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(54) Title: INCREASED WAX PRODUCTION IN PLANTS

(57) Abstract: The invention provides genes that activate wax biosynthesis in plants. Also provided are constructs comprising these sequences, plants transformed therewith and methods of use thereof. The invention allows the modification of plants for increased wax production, particularly in leaves. The inventors have demonstrated increased drought tolerance in connection with increased leaf wax production engineered in plants.



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INCREASED WAX PRODUCTION IN PLANTS

CROSS REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U. S. Provisional Patent Application Serial No. 60/619,801, filed October 18, 2004, the entire contents of which are herein
5 specifically incorporated by reference.

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates generally to the field of molecular biology. More specifically, the invention relates to plant genes that activate wax biosynthesis
10 and methods of use thereof.

2. Description of the Related Art

A cuticle layer covers most primary aerial organs of vascular plants and forms the contact zone between the plant and the environment (Kerstiens, 1996). Cuticular waxes are the major constituents of plant cuticle and play an important role in
15 protecting aerial organs from damage caused by environmental stresses. Cuticular waxes are complex mixtures of very long chain fatty acids, alkanes, primary and/or secondary alcohols, aldehydes, ketones, esters, triterpenes, sterols, and flavonoids. Wax compounds can be embedded within the cutin polymer framework and form intracuticular wax. In many plants, however, more waxes are loaded outside of the
20 cuticle membrane and form an epicuticular wax layer that give the plant surface a glaucous or grey appearance (Jenks and Ashworth, 1999; Post-Beittenmiller, 1996).

Plant cuticular wax biosynthesis and its loading to the plant surface is a complicated but actively regulated process (Broun *et al.*, 2004; Jenks *et al.*, 2002). Mutant analysis has contributed to the identification of some of the components and
25 genes involved in wax deposition. Mutants with reduced wax accumulation or altered wax composition are in general characterized by a bright green phenotype which can be detected visually (Aarts *et al.*, 1995). In *Arabidopsis*, 120 cuticular wax mutants representing a total of 31 recessive mutant loci have been identified, although dominant wax gene mutations have not been reported (Jenks *et al.*, 2002). Wax-

deficient mutants have also been identified in other species, including maize, sorghum, barley and rape (Kunst and Samuels, 2003).

Studies on the *eceriferum* (*cer*) mutants and T-DNA insertional mutants in *Arabidopsis* and *glossy* (*gl*) mutants in maize led to the identification and isolation of a number of wax-related genes. To date, 12 genes associated with wax production or regulation have been identified by molecular-genetic approaches. Among these genes, *CER1*, *CER2*, *CER6/CUT1*, 3-ketoacyl-CoA synthase (*KCSI*), *FIDDLEHEAD* (*FDH*), *GL1*, *GL8* and *WAX2*, may encode metabolic enzymes or be involved in transport of wax compounds (Aarts *et al.*, 1995; Chen *et al.*, 2003; Fiebig *et al.*, 2000; Hansen *et al.*, 1997; Millar *et al.*, 1999; Negruk *et al.*, 1996; Pruitt *et al.*, 2000; St-Pierre *et al.*, 1998; Todd *et al.*, 1999; Xia *et al.*, 1996, 1997; Xu *et al.*, 1997), while *CER3*, *GL2*, *GL15* and *WIN1/SHINE1* appear to encode regulatory proteins (Aharoni *et al.*, 2004; Broun *et al.*, 2004; Hannoufa *et al.*, 1996; Moose and Sisco, 1996; Tacke *et al.*, 1995). Mutations in most of these genes showed altered wax accumulation (Jenks *et al.*, 2002), cosuppression of some of the genes resulted in waxless stems in *Arabidopsis* (Millar *et al.*, 1999; Todd *et al.*, 1999), and overexpression of some of the genes in *Arabidopsis* mutant background complemented corresponding mutant phenotypes (Fiebig *et al.*, 2000; Hannoufa *et al.*, 1996). However, only limited information is available on the effects of overexpression of these genes in a wild-type background.

Overexpression of the condensing enzyme gene *CER6/CUT1* under the control of CaMV35S promoter failed to promote wax deposition (Millar *et al.*, 1999), while under the control of epidermis-specific *CER6* promoter, *CER6/CUT1* overexpression led to increased wax load in stems of *Arabidopsis* (Hooker *et al.*, 2002). The only report of increased wax accumulation in leaf tissues of *Arabidopsis* was by the overexpression of a transcriptional activator (Broun *et al.*, 2004; Aharoni *et al.*, 2004).

Transcription factors are regulatory proteins that modulate gene expression through sequence-specific DNA binding and/or protein-protein interactions. They are capable of activating or repressing transcription of target genes as switches of the regulatory cascade. Most of the transcription factors are grouped into gene families according to their well-conserved DNA-binding domains.

APETALA 2 (AP2)/Ethylene-responsive element binding factors (ERF or EREBP) domain-containing transcription factor is a group of transcriptional regulators that are specifically found in plants (Okamuro *et al.*, 1997; Riechmann *et al.*, 2000). The AP2 domains in these proteins play a major role in specific promoter DNA sequence/element binding and transcriptional activation (Okamuro *et al.*, 1997; Sakuma *et al.*, 2002). This gene family has been further grouped into three major subfamilies and some smaller groups based on their functions and sequence similarities (Dubouzet *et al.*, 2003; Riechmann *et al.*, 2000). The AP2 subfamily genes (containing double AP2 domains) were thought to developmentally control flowering time in plants (Jofuku *et al.*, 1994; Schultz and Haughn, 1991). The genes in ERF subfamily have been found to be involved in plant response to pathogen infection and mediate disease resistance (Chakravarthy *et al.*, 2003; Gutterson and Reuber, 2004; Onate-Sanchez and Singh, 2002).

Recently, a new group of AP2 domain-containing transcription factors, dehydration-response element binding protein (DREB)/C-repeat binding factor (CBF), have been identified and characterized (Novillo *et al.*, 2004; Shinzaki *et al.*, 2003; Thomashow, 1999). They are mainly involved in the regulation of abiotic stress inducible genes; overexpression of some members from this subfamily in transgenic *Arabidopsis* induced a host of genes and conferred stress tolerance (Gilmour *et al.*, 2000; Haake *et al.*, 2002; Jaglo-Ottosen *et al.*, 1998; Kasuga *et al.*, 1999; Stockinger *et al.*, 1997).

While the foregoing studies have provided a further understanding of the metabolism of wax in plants, the prior art has generally failed to provide wax biosynthesis activating genes, for example, that would yield drought tolerance. The identification of such genes would allow the creation of novel plants with improved phenotypes and methods for use thereof. There is, therefore, a great need in the art for the identification of plant genes that activate wax biosynthesis and methods for their use.

SUMMARY OF THE INVENTION

In one aspect, the invention provides an isolated nucleic acid sequence that activates wax biosynthesis. In certain embodiments, the nucleic acid sequence is operably linked to a heterologous promoter. In one embodiment, the nucleic acid

sequence is further defined as selected from the group consisting of: (a) a nucleic acid sequence encoding the polypeptide of SEQ ID NO:1; (b) a nucleic acid sequence comprising the sequence of SEQ ID NO:2; (c) a nucleic acid sequence hybridizing to SEQ ID NO:2 under conditions of 5X SSC, 50% formamide and 42°C; and (d) a
 5 nucleic acid sequence encoding a polypeptide with at least 65% amino acid identity to SEQ ID NO:1.

In another aspect, the invention provides an isolated nucleic acid sequence that activates wax biosynthesis. In certain embodiments, the nucleic acid sequence is operably linked to a heterologous promoter. In one embodiment, the nucleic acid is
 10 selected from the group consisting of: (a) a nucleic acid sequence encoding the polypeptide of SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID
 15 NO:43, and/or SEQ ID NO:45; (b) a nucleic acid sequence comprising the sequence of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, and/or SEQ ID NO:46; (c) a nucleic acid sequence hybridizing to SEQ ID NO:6, SEQ
 20 ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, and/or SEQ ID NO:46
 25 under conditions of 5X SSC, 50% formamide and 42°C; and (d) a nucleic acid sequence encoding a polypeptide with at least 65% amino acid identity to SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID
 30 NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, and/or SEQ ID NO:45.

In still yet another aspect, the invention provides a recombinant vector comprising an isolated nucleic acid sequence provided herein. The recombinant

vector may comprise at least one additional sequence chosen from the group consisting of: a regulatory sequence, a selectable marker, a leader sequence and a terminator. The additional sequence may be a heterologous sequence. The promoter may be an epidermis specific promoter, developmentally-regulated, organelle-specific, inducible, tissue-specific, constitutive, cell-specific, seed-specific or germination-specific promoter. Such an epidermis-specific promoter directs expression preferentially to the plant surface, for example, to leaves or the leaf surface. The promoter may also be drought-inducible promoter. The recombinant vector may be defined as an isolated expression cassette.

Yet another aspect of the invention is an isolated polypeptide having at least 65% amino acid identity to the amino acid sequence of SEQ ID NO:1 or a fragment thereof having wax biosynthesis activity. Also provided is an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment thereof having wax biosynthesis activity.

In still yet another aspect, the invention provides a transgenic plant transformed with a selected DNA comprising a nucleic acid sequence provided by the invention. The plant may be a dicotyledonous or monocotyledonous plant, and may be an R0 transgenic plant and/or further defined as a progeny plant of any generation of an R0 transgenic plant that has inherited the selected DNA from the R0 transgenic plant. Seed of such a transgenic plant comprising the selected DNA are also provided, as are cells of such a plant. Such a cell may express a protein encoded by the selected DNA and may have inherited the selected DNA from a progenitor of the cell and/or been transformed with the selected DNA. The host cell may be a plant cell.

In still yet another aspect, a method of increasing drought tolerance in a plant is provided comprising increasing leaf wax production in the plant. The method may comprise altering the wax content or composition of a plant by introducing into the plant a recombinant vector provided herein, wherein the nucleic acid is expressed in the plant. The coding sequence may be operably linked to a heterologous promoter functional in the plant and may be in sense orientation. Wax biosynthesis may be up-regulated in the plant and the plant may exhibit increased tolerance to water deficit

relative to a plant of the same genotype lacking the coding sequence. Introducing the coding sequence may comprise plant breeding and/or genetic transformation.

In still yet another aspect, the invention provides a method of making food for human or animal consumption comprising: (a) obtaining the plant of claim 13; (b) growing the plant under plant growth conditions to produce plant tissue from the plant; and (c) preparing food for human or animal consumption from the plant tissue. Preparing food may comprise harvesting the plant tissue. The food may be starch, protein, meal, flour or grain.

The use of the word “a” or “an” when used in conjunction with the term “comprising” in the claims and/or the specification may mean “one,” but it is also consistent with the meaning of “one or more,” “at least one,” and “one or more than one.”

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein:

FIG. 1. Alignment of *Medicago truncatula* WXP1, Mt77128 (WXP2 thereafter) and 11 other AP2 domain-containing transcription factors that either have been characterized or are related. The alignment consists of the following predicted protein sequences: *Arabidopsis* WIN1/SHINE1 and At5g11190 (Aharoni *et al.*, 2004; Broun *et al.*, 2004), *Arabidopsis* ERF1 (Fujimoto *et al.*, 2000), tomato ERF-like gene LePti4 (Gu *et al.*, 2002), tobacco ERF-like gene NtERF1 (Ohme-Takagi and Shinshi, 1995), WXP1 and its paralog, Mt77128 (WXP2, deduced amino acid sequence based

on TIGR sequence TC77128), most similar *WXP1* sequence found in *Arabidopsis* AtRAP2.4 (At1g78080), and characterized sequences *Arabidopsis* CBF1 (Stockinger *et al.*, 1997), CBF2 (Novillo *et al.*, 2004), CBF3 (Gilmour *et al.*, 2000), and CBF4 (Haake *et al.*, 2002). Sequences were aligned with CLUSTAL W using default parameters. Identical and similar amino acid residues are shown on black and gray background, respectively. Gaps required for optimal alignment are indicated by dashes. AP2 domains aligned with the Pfam seed sequences (<http://pfam.wustl.edu/cgi-bin/getdesc?name=AP2>) are indicated by stars under the sequences.

FIG. 2. Phylogenetic analysis of *WXP1* and 11 other AP2 domain-containing transcription factors that either have been characterized or are related. The root tree was constructed using CLUSTAL W and showed in TreeView.

FIG. 3. Changes of *WXP1* transcript level in *Medicago truncatula* in response to drought, cold and ABA treatment. CT, control; RC, recovered 24 hrs after transferring the plants back to normal growth conditions; d, days after watering was ceased; h, hours after treatment was applied.

FIG. 4. Phenotype of *WXP1* transgenic alfalfa lines after 60 days of growth in the greenhouse. VCTR, empty vector control; T, transgenic lines overexpressing *WXP1*.

FIG. 5. Leaf surfaces of transgenic alfalfa plants overexpressing *WXP1*. (A) Adaxial surface of transgenic and control plants. (B) Adaxial and abaxial sides of the same trifoliolate from control plant. (C) Adaxial and abaxial sides of the same trifoliolate from transgenic line 47. VCTR, empty vector control; T, transgenic lines overexpressing *WXP1*.

FIG. 6. Epicuticular wax crystallization patterns on adaxial and abaxial leaf surfaces of transgenic and control alfalfa plants viewed by scanning electron microscope. (A,C,E,G) empty vector control (VCTR). (B, D, F, H) Transgenic line 47. Images were taken at $\times 8,000$ magnification.

FIG. 7. Cuticular wax accumulation in transgenic and control alfalfa plants. (A) Wax constituents in leaves. (B) Wax constituents in stems. (C) Total wax accumulated on leaves and stems. Acids, long-chain fatty acids; Alk, Alkanes; Alc, primary alcohols; Ald, aldehydes; Terp, terpenoids; Unk, Unknown compounds; WT, wild type; VCTR, empty vector control; T, transgenic lines overexpressing *WXP1*.

FIG. 8. Leaf cuticular wax profile of transgenic alfalfa plants overexpressing *WXP1*.

FIG. 9. Chlorophyll leaching from leaves and stems of transgenic and control alfalfa plants. VCTR, empty vector control; T-47, transgenic line overexpressing *WXP1*.

FIG. 10. Effects of drought stress on transgenic and control alfalfa plants. (A) Three days of no-watering in growth chamber. Control plants (left three plants) wilted while transgenics (right three plants) still look normal. (B) After 10 days of no-watering, normal watering scheme was resumed, and the dried plants started recovering. Transgenic plants (right three) recovered much better and quicker than the control plants (left three).

FIG. 11. Growth and development of transgenic alfalfa plants overexpressing *WXP1*. (A) Days to flowering after transplanting. (B) Plant height. (C) Plant fresh weight. (D) Plant dry weight. WT, wild type; VCTR, empty vector control.

FIG. 12. Scanning electron microscopy of stem surfaces of top-1st and top-2nd internodes of vector control (A, C) and transgenic line T-47 (B, D). Images were taken at $\times 8,000$ magnification.

FIG. 13. Stem cuticular wax profile of transgenic alfalfa plants overexpressing *WXP1*.

FIG. 14. Water loss rate ($\pm$ SD) of detached leaves from transgenic and control alfalfa plants. (A) Top-2nd trifoliate. (B) Top-3rd trifoliate. VCTR, empty vector control. T-47, transgenic line overexpressing *WXP1*.

FIG. 15. Northern hybridization analysis of the expression of wax related genes in *WXP1* transgenic alfalfa plants. FAE, fatty acid elongase; CYP450, cytochrome P450 monooxygenase; LTP, lipid transfer protein; G3PD, glycerol-3-phosphate dehydrogenase; KAR, β -keto acyl reductase; ECH/ECI, Enoyl-CoA hydratase/isomerase. VCTR, empty vector control; T, transgenic lines overexpressing *WXP1*.

FIG. 16. Morphological changes induced by transgenic expression of *WXP1* and *WXP2* in *Arabidopsis*. A, 4-week-old vector control plants; B, C, D, 4-week-old plants from line 4, 55, and 57 overexpressing *WXP1*; E, 6-week-old plants of vector

control (left) and transgenic plants from line 4 (middle) and line 14 (right) overexpressing *WXP2*.

FIG. 17. Whole plant drought tolerance of 35S::*WXP1* and 35S::*WXP2* transgenic *Arabidopsis* plants. (A) Phenotype and survival rate (%) of different transgenic lines. (B) Transgenic lines that harbored either *WXP1* or *WXP2* from different replicates.

FIG. 18. Assay for fresh weight loss of detached leaves (FWLDL) for transgenic *Arabidopsis* plants overexpressing *WXP1* (A) and *WXP2* (B).

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DETAILED DESCRIPTION OF THE INVENTION

The invention overcomes the limitations of the prior art by providing coding sequences that activate wax biosynthesis. As very limited information is available on plant genes conferring increased wax production, the invention represents a major advance and allows the creation of transgenic plants modified for increased plant wax accumulation. By introduction of one or more heterologous wax biosynthesis activating nucleic acid sequences into a plant, wax biosynthesis and environmental stress tolerance may be up-regulated in accordance with the invention.

Drought tolerance in particular is an important target for improvement in plants and for the implementation of sustainable farming techniques. Since cuticular waxes play a pivotal role in limiting transpirational water loss across the plant surface, genetic engineering of plant waxes represents a significant advance for increasing tolerance to environmental stresses in crops of agronomic importance (Millar *et al.*, 1999; Vogg *et al.*, 2004).

The inventors report the characterization and transgenic expression of novel genes that activate wax production. Overexpression of a gene designated *WXP1* under the control of CaMV35S promoter in particular was shown to increase cuticular wax loading on leaf surfaces of transgenic alfalfa. Furthermore, it was demonstrated that the transgenic plants had reduced water loss, enhanced drought tolerance and reduced chlorophyll leaching. The plants with increased cuticular waxes showed delayed wilting after watering was ceased and better recovery when re-watered. To

the inventors' knowledge, this is the first report of improved drought tolerance by genetic manipulation of wax biosynthesis in an agronomically important species.

Scanning electron microscopy on transgenic alfalfa plant tissues revealed earlier accumulation of wax crystals on the adaxial surface of newly expanded leaves and higher densities of wax crystalline structures on both the adaxial and abaxial surfaces of mature leaves. GC-MS analysis revealed that total leaf wax accumulation per surface area increased 29.6 – 37.7% in the transgenic alfalfa lines, and the increase was mainly contributed by C30 primary alcohol.

It was further demonstrated that overexpression of *WXP1* and a second gene designated *WXP2* under the control of a strong constitutive promoter led increased cuticular wax loading on leaf surfaces of transgenic *Arabidopsis*. Total leaf wax content per gram of fresh weight in 3-week-old transgenic *Arabidopsis* plants expressing *WXP1* and *WXP2* increased by 32.36% and 16.96%, respectively. In 6-week-old transgenic *Arabidopsis* plants expressing *WXP1* and *WXP2*, leaf wax accumulation per surface area averagely increased by 27.51% and 23.61%, respectively.

I. Plant Transformation Constructs and Nucleic Acids

In one aspect of the invention, plant transformation vectors comprising one or more coding sequences that activate wax biosynthesis are provided. Two exemplary coding sequences for use with the invention are the *Medicago truncatula* wax biosynthesis activating sequences of SEQ ID NO:2 and SEQ ID NO:6. Such coding sequences may encode polypeptides having the amino acid sequences of SEQ ID NO:1 and SEQ ID NO:5. Sequences that hybridize to this coding sequence under stringent conditions are also provided by the invention. An example of such conditions include a wash of 5X SSC, 50% formamide and 42°C for 10 minutes. It will be understood by those of skill in the art that stringency conditions may be increased by increasing temperature, such as to about 60°C or decreasing salt, such as to about 1X SSC, or may be decreased by increasing salt, for example to about 10X SSC, or decreasing temperature, such as to about 25 °C.

Further provided by the invention are polypeptides encoded by coding sequences that activate wax biosynthesis. In specific embodiments, such polypeptides

may be defined as having at least 45%, 60%, 70, 80%, 90%, 95%, 98% or 99% sequence identity to a polypeptide sequence of SEQ ID NO:1 and SEQ ID NO:5. In further embodiments, the invention provides nucleic acids encoding these polypeptides.

5 Still further contemplated for use with the invention for modification of plant wax biosynthesis are additional sequences from species including *Medicago truncatula* (WXP2; SEQ ID NO:5 and SEQ ID NO:6), *Arabidopsis thaliana* (AtTC261112; SEQ ID NO:7 and SEQ ID NO:8) (AtTC262267; SEQ ID NO:9 and SEQ ID NO:10), cotton (CTC28125; SEQ ID NO:11 and SEQ ID NO:12)
 10 (CTC37888; SEQ ID NO:13 and SEQ ID NO:14) (CTC38239; SEQ ID NO:15 and SEQ ID NO:16), soybean (GmTC215663; SEQ ID NO:17 and SEQ ID NO:18) (GmTC215664; SEQ ID NO:19 and SEQ ID NO:20) (GmTC225042; SEQ ID NO:21 and SEQ ID NO:22) (GmTC225047; SEQ ID NO:23 and SEQ ID NO:24) (GmTC228532; SEQ ID NO:25 and SEQ ID NO:26), tomato (LeTC137279; SEQ ID
 15 NO:27 and SEQ ID NO:28) (LeTC148534; SEQ ID NO:29 and SEQ ID NO:30), *Lotus japonicus* (Partial, LjTC16026; SEQ ID NO:31 and SEQ ID NO:32), lettuce (LsTC8978; SEQ ID NO:33 and SEQ ID NO:34), poplar (PpITC2364; SEQ ID NO:35 and SEQ ID NO:36), sorghum (SbTC103215; SEQ ID NO:37 and SEQ ID NO:38), potato (StTC102572; SEQ ID NO:39 and SEQ ID NO:40) (StTC104330;
 20 SEQ ID NO:41 and SEQ ID NO:42), grape (VvTC45892; SEQ ID NO:43 and SEQ ID NO:44) and maize (ZmTC270551; SEQ ID NO:45 and SEQ ID NO:46).

In certain embodiments, the invention therefore provides an isolated polypeptide comprising the amino acid sequence of SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17,
 25 SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, and/or SEQ ID NO:45 and biologically active fragments thereof. Also provided polypeptides having at least about 45, 55, 65, 70, 80, 90, 95 98 and about 99% amino acid identity to these
 30 sequences are provided. Still further provided are nucleic acids encoding any of these polypeptides.

In one embodiment of the invention, such a nucleic acid sequence may comprise the nucleic acid sequence of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10,

SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, and/or SEQ ID NO:46. The invention also provides
5 nucleic acids hybridizing to these nucleic acid sequences under wash conditions of 5X SSC, 50% formamide and 42°C for 10 minutes.

Nucleic acids provided by the invention include those encoding active wax biosynthesis fragments. Those of skill in the art will immediately understand in view of the disclosure that such fragments may be prepared by placing fragments of wax
10 biosynthesis activating sequences in frame in an appropriate expression vector, for example, comprising a plant promoter. Using the assays described in the working examples, wax biosynthesis activity can be confirmed for any given fragment. Fragments of nucleic acids may be prepared according to any of the well known techniques including partial or complete restriction digests and manual shearing.

Sequences provided by the invention may be defined as encoding a
15 transcription factor that increases wax biosynthesis. In certain further aspects of the invention, a plant wax biosynthesis gene may be characterized as from a monocotyledonous or dicotyledonous plant. Coding sequences may be provided operably linked to a heterologous promoter, in either sense or antisense orientation.
20 Expression constructs are also provided comprising these sequences, including antisense oligonucleotides thereof, as are plants and plant cells transformed with the sequences.

The construction of vectors which may be employed in conjunction with plant transformation techniques using these or other sequences according to the invention
25 will be known to those of skill of the art in light of the present disclosure (see, for example, Sambrook *et al.*, 1989; Gelvin *et al.*, 1990). The techniques of the current invention are thus not limited to any particular nucleic acid sequences.

One important use of the sequences provided by the invention will be in the alteration of plant phenotypes by genetic transformation with coding sequences that
30 activate wax biosynthesis. The wax biosynthesis activating sequence may be provided with other sequences and may be in sense or antisense orientation with respect to a promoter sequence. Where an expressible coding region that is not

necessarily a marker coding region is employed in combination with a marker coding region, one may employ the separate coding regions on either the same or different DNA segments for transformation. In the latter case, the different vectors are delivered concurrently to recipient cells to maximize cotransformation.

5 The choice of any additional elements used in conjunction with a wax biosynthesis activating sequences will often depend on the purpose of the transformation. One of the major purposes of transformation of crop plants is to add commercially desirable, agronomically important traits to the plant, as described above.

10 Vectors used for plant transformation may include, for example, plasmids, cosmids, YACs (yeast artificial chromosomes), BACs (bacterial artificial chromosomes) or any other suitable cloning system, as well as fragments of DNA therefrom. Thus when the term "vector" or "expression vector" is used, all of the foregoing types of vectors, as well as nucleic acid sequences isolated therefrom, are
15 included. It is contemplated that utilization of cloning systems with large insert capacities will allow introduction of large DNA sequences comprising more than one selected gene. In accordance with the invention, this could be used to introduce genes corresponding to an entire biosynthetic pathway into a plant. Introduction of such sequences may be facilitated by use of bacterial or yeast artificial chromosomes
20 (BACs or YACs, respectively), or even plant artificial chromosomes. For example, the use of BACs for *Agrobacterium*-mediated transformation was disclosed by Hamilton *et al.* (1996).

 Particularly useful for transformation are expression cassettes which have been isolated from such vectors. DNA segments used for transforming plant cells
25 will, of course, generally comprise coding sequence which one desires to introduce into and have expressed in the host cells. These DNA segments can further include structures such as promoters, enhancers, polylinkers, or even regulatory genes as desired. The DNA segment or gene chosen for cellular introduction will often encode a protein which will be expressed in the resultant recombinant cells resulting in a
30 screenable or selectable trait and/or which will impart an improved phenotype to the resulting transgenic plant. Preferred components that may be included with plant transformation vectors are as follows.

A. Regulatory Elements

Exemplary promoters for expression of a nucleic acid sequence in plants include the CaMV 35S promoter (Odell *et al.*, 1985), CaMV 19S (Lawton *et al.*, 1987), *nos* (Ebert *et al.*, 1987), *Adh* (Walker *et al.*, 1987), sucrose synthase (Yang and Russell, 1990), α -tubulin, actin (Wang *et al.*, 1992), *cab* (Sullivan *et al.*, 1989), PEPCase (Hudspeth and Grula, 1989) or R gene complex associated promoters (Chandler *et al.*, 1989). Tissue specific promoters such as root cell promoters (Conkling *et al.*, 1990) and tissue specific enhancers (Fromm *et al.*, 1986) are also contemplated to be useful, as are inducible promoters such as ABA- and turgor-inducible promoters. In one embodiment of the invention, the native promoter of a wax biosynthesis activating sequence is used. In certain aspects of the invention, it may be beneficial to use an epidermis-specific promoter or a promoter inducible by environmental stress, such as water deficit.

The DNA sequence between the transcription initiation site and the start of the coding sequence, *i.e.*, the untranslated leader sequence, can also influence gene expression. One may thus wish to employ a particular leader sequence with a transformation construct of the invention. Preferred leader sequences are contemplated to include those which comprise sequences predicted to direct optimum expression of the attached gene, *i.e.*, to include a preferred consensus leader sequence which may increase or maintain mRNA stability and prevent inappropriate initiation of translation. The choice of such sequences will be known to those of skill in the art in light of the present disclosure. Sequences that are derived from genes that are highly expressed in plants will typically be preferred.

It is envisioned that wax biosynthesis activating sequences may be introduced under the control of novel promoters or enhancers, *etc.*, or homologous or tissue specific promoters or control elements. Vectors for use in tissue-specific targeting of genes in transgenic plants will typically include tissue-specific promoters and may also include other tissue-specific control elements such as enhancer sequences. Promoters which direct specific or enhanced expression in certain plant tissues will be known to those of skill in the art in light of the present disclosure. These include, for example, the *rbcS* promoter, specific for green tissue; the *ocs*, *nos* and *mas* promoters which have higher activity in roots or wounded leaf tissue.

B. Terminators

Transformation constructs prepared in accordance with the invention will typically include a 3' end DNA sequence that acts as a signal to terminate transcription and allow for the poly-adenylation of the mRNA produced by coding sequences operably linked to a promoter. Alternatively, a heterologous 3' end may enhance the expression of sense or antisense wax biosynthesis coding sequences. Examples of terminators that are deemed to be useful in this context include those from the nopaline synthase gene of *Agrobacterium tumefaciens* (nos 3' end) (Bevan *et al.*, 1983), the terminator for the T7 transcript from the octopine synthase gene of *Agrobacterium tumefaciens*, and the 3' end of the protease inhibitor I or II genes from potato or tomato. Regulatory elements such as an Adh intron (Callis *et al.*, 1987), sucrose synthase intron (Vasil *et al.*, 1989) or TMV omega element (Gallie *et al.*, 1989), may further be included where desired.

C. Transit or Signal Peptides

Sequences that are joined to the coding sequence of an expressed gene, which are removed post-translationally from the initial translation product and which facilitate the transport of the protein into or through intracellular or extracellular membranes, are termed transit (usually into vacuoles, vesicles, plastids and other intracellular organelles) and signal sequences (usually to the endoplasmic reticulum, golgi apparatus and outside of the cellular membrane). By facilitating the transport of the protein into compartments inside and outside the cell, these sequences may increase the accumulation of gene product protecting them from proteolytic degradation. These sequences also allow for additional mRNA sequences from highly expressed genes to be attached to the coding sequence of the genes. Since mRNA being translated by ribosomes is more stable than naked mRNA, the presence of translatable mRNA in front of the gene may increase the overall stability of the mRNA transcript from the gene and thereby increase synthesis of the gene product. Since transit and signal sequences are usually post-translationally removed from the initial translation product, the use of these sequences allows for the addition of extra translated sequences that may not appear on the final polypeptide. It further is contemplated that targeting of certain proteins may be desirable in order to enhance the stability of the protein (U.S. Patent No. 5,545,818, incorporated herein by reference in its entirety).

Additionally, vectors may be constructed and employed in the intracellular targeting of a specific gene product within the cells of a transgenic plant or in directing a protein to the extracellular environment. This generally will be achieved by joining a DNA sequence encoding a transit or signal peptide sequence to the coding sequence of a particular gene. The resultant transit, or signal, peptide will transport the protein to a particular intracellular, or extracellular destination, respectively, and will then be post-translationally removed.

D. Marker Genes

By employing a selectable or screenable marker protein, one can provide or enhance the ability to identify transformants. "Marker genes" are genes that impart a distinct phenotype to cells expressing the marker protein and thus allow such transformed cells to be distinguished from cells that do not have the marker. Such genes may encode either a selectable or screenable marker, depending on whether the marker confers a trait which one can "select" for by chemical means, *i.e.*, through the use of a selective agent (*e.g.*, a herbicide, antibiotic, or the like), or whether it is simply a trait that one can identify through observation or testing, *i.e.*, by "screening" (*e.g.*, the green fluorescent protein). Of course, many examples of suitable marker proteins are known to the art and can be employed in the practice of the invention.

Included within the terms selectable or screenable markers also are genes which encode a "secretable marker" whose secretion can be detected as a means of identifying or selecting for transformed cells. Examples include markers which are secretable antigens that can be identified by antibody interaction, or even secretable enzymes which can be detected by their catalytic activity.

Many selectable marker coding regions are known and could be used with the present invention including, but not limited to, *neo* (Potrykus *et al.*, 1985), which provides kanamycin resistance and can be selected for using kanamycin, G418, paromomycin, *etc.*; *bar*, which confers bialaphos or phosphinothricin resistance; a mutant EPSP synthase protein (Hinchee *et al.*, 1988) conferring glyphosate resistance; a nitrilase such as *bxn* from *Klebsiella ozaenae* which confers resistance to bromoxynil (Stalker *et al.*, 1988); a mutant acetolactate synthase (ALS) which confers resistance to imidazolinone, sulfonylurea or other ALS inhibiting chemicals (European Patent Application 154, 204, 1985); a methotrexate resistant DHFR

(Thillet *et al.*, 1988), a dalapon dehalogenase that confers resistance to the herbicide dalapon; or a mutated anthranilate synthase that confers resistance to 5-methyl tryptophan.

Screenable markers that may be employed include a β -glucuronidase (GUS) or
 5 *uidA* gene which encodes an enzyme for which various chromogenic substrates are
 known; an R-locus gene, which encodes a product that regulates the production of
 anthocyanin pigments (red color) in plant tissues (Dellaporta *et al.*, 1988); a
 β -lactamase gene (Sutcliffe, 1978), which encodes an enzyme for which various
 chromogenic substrates are known (*e.g.*, PADAC, a chromogenic cephalosporin); a
 10 *xyIE* gene (Zukowsky *et al.*, 1983) which encodes a catechol dioxygenase that can
 convert chromogenic catechols; an α -amylase gene (Ikuta *et al.*, 1990); a tyrosinase
 gene (Katz *et al.*, 1983) which encodes an enzyme capable of oxidizing tyrosine to
 DOPA and dopaquinone which in turn condenses to form the easily-detectable
 compound melanin; a β -galactosidase gene, which encodes an enzyme for which there
 15 are chromogenic substrates; a luciferase (*lux*) gene (Ow *et al.*, 1986), which allows for
 bioluminescence detection; or a gene encoding for green fluorescent protein (Sheen *et al.*, 1995; Haseloff *et al.*, 1997; Reichel *et al.*, 1996; Tian *et al.*, 1997; WO 97/41228).

II. Methods for Genetic Transformation

20 Suitable methods for transformation of plant or other cells for use with the
 current invention are believed to include virtually any method by which DNA can be
 introduced into a cell, such as by direct delivery of DNA such as by PEG-mediated
 transformation of protoplasts (Omirulleh *et al.*, 1993), by desiccation/inhibition-
 mediated DNA uptake (Potrykus *et al.*, 1985), by electroporation (U.S. Patent No.
 25 5,384,253, specifically incorporated herein by reference in its entirety), by agitation
 with silicon carbide fibers (Kaeppeler *et al.*, 1990; U.S. Patent No. 5,302,523,
 specifically incorporated herein by reference in its entirety; and U.S. Patent No.
 5,464,765, specifically incorporated herein by reference in its entirety), by
Agrobacterium-mediated transformation (U.S. Patent No. 5,591,616 and U.S. Patent
 30 No. 5,563,055; both specifically incorporated herein by reference) and by acceleration
 of DNA coated particles (U.S. Patent No. 5,550,318; U.S. Patent No. 5,538,877; and
 U.S. Patent No. 5,538,880; each specifically incorporated herein by reference in its

entirety), *etc.* Through the application of techniques such as these, the cells of virtually any plant species may be stably transformed, and these cells developed into transgenic plants.

A. *Agrobacterium*-mediated Transformation

5 *Agrobacterium*-mediated transfer is a widely applicable system for introducing genes into plant cells because the DNA can be introduced into whole plant tissues, thereby bypassing the need for regeneration of an intact plant from a protoplast. The use of *Agrobacterium*-mediated plant integrating vectors to introduce DNA into plant cells is well known in the art. See, for example, the methods described by Fraley *et*
10 *al.*, (1985), Rogers *et al.*, (1987) and U.S. Patent No. 5,563,055, specifically incorporated herein by reference in its entirety.

Agrobacterium-mediated transformation is most efficient in dicotyledonous plants and is the preferable method for transformation of dicots, including *Arabidopsis*, tobacco, tomato, alfalfa and potato. Indeed, while *Agrobacterium*-
15 mediated transformation has been routinely used with dicotyledonous plants for a number of years, it has only recently become applicable to monocotyledonous plants. Advances in *Agrobacterium*-mediated transformation techniques have now made the technique applicable to nearly all monocotyledonous plants. For example, *Agrobacterium*-mediated transformation techniques have now been applied to rice
20 (Hiei *et al.*, 1997; U.S. Patent No. 5,591,616, specifically incorporated herein by reference in its entirety), wheat (McCormac *et al.*, 1998), barley (Tingay *et al.*, 1997; McCormac *et al.*, 1998) and maize (Ishida *et al.*, 1996).

Modern *Agrobacterium* transformation vectors are capable of replication in *E. coli* as well as *Agrobacterium*, allowing for convenient manipulations as described
25 (Klee *et al.*, 1985). Moreover, recent technological advances in vectors for *Agrobacterium*-mediated gene transfer have improved the arrangement of genes and restriction sites in the vectors to facilitate the construction of vectors capable of expressing various polypeptide coding genes. The vectors described (Rogers *et al.*, 1987) have convenient multi-linker regions flanked by a promoter and a
30 polyadenylation site for direct expression of inserted polypeptide coding genes and are suitable for present purposes. In addition, *Agrobacterium* containing both armed and disarmed Ti genes can be used for the transformations. In those plant strains

where *Agrobacterium*-mediated transformation is efficient, it is the method of choice because of the facile and defined nature of the gene transfer.

B. Electroporation

To effect transformation by electroporation, one may employ either friable
5 tissues, such as a suspension culture of cells or embryogenic callus or alternatively
one may transform immature embryos or other organized tissue directly. In this
technique, one would partially degrade the cell walls of the chosen cells by exposing
them to pectin-degrading enzymes (pectolyases) or mechanically wounding in a
controlled manner. Examples of some species which have been transformed by
10 electroporation of intact cells include maize (U.S. Patent No. 5,384,253; Rhodes *et al.*, 1995; D'Halluin *et al.*, 1992), wheat (Zhou *et al.*, 1993), tomato (Hou and Lin, 1996), soybean (Christou *et al.*, 1987) and tobacco (Lee *et al.*, 1989).

One also may employ protoplasts for electroporation transformation of plants
(Bates, 1994; Lazzeri, 1995). For example, the generation of transgenic soybean
15 plants by electroporation of cotyledon-derived protoplasts is described by Dhir and
Widholm in Intl. Patent Appl. Publ. No. WO 9217598 (specifically incorporated
herein by reference). Other examples of species for which protoplast transformation
has been described include barley (Lazzeri, 1995), sorghum (Battraw *et al.*, 1991),
maize (Bhattacharjee *et al.*, 1997), wheat (He *et al.*, 1994) and tomato (Tsukada,
20 1989).

C. Microprojectile Bombardment

Another method for delivering transforming DNA segments to plant cells in
accordance with the invention is microprojectile bombardment (U.S. Patent No.
5,550,318; U.S. Patent No. 5,538,880; U.S. Patent No. 5,610,042; and PCT
25 Application WO 94/09699; each of which is specifically incorporated herein by
reference in its entirety). In this method, particles may be coated with nucleic acids
and delivered into cells by a propelling force. Exemplary particles include those
comprised of tungsten, platinum, and preferably, gold. It is contemplated that in some
instances DNA precipitation onto metal particles would not be necessary for DNA
30 delivery to a recipient cell using microprojectile bombardment. However, it is
contemplated that particles may contain DNA rather than be coated with DNA.

Hence, it is proposed that DNA-coated particles may increase the level of DNA delivery via particle bombardment but are not, in and of themselves, necessary.

For the bombardment, cells in suspension are concentrated on filters or solid culture medium. Alternatively, immature embryos or other target cells may be arranged on solid culture medium. The cells to be bombarded are positioned at an appropriate distance below the macroprojectile stopping plate.

An illustrative embodiment of a method for delivering DNA into plant cells by acceleration is the Biolistics Particle Delivery System, which can be used to propel particles coated with DNA or cells through a screen, such as a stainless steel or Nytex screen, onto a filter surface covered with monocot plant cells cultured in suspension. The screen disperses the particles so that they are not delivered to the recipient cells in large aggregates. Microprojectile bombardment techniques are widely applicable, and may be used to transform virtually any plant species. Examples of species for which have been transformed by microprojectile bombardment include monocot species such as maize (PCT Application WO 95/06128), barley (Ritala *et al.*, 1994; Hensgens *et al.*, 1993), wheat (U.S. Patent No. 5,563,055, specifically incorporated herein by reference in its entirety), rice (Hensgens *et al.*, 1993), oat (Torbet *et al.*, 1995; Torbet *et al.*, 1998), rye (Hensgens *et al.*, 1993), sugarcane (Bower *et al.*, 1992), and sorghum (Casa *et al.*, 1993; Hagio *et al.*, 1991); as well as a number of dicots including tobacco (Tomes *et al.*, 1990; Busing and Benbow, 1994), soybean (U.S. Patent No. 5,322,783, specifically incorporated herein by reference in its entirety), sunflower (Knittel *et al.*, 1994), peanut (Singsit *et al.*, 1997), cotton (McCabe and Martinell, 1993), tomato (VanEck *et al.*, 1995), and legumes in general (U.S. Patent No. 5,563,055, specifically incorporated herein by reference in its entirety).

D. Other Transformation Methods

Transformation of protoplasts can be achieved using methods based on calcium phosphate precipitation, polyethylene glycol treatment, electroporation, and combinations of these treatments (see, *e.g.*, Potrykus *et al.*, 1985; Lorz *et al.*, 1985; Omirulleh *et al.*, 1993; Fromm *et al.*, 1986; Uchimiya *et al.*, 1986; Callis *et al.*, 1987; Marcotte *et al.*, 1988).

Application of these systems to different plant strains depends upon the ability to regenerate that particular plant strain from protoplasts. Illustrative methods for the

regeneration of cereals from protoplasts have been described (Toriyama *et al.*, 1986; Yamada *et al.*, 1986; Abdullah *et al.*, 1986; Omirulleh *et al.*, 1993 and U.S. Patent No. 5,508,184; each specifically incorporated herein by reference in its entirety). Examples of the use of direct uptake transformation of cereal protoplasts include
5 transformation of rice (Ghosh-Biswas *et al.*, 1994), sorghum (Battraw and Hall, 1991), barley (Lazerri, 1995), oat (Zheng and Edwards, 1990) and maize (Omirulleh *et al.*, 1993).

To transform plant strains that cannot be successfully regenerated from protoplasts, other ways to introduce DNA into intact cells or tissues can be utilized.
10 For example, regeneration of cereals from immature embryos or explants can be effected as described (Vasil, 1989). Also, silicon carbide fiber-mediated transformation may be used with or without protoplasting (Kaeppeler, 1990; Kaeppeler *et al.*, 1992; U.S. Patent No. 5,563,055, specifically incorporated herein by reference in its entirety). Transformation with this technique is accomplished by agitating
15 silicon carbide fibers together with cells in a DNA solution. DNA passively enters as the cells are punctured. This technique has been used successfully with, for example, the monocot cereals maize (PCT Application WO 95/06128, specifically incorporated herein by reference in its entirety; (Thompson, 1995) and rice (Nagatani, 1997).

E. Tissue Cultures

20 Tissue cultures may be used in certain transformation techniques for the preparation of cells for transformation and for the regeneration of plants therefrom. Maintenance of tissue cultures requires use of media and controlled environments. "Media" refers to the numerous nutrient mixtures that are used to grow cells *in vitro*, that is, outside of the intact living organism. The medium usually is a suspension of
25 various categories of ingredients (salts, amino acids, growth regulators, sugars, buffers) that are required for growth of most cell types. However, each specific cell type requires a specific range of ingredient proportions for growth, and an even more specific range of formulas for optimum growth. Rate of cell growth also will vary among cultures initiated with the array of media that permit growth of that cell type.

30 Nutrient media is prepared as a liquid, but this may be solidified by adding the liquid to materials capable of providing a solid support. Agar is most commonly used

for this purpose. Bactoagar, Hazelton agar, Gelrite, and Gelgro are specific types of solid support that are suitable for growth of plant cells in tissue culture.

Some cell types will grow and divide either in liquid suspension or on solid media. As disclosed herein, plant cells will grow in suspension or on solid medium, but regeneration of plants from suspension cultures typically requires transfer from liquid to solid media at some point in development. The type and extent of differentiation of cells in culture will be affected not only by the type of media used and by the environment, for example, pH, but also by whether media is solid or liquid.

Tissue that can be grown in a culture includes meristem cells, Type I, Type II, and Type III callus, immature embryos and gametic cells such as microspores, pollen, sperm and egg cells. Type I, Type II, and Type III callus may be initiated from tissue sources including, but not limited to, immature embryos, seedling apical meristems, root, leaf, microspores and the like. Those cells which are capable of proliferating as callus also are recipient cells for genetic transformation.

Somatic cells are of various types. Embryogenic cells are one example of somatic cells which may be induced to regenerate a plant through embryo formation. Non-embryogenic cells are those which typically will not respond in such a fashion. Certain techniques may be used that enrich recipient cells within a cell population. For example, Type II callus development, followed by manual selection and culture of friable, embryogenic tissue, generally results in an enrichment of cells. Manual selection techniques which can be employed to select target cells may include, *e.g.*, assessing cell morphology and differentiation, or may use various physical or biological means. Cryopreservation also is a possible method of selecting for recipient cells.

Manual selection of recipient cells, *e.g.*, by selecting embryogenic cells from the surface of a Type II callus, is one means that may be used in an attempt to enrich for particular cells prior to culturing (whether cultured on solid media or in suspension).

Where employed, cultured cells may be grown either on solid supports or in the form of liquid suspensions. In either instance, nutrients may be provided to the cells in the form of media, and environmental conditions controlled. There are many types of tissue culture media comprised of various amino acids, salts, sugars, growth

regulators and vitamins. Most of the media employed in the practice of the invention will have some similar components, but may differ in the composition and proportions of their ingredients depending on the particular application envisioned. For example, various cell types usually grow in more than one type of media, but will exhibit different growth rates and different morphologies, depending on the growth media. In some media, cells survive but do not divide. Various types of media suitable for culture of plant cells previously have been described. Examples of these media include, but are not limited to, the N6 medium described by Chu *et al.* (1975) and MS media (Murashige and Skoog, 1962).

10

III. Production and Characterization of Stably Transformed Plants

After effecting delivery of exogenous DNA to recipient cells, the next steps generally concern identifying the transformed cells for further culturing and plant regeneration. In order to improve the ability to identify transformants, one may desire to employ a selectable or screenable marker gene with a transformation vector prepared in accordance with the invention. In this case, one would then generally assay the potentially transformed cell population by exposing the cells to a selective agent or agents, or one would screen the cells for the desired marker gene trait.

A. Selection

It is believed that DNA is introduced into only a small percentage of target cells in any one study. In order to provide an efficient system for identification of those cells receiving DNA and integrating it into their genomes one may employ a means for selecting those cells that are stably transformed. One exemplary embodiment of such a method is to introduce into the host cell, a marker gene which confers resistance to some normally inhibitory agent, such as an antibiotic or herbicide. Examples of antibiotics which may be used include the aminoglycoside antibiotics neomycin, kanamycin and paromomycin, or the antibiotic hygromycin. Resistance to the aminoglycoside antibiotics is conferred by aminoglycoside phosphotransferase enzymes such as neomycin phosphotransferase II (NPT II) or NPT I, whereas resistance to hygromycin is conferred by hygromycin phosphotransferase.

30

Potentially transformed cells then are exposed to the selective agent. In the population of surviving cells will be those cells where, generally, the resistance-conferring gene has been integrated and expressed at sufficient levels to permit cell survival. Cells may be tested further to confirm stable integration of the exogenous DNA.

One herbicide which constitutes a desirable selection agent is the broad spectrum herbicide bialaphos. Bialaphos is a tripeptide antibiotic produced by *Streptomyces hygroscopicus* and is composed of phosphinothricin (PPT), an analogue of L-glutamic acid, and two L-alanine residues. Upon removal of the L-alanine residues by intracellular peptidases, the PPT is released and is a potent inhibitor of glutamine synthetase (GS), a pivotal enzyme involved in ammonia assimilation and nitrogen metabolism (Ogawa *et al.*, 1973). Synthetic PPT, the active ingredient in the herbicide Liberty™ also is effective as a selection agent. Inhibition of GS in plants by PPT causes the rapid accumulation of ammonia and death of the plant cells.

The organism producing bialaphos and other species of the genus *Streptomyces* also synthesizes an enzyme phosphinothricin acetyl transferase (PAT) which is encoded by the *bar* gene in *Streptomyces hygroscopicus* and the *pat* gene in *Streptomyces viridochromogenes*. The use of the herbicide resistance gene encoding phosphinothricin acetyl transferase (PAT) is referred to in DE 3642 829 A, wherein the gene is isolated from *Streptomyces viridochromogenes*.

Another example of a herbicide which is useful for selection of transformed cell lines in the practice of the invention is the broad spectrum herbicide glyphosate. Glyphosate inhibits the action of the enzyme EPSPS which is active in the aromatic amino acid biosynthetic pathway. Inhibition of this enzyme leads to starvation for the amino acids phenylalanine, tyrosine, and tryptophan and secondary metabolites derived thereof. U.S. Patent No. 4,535,060 describes the isolation of EPSPS mutations which confer glyphosate resistance on the *Salmonella typhimurium* gene for EPSPS, *aroA*. The EPSPS gene was cloned from *Zea mays* and mutations similar to those found in a glyphosate resistant *aroA* gene were introduced *in vitro*. Mutant genes encoding glyphosate resistant EPSPS enzymes are described in, for example, International Patent WO 97/4103. The best characterized mutant EPSPS gene conferring glyphosate resistance comprises amino acid changes at residues 102 and

106, although it is anticipated that other mutations will also be useful (PCT/WO97/4103).

To use a *bar*-bialaphos or the EPSPS-glyphosate selective system, for example, transformed tissue can be cultured for 0 - 28 days on nonselective medium and subsequently transferred to medium containing from 1-3 mg/l bialaphos or 1-3 mM glyphosate as appropriate. While ranges of 1-3 mg/l bialaphos or 1-3 mM glyphosate may be preferred, it is proposed that ranges of 0.1-50 mg/l bialaphos or 0.1-50 mM glyphosate will find utility.

B. Regeneration and Seed Production

Cells that survive the exposure to the selective agent, or cells that have been scored positive in a screening assay, may be cultured in media that supports regeneration of plants. In an exemplary embodiment, MS and N6 media may be modified by including further substances such as growth regulators. One such growth regulator is dicamba or 2,4-D. However, other growth regulators may be employed, including NAA, NAA + 2,4-D or picloram. Media improvement in these and like ways has been found to facilitate the growth of cells at specific developmental stages. Tissue may be maintained on a basic media with growth regulators until sufficient tissue is available to begin plant regeneration efforts, or following repeated rounds of manual selection, until the morphology of the tissue is suitable for regeneration, at least 2 wk, then transferred to media conducive to maturation of embryoids. Cultures are transferred every 2 wk on this medium. Shoot development will signal the time to transfer to medium lacking growth regulators.

The transformed cells, identified by selection or screening and cultured in an appropriate medium that supports regeneration, will then be allowed to mature into plants. Developing plantlets are transferred to soilless plant growth mix, and hardened, *e.g.*, in an environmentally controlled chamber, for example, at about 85% relative humidity, 600 ppm CO₂, and 25-250 microeinsteins m⁻² s⁻¹ of light. Plants may be matured in a growth chamber or greenhouse. Plants can be regenerated from about 6 wk to 10 months after a transformant is identified, depending on the initial tissue. During regeneration, cells are grown on solid media in tissue culture vessels. Illustrative embodiments of such vessels are petri dishes and Plant Cons. Regenerating plants can be grown at about 19 to 28°C. After the regenerating plants

have reached the stage of shoot and root development, they may be transferred to a greenhouse for further growth and testing.

Seeds on transformed plants may occasionally require embryo rescue due to cessation of seed development and premature senescence of plants. To rescue
5 developing embryos, they are excised from surface-disinfected seeds 10-20 days post-pollination and cultured. An embodiment of media used for culture at this stage comprises MS salts, 2% sucrose, and 5.5 g/l agarose. In embryo rescue, large embryos (defined as greater than 3 mm in length) are germinated directly on an appropriate media. Embryos smaller than that may be cultured for 1 wk on media
10 containing the above ingredients along with 10^{-5} M abscisic acid and then transferred to growth regulator-free medium for germination.

C. Characterization

To confirm the presence of the exogenous DNA or "transgene(s)" in the regenerating plants, a variety of assays may be performed. Such assays include, for
15 example, "molecular biological" assays, such as Southern and Northern blotting and PCRTM; "biochemical" assays, such as detecting the presence of a protein product, e.g., by immunological means (ELISAs and Western blots) or by enzymatic function; plant part assays, such as leaf or root assays; and also, by analyzing the phenotype of the whole regenerated plant.

20 D. DNA Integration, RNA Expression and Inheritance

Genomic DNA may be isolated from cell lines or any plant parts to determine the presence of the exogenous gene through the use of techniques well known to those skilled in the art. Note, that intact sequences will not always be present, presumably due to rearrangement or deletion of sequences in the cell. The presence of DNA
25 elements introduced through the methods of this invention may be determined, for example, by polymerase chain reaction (PCRTM). Using this technique, discrete fragments of DNA are amplified and detected by gel electrophoresis. This type of analysis permits one to determine whether a gene is present in a stable transformant, but does not prove integration of the introduced gene into the host cell genome. It is
30 typically the case, however, that DNA has been integrated into the genome of all transformants that demonstrate the presence of the gene through PCRTM analysis. In addition, it is not typically possible using PCRTM techniques to determine whether

transformants have exogenous genes introduced into different sites in the genome, *i.e.*, whether transformants are of independent origin. It is contemplated that using PCRTM techniques it would be possible to clone fragments of the host genomic DNA adjacent to an introduced gene.

5 Positive proof of DNA integration into the host genome and the independent identities of transformants may be determined using the technique of Southern hybridization. Using this technique specific DNA sequences that were introduced into the host genome and flanking host DNA sequences can be identified. Hence the Southern hybridization pattern of a given transformant serves as an identifying
10 characteristic of that transformant. In addition it is possible through Southern hybridization to demonstrate the presence of introduced genes in high molecular weight DNA, *i.e.*, confirm that the introduced gene has been integrated into the host cell genome. The technique of Southern hybridization provides information that is obtained using PCRTM, *e.g.*, the presence of a gene, but also demonstrates integration
15 into the genome and characterizes each individual transformant.

It is contemplated that using the techniques of dot or slot blot hybridization which are modifications of Southern hybridization techniques one could obtain the same information that is derived from PCRTM, *e.g.*, the presence of a gene.

Both PCRTM and Southern hybridization techniques can be used to
20 demonstrate transmission of a transgene to progeny. In most instances the characteristic Southern hybridization pattern for a given transformant will segregate in progeny as one or more Mendelian genes (Spencer *et al.*, 1992) indicating stable inheritance of the transgene.

Whereas DNA analysis techniques may be conducted using DNA isolated
25 from any part of a plant, RNA will only be expressed in particular cells or tissue types and hence it will be necessary to prepare RNA for analysis from these tissues. PCRTM techniques also may be used for detection and quantitation of RNA produced from introduced genes. In this application of PCRTM it is first necessary to reverse transcribe RNA into DNA, using enzymes such as reverse transcriptase, and then
30 through the use of conventional PCRTM techniques amplify the DNA. In most instances PCRTM techniques, while useful, will not demonstrate integrity of the RNA product. Further information about the nature of the RNA product may be obtained

by Northern blotting. This technique will demonstrate the presence of an RNA species and give information about the integrity of that RNA. The presence or absence of an RNA species also can be determined using dot or slot blot Northern hybridizations. These techniques are modifications of Northern blotting and will only demonstrate the presence or absence of an RNA species.

E. Gene Expression

While Southern blotting and PCR™ may be used to detect the gene(s) in question, they do not provide information as to whether the corresponding protein is being expressed. Expression may be evaluated by specifically identifying the protein products of the introduced genes or evaluating the phenotypic changes brought about by their expression.

Assays for the production and identification of specific proteins may make use of physical-chemical, structural, functional, or other properties of the proteins. Unique physical-chemical or structural properties allow the proteins to be separated and identified by electrophoretic procedures, such as native or denaturing gel electrophoresis or isoelectric focusing, or by chromatographic techniques such as ion exchange or gel exclusion chromatography. The unique structures of individual proteins offer opportunities for use of specific antibodies to detect their presence in formats such as an ELISA assay. Combinations of approaches may be employed with even greater specificity such as western blotting in which antibodies are used to locate individual gene products that have been separated by electrophoretic techniques. Additional techniques may be employed to absolutely confirm the identity of the product of interest such as evaluation by amino acid sequencing following purification. Although these are among the most commonly employed, other procedures may be additionally used.

Very frequently the expression of a gene product is determined by evaluating the phenotypic results of its expression. These assays also may take many forms including but not limited to analyzing changes in the chemical composition, morphology, or physiological properties of the plant. Chemical composition may be altered by expression of genes encoding enzymes or storage proteins which change amino acid composition and may be detected by amino acid analysis, or by enzymes which change starch quantity which may be analyzed by near infrared reflectance

spectrometry. Morphological changes may include greater stature or thicker stalks. Most often changes in response of plants or plant parts to imposed treatments are evaluated under carefully controlled conditions termed bioassays.

5 IV. Breeding Plants of the Invention

In addition to direct transformation of a particular plant genotype with a construct prepared according to the current invention, transgenic plants may be made by crossing a plant having a selected DNA of the invention to a second plant lacking the construct. For example, a selected wax biosynthesis coding sequence can be
10 introduced into a particular plant variety by crossing, without the need for ever directly transforming a plant of that given variety. Therefore, the current invention not only encompasses a plant directly transformed or regenerated from cells which have been transformed in accordance with the current invention, but also the progeny of such plants. As used herein the term "progeny" denotes the offspring of any
15 generation of a parent plant prepared in accordance with the instant invention, wherein the progeny comprises a selected DNA construct prepared in accordance with the invention. "Crossing" a plant to provide a plant line having one or more added transgenes relative to a starting plant line, as disclosed herein, is defined as the techniques that result in a transgene of the invention being introduced into a plant line
20 by crossing a starting line with a donor plant line that comprises a transgene of the invention. To achieve this one could, for example, perform the following steps:

- (a) plant seeds of the first (starting line) and second (donor plant line that comprises a transgene of the invention) parent plants;
- (b) grow the seeds of the first and second parent plants into plants that
25 bear flowers;
- (c) pollinate a flower from the first parent plant with pollen from the second parent plant; and
- (d) harvest seeds produced on the parent plant bearing the fertilized flower.

30 Backcrossing is herein defined as the process including the steps of:

(a) crossing a plant of a first genotype containing a desired gene, DNA sequence or element to a plant of a second genotype lacking the desired gene, DNA sequence or element;

5 (b) selecting one or more progeny plant containing the desired gene, DNA sequence or element;

(c) crossing the progeny plant to a plant of the second genotype; and

(d) repeating steps (b) and (c) for the purpose of transferring a desired DNA sequence from a plant of a first genotype to a plant of a second genotype.

10 Introgression of a DNA element into a plant genotype is defined as the result of the process of backcross conversion. A plant genotype into which a DNA sequence has been introgressed may be referred to as a backcross converted genotype, line, inbred, or hybrid. Similarly a plant genotype lacking the desired DNA sequence may be referred to as an unconverted genotype, line, inbred, or hybrid.

15 V. Definitions

Expression: The combination of intracellular processes, including transcription and translation undergone by a coding DNA molecule such as a structural gene to produce a polypeptide.

20 **Genetic Transformation:** A process of introducing a DNA sequence or construct (e.g., a vector or expression cassette) into a cell or protoplast in which that exogenous DNA is incorporated into a chromosome or is capable of autonomous replication.

25 **Heterologous:** A sequence which is not normally present in a given host genome in the genetic context in which the sequence is currently found. In this respect, the sequence may be native to the host genome, but be rearranged with respect to other genetic sequences within the host sequence. For example, a regulatory sequence may be heterologous in that it is linked to a different coding sequence relative to the native regulatory sequence.

30 **Obtaining:** When used in conjunction with a transgenic plant cell or transgenic plant, obtaining means either transforming a non-transgenic plant cell or plant to create the transgenic plant cell or plant, or planting transgenic plant seed to

produce the transgenic plant cell or plant. Such a transgenic plant seed may be from an R₀ transgenic plant or may be from a progeny of any generation thereof that inherits a given transgenic sequence from a starting transgenic parent plant.

Promoter: A recognition site on a DNA sequence or group of DNA sequences that provides an expression control element for a structural gene and to which RNA polymerase specifically binds and initiates RNA synthesis (transcription) of that gene.

R₀ transgenic plant: A plant that has been genetically transformed or has been regenerated from a plant cell or cells that have been genetically transformed.

Regeneration: The process of growing a plant from a plant cell (e.g., plant protoplast, callus or explant).

Selected DNA: A DNA segment which one desires to introduce or has introduced into a plant genome by genetic transformation.

Transformation construct: A chimeric DNA molecule which is designed for introduction into a host genome by genetic transformation. Preferred transformation constructs will comprise all of the genetic elements necessary to direct the expression of one or more exogenous genes. In particular embodiments of the instant invention, it may be desirable to introduce a transformation construct into a host cell in the form of an expression cassette.

Transformed cell: A cell the DNA complement of which has been altered by the introduction of an exogenous DNA molecule into that cell.

Transgene: A segment of DNA which has been incorporated into a host genome or is capable of autonomous replication in a host cell and is capable of causing the expression of one or more coding sequences. Exemplary transgenes will provide the host cell, or plants regenerated therefrom, with a novel phenotype relative to the corresponding non-transformed cell or plant. Transgenes may be directly introduced into a plant by genetic transformation, or may be inherited from a plant of any previous generation which was transformed with the DNA segment.

Transgenic plant: A plant or progeny plant of any subsequent generation derived therefrom, wherein the DNA of the plant or progeny thereof contains an introduced exogenous DNA segment not naturally present in a non-transgenic plant of

the same strain. The transgenic plant may additionally contain sequences which are native to the plant being transformed, but wherein the "exogenous" gene has been altered in order to alter the level or pattern of expression of the gene, for example, by use of one or more heterologous regulatory or other elements.

5 **Vector:** A DNA molecule designed for transformation into a host cell. Some vectors may be capable of replication in a host cell. A plasmid is an exemplary vector, as are expression cassettes isolated therefrom.

Wax Biosynthesis Gene: A nucleic acid sequence that confers wax biosynthesis when expressed in a plant.

10 **Wax Biosynthesis Activating Sequences:** A nucleic acid sequence that confers activation of wax biosynthesis when expressed in a plant.

VI. Examples

The following examples are included to demonstrate preferred embodiments of the invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by
15 the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar
20 result without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit,
25 scope and concept of the invention as defined by the appended claims.

EXAMPLE 1

Sequence Analysis and Expression Pattern of *WXP1* and *WXP2*

A number of AP2 domain-containing transcription factor genes were
30 identified from *M. truncatula* and characterized by sequence alignment, northern hybridization analysis and transgenic overexpression. One of the genes, *WXP1*, has

an open reading frame (ORF) encoding 371 aa with an estimated molecular mass of 41.3 kD and a theoretical pI of 5.55. It is one of the longest peptides among 80 AP2 domain-containing transcription factors identified in *M. truncatula*. Another gene, *WXP2*, has an open reading frame (ORF) encoding 340 aa with an estimated molecular mass of 37.8 kD and a theoretical pI of 5.36. The deduced amino acid sequences of these genes contain one conserved AP2 domain when analyzed by Pfam (Bateman *et al.*, 2002). When aligned with well-characterized AP2 domain-containing transcription factors from *Arabidopsis* (At), tomato (Le) and tobacco (Nt), the AP2 domain of *WXP1* and *WXP2* shared high similarity to that of the other proteins (FIG. 1). All these AP2 domains have a 100% identical WLQ motif in the middle and an extremely conserved YRG motif in the front. But they are clearly divided into three groups by the diversification of the RAYD, LAYD and RAHD motifs. *WXP1* and *WXP2* contain LAYD motifs that are found in three ERFs (LePt4, NtERF1, AtERF1), and AtRAP2.4 (At1g78080) (FIG. 1).

Comparison of *WXP1* to predicted protein sequences of AP2 domain-containing transcription factors from different species revealed that Mt77128 (*WXP2*) from *M. truncatula* is the closest homolog with 53.4% identity to *WXP1*, and AtRAP2.4 from *Arabidopsis* is the closest ortholog with 48.8% identity to *WXP1*. When compared with amino acid sequences of other stress or wax inducing genes, *WXP1* is only 19.4-22.8% identical to AtCBFs, 16.7% to AtDREB2A, 20.6% to AtDREB2B, 22.8-25.3% to the three ERFs, 28.6% to AtWIN1 and 14.0% to ZmGlossy15. Phylogenetic analysis showed that *WXP1* and *WXP2* are distinct from most of the known AP2 domain transcription factors based on analysis of their complete protein sequences (FIG. 2).

The expression pattern of the *WXP1* gene in *M. truncatula* may also be detected by northern hybridization analysis. Drought treatment slightly induced its expression in shoot tissues but suppressed its expression in root tissues (FIG. 3). *WXP1* transcript in shoot was quickly induced to a high level after transferring plants to 4°C, however, no such change was observed in root after the same cold treatment (FIG. 3). Abscissic acid (ABA) treatment induced *WXP1* expression in both shoot and root in a relatively short time (FIG. 3). The induction or suppression of *WXP1* in *M. truncatula* was reversible, as transcription would go back to normal levels when the stimuli were withdrawn.

EXAMPLE 2

Growth and development of transgenic alfalfa plants overexpressing *WXP1*

The ORF of *WXP1* was placed under the control of CaMV 35S promoter by replacing the *gusA* gene of the binary vector pCAMBIA3301. The resulting vector pC35S-*WXP1* was introduced into alfalfa by *Agrobacterium*-mediated transformation and 60 independent transgenic plants were produced. PCR screening indicated that more than 98% of the regenerated plants contained the target gene, and northern hybridization analysis revealed that 60% of the plants expressed the transgene with various mRNA levels. To facilitate the analysis, three transgenic lines (18, 41, 47) were focused on for agronomic and biochemical analysis and five lines for gene regulation assay. These lines were selected because they showed different transgene expression levels. Lines 4, 18, 41, 47 and 45 showed very low, low, medium, high, and extremely high levels of *WXP1* expression, respectively (see FIG. 15). All the lines were vegetatively propagated by cuttings of young shoots.

Even though the overexpression of *WXP1* did not result in severe growth retardation, which was typical in AP2 domain-containing gene overexpressed *Arabidopsis* (Broun *et al.*, 2004; Gilmour *et al.*, 2000; Jaglo-Ottosen *et al.*, 1998; Liu *et al.*, 1998), the alfalfa transgenic lines tended to grow relatively slowly (FIG. 4). When compared with wild-type and empty vector control plants, flowering time of the transgenic plants was delayed 5 – 28 d (FIG. 11A), height of the transgenic lines were 2 – 32% shorter at flowering time (FIG. 11B), and trifoliates of the transgenic lines were smaller (FIG. 5A). Even though the transgenic lines produced more branches, fresh and dry matter production of the transgenic lines decreased 5.6 – 26.8% and 6.7 – 42.7%, respectively (FIGs. 11C and 11D).

The most striking phenotypic change in the *WXP1* overexpressed alfalfa plants was the more glaucous appearance in the leaves (FIG. 5A). The increased glaucousness occurred in most of the transgenic lines with low to extremely high *WXP1* overexpression levels, represented by line 18, 27, 41, 47, 33, and 45.

Observation of leaf surfaces under a stereomicroscope with strong light indicated that glaucousness was added to both sides of the *WXP1* overexpressed alfalfa leaves; however, the change of glaucous appearance on the adaxial and abaxial surfaces of the transgenic lines was not equal. By observing leaflets excised from the

same trifoliolate, the adaxial side showed less light refraction and thus less glaucous than the abaxial side in control leaflets (FIG. 5B), however, the difference between the two sides was drastically reduced in transgenic leaflets (FIGs. 5C). Compared with control leaflets, the transgenic leaflets showed more obvious increase of glaucousness in the adaxial surface than the abaxial side (FIGs. 5B and 5C). The results indicate that the difference in glaucousness between transgenic and control lines was more prominent on the adaxial side than that on the abaxial side.

EXAMPLE 3

Impact of *WXP1* overexpression on cuticular wax production in transgenic alfalfa

To confirm the increased glaucous appearance on leaves was caused by alteration of epicuticular wax production, *WXP1* transgenic alfalfa lines and empty vector control plants were examined by scanning electron microscopy (SEM). When the adaxial and abaxial surfaces of the same leaflet from a control plant were compared, the time of epicuticular wax loading, wax crystal type, crystal size, and crystal density was different between the two sides (FIGs. 6A, 6C, 6E and 6G). It was evident that the abaxial surface started loading epicuticular wax earlier than the adaxial surface (FIGs. 6A and 6C). Comparison of leaf epicuticular wax crystalline pattern between control and transgenic lines showed that overexpression of *WXP1* in alfalfa resulted in increased wax loading on both sides and earlier accumulation of epicuticular waxes on the adaxial surface. When the new leaf became fully expanded (top-1st), there were no visible wax crystals on the adaxial leaf surface of control plants (FIG. 6A), in contrast, wax crystal structures were already developed on the adaxial leaf surface of transgenic plants (FIGs. 6B).

Wax crystal density of newly expanded leaf showed some difference on the abaxial side, with a better coverage of wax crystals in transgenics than in the control plant (FIGs. 6C and 6D). For the top-2nd trifoliolate, differences in wax crystal density on the adaxial leaf surfaces were apparent between the transgenic and control plants, with much more wax crystals present in the transgenic plant (FIGs. 6E and 6F). On the abaxial surface of the top-2nd trifoliolate, the long coiled crystalline structure in transgenic line was reduced, but the loss of the long coiled crystals was

complemented by the increase in density of tubular and plate-like wax crystals (FIGs. 6G and 6H). Thus the SEM examination is in agreement with the increased leaf surface glaucous appearance observed under light microscope. Stem surfaces of both control and transgenic alfalfa lines did not show visible wax crystalline structure (FIG. 12).

To further determine whether the crystal pattern alteration in transgenic alfalfa was the result of qualitative or quantitative changes in wax loading, leaf samples from the top four fully expanded trifoliate and stem samples from the top four internodes of wild-type, empty vector control and *WXP1* transgenic plants were subjected to gas chromatography (GC)-mass spectrometry (MS) analysis. As shown in FIGs. 7A and 7B, primary alcohol is the major constituent of alfalfa leaf wax while alkane is the most abundant wax compound in stem. Total primary alcohol content per leaf area dramatically increased in the *WXP1* overexpressed lines (FIG. 7A), while only a slight increase in alkane content was observed in stems of the transgenics (FIG. 7B). No new compound was identified in the transgenic lines.

Total wax accumulation per leaf area was significantly higher in the transgenic lines than that in the control plants (FIG. 7C). The biggest change of cuticular wax deposition was in transgenic line 47, which had an increase of 37.7% in its wax load. Lines 18 and 41 showed increases in their total wax load by 29.6% and 35.3%, respectively. The increase was mainly contributed by C30 primary alcohol, which is the major component of alfalfa leaf wax (FIG. 8A). C30 alcohol in transgenic lines 18, 41 and 47 was 34.5%, 43.0% and 45.3% more than that of the control plants, respectively. Other carbon length alcohols did not show significant changes (FIG. 8A). Alkanes were not significantly affected by the overexpression of *WXP1*, even though C31 alkane had minor increase in the transgenic lines (FIG. 8B). The total amount of long chain fatty acids did not show significant change. The abundance of unsaturated octadecenoic acid (C18:1) was apparently increased in the transgenic lines, while saturated fatty acid (C18:0) remained essentially the same, and the amount of C22 and C24 fatty acids slightly decreased (FIG. 8C). In leaf cuticular wax extracts, C30 aldehyde was the only aldehyde component detected. The amount of this compound decreased in line 47, but no significant change was found in other transgenic lines (FIG. 8D).

In contrast to leaves, cuticular wax accumulation in stems was not significantly altered in the transgenic alfalfa plants (FIG. 7C). The most abundant stem wax component, C31 alkane, increased gradually with the increased transgene expression level from transgenic line 18 to line 47. However, C29 alkane, the second important stem wax component, was negatively affected by the level of transgene expression (FIG. 13A). For the minor components of alfalfa stem wax, only C20 fatty acid and triterpenoids were reduced in the transgenics. No consistent and significant changes were observed for the other components (FIG. 13B-D).

EXAMPLE 4

Water loss rate, chlorophyll extraction rate and drought tolerance of transgenic alfalfa overexpressing *WXP1*

Detached alfalfa trifoliate leaves were subjected to leaf water loss rate assay. Both top-2nd and top-3rd trifoliate leaves from the transgenic lines showed lower water evaporation rate than that from the vector control (FIG. 14).

Chlorophyll efflux analysis revealed that rates of chlorophyll extraction from the top-1st through top-4th leaves during a 6 h period were much slower in the transgenic plants than in the control plants (FIGs. 9A, 9C, 9E and 9G), indicating reduced epidermal permeability in transgenic leaves. In stems, the difference on chlorophyll extraction rate between transgenic and control plants was relatively small, with no difference in the 4th internode (FIGs. 9B, 9D, 9F and 9H). In addition, little difference in epidermal permeability was observed between leaves at different positions in the stem, although they represented different developmental stages. However, different internodes showed substantial differences, with older internodes having much less chlorophyll efflux than younger internodes (FIG. 9).

Transgenic lines and control plants were subjected to drought stress in growth chambers and greenhouse. In the growth chamber experiments, watering was ceased for three replicates of 20-day-old alfalfa plants and the chambers were dehumidified. Three days after watering was stopped, all the control plants became wilted while all the transgenic plants still kept their whole plant turgor (FIG. 10A). The transgenics began to wilt one day later than the control plants. After one more week in the same

chambers without watering, all the plants became dehydrated and dead-like. Upon resumption of the normal watering scheme, the transgenic plants recovered much faster and better than the control plants (FIG. 10B). In the greenhouse experiments, it took longer time to completely dehydrate the plants because of the fluctuations in relative humidity. Nevertheless, after three cycles of drought stress-rewatering in a period of four weeks, most of the transgenic plants survived while the control plants failed to recover.

EXAMPLE 5

Overexpression of *WXP1* induced/suppressed a number of genes related to wax biosynthesis

Northern hybridization analysis was used to characterize the expression of a number of wax related genes in the transgenic plants. Total RNA was isolated from leaves of transgenic lines 4, 18, 41, 47, and 45; these lines had increasing levels of *WXP1* expression (FIG. 15). A list of candidate genes (Table 1) were selected based on TBLASTN search against TIGR *Medicago truncatula* Gene Index database (Quackenbush *et al.*, 2000) with a set of query genes that were implicated in cuticular wax loading or cutin biosynthesis in *Arabidopsis* and maize. All the DNA probes used for hybridization were selected from *M. truncatula* cDNA libraries.

Because of the potential role of fatty acid elongase (FAE) in wax production, and the fact that a putative fatty acid elongase gene was strongly down-regulated by *WIN1* overexpression in *Arabidopsis* (Broun *et al.*, 2004), five *M. truncatula* cDNA clones predicted to encode different FAE-like proteins were tested. Totally seven bands were revealed by northern hybridization with these five FAE-like genes. Transcript levels in three of the FAE-like genes, MtTC79579, MtTC80406 and MtTC87247, were enhanced in the transgenic lines with increasing levels of *WXP1* expression (Table 1, FIG. 15). This is opposite to the effect of *WIN1* overexpression. No obvious changes were detected for the other FAE-like genes in the transgenic lines (Table 1).

KCS and CER6 have been implicated in the synthesis of very-long-chain fatty acids (VLCFA) precursors for wax production (Kunst and Samuels, 2003). No

change in the expression level of KCS- or CER6-like genes (TC78487, TC82553 and TC81348) was observed in the alfalfa transgenic plants (Table 1).

The *LACERATA* (*LCR*) gene encodes cytochrome P450 monooxygenase, which catalyzes ω -hydroxylation of fatty acids ranging from C12 to C18:1 (Wellesen
 5 *et al.*, 2001). Overexpression of *WXP1* in alfalfa up-regulated two *LCR*-like genes, TC81689 and TC84740. Two bands showed up when another *LCR*-like gene (TC80834) was used as probe, the intensity of the upper band was increased, while the intensity of the lower band was decreased with the expression of *WXP1* (Table 1, FIG. 15).

10 *Arabidopsis* WAX2 is predicted to have a metabolic function associated with both cuticle membrane and wax synthesis. Transcript level of one *WAX2*-like gene (TC82822) was negatively affected by the overexpression of the *WXP1*, while the mRNA level of the other *WAX2*-like gene (TC87337) did not change (Table 1, FIG. 15). Maize GL8 functions as a β -ketoacyl-reductase (*KAR*) in wax production (Kunst
 15 and Samuels, 2003). The intensity of the *KAR* bands showed a negative correlation with expression of *WXP1* (Table 1, FIG. 15).

The expression pattern of a number of other genes that were up-regulated by *WIN1* in *Arabidopsis* was also analyzed. Among these genes, the expression of the two glycerol-3-phosphate dehydrogenase genes was positively correlated with *WXP1*
 20 expression, while the phospholipid/glycerol acyltransferase gene was negatively correlated with *WXP1* expression. The transcript levels of enoyl-CoA hydratase/isomerase and long-chain acyl-CoA synthase (*LACS*) were not affected by the overexpression of *WXP1* in alfalfa (Table 1, FIG. 15).

Table 1 Expression of wax related genes in transgenic alfalfa plants overexpressing *WXP1*

MtGI #	Putative protein function/annotation	<i>Arabidopsis</i> /maize ortholog	Transcript level
TC79579	Fatty acid elongase (FAE)	CER2	Up
TC80406	Fatty acid elongase (FAE)	CER2	Up
TC87247	Fatty acid elongase (FAE)	CER2	Up
TC88787	Fatty acid elongase (FAE)	CER2	None/None
TC92418	Fatty acid elongase (FAE)	CER2	None/None
TC78487	β -ketoacyl-CoA synthase	KCS1	None
TC82553	β -ketoacyl-CoA synthase very-long-chain fatty acid	KCS1	None
TC81348	condensing enzyme	CER6/CUT1	None
TC77258	Cytochrome P450 monooxygenase	LCR	None
TC80834	Cytochrome P450 monooxygenase	LCR	Up/Down
TC81689	Cytochrome P450 monooxygenase	LCR	Up
TC84740	Cytochrome P450 monooxygenase	LCR	Up
TC82822	Sterol desaturase	WAX2/CER1	Down
TC87337	Sterol desaturase	WAX2/CER1	None
TC80863	Lipid transfer protein	GL1	None
TC88304	β -keto acyl reductase Glycerol-3-phosphate	GL8	Down/Down
TC79586	dehydrogenase Glycerol-3-phosphate	At2G41540	Up
TC91553	dehydrogenase Phospholipid/glycerol	At2G41540	Up
BI271665	acyltransferase	At 2G38110	Down
TC89646	Enoyl-CoA hydratase/isomerase Long-chain acyl-CoA synthase	At4G14440	None/None
TC86198	(LACS)	At3g16170	None

5

EXAMPLE 6

***WXP1* is a novel transcription factor gene that activates wax production in leaves of transgenic alfalfa plants**

The *WXP1* gene cloned from *M. truncatula* encodes one of the longest
10 peptides of all the predicted AP2 domain-containing transcription factors in *M.*

truncatula. Sequence analysis revealed *WXP1* is very different from other well-characterized transcription factors related to abiotic stress or wax accumulation, e.g. DREB/CBF from *Arabidopsis* (Jaglo-Ottosen *et al.*, 1998; Liu *et al.*, 1998), WIN1 from *Arabidopsis* (Broun *et al.*, 2004) and GL15 from maize (Moose and Sisco, 1996). Northern hybridization analysis showed that the expression of *WXP1* is inducible by cold or drought stress, which is similar to some members of the DREB/CBF family. The response to environmental stress has not been reported for WIN1 and GL15. As discussed below, functional characterization of *WXP1* in transgenic alfalfa plants further proved its novelty.

Because of the unique characteristics and modes of action of transcription factors, it has been realized that the overexpression strategy is particularly effective in revealing transcription factor function (Zhang, 2003). Different from DREB/CBF genes, constitutive expression of the *WXP1* gene in alfalfa resulted in a significant increase in wax accumulation on the leaf surfaces. Wax crystals were produced earlier on the adaxial side of newly expanded transgenic leaves, and higher density of wax crystals were found on both adaxial and abaxial sides of mature transgenic leaves. It is important to be able to alter wax accumulation in leaf tissues of crops, because leaves are the primary photosynthetic organs, comprise the primary biomass of most agronomic crops, and are often severely affected by environmental stresses (Jenks *et al.*, 2002). Most of the *Arabidopsis* mutants have alterations in their stem waxes (Jenks *et al.*, 1995; Koornneef *et al.*, 1989). Visual screening of mutagenesis populations of *Arabidopsis* to find mutants having increased leaf glaucousness due to changes in cuticular waxes have had limited success (Jenks *et al.*, 2002). Thus, overexpression of transcription factor genes is indicated to be an effective approach to turn on the wax biosynthetic pathway and lead to increased wax production in leaves.

EXAMPLE 7

***WXP1* overexpression affected the expression of genes that are potentially related to wax or cutin biosynthesis**

Among the candidate enzymatic genes analyzed, several of them seemed to correlate their expression with the expression of *WXP1*. In contrast to *Arabidopsis* WIN1, which down-regulated the expression of a fatty acid elongase gene, *WXP1* up-

regulated three fatty acid elongase genes. It is known that acyl chain extensions are carried out by several distinct elongases with unique substrate chain specificities (Kunst and Samuels, 2003). Thus *WXP1* may positively impact the fatty acid elongation process for the production of VLCFA chains that are used for the production of aliphatic wax components. The transcript levels of genes coding for cytochrome P450 monooxygenase and glycerol-3-phosphate dehydrogenase were also up-regulated in the *WXP1* transgenics. Glycerol-3-phosphate dehydrogenase is essential for phospholipid synthesis through both the prokaryotic and the eukaryotic glycerolipid pathway (Wei *et al.*, 2001). Cytochrome P450 monooxygenase functions as a fatty acid ω -hydroxylase that is required in the formation of cutin monomers (Wellesen *et al.*, 2001). The results indicate that besides its function in wax biosynthesis, *WXP1* may also play a role in the biosynthesis of cutin, which may act as a barrier that mechanically isolates epidermis cells of adjoining organs.

Fatty acids produced in the plastid from de novo synthesis are directed to at least three biosynthetic pathways that lead to the production of waxes, cutin/suberin, and glycerolipids respectively (Post-Beittenmiller, 1996). This is accomplished by a partition occurred after their biosynthesis, which delivers C16:0 and unsaturated C18:1 fatty acids as precursors to produce glycerolipids or cutin/suberin and saturated C18:0 fatty acid as precursor to produce waxes (Post-Beittenmiller, 1996). In our transgenic *WXP1* plants, no significant change was observed for the amount of saturated C18:0 fatty acid and total fatty acids, indicating the increase of wax accumulation, particularly the increase of primary alcohols in leaves, is most probably due to the regulation of genes that control fatty acid elongation and the acyl-reduction pathway. On the other hand, the amount of unsaturated C18:1 fatty acid in leaves was more than double that of controls. The increase of C18:1 fatty acid is consistent with the up-regulation of cytochrome P450 monooxygenase, because the enzyme is directly involved in the biosynthesis of cutin (Wellesen *et al.*, 2001). Further research with respect to *WXP1* expression and cuticle composition is required to elucidate the potential role of *WXP1* on cutin biosynthesis.

EXAMPLE 8

Overexpression of *WXPI* confers drought tolerance in transgenic plants

Overexpression of transcription factor genes *DREB/CBF* in *Arabidopsis* activated C-repeat/DRE containing downstream genes that are involved in cold acclimation and drought adaptation. Although the transgenic *Arabidopsis* showed increased stress tolerance, no additional waxes were produced (Jaglo-Ottosen *et al.*, 1998; Kasuga *et al.*, 1999). Thus, the mechanism of *DREB/CBF* genes on drought tolerance improvement is different from that of *WXPI*. Overexpression of *WXPI* activated wax production and led to the glaucousness appearance in leaves of transgenic alfalfa. Glaucousness resulting from wax accumulation has been considered a beneficial trait for the adaptation of plants to water-limited environments (Jefferson, 1994). Under severe water deficient conditions, plant stomata normally close. The survival of a plant will then depend largely on its ability to restrict water loss through leaf epidermis (Rawson and Clarke, 1988). The transgenic alfalfa plants showed reduced water loss and decreased epidermal permeability, therefore the transgenic plants were much more drought tolerant than the control plants. Genetic and mutant studies have suggested that wax accumulation is a potential drought adaptation trait (Jefferson, 1994). The studies here using isogenic lines (wild type control, empty vector control and transgenic plants) clearly demonstrate the positive effects of cuticular waxes on drought tolerance. Despite the fact that both drought tolerance and wax accumulation are complicated traits that are under the control of multiple genes. The results demonstrated for the first time that overexpression of a single transcription factor gene, *WXPI*, could increase wax production and improve plant drought tolerance.

Alfalfa is the fourth most widely grown crop in the United States behind only corn, wheat and soybeans. It contributes enormously to world's dairy, beef and wool production, although the contribution often goes unrecognized. As a perennial forage crop, alfalfa is a fairly hardy species and has a relative high level of drought tolerance compared to many food crops. Even so, increased wax loading by overexpression of *WXPI* on alfalfa leaves further enhances its drought and dehydration tolerance. Thus, manipulation of wax production by transgenic expression of *WXPI* or its orthologs has significant potential for the genetic improvement of other forage, food or horticultural crops. Although transgenic alfalfa plants showed moderate slow growth

due to increased wax accumulation, the problem may be overcome by the use of epidermis-specific promoter (Hooker *et al.*, 2002) or drought inducible promoter (Kasuga *et al.*, 1999; Kasuga *et al.*, 2004). The use of stress-inducible promoter has been shown to minimize the negative effects of *DREB/CBF* overexpression on plant growth (Kasuga *et al.*, 1999; Kasuga *et al.*, 2004).

EXAMPLE 9

Growth and development of *Arabidopsis* plants overexpressing *WXP1* and *WXP2*

Expression of *WXP1* and *WXP2* did not lead to severe growth retardation of transgenic *Arabidopsis* as *AtWIN1/AtSHINE1* (Aharoni *et al.*, 2004; Broun *et al.*, 2004), *AtCBF1/DREB1b* (Jaglo-Ottosen *et al.*, 1998), *AtCBF3/DREB1a* (Kasuga *et al.*, 1999; Gilmour *et al.*, 2000) and *AtCBF4* (Haake *et al.*, 2002) did. The transgenic plants with *WXP1* gene overexpression did not show a significant change in plant growth rate and flowering date. Nevertheless, the plants have broader leaves with more glaucous leaf surfaces compared with the control plant (Figure 16A-16D). The transgenic plants with *WXP2* overexpression showed more morphological changes. The plants were shorter and flowered one week later than the control (Figure 16E).

EXAMPLE 10

WXP1 and *WXP2* overexpression in *Arabidopsis* resulted in leaf cuticular wax alteration

WXP1 and *WXP2* conferred cuticular wax alteration in rosette leaves when overexpressed in *Arabidopsis* (Tables 2 and 3). The total wax for the 3-week-old plants increased 32.36% with *WXP1* overexpression and 16.96% with *WXP2* overexpression. For the 6-week-old plants, total leaf wax increased 27.51% and 23.61% with the overexpression of *WXP1* and *WXP2*, respectively.

The wax increase in both cases was contributed by the increase of alkanes that are the major cuticular wax components of *Arabidopsis*. For the 3-week-old plants, the total alkanes accumulation in leaves changed from 52.95 ± 3.11 micrograms per gram fresh weight ($\mu\text{g/g FW}$) in the vector control plants to 77.14 ± 2.11 $\mu\text{g/g FW}$ in the *WXP1* overexpressors and 72.38 ± 2.78 $\mu\text{g/g FW}$ in the *WXP2* overexpressors, increased by 45.68% and 36.71%, respectively (Table 2). For the rosette leaves from the 6-week-old *Arabidopsis* plants, the total alkanes changed from 0.391 ± 0.0319

micrograms per square centimeter leaf surface (including both adaxial and abaxial sides, $\mu\text{g}/\text{cm}^2$) in the vector control plants to $0.543 \pm 0.0393 \mu\text{g}/\text{cm}^2$ in the *WXP1* overexpressors and $0.541 \pm 0.0259 \mu\text{g}/\text{cm}^2$ in the *WXP2* overexpressors, increase by 36.38% and 38.19%, respectively (Table 3).

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Table 2. Cuticular Wax Composition Change of Rosette Leaves from 3-week-old Transgenic *Arabidopsis* Plants

Compound Class	VC ^a ($\mu\text{g}/\text{g}$ FW) n=6	MtWXP1 ($\mu\text{g}/\text{g}$ FW) n=5 $\times$ 4 ^b	Average Percent increase	MtWXP2 ($\mu\text{g}/\text{g}$ FW) n= 5 $\times$ 4 ^b	Average percent increase
Fatty acids	11.85 $\pm$ 0.41	15.54 $\pm$ 0.38	31.14**	11.88 $\pm$ 0.73	0.22
Aldehydes	8.82 $\pm$ 0.78	9.45 $\pm$ 0.30	7.14	9.53 $\pm$ 0.58	8.01
Prim. alcohols	23.18 $\pm$ 1.01	24.80 $\pm$ 0.82	6.99	19.73 $\pm$ 1.03	-14.88*
Sec. alcohols	4.96 $\pm$ 0.17	7.75 $\pm$ 0.20	56.40**	5.49 $\pm$ 0.37	10.81
Alkanes	52.95 $\pm$ 3.11	77.14 $\pm$ 2.11	45.68**	72.38 $\pm$ 2.78	36.71**
Cholesterol	2.21 $\pm$ 0.09	1.68 $\pm$ 0.06	-24.00**	1.28 $\pm$ 0.05	-42.36**
Sterols	4.65 $\pm$ 0.24	4.02 $\pm$ 0.13	-13.72	3.52 $\pm$ 0.14	-24.33*
Sitosterol	9.60 $\pm$ 0.31	12.67 $\pm$ 0.53	31.91**	13.63 $\pm$ 0.79	41.90**
Unidentified	3.40 $\pm$ 0.14	3.25 $\pm$ 0.20	-4.43	3.52 $\pm$ 0.18	3.48
Total	121.64 $\pm$ 3.47	156.30 $\pm$ 3.23	28.50**	140.96 $\pm$ 5.23	15.89*
Total Wax	101.76 $\pm$ 3.40	134.69 $\pm$ 2.67	32.36**	119.02 $\pm$ 4.44	16.96*

^aEmpty vector control.

^bData from five independent transgenic lines, which had four replicates for each line.

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Table 3. Cuticular Wax Composition Change of Rosette Leaves from 6-week-old Transgenic *Arabidopsis* Plants

Compound Class	VC ^a ($\mu\text{g}/\text{cm}^2$) n=6	MtWXP1 ($\mu\text{g}/\text{cm}^2$) n=5 $\times$ 4 ^b	Average percent increase	MtWXP2 ($\mu\text{g}/\text{cm}^2$) n= 5 $\times$ 4 ^b	Average percent increase
Fatty acids	0.116 $\pm$ 0.0047	0.120 $\pm$ 0.0105	3.35	0.133 $\pm$ 0.0051	14.83*
Aldehydes	0.021 $\pm$ 0.0032	0.017 $\pm$ 0.0022	-18.46**	0.038 $\pm$ 0.0033	80.33**
Prim. alcohols	0.092 $\pm$ 0.0108	0.120 $\pm$ 0.0113	30.70**	0.055 $\pm$ 0.0033	-40.24**
Alkanes	0.391 $\pm$ 0.0319	0.534 $\pm$ 0.0393	36.38**	0.541 $\pm$ 0.0259	38.19**
Cholesterol	0.032 $\pm$ 0.0038	0.020 $\pm$ 0.0017	-36.61**	0.023 $\pm$ 0.0014	-28.29**
Unidentified	0.037 $\pm$ 0.0034	0.033 $\pm$ 0.0032	-12.18*	0.044 $\pm$ 0.0032	18.35**
Total	0.689 $\pm$ 0.0352	0.844 $\pm$ 0.0605	22.43**	0.834 $\pm$ 0.0338	20.95**
Total Wax	0.620 $\pm$ 0.0395	0.791 $\pm$ 0.0576	27.51**	0.767 $\pm$ 0.0311	23.61**

^aEmpty vector control.

^bData from five independent transgenic lines, which had four replicates for each line.

EXAMPLE 11

Arabidopsis plants overexpressing *WXP1* and *WXP2* were more drought tolerant

Overexpression of both *WXP1* and *WXP2* in *Arabidopsis* lead to increased drought tolerance as demonstrated by whole plant drought tolerance assays and the fresh weigh loss of detached leaves (FWLDL). When compared with the vector controls, the transgenic lines survived a two week drought stress much better (Figure 17). From two independent studies that had three replicates each, only 7.6% of control plants survived. However, the survival rate for the *WXP1* transgenic lines were from 63.9% to 80.6%, and the survival rate for the *WXP2* transgenic lines were 100%.

The assay for fresh weight loss of detached leaves may partially explain the results from the whole plant drought tolerance assay. The fresh weight loss from both *WXP1* and *WXP2* transgenic lines were significantly lower than that of controls at most of the timepoints (Figure 18A and 18B).

EXAMPLE 12

Materials and Methods

A. Construction of pC35S-*WXP1* vector and alfalfa transformation

The coding sequence of *WXP1* from *Medicago truncatula* (genotype Jemalong A17) was PCR amplified using primers 5'-GGTACCATGGATTTCTTCAACA-3' (forward) (SEQ ID NO:3) and 5'-AACCGGTCACCA AATTCATCCA-3' (reverse) (SEQ ID NO:4) and digested by Nco I and BstE II. The fragment was inserted into pCAMBIA 3301 vector by replacing the *gusA* gene. The resulting binary vector pC35S-*WXP1* was transferred into *Agrobacterium tumefaciens* stain C58C1 using freezing/heat shock method.

An alfalfa genotype, Regen SY-4D, was used for *Agrobacterium*-mediated transformation to generate transgenic plants (Austin *et al.*, 1995). Vegetatively propagated plants from the original Regen SY-4D clone were used as wild type control. Alfalfa lines transformed with the original pCAMBIA3301 vector were used as vector control.

B. Growth and treatments of *Medicago truncatula*

Medicago truncatula (genotype Jemalong A17) seeds were pre-germinated and planted in 4.5-inch pots filled with Turface MVP clay (Profile Products LLC, Buffalo Grove, IL). Plants were grown in greenhouse at 24/22 °C with 16h/8h photoperiod and a relative humidity at ~70-80%. Four-week-old plants were used for drought, cold and ABA treatment. For drought stress treatment, watering was ceased for one to four days, which represented very mild, mild, moderate, and severe drought stresses, respectively. Additional severely stressed plants were re-watered and tissue was sampled 24 hours later as recovering treatment. Cold treatment was performed by transferring plants to a 4°C cold room, and leaf tissues were collected at different timepoints. Samples from recovered tissues were also collected 24h after transferring the plants back to greenhouse. For ABA treatment, 100 µM ABA (mixed isomers) in 0.02% Tween-20 water solution was sprayed on leaves and also poured into the pots. Controls were sprayed and watered with the same solution without ABA. Leaf tissues were sampled at different timepoints.

C. Growth and treatments of alfalfa

The transgenic or control plants were propagated using shoot cuttings. Root system was developed and seedlings established 2-3 weeks after transferring the shoot cuttings to Oasis® Rootcubes® Growing Medium (Smithers-Oasis U.S.A., Kent, OH). Seedlings were transplanted to 4.5-inch pots filled with BM-7 bark mix (Berger, Saint-Modeste, Quebec, Canada). For the analysis of wax content, chlorophyll leaching and leaf water loss rate, samples were taken from plants with 6-7 fully expanded trifoliate leaves on the major stem and 4-6 trifoliate leaves on the branches. All the plants were grown in a greenhouse at 23/19°C with 14h/10h photoperiod and relative humidity at ~50%. Flowering time was measured as the days from transplanting to the emergence of first flower. Plant height and fresh weight was measured when all the plants flowered after 80 days of growth. Dry matter data was obtained by drying individual plants for 48 hours at 60 °C.

For THE drought tolerance study, seedlings were transplanted to 4.5-inch pots and grown in growth chamber at 23/19°C with 16h/8h photoperiod and relative humidity at 60%. After twenty days of growth, plants (in three replicates) were drought stressed by stopping watering. In the mean time, humidity was re-set to 20%

and photoperiod was re-set to 8h/16h in order to keep the stomata closed most of the time. Drought tolerance phenotype was recorded 3-4 days later. When the control plants became totally dried or dead-like (~10 days after watering was withheld), all the pots were re-watered and humidity was re-set to 60%. Plant recovery was scored
5 two weeks later. In the greenhouse experiment, the plants were subjected to three cycles of drought-recovery treatment. During each cycle of drought-recovery treatment, plants were drought stressed for 7 days and watering resumed for 2 days.

D. Growth and treatments of *Arabidopsis*

Arabidopsis thaliana ecotype Colombia was used to generate transgenic
10 plants. All genes, including the *GusA* (as empty vector control (VC)) and *WXP1* and *WXP2* were driven by a CaMV 35S promoter.

T2 and T3 seeds were sterilized with 20% bleach (with 0.1% SDS) and rinsed three times in sterile water. The re-suspended seeds in 0.1 % agarose solution were uniformly spread on MS medium with appropriate selection. Plates were put under 4
15 °C for 48-72 h before growing the plants under 22 °C with 16/8 h light/dark. One week old seedlings were transferred to appropriate medium or to soil.

For normal growth and detached leaf sampling, Sunshine Professional Mix 350 soil (Sun Gro Horticulture Distribution Inc., Bellevue, WA) was used. For whole plant drought and freezing tolerance assay, a customized soil mixture comprised five
20 parts (in volume) of Sunshine Professional Growth Mix #4, four parts of medium size vermiculite (both from Sun Gro Horticulture Distribution Inc., Bellevue, WA) and one part of washed medium sand (No. 1962, The Quikrete Companies, Inc., Atlanta, GA) was used. Before and after stress treatment and for leaf sampling, all the plants were grown in growth chamber under long-day conditions (16 h light/8 h dark) with
25 cool-white light ($125 \mu\text{mol m}^{-2} \text{s}^{-1}$), consistent temperature (22 °C) and relative humidity (80%) except for during the period of whole plant drought stress treatment.

E. Whole Plant Drought Tolerance Assay of Transgenic *Arabidopsis*

One week after transplanting, transgenic *Arabidopsis* plants (12 individuals/4.5-inch-pot) were subjected to drought stress by stopping watering. Three
30 days later, the growth chamber was re-set to short-day condition (8 h light/16 h dark) and a relative humidity of 20%. Plants were rehydrated and observed for recovery after 1 week. The study was repeated twice.

F. Assay for fresh weight loss from detached leaves of transgenic *Arabidopsis* plants

For water loss measurements, leaves of 4-week-old plants were detached and weighed immediately in a plastic weighing boat (pre-dried overnight at 65 °C and pre-weighted). The weighing boats with the leaves then were placed on a laboratory bench in a dark room (room temperature was at 22±1 °C and relative humidity at 23±1%) and weighed at designated time intervals. The percentage loss of fresh weight was calculated based on the initial weight of the samples. There were three replicates for each line. The same design repeated twice in similar conditions.

G. RNA gel blotting and hybridization

Total RNA was extracted with MRC Tri-Reagent[®] (Molecular Research Center, Inc., Cincinnati, Ohio). Twenty micro-grams of RNA were loaded in each lane of 1.2% agarose gels with formaldehyde. *WXPI* cDNA and other wax biosynthesis related genes from *M. truncatula* was ³²P labeled using the RanPrime DNA Labeling System (Invitrogen, Carlsbad, CA) as instructed by the manufacturer. Northern hybridization was conducted using High Efficiency Hybridization System and Washing/Pre-Hyb solution (Molecular Research Center) following manufacturer's instructions.

H. Bioinformatics

Multiple sequence alignment was performed with Clustal W (Thompson *et al.*, 1994) version 1.82 through EMBL-EBI Sequence Analysis launcher using default parameters (www.ebi.ac.uk/clustalw/). Rooted phylogenetic tree was displayed by TreeView program with PHYLIP method. Sequence similarity was calculated with MegAlign program of DNASTAR (Madison, WI). The box-shade in sequence alignment was created using BOXSHADE 3.21 (www.ch.embnet.org/software/BOX-form.html).

I. Scanning electron microscopy (SEM)

The top-first and the second trifoliates and the top two internodes from the major stem were harvested and air-dried at room temperature. The middle section between the leaf edge and the major vein of the leaflets or the middle of the internodes were mounted on stubs and coated with ~20 nm of 60/40 Gold-Palladium particles using a Hummer VI sputtering system (Anatech LTD, Springfield, VA).

Coated surfaces were viewed using a JEOL JSM-840 scanning electron microscope at 15 kV (Peabody, MA).

J. GC-MS analysis of cuticular wax composition

Leaf cuticular wax samples were collected from top four fully expanded trifoliates excised from the major stems. The stem cuticular wax samples were collected from the top four internodes. One leaflet was excised from each trifoliolate and the four leaflets were combined as one leaf sample. Four internodes were combined as one stem sample. Each sample was inserted into a 20-mL glass tube, and 10-mL (for leaves) or 5-mL (for stem) of hexane (Sigma-Aldrich, Inc., St. Louis, MO) was added. Tissues were agitated for 2 min on a rotator at 50 rpm, and the solvent was decanted into new glass tubes. Tissues and tubes were given a 10-s rinse with the same amount of hexane, and the solution was combined in the new tubes. The hexane-soluble wax extracts were evaporated to a small volume (~ 1mL) under a nitrogen stream and then transferred into 2-mL auto-sampler vials. After complete evaporation in the 2-mL vials, the extracts were resuspended/derivatized in 15.0 μ L of 70% Pyridine and 30% MSTFA (with 0.01 μ g/ μ L of cholesterol as internal standard) for every one cm^2 leaf section or one cm^2 stem surface area. Derivatization was performed for 60 min at 50°C. One microliter of the solution was injected onto an Agilent 6890 gas chromatograph in splitless mode. The injector was held at 280°C, the oven programmed from 120°C (2 min) to 315°C (8min) at 5°C/min, and the transfer arm held at 250 °C. The GC was coupled to an Agilent 5973 MSD using electron impact ionization with scanning masses 50-650. Duplicate injections were performed for each sample, and the average value of the two injections was used for statistical analysis. The peak value was extracted using Agilent Chemstation integration of the total ion chromatogram.

Quantification was based on peak areas and the variation in MS sensitivity was adjusted based on the internal standard as described by Jenks *et al* (1995) and Bergman *et al* (1991) with modifications. Dose responsive curve for correction were developed for each class of wax components: heptadecanoic acid for free fatty acids, tridecanal for aldehydes, hentriacontane for alkanes, docosanol for primary alcohols, and cholesterol for sterols, triterpenes and other unknown peaks. The amount of each cuticular wax component and total wax composition was expressed per unit of leaf or stem surface area. Leaf areas were determined using computer digitization of the leaf

images by scanning (NIH ImageJ 1.31t). Stem surface areas were calculated as the surfaces of right circular cylinders for every internodes (Chen *et al.*, 2003). All values represent averages of six replicate plant samples $\pm$ SD.

K. Quantification of epidermal traits

5 To quantify leaf water loss rate, the top-2nd and top-3rd trifoliates were detached from 20-d old alfalfa after the plants were kept in constant dark for 10 hours. Dehydration and measurement was performed in a dark room at 23°C. Humidity was around 50% in the room.

Epidermis permeability was measured using chlorophyll extraction assay.
10 Three trifoliates or stem internode segments from the same stem position were collected from 20-d old alfalfa plants, and immersed in 50-mL tubes with 15 mL (for trifoliates) or 10 mL (for internodes) of 80% ethanol. Tubes were agitated gently on a rotator platform at 50 rpm. Aliquots of 1000 μ L were taken out for chlorophyll quantification and poured back to the same tube at every timepoint. The amount of
15 chlorophyll extracted into the solution was quantified using a U-60 spectrophotometer (Beckman, Fullerton, CA) and calculated from light absorption at wavelength of 647 and 664 nm as described by Lolle *et al.* (1997). Chlorophyll extracted at each time point was expressed as a percentage of total chlorophyll extracted after 48 hours of immersion.

20 * * *

All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations
25 may be applied to the compositions and methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such
30 similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

REFERENCES

The references listed below are incorporated herein by reference to the extent that they supplement, explain, provide a background for, or teach methodology, techniques, and/or compositions employed herein.

- 5 U.S. Patent No. 4,535,060; U.S. Patent No. 5,302,523; U.S. Patent No. 5,322,783; U.S. Patent No. 5,384,253; U.S. Patent No. 5,464,765; U.S. Patent No. 5,508,184; U.S. Patent No. 5,508,468; U.S. Patent No. 5,538,877; U.S. Patent No. 5,538,880; U.S. Patent No. 5,545,818; U.S. Patent No. 5,550,318; U.S. Patent No. 5,563,055; U.S. Patent No. 5,591,616; U.S. Patent No. 5,610,042
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CLAIMS

1. An isolated nucleic acid sequence selected from the group consisting of:
 - (a) a nucleic acid sequence encoding the polypeptide of SEQ ID NO:1;
 - (b) a nucleic acid sequence encoding the polypeptide of SEQ ID NO:5,
 5 SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, or SEQ ID NO:45, wherein
 10 the nucleic acid is operably linked to a heterologous promoter functional in plants;
 - (c) a nucleic acid sequence comprising the sequence of SEQ ID NO:2;
 - (d) a nucleic acid sequence comprising the sequence of SEQ ID NO:6,
 15 SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, or SEQ ID NO:46, wherein
 20 the nucleic acid sequence is operably linked to a heterologous promoter functional in plants;
 - (e) a nucleic acid sequence that hybridizes to SEQ ID NO:2 under conditions of 5X SSC, 50% formamide and 42°C and encodes a polypeptide that activates wax biosynthesis;
 - (f) a nucleic acid sequence that hybridizes to SEQ ID NO:6, SEQ ID
 25 NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16, SEQ ID NO:18, SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:24, SEQ ID NO:26, SEQ ID NO:28, SEQ ID NO:30, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:36, SEQ ID NO:38, SEQ ID NO:40, SEQ ID NO:42, SEQ ID NO:44, and/or SEQ ID NO:46 under
 30 conditions of 5X SSC, 50% formamide and 42°C and encodes a polypeptide that activates wax biosynthesis, wherein the nucleic acid sequence is operably linked to a heterologous promoter functional in plants;

- (g) a nucleic acid sequence encoding a polypeptide with at least 65% amino acid identity to SEQ ID NO:1 that activates wax biosynthesis;
- (h) a nucleic acid sequence encoding a polypeptide with at least 65% amino acid identity to SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19, SEQ ID NO:21, SEQ ID NO:23, SEQ ID NO:25, SEQ ID NO:27, SEQ ID NO:29, SEQ ID NO:31, SEQ ID NO:33, SEQ ID NO:35, SEQ ID NO:37, SEQ ID NO:39, SEQ ID NO:41, SEQ ID NO:43, or SEQ ID NO:45 that activates wax biosynthesis, wherein the nucleic acid sequence is operably linked to a heterologous promoter functional in plants; and
- (i) the complement of a sequence of (a)-(h).
2. The isolated nucleic acid sequence of claim 1, wherein the nucleic acid sequence is operably linked to a heterologous promoter.
3. A recombinant vector comprising the isolated nucleic acid sequence of claim 1.
4. The recombinant vector of claim 3, further comprising at least one additional sequence chosen from the group consisting of: a regulatory sequence, a selectable marker, a leader sequence and a terminator.
5. The recombinant vector of claim 4, wherein the additional sequence is a heterologous sequence.
6. The recombinant vector of claim 3, wherein the promoter is a plant developmentally-regulated, organelle-specific, inducible, tissue-specific, constitutive, cell-specific, seed specific, or germination-specific promoter.
7. The recombinant vector of claim 3, wherein the promoter is a drought-inducible promoter.
8. The recombinant vector of claim 3, defined as an isolated expression cassette.
9. An isolated polypeptide having at least 65% amino acid identity to the amino acid sequence of SEQ ID NO:1 or a fragment thereof having wax biosynthesis activity.

10. The isolated polypeptide of claim 9, comprising the amino acid sequence of SEQ ID NO:1 or a fragment thereof having wax biosynthesis activity.
11. A transgenic plant transformed with a selected DNA comprising the nucleic acid sequence of claim 1.
- 5 12. The transgenic plant of claim 11, further defined as a dicotyledonous plant.
13. The transgenic plant of claim 11, further defined as a monocotyledonous plant.
14. The transgenic plant of claim 11, further defined as an R₀ transgenic plant.
15. The transgenic plant of claim 11, further defined as a progeny plant of any generation of an R₀ transgenic plant, wherein the transgenic plant comprises the selected DNA.
- 10 16. A seed of the transgenic plant of claim 11, wherein the seed comprises the selected DNA.
17. A host cell transformed with a selected DNA comprising the nucleic acid sequence of claim 1.
- 15 18. The host cell of claim 17, wherein the host cell expresses a protein encoded by the selected DNA.
19. The host cell of claim 17, wherein the host cell is a plant cell.
20. A method of increasing drought tolerance in a plant comprising increasing leaf wax production in the plant.
- 20 21. A method of altering the wax content of a plant comprising introducing into the plant the recombinant vector of claim 3, wherein the nucleic acid is expressed in the plant.
22. The method of claim 21, wherein the coding sequence is operably linked to a heterologous promoter functional in the plant.
- 25 23. The method of claim 21, wherein the plant exhibits increased tolerance to water deficit relative to a plant of the same genotype lacking the coding sequence.
24. The method of claim 21, wherein introducing the coding sequence comprises plant breeding.

25. The method of claim 21, wherein introducing the coding sequence comprises genetic transformation.
26. A method of producing food for human or animal consumption comprising:
- 5 (a) obtaining the plant of claim 11;
- (b) growing the plant under plant growth conditions to produce plant tissue from the plant; and
- (c) preparing food for human or animal consumption from the plant tissue.
27. The method of claim 26, wherein preparing food comprises harvesting the plant tissue.
- 10 28. The method of claim 27, wherein the food is starch, protein, meal, flour or grain.

```

AtWIN1      1 -----
At5g11190   1 -----
LePti4      1 -----
NtERF1      1 -----
AtERF1      1 ----MSMTADSQSDYA-----FLESRRHILGESEPIlseSTASSVTQSCVTGQSI-----KPVYGRNPsfSKLY
Mt77128     1 MAAMNYYSNMQ-QFQFHDSDPFRGELMEVPEPFK-----SPSST-----STSPSSS-----TPSP-----SYSSSLSSP
AtRAP2.4    1 MAAMNLYTCsr-SFQ--DSG---GELMDA VPFKSVSDSPSSS-----SAASASA-----FLHP-----SAFSLPLP
MtWXP1      1 ----MDFFNNSQDPFN-----GELMEVPEPFKSSSISPSPPSLNSHLPSTSSSSSPFYSSTPPFFQPSFSPQSSSSSSSFPQP
AtCBF2      1 -----
AtCBF3      1 -----
AtCBF1      1 -----
AtCBF4      1 -----

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At5g11190   1 -----
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AtERF1      62 PCFTS-----GDLPLK-----ENDSEDVLYGLNDAFHGGWEPSSSSSEDRSSFSPVKIETPESFAAVDSIPV
Mt77128     61 SFYTFQ-----NFIG--AQPSSS-----FSSPSLIGLNHTPSQINQIQVQIQQNFTQHQQIQQQQRCLSNLTLSPK
AtRAP2.4    60 GYYP-----TFLTQPSYGSDL-----QQTGSLIGLNHSSSQIHQIQSQIHHPPLPPTHNNNNSFSNLLRPKPLLMKQSG
MtWXP1      79 NFYFNGYCSSMMNYQPSLGNSTSQNNFIFGEQPPQPNVGLNLTTPSQINQIQAQIQFQ---QTQSNSSSLNPLGPKPIPMKQP-
AtCBF2      1 -----MNSF-SAFSEFG--SDYESPVSSGGDYSPK
AtCBF3      1 -----MNSF-SAFSEFG--SDYESVSSGGDYIPT
AtCBF1      1 -----MNSF-SAFSEFG--SDYEP---QGGDYCPT
AtCBF4      1 -----MNFYSTFPSPLSISDHRSPVSDSSECSPK

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                YRG motif          WLQ motif      RAYD motif
AtWIN1      1 -----MVOTKRGVQRHGWGKVAEIRHP-LLKRRWLGTFTAEAAARAYDEAAVLISGRNATNFFLNNNNTGETSEGK
At5g11190   1 -----MVESRKRGVQRQWGWVSEIRHP-LLKRRWLGTFTAEAAARAYDOALLNQNATNFFVVK--SEEGSD
LePti4      87 PSPAETTAPAAETPKGRHYRGVQRQWGWKVAEIRHP-LLKRRWLGTFTAEAAARAYDAAYRGRSKALLNFP-----HRI
NtERF1      80 VAPPAETTAAQAVVPGRHYRGVQRQWGWKVAEIRHP-LLKRRWLGTFTAEAAALAYDAAYRGRSKALLNFP-----HRI
AtERF1      129 KKEKTSFVSAAVTAAGRHYRGVQRQWGWKVAEIRHP-LLKRRWLGTFTAEAAALAYDAAYRGRSKALLNFP-----LRI
Mt77128     132 SIPMKHVGGSSV-SKPTGRHYRGVQRHGWKVAEIRLP-KNTRWLGTFTAEAAALAYDAAYLRGDFALNFFNLKHQSGIIGGEF
AtRAP2.4    133 VAGSCFAYGSGVPSKPTGRHYRGVQRHGWKVAEIRLP-KNTRWLGTFTAEAAALAYDAAYLRGDFALNFFNLRHNGSHIGGEF
MtWXP1      164 -----GVPPKPTGRHYRGVQRHGWKVAEIRLP-KNTRWLGTFTAEAAALAYDAAYLRGDFALNFFNMKDQ--QGGGMF
AtCBF2      32 SCPKKPAIRKKFRETHHPHYRGVQRNSGKWVCEIRP-NKKTRWLGTFTAEAAARAHDAALALRGRSACLNFDASAWR----LRI
AtCBF3      32 SCPKKPAIRKKFRETHHPHYRGVQRNSGKWVCEIRP-NKKTRWLGTFTAEAAARAHDAALALRGRSACLNFDASAWR----LRI
AtCBF1      29 SCPKKPAIRKKFRETHHPHYRGVQRNSGKWVCEIRP-NKKTRWLGTFTAEAAARAHDAALALRGRSACLNFDASAWR----LRI
AtCBF4      35 SCPKKPAIRKKFRETHHPHYRGVQRNSGKWVCEIRP-NKKTRWLGTFTAEAAARAHDAALALRGRSACLNFDASAWR----LRI

```

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AtWIN1      77 TIASSTMSSTSSSLSSSAKLRCKCKSPSPSLTCLRLDT---ASHGKVWOKRAGS-----KSDSSWVTVELG
At5g11190   75 KIVN-SPLMSP---KSLSEENAKLRCKCKDLTPSLTCLRLDT---SSHGKVWOKRAGS-----KTSPTWVRLLEL
LePti4      167 GLNE-----PEPVRITAKRRASPEPASSG-----NGSKRRKAV-----QKCDGEASRSS
NtERF1      160 GLNE-----PEPVRITAKRRS-PEPASSISSA-----LENGSPKRRKAVAA-----KKAELEWQSRSN
AtERF1      209 NSGE-----PDPVRKERRS-----FSSN-----ENGAPKKRTVA-----AGGCDKG--
Mt77128     220 GBFK-----PLPSSDAKLQAI CEGLAEQKQKGAEKPKKMPASKAKASSVVSKE-----ESVDDLKIDSEPEECKVE
AtRAP2.4    222 GBYK-----PLPSSDAKLQAI CECKSMAEIQKQDKSTK-----SKKREKVSSP-----DLSEKVKAE---ENSVSIG
MtWXP1      241 GBFK-----VLSSDAKLQAI CESLGNSSDNVNKKQKGSKKSLKEVEAQPPQPLVVVENNDDNNTNTNKVVVEC
AtCBF2      116 PST-----CADEQKAAAPALNFQDEMCHMTTDAHGLDMEELAEIYTPE-----QSQDAFYMDE
AtCBF3      116 PST-----CADEQKAAAPALAFQDEMCDATTD-HGFDMEELAEIYTAE-----QSENAFYMHD
AtCBF1      113 PST-----CADEQKAAAPALAFQDECDTTTTNHGLDMEELAEIYTPE-----QSEGAFFYMDE
AtCBF4      119 PETT-----CPMEQKAAAPALAFQDETTTEGSK-TAAEAEEAAGEGVREGERR-----ABEQNGCFYMD

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AtWIN1      148 PASSSQETSKASQALAPITTEVEIGGSRPEVLDEEKVALQMIEELLNTN---- 199
At5g11190   141 --MVNEAVDLGLTTNKQNVKEEEE-EETAIISDEQDLAMEMIEELLNWS---- 189
LePti4      215 VMQVGCQIEQLTGVHQLLTI----- 234
NtERF1      214 AMQVGCQMEQFPVHQLLWS----- 233
AtERF1      250 -LTMKCEVVEVARLRLGL----- 268
Mt77128     289 AVSYITEEGS-ESSPLSDLTFGDVGEF-QWEGDSNFNLLKPSYEIDWDSL-- 340
AtRAP2.4    282 GSPPVTEFEESTA-SSPLSDLTfADPEEPPQWN---ETFSLEKPSYEIDWDSILA 334
MtWXP1      319 GSSSSHSEGGSDSSPLSDLTfGEFAEP-QWNGTFQFNLOKPSYEIDWASL-- 371
AtCBF2      174 EAMGMSLLDNMAGNLPPSPVQWNNYFVVGDD-VSLWS----- 216
AtCBF3      173 EAMFEMPILLANMAGNLPLPSVQWNNHNVVGDDVDVLSW----- 216
AtCBF1      171 ETMFGMPLLDNMAGNLPPSPVQWNNHNYVGGDG-VSLWS----- 213
AtCBF4      181 EALLGMPNFFENMAGNLPPPEVGWNHN-DFGVGV-VSLWSFDE----- 224

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FIG. 1

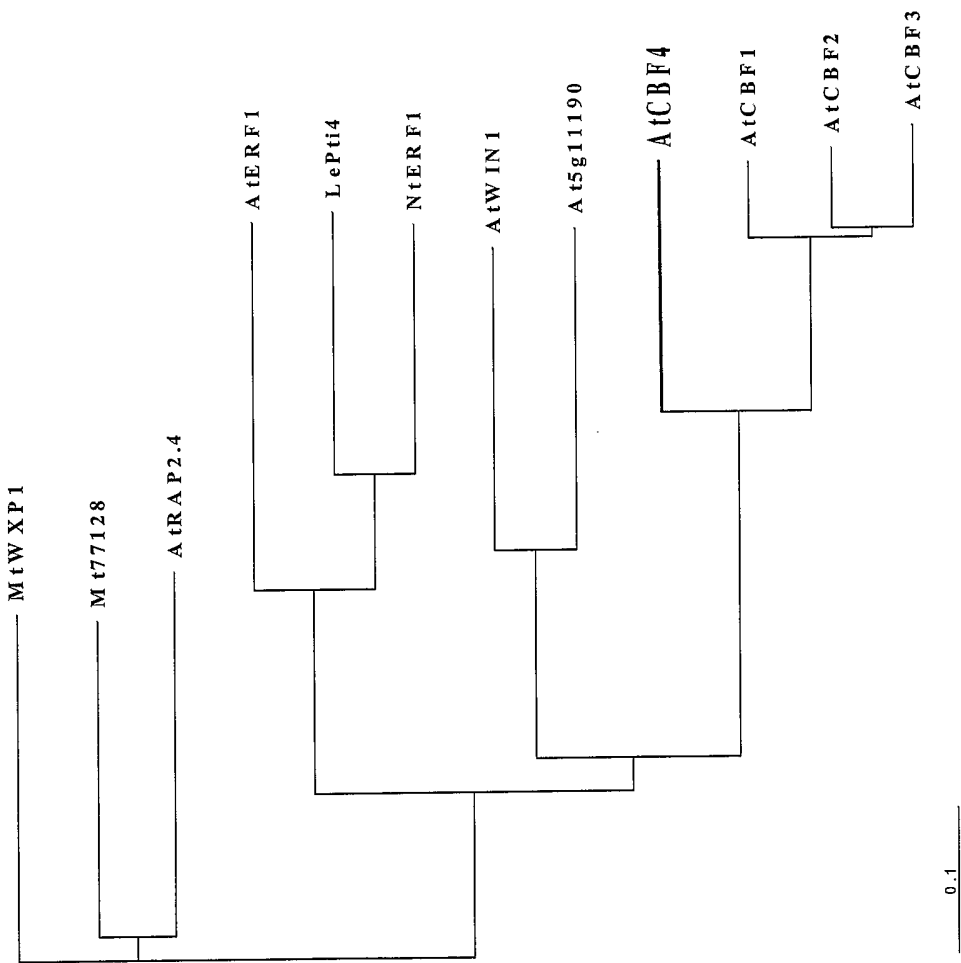


FIG. 2

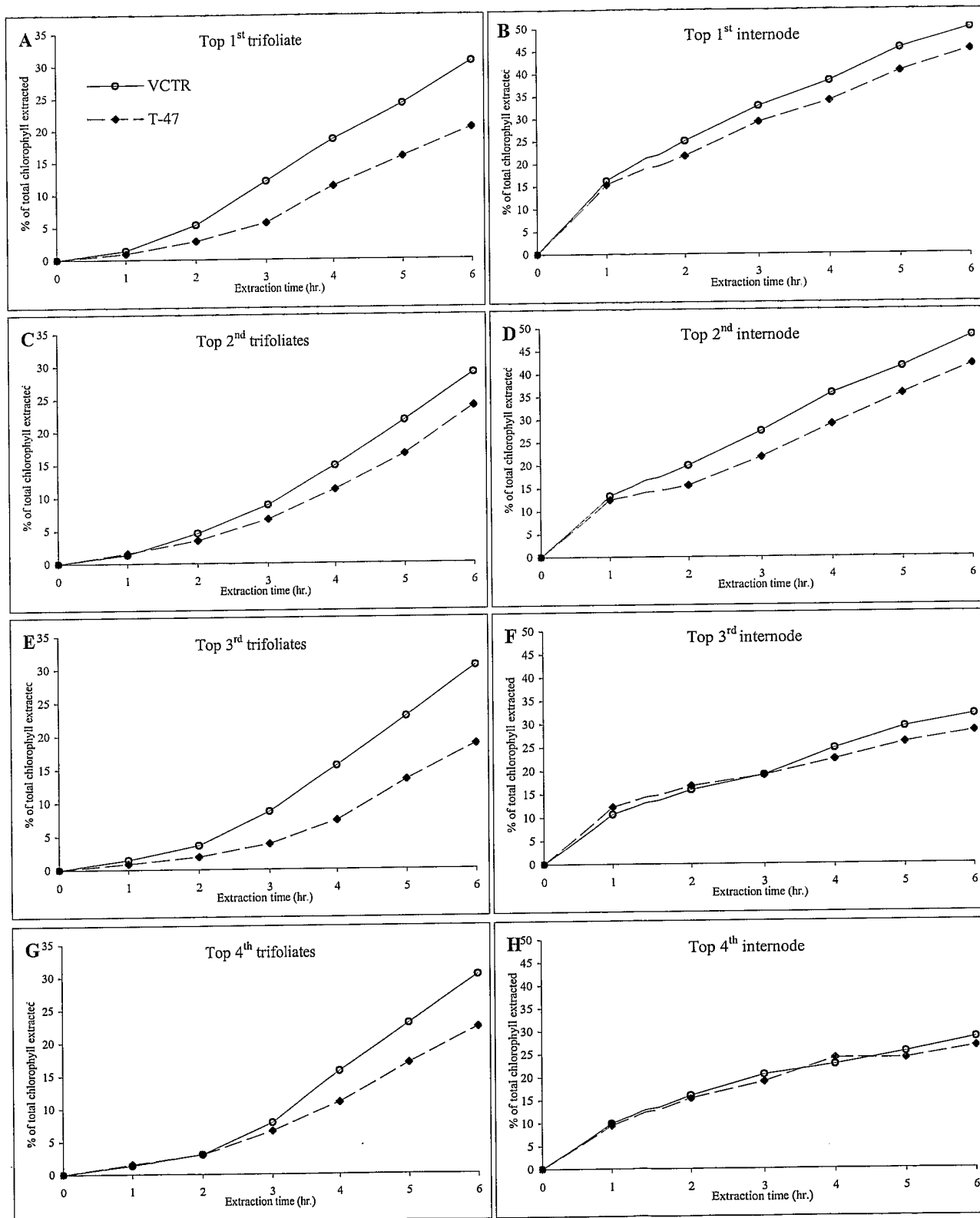


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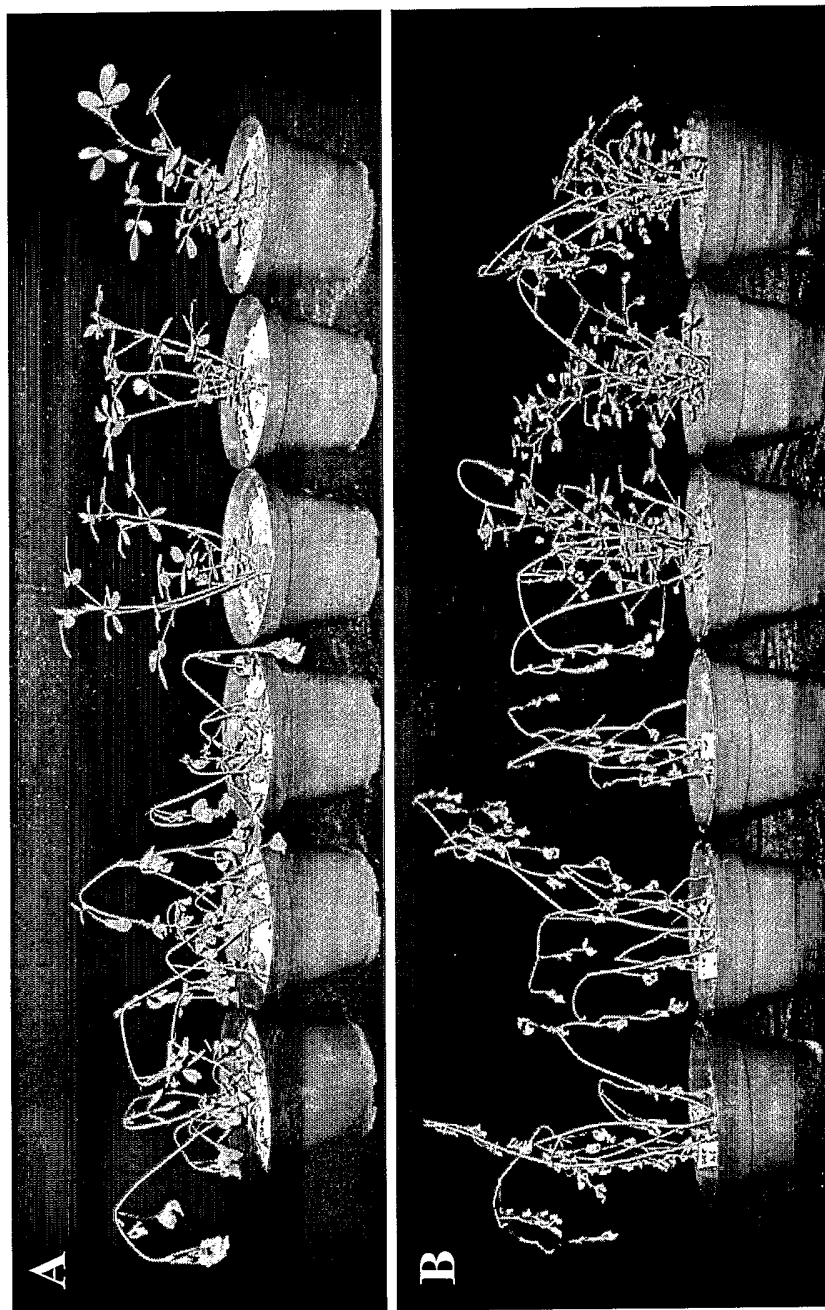
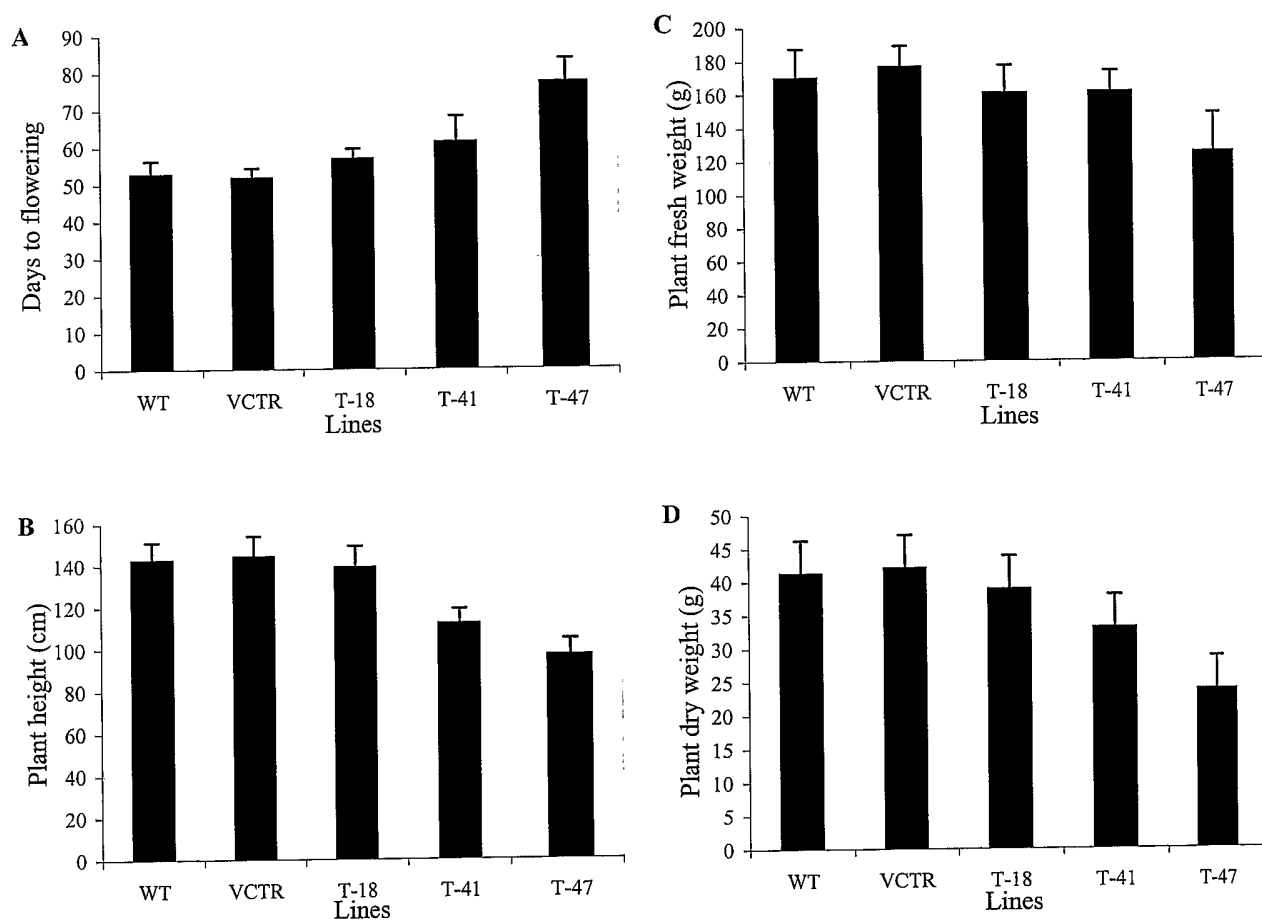
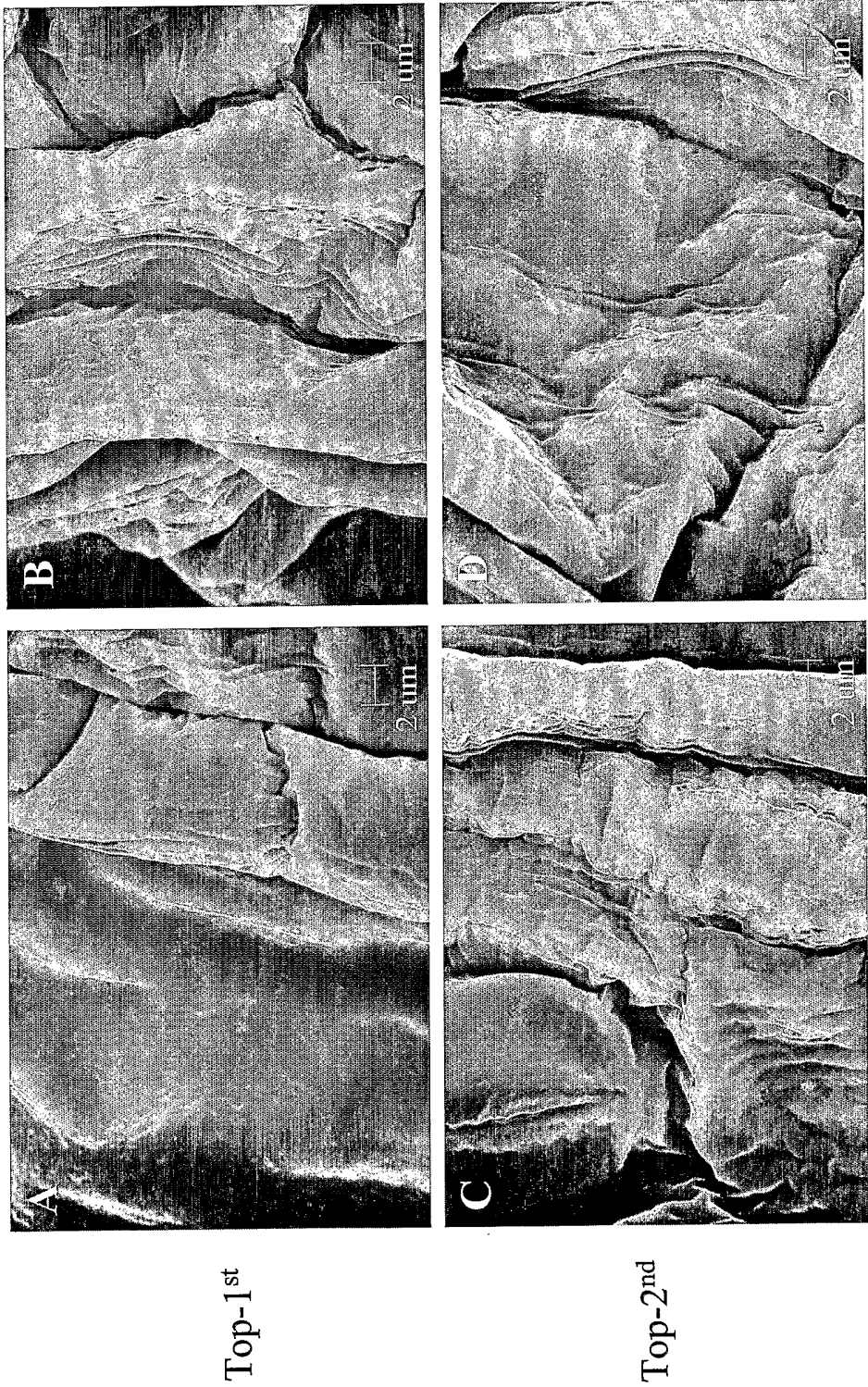


FIG. 10

**FIG. 11**



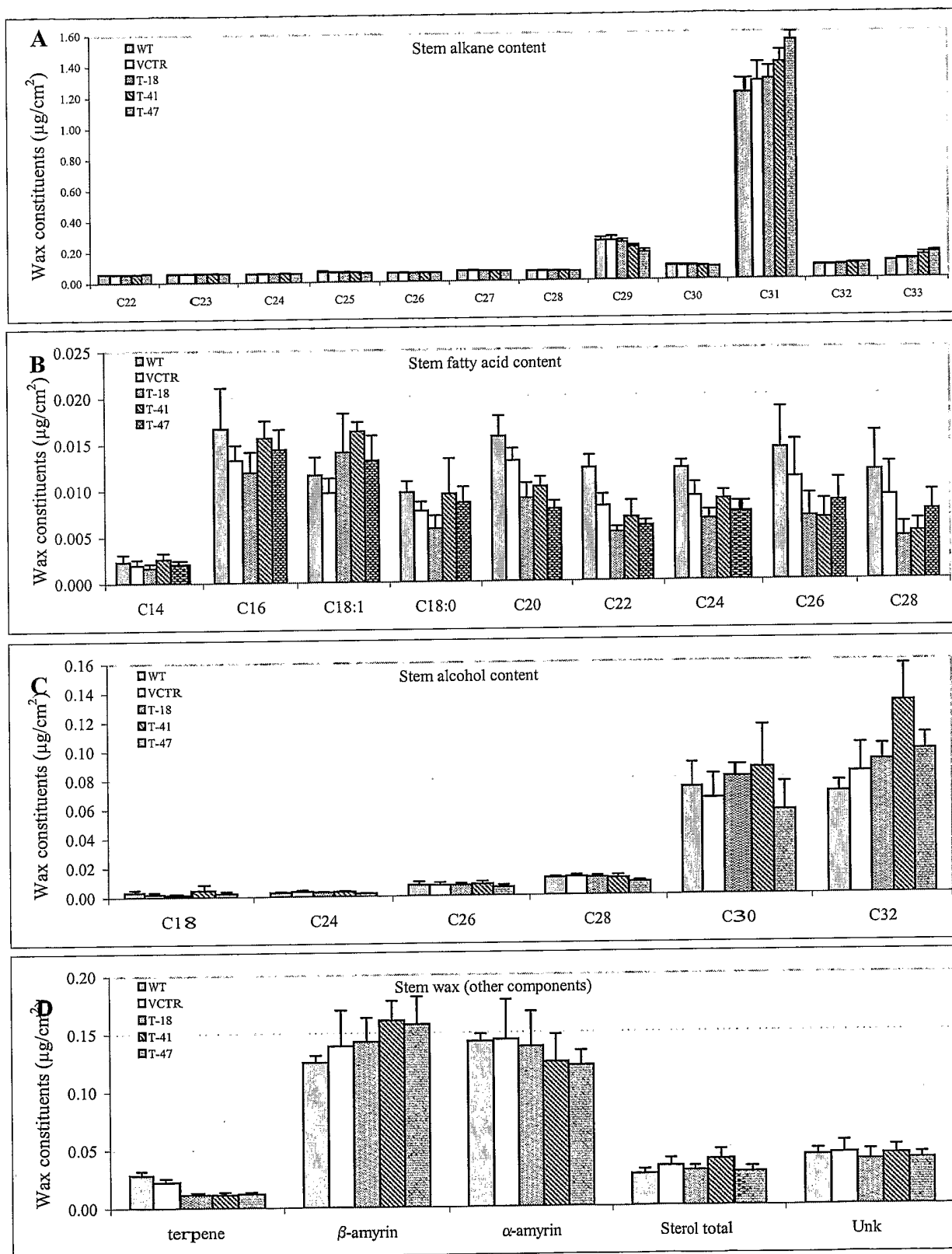


FIG. 13

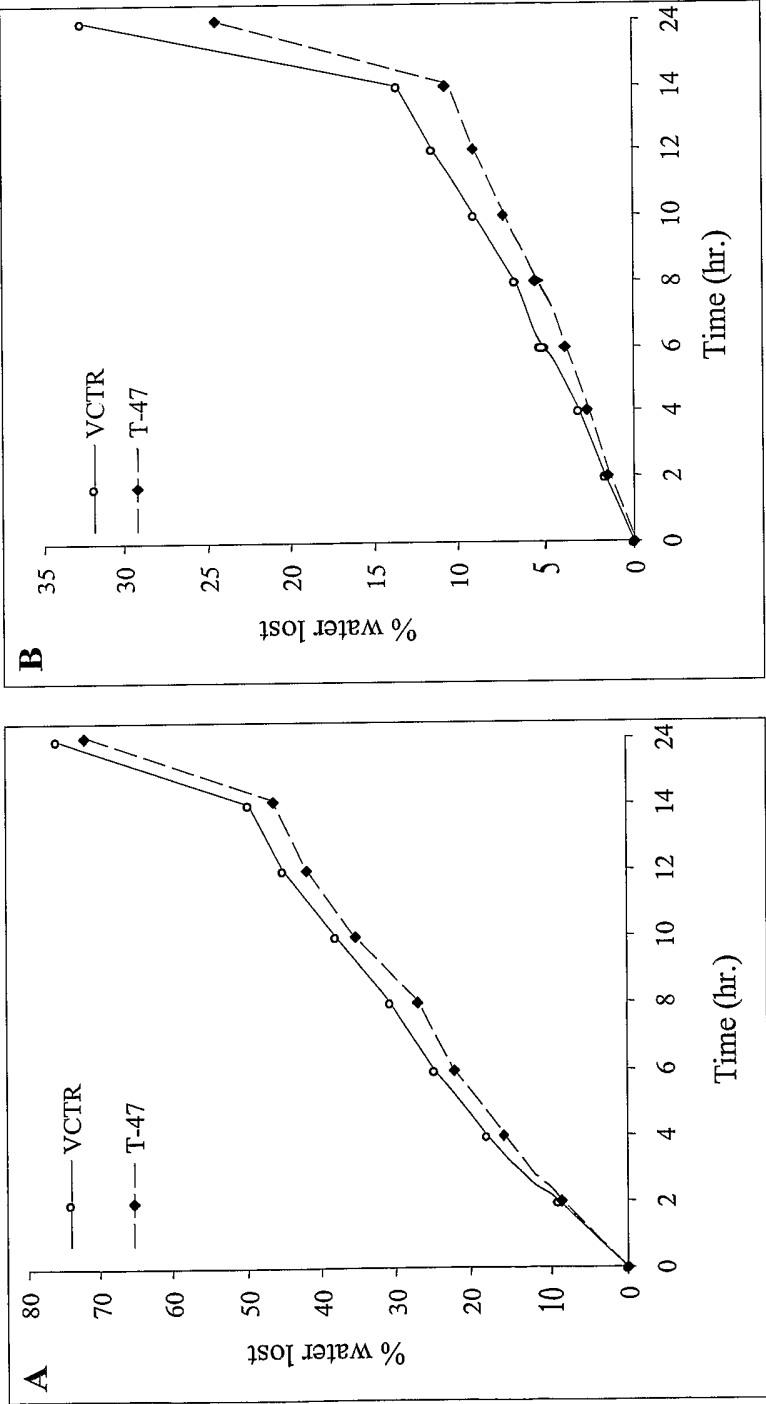


FIG. 14

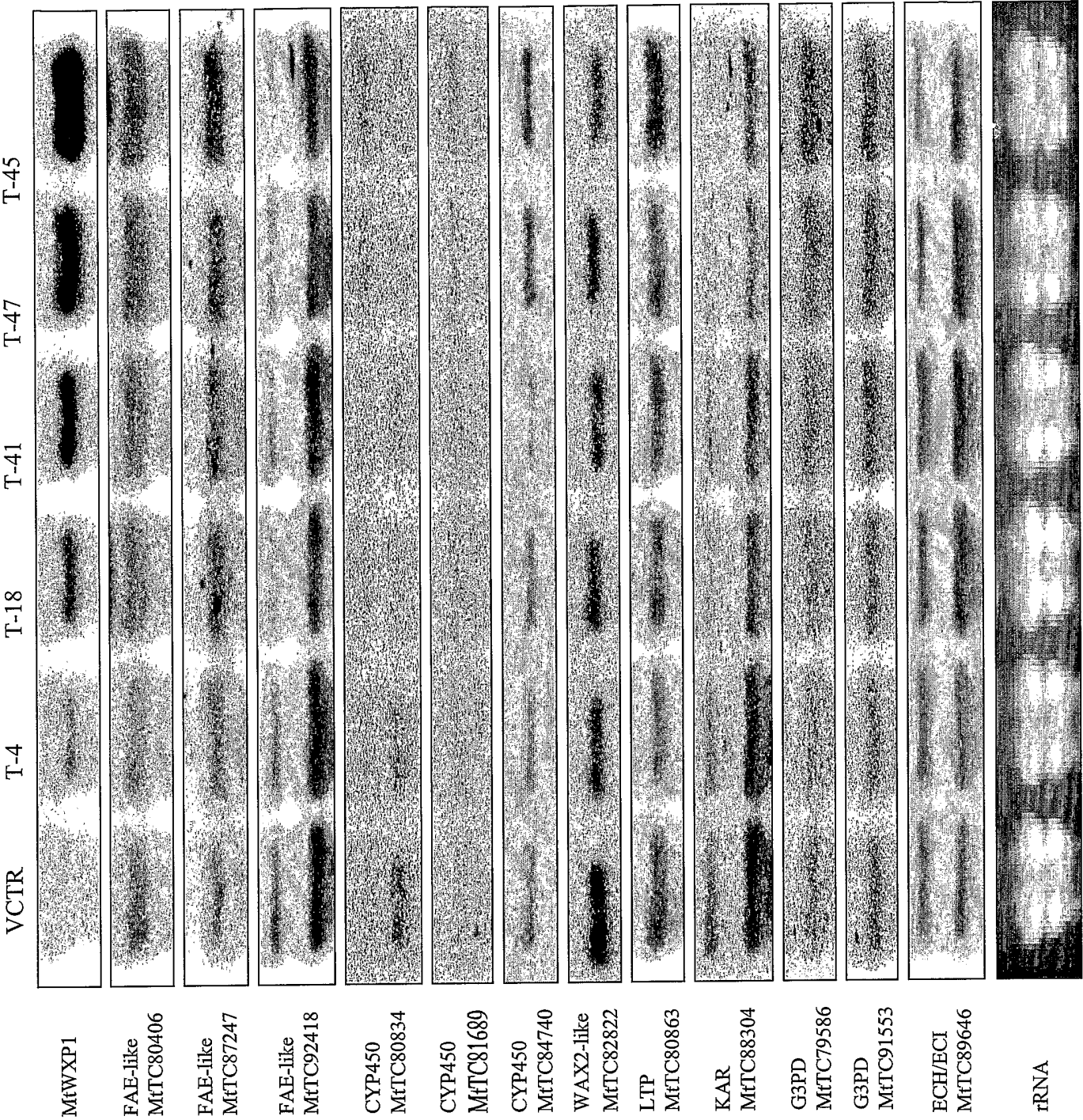


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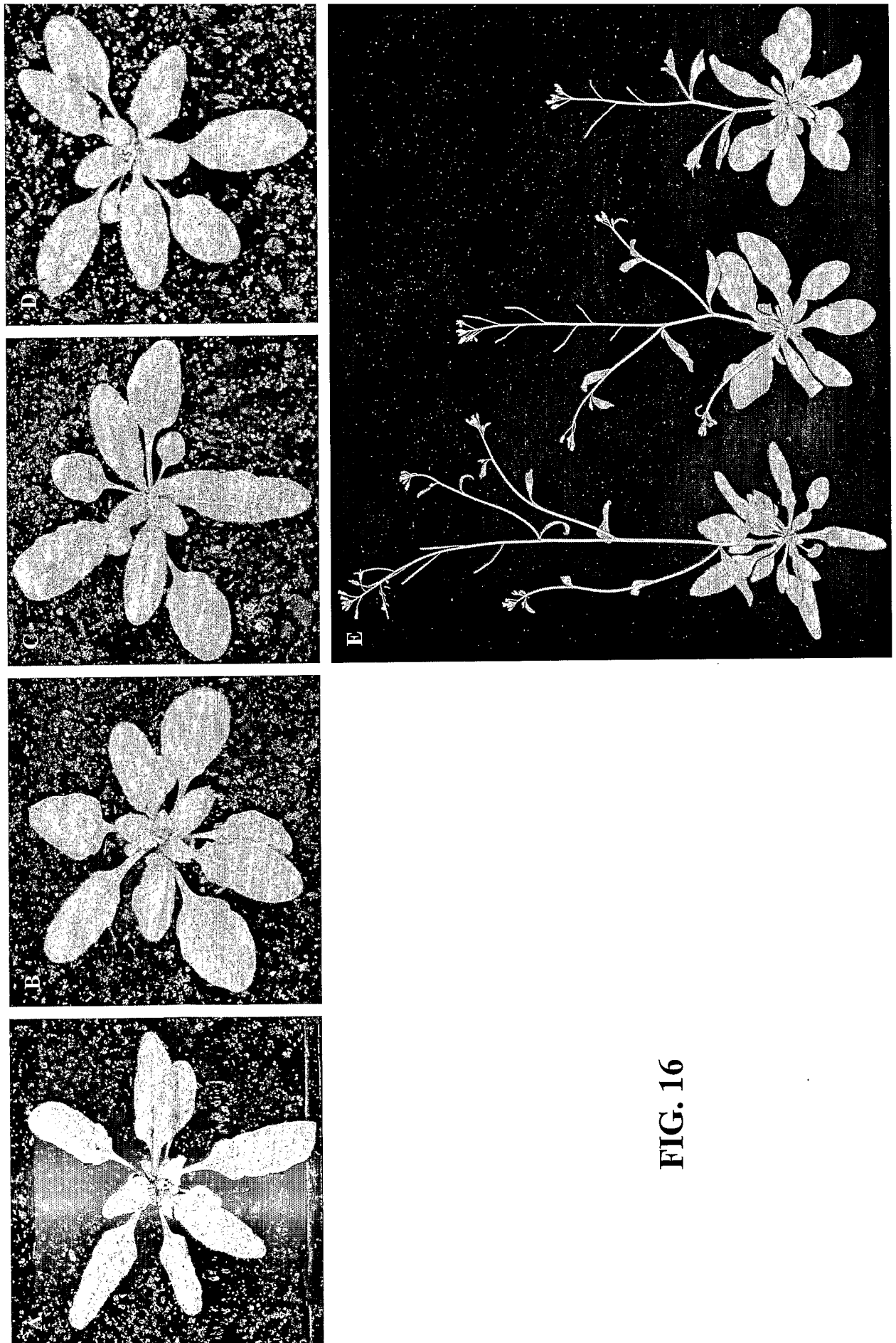


FIG. 16

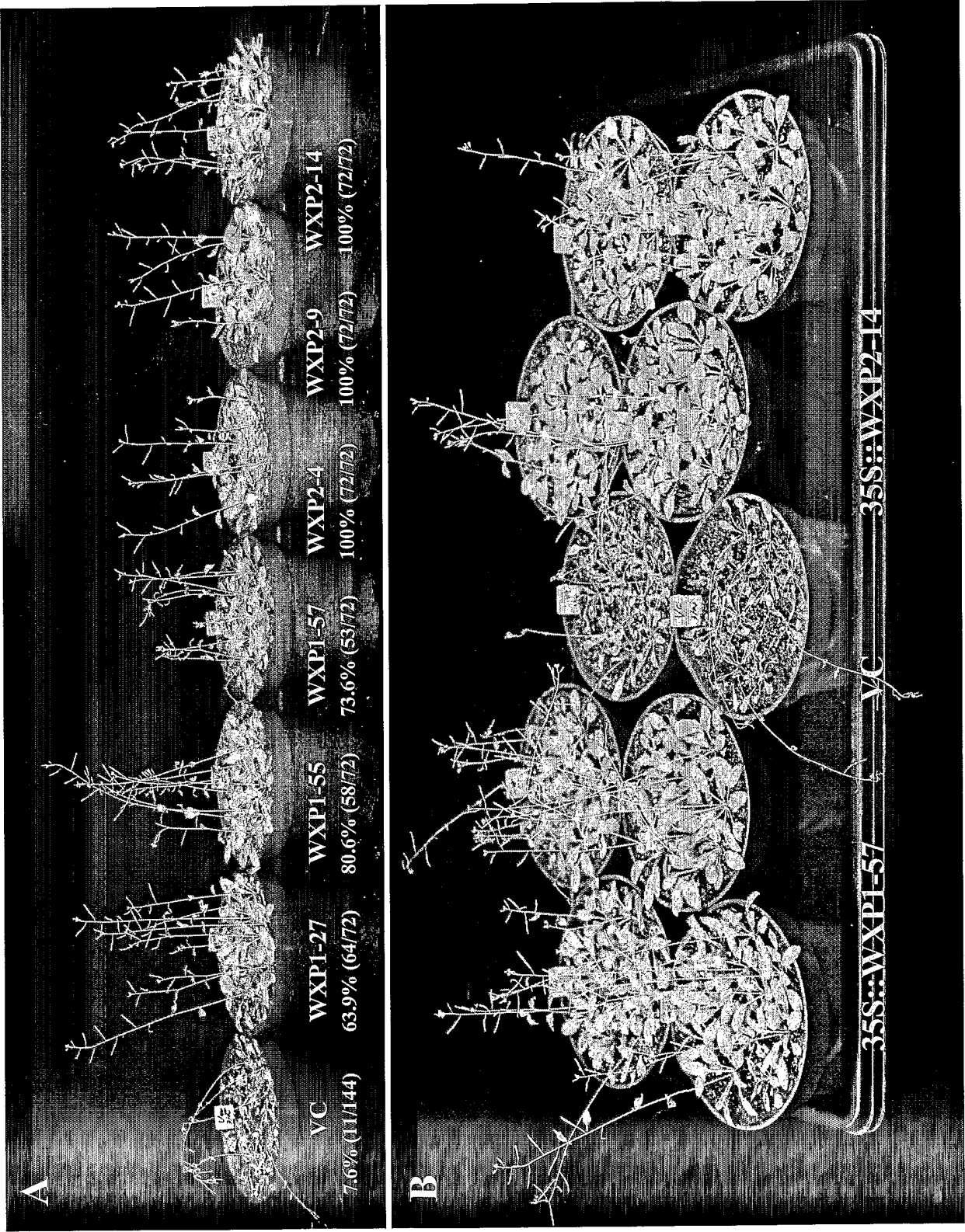


FIG. 17

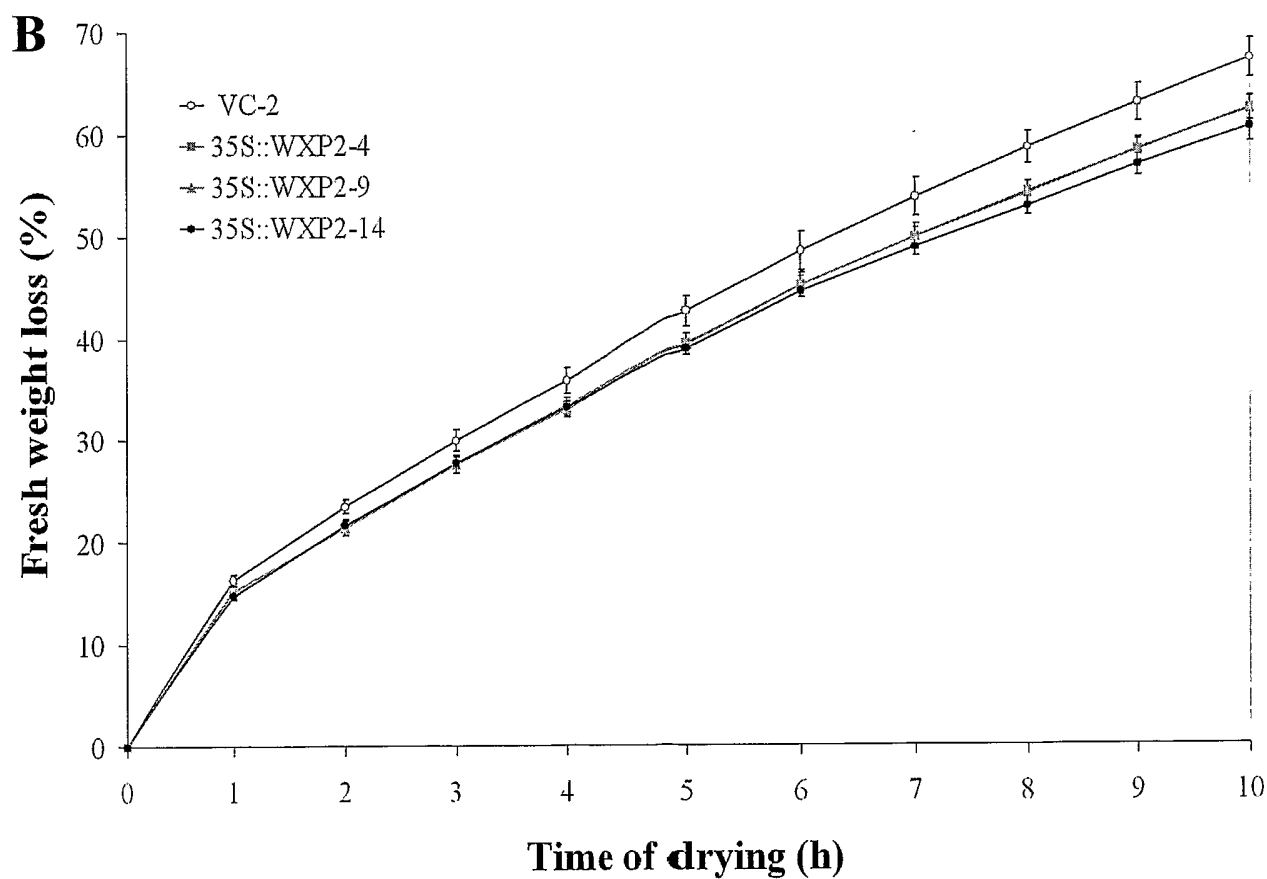
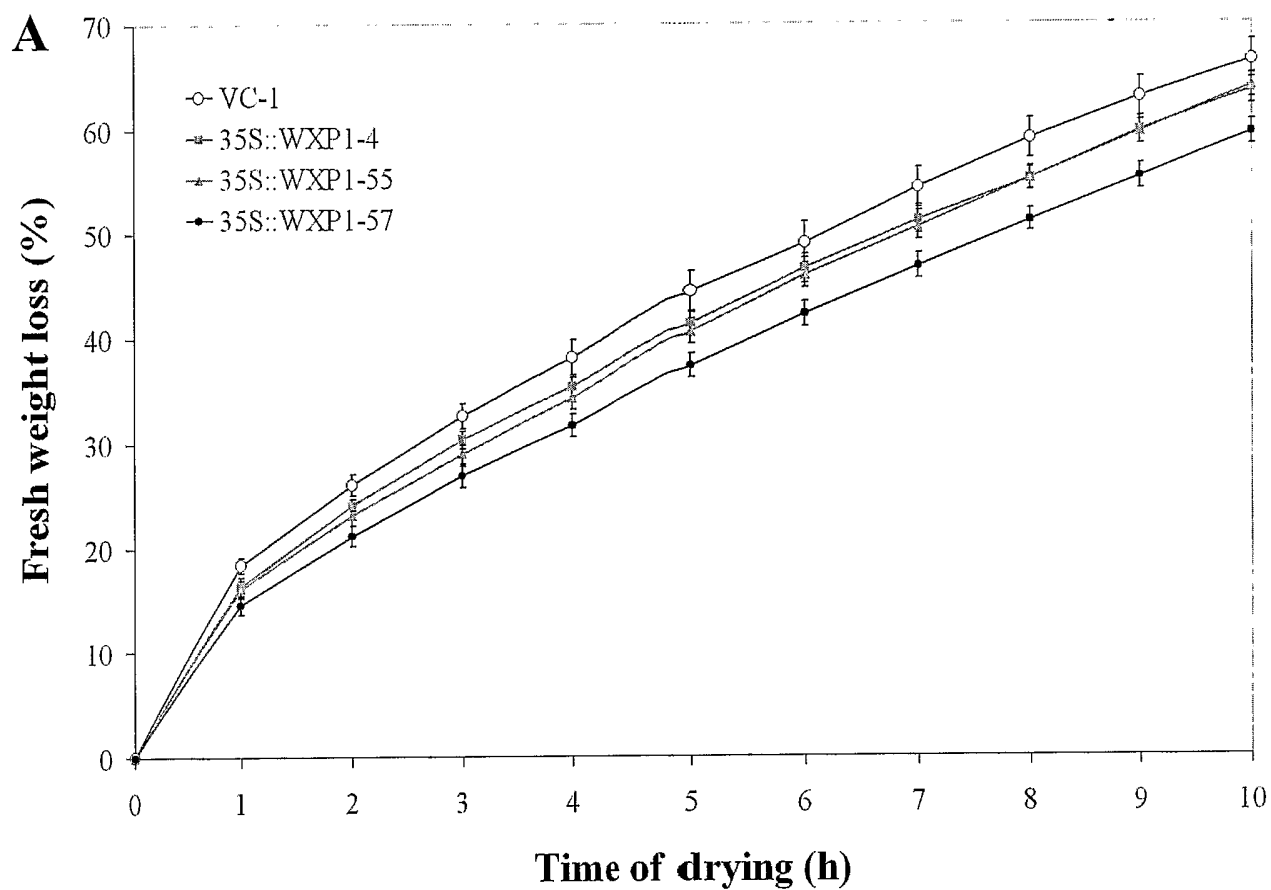


FIG. 18

SEQUENCE LISTING

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WANG, ZENGYU

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 acttttgaca ctgctgaaga agctgctttg gcttatgata gagctgctta tagattaaga 660
 ggtgattttg ctagacttaa tttcccaaac atgaaagacc aacaacaagg tggatggtt 720
 ggtgagttta aggttttgca ttcattctatt gatgctaaac ttgatgctat ttgtgagagt 780
 ttaggtaaca acaatagtag tgataatgtc aagaacaaga aacaagggaa ggggttcaaag 840
 aagagtttga agaagaggt tgaagctcaa cctcaacctc aaccattggt tggtgttgaa 900
 aataataatg atgataacaa caataccaat accaacaagg ttgtgttgta atgtggttct 960
 tctttgtctt cacatagtgga aggtggttct gatgattctt ctccactttc tgatcttact 1020
 tttggtgaat ttgctgagcc acaatgggaa aatggttttg aacaatttaa tttgcagaaa 1080

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1116

<210> 3

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Primer

<400> 3

ggtaccatgg atttcttcaa ca

22

<210> 4

<211> 22

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Synthetic
Primer

<400> 4

aaccggtcac caaattcatc ca

22

<210> 5

<211> 340

<212> PRT

<213> Medicago truncatula

<400> 5

Met Ala Ala Met Met Asn Tyr Tyr Ser Asn Met Gln Gln Phe Gln Phe
1 5 10 15

His Asp Ser Asp Pro Phe Arg Gly Glu Leu Met Glu Val Leu Glu Pro
20 25 30

Phe Ile Lys Ser Pro Ser Ser Thr Ser Thr Ser Pro Ser Ser Ser Thr
35 40 45

Pro Ser Pro Ser Tyr Ser Ser Ser Leu Ser Ser Pro Ser Phe Tyr Thr
50 55 60

Glu Gln Asn Phe Ile Gly Phe Ala Gln Pro Ser Ser Ser Phe Ser Ser
65 70 75 80

Pro Ser Leu Leu Gly Leu Asn His Leu Thr Pro Ser Gln Ile Asn Gln
85 90 95

Ile Gln Val Gln Ile Gln Gln Gln Asn Phe Thr Met Gln His Gln Gln
100 105 110

Ile Gln Gln Gln Gln Gln Arg Cys Leu Ser Asn Thr Leu Ser Phe Leu
115 120 125

Ser Pro Lys Ser Ile Pro Met Lys His Val Gly Gly Ser Ser Val Ser
130 135 140

Lys Pro Thr Lys Leu Tyr Arg Gly Val Arg Gln Arg His Trp Gly Lys
 145 150 155 160
 Trp Val Ala Glu Ile Arg Leu Pro Lys Asn Arg Thr Arg Leu Trp Leu
 165 170 175
 Gly Thr Phe Asp Thr Ala Glu Glu Ala Ala Leu Ala Tyr Asp Lys Ala
 180 185 190
 Ala Tyr Lys Leu Arg Gly Asp Phe Ala Arg Leu Asn Phe Pro Asn Leu
 195 200 205
 Lys His Gln Gly Ser Ile Ile Gly Gly Glu Phe Gly Glu Phe Lys Pro
 210 215 220
 Leu Pro Ser Ser Val Asp Ala Lys Leu Gln Ala Ile Cys Glu Gly Leu
 225 230 235 240
 Ala Glu Met Gln Lys Gln Gly Lys Ala Glu Lys Pro Lys Lys Met Pro
 245 250 255
 Ala Ser Lys Ala Lys Ala Ser Ser Lys Val Val Ser Lys Glu Ser Val
 260 265 270
 Asp Asp Leu Lys Lys Asp Ser Glu Pro Glu Glu Cys Cys Lys Val Glu
 275 280 285
 Ala Val Ser Val Ile Thr Glu Ser Glu Gly Ser Glu Gly Ser Ser Pro
 290 295 300
 Leu Ser Asp Leu Thr Phe Gly Asp Val Gly Glu Pro Gln Trp Glu Gly
 305 310 315 320
 Asp Ser Glu Asn Phe Asn Leu Leu Lys Tyr Pro Ser Tyr Glu Ile Asp
 325 330 335
 Trp Asp Ser Leu
 340

<210> 6

<211> 1023

<212> DNA

<213> Medicago truncatula

<400> 6

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 tcaacttcac cttcatcttc aacaccatca cttctttatt cttcttctct ctcttcacct 180
 tctttctaca cagagcaaaa cttcataggc ttgtctcaac catcttcttc tttttcttct 240
 ccttctcttc ttggtctcaa ccacttgaca ccatacacia tcaaccaa at ccaagtacaa 300
 atccaacaac aaaacttcac catgcaacat caacaaattc aacagcaaca acaacgttgt 360
 cttagtaaca ctttgagttt tcttagtcca aagtcaattc ctatgaagca tgtgggttgt 420
 agtagtggtt caaaacccac aaagctatac agaggagtga gacaaaggca ctggggaaaa 480
 tgggttgctg agataagact acctaagaac cgaacaagat tatggcttgg tacttttgac 540
 acagctgaag aagcagcttt ggcttatgat aaagctgctt ataagctacg tgggtgacttt 600
 gctaggctaa attttccaaa ttgaaacac caaggttcaa ttattgggtg tgaatttggt 660
 gagtttaaac ctcttccttc ttctgttgat gctaagcttc aagctatttg tgaaggttta 720
 gctgagatgc agaaacaggg gaaggctgag aagcctaaga agatgccggc gtcgaaggcg 780
 aaagcctctt caaaggtggt ttctaaggag tctgttgatg atttgaagaa ggattcagag 840

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ccagaggagt gttgtaaggt tgaagcagtt tcagtgatta ctgagagtga aggttctgaa 900
ggttcttcac cactttcaga tcttactttt ggtgatgttg gtgaaccaca gtgggagggt 960
gattcagaaa attttaattt gctcaagtac ccttcttatg agattgattg ggattctctg 1020
tga                                         1023

```

<210> 7

<211> 334

<212> PRT

<213> Arabidopsis thaliana

<400> 7

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Met Ala Ala Ala Met Asn Leu Tyr Thr Cys Ser Arg Ser Phe Gln Asp
  1              5              10              15

```

```

Ser Gly Gly Glu Leu Met Asp Ala Leu Val Pro Phe Ile Lys Ser Val
      20              25              30

```

```

Ser Asp Ser Pro Ser Ser Ser Ser Ala Ala Ser Ala Ser Ala Phe Leu
      35              40              45

```

```

His Pro Ser Ala Phe Ser Leu Pro Pro Leu Pro Gly Tyr Tyr Pro Asp
      50              55              60

```

```

Ser Thr Phe Leu Thr Gln Pro Phe Ser Tyr Gly Ser Asp Leu Gln Gln
      65              70              75              80

```

```

Thr Gly Ser Leu Ile Gly Leu Asn Asn Leu Ser Ser Ser Gln Ile His
      85              90              95

```

```

Gln Ile Gln Ser Gln Ile His His Pro Leu Pro Pro Thr His His Asn
      100             105             110

```

```

Asn Asn Asn Ser Phe Ser Asn Leu Leu Ser Pro Lys Pro Leu Leu Met
      115             120             125

```

```

Lys Gln Ser Gly Val Ala Gly Ser Cys Phe Ala Tyr Gly Ser Gly Val
      130             135             140

```

```

Pro Ser Lys Pro Thr Lys Leu Tyr Arg Gly Val Arg Gln Arg His Trp
      145             150             155             160

```

```

Gly Lys Trp Val Ala Glu Ile Arg Leu Pro Arg Asn Arg Thr Arg Leu
      165             170             175

```

```

Trp Leu Gly Thr Phe Asp Thr Ala Glu Glu Ala Ala Leu Ala Tyr Asp
      180             185             190

```

```

Lys Ala Ala Tyr Lys Leu Arg Gly Asp Phe Ala Arg Leu Asn Phe Pro
      195             200             205

```

```

Asn Leu Arg His Asn Gly Ser His Ile Gly Gly Asp Phe Gly Glu Tyr
      210             215             220

```

```

Lys Pro Leu His Ser Ser Val Asp Ala Lys Leu Glu Ala Ile Cys Lys
      225             230             235             240

```

```

Ser Met Ala Glu Thr Gln Lys Gln Asp Lys Ser Thr Lys Ser Ser Lys
      245             250             255

```

```

Lys Arg Glu Lys Lys Val Ser Ser Pro Asp Leu Ser Glu Lys Val Lys

```

260 265 270

Ala Glu Glu Asn Ser Val Ser Ile Gly Gly Ser Pro Pro Val Thr Glu
275 280 285

Phe Glu Glu Ser Thr Ala Gly Ser Ser Pro Leu Ser Asp Leu Thr Phe
290 295 300

Ala Asp Pro Glu Glu Pro Pro Gln Trp Asn Glu Thr Phe Ser Leu Glu
305 310 315 320

Lys Tyr Pro Ser Tyr Glu Ile Asp Trp Asp Ser Ile Leu Ala
325 330

<210> 8

<211> 1005

<212> DNA

<213> Arabidopsis thaliana

<400> 8

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gcagcgtctg cgtctgctgt tcttcacccc tctgctgttt ctctccctcc tctccccggt 180
tattaccggt attcaacggt cttgacccaa ccgttttcat acgggtcggg tcttcaacaa 240
accgggtcat taatcggact caacaacctc tcttcttctc agatccacca gatccagtct 300
cagatccatc atcctcttcc tccgacgcat cacaacaaca acaactcttt ctccaatctt 360
ctcagcccaa agccgttact gatgaagcaa tctggagtcg ctggatcttg tttcgcttac 420
ggttcagggt ttccttcgaa gccgacgaag ctttacagag gtgtgaggca acgtcactgg 480
ggaaaatggg tggctgagat ccgtttgccg agaaatcgga ctgctctctg gcttgggact 540
tttgacacgg cggaggaagc tgcgttgccg tatgataagg cggcgtacaa gctgcgcggc 600
gatttcgccc ggcttaactt ccctaacctc cgtcataacg gatctcacat cggaggcgat 660
ttcggatgaat ataaacctct tcaactctca tgcgacgcta agcttgaagc tatttgtaaa 720
agcatggcgg agactcagaa acaggacaaa tcgacgaaat catcgaagaa acgtgagaag 780
aaggtttctg cgccagatct atcggagaaa gtgaaggcgg aggagaattc ggtttcgatc 840
ggtggatctc caccggtgac ggagtttgaa gagtccaccg ctggatcttc gccgttgctg 900
gacttgacgt tcgctgaccc ggaggagccg ccgcagtggg acgagacggt ctcgttggag 960
aagtatccgt cgtacgagat cgattgggat tcgattctag cttag 1005

```

<210> 9

<211> 261

<212> PRT

<213> Arabidopsis thaliana

<400> 9

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

```

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

<210> 10
 <211> 786
 <212> DNA
 <213> Arabidopsis thaliana

<400> 10
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 ggtgaattaa tggaagcgct tttacctttt atcaaaagcc cttccaacga ttcattccgcg 120
 tttgcgttct ctctaccgcg tccaatttca tacgggtcgg atctccactc attttctcac 180
 catcttagtc ctaaaccggt ctcaatgaaa caaacgggta cttccgcggc taaaccgacg 240
 aagctataca gaggagttag acaacgtcac tgggggaaaat ggggtggctga gattcgttta 300
 ccgagggaatc gaactcgact ttggctcgga acattcgaca cggcggagga agctgcttta 360
 gcttatgaca aggcggcgta taagctccga ggagattttg cgcggcttaa tttccctgat 420
 ctccgtcata acgacgagta tcaacctctt caatcatcag tcgacgctaa gcttgaagct 480
 atttgtcaaa acttagctga gacgacgcag aaacagggtga gatcaacgaa gaagtcttct 540
 tctcggaaac gttcatcaac cgtcgcagtg aaactaccgg aggaggacta ctctagcgcc 600
 ggatcttcgc cgctgttaac ggagagttat ggaatctgggtg gatcttcttc gccgttgctg 660
 gagctgacgt ttggtgatac ggaggaggag attcagccgc cgtggaacga gaacgcgttg 720
 gagaagtatc cgtcgtacga gatcgattgg gatctcgattc ttcagtgttc gagtcttgta 780
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<210> 11
 <211> 352

<212> PRT

<213> Gossypium hirsutum

<400> 11

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Met Ala Ala Thr Met Asp Phe Cys Ser Ser Ser Ser Asp Ile Tyr Gly
 1           5           10           15

Gly Glu Leu Met Glu Ala Leu Glu Pro Phe Met Lys Ser Ala Ser Ser
      20           25           30

Ser Ser Ser Ser Ser Pro Ser Pro Ser Pro Ser Pro Ser Ser Tyr Ser
      35           40           45

Pro Ser Pro Ser Pro Ser Pro Ser Pro Ser Thr Ser Tyr Leu Ser Phe
      50           55           60

Ser Ser Ser Gln Thr Gln Pro Asn Phe Tyr Ala Asp Gly Cys Cys Tyr
      65           70           75           80

Ser Pro Ala Val Asp Gln Phe Leu Gly Val Gln Gln Pro Gln Leu Gly
      85           90           95

Ser Thr Ile Gly Leu Asn Asn Leu Thr Gln Ala Gln Ile Asn Gln Ile
      100          105          110

Gln Ala Gln Phe Leu Phe Gln Asn Asn Gln Pro Ser Tyr Leu Tyr Gln
      115          120          125

Asn Pro Gln Leu Asn Ala Asn Pro Asn Thr Asn His Met Leu Ser Phe
      130          135          140

Leu Gly Pro Lys Pro Val Pro Met Lys Gln Met Gly Ser Pro Pro Lys
      145          150          155          160

Pro Thr Lys Leu Tyr Arg Gly Val Arg Gln Arg His Trp Gly Lys Trp
      165          170          175

Val Ala Glu Ile Arg Leu Pro Lys Asn Arg Thr Arg Leu Trp Leu Gly
      180          185          190

Thr Phe Asp Thr Ala Glu Glu Ala Ala Leu Ala Tyr Asp Lys Ala Ala
      195          200          205

Tyr Lys Leu Arg Gly Asp Phe Ala Arg Leu Asn Phe Pro Asn Leu Arg
      210          215          220

His Gln Gly Ser His Ile Gly Gly Asp Phe Gly Glu Tyr Lys Pro Leu
      225          230          235          240

His Ser Ser Val Asp Ala Lys Leu Gln Ala Ile Cys Glu Ser Leu Glu
      245          250          255

Leu Asn Gln Lys Gln Gly Asn Asn Asn Lys Lys Lys Lys Ser Ser Lys
      260          265          270

Glu Asn Lys Val Gln Leu Ala Glu Pro Glu Glu Lys Thr Val Lys Val
      275          280          285

Glu Asn Ser Pro Ser Ser Leu Ser Pro Val Leu Ser Glu Asn Glu Gly
      290          295          300

```

Ser Thr Glu Ser Ser Pro Leu Ser Asp Leu Thr Phe Ser Asp Phe Asn
 305 310 315 320

Glu Gln Pro Trp Pro Glu Val Val Thr Ser Ser Glu Ser Phe Met Leu
 325 330 335

Ser Lys Tyr Pro Ser Tyr Glu Ile Asp Trp Asp Ser Ile Leu Lys Ala
 340 345 350

<210> 12

<211> 1059

<212> DNA

<213> Gossypium hirsutum

<400> 12

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gaagcactcg aaccttttat gaaaagtgc tcttcctctt cctcttcttc cccttccctt 120
tctccttccc cttcttctta ttctccctct ccttctcctt ctcttctctc ttctacttca 180
tacctctctt tctcttcttc ccaaacaaca cccaattttt acgcagatgg ttgttggtat 240
tccccagcag ttgatcaatt tttagggtgt cagcagcctc aacttgggtc aaccattggg 300
ctaaacaact taacccaagc tcagatcaac caaattcagg ctcaattcct cttccagaac 360
aatcaaccaa gctaccttta ccaaaatcct caacttaacg ccaaccccaa caccaaccac 420
atgctcagct ttcttggtcc caagccagtc cccgatgaaac agatgggttc accacaaaaa 480
cccaccaagc tctacagggg agttaggcaa cgctactggg gaaaatgggt cgctgagatc 540
cggctaccta agaaccggac acgtcttctg ttaggcactt tcgatacagc cgaggaagca 600
gccttggtct atgataaagc agcctatata cttaggggtg attttgcgag actcaacttc 660
cctaaccctt gtcaccaagg ttcccatatc ggtggcgact tcggtgaata caagcctttg 720
cattcctctg ttgatgctaa gcttcaagct atctgtgaaa gtttggaact caacaaaaag 780
caagggaaca acaacaagaa gaagaaatca tcgaaggaga acaaagtcca attggctgag 840
cctgaggaaa agacagtga ggtggagaa tccccctcat ctttatctcc ggttctgtcg 900
gagaaatgagg ggtcgacaga atcttcacct ttgtcggatc ttaccttctc ggacttcaac 960
gagcagccat ggctgaagt tgttacttcc tcggagtctt ttatgttgtc caaataccca 1020
tcatatgaga tcgattggga ttctattcta aaagccttag 1059
  
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<210> 13

<211> 332

<212> PRT

<213> Gossypium hirsutum

<400> 13

```

Met Ala Ala Val Ile Asp Met Tyr Asn Ser Ser Arg Asn Ser Val Phe
  1 5 10 15

Tyr Asp Pro Leu Arg Glu Glu Leu Met Lys Ala Leu Glu Pro Phe Met
  20 25 30

Lys Ser Ala Ser Ser Ser Ser Ser Pro Leu Gln Pro Asn Leu Tyr Pro
  35 40 45

Glu Tyr Cys Ser Pro Leu Asn Ser Ser Thr His Leu Phe Ser Asn His
  50 55 60

Gly Phe Ser Asn Tyr Asn Lys Asn Met Leu Gly Phe Glu Gln Thr Gly
  65 70 75 80

Ser Ser Leu Gly Leu Asn Gln Leu Thr Pro Ser Gln Ile Leu Gln Ile
  
```

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

<210> 14

<211> 999

<212> DNA

<213> Gossypium hirsutum

<400> 14

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agagaagagt	taatgaaagc	acttgaacct	tttatgaaaa	gtgcttcttc	ttcttcttct	120
cccttacagc	ccaatttgta	ccctgaatat	tgctcccat	tgaacagtag	taccacttg	180
ttttcaaacc	atgggttctc	taactataac	aagaacatgt	tgggttttga	gcaaacaggt	240
agttcacttg	ggcttaacca	actcaccct	tctcaaatcc	ttcaaattca	atcccaaata	300
ttacatcaac	aacaacagca	acaggttgct	tccatggcgg	ctgttgcaac	accaccacca	360
acaacctttg	agaacccaag	ggtgggcttt	ctttcaccaa	agcctgttcc	tatgaaacat	420

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gtctcttcca cccacacaaa agctgcgaag ctttataggg gtgtgagaca gaggcattgg 480
ggcaaatggg tggctgaaat tagactccct aagaaccgaa cacgactttg gcttggcact 540
ttcgacacag ctgaagaagc agccttggct tacgacaagg ctgcttacaa gcttagagga 600
gagttcgcta ggctgaattt tccccacctt aagcaccaag gagctcatgt ttttggcgag 660
tttggtgact acaaaccctt ccatgcctcg gttgatgcta agctccaggc tatctgtcaa 720
agcttacaag cacaaggcaa tccagtgaag acgaagacct tccccaaggc cgcggagttt 780
cattaccca tcaaaaccga ggagttcgat cataaacaag acaactcatc atcatcatca 840
tcatcagttg atgaatcatc attagcagga tcattatctt caccagaatc tgatatcact 900
ttctttgatt tctctgattc caaatgggaa gacaatgaaa ttgaaaactt caatttagat 960
aagttcccat cactggaaat cgattgggaa gccatttga 999

```

<210> 15

<211> 350

<212> PRT

<213> Gossypium hirsutum

<400> 15

```

Met Ala Ala Ala Met Asp Phe Ile Ser Ser Gly Val Asp Gln Ser Asp
  1             5             10             15

```

```

Leu Tyr Gly Gly Glu Leu Met Glu Val Leu Glu Pro Phe Met Lys Ser
      20             25             30

```

```

Val Ser Ser Ser Ser Pro Ser Pro Ser Pro Ser Pro Ser Ser Ser
      35             40             45

```

```

Leu Pro Ser Thr Ser Tyr Leu Ser Phe Ser Ser Ser Glu Thr Gln Pro
      50             55             60

```

```

Asn Phe Tyr Pro Asp Ser Cys Cys Tyr Pro Tyr Pro Thr Pro Met Asp
      65             70             75             80

```

```

Ser Val Ser Cys Pro Gln Gln Pro Gln Thr Gly Ser Thr Ile Gly Leu
      85             90             95

```

```

Asn Ser Leu Thr Gln Ala Gln Ile His Gln Ile Gln Leu Gln Phe His
     100             105             110

```

```

Leu His Asn Asn Gln Pro Ser Tyr Leu Cys Gln Ser Ser Gln Pro Asn
     115             120             125

```

```

Thr Ile Ser Ala Asn Ser Asn Pro Met Val Ser Phe Leu Cys Pro Lys
     130             135             140

```

```

Pro Val Pro Met Lys His Val Gly Ala Pro Ser Lys Pro Thr Lys Leu
     145             150             155             160

```

```

Tyr Arg Gly Val Arg Gln Arg His Trp Gly Lys Trp Val Ala Glu Ile
     165             170             175

```

```

Arg Leu Pro Arg Asn Arg Thr Arg Leu Trp Leu Gly Thr Phe Asp Thr
     180             185             190

```

```

Ala Glu Glu Ala Ala Leu Ala Tyr Asp Lys Ala Ala Tyr Lys Leu Arg
     195             200             205

```

```

Gly Asp Phe Ala Arg Leu Asn Phe Pro Asn Leu Arg His His Gly Ser
     210             215             220

```

```

His Val Gly Asp Tyr Lys Pro Leu Pro Ser Ser Val Asp Ala Lys Leu

```

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<210> 16
<211> 1053
<212> DNA
<213> Gossypium hirsutum
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<400> 16						
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gaattgatgg	aagttcttga	a ccttttatg	aaaagtgttt	cctcctcttc	ccccccccc	120
tccccctccc	cttccccctt	t tctcttcct	tctacttctt	acctttcttt	ctcttcttcc	180
gaaacacagc	ctaattttta	c ccagatagc	tggtgttacc	cttaccctac	accaatggac	240
tcagtatcat	gtccccaaca	g cctcaaact	ggttcaacaa	ttgggctaaa	tagcttgaca	300
caagctcaga	tccatcagat	c cagcttcaa	ttccacctcc	acaacaacca	accaagctac	360
ctttgccaaa	gttcccaacc	c aacaccatc	agcgccaata	gtaacccgat	ggttagcttt	420
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caagccattt	gtgagagctt	g gtacaaaac	cctaagcaag	ggagcaagaa	gaaatcattc	780
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agtgaagggt	cggctgtatc	t tcaaccttta	tcagatctta	cattctcgga	tttcgacgaa	960
caaccatggc	cggaagtcgt	t tcttcttcg	gaaactttta	tggtgtccaa	gtacccttca	1020
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<210> 17
<211> 321
<212> PRT
<213> Glycine max
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<400> 17
Met Ser Val Val Gly Lys Leu Asn Phe Tyr Met Ala Ala Thr Met Asn
  1              5              10              15

Phe Tyr Asn Glu Thr Ser Gln Gln Val Gln Ser Asp Pro Phe Arg Gly
          20          25          30

```

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

<210> 18

<211> 966

<212> DNA

<213> Glycine max

<400> 18

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tttatgaaaa cttgtccttc ctcaactccc tctattctct cctcagattc accatcacct 180
tcctcttact ctcttttact acctccacac cccagtttct ccacatacac cccctctgcc 240
tacttattcc aaaaccaaca acccttaata ggctttgagc aacaaccaag ttcccttctc 300
gggctcaacc acctaagcac gtctcagatt tcccagatcc aagcccaagc ccaagcccag 360
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ctgaacttcc cgaacctgaa aggctcgtgc cccggcgagg agtacaagcc tatgcaggct 660
gcggtggacg ctaagctcga cgcaatctgc gcgaacttgg cggaaatgca gaagcaaggg 720
aagaacgaga agggtgccag gtccgggaag aagtcgaagc aaggtccgaa cctggaggcg 780
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tctgcggtat cttctgcttt gtctgatctt acctttgatg taaccgagcc gcaatgggag 900
gatgcttcag cacattttta tttgcaaaag tttccttctt atgagatcga ttggggattct 966
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```

<210> 19

<211> 308

<212> PRT

<213> Glycine max

<400> 19

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Met Asn Phe Tyr Asn Gly Thr Ser Gln Glu Gln Val Glu Ser Asp Pro
  1             5             10             15

Phe Arg Gly Glu Leu Met Glu Val Leu Glu Pro Phe Met Lys Thr Ser
      20             25             30

Pro Ser Ser Thr Thr Pro Ser Ile Ile Leu Ser Ser Asp Ser Pro Ser
      35             40             45

Ser Ser Ser Phe Asn Phe Pro Ser Ser Ser Leu Leu Ser Pro His Pro
      50             55             60

Asn Phe Tyr Thr His Thr Pro Pro Pro Ser Tyr Leu Leu Gln Ser Gln
      65             70             75             80

Gln Ser Leu Ile Gly Phe Glu Gln Pro Pro Ser Ser Leu Leu Gly Leu
      85             90             95

Asn His Leu Ser Pro Ser Gln Ile Ser Gln Ile Gln Ala Gln Ile Glu
      100            105            110

Ala Gln Gln Ser Gln Asn Gln Asn Pro His Ser Leu Asn Phe Leu Gly
      115            120            125

Pro Lys Pro Val Pro Met Lys His Val Gly Gly Pro Pro Lys Pro Thr
      130            135            140

Lys Leu Tyr Arg Gly Val Arg Gln Arg His Trp Gly Lys Trp Val Ala
      145            150            155            160

Glu Ile Arg Leu Pro Lys Asn Arg Thr Arg Leu Trp Leu Gly Thr Phe
      165            170            175

```

Asp Thr Ala Glu Glu Ala Ala Leu Ala Tyr Asp Lys Ala Ala Tyr Arg
 180 185 190
 Leu Arg Gly Asp Phe Ala Arg Leu Asn Phe Pro Ser Leu Lys Gly Ser
 195 200 205
 Cys Pro Gly Glu Glu Tyr Lys Pro Val His Ser Ala Val Asp Ala Lys
 210 215 220
 Leu Asp Ala Ile Cys Ala Asn Leu Ala Glu Met Gln Lys Gln Gly Lys
 225 230 235 240
 Thr Glu Lys Gly Ala Arg Ser Ala Lys Lys Ser Lys Gln Gly Pro Asn
 245 250 255
 Gln Glu Ala Lys Pro Glu Pro Gln Ala Ser Ala Glu Ser Glu Gly Ser
 260 265 270
 Ala Asp Ser Ser Pro Leu Ser Asp Leu Thr Phe Asp Val Thr Glu Pro
 275 280 285
 Gln Trp Glu His Phe Asn Leu Gln Lys Phe Pro Ser Tyr Glu Ile Asp
 290 295 300
 Trp Asp Ser Leu
 305

<210> 20
 <211> 927
 <212> DNA
 <213> Glycine max

<400> 20
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 attctctctt cagattcacc ttcatcttca tcttttaatt tcccttcctc ttctttactt 180
 tctccacacc ccaatttcta cacacacact cccccccctt cctacttact ccaaagccaa 240
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 ccgtctcaga tttctcagat ccaggcccag atcgaggccc aacagagcca aaaccagaac 360
 ccacactctc tcaactttct cggcccgaag cccgtcccaa tgaagcacgt gggcgggcct 420
 ccgaagccca cgaagctgta ccggggcgta aggcagaggc attgggggaa gtgggtggcg 480
 gagatcaggc tcccgaagaa ccgaaccagg ctctggctcg gaaccttga caggcgag 540
 gaagctgctt tggcttacga caaagccgct tataggctcc gaggcgactt cgcgaggctg 600
 aacttcccga gcctgaaagg ctctgtcccc ggggaggagt acaagcctgt gcattccgctg 660
 gtggacgcta agctcgacgc gatttgcgcc aacttggcgg aaatgcagaa gcaagggaag 720
 acggagaaaag gtgccaggtc agcgaagaaa tcgaagcaag gtccgaacca ggaggccaag 780
 ccggaacctc aagcttccgc tgaaagttag ggttctgcgg attcttctcc gctgtctgat 840
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 tatgagatcg attgggattc tctctga 927

<210> 21
 <211> 314
 <212> PRT
 <213> Glycine max

<400> 21
 Met Ala Ala Met Met Asp Phe Tyr Ser Ser Ser Thr Glu Phe Gln Leu
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His Ser Asp Pro Phe Arg Gly Glu Leu Met Glu Val Leu Glu Pro Phe
 20 25 30
 Met Lys Ser Pro Phe Ser Thr Pro Ser Pro Ser Asn Ser Cys Phe Leu
 35 40 45
 Ser Thr Ser Tyr Ser Pro Ser Pro Asn Asn Tyr Ser Pro Ser Leu Tyr
 50 55 60
 Ser Asn Gly Leu Ser Ser Ile Pro Asn Thr Thr Gln Asn Leu Ile Gly
 65 70 75 80
 Phe Gly Gln Gly Gln Pro Thr Ser Leu Val Gly Leu Asn His Leu Thr
 85 90 95
 Pro Ser Gln Ile Ser Gln Ile Gln Ala Gln Ile Gln Ile Gln Asn His
 100 105 110
 Ser Asn Thr Leu Ser Phe Leu Gly Pro Lys Pro Ile Pro Met Lys His
 115 120 125
 Val Gly Met Pro Pro Lys Pro Thr Lys Leu Tyr Arg Gly Val Arg Gln
 130 135 140
 Arg His Trp Gly Lys Trp Val Ala Glu Ile Arg Leu Pro Lys Asn Arg
 145 150 155 160
 Thr Arg Leu Trp Leu Gly Thr Phe Asp Thr Ala Glu Glu Ala Ala Leu
 165 170 175
 Ala Tyr Asp Lys Ala Ala Tyr Lys Leu Arg Gly Asp Phe Ala Arg Leu
 180 185 190
 Asn Phe Pro Asn Leu Arg His Gln Gly Ser Ser Val Gly Gly Asp Phe
 195 200 205
 Gly Glu Tyr Lys Pro Leu His Ser Ala Val Asp Ala Lys Leu Gln Ala
 210 215 220
 Ile Cys Glu Gly Leu Ala Glu Leu Gln Lys Gln Gly Lys Thr Glu Lys
 225 230 235 240
 Pro Pro Arg Lys Thr Arg Ser Lys Leu Ala Ser Pro Pro Glu Asn Asp
 245 250 255
 Asn Asn Asn Asp Asn Asn Ser Cys Lys Val Glu Ala Ala Ser Ser Ser
 260 265 270
 Ser Glu Gly Ser Ser Pro Leu Ser Val Leu Thr Phe Ala Asp Val Ser
 275 280 285
 Glu Pro Gln Trp Glu Gly Asp Ser Asp Asn Phe Asn Leu Gln Lys Tyr
 290 295 300
 Pro Ser Tyr Glu Ile Asp Trp Asp Ser Leu
 305 310

<210> 22

<211> 945

<212> DNA

<213> Glycine max

<400> 22

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tctccttcaa attccttgTT tctttctacc tcttactctc cttcccccaa caactactct 180
ccctccctat actcaaacgg gttatcatcc atacccaaca ccacccaaaa cttaattggt 240
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ccgaagccca tccctatgaa gcacgtgggc atgcctccga agcccacgaa gctatacaga 420
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gccgcgtaca agctccgagg tgacttcgcc aggctcaact tccccaacct ccgacaccag 600
ggttcctccg tcggtggtga cttcggggag tataagcctc ttcattccgc cgttgacgcc 660
aagcttcagg ccatttgCGa gggcctggct gagctgcaga aacaggggaa gaccgagaag 720
cctccgagga aaacgcgac caaactcgct tctccgccag agaacgacaa caacaacgac 780
aacaactctt gtaaggTCga agctgcttcc tctcttctg aaggttcttc gcCgctttcg 840
gttctgactt tcgctgacgt cagcgagccg cagtgggaag gtgattcGga taattttaat 900
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```

<210> 23

<211> 302

<212> PRT

<213> Glycine max

<400> 23

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Met Ala Ala Leu Met Asp Phe Tyr Ser Ser Ser Pro Glu Phe Gln Leu
 1                5                10                15

His Ser Asp Pro Phe Arg Gly Glu Leu Met Glu Val Leu Glu Pro Phe
      20                25                30

Met Lys Ser Pro Ser Pro Asn Tyr Phe Pro Ser Ser Pro Ser Leu Pro
      35                40                45

Asn Leu Tyr Ser Asn Gly Leu Ser Ser Asn Thr Gln Ser Leu Ile Gly
      50                55                60

Phe Gly Gln Ala Gln Pro Thr Ser Leu Val Gly Leu Asn His Leu Thr
      65                70                75                80

Pro Ser Gln Ile Ser Gln Ile Gln Ala Gln Ile Gln Ile Gln Ala Gln
      85                90                95

Gln His Gln Asn Arg Ser Asn Thr Leu Ser Phe Leu Gly Pro Lys Pro
      100                105                110

Ile Pro Met Lys His Ala Gly Met Pro Pro Lys Pro Thr Lys Leu Tyr
      115                120                125

Arg Gly Val Arg Gln Arg His Trp Gly Lys Trp Val Ala Glu Ile Arg
      130                135                140

Leu Pro Lys Asn Arg Thr Arg Leu Trp Leu Gly Thr Phe Asp Thr Ala
      145                150                155                160

Glu Glu Ala Ala Leu Ala Tyr Asp Lys Ala Ala Tyr Lys Leu Arg Gly
      165                170                175

```

Asp Phe Ala Arg Leu Asn Phe Pro Asn Leu Arg His Gln Gly Ser Ser
 180 185 190
 Val Gly Gly Asp Phe Gly Glu Tyr Lys Pro Leu His Ser Ala Val Asp
 195 200 205
 Ala Lys Leu Gln Ala Ile Cys Glu Gly Leu Ala Glu Leu Gln Lys Gln
 210 215 220
 Gly Lys Thr Glu Lys Pro Pro Arg Lys Ser Arg Ser Lys Leu Ala Glu
 225 230 235 240
 Lys Val Val Ser Asp Lys Glu Asn Asn Asn Ser Cys Lys Val Glu Ala
 245 250 255
 Ala Ser Trp Ser Ser Glu Gly Ser Ser Pro Leu Ser Asp Leu Thr Phe
 260 265 270
 Ala Asp Val Ser Glu Ala Gln Trp Glu Gly Asp Ser Asp Asn Tyr Asn
 275 280 285
 Leu Gln Lys Tyr Pro Ser Tyr Glu Ile Asp Trp Asp Ser Leu
 290 295 300

<210> 24
 <211> 909
 <212> DNA
 <213> Glycine max

<400> 24
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 ttcccttctt cccctccct ccctaattct tactcaaacg gggtatccag caacacccaa 180
 agcttaattg gcttcgggca agcgcaacc acatctcttg tgggcctgaa ccacctaac 240
 ccattctcaga tctctcagat ccaagcccaa atccagatcc aggccagca gcatcagaat 300
 cgcagcaaca ccttgagctt ccttggggcg aagcccatcc ccatgaagca cgcgggcatg 360
 cctccgaagc ccacgaagct ttacagaggg gtgagacaga ggactgggg aaagtgggtg 420
 gctgagatca gacttcccaa gaaccggacc aggtgtggc tgggaacctt cgacacgcc 480
 gaggaagccg ctctggcata cgacaaggcc gcgtacaagc tccgaggtga cttcgcagg 540
 ctcaacttcc caaacctgcg acaccaggtt tctccgctcg gtggtgattt cgggagtag 600
 aagcctcttc attccgctgt tgacgccaa cttcaggcca tttgcgaagg cctggctgag 660
 ctgcagaaac aggggaagac cgagaagcct ccgaggaagt cgcgttcaa actcgcggag 720
 aaggttggtt ccgacaagga gaacaacaac tcttgtaagg tggaagctgc gtcctggctg 780
 tcggaaggtt cttcgccgct ttcggtatct acgtttgctg acgtgagcga ggctcagtgg 840
 gaaggtgatt cggataatta taatctccag aagtaccctt cttatgagat cgattgggat 900
 tctctgtga 909

<210> 25
 <211> 312
 <212> PRT
 <213> Glycine max

<400> 25
 Met Gly Thr Ala Ile Asp Met Tyr Asn Ser Ser Asn Ile Val Ala Asp
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 Phe Leu Asp Pro Tyr Ser Glu Glu Leu Met Lys Ala Leu Lys Pro Phe
 20 25 30

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

<210> 26

<211> 939

<212> DNA

<213> Glycine max

<400> 26

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tcttcttctt cttcactcga atcacagcct tgttcttttt catctaattc tctccctact 180
tcgtatccct cttccaacca aatcaagctc aaccaactca cccagacca aattgttcag 240
attcaggccc aaatccacat tcagcagcag cagcagcagc tggcccaaac ccaaaccac 300
ctgggcccac aacgcgtccc catgaagcac gctggcacgg ccgcgaaacc cacgaagctc 360
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aaccgcacgc gcctctggct aggaacattc gacaccgcag aggaagcagc attagcgtac 480
gacaacgcag cgtttaagct cagaggcgag ttgcgcgctc tcaattttcc tcatctaaga 540
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```

<210> 27

<211> 366

<212> PRT

<213> *Lycopersicon esculentum*

<400> 27

```

Met Ala Ala  Met Asp Phe Trp Asn Ser Thr Cys Val Asp Phe Glu Leu
  1              5              10              15

```

```

Ser Ser Glu  Pro Val Thr Ser Gly Gly Glu Leu Met Glu Ala Leu Glu
      20              25              30

```

```

Pro Phe Met   Lys Ser Ala Ser Ser Ser Pro Ser Pro Pro Pro Ser Thr
  35              40              45

```

```

Phe Pro Pro  Val Phe Pro Ser Ser Ser Ser Thr Phe Pro Ser Ser
  50              55              60

```

```

Asp Phe Gln  Ser Phe Pro Ser Phe Pro Pro Pro Thr Pro Pro Ile Ser
  65              70              75              80

```

```

Tyr Pro Tyr  Thr Ser Ser Phe Tyr Pro Ser Thr Met Ser Ser Glu Val
      85              90              95

```

```

Cys Ser Thr  Ser Thr Glu Met Asn Ser Gln Ile Phe Ser Thr Gly Phe
  100              105              110

```

```

Ser Gly Tyr  Gly Met Glu Gln Gln Gly Ser Ile Gly Leu Asn Gln Leu
  115              120              125

```

```

Thr Pro Ile  Gln Ile Gln Gln Ile Gln Ala Gln Ile Asn Phe Gln Asn
  130              135              140

```

```

Gln Gln Gln  Gln Gln Gln Gln Gln Met Met Leu Gln Thr Ala His His
  145              150              155              160

```

```

Ala Ser Thr  Met Asn Phe Leu Ala Pro Lys Pro Val Pro Met Lys Gln
      165              170              175

```

```

Ser Gly Ser  Pro Pro Lys Pro Thr Lys Leu Tyr Arg Gly Val Arg Gln
      180              185              190

```

```

Arg His Trp  Gly Lys Trp Val Ala Glu Ile Arg Leu Pro Lys Asn Arg

```

195	200	205
Thr Arg Leu Trp Leu Gly	Thr Phe Asp Thr Ala Glu	Glu Ala Ala Leu
210	215	220
Ala Tyr Asp Lys Ala Ala Tyr Met Leu Arg Gly Asp Phe Ala Arg Leu		
225	230	240
Asn Phe Pro Gln Leu Arg His Asn Gly Asn Leu Ile Gly Gly Asp Phe		
245	250	255
Gly Glu Tyr Asn Pro Leu His Ser Ser Val Asp Ala Lys Leu Lys Asp		
260	265	270
Ile Cys Gln Ser Leu Ala Gln Gly Lys Ser Ile Asp Ser Lys Lys Lys		
275	280	285
Lys Thr Lys Gly Leu Ser Ala Glu Lys Ala Ala Val Val Lys Met Glu		
290	295	300
Glu Glu Glu Ser Lys Thr Ala Glu Val Gly Ser Glu Ser Asp Gly Ser		
305	310	315
His Ser Gly Ser Gly Gly Ser Ser Pro Val Thr Glu Leu Ile Phe Pro		
325	330	335
Glu Phe Thr Glu Glu Glu Pro Thr Trp Asp Met Ser Glu Asn Phe Leu		
340	345	350
Leu Gln Lys Tyr Pro Ser His Glu Ile Asp Trp Ala Ser Leu		
355	360	365

<210> 28

<211> 1101

<212> DNA

<213> Lycopersicon esculentum

<400> 28

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tctccttctc ctccaccttc tacttttccct cctgttttcc cttcttcttc ttcttcaact 180
tttccttctt cagattttca atcttttccct tcatttcctc ctccaactcc tcccatttca 240
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aactttcaaa accaacaaca acagcagcag cagcagatga tggtacagac tgcccatcat 480
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aacttccctc aactccgcca caacggcaac ctaatcggcg gcgacttttg tgaatacaat 780
ccattgcatt cctcagttga tgctaagcta aaggacatat gccaaagctt ggcacagggg 840
aagagcattg actctaagaa gaagaaaacc aaagggttgt cggcggagaa agcggcggtg 900
gtgaagatgg aggaagagga gagcaaaaca gcagaagttg gatccgaaag tgacgggtcc 960
cattccggtt ccggtggatc atcgccggtg accgaactga tattcccgga gttcactgag 1020
gaagagccaa cttgggacat gtcagaaaat tttttgttgc agaagtatcc atctcatgaa 1080
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<210> 29

<211> 367

<212> PRT

<213> Lycopersicon esculentum

<400> 29

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Met Ala Ala Ala Ile Asp Val Tyr Ser Ser Ser Ser Asn Leu Ser Asp
 1           5           10           15

Pro Leu Thr Glu Glu Leu Met Lys Ala Leu Glu Pro Phe Met Lys Gly
           20           25           30

Val Ser Tyr Ser Ser Ser Ser Ser Pro Ser Thr Ser Ser Ser Ser Phe
           35           40           45

Tyr Thr Phe Gly Glu Pro Asn Leu Tyr Thr Asp Phe Cys Thr Ile Pro
 50           55           60

Ser Thr Ala Thr Gln Met Phe Ser Gln Gly Tyr Ser Cys Phe Asp Asn
 65           70           75           80

Met Gly Val Ala Glu Thr Gly Ser Ile Gly Leu Asn His Leu Thr Pro
           85           90           95

Ser Gln Ile Leu Gln Ile Gln Ala Gln Ile Gln Phe Gln Asn Gln Gln
          100          105          110

Gln Gln Leu Gln Leu Leu His Gln Gln Gln Gln Ser Leu Gly Leu Leu
          115          120          125

Thr Pro Thr Ser Thr Tyr Ser Lys Asn Leu Asn Ser Thr Asn Phe Leu
          130          135          140

Gly Met Lys Pro Val Pro Met Lys Gln Thr Gly Ala Thr Ser Ser Gln
          145          150          155          160

Lys Ala Thr Lys Leu Tyr Arg Gly Val Arg Gln Arg His Trp Gly Lys
          165          170          175

Trp Val Ala Glu Ile Arg Leu Pro Lys Asn Arg Thr Arg Leu Trp Leu
          180          185          190

Gly Thr Phe Asp Thr Ala Glu Glu Ala Ala Leu Ala Tyr Asp Lys Ala
          195          200          205

Ala Tyr Lys Leu Arg Gly Glu Phe Ala Arg Leu Asn Phe Pro His Leu
          210          215          220

Arg His Gln Leu Asn Asn Glu Phe Ser Asp Phe Lys Pro Leu His Ser
          225          230          235          240

Ser Val Asp Ala Lys Leu Gln Ala Ile Cys Gln Ser Leu Ala Asn Pro
          245          250          255

Lys Ser Asp Asp Ser Cys Ser Lys Ser Asn Ser Lys Pro Arg Lys Ser
          260          265          270

Lys Thr Ala Ala Val Ser Val Asp Ser Asn Ser Ala Gln Glu Ser Ser
          275          280          285

Ser Lys Ser Glu Ile Thr Thr Asp Asp Ser Leu Lys Glu Glu Phe Ser
          290          295          300

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Tyr Pro Glu Asn Gly Thr Ile Lys Ile Glu Ala Ser Ser Ser Ser Ser
 305 310 315 320
 Pro Pro Thr Pro Ser Glu Glu Ser Ser Ser Ser Ser Glu Ser Asp Ile
 325 330 335
 Thr Phe Leu Asp Phe Ala Glu Pro Ser Phe Asp Glu Ser Glu Asn Phe
 340 345 350
 Phe Leu Pro Lys Tyr Pro Ser Val Glu Ile Asp Trp Ala Ala Leu
 355 360 365

<210> 30
 <211> 1104
 <212> DNA
 <213> *Lycopersicon esculentum*

<400> 30
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 gaacttatga aagcacttga accttttatg aaagggtgtt cttattcttc ttcttcttca 120
 ccttcaactt cgtcttcttc tttctatact tttgggtgagc ctaatttgta tactgatttc 180
 tgcacaatcc catcaacggc taccctaatg ttttctcaag ggtattcgtg ttttgacaat 240
 atgggtgtag ctgaaacagg ttcaattggg cttaaccatc taaccccttc tcagatcttg 300
 caaatccaag ctcatatcca atttcaaaat caacagcaac aactacaact attacatcaa 360
 caacaacaga gcttaggttt gttaacaccc acttcaacat attctaagaa tctgaattct 420
 actaactttc ttggtagtaa accagtccca atgaagcaaa cgggtgctac ttcttcacag 480
 aaggctacta agctttatcg tggagttaga caacgccatt ggggcaa atg ggttgctgaa 540
 attagacttc ctaagaacag aactaggctt tggtaggca cttttgatc agctgaagag 600
 gctgctttgg cttatgacaa agctgcttat aagctaagag gtgagtt tgc taggcttaat 660
 tttccacatc taaggcatca attaaacaat gaattctctg atttcaagcc tttgcattcc 720
 tctgtggatg ctaaacttca agccatttgc caaagcttgg ctaatcccaa atcagatgac 780
 tcgtgttcta aatctaattc caagccaaga aagtccaaaa ctgcagcagt ttcagtggat 840
 tcaaattcag ctcaagaatc ttcataaag tcagaaatca ccacagatga ttcattgaaa 900
 gaagaattca gctatccaga aaatgggtact atcaagattg aggcttcatc atcatcatca 960
 cccctacac cctctgagga atcatcatct tcgtctgagt ctgatat tac tttcttggat 1020
 ttcgctgaac catctttcga tgaatcagaa aacttctttt taccctagta cccttccgtg 1080
 gagattgatt gggcagctct ttga 1104

<210> 31
 <211> 226
 <212> PRT
 <213> *Lotus japonicus*

<400> 31
 Met Ala Thr Met Met Asp Phe Phe Ser Ser Thr Glu Phe His Ser Asp
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 Pro Phe Arg Gly Glu Leu Met Glu Val Leu Glu Pro Phe Ile Lys Ser
 20 25 30
 Ser Ser Pro Thr Pro Thr Ser Thr Ser Thr Phe Ser Pro Ser Ser Ser
 35 40 45
 Ser Ser Ser Ser Ser Ser Ser Ser Pro Ser Pro Ser Ser Ser Asn Ser
 50 55 60
 Ser Leu Pro Cys Ser Ser Pro Pro Asn Ser Phe Tyr Ser Ser Pro Ser
 65 70 75 80

Val Thr Pro Phe Phe Ser Asp Gly Cys Ser Thr Ser Met Thr His Val
85 90 95

Phe Ala Asn Gly Leu Ser Phe Val Gln Pro Ala Thr Ser Pro Pro Leu
100 105 110

Leu Gly Phe Asn His Leu Thr Pro Ser Gln Ile His Gln Ile Gln Ser
115 120 125

Gln Ile Gln Ile Gln Gln Asn Asn His Leu Pro Trp Gln Asn Gln Asn
130 135 140

Gln Asn His His Leu Asn Thr Thr Leu Ser Phe Leu Ser Pro Lys Pro
145 150 155 160

Ile Pro Met Lys His Val Ala Asn Pro Pro Lys Pro Thr Lys Leu Tyr
165 170 175

Arg Gly Val Arg Gln Arg His Trp Gly Lys Trp Val Ala Glu Ile Arg
180 185 190

Leu Pro Lys Asn Arg Thr Arg Leu Trp Leu Gly Thr Phe Asp Thr Ala
195 200 205

Glu Glu Ala Ala Leu Ala Tyr Asp Lys Ala Ala Tyr Lys Leu Arg Gly
210 215 220

Asp Phe
225

<210> 32

<211> 678

<212> DNA

<213> Lotus japonicus

<400> 32

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tcaaccttct ctctctcttc ttctctctct tctctctctt cttctctctc ctctccatct 180
tcatcaaatt catctctacc ctgttcttct cccctaatt ccttctactc ttctccttct 240
gtcactcctt tcttctcaga tggttgctca acatcgatga cccatgtatt tgcaaacggg 300
ttatcctttg tgcaaccagc aacttctcct cctcttcttg gcttcaacca cctaacccca 360
tctcagatcc atcagatcca atctcagatc cagatccaac agaacaacca cctcccatgg 420
cagaatcaga atcagaatca ccatctcaac accacactca gcttcttag tccgaagccg 480
attccgatga agcacgtggc gaatcctcca aaaccacga agctatacag aggagtggg 540
cagaggcact ggggaaaatg ggtcgctgag atcaggcttc caaagaatcg gaccaggctc 600
tggttgggga catttgacac ggcggaggaa gctgctctgg cgtacgacaa ggctgcgtac 660
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678

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<210> 33

<211> 333

<212> PRT

<213> Lactuca sativa

<400> 33

Met Ala Ser Thr Ala Met Asp Phe Trp Asn Asn Asn Glu Phe Gln Gln
1 5 10 15

- 25 -

<210> 34
 <211> 1002
 <212> DNA
 <213> *Lactuca sativa*

<400> 34
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 ggtgaattaa tggaggcact tgaacctttt tataaaaagtg cctcaccttc ctcttcttct 120
 tattctcaaa tggcgtcttc taattaccaa aatacccttc ctttctctac accttctgcc 180
 tcttatccat acccttcttc atctttcgac tcatttcctt catcttcttc ttacgtcccc 240
 accacccatc aacaaaccgg tttcttcccg gattactcaa tccaagaccg gtttgtgtac 300
 gaccaaccgg gttcttccct cgggttgaac ttgaaccagt tatccgaatc ccagatttac 360
 cagatccaaa cccagatgga aatacccaca caatggccac agactaactt gaatttcatg 420
 gctcaagatc ctgatