS region) | Prot ACC number     | SEQ ID NO | PRO0129* | PRO0170* | PRO0061_2* | PRO0123* | PRO0207* | PRO0110* |
|----------------------------------------|-------------------------------------------------------------------------------------------|---------------------|-----------|----------|----------|------------|----------|----------|----------|
| CDS1536<br>STZ<br>Arabidopsis          | X95573                                                                                    | CAA64820            | 1 + 2     | CD4398   | CD11371  | CD11382    | CD10960  | CD10959  | CD10319  |
| CDS2200<br>Paralog<br>Arabidopsis      | AF022658 NM_120516                                                                        | AAB80922.1At5g04340 | 28 + 29   | CD11576  |          |            | CD11413  |          | CD11540  |
| CDS2205<br>Paralog<br>Arabidopsis      | NM_123683                                                                                 | At5g43170           | 32 + 33   | CD11325  |          |            | CD11414  |          | CD11387  |
| CDS2775<br>Ortholog<br>Oryza<br>sativa | AF332876                                                                                  | AAK01713.1          | 36 + 37   | CD09948  |          |            |          |          | CD10319  |
| CDS1677<br>Homolog<br>Arabidopsis      | AL132966 REGION:<br>116202..116729                                                        | CAB67667            | 38 + 39   | CD06462  |          |            | CD       |          | CD       |
| CDS3337<br>Homolog<br>Sugarcane        | CA279020                                                                                  |                     | 40        | CD       |          |            | CD       |          | CD       |
| CDS2416<br>Homolog<br>Arabidopsis      | AF254447                                                                                  | At3g57670           | 41 + 42   | CD       |          |            | CD       |          | CD       |
| CDS2377<br>Homolog<br>Arabidopsis      | AJ311810                                                                                  | CAC86167            | 43 + 44   | CD       |          |            | CD       |          | CD       |
| CDS<br>Homolog<br>Arabidopsis          | AL355775 REGION:<br>complement(7957..8451)                                                | CAB90935            | 45 + 46   | CD       |          |            | CD       |          | CD       |
| CDS<br>Homolog<br>Arabidopsis          | AL391143 REGION:<br>complement(31730..32938)                                              | CAC01747            | 47 + 48   | CD       |          |            | CD       |          | CD       |
| CDS3641<br>Homolog<br>Arabidopsis      | X98678                                                                                    | CAA67236            | 49 + 50   | CD       |          |            | CD       |          | CD       |

**Table 10:** examples promoters used in combination with 2xC2H2 for the methods of the present invention.

| Promoter  | Preferred expression type                              | Origin species        | Gene                          |
|-----------|--------------------------------------------------------|-----------------------|-------------------------------|
| PRO0151   | Seeds (mainly embryo and aleurone). Strong expression. | Oryza sativa          | WSI18                         |
| PRO0110   | Root                                                   | Oryza sativa          | RCc3                          |
| PRO0207   | Green tissue. Moderate expresssion levels              | Saccharum officinarum | Prp                           |
| PRO0123   | Green tissue. Strong expression levels.                | Oryza sativa          | Protochlorophyllide reductase |
| PRO0090   | Seed specific (mainly endosperm)                       | Oryza sativa          | Prolamin RP6                  |
| PRO0170   | Constitutive. Strong Expression.                       | Oryza sativa          | High Mobility Group protein   |
| PRO0218   | Seeds (mainly embryo and aleurone)                     | Oryza sativa          | oleosine 18kda                |
| PRO0061_2 | Young expanding tissues                                | Oryza sativa          | beta-expansine EXPB9          |
| PRO0129   | Constitutive. High expression levels.                  | Oryza sativa          | GOS2                          |

5

**Example 8: use of the invention in corn.**

The methods of the invention described herein are also used in maize. To this aim, an STZ encoding gene, for example a maize or other STZ ortholog, is cloned under control of a promoter operable in maize, in a plant transformation vector suitable for Agrobacterium-mediated corn transformation. Methods to use for corn transformation have been described in literature (Ishida et al., Nat Biotechnol. 1996 Jun;14(6):745-50; Frame et al., Plant Physiol. 2002 May;129(1):13-22).

Transgenic plants made by these methods are grown in the greenhouse for T1 seed production. Inheritability and copy number of the transgene are checked by quantitative real-time PCR and Southern blot analysis and expression levels of the transgene are determined by reverse PCR and Northern analysis. Transgenic lines with single copy insertions of the transgene and with varying levels of transgene expression are selected for T2 seed production.

20

Progeny seeds are germinated and grown in the greenhouse in conditions well adapted for maize (16:8 photoperiod, 26-28°C daytime temperature and 22-24°C nighttime temperature)

as well under water-deficient, nitrogen-deficient, and excess NaCl conditions. Null segregants from the same parental line, as well as wild type plants of the same cultivar are used as controls. The progeny plants resulting from the selfing or the crosses are evaluated on different biomass and developmental parameters, including, plant height, stalk/stem thickness, stem size, number of leaves, total above ground area, leaf greenness, time to maturity, time to silking, flowering time, time to flower, ear number, ear length, row number, kernel number, kernel size, kernel oil content, grain maturity, harvesting time. The seeds of these lines are also checked on various parameters, such as grain size, total grain yield per plant, and grain quality (starch content, protein content and oil content).

10

Lines that are most significantly improved compared to corresponding control lines are selected for further field-testing and marker-assisted breeding, with the objective of transferring the field-validated transgenic traits into commercial germplasm. The testing of maize for growth and yield-related parameters in the field is conducted using well-established protocols.

15

The corn plants are particularly evaluated on yield parameters, such as for example, amount of plants per acre, amount of ears per plant, amount of rows per ear, amount of seeds per row and TKW. Subsequent improvements for introgressing specific loci (such as transgene containing loci) from one germplasm into another is also conducted using well-established protocols.

20

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<120> Plants having modified growth characteristics and a method for making the same

<130> CD-070-PCT

<160> 50

<170> PatentIn version 3.1

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|     |     |     |     | 180 |     |     |     |     | 185 |     |     |     |     | 190 |     |
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| tggaagcact | taactcacc  | accactgcta  | ctcctttcac | accctttgag | gaaccaaadc | 180  |
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 Lys Thr Thr Thr Thr Ala Thr Ala Ala Ser Asp Asp Asn Asn Pro Ser  
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 Thr Ser Thr Ser Thr Gly Ala Val Asn Ile Ser Ala Leu Asn Pro Thr  
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 Gly Arg Ser His Val Cys Ser Ile Cys His Lys Ala Phe Pro Thr Gly  
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 Asp Phe Asp Leu Asn Met Pro Ala Ser Pro Glu Leu Gln Leu Gly Leu  
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<213> Oryza sativa

<400> 18  
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gtggaagcat gtcgagcgcg tcgtccatgg aagcgtcca cgccgcggtg ctcaaggagg 180  
agcagcagca gcacgaggtg gaggaggcga cggtcgtgac gagcagcagc gccacgagcg 240  
gggaggaggg cggacacctg ccccaggggt gggcgaagcg gaagcggtcg cgccgccagc 300  
gatcggagga ggagaacctc gcgctctgcc tcctcatgct cgcccgcggc ggccaccacc 360  
gcgtccaggc gccgcctccg ctctcggctt cggcgccccc gccggcaggt gcggagttca 420  
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gccaccgggt caagctgccg actccgcccg cagctcccgt cttggctccc gccccgctcg 540  
ccgccttgct gccttccgcc gaggaccgcg agccagccac gtcattccacc gccgcgtcct 600  
ccgacggcat gaccaacaga gtccacaggt gttccatctg ccagaaggag ttccccaccg 660  
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gcgcattctc aaccgagctc ctggccacgg tggccgccga gtccgaggtg ggaagctccg 780  
gcaacggcca gtccgccacc cgggcgttcg acctcaacct cccggccgtg ccggagttcg 840  
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ccctcgcctt caagaagccc cggcttctca ccgcgtaatt cagcagctgc acggatccga 960  
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agccatacat acagtcatac agatataggt ctagctcttc cttggttcct tgtaacactg 1140  
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aaaaaaaaaaa aaa 1213

<210> 19  
<211> 269  
<212> PRT  
<213> Oryza sativa

<400> 19

Met Ser Ser Ala Ser Ser Met Glu Ala Leu His Ala Ala Val Leu Lys  
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Glu Glu Gln Gln Gln His Glu Val Glu Glu Ala Thr Val Val Thr Ser  
20 25 30  
Ser Ser Ala Thr Ser Gly Glu Glu Gly Gly His Leu Pro Gln Gly Trp  
35 40 45  
Ala Lys Arg Lys Arg Ser Arg Arg Gln Arg Ser Glu Glu Glu Asn Leu  
50 55 60  
Ala Leu Cys Leu Leu Met Leu Ala Arg Gly Gly His His Arg Val Gln  
65 70 75 80

Ala Pro Pro Pro Leu Ser Ala Ser Ala Pro Pro Pro Ala Gly Ala Glu  
85 90 95

Phe Lys Cys Ser Val Cys Gly Lys Ser Phe Ser Ser Tyr Gln Ala Leu  
100 105 110

Gly Gly His Lys Thr Ser His Arg Val Lys Leu Pro Thr Pro Pro Ala  
115 120 125

Ala Pro Val Leu Ala Pro Ala Pro Val Ala Ala Leu Leu Pro Ser Ala  
130 135 140

Glu Asp Arg Glu Pro Ala Thr Ser Ser Thr Ala Ala Ser Ser Asp Gly  
145 150 155 160

Met Thr Asn Arg Val His Arg Cys Ser Ile Cys Gln Lys Glu Phe Pro  
165 170 175

Thr Gly Gln Ala Leu Gly Gly His Lys Arg Lys His Tyr Asp Gly Gly  
180 185 190

Val Gly Ala Gly Ala Gly Ala Ser Ser Thr Glu Leu Leu Ala Thr Val  
195 200 205

Ala Ala Glu Ser Glu Val Gly Ser Ser Gly Asn Gly Gln Ser Ala Thr  
210 215 220

Arg Ala Phe Asp Leu Asn Leu Pro Ala Val Pro Glu Phe Val Trp Arg  
225 230 235 240

Pro Cys Ser Lys Gly Lys Lys Met Trp Asp Glu Glu Glu Glu Val Gln  
245 250 255

Ser Pro Leu Ala Phe Lys Lys Pro Arg Leu Leu Thr Ala  
260 265

&lt;210&gt; 20

&lt;211&gt; 1020

&lt;212&gt; DNA

&lt;213&gt; Petunia x hybrida

&lt;400&gt; 20

|             |            |             |             |             |            |     |
|-------------|------------|-------------|-------------|-------------|------------|-----|
| ttcactcacc  | aaaacaactt | ctctacctct  | tctacttgca  | cattcaaatt  | ctttcattac | 60  |
| tacttatctc  | tactaatctt | gattcgattt  | tagtaaatca  | aacaagagaa  | tcttttcagt | 120 |
| aatacaaaca  | agaaaatttt | ctctctatac  | ttgattgagt  | ttagtaaggc  | aaacaagaaa | 180 |
| actatcatgg  | cacttgaagc | attgaattct  | ccaactacaa  | caacaccacc  | atcattccaa | 240 |
| tttgagaaca  | acgggcttaa | gtaccttgag  | agttggacaa  | aaggtaaaaag | atcaaaaagg | 300 |
| caacgcagca  | tggaacgaca | gtgtactgaa  | gaagagtatt  | tagcactttg  | tcttatcatg | 360 |
| ctagcacgta  | gcgatggttc | tggttaataac | tcacggcttc  | taccaccacc  | accactacca | 420 |
| ccatcagttc  | cagtaacgtc | gcaaataaac  | gcgacgttat  | tggaacagaa  | gaatttgtac | 480 |
| aagtgttccg  | tttgtggtaa | agggtttggg  | tcttatcaag  | cttttaggtgg | acataaagca | 540 |
| agtcaccgga  | aacttgtcag | catgggagga  | gatgaacaat  | ctactacttc  | cactactact | 600 |
| aacgtaacgg  | gaactagttc | cgctaacggt  | aacggtaacg  | gaagaactca  | cgaatgttca | 660 |
| atthgtcaca  | agtgcctttc | tactggacaa  | gcttttaggtg | gtcataaaaag | gtgccactat | 720 |
| gacgggtggt  | acggtaacgg | taacggaagt  | gtaagtgttg  | gggtgacgtc  | atctgaaggt | 780 |
| gtgggggtcca | ctattagtca | tcaccgtgac  | tttgacttga  | atattcccgc  | gttgccggag | 840 |
| ttttggccgg  | gatttggttc | cggcgaggat  | gaggtggaga  | gtcctcatcc  | agcaaagaag | 900 |

tcaaggctat ctcttccacc taaacttgaa ttattcaaag gattatagag ggaatattga 960  
 tttgttacag gaagatttat taggattcac gaattttttg ttgactagtt tatgtaatat 1020

<210> 21  
 <211> 253  
 <212> PRT  
 <213> Petunia x hybrida

<400> 21  
 Met Ala Leu Glu Ala Leu Asn Ser Pro Thr Thr Thr Thr Pro Pro Ser  
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 Phe Gln Phe Glu Asn Asn Gly Leu Lys Tyr Leu Glu Ser Trp Thr Lys  
 20 25 30  
 Gly Lys Arg Ser Lys Arg Gln Arg Ser Met Glu Arg Gln Cys Thr Glu  
 35 40 45  
 Glu Glu Tyr Leu Ala Leu Cys Leu Ile Met Leu Ala Arg Ser Asp Gly  
 50 55 60  
 Ser Val Asn Asn Ser Arg Ser Leu Pro Pro Pro Pro Leu Pro Pro Ser  
 65 70 75 80  
 Val Pro Val Thr Ser Gln Ile Asn Ala Thr Leu Leu Glu Gln Lys Asn  
 85 90 95  
 Leu Tyr Lys Cys Ser Val Cys Gly Lys Gly Phe Gly Ser Tyr Gln Ala  
 100 105 110  
 Leu Gly Gly His Lys Ala Ser His Arg Lys Leu Val Ser Met Gly Gly  
 115 120 125  
 Asp Glu Gln Ser Thr Thr Ser Thr Thr Thr Asn Val Thr Gly Thr Ser  
 130 135 140  
 Ser Ala Asn Val Asn Gly Asn Gly Arg Thr His Glu Cys Ser Ile Cys  
 145 150 155 160  
 His Lys Cys Phe Pro Thr Gly Gln Ala Leu Gly Gly His Lys Arg Cys  
 165 170 175  
 His Tyr Asp Gly Gly Asn Gly Asn Gly Asn Gly Ser Val Ser Val Gly  
 180 185 190  
 Val Thr Ser Ser Glu Gly Val Gly Ser Thr Ile Ser His His Arg Asp  
 195 200 205  
 Phe Asp Leu Asn Ile Pro Ala Leu Pro Glu Phe Trp Pro Gly Phe Gly  
 210 215 220  
 Ser Gly Glu Asp Glu Val Glu Ser Pro His Pro Ala Lys Lys Ser Arg  
 225 230 235 240  
 Leu Ser Leu Pro Pro Lys Leu Glu Leu Phe Lys Gly Leu  
 245 250

<210> 22  
 <211> 786  
 <212> DNA  
 <213> Triticum aestivum

<400> 22  
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 caggggtggg ccaagaggaa gcgatcgcg cgcagcgct ccgaggagga gaacctcgcg 180  
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 tcgttcgctg cgccgggtgcc tgccgagttc aagtgtccg tctgcggcaa gtccttcagc 300  
 tcctaccagg cgctcggagg ccacaagacg agccaccggg tgaagcagcc gtctcctccc 360  
 tctgatgccg ctgctgcccc actcgtggcc ctcccggccg tcgcgcccat cctgccgtcc 420  
 gccgagccgg ccacgtcgtc caccgcccgc tcctccgacg gcgcgaccaa cagagtccac 480  
 aggtgctcca tctgccaaaa ggagttcccg actgggcagg cgctcggcgg gcacaagagg 540  
 aagcactacg acggaggcgt gggcgccgce gcctcgtcga ccgagcttct ggccgcccgc 600  
 gccgcccagt ctgaggtggg gagcaccggc aacgggagct ccgcccggcg ggccttcgac 660  
 ctgaacattc cggccgtgcc ggagttcgtg tggaggccgt gcgccaaggg caagatgatg 720  
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 gcttga 786

<210> 23  
 <211> 261  
 <212> PRT  
 <213> Triticum aestivum

<400> 23  
 Met Ser Ser Ser Ala Met Glu Ala Leu His Ala Leu Ile Pro Glu Gln  
 1 5 10 15  
 His Gln Leu Asp Val Glu Ala Ala Ala Val Ser Ser Ala Thr Ser  
 20 25 30  
 Gly Glu Glu Ser Gly His Val Leu Gln Gly Trp Ala Lys Arg Lys Arg  
 35 40 45  
 Ser Arg Arg Gln Arg Ser Glu Glu Glu Asn Leu Ala Leu Cys Leu Leu  
 50 55 60  
 Met Leu Ser Arg Gly Gly Lys Gln Arg Val Gln Ala Pro Gln Pro Glu  
 65 70 75 80  
 Ser Phe Ala Ala Pro Val Pro Ala Glu Phe Lys Cys Ser Val Cys Gly  
 85 90 95  
 Lys Ser Phe Ser Ser Tyr Gln Ala Leu Gly Gly His Lys Thr Ser His  
 100 105 110  
 Arg Val Lys Gln Pro Ser Pro Pro Ser Asp Ala Ala Ala Pro Leu  
 115 120 125  
 Val Ala Leu Pro Ala Val Ala Ala Ile Leu Pro Ser Ala Glu Pro Ala  
 130 135 140  
 Thr Ser Ser Thr Ala Ala Ser Ser Asp Gly Ala Thr Asn Arg Val His  
 145 150 155 160  
 Arg Cys Ser Ile Cys Gln Lys Glu Phe Pro Thr Gly Gln Ala Leu Gly

```

<400> 25
Met Ala Leu Glu Ala Leu Asn Ser Pro Thr Gly Thr Pro Thr Pro Pro
1          5          10          15

Pro Phe Gln Phe Glu Ser Asp Gly Gln Gln Leu Arg Tyr Ile Glu Asn
          20          25          30

Trp Arg Lys Gly Lys Arg Ser Lys Arg Ser Arg Ser Met Glu His Gln

```

|                                                                 |     |         |
|-----------------------------------------------------------------|-----|---------|
| 35                                                              | 40  | 45      |
| Pro Thr Glu Glu Glu Tyr Leu Ala Leu Cys Leu Ile Met Leu Ala Arg |     |         |
| 50                                                              | 55  | 60      |
| Ser Gly Gly Ser Val Asn His Gln Arg Ser Leu Pro Pro Pro Ala Pro |     |         |
| 65                                                              | 70  | 75 80   |
| Val Met Lys Leu His Ala Pro Ser Ser Ser Ser Ala Ala Glu Glu Glu |     |         |
|                                                                 | 85  | 90 95   |
| Lys Glu Lys Met Val Tyr Lys Cys Ser Val Cys Gly Lys Gly Phe Gly |     |         |
|                                                                 | 100 | 105 110 |
| Ser Tyr Gln Ala Leu Gly Gly His Lys Ala Ser His Arg Lys Leu Val |     |         |
|                                                                 | 115 | 120 125 |
| Pro Gly Gly Asp Asp Gln Ser Thr Thr Ser Thr Thr Thr Asn Ala Thr |     |         |
|                                                                 | 130 | 135 140 |
| Gly Thr Thr Thr Ser Val Asn Gly Asn Gly Asn Arg Ser Gly Arg Thr |     |         |
| 145                                                             | 150 | 155 160 |
| His Glu Cys Ser Ile Cys His Lys Cys Phe Pro Thr Gly Gln Ala Leu |     |         |
|                                                                 | 165 | 170 175 |
| Gly Gly His Lys Arg Cys His Tyr Asp Gly Gly Ile Gly Asn Gly Asn |     |         |
|                                                                 | 180 | 185 190 |
| Ala Asn Ser Gly Val Ser Ala Ser Val Gly Val Thr Ser Ser Glu Gly |     |         |
|                                                                 | 195 | 200 205 |
| Val Gly Ser Thr Val Ser His Arg Asp Phe Asp Leu Asn Ile Pro Ala |     |         |
|                                                                 | 210 | 215 220 |
| Leu Pro Glu Phe Trp Leu Gly Phe Gly Ser Gly Glu Asp Glu Val Glu |     |         |
| 225                                                             | 230 | 235 240 |
| Ser Pro His Pro Ala Lys Lys Ser Arg Leu Cys Leu Pro Pro Lys Tyr |     |         |
|                                                                 | 245 | 250 255 |
| Glu Leu Phe Gln His                                             |     |         |
|                                                                 | 260 |         |

<210> 26  
 <211> 1068  
 <212> DNA  
 <213> Arabidopsis thaliana

<400> 26  
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 tggccctcga agcgatgaac actccaactt cttctttcac cagaatcgaa acgaaagaag 180  
 atttgatgaa cgacgccgtt ttcattgagc cgtggcttaa acgcaaacgc tccaaacgtc 240  
 agcgtttctca cagcccttct tcgtcttctt cctcaccgcc tcgatctcga cccaaatccc 300  
 agaatcaaga tcttacggaa gaagagtatc tcgctctttg tctcctcatg ctcgctaaag 360  
 atcaaccgtc gcaaacgcga tttcatcaac agtcgcaatc gttaacgccg ccgccagaat 420  
 caaagaacct tccgtacaag tgtaacgtct gtgaaaaagc gtttccttcc tatcaggctt 480

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taggcgggtca caaagcaagt caccgaatca aaccaccaac cgtaatctca acaaccgccg 540
atgattcaac agctccgacc atctccatcg tcgccggaga aaaacatccg attgctgcct 600
ccggaaagat ccacgagtgt tcaatctgtc ataaagtgtt tccgacgggt caagcttttag 660
gcggtcacaa acgttgtcac tacgaaggca acctcggcgg cggaggagga ggaggaagca 720
aatcaatcag tcacagtga agcgtgtcga gcacgggtatc ggaagaaagg agccaccgtg 780
gattcatcga tctaaacctt ccggcgttac ctgaactcag ccttcatcac aatccaatcg 840
tcgacgaaga gatcttgagt ccgttgaccg gtaaaaaacc gcttttgttg accgatcacg 900
accaagtcac caagaaagaa gatttatctt taaaaatcta atactcgact attaattctt 960
gtgtgatttt ttctgttaca accatagttt cattttcatt ttttttagtta caaattttta 1020
attgttctga tttggattga atattggtat attgttaggg gttgatac 1068

```

&lt;210&gt; 27

&lt;211&gt; 273

&lt;212&gt; PRT

&lt;213&gt; Arabidopsis thaliana

&lt;400&gt; 27

```

Met Ala Leu Glu Ala Met Asn Thr Pro Thr Ser Ser Phe Thr Arg Ile
1           5           10           15

```

```

Glu Thr Lys Glu Asp Leu Met Asn Asp Ala Val Phe Ile Glu Pro Trp
20           25           30

```

```

Leu Lys Arg Lys Arg Ser Lys Arg Gln Arg Ser His Ser Pro Ser Ser
35           40           45

```

```

Ser Ser Ser Ser Pro Pro Arg Ser Arg Pro Lys Ser Gln Asn Gln Asp
50           55           60

```

```

Leu Thr Glu Glu Glu Tyr Leu Ala Leu Cys Leu Leu Met Leu Ala Lys
65           70           75           80

```

```

Asp Gln Pro Ser Gln Thr Arg Phe His Gln Gln Ser Gln Ser Leu Thr
85           90           95

```

```

Pro Pro Pro Glu Ser Lys Asn Leu Pro Tyr Lys Cys Asn Val Cys Glu
100          105          110

```

```

Lys Ala Phe Pro Ser Tyr Gln Ala Leu Gly Gly His Lys Ala Ser His
115          120          125

```

```

Arg Ile Lys Pro Pro Thr Val Ile Ser Thr Thr Ala Asp Asp Ser Thr
130          135          140

```

```

Ala Pro Thr Ile Ser Ile Val Ala Gly Glu Lys His Pro Ile Ala Ala
145          150          155          160

```

```

Ser Gly Lys Ile His Glu Cys Ser Ile Cys His Lys Val Phe Pro Thr
165          170          175

```

```

Gly Gln Ala Leu Gly Gly His Lys Arg Cys His Tyr Glu Gly Asn Leu
180          185          190

```

```

Gly Gly Gly Gly Gly Gly Gly Ser Lys Ser Ile Ser His Ser Gly Ser
195          200          205

```

```

Val Ser Ser Thr Val Ser Glu Glu Arg Ser His Arg Gly Phe Ile Asp
210          215          220

```

Leu Asn Leu Pro Ala Leu Pro Glu Leu Ser Leu His His Asn Pro Ile  
225 230 235 240

Val Asp Glu Glu Ile Leu Ser Pro Leu Thr Gly Lys Lys Pro Leu Leu  
245 250 255

Leu Thr Asp His Asp Gln Val Ile Lys Lys Glu Asp Leu Ser Leu Lys  
260 265 270

Ile

<210> 28

<211> 976

<212> DNA

<213> Arabidopsis thaliana

<400> 28

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ctgtttcaag attcagcact aggggtttcat ggaagcaaag gcaaacgatc taagcgatca 180
agatctgaat tcgaccgtca gagtctcacg gaggatgaat atatcgcttt atgtctcatg 240
cttcttgctc gcgacggaga tagaaaccgt gaccttgacc tgccttcttc ttcgtcttca 300
cctcctctgc ttcctcctct tcctactccg atctacaagt gtagcgtctg tgacaaggcg 360
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actcaatctg ccggaggaga tgagctgtcg acatcgctcg cgataaccac gtctggtata 480
tccggtggcg ggggaggaag tgtgaagtcg cacgtttgct ctatctgtca taaatcgctc 540
gccaccggtc aagctctcgg cggccacaaa cggtgccact acgaaggaaa gaacggagggc 600
ggtgtgagta gtagcgtgtc gaattctgaa gatgtggggg ctacaagcca cgtcagcagt 660
ggccaccgtg ggtttgacct caacataccg ccgataccgg aattctcgat ggtcaacgga 720
gacgaagagg tgatgagtc tttgcccggc aagaaactcc ggtttgactt cccggagaaa 780
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gtatatacaa atatcgattt tgattgatgt tcttcttcac tgaaaaatta tgattctttg 900
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ttgcttggat acatca 976

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<210> 29

<211> 238

<212> PRT

<213> Arabidopsis thaliana

<400> 29

Met Ala Leu Glu Thr Leu Thr Ser Pro Arg Leu Ser Ser Pro Met Pro  
1 5 10 15

Thr Leu Phe Gln Asp Ser Ala Leu Gly Phe His Gly Ser Lys Gly Lys  
20 25 30

Arg Ser Lys Arg Ser Arg Ser Glu Phe Asp Arg Gln Ser Leu Thr Glu  
35 40 45

Asp Glu Tyr Ile Ala Leu Cys Leu Met Leu Leu Ala Arg Asp Gly Asp  
50 55 60

Arg Asn Arg Asp Leu Asp Leu Pro Ser Ser Ser Ser Ser Pro Pro Leu  
65 70 75 80

Leu Pro Pro Leu Pro Thr Pro Ile Tyr Lys Cys Ser Val Cys Asp Lys  
                     85                    90                    95  
 Ala Phe Ser Ser Tyr Gln Ala Leu Gly Gly His Lys Ala Ser His Arg  
                     100                    105                    110  
 Lys Ser Phe Ser Leu Thr Gln Ser Ala Gly Gly Asp Glu Leu Ser Thr  
                     115                    120                    125  
 Ser Ser Ala Ile Thr Thr Ser Gly Ile Ser Gly Gly Gly Gly Gly Ser  
                     130                    135                    140  
 Val Lys Ser His Val Cys Ser Ile Cys His Lys Ser Phe Ala Thr Gly  
                     145                    150                    155                    160  
 Gln Ala Leu Gly Gly His Lys Arg Cys His Tyr Glu Gly Lys Asn Gly  
                     165                    170                    175  
 Gly Gly Val Ser Ser Ser Val Ser Asn Ser Glu Asp Val Gly Ser Thr  
                     180                    185                    190  
 Ser His Val Ser Ser Gly His Arg Gly Phe Asp Leu Asn Ile Pro Pro  
                     195                    200                    205  
 Ile Pro Glu Phe Ser Met Val Asn Gly Asp Glu Glu Val Met Ser Pro  
                     210                    215                    220  
 Met Pro Ala Lys Lys Leu Arg Phe Asp Phe Pro Glu Lys Pro  
                     225                    230                    235

<210> 30  
 <211> 718  
 <212> DNA  
 <213> Arabidopsis thaliana

<400> 30  
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 cgtacaaaac gtcaccgtat agatcaacca aacctcctc cttctgaaga agagtatctc 180  
 gctctttgcc tccttatgct cgctcgtggc tcctccgata atcactctcc accgtcggat 240  
 catcactctc tttctccact gtccgatcat cagaaagatt acaagtgttc cgtctgtggc 300  
 aaatctttcc cgtcttacca agcgtaggt ggacacaaaa caagtcaccg gaaaccggtt 360  
 agtgtcgatg ttaataatag taacggaacc gttactaata acggaaatat tagtaacggt 420  
 ttagttggtc aaagtgggaa gactcataac tgctctatat gttttaagtc gtttccctct 480  
 ggtcaagcat tgggtgggtca caaacgttgt cactatgatg gtggtaacgg taacagtaac 540  
 ggtgacaata gccacaagtt tgacctaaat ttaccggtg atcaagttag tgatgagaca 600  
 attggaaaaa gtcaactctc cgggtgaagaa acaaagtcgg tgttgtgatt attattattt 660  
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<210> 31  
 <211> 215  
 <212> PRT  
 <213> Arabidopsis thaliana

<400> 31  
 Met Ala Leu Asp Thr Leu Asn Ser Pro Thr Ser Thr Thr Thr Thr Thr  
 1                    5                    10                    15

Ala Pro Pro Pro Phe Leu Arg Cys Leu Asp Glu Thr Glu Pro Glu Asn  
 20 25 30

Leu Glu Ser Trp Thr Lys Arg Lys Arg Thr Lys Arg His Arg Ile Asp  
 35 40 45

Gln Pro Asn Pro Pro Pro Ser Glu Glu Glu Tyr Leu Ala Leu Cys Leu  
 50 55 60

Leu Met Leu Ala Arg Gly Ser Ser Asp His His Ser Pro Pro Ser Asp  
 65 70 75 80

His His Ser Leu Ser Pro Leu Ser Asp His Gln Lys Asp Tyr Lys Cys  
 85 90 95

Ser Val Cys Gly Lys Ser Phe Pro Ser Tyr Gln Ala Leu Gly Gly His  
 100 105 110

Lys Thr Ser His Arg Lys Pro Val Ser Val Asp Val Asn Asn Ser Asn  
 115 120 125

Gly Thr Val Thr Asn Asn Gly Asn Ile Ser Asn Gly Leu Val Gly Gln  
 130 135 140

Ser Gly Lys Thr His Asn Cys Ser Ile Cys Phe Lys Ser Phe Pro Ser  
 145 150 155 160

Gly Gln Ala Leu Gly Gly His Lys Arg Cys His Tyr Asp Gly Gly Asn  
 165 170 175

Gly Asn Ser Asn Gly Asp Asn Ser His Lys Phe Asp Leu Asn Leu Pro  
 180 185 190

Ala Asp Gln Val Ser Asp Glu Thr Ile Gly Lys Ser Gln Leu Ser Gly  
 195 200 205

Glu Glu Thr Lys Ser Val Leu  
 210 215

<210> 32  
 <211> 702  
 <212> DNA  
 <213> Arabidopsis thaliana

<400> 32  
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 tctgatcttc atcataacca ccgtctcact gaggaagagt atctagcttt ctgtctcatg 180  
 cttcttgctc gggatggcgg cgatcttgac tctgtgacgg ttgcggagaa gccgagttat 240  
 aagtgtggcg tttgttacia gacgttttcg tcttaccag ctctcggcgg tcataaagcg 300  
 agccaccgga gcttatacgg tgggtggagag aatgataaat cgacaccatc caccgccgtg 360  
 aaatctcaag tttgttcggg ttgcgggaaa tctttcgcca ccggtcaagc tctcggcggc 420  
 cacaagcggg gccactacga tgggtggcgtt tcgaactcgg aagggtgtggg gtctactagc 480  
 cacgtcagca gtagtagcca ccgtggattt gaccttaata ttataccggg gcagggattt 540  
 tcgccggacg acgaagtgat gagtccgatg gcgactaaga agcctcgcct gaagtaagtc 600  
 tttgttgaag acctggaagt ttatcaaag taaatatcaa atttcaattt caaggaacag 660  
 ttttgttgat tctattacca atacacaata cgattcaatt cc 702

<210> 33  
 <211> 193  
 <212> PRT  
 <213> Arabidopsis thaliana

<400> 33  
 Met Ala Leu Glu Ala Leu Asn Ser Pro Arg Leu Val Glu Asp Pro Leu  
 1 5 10 15  
 Arg Phe Asn Gly Val Glu Gln Trp Thr Lys Cys Lys Lys Arg Ser Lys  
 20 25 30  
 Arg Ser Arg Ser Asp Leu His His Asn His Arg Leu Thr Glu Glu Glu  
 35 40 45  
 Tyr Leu Ala Phe Cys Leu Met Leu Leu Ala Arg Asp Gly Gly Asp Leu  
 50 55 60  
 Asp Ser Val Thr Val Ala Glu Lys Pro Ser Tyr Lys Cys Gly Val Cys  
 65 70 75 80  
 Tyr Lys Thr Phe Ser Ser Tyr Gln Ala Leu Gly Gly His Lys Ala Ser  
 85 90 95  
 His Arg Ser Leu Tyr Gly Gly Gly Glu Asn Asp Lys Ser Thr Pro Ser  
 100 105 110  
 Thr Ala Val Lys Ser His Val Cys Ser Val Cys Gly Lys Ser Phe Ala  
 115 120 125  
 Thr Gly Gln Ala Leu Gly Gly His Lys Arg Cys His Tyr Asp Gly Gly  
 130 135 140  
 Val Ser Asn Ser Glu Gly Val Gly Ser Thr Ser His Val Ser Ser Ser  
 145 150 155 160  
 Ser His Arg Gly Phe Asp Leu Asn Ile Ile Pro Val Gln Gly Phe Ser  
 165 170 175  
 Pro Asp Asp Glu Val Met Ser Pro Met Ala Thr Lys Lys Pro Arg Leu  
 180 185 190

Lys

<210> 34  
 <211> 1157  
 <212> DNA  
 <213> Arabidopsis thaliana

<400> 34  
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 attaaatctt atggctctcg agactctcaa ttctccaaca gctaccacca ccgctcggcc 120  
 tcttctccgg tatcgtgaag aaatggagcc tgagaatctc gagcaatggg ctaaaagaaa 180  
 acgaacaaaa cgtcaacggt ttgatcacgg tcatcagaat caagaaacga acaagaacct 240  
 tccttctgaa gaagagtatc tcgctctttg tctcctcatg ctcgctcgtg gctccgccgt 300  
 acaatctcct cctcttcctc ctctaccgtc acgtgcgtca ccgtccgata accgagatta 360  
 caagtgtacg gtctgtggga agtccttttc gtcataccaa gccttaggtg gacacaagac 420

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gagtcaccgg aaaccgacga acactagtat cacttccggt aaccaagaac tgtctaataa 480
cagtcacagt aacagcggtt ccgttggtat taacggtacc gtgaacactg gtaacggtgt 540
tagtcaaagc ggaaagattc acacttgctc aatctgtttc aagtcgtttg cgtctggtca 600
agccttaggt ggacacaaac ggtgtcacta tgacggtggc aacaacggta acggtaacgg 660
aagtagcagc aacagcgtag aactcgtcgc tggtagtgac gtcagcgatg ttgataatga 720
gagatgggtcc gaagaaagtg cgatcggtgg ccaccgtgga tttgacctaa acttaccggc 780
tgatcaagtc tcagtgcgca cttcttaacg ttgactgagt ttgaggaaaa agtcaactat 840
caagcgaaga aagggttagt ggacggtgaa gattaacggt cgtttctttc cagttgcttc 900
ggtttgagct tgactgggtc tgtaatgaaa atgattggag tggacttggc attattatta 960
ttatttttaa aaagaaatgt taatttggtg ttggatttgt ttatagatag aggaaacaat 1020
tgggatacac aaatatTTTT tttttttaca aagaaaataa taatgcagag atggatgatt 1080
ggatcgtaga cgttattata tagtggacca ttctgtaatc gtgaattatt attatttgtt 1140
agaaatttaa ttttcgt 1157

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<210> 35  
 <211> 245  
 <212> PRT  
 <213> Arabidopsis thaliana

<400> 35  
 Met Ala Leu Glu Thr Leu Asn Ser Pro Thr Ala Thr Thr Thr Ala Arg  
 1 5 10 15  
 Pro Leu Leu Arg Tyr Arg Glu Glu Met Glu Pro Glu Asn Leu Glu Gln  
 20 25 30  
 Trp Ala Lys Arg Lys Arg Thr Lys Arg Gln Arg Phe Asp His Gly His  
 35 40 45  
 Gln Asn Gln Glu Thr Asn Lys Asn Leu Pro Ser Glu Glu Glu Tyr Leu  
 50 55 60  
 Ala Leu Cys Leu Leu Met Leu Ala Arg Gly Ser Ala Val Gln Ser Pro  
 65 70 75 80  
 Pro Leu Pro Pro Leu Pro Ser Arg Ala Ser Pro Ser Asp His Arg Asp  
 85 90 95  
 Tyr Lys Cys Thr Val Cys Gly Lys Ser Phe Ser Ser Tyr Gln Ala Leu  
 100 105 110  
 Gly Gly His Lys Thr Ser His Arg Lys Pro Thr Asn Thr Ser Ile Thr  
 115 120 125  
 Ser Gly Asn Gln Glu Leu Ser Asn Asn Ser His Ser Asn Ser Gly Ser  
 130 135 140  
 Val Val Ile Asn Val Thr Val Asn Thr Gly Asn Gly Val Ser Gln Ser  
 145 150 155 160  
 Gly Lys Ile His Thr Cys Ser Ile Cys Phe Lys Ser Phe Ala Ser Gly  
 165 170 175  
 Gln Ala Leu Gly Gly His Lys Arg Cys His Tyr Asp Gly Gly Asn Asn  
 180 185 190  
 Gly Asn Gly Asn Gly Ser Ser Ser Asn Ser Val Glu Leu Val Ala Gly  
 195 200 205

Ser Asp Val Ser Asp Val Asp Asn Glu Arg Trp Ser Glu Glu Ser Ala  
 210 215 220

Ile Gly Gly His Arg Gly Phe Asp Leu Asn Leu Pro Ala Asp Gln Val  
 225 230 235 240

Ser Val Thr Thr Ser  
 245

<210> 36  
 <211> 1213  
 <212> DNA  
 <213> Oryza sativa

<400> 36  
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 cgaagcttag ctagcggata gaacaagtcg tcgatctgct tgctgctttt gtgaattgcg 120  
 gtggaagcat gtcgagcgcg tcgtccatgg aagcgctcca cgccgcggtg ctcaaggagg 180  
 agcagcagca gcacgaggtg gaggaggcga cggtcgtgac gagcagcagc gccacgagcg 240  
 gggaggaggg cggacacctg ccccaggggt gggcgaagcg gaagcggtcg cgccgccagc 300  
 gatcggagga ggagaacctc gcgtctgtgc tcctcatgct cgcccgcggc ggccaccacc 360  
 gcgtccaggc gccgcctccg ctctcggtt cggcgcccc gccggcaggt gcggagttea 420  
 agtgctccgt ctgcggcaag tccttcagct cctaccaggc gctcggcggc cacaagacga 480  
 gccaccgggt caagctgccg actccgccc cagctcccgt cttggctccc gccccgtcg 540  
 ccgccttgct gccttccgce gaggaccgcg agccagccac gtcattccacc gccgcgtcct 600  
 ccgacggcat gaccaacaga gtccacaggt gttccatctg ccagaaggag ttccccaccg 660  
 ggcaggcgct cggcgggcac aagaggaagc actacgacgg tggcgtaggc gccggcgccg 720  
 gcgcattctc aaccgagctc ctggccacgg tggccgcca gtccgaggtg ggaagctccg 780  
 gcaacggcca gtccgccacc cgggcgttcg acctcaacct cccggcgtg ccggagttec 840  
 tgtggcgccg gtgctccaag ggcaagaaga tgtgggacga ggaggaggag gtccagagcc 900  
 ccctcgctt caagaagccc cggcttctca ccgcgtaatt cagcagctgc acggatccga 960  
 tccgtcagag tttttgtcta gggagtgaat ttcagtcgaa acacactatt cgttgattcg 1020  
 ttttgtgccg ctattgttta atttgttctt gcttttgtac agagcaagcg agtgatacat 1080  
 agccatacat acagtcatac agatataggt ctagctcttc cttggttctt tgtaacactg 1140  
 gaactgtacc tgtatctttt acactttgtt ctttgacagt catatattgt agaccaaaaa 1200  
 aaaaaaaaaaaa aaa 1213

<210> 37  
 <211> 269  
 <212> PRT  
 <213> Oryza sativa

<400> 37  
 Met Ser Ser Ala Ser Ser Met Glu Ala Leu His Ala Ala Val Leu Lys  
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 Glu Glu Gln Gln Gln His Glu Val Glu Glu Ala Thr Val Val Thr Ser  
 20 25 30  
 Ser Ser Ala Thr Ser Gly Glu Glu Gly Gly His Leu Pro Gln Gly Trp  
 35 40 45  
 Ala Lys Arg Lys Arg Ser Arg Arg Gln Arg Ser Glu Glu Glu Asn Leu  
 50 55 60  
 Ala Leu Cys Leu Leu Met Leu Ala Arg Gly Gly His His Arg Val Gln  
 65 70 75 80

Ala Pro Pro Pro Leu Ser Ala Ser Ala Pro Pro Pro Ala Gly Ala Glu  
85 90 95

Phe Lys Cys Ser Val Cys Gly Lys Ser Phe Ser Ser Tyr Gln Ala Leu  
100 105 110

Gly Gly His Lys Thr Ser His Arg Val Lys Leu Pro Thr Pro Pro Ala  
115 120 125

Ala Pro Val Leu Ala Pro Ala Pro Val Ala Ala Leu Leu Pro Ser Ala  
130 135 140

Glu Asp Arg Glu Pro Ala Thr Ser Ser Thr Ala Ala Ser Ser Asp Gly  
145 150 155 160

Met Thr Asn Arg Val His Arg Cys Ser Ile Cys Gln Lys Glu Phe Pro  
165 170 175

Thr Gly Gln Ala Leu Gly Gly His Lys Arg Lys His Tyr Asp Gly Gly  
180 185 190

Val Gly Ala Gly Ala Gly Ala Ser Ser Thr Glu Leu Leu Ala Thr Val  
195 200 205

Ala Ala Glu Ser Glu Val Gly Ser Ser Gly Asn Gly Gln Ser Ala Thr  
210 215 220

Arg Ala Phe Asp Leu Asn Leu Pro Ala Val Pro Glu Phe Val Trp Arg  
225 230 235 240

Pro Cys Ser Lys Gly Lys Lys Met Trp Asp Glu Glu Glu Glu Val Gln  
245 250 255

Ser Pro Leu Ala Phe Lys Lys Pro Arg Leu Leu Thr Ala  
260 265

<210> 38  
<211> 528  
<212> DNA  
<213> Arabidopsis thaliana

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ctaagtatgt tatctcgaag tttcgtgggc aaacaaatcg atgtaaagca atctaccgga 120  
agcaaaacga accataataa ccaacttcgaa tgcaaaacgt gtaaccggaa atttgattcc 180  
ttccaagctc ttggaggtca tagagctagc cacaagaaac ctaagctgat cgttgaccaa 240  
gaacaggtga agcatcgtaa caaagagaat gatatgcata agtgtacaat ttgcgatcaa 300  
atgtttggga ccggtcaagc tctaggcggc cacatgagaa agcataggac gagcatgata 360  
accgagcaat cgattgtccc ttctgtgggt tattccagac cggtttttaa tcgttgacgt 420  
agcagcaagg agatcttgga cttaaatac actccattgg aaaatgatct tgtgttaatc 480  
tttgggaaga atttggttcc acaaattgat ttgaagtttg tgaattag 528

<210> 39  
<211> 175  
<212> PRT  
<213> Arabidopsis thaliana

&lt;400&gt; 39

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Met Lys Arg Asp Arg Ser Asp Tyr Glu Glu Ser Met Lys His Ile Asp
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          20          25          30
Ile Asp Val Lys Gln Ser Thr Gly Ser Lys Thr Asn His Asn Asn His
          35          40          45
Phe Glu Cys Lys Thr Cys Asn Arg Lys Phe Asp Ser Phe Gln Ala Leu
          50          55          60
Gly Gly His Arg Ala Ser His Lys Lys Pro Lys Leu Ile Val Asp Gln
65          70          75          80
Glu Gln Val Lys His Arg Asn Lys Glu Asn Asp Met His Lys Cys Thr
          85          90          95
Ile Cys Asp Gln Met Phe Gly Thr Gly Gln Ala Leu Gly Gly His Met
          100          105          110
Arg Lys His Arg Thr Ser Met Ile Thr Glu Gln Ser Ile Val Pro Ser
          115          120          125
Val Val Tyr Ser Arg Pro Val Phe Asn Arg Cys Ser Ser Ser Lys Glu
          130          135          140
Ile Leu Asp Leu Asn Leu Thr Pro Leu Glu Asn Asp Leu Val Leu Ile
145          150          155          160
Phe Gly Lys Asn Leu Val Pro Gln Ile Asp Leu Lys Phe Val Asn
          165          170          175

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&lt;210&gt; 40

&lt;211&gt; 820

&lt;212&gt; DNA

&lt;213&gt; Saccharum officinarum

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (406)..(406)

&lt;223&gt; n can be any nucleotide

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (581)..(582)

&lt;223&gt; n can be any nucleotide

&lt;220&gt;

&lt;221&gt; misc\_feature

&lt;222&gt; (589)..(589)

&lt;223&gt; n can be any nucleotide

&lt;400&gt; 40

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cctaaccagc attagctttt caaatcaaca agcctcgccg tgaccgatcg atggccatca      60
cccacgacga ctacgtctcc ctctgcctca tggcgctcgc agccgcggga ggcggaggcc      120

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|             |            |            |            |             |             |     |
|-------------|------------|------------|------------|-------------|-------------|-----|
| aagctgggttt | aacaacgcag | tacgctctga | acacggctgc | ctggacagcg  | acggcgcaag  | 180 |
| agtccgagct  | ccgcttccgg | tgctccgtct | gtggcaaggc | cttcgcgtcg  | caccaggcac  | 240 |
| tgggcgggca  | caaggccagc | caccgcaage | cgacgctcgt | acaggcacat  | gcgtcgtcct  | 300 |
| cagccggagg  | cgcggcgtcg | tcgtcggtaa | caatgacctc | ggccgtaggc  | agcagtgggc  | 360 |
| aggggaggca  | caggtgcacg | gtgtgccatc | ggagcttcgc | gacgngcaa   | gcgctcggcg  | 420 |
| ggcacaagag  | gtgccattac | tgggacgggc | tctcgggtgc | gctcaccgcg  | tcgtcggcgc  | 480 |
| categgggtc  | cgggtcgacc | gtcaagggct | ttgatctgaa | tttgggtgccg | gtgccgcccg  | 540 |
| cgatggccgc  | caacgctgcg | acaagggtgg | gagaggagaa | nnaagtcana  | aacccttggc  | 600 |
| ggtcaagaga  | aggcggcttg | ccggtccgct | ttggacccta | atttaacgat  | ttagaagtcc  | 660 |
| tttttttaat  | aattaagagt | tcttttgaag | aaggttgtaa | agttttcgaa  | ccttggttctt | 720 |
| ttaatggatt  | tgggtgctgg | cgaaatttta | aaactggatt | taaatttgcg  | ctcactcttt  | 780 |
| ttttttatatt | tttacaccct | tttttttttt | tagaagaaga |             |             | 820 |

&lt;210&gt; 41

&lt;211&gt; 1509

&lt;212&gt; DNA

&lt;213&gt; Arabidopsis thaliana

&lt;400&gt; 41

|            |            |            |            |            |             |      |
|------------|------------|------------|------------|------------|-------------|------|
| ttcctttctc | ttcctctctc | tctctcttca | ccatgactga | tccttattcc | aattttcttca | 60   |
| cagactgggt | caagtcta   | ccttttccac | attaccctaa | ttcctccact | aaccctctc   | 120  |
| ctcatcctct | tcctcctgtt | actcctccct | cttccttctt | cttcttccct | caatccggag  | 180  |
| acctccgcg  | tccaccgcg  | ccaccaactc | ctcctccttc | tcctcctctc | cgagaagccc  | 240  |
| tcctctcct  | cagcctcagc | cccgccaa   | aacaacaaga | ccaccatcac | aaccatgacc  | 300  |
| accttattca | agaaccacct | tcaacctcca | tggatgtcga | ctacgatcat | caccatcaag  | 360  |
| atgatcatca | taacctcgat | gacgatgacc | atgacgtcac | cgttgctctt | cacataggcc  | 420  |
| ttccaagccc | tagtgctcaa | gagatggcct | ccttgctcat | gatgtcttct | tcttcctctt  | 480  |
| cctcgaggac | cactcatcat | cacgaggaca | tgaatcaca  | gaaagacctc | gaccatgagt  | 540  |
| acagccacgg | agctgtcggg | ggaggagaag | atgacgatga | agattcagtc | ggcggagacg  | 600  |
| gcggtgtgag | aatcagcaga | ctcaacaagg | gtcaatattg | gatccctaca | ccttctcaga  | 660  |
| ttctcattgg | ccctactcag | ttctcatgtc | ctgtttgctt | caaaaccttc | aacagataca  | 720  |
| ataacatgca | gatgcata   | tggggacatg | gatcacaata | cagaaaagga | cctgaatctc  | 780  |
| taaggggaac | acaaccaaca | ggaatgctaa | ggcttccgtg | ctattgctgc | gccccaggct  | 840  |
| gtcgcaacaa | cattgaccat | ccaagggcaa | agcctctcaa | agacttcaga | acccttcaaa  | 900  |
| cacattacaa | gagaaaacat | gggatcaaac | ccttcatgtg | taggaaatgt | ggaaaggctt  | 960  |
| tcgcagtcg  | aggggactgg | agaacacatg | agaagaattg | tggcaaactt | tgggtattgca | 1020 |
| tatgtggatc | tgatttcaag | cacaagagat | ctctcaaaga | tcacatcaag | gcttttggga  | 1080 |
| atggtcatgg | agcctacgga | attgatgggt | ttgatgaaga | agatgagcct | gcctctgagg  | 1140 |
| tagaacaatt | agacaatgat | catgagtcaa | tgcagtctaa | atagcttata | tatattacta  | 1200 |
| taagtactaa | gtaattcggg | atatatatta | attataagaa | acctaaatct | atggaccaag  | 1260 |
| ttttgatgga | ggtagggctt | ttcaaactaa | aagctatatc | atctaattga | tcataggaaa  | 1320 |
| aaaatgaatc | aagagcactt | ggaaaatttt | aaattgtatc | tttagcttcc | tagttaaatt  | 1380 |
| tattgcaaga | caatgtagca | gtctaacc   | tgaggttccc | aacggtttat | ttctatttgt  | 1440 |
| atattatttt | gtcattagct | tcacctttcg | ttaattcgaa | ggacataact | tataaatggt  | 1500 |
| ttaattatg  |            |            |            |            |             | 1509 |

&lt;210&gt; 42

&lt;211&gt; 383

&lt;212&gt; PRT

&lt;213&gt; Arabidopsis thaliana

&lt;400&gt; 42

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Thr | Asp | Pro | Tyr | Ser | Asn | Phe | Phe | Thr | Asp | Trp | Phe | Lys | Ser | Asn |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Pro | Phe | His | His | Tyr | Pro | Asn | Ser | Ser | Thr | Asn | Pro | Ser | Pro | His | Pro |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |

Leu Pro Pro Val Thr Pro Pro Ser Ser Phe Phe Phe Phe Pro Gln Ser  
 35 40 45  
 Gly Asp Leu Arg Arg Pro Pro Pro Pro Thr Pro Pro Pro Ser Pro  
 50 55 60  
 Pro Leu Arg Glu Ala Leu Pro Leu Leu Ser Leu Ser Pro Ala Asn Lys  
 65 70 75 80  
 Gln Gln Asp His His His Asn His Asp His Leu Ile Gln Glu Pro Pro  
 85 90 95  
 Ser Thr Ser Met Asp Val Asp Tyr Asp His His His Gln Asp Asp His  
 100 105 110  
 His Asn Leu Asp Asp Asp Asp His Asp Val Thr Val Ala Leu His Ile  
 115 120 125  
 Gly Leu Pro Ser Pro Ser Ala Gln Glu Met Ala Ser Leu Leu Met Met  
 130 135 140  
 Ser Ser Ser Ser Ser Ser Ser Arg Thr Thr His His His Glu Asp Met  
 145 150 155 160  
 Asn His Lys Lys Asp Leu Asp His Glu Tyr Ser His Gly Ala Val Gly  
 165 170 175  
 Gly Gly Glu Asp Asp Asp Glu Asp Ser Val Gly Gly Asp Gly Gly Cys  
 180 185 190  
 Arg Ile Ser Arg Leu Asn Lys Gly Gln Tyr Trp Ile Pro Thr Pro Ser  
 195 200 205  
 Gln Ile Leu Ile Gly Pro Thr Gln Phe Ser Cys Pro Val Cys Phe Lys  
 210 215 220  
 Thr Phe Asn Arg Tyr Asn Asn Met Gln Met His Met Trp Gly His Gly  
 225 230 235 240  
 Ser Gln Tyr Arg Lys Gly Pro Glu Ser Leu Arg Gly Thr Gln Pro Thr  
 245 250 255  
 Gly Met Leu Arg Leu Pro Cys Tyr Cys Cys Ala Pro Gly Cys Arg Asn  
 260 265 270  
 Asn Ile Asp His Pro Arg Ala Lys Pro Leu Lys Asp Phe Arg Thr Leu  
 275 280 285  
 Gln Thr His Tyr Lys Arg Lys His Gly Ile Lys Pro Phe Met Cys Arg  
 290 295 300  
 Lys Cys Gly Lys Ala Phe Ala Val Arg Gly Asp Trp Arg Thr His Glu  
 305 310 315 320  
 Lys Asn Cys Gly Lys Leu Trp Tyr Cys Ile Cys Gly Ser Asp Phe Lys  
 325 330 335  
 His Lys Arg Ser Leu Lys Asp His Ile Lys Ala Phe Gly Asn Gly His

340 345 350

Gly Ala Tyr Gly Ile Asp Gly Phe Asp Glu Glu Asp Glu Pro Ala Ser  
355 360 365

Glu Val Glu Gln Leu Asp Asn Asp His Glu Ser Met Gln Ser Lys  
370 375 380

<210> 43  
<211> 1303  
<212> DNA  
<213> Arabidopsis thaliana

<400> 43

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| atacgggtgct | tttattcttg  | atcttcttct  | tcttctttgt  | cttctcctca | gagtcatgtc | 120  |
| taatccagct  | tgttcgaatc  | tcttcaacaa  | tggatgtgac  | cataatagct | tcaactattc | 180  |
| cacttctctc  | tcttacattt  | acaactctca  | cggtagctac  | tattactcta | ataccacaaa | 240  |
| ccctaattac  | attaatcata  | ctcataccac  | ttccacttcc  | cctaactcac | ccccactaag | 300  |
| agaagctctt  | cctcttctta  | gcttaagccc  | cataaggcac  | caagaacaac | aagaccaaca | 360  |
| ctatttcatg  | gacacccatc  | aaattagctc  | ttcaaacttt  | cttgatgac  | ctcttgtgac | 420  |
| tgtggatctt  | catctagggt  | taccaaacta  | cgggtgttgg  | gagagcatta | ggagcaatat | 480  |
| tgctcctgat  | gcaaccacgg  | acgagcaaga  | tcaagatcat  | gaccgaggag | tagaagtcac | 540  |
| agttgagtc   | caccttgatg  | atgatgatga  | tcatcatgga  | gatctacaca | gaggtcatca | 600  |
| ctattggatt  | cctactcctt  | ctcagatttt  | gatttggtcct | acacagttca | cttgtcctct | 660  |
| ttgcttcaag  | acattcaaca  | gatacaacaa  | catgcagatg  | cacatgtggg | gacacggctc | 720  |
| acaatacaga  | aagggaccag  | aatccttaag  | aggaacccaa  | ccaacaggaa | tgctaagact | 780  |
| accatgtttc  | tgctgtgcac  | ccggttgcaa  | gaacaacatt  | gaccaccac  | gagccaagcc | 840  |
| tcttaaggac  | tttcgaaccc  | tccaaacaca  | ttacaaacgt  | aaacatgggt | ctaaaccatt | 900  |
| tgcttgtcgt  | atgtgtggta  | aggcctttgc  | agtgaaagga  | gattggagaa | cgcatgagaa | 960  |
| gaattgtgga  | aagctttggg  | attgctcttg  | tggctcggat  | tttaagcaca | agaggtcgct | 1020 |
| taaggaccat  | gtcaaggcct  | ttggaaatgg  | tcatgttcct  | tgtgggattg | atagttttgg | 1080 |
| aggagatcat  | gaggactact  | atgatgctgc  | ttctgatatc  | gagcaataag | atgatagcaa | 1140 |
| caacaatgag  | tgtttaattag | gggttttgg   | tatttttcct  | ctcatgcatt | agttgattgt | 1200 |
| atgcacgtgt  | tcttttagttt | tggttcttcgg | atctttgttt  | tattttgttt | tgagctgttt | 1260 |
| tttttttaat  | tactaagaag  | ttaattatca  | tctaaagatt  | ttc        |            | 1303 |

<210> 44  
<211> 337  
<212> PRT  
<213> Arabidopsis thaliana

<400> 44

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
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| Met | Ser | Asn | Pro | Ala | Cys | Ser | Asn | Leu | Phe | Asn | Asn | Gly | Cys | Asp | His |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     | 15  |     |     |
| Asn | Ser | Phe | Asn | Tyr | Ser | Thr | Ser | Leu | Ser | Tyr | Ile | Tyr | Asn | Ser | His |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |
| Gly | Ser | Tyr | Tyr | Tyr | Ser | Asn | Thr | Thr | Asn | Pro | Asn | Tyr | Ile | Asn | His |
|     |     | 35  |     |     |     |     | 40  |     |     |     |     | 45  |     |     |     |
| Thr | His | Thr | Thr | Ser | Thr | Ser | Pro | Asn | Ser | Pro | Pro | Leu | Arg | Glu | Ala |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |
| Leu | Pro | Leu | Leu | Ser | Leu | Ser | Pro | Ile | Arg | His | Gln | Glu | Gln | Gln | Asp |
| 65  |     |     |     |     | 70  |     |     |     | 75  |     |     |     |     |     | 80  |

Gln His Tyr Phe Met Asp Thr His Gln Ile Ser Ser Ser Asn Phe Leu  
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Lys Lys Lys Thr Gln Glu Ser Pro Glu Pro Met Lys Lys Ile Leu Phe  
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Arg Cys Glu Glu Cys Gly Lys Gly Phe Arg Tyr Glu Lys Tyr Phe Lys  
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Arg His Arg Thr Ala Val Thr Thr Ile Ser Pro Val Ala Ala Thr Ala

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## Claims

1. Method for increasing plant yield relative to corresponding wild type plants, comprising modifying expression in a plant of a nucleic acid sequence encoding a 2xC2H2 zinc finger protein and/or modifying in a plant level and/or activity of a 2xC2H2 zinc finger protein.  
5
2. Method for increasing leaf surface area relative to corresponding wild type plants, comprising modifying expression in a plant of a nucleic acid sequence encoding a 2xC2H2 zinc finger protein and/or modifying in a plant level and/or activity of a 2xC2H2 zinc finger protein.  
10
3. Method for prolonging vegetative growth phase of a plant relative to corresponding wild type plants, comprising modifying expression in a plant of a nucleic acid sequence encoding a 2xC2H2 zinc finger protein and/or modifying in a plant level and/or activity of a 2xC2H2 zinc finger protein.  
15
4. Method according to any of claims 1 to 3, wherein said modifying expression, level and/or activity is effected by recombinant means and/or chemical means.
- 20 5. Method according to any of claims 1 to 4, wherein said 2xC2H2 zinc finger protein comprises a QALGGH motif.
6. Method according to any of claims 1 to 4, wherein said 2xC2H2 zinc finger protein comprises a NNM(W)QMH motif.  
25
7. Method according to any of claims 1 to 6, wherein said 2xC2H2 zinc finger protein comprises an EAR motif.
8. Method according to any of claims 1 to 7, wherein said 2xC2H2 zinc finger protein further comprises a B-box.  
30
9. Method according to any of claims 1 to 8, wherein said 2xC2H2 zinc finger protein further comprises an L-box.
- 35 10. Method according to any of claims 1 to 9, wherein said 2xC2H2 zinc finger protein is derived from a dicotyledonous plant, preferably from the family *Brassicaceae*, further preferably from *Arabidopsis thaliana*, more preferably the nucleic acid is as represented by

SEQ ID NO 2 or a homologue, derivative or active fragment thereof and/or wherein said nucleic acid is as represented by SED ID NO 1 or a portion thereof or sequences capable of hybridising therewith .

- 5 11. Method according to claim 10, wherein said homologue, derivative or active fragment has, in increasing order of preference, at least 30%, 31%, 32%, 33%, 34%, 35%, 36%, 37%, 38%, 39%, 40%, 41%, 42%, 43%, 44%, 45%, 46%, 47%, 48%, 49%, 50%, 52%, 54%, 56%, 58%, 60%, 62%, 64%, 66%, 68%, 70%, 72%, 74%, 76%, 78%, 80%, 82%, 84%, 86%, 88%, 90%, 92%, 94%, 96%, 98% sequence identity with the sequence of SEQ ID NO
- 10 2.
12. Method according to any of claims 1 to 11, wherein said plant is a monocot.
13. Method according to any of claims 1 to 12, wherein said modifying expression is effected
- 15 by introducing into a plant a nucleic acid capable of modifying expression of a gene encoding a 2xC2H2 zinc finger protein and/or capable of modifying level and/or activity of a 2xC2H2 zinc finger protein.
14. Method according to claim 13, wherein said nucleic acid capable of modifying expression is
- 20 a nucleic acid encoding a 2xC2H2 protein, such as a 2xC2H2 protein as defined in any of claims 5 to 11.
15. Method according to claims 13 or 14, wherein said nucleic acid introduced into a plant is an alternative splice variant of a nucleic acid as defined in claim 14.
- 25 16. Method according to claims 13 or 15, wherein said nucleic acid introduced into a plant is an allelic variant of a nucleic acid as defined in claim 14.
17. Method according to claims 13 or 16, wherein said nucleic acid introduced into a plant is
- 30 comprised on at least part of a chromosome.
18. Method according to any of claims 1 to 17, wherein said modifying expression comprises increased expression.
- 35 19. Method according to any of claims 1 to 18, wherein expression of said nucleic acid is driven by a plant promoter, preferably a constitutive promoter, such as a GOS2 promoter.

20. Method according to any of claims 1 to 18, wherein expression of said nucleic acid is driven by a plant promoter, preferably a tissue preferred promoter, such as seed-preferred promoter.
- 5 21. Method according to any of claims 1 to 20, wherein said increased yield comprises increased above ground biomass.
22. Method according to any of claim 1 to 20, wherein said increased yield comprises increased seed yield.
- 10 23. Method according to any of claim 1 to 20, wherein said increased yield comprises increased root yield.
24. Construct comprising:
- 15 (i) A nucleic acid capable of modifying expression of a nucleic acid encoding a 2xC2H2 zinc finger protein and/or capable of modifying level and/or activity of a 2xC2H2 zinc finger protein;
- (ii) One or more plant control sequence capable of driving expression of the nucleic acid sequence of (i); and optionally
- 20 (iii) A transcription termination sequence.
25. Construct according to claim 24, wherein said nucleic acid of (i) is a nucleic acid as defined in any of claims 14 to 17.
- 25 26. Construct according to claim 24 or 25, wherein said control sequences of (ii) is at least a constitutive promoter, such as a GOS2 promoter.
27. Construct according to claim 24 or 25, wherein said control sequences of (ii) is at least a tissue preferred promoter, such as seed-preferred promoter.
- 30 28. Host cell comprising a construct according to any of claims 24 to 27.
29. Method for the production of a transgenic plant having increased yield, increased leaf surface area and/or prolonged vegetative growth, which method comprises
- 35 (i) introducing into a plant or plant cell a 2xC2H2 zinc finger nucleic acid;
- (ii) Cultivating the plant or plant cell under conditions promoting plant growth.

30. Plant obtainable by a method according to any of claims 1 to 23 and 29, which plant has increased yield, modified leaf surface area and/or prolonged vegetative growth, relative to corresponding wild type plants.

5

31. Transgenic plant having increased yield, increased leaf surface area and/or prolonged vegetative growth, which transgenic plant has modified expression of a nucleic acid encoding a 2xC2H2 zinc finger protein and/or modified level and/or activity of a 2xC2H2 zinc finger protein, relative to corresponding wild type plants.

10

32. Plant part, preferably a harvestable part, a propagule or progeny of a plant as defined in claim 30 or 31, which progeny has modified expression of a nucleic acid encoding 2xC2H2 zinc finger protein and/or modified level and/or activity of a 2xC2H2 zinc finger protein, relative to corresponding wild type plants.

15

33. Plant or plant part according to any of claims 30 to 32, which plant is a monocotyledonous plant, preferably a cereal.

34. Plant or plant part according to any of claims 30 to 33 selected from rice, maize, wheat, barley, millet, oats, rye, sorghum, soybean, sunflower, canola, sugarcane, alfalfa, leguminosae (bean, pea), flax, lupinus, rapeseed, tobacco, tomato, potato, squash, papaya, poplar and cotton.

20

35. Use of a nucleic acid encoding a 2xC2H2 protein, of a 2xC2H2 protein and/or of a construct as defined in any of claims 24 to 27 to increase plant yield.

25

36. A yield regulating composition comprising a nucleic acid encoding a 2xC2H2 protein, and/or comprising a 2xC2H2 protein, and/or comprising a construct as defined in any one of claims 24 to 27.

30

37. Use of a nucleic acid encoding a 2xC2H2 protein, of a 2xC2H2 protein and/or of a construct as defined in any of claims 24 to 27 to increase leaf surface area.

38. Use of a nucleic acid encoding a 2xC2H2 protein, of a 2xC2H2 protein and/or of a construct as defined in any of claims 24 to 27 to prolong vegetative growth.

35

39. Use of a nucleic acid encoding a 2xC2H2 protein, of a 2xC2H2 protein and/or of a construct as defined in any of claims 24 to 27 as target for an agrochemical.

5 40. Use of a nucleic acid encoding a 2xC2H2 protein, of a 2xC2H2 protein and/or of a construct as defined in any of claims 24 to 27 in a breeding program.

41. Use of a plant as defined in any of claims 30 to 34 to produce enzymes, pharmaceuticals or agrochemicals.

10 42. Use of a plant as defined in any one of claims 30 to 34 to produce food or feed products.

15

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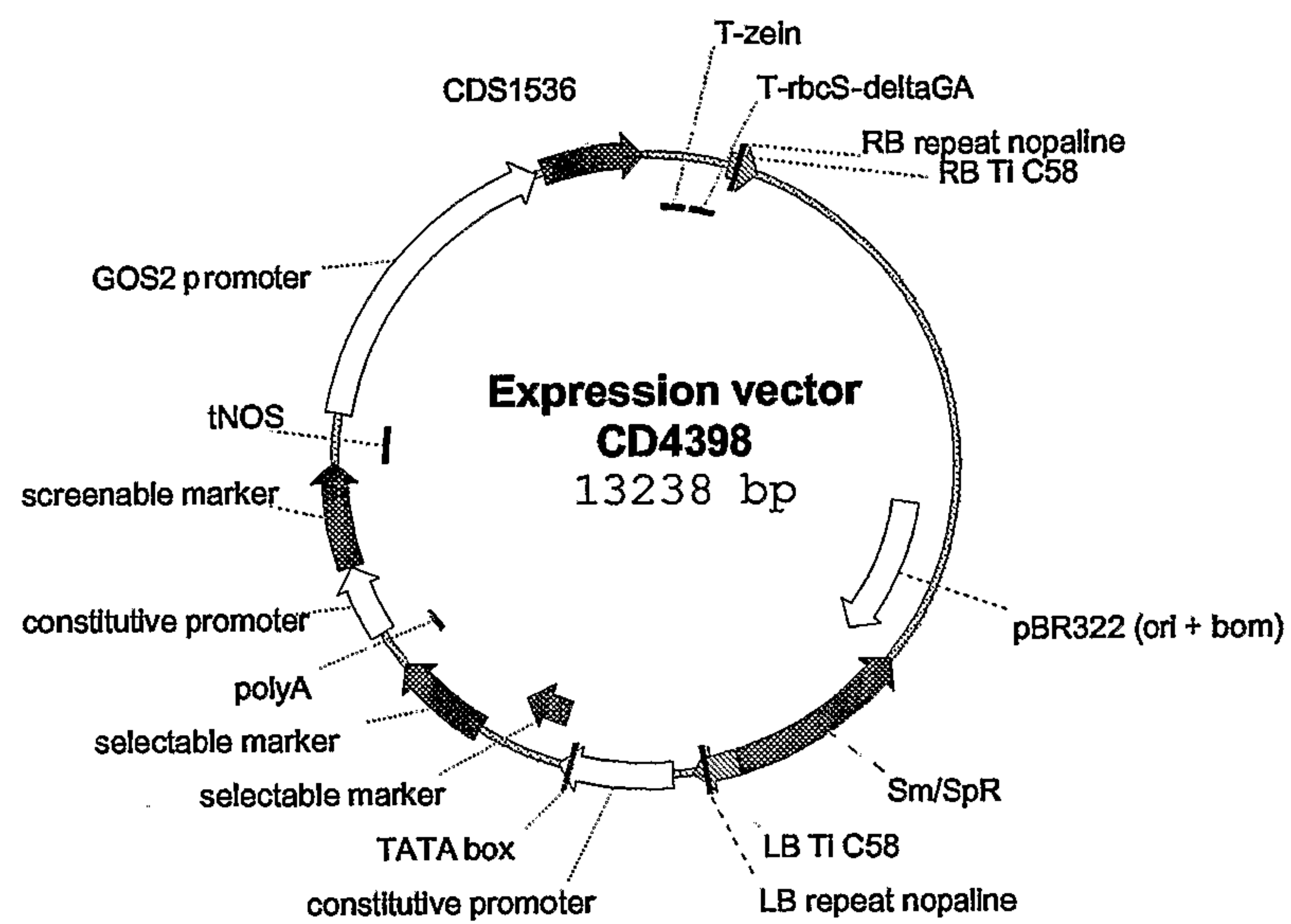
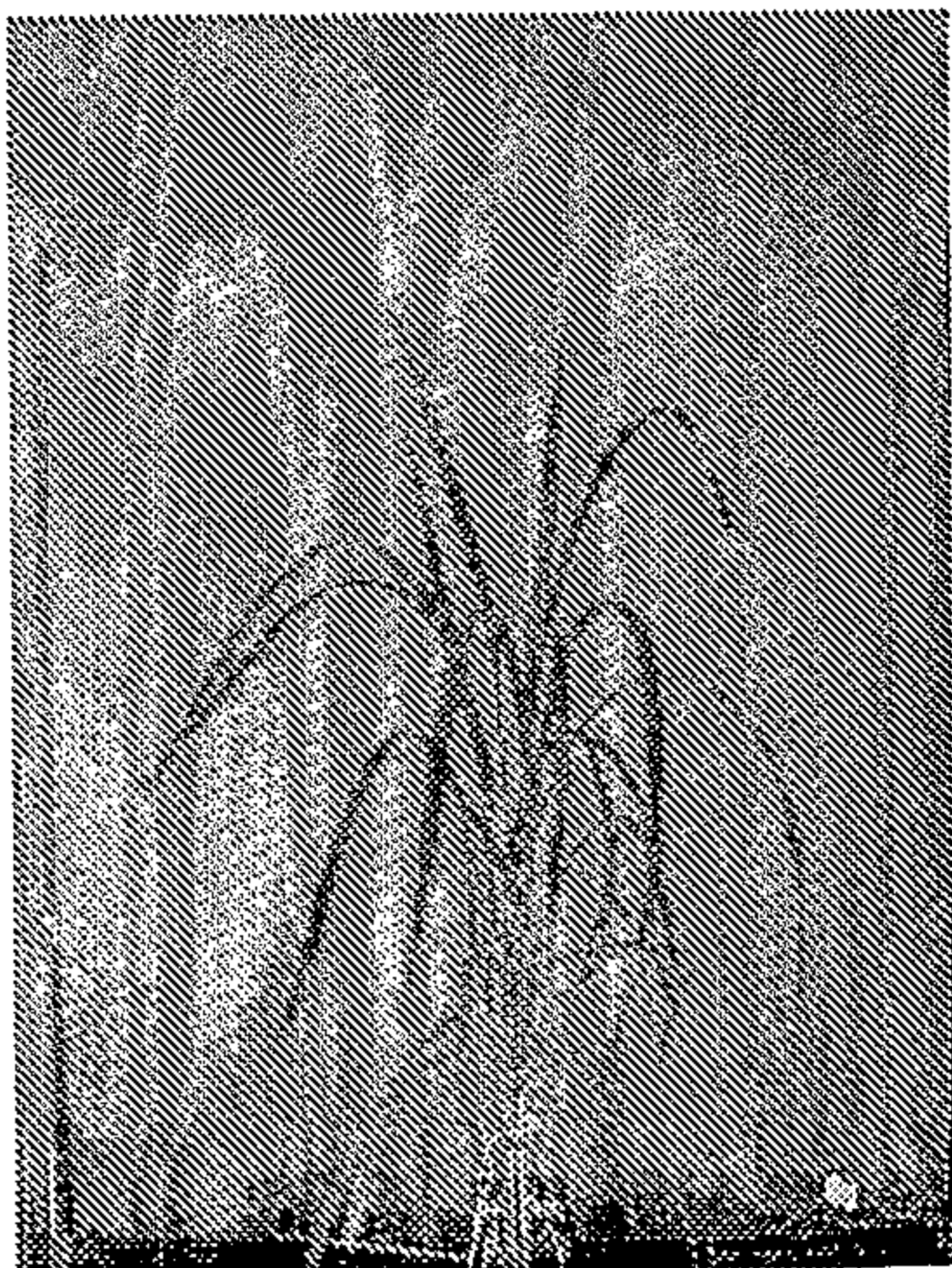


FIGURE 1

A



B

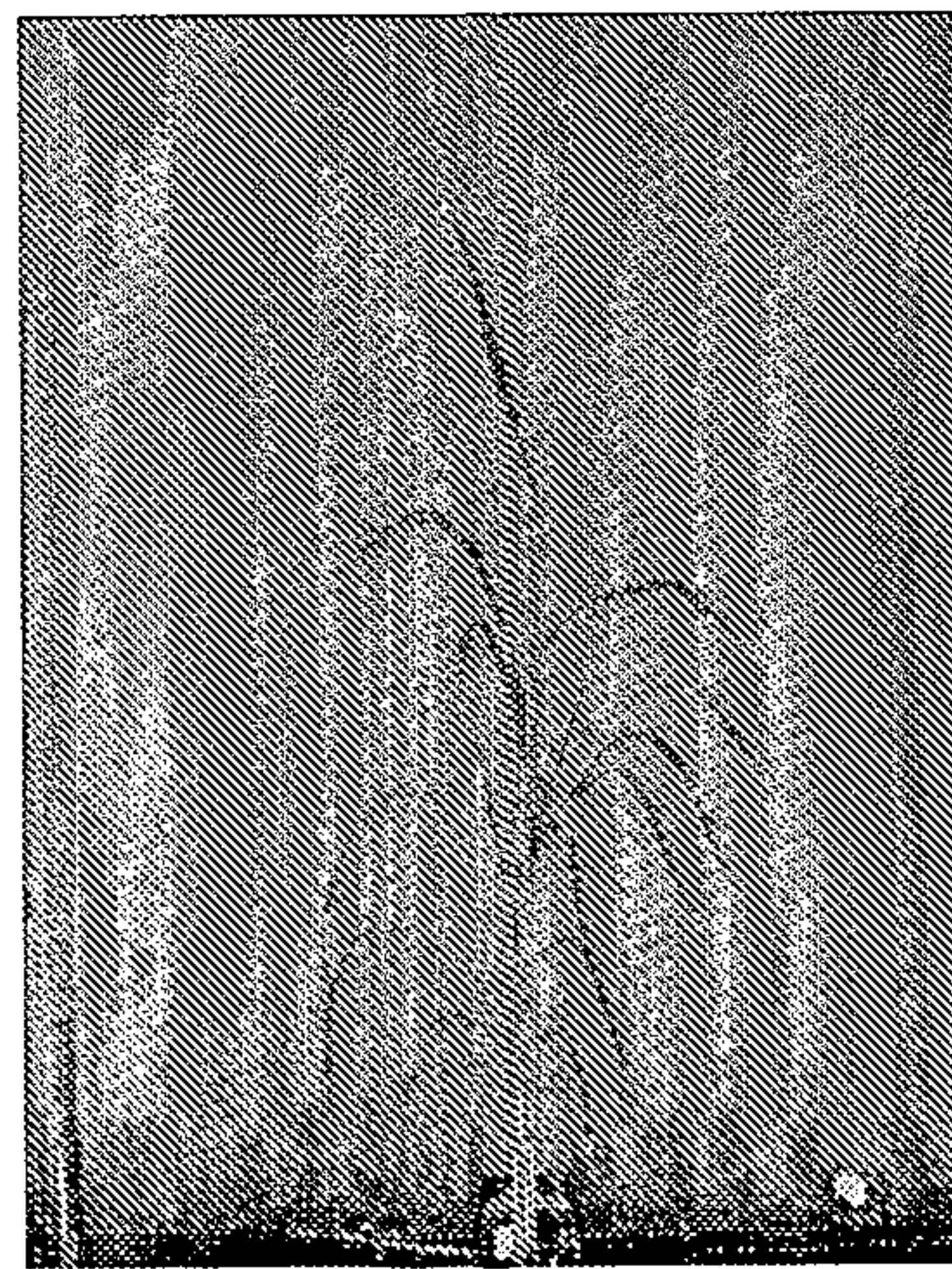


FIGURE 2

### FIGURE 3

**3/17****ORTHOLOGS OF STZ in OTHER PLANT SPECIES**

**SEQ ID NO 10:** Dg\_AF119050\_1 *Datisca glomerata* zinc-finger protein 1 (zfp1) mRNA, complete cds

GGCACGAGGACAAATTCTCTCTCTATCCTCTGAATATCTTTGGTTTGTGAACTGAGAAGCTA  
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ACCCAGCTTGAATTACCTTGAGCCATGGACCAAGCGTAAGCGTTCCAAGCGTACGCGCTTA  
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TTTGCCACCACGAATTCAGCCACCGTCACTACCACCACAGCCTCCGGAGGTGGTGGCAGGTC  
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GCTGCCACTACGAAGGCAGTATCGGCGGCAATAGTATTACCACCACAACAATACCACCAAC  
AGCGGAAGCAACGGTGGCATGAGCATGACCTCCGAAGTAGGTTCCACACACACAGTCAGCCA  
CAGTCACCGTGACTTCGATCTCAACATCCCGGCCTTGCCGGAGTTTCGGTCTGAATTTCTTCA  
TATCCGGGGATGACGAGGTGAGAGTCTCATCCGGCCAAGAAACCCCGTATATTGATGAAA  
**TAAA**ACATTTCTCAAGATCACTGAACCAGGCTTTAGTTTCTTTATAGGAGGAGATTTAAAAA  
AGTAGTATCTCTCTTTCTTTATCCGTAGGATAATTAATATATTTTCGTGTACATAAATTTGTA  
GTTCTTTAACACACTCTGTTTCATTTTCTTGCTTTGCTCAACTTTGTATTGGTTATTTCAAT  
ATGAAAATTCAATT

**SEQ ID NO 11:** Dg\_AF119050\_1 *Datisca glomerata*, STZ ortholog, protein

MALEALNSPTTATPVFHYDDPSLNYLEPWTKRKRSKRTRLDSPHTEEEYLAFLIMLARGRV  
ASANRRDSQSSIIQIQPEATTSATKVSYSKCSVCDKAFSSYQALGGHKASHRKLGGEDQSTSF  
ATTNSATVTTTTASGGGGRSHECSICHKSEPTGQALGGHKRCHYECSIGGNSIHNNNTTNS  
GSNGGMSMTSEVGSTHTVSHSHRDFDLNIPALPEFRSNFFISGDDEVESPHPAKKPRIIMK

**SEQ ID NO 12:** Gm\_T09602\_U68763.1\_GMU68763 *Glycine max* (soybean) probable zinc finger protein SCOF-1 mRNA, complete cds

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**FIGURE 3 (continued)**

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**SEQ ID NO 13:** Gm\_T09602 Glycine max (soybean) probable zinc finger protein SCOF-1, STZ ortholog, protein  
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 HVSPPLQPQPQPTPDPSTKLSYKCSVCDKSFPSYQALGGHKASHRKLAGAAEDQPPSTTTS  
 SAAATSSASGGKAHECSICHKSFPTGQALGGHKRCHYEGNGNGNNNSNSVVTVASEGVGST  
 HTVSHGHRDFDLNIPAFPDFSTKVGEDEVESPHPMKKPRLFVIPKIEIPQFQ

**SEQ ID NO 14:** Ms\_CAB77055\_Y18788.1\_MSY18788 Medicago sativa putative TFIIIA (or kruppel)-like zinc finger protein mRNA  
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 TACAAATTGGGATTGGTTTTAGAATTTAGGACGGTTGTAGACAATGGAAATTCAATTCAATT  
 ATTTAATTTTGTGT

**SEQ ID NO 15:** Ms\_CAB77055\_Medicago sativa putative TFIIIA (or kruppel)-like zinc finger protein, STZ ortholog, protein  
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 GNNNDKKS DSVATPLTTVKLSHKCSVCNKAFFSSYQALGGHKASHRKAVMSATTAEDQITTS  
 SAVTTSSASNGKNKTHECSICHKSFPTGQALGGHKRCHYEGSVGAGAGAGSNAVTAASEGVGL  
 SHSHHRDFDLNLPAPDFSKKFFVDDEVFSPLPAKKPCLFKLEIPSHY

**SEQ ID NO 16:** Nt\_AAC06243\_AF053077 Nicotiana tabacum osmotic stress-induced zinc-finger protein (zfp) mRNA, complete cds  
 TTTTCCCTCGAATTTGATAACTAAAGAGAATATTATGACTCTTGAAGCTTTGAAGTCACCTA  
 CGGCGGCAACGCCGACTCTACCACCACGCTATGAAGATGATGATGAAATTCATAATTTGGAT  
 TCTTGGGCTAAAGGAAAACGATCAAAACGGCCCCGTATTGATGCCCCACCGACTGAAGAAGA  
 GTATTTAGCCCTCTGTCTCATCATGCTCGCTCGCAGCGGAACCGGAACCAGAACCGGTTTAA  
 CTGATGCTACTACTTCCCAACAACCTGCCGATAAAAAAACC GCCGAGTTGCCGCCGGTTCAT  
 AAGAAAGAGGTGGCAACAGAGCAAGCAGAGCAATCTTACAAGTG TAGCGTGTGTGACAAGGC  
 TTTTCTTCTTATCAAGCACTCGGTGGGCATAAAGCAAGTCACCGTAAACTACTACTACTG  
 CTACCGCCGCCTCTGATGATAACAATCCTTCAACTTCAACTTCCACTGGCGCCGTTAATATC  
 TCTGCTCTTAATCCAATGGTTCGTTCACACGTCTGTTCTATTTGCCACAAGGCTTTTCCTAC  
 TGGCCAAGCTTTGGGTGGGCACAAGCGCCGCCACTATGAAGGCAAACTCGGTGGTAACAGCC  
 GCGACTTAGGCGGCGGCGGCGGCGGTCATAGTGGAAGCGTCTTGACTACTTCAGACGGC

FIGURE 3 (continued)

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GGCGCGTCGACTCACACGCTACGTGACTTTGACCTGAACATGCCTGCTTCGCCGGAATTGCA  
 ACTGGGTCTGAGTATTGATTGTGGACGGAAAAGTCAACTGTTGCCGATGGTCCAAGAGGTGG  
 AAAGTCCTATGCCTGCAAAGAAACCGCGTTTATTGTTTTTCGTTGGGT**TGA**AACTTCTTTAGG  
 GGAATTGAATTGATTGTGTTTTAGCCAAATTAGTAAATTGGTTCATGTGATTTTATTTTAG  
 GAAAAGGAATTATTGATTGTTTTACCCGTTTATTCTTAGGGTGGTATTATGTACAGGGAGTG  
 AATCATTCATTGGTTTTACACTTTCTTAATTATATATTCTTTTTTTTTTACACATAAAAAAA  
 AAAAAA

**SEQ ID NO 17:** Nt\_AAC06243\_Nicotiana tabacum osmotic stress-  
 induced zinc-finger protein, STZ otholog, protein  
 MTLEALKSPTAATPTLPPRYEDDDEIHNLD SWAKGKRSKRPRIDAPPTEEY LALCLIMLAR  
 SGTGTRTGLTDATTSQQPADKKTAE LPPVHKKEVATEQAEQSYKCSVCDKAFSSYQALGGHK  
 ASHRKTTTTATAASDDNNPSTSTSTGAVNISALNPTGRSHVCSICHKAFPTGQALGGHKRRH  
 YEGKLGGNSRDLGGGGGGHSGSVLTTS DGGASTHTLRDFDLNMPASPELQLGLSIDCGRKS  
 QLLPMVQEVESPMPAKKPRL LFSLG

**SEQ ID NO 18:** Os\_AF332876 Oryza sativa zinc finger  
 transcription factor ZF1 mRNA, complete cds  
 AATTCGGCACGAGGCCACACAGCAACCAGCCAGCTGCCACACTAGCTTGAGGCGAGCGAGCG  
 AAGCTTAGCTAGCGGATAGAACAAGTCGTCGATCTGCTTGCTGCTTTTGTGAATTGCGGTGG  
 AAG**ATG**TCGAGCGCGTCGTC CATGGAAGCGCTCCACGCCGCGGTGCTCAAGGAGGAGCAGC  
 AGCAGCACGAGGTGGAGGAGGCGACGGTCTGTACGAGCAGCAGCGCCACGAGCGGGGAGGAG  
 GGCGGACACCTGCCCCAGGGGTGGGCGAAGCGGAAGCGGTGCGCGCCGCGAGCGATCGGAGGA  
 GGAGAACCTCGCGCTCTGCCTCCTCATGCTCGCCCGCGGGCGGCCACCACCGCGTCCAGGCGC  
 CGCCTCCGCTCTCGGCTTCGGCGCCCCCGCCGGCAGGTGCGGAGTTCAAGTGCTCCGTCTGC  
 GGCAAGTCCTTCAGCTCCTACCAGGCGCTCGGCGGCCACAAGACGAGCCACCAGGGTCAAGCT  
 GCCGACTCCGCCCCGAGCTCCCGTCTTGGCTCCCGCCCCCGTCCGCGCCTTGCTGCCTTCCG  
 CCGAGGACCGCGAGCCAGCCACGTCATCCACCGCCGCGTCTCCGACGGCATGACCAACAGA  
 GTCCACAGGTGTTCCATCTGCCAGAAGGAGTTCCCCACCGGGCAGGCGCTCGGCGGGCACA  
 GAGGAAGCACTACGACGGTGGCGTAGGCGCCGGCGCCGGCGCATCTTCAACCGAGCTCCTGG  
 CCACGGTGGCCGCCGAGTCCGAGGTGGGAAGCTCCGGCAACGGCCAGTCCGCCACCCGGGCG  
 TTCGACCTCAACCTCCCGGCCGTGCCGGAGTTCGTGTGGCGGCCGTGCTCCAAGGGCAAGAA  
 GATGTGGGACGAGGAGGAGGAGGTCCAGAGCCCCCTCGCCTTCAAGAAGCCCCGGCTTCTCA  
 CCGCG**TAA**TTTCAGCAGCTGCACGGATCCGATCCGTCAGAGTTTTTGTCTAGGGAGTGAAATT  
 CAGTCGAAACACACTATTCGTTGATTCGTTTTGTGCCGCTATTGTTTAATTTGTTCTGCTT  
 TTGTACAGAGCAAGCGAGTGATACATAGCCATACATACAGTCATACAGATATAGGTCTAGCT  
 CTTCTTGGTTCTTTGTAACACTGGAAGTGTACCTGTATCTTTTACACTTTGTTCTTTGACA  
 GTCATATATTGTAGACCAAAAAAAAAAAAAAAAAA

**SEQ ID NO 19:** Os\_AF332876 Oryza sativa seedling zinc finger  
 transcription factor ZF1, STZ otholog, protein  
 MSSASSMEALHAAVLKEEQQQHEVEEATVVTSSSATSGEEGGHLPQGWAKRKRSRRQRSEEE  
 NLALCLMLLARGGHRVQAPPLSASAPPPAGAEFKCSVCGKSFSSYQALGGHKTS HRVKLP  
 TPPAAPVLAPAPVAALLPSAEDREPATSSSTAASSDGMTNRVHRC SICQKEFPTGQALGGHKR  
 KHYDGGVGAGAGASSTELLATVAAESEVGSSGNGQSATRAFDLNLPAVPEFVWRPCSKGKKM  
 WDEEEEVQSPLAFKKPRL LTA

**FIGURE 3 (continued)**

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**SEQ ID NO 20:** Ph\_BAA05079\_D26086.1 [Petunia x hybrida].  
 PETZFP4 zinc-finger protein gene  
 TTCACTCACCAAACAACCTTCTCTACCTCTTCTACTTGCACATTCAAATTCTTTTCATTACTA  
 CTTATCTCTACTAATCTTGATTCGATTTTAGTAAATCAAACAAGAGAATCTTTTCAGTAATA  
 CAAACAAGAAAATTTTCTCTCTATACTTGATTGAGTTTAGTAAGGCAAACAAGAAAACCTATC  
**ATGG**CACTTGAAGCATTGAATTCCTCAACTACAACAACACCACCATCATTCCAATTTGAGAA  
 CAACGGGCTTAAGTACCTTGAGAGTTGGACAAAAGGTAAAAGATCAAAAAGGCAACGCAGCA  
 TGGAACGACAGTGTACTGAAGAAGAGTATTTAGCACTTTGTCTTATCATGCTAGCACGTAGC  
 GATGGTTCTGTTAATAACTCACGGTCTCTACCACCACCACCCTACCACCATCAGTTCCAGT  
 AACGTCGCAAATAAACGCGACGTTATTGGAACAGAAGAATTTGTACAAGTGTTCCGTTTGTG  
 GTAAAGGGTTTGGGTCTTATCAAGCTTTAGGTGGACATAAAGCAAGTCACCGGAAACTTGTC  
 AGCATGGGAGGAGATGAACAATCTACTACTTCCACTACTACTAACGTAACGGGAACTAGTTC  
 CGCTAACGTTAACGGTAACGGAAGAAGTACGAATGTTCAATTTGTCACAAGTGCTTTTCTA  
 CTGGACAAGCTTTAGGTGGTCATAAAAGGTGCCACTATGACGGTGGTAACGGTAACGGTAAC  
 GGAAGTGTAAGTGTTGGGGTGACGTCATCTGAAGGTGTGGGGTCCACTATTAGTCATCACCG  
 TGACTTTGACTTGAATATTCCCGCGTTGCCGGAGTTTGGCCGGGATTTGGTTCCGGCGAGG  
 ATGAGGTGGAGAGTCCTCATCCAGCAAAGAAGTCAAGGCTATCTCTTCCACCTAAACTTGAA  
 TTATTCAAAGGATTAT**TAG**AGGGAATATTGATTGTGTTACAGGAAGATTTATTAGGATTCACGA  
 ATTTTTTGTGACTAGTTTATGTAATAT

**SEQ ID NO 21:** Ph\_BAA05079 [Petunia x hybrida] zinc-finger  
 protein, STZ ortholog, protein  
 MALEALNSPTTTTPPSFQFENGLKYLESWTKGKRSKRQRSMERQCTEEYLLALCLIMLARS  
 DGSVNNSRSLPPPPLPPSVPVTSQINATLLEQKNLYKCSVCGKGFSGSYQALGGHKASHRKLV  
 SMGGDEQSTTSTTTNVTGTSSANVNGNGRTHECSICHKCFPTGQALGGHKRCHYDGGNGNGN  
 GSVSVGVTSSEGVGSTISHHRDFDLNIPALPEFWPGFGSGEDEVESPHPAKKSRLSLPPKLE  
 LFKGL

**SEQ ID NO 22:** Ta\_BAA03901 Triticum aestivum gene for zinc-  
 finger protein WZF1, complete cds  
**ATG**TCGTCGTCGGCCATGGAAGCGCTCCACGCCCTGATCCCGGAGCAGCACCAGCTGGACGT  
 TGAGGCGGCTGCGGCTGTCAGCAGCGCCACCAGCGGCGAGGAGAGCGGCCACGTGCTGCAGG  
 GGTGGGCCAAGAGGAAGCGATCGCGCCGCCAGCGCTCCGAGGAGGAGAACCTCGCGCTCTGC  
 CTCCTCATGCTCTCGCGCGGCGGCAAGCAGCGTGTTAGGCGCCGCAGCCGGAGTCGTTTCGC  
 TCGCGCCGGTGCTGCGGAGTTCAAGTGCTCCGTCTGCGGCAAGTCCTTCAGCTCCTACCAGG  
 CGCTCGGAGGCCACAAGACGAGCCACCGGGTGAAGCAGCCGTCTCCTCCCTCTGATGCCGCT  
 GCTGCCCCACTCGTGGCCCTCCCGGCCGTGCGCGCCATCCTGCCGTCCGCCGAGCCGGCCAC  
 GTCGTCCACCGCCGCGTCCTCCGACGGCGCGACCAACAGAGTCCACAGGTGCTCCATCTGCC  
 AAAAGGAGTTCCCGACTGGGCAGGCGCTCGGCGGGCACAAGAGGAAGCACTACGACGGAGGC  
 GTGGGCGCCGCCGCTCGTCGACCGAGCTTCTGGCCGCCGCGGCCGCGGAGTCTGAGGTGGG  
 GAGCACCGGCAACGGGAGCTCCGCCGCCGGGCCTTCGACCTGAACATTCCGGCCGTGCCGG  
 AGTTCGTGTGAGAGCCGTGCGCCAAGGGCAAGATGATGTGGGAGGACGATGAGGAGGTGCAG  
 AGCCCCCTCGCCTTCAAGAAGCCTCGGCTTCTCACCGCT**TGA**

FIGURE 3 (continued)

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**SEQ ID NO 23:** Ta\_BAA03901\_WZF1 *Triticum aestivum*, STZ ortholog, protein

MSSSAMEALHALIPEQHQLDVEAAAAVSSATSGEESGHVLQGWAKRKRSRRQRSEENLALC  
LLMLSRGGKQRVQAPQPESFAAPVPAEFKCSVCGKSFSSYQALGGHKTSHRVKQPSPPSDAA  
AAPLVALPAVAAILPSAEPATSSSTAASSDGATNRVHRCSICQKEFPTGQALGGHKRKHVDGG  
VGAAASSTELLAAAAAESEVGSTGNGSSAARAFDLNIPAVPEFVWRPCAKGKMMWEDDEEVQ  
SPLAFKKPRLTA

**SEQ ID NO 24:** Ca AF539746 *Capsicum annum* zinc finger protein mRNA, complete cds

AAAATCTTCGCTACTTACTTACATCTTCTAGAATAGTCACTAGAACCAGTAACTTTATACAA  
CGGATATCGATATGGCACTTGAAGCTTTGAATTCTCCAACCTGGTACACCAACTCCGCCACCG  
TTTCAATTTGAGAGCGACGGCCAACAGCTTCGATATATCGAAACTGGAGGAAGGGAAAGAG  
ATCTAAAAGGTCACGCAGCATGGAGCACCAGCCTACTGAGGAAGAATACTTAGCGCTTTGTT  
TGATCATGCTTGCACGTAGCGGTGGCTCCGTTAATCATCAACGATCTCTACCACCGCCGGCT  
CCGGTGATGAAACTGCACGCGCCGTCGTCATCATCGGCGGCGGAGGAGGAGAAGGAGAAGAT  
GGTGATAAGTGTTCCGGTTTGTGGTAAGGGATTTGGGTCTTATCAAGCTTTAGGTGGACACA  
AAGCTAGTCACCGGAAACTCGTACCCGGCGGAGATGATCAGTCAACTACCTCCACAACCACT  
AACGCAACCGGAACAACAACCTCCGTTAACGGCAACGGCAACAGAAGTGGAAGGACTCACGA  
GTGTTTCGATTTGTCACAAGTGTTTTCCCACTGGACAAGCTTTAGGTGGACACAAAAGGTGTC  
ACTACGACGGCGGTATCGGTAACGGAAACGCTAACAGTGGCGTTAGTGCTAGCGTTGGAGTG  
ACGTCATCGGAGGGTGTTGGGTCCACAGTCAGTCACCGGGATTTGCGACTTGAACATTCGGC  
GTTGCCGGAATTCTGGCTGGGATTTGGTTCGGCGAAGATGAGGTGGAGAGTCCACATCCGG  
CGAAGAAATCGCGGTTATGTTTGCTCCAAAATATGAATTATTTCAACATTAATGGGAATTT  
GATTGTTAGGATTTACTATTTTGGTAGACAAAATTATACTATGTAAGTTTTAATTTTCATTG  
TGGGTGGGAGCAAAATTTTTAATTTTTTGTCTATAGACCTAGCTAGTTACTAATAGCAAAAA  
TTCAATTGATTGATTTAAAAA

**SEQ ID NO 25:** Ca AF539746-capsicum annum, STZ ortholog, protein  
MALEALNSPTGTPTPPPFQFESDQQLRYIENWRKGRSKRSRSMEHQPTEEYLLALCLIML  
ARSGGSVNHQRLPPPAPVMKLHAPSSSSAAEEKEKMKVYKCSVCGKGFGSYQALGGHKASH  
RKLVPGGDDQSTTSTTTNATGTTTSVNGNGNRSGRTHECSICHKCFPTGQALGGHKRCHYDG  
GIGNGNANSVGSASVGVTSSEGVGSTVSHRDFDLNIPALPEFWLGFSGEDEVESPHPAKKS  
RLCLPPKYELFQH

#### PARALOGS OF STZ IN ARABIDOPSIS THALIANA

**SEQ ID NO 26:** gi\_18402298\_ref\_NM\_112848.1 mRNA

ACTTCACTCTCTAATTTCTTCTCTCTATCTCTCACCATATTCGCGATTAAAACTCTCAAC  
TTTTCTCTCAAATTTCTGATCCTTTGATCCAACAGTTAGAAGAAGATTCATCTGATCATGGC  
CCTCGAAGCGATGAACACTCCAACCTTCTTCTTACCAGAATCGAAACGAAAGAAGATTTGA  
TGAACGACGCCGTTTTTCATTGAGCCGTGGCTTAAACGCAAACGCTCCAAACGTCAGCGTTCT  
CACAGCCCTTCTTCGTCTTCTTCTCCTCACCGCCTCGATCTCGACCCAAATCCCAGAATCAAGA  
TCTTACGGAAGAAGAGTATCTCGCTCTTTGTCTCCTCATGCTCGCTAAAGATCAACCGTCGC  
AAACGCGATTTTCATCAACAGTCGCAATCGTTAACGCCGCCGCGCAGAATCAAAGAACCTTCGG  
TACAAGTGTAACGTCTGTGAAAAAGCGTTTCCTTCTATCAGGCTTTAGGCGGTACAAAGC  
AAGTCACCGAATCAAACCAACCGTAATCTCAACAACCGCCGATGATTCAACAGCTCCGA

**FIGURE 3 (continued)**

**FIGURE 3 (continued)**

**9/17****SEQ ID NO 30:** gi\_30693252\_ref\_NM\_114853.2\_mRNA

ATGGCTCTCGACACTCTCAATTCTCCACCTCCACCACCACAACCACCGCTCCTCCTCCTTT  
CCTCCGTTGCCTCGACGAAACCGAGCCCGAAAACCTCGAATCATGGACCAAAGAAAACGTA  
CAAAACGTCACCGTATAGATCAACCAAACCTCCTCCTTCTGAAGAAGAGTATCTCGCTCTT  
TGCTCCTTATGCTCGCTCGTGGCTCCTCCGATCATCACTCTCCACCGTCGGATCATCACTC  
TCTTTCTCCACTGTCCGATCATCAGAAAGATTACAAGTGTTCCGTCTGTGGCAAATCTTTCC  
CGTCTTACCAAGCGTTAGGTGGACACAAAACAAGTCACCGGAAACCGGTTAGTGTGATGTT  
AATAATAGTAACGGAACCGTTACTAATAACGGAATATTAGTAACGGTTTAGTTGGTCAAAG  
TGGAAGACTCATAACTGCTCTATATGTTTTAAGTCGTTTCCCTCTGGTCAAGCATTGGGTG  
GTCACAAACGTTGTCACCTATGATGGTGGTAACGGTAACAGTAACGGTGACAATAGCCACAAG  
TTTGACCTAAATTTACCGGCTGATCAAGTTAGTGATGAGACAATTGGAAAAAGTCAACTCTC  
CGGTGAAGAAACAAAGTCGGTGTTGTGATTATTATTATTTTTTACCGATCGGGATTAGCTAG  
TGTTGATCATTAGCTGAGTCTGTAATGAAAATGAT

**SEQ ID NO 31:** Translation of gi\_30693252\_ref\_NM\_114853.2

MALDTLNSPTSTTTTTAPPPFLRCLDETEPENLESWTKRKRTRHRIDQPNPPPSEEEYLAL  
CLLMLARGSSDHHSPPSDHHSLSPLSDHQDYKCSVCGKSFPSYQALGGHKTSRKPVSVDV  
NNSNGTVTNNGNISNGLVGQSGKTHNCISCFKSFPSGQALGGHKRCHYDGGNGNSNGDNSHK  
FDLNLPADQVSDETIGKSQLSGEETKSVL

**SEQ ID NO 32:** gi\_30694224\_ref\_NM\_123683.2\_mRNA

AAATTTTCTATAGCAATGGCGCTTGAAGCTCTTAATTCACCAAGATTGGTCGAGGATCCCTT  
AAGATTCAATGGCGTTGAGCAGTGGACCAAATGTAAGAAACGATCCAAACGTTTCGAGATCTG  
ATCTTCATCATAACCACCGTCTCACTGAGGAAGAGTATCTAGCTTTCTGTCTCATGCTTCTT  
GCTCGGGATGGCGGCGATCTTGACTCTGTGACGGTTGCGGAGAAGCCGAGTTATAAGTGTGG  
CGTTTGTTACAAGACGTTTTTCGTCTTACCAAGCTCTCGGCGGTCATAAAGCGAGCCACCGGA  
GCTTATACGGTGGTGGAGAGAATGATAAATCGACACCATCCACCGCCGTGAAATCTCACGTT  
TGTTCCGTTTGCGGGAAATCTTTCGCCACCGGTCAAGCTCTCGGCGGCCACAAGCGGTGCCA  
CTACGATGGTGGCGTTTTCGAACTCGGAAGGTGTGGGGTCTACTAGCCACGTCAGCAGTAGTA  
GCCACCGTGGATTTGACCTTAATATTATACCGGTGCAGGGATTTTCGCCGGACGACGAAGTG  
ATGAGTCCGATGGCGACTAAGAAGCCTCGCCTGAAGTAAGTCTTTGTTGAAGACCTGGAAGT  
TTATCAAATGTAAATATCAAATTTCAATTTCAAGGAACAGTTTTGTTGATTCTATTACCAAT  
ACACAATACGATTCAATTCC

**SEQ ID NO 33:** Translation of gi\_30694224\_ref\_NM\_123683.2\_

MALEALNSPRLVEDPLRFNGVEQWTKCKKRSKRSRSDLHHNHRLTEEEYLAFCLMLLARDGG  
DLDSVTVAEKPSYKCGVCYKTFSSYQALGGHKASHRSLYGGGENDKSTPSTAVKSHVCSVCG  
KSFATGQALGGHKRCHYDGGVSNSEGVGSTSHVSSSSHRGFDLNIIPVQGFSPDDEVMSMA  
TKKPRLK

**FIGURE 3 (continued)**

**10/17****SEQ ID NO 34:** gi\_30698307\_ref\_NM\_126145.2\_mRNA

CACACTTCACTCTTTCTTCATCTTCTTCTTCTTAAATAGCTCGAAATCACATCTCACAGAAT  
TAAATCTTATGGCTCTCGAGACTCTCAATTCTCCAACAGCTACCACCACCGCTCGGCCTCTT  
CTCCGGTATCGTGAAGAAATGGAGCCTGAGAATCTCGAGCAATGGGCTAAAAGAAAACGAAC  
AAAACGTCAACGTTTTTGATCACGGTCATCAGAATCAAGAAACGAACAAGAACCTTCCTTCTG  
AAGAAGAGTATCTCGCTCTTTGTCTCCTCATGCTCGCTCGTGGCTCCGCCGTACAATCTCCT  
CCTCTTCCTCCTCTACCGTCACGTGCGTCACCGTCCGATCACCGAGATTACAAGTGTACGGT  
CTGTGGGAAGTCCTTTTCGTCATACCAAGCCTTAGGTGGACACAAGACGAGTCACCGGAAAC  
CGACGAACACTAGTATCACTTCCGGTAACCAAGAACTGTCTAATAACAGTCACAGTAACAGC  
GGTTCGGTTGTTATTAACGTTACCGTGAACACTGGTAACGGTGTAGTCAAAGCGGAAAGAT  
TCACACTTGCTCAATCTGTTTCAAGTCGTTTTCGCTCTGGTCAAGCCTTAGGTGGACACAAAC  
GGTGTCACTATGACGGTGGCAACAACGGTAACGGTAACGGAAGTAGCAGCAACAGCGTAGAA  
CTCGTCGCTGGTAGTGACGTCAGCGATGTTGATAATGAGAGATGGTCCGAAGAAAGTGCGAT  
CGGTGGCCACCGTGGATTTGACCTAACTTACCGGCTGATCAAGTCTCAGTGACGACTTCTT  
AACGTTGACTGAGTTTGAGGAAAAAGTCAACTATCAAGCGAAGAAAGGGTTAGTGGACGGTG  
AAGATTAACGGTCGTTTCTTTCCAGTTGCTTCGGTTTGAGCTTGACTGGGTCTGTAATGAAA  
ATGATTGGAGTGGACTTGGCATTATTATTATTATTTTAAAAAGAAATGTTAATTTGTTGTT  
GGATTTGTTTATAGATAGAGGAAACAATTGGGATACACAAATATTTTTTTTTTTTACAAAGA  
AAATAATAATGCAGAGATGGATGATTGGATCGTACACGTTATTATATAGTGGACCATTTCTGT  
AATCGTGAATTATTATTATTGTTAGAAATTTAATTTTCGT

**SEQ ID NO 35:** Translation of gi\_30698307\_ref\_NM\_126145.2\_

MALETILNSPTATTTARPLLRYREEMEPENLEQWAKRKRTKRQRFDHGHQONQETNKNLPSEEE  
YLALCLLMLARGSAVQSPPLPPLPSRASPSDHRDYKCTVCGKSFSSYQALGGHKTSHRKPTN  
TSITSGNQELSNNSHSNSGVSVINVTVNTGNVGSQSGKIHTCSICFKSFASGQALGGHKRCH  
YDGGNNGNGNGSSSNSVELVAGSDVSDVDNERWSEESAIGGHRGFDLNLPADQVSVTTS

**OTHER GENES IN EVALUATION****SEQ ID NO 36:** gi\_12698881\_ref\_AF\_332876.1\_mRNA, 2xC2H2,  
Oryza sativa

AATTCGGCACGAGGCCACACAGCAACCAGCCAGCTGCCACACTAGCTTGAGGCGAGCGAGCG  
AAGCTTAGCTAGCGGATAGAACAAGTCGTCGATCTGCTTGCTGCTTTTGTGAATTGCGGTGG  
AAGCATGTGAGCGCGTCGTCATGGAAGCGCTCCACGCCGCGGTGCTCAAGGAGGAGCAGC  
AGCAGCACGAGGTGGAGGAGGCGACGGTCGTGACGAGCAGCAGCGCCACGAGCGGGGAGGAG  
GGCGGACACCTGCCCCAGGGGTGGGCGAAGCGGAAGCGGTGCGCGCCGCCAGCGATCGGAGGA  
GGAGAACCTCGCGCTCTGCCTCCTCATGCTCGCCCGCGGCGGCCACCACCGCGTCCAGGCGC  
CGCCTCCGCTCTCGGCTTCGGCGCCCCCGCCGGCAGGTGCGGAGTTCAAGTGCTCCGTCTGC  
GGCAAGTCCTTCAGCTCCTACCAGGCGCTCGGCGGCCACAAGACGAGCCACCGGGTCAAGCT  
GCCGACTCCGCCCGCAGCTCCCGTCTTGGCTCCCGCCCCCGTCGCCGCTTGCTGCCTTCCG  
CCGAGGACCGCGAGCCAGCCACGTCATCCACCGCCGCGTCTCCGACGGCATGACCAACAGA  
GTCCACAGGTGTTCCATCTGCCAGAAGGAGTTCCCCACCGGGCAGGCGCTCGGCGGGCACAA  
GAGGAAGCACTACGACGGTGGCGTAGGCGCCGGCGCCGGCGCATCTTCAACCGAGCTCCTGG  
CCACGGTGGCCGCCGAGTCCGAGGTGGGAAGCTCCGGCAACGGCCAGTCCGCCACCCGGGCG  
TTCGACCTCAACCTCCCGGCCGTGCCGGAGTTGCTGTGGCGGCCGTGCTCCAAGGGCAAGAA  
GATGTGGGACGAGGAGGAGGAGGTCCAGAGCCCCCTCGCCTTCAAGAAGCCCCGGCTTCTCA  
CCGCGTAATTCAGCAGCTGCACGGATCCGATCCGTCAGAGTTTTTGTCTAGGGAGTGAAATT  
CAGTCGAAACACACTATTCGTTGATTCGTTTTGTGCCGCTATTGTTTAATTTGTTCTGCTT

**FIGURE 3 (continued)**

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TTGTACAGAGCAAGCGAGTGATACATAGCCATACATACAGTCATACAGATATAGGTCTAGCT  
CTTCCTTGGTTCTTTGTAACTGGAAGTGTACCTGTATCTTTTACACTTTGTTCTTTGACA  
GTCATATATTGTAGACCAAAAAAAAAAAAAAAAAAAAAA

**SEQ ID NO 37:** gi\_12698882\_ref\_AAK01713.1, 2xC2H2, *Oryza sativa*  
MSSASSMEALHAAVLKEEQQHEVEEATVVTSSSATS GEEGGHLPQGWAKRKRSRRQRSEEE  
NLALCLLMLARGGHRVQAPPPLSASAPPPAGAEFKCSVCGKSFSSYQALGGHKTSHRVKLP  
TPPAAPVLAPAPVAALLPSAEDREPATSSTAASSDGMTNRVHRC SICQKEFPTGQALGGHKR  
KHYDGGVGAGAGASSTELLATVAAESEVGSSGNGQSATRAFDLNLPAVPEFVWRPCSKGKKM  
WDEEEEVQSPLAFKKPRLTA

**SEQ ID NO 38:** gi\_6434215\_ref\_AL132966.1\_region 116202 ...  
116729, 2xC2H2, *Arabidopsis thaliana*

ATGAAGAGAGACCGGTCCGATTACGAAGAATCCATGAAGCATATAGACATAGTAGAAAGTCT  
AATGATGTTATCTCGAAGTTTCGTGGTCAAACAAATCGATGTAAAGCAATCTACCGGAAGCA  
AAACGAACCATAATAACCACTTCGAATGCAAACGTGTAACCGGAAATTTGATTCCCTCCAA  
GCTCTTGGAGGTCATAGAGCTAGCCACAAGAAACCTAAGCTGATCGTTGACCAAGAACAGGT  
GAAGCATCGTAACAAAGAGAATGATATGCATAAGTGTACAATTTGCGATCAAATGTTTGGGA  
CCGGTCAAGCTCTAGGCGGTACATGAGAAAGCATAGGACGAGCATGATAACCGAGCAATCG  
ATTGTCCCTTCTGTGGTTTATTCAGACCGGTTTTTAATCGTTGCAGTAGCAGCAAGGAGAT  
CTTGGACTTAAATCTAACTCCATTGGAAAATGATCTTGTGTTAATCTTTGGGAAGAATTG  
TTCCACAAATTGATTTGAAGTTTGTGAATTAG

**SEQ ID NO 39:** gi\_6729511\_ref\_CAB67667.1, 2xC2H2, *Arabidopsis thaliana*  
MKRDRSDYEESMKHIDIVESLMMLSRSFVVKQIDVKQSTGSKTNHNNHFECKTCNRKFDSEFQ  
ALGGHRASHKKPKLIVDQEQVKHRNKENDMHKCTICDQMFGTGQALGGHMRKHRTSMITEQS  
IVPSVVYSRPVFNRCSSSKEILDNLTPLENDLVLI FGKNLVPQIDLKFDVN

**SEQ ID NO 40:** ref\_CA279020, 2xC2H2, sugar cane  
CCTAACCAGCATTAGCTTTTCAAATCAACAAGCCTCGCCGTGACCGATCGATGGCCATCACC  
CACGACGACTACGTCTCCCTCTGCCTCATGGCGCTCGCAGCCGCGGGAGGCGGAGGCCAAGC  
TGGTTTAAACAACGCAGTACGCTCTGAACACGGCTGCCTGGACAGCGACGGCGCAAGAGTCCG  
AGCTCCGCTTCCGGTGCTCCGTCTGTGGCAAGGCCTTCGCGTTCGCACCAGGCACTGGGCGGG  
CACAAGGCCAGCCACCGCAAGCCGACGCTCGTACAGGCACATGCGTCGTCTCAGCCGGAGG  
CGCGGCGTTCGTTCGTAACAATGACCTCGGCCGTAGGCAGCAGTGGGCAGGGGAGGCACA  
GGTGCACGGTGTGCCATCGGAGCTTCGCGACGGNGCAAGCGCTCGGCGGGCACAAGAGGTGC  
CATTACTGGGACGGGCTCTCGGTGTGCTCACCGCGTTCGTCGGCGCCATCGGGGTCCGGGTC  
GACCGTCAAGGGCTTTGATCTGAATTTGGTGCCGGTGCCGCCCGCGATGGCCGCCAACGCTG  
CGACAAGGTGGGGAGAGGAGAANNAAGTCANAAACCCTTGCGGTCAAGAGAAGGCGGCTTG  
CCGGTCCGTCTTGGACCCTAATTTAACGATTTAGAAGTCCTTTTTTTTAATAATTAAGAGTTC  
TTTTGAAGAAGGTTGTAAAGTTTTCGAACCTTGTTCTTTTAATGGATTTGGGTGCTGGCGAA  
ATTTTAAACTGGATTTAAATTTGCGCTCACTCTTTTTTTTTTATTTTTTACACCCTTTTTTT  
TTTTTAGAAGAAGA

**FIGURE 3 (continued)**

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**SEQ ID NO 41:** gi\_18027011\_ref\_AF254447.1, 2xC2H2, *Arabidopsis thaliana*  
TTCCTTTCTCTTCCTCTCTCTCTCTCTTCACCATGACTGATCCTTATTCCAATTTCTTCACA  
GACTGGTTCAAGTCTAATCCTTTTCACCATTACCCTAATTCCTCCACTAACCCCTCTCCTCA  
TCCTCTTCCTCCTGTTACTCCTCCCTCTTCCTTCTTCTTCTTCCCTCAATCCGGAGACCTCC  
GCCGTCCACCGCCGCCACCAACTCCTCCTCCTTCTCCTCCTCTCCGAGAAGCCCTCCCTCTC  
CTCAGCCTCAGCCCCGCCAACAAACAACAAGACCACCATCACAAACCATGACCACCTTATTCA  
AGAACCACCTTCAACCTCCATGGATGTGCGACTACGATCATCACCATCAAGATGATCATCATA  
ACCTCGATGACGATGACCATGACGTCACCGTTGCTCTTCACATAGGCCTTCCAAGCCCTAGT  
GCTCAAGAGATGGCCTCTTTGCTCATGATGTCTTCTTCTTCTCCTCTTCCTCGAGGACCACTCA  
TCATCACGAGGACATGAATCACAAAGAAAGACCTCGACCATGAGTACAGCCACGGAGCTGTGCG  
GAGGAGGAGAAGATGACGATGAAGATTGAGTCGGCGGAGACGGCGGGCTGTAGAATCAGCAGA  
CTCAACAAGGGTCAATATTGGATCCCTACACCTTCTCAGATTCTCATTTGGCCCTACTCAGTT  
CTCATGTCTGTTTGCTTCAAAACCTTCAACAGATACAATAACATGCAGATGCATATGTGGG  
GACATGGATCACAAATACAGAAAAGGACCTGAATCTCTAAGGGGAACACAACCAACAGGAATG  
CTAAGGCTTCCGTGCTATTGCTGCGCCCCAGGCTGTGCGCAACAACATTGACCATCCAAGGGC  
AAAGCCTCTCAAAGACTTCAGAACCTTCAAACACATTACAAGAGAAAACATGGGATCAAAC  
CTTTCATGTGTAGGAAATGTGGAAAGGCTTTCGCGAGTCCGAGGGGACTGGAGAACACATGAG  
AAGAATTGTGGCAAACCTTTGGTATTGCATATGTGGATCTGATTTCAAGCACAAGAGATCTCT  
CAAAGATCACATCAAGGCTTTTGGGAATGGTCATGGAGCCTACGGAATTGATGGGTTTGATG  
AAGAAGATGAGCCTGCCTCTGAGGTAGAACAATTAGACAATGATCATGAGTCAATGCAGTCT  
AAATAGCTTATATATATTACTATAAGTACTAAGTAATTCGGTATATATATTAATTATAAGAA  
ACCTAAATCTATGGACCAAGTTTTGATGGAGGTAGGGCTTTTCAAACCTAAAAGCTATATCAT  
CTAATTGATCATAGGAAAAAATGAATCAAGAGCACTTGGAAAAATTTTAAATTGTATCTTTA  
GCTTCCTAGTTAAATTTATTGCAAGACAATGTAGCAGTCTAACCAATGAGGTTCCCAACGGT  
TTATTTCTATTTGTATATTATTTGTCATTAGCTTCACCTTTCGTTAATTCGAAGGACATAA  
CTTATAAATGTTTAAATTATG

**SEQ ID NO 42:** At3g57670, 2xC2H2, *Arabidopsis thaliana*  
MTDPYSNFFTDWFKSNPFHHYPNSSTNPSPHPLPPVTPPSSFFFFPQSGDLRRPPPPPTPPP  
SPPLREALPLLSLSPANKQQDHHHNHDHLIQEPPSTSMVDVYDHHHQDDHHNLDDDDHDVTV  
ALHIGLPSPSAQEMASLLMMSSSSSSSRTHHHHEDMNHKKDLDEYSHGAVGGGEDDDDEDSV  
GGDGGCRISRLNKGQYWIPTPSQILIGPTQFSCPVCFKTFNRYNNMQMHMWGHGSQYRKGP  
SLRGTQPTGMLRLPCYCCAPGCRNNIDHPRAKPLKDFRTLQTHYKRKHGIKPFMCRCCKGAF  
AVRGDWRTHEKNCGLWYCICGSDFKHKRSCLKDHIAFGNGHGAYGIDGFDEEDEPASEVEQ  
LDNDHESMQSK

**SEQ ID NO 43:** gi\_18676370\_ref\_AJ311810.2, 2xC2H2, *Arabidopsis thaliana*  
ATCTACACACTACTACTCACATCTCATCTCTCTCTAGCACATAACCATCAAACCATATAGAT  
ACGGTGCTTTTATTCTTGATCTTCTTCTTCTTCTTGTCTTCTCCTCAGAGTCATGTCTAAT  
CCAGCTTGTTTGAATCTCTTCAACAATGGATGTGACCATAATAGCTTCAACTATTCCACTTC  
TCTCTTTACATTTACAACCTCTCACGGTAGCTACTATTACTCTAATACCACAAACCCTAATT  
ACATTAATCATACTCATACCACTTCCACTTCCCCTAACTCACCCCCACTAAGAGAAGCTCTT  
CCTCTTCTTAGCTTAAGCCCCATAAGGCACCAAGAACAACAAGACCAACACTATTTTCATGGA  
CACCCATCAAATTAGCTCTTCAAACCTTCTTGATGATCCTCTTGTGACTGTGGATCTTCATC  
TAGGGTTACCAAACCTACGGTGTTGGTGAGAGCATTAGGAGCAATATTGCTCCTGATGCAACC

**FIGURE 3 (continued)**

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ACGGACGAGCAAGATCAAGATCATGACCGAGGAGTAGAAGTCACAGTTGAGTCCCACCTTGA  
 TGATGATGATGATCATCATGGAGATCTACACAGAGGTCATCACTATTGGATTCCTACTCCTT  
 CTCAGATTTTGGATTGGTCCTACACAGTTCACCTTGTCTCTTTGCTTCAAGACATTCAACAGA  
 TACAACAACATGCAGATGCACATGTGGGGACACGGCTCACAATACAGAAAGGGACCAGAATC  
 CTTAAGAGGAACCCAACCAACAGGAATGCTAAGACTACCATGTTTCTGCTGTGCACCCGGTT  
 GCAAGAACAACATTGACCACCCACGAGCCAAGCCTCTTAAGGACTTTCGAACCCTCCAAACA  
 CATTACAAACGTAAACATGGGTCTAAACCATTGCTTGTCTGTATGTGTGGTAAGGCCTTTGC  
 AGTGAAAGGAGATTGGAGAACGCATGAGAAGAATTGTGGAAAGCTTTGGTATTGCTCTTGTG  
 GCTCGGATTTTAAGCACAAGAGGTCGCTTAAGGACCATGTCAAGGCCTTTGGAAATGGTCAT  
 GTTCCTTGTGGGATTGATAGTTTTGGAGGAGATCATGAGGACTACTATGATGCTGCTTCTGA  
 TATCGAGCAATAAGATGATAGCAACAACAATGAGTGTTAATTAGGGGTTTTGTTTATTTTTC  
 CTCTCATGCATTAGTTGATTGTATGCACGTGTTCTTTAGTTTTGTTCTTCGGATCTTTGTTT  
 TATTTTGTTTTGAGCTGTTTTTTTTTTTAATTACTAAGAAGTTAATTATCATCTAAAGATTTT  
 C

**SEQ ID NO 44:** gi\_18376498\_ref\_CAC86167.1, 2xC2H2, Arabidopsis thaliana

MSNPACSNLFNNGCDHNSFNYSTSLSYIYNHSGSYYYSNNTNPNYINHTHTTSTSPNSPPLR  
 EALPLLSLSPIRHQEQDQHYFMDTHQISSSNFLDDPLVTVDLHLGLPNYGVGESIRSNIAP  
 DATTDEQDQDHDGRGVEVTVESHLDDDDHHGDLHRGHHYWIPTPSQILIGPTQFTCPLCFKT  
 FNRYNNMQMHMWHGHSQYRKGPESLRGTQPTGMLRLPCFCCAPGCKNNIDHPRAKPLKDFRT  
 LQTHYKRKHGSKPFACRMCGKAFVKGDWRTHEKNCCKLWYCSCGSDFKHKRSCLKDHVKAFC  
 NGHVP CGIDSFGGDHEDYYDAASDIEQ

**SEQ ID NO 45:** gi\_7798991\_ref\_AL355775.1\_region 7957 ... 8451, 2xC2H2, Arabidopsis thaliana

ATGGTTGCGAGAAGTGAGGAAGTTGAGATAGTGGAAGATACGGCGGCGAAATGTTTGATGTT  
 GTTATCAAGAGTTGGAGAATGCGGCGGAGGAGGAGAGAAACGAGTTTTCCGATGCAAGACTT  
 GTCTTAAAGAGTTTTCGTCGTTTTCAAGCTTTGGGAGGTCATCGTGCAAGCCACAAGAACTC  
 ATTAACAGTAGCGATCCATCACTTCTTGGATCCTTGTCTAACAAGAAAACCTAAAACGGCGAC  
 GTCTCATCCTTGTCCGATATGTGGCGTGGAGTTTCCGATGGGGCAAGCTCTTGGTGGTCACA  
 TGAGGAGACATAGGAGTGAGAAAGCCTCACCAGGCACGTTGGTTACACGTTCTTTTTTACCG  
 GAGACGACGACGGTGACGACTTTGAAAAAATCGAGTAGTGGAAGAGAGTGGCTTGTTTGGA  
 CTTAGATTCGATGGAGAGTTTAGTCAATTGGAAGTTGGAGTTGGGAAGAACGATTTCTTGA

**SEQ ID NO 46:** gi\_7798996\_ref\_CAB90935.1, 2xC2H2, Arabidopsis thaliana

MVARSEEVEIVEDTAAKCLMLLSRVGECGGGGEKRVFRCKTCLKEFSSFQALGGHRASHKKL  
 INSSDPSLLGSLSNKKTKTATSHPCPICGVEFPMGQALGGHMRRHRSEKASPGTLVTRSFLP  
 ETTTVTTLKKSSSGKRVACLDLDSMESLVNWKLELGRTIS

**SEQ ID NO 47:** gi\_9755794\_ref\_AL391143.1\_region 31730 ... 32938, 2xC2H2, Arabidopsis thaliana

ATGGAAGACGAACATCAAGATCTCCATAAACCCATTAATGGAGCTTTGCGAGACCTCAAGAT  
 TACTCGGTCACAGAAAGAAACAGAAAAGTCTACGAACCAACAGCAAGATGTTACTTGTACT  
 ATGGTCTAAGGGAAAACCTCGAAGAAGAAAACCCAGGAATCTCCGGAACCAATGAAGAAGATT  
 TTGTTTCGATGCGAAGAATGTGGAAAAGGGTTTCGGTACGAGAAATATTTTAAGAATCATCG

**FIGURE 3 (continued)**

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CTCGATGATGCATTTATCGCCGAACGAGAAGGTTTGTGAAGAATCCTTGATGACTCTGTCTC  
GTAGCCTTGGGTTTGTGAAGAAGAAGAAAAGATCAAGACTTGGTAGGTCTGGGAAGACTTTA  
TTTACTACGTTTCTTGAACCGAGTTCTATTTTTTGTGCGACTGATGAAGAATTAGAAGTGGC  
GGATTGTTTGATTCTATTGTCTAAGAGTGCTCCCAAGGTTGTAGACGAATTGAAAAGTCTTT  
CTGAGGCAGTACGTGTTACTCCTGAAACACCTGAAAGTAGCTATGATTTGGGTTGTTTGCTC  
AACAAAGAAACCGAGAAAAGGTGGTGAATTGGAATCTGGGGTTTTAAGTAATGAGCAAAGACT  
TATGGAAGAAGGGTTTAGTAGTTATGGAACATCGAAAGAACCAGCTAGCTTCTTGAGAGACG  
AAAACAGATTGGATCAGCAGAAACGGAGAAAAGATGGTGAATTTGAATCCGGACTTTTGAGT  
AATGAGCAAAGACTGCTAGAAGAAGAGATTACTACTCCTGTGACATTCAAAGGTCCAGCGAG  
TTCCTTGAGACACAAGTGTGCTTTGGATCGAAATGGAGGTGAATTTGGTCCTGAGTTTTTGA  
GTAATGAGCAAACACTGATGGAAGAAACATGGAAAGAACCAGTGAGTTTCTTAGAAGATAAG  
CATGAATTTGATCAGCGGAAAATGCGAGAAGCTGGCGACTTTGAATCTAGGTTTTACAGAAT  
TGAGCTTGGAGTAGGAGCTATGGAGTGTAATCTTTCAGATACTGATATGCTCACGCAATCTG  
ATAAGAAGAACGTTGAGCATCGATGCAGGTTGTGCAACAAGATATTCTCGTCTTATCAAGCT  
CTAGGGGGTCATCAGACGTTTCATCGGATGAGCAAATGTAAGAACAAGAAGAATGGCATAGA  
GGAATCAGTTGAACCCAGGATGACTCTGTGA

**SEQ ID NO 48:** gi\_9755803\_ref\_CAC01747.1, 2xC2H2, *Arabidopsis thaliana*

MEDEHQDLHKPINGALRDLKITRSQKETEKSTNQOQDVTCYYGLRENSKKKTQESPEPMKKI  
LFRCEECKGKGFYKYEKYNHRSMMHLSPNEKVCEESLMTLSRSLGFVKKKRSRLGRSGKTL  
FTTFLEPSSIIFDATDEELEVADCLILLSKSA PKVVDELKSLSEAVRVTPETPESSYDLGCLL  
NKKPRKGGELESGVLSNEQRLMEEGFSSYGTSKEPASFLRDENRLDQKRRKDGEFESGLLS  
NEQRLLEEEITTPVTFKGPASSLRHKCALDRNGGEFGPEFLSNEQTLMEETWKEPVSFLEDK  
HEFDQORKMREAGDFESRFYRIELGVGAMECTSSD TDMLTQSDKKNVEHRCRLCNKIESSYQA  
LGGHQTFHRMSKCKNKNKNGIEESVEPRMTL

**SEQ ID NO 49:** gi\_1418338\_ref\_X98678.1, 2xC2H2, *Arabidopsis thaliana*

CTTGTTAGTTCACCTCCACATAATAAACACCAAAGATTTCAATCTCTTCTCCATAATTTCGAA  
GTTTCTTGAATTGGGTTTGTCTTCTTGATTTGTTTCTTGAATTGGGTTTTGGTCTTCTTTTCT  
TACTATATTTGGATATGATGATGGGTCAAGATGAGGTTGGGAGTGATCAGACGCAAATCATA  
AAAGGGAAACGTACGAAGCGACAAAGATCGTCTTTCGACGTTTGTGGTGACGGCGGCGACAAC  
AGTGACTTCAACAAGTTCATCGGCCGGTGGAAGTGGAGGAGAAAGAGCTGTTTCAGATGAAT  
ACAACCTCGGCGGTTTCGTCTCCGGTGACTACTGATTGTACGCAAGAAGAAGAAGACATGGCG  
ATTTGTCTCATCATGTTAGCTCGTGGGACAGTTCTTCCATCGCCGGATCTCAAGAACTCGAG  
AAAAATTCATCAGAAGATTTTCGTGCGAGAATTCTAGTTTCTATGTGTACGAGTGTAACCGT  
GTAACCGGACGTTTTTCGTGCTTCCAAGCACTTGGTGGACACAGAGCGAGCCACAAGAAGCCG  
AGGACGTCGACTGAGGAAAAGACTAGACTACCCCTGACGCAACCCAAGTCTAGTGCATCAGA  
AGAAGGGCAAAACAGTCATTTCAAAGTTTCCGGCTCAGCCCTAGCTTCACAGGCAAGTAACA  
TCATCAACAAGGCAAACAAAGTACACGAGTGTTCCATCTGCGGTTCTGAGTTCACTTCCGGG  
CAAGCTCTCGGTGGTCACATGAGGCGGCACAGGACAGCCGTAACCACGATTAGCCCCGTTGC  
AGCCACCGCAGAAGTAAGCAGAAACAGTACAGAGGAAGAGATTGAGATCAATATAGGCCGTT  
CGATGGAACAGCAGAGGAAATATCTACCGTTGGATCTTAATCTACCAGCACCAGGAGATGAT  
CTAAGAGAGTCCAAGTTTCAAGGGATAGTATTCTCAGCAACACCAGCGTTAATAGATTGTCA  
TTACTAGTTGTTTTTTTTTACTACATAATATGATGAAATATTTGTGAATTCTTCTTACTTACT  
ACTATATTGTTGATCAAAAAAAAAAAAAAAAAA

**FIGURE 3 (continued)**

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**SEQ ID NO 50:** gi\_1418339\_ref\_CAA67236.1, 2xC2H2, Arabidopsis thaliana

MGQDEVGSDQTQIIKGKRTKRQRSSSTFVVTAATTVTSTSSSAGGSGGERAVSDEYN SAVSS  
PVTTDCTQEEEDMAICLIMLARGTVLPSPDLKNSRKIHQKISSENSSFYVYECKTCNRTFSS  
FQALGGHRASHKKPRTSTEEKTRLPLTQPKSSASEEGQNSHFKVSGSALASQASNIINKANK  
VHECSICGSEFTSGQALGGHMRRHRTAVTTISPVAATAEVSRNSTEEIEINIGRSMEQQRK  
YLPLDLNLPAPGDDLRESKFQGIVFSATPALIDCHY

**FIGURE 3 (continued)**

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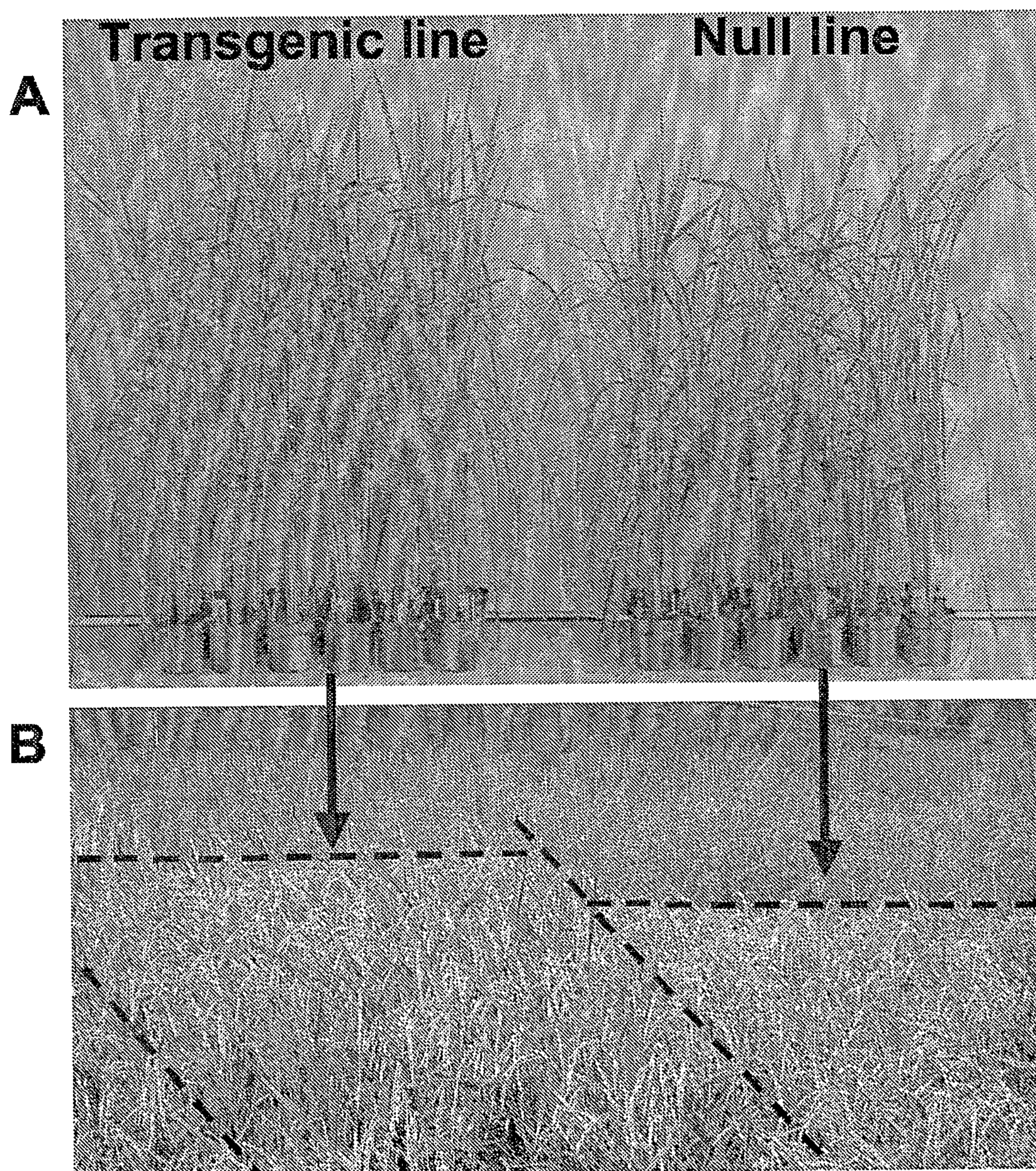


FIGURE 4

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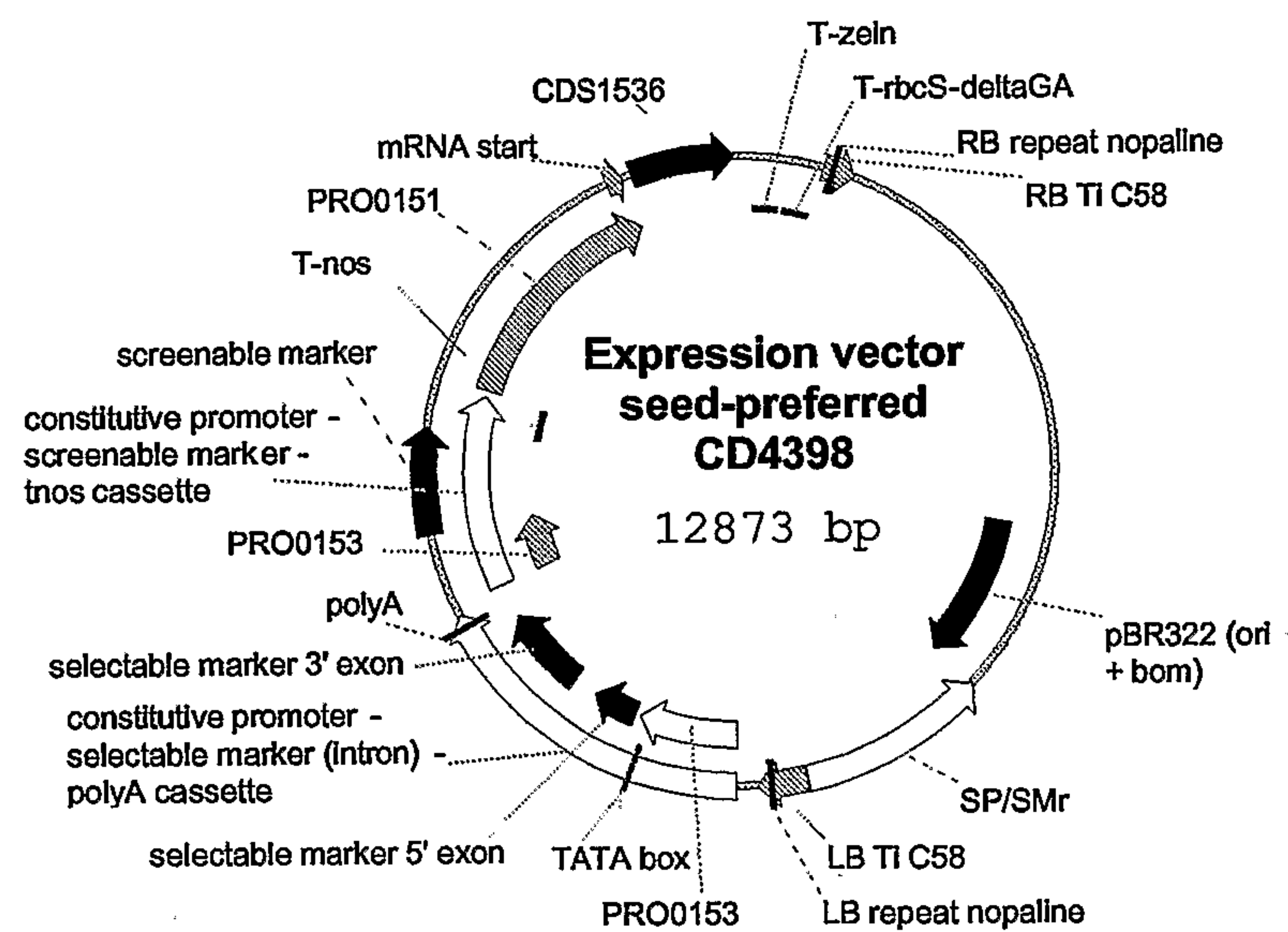


FIGURE 5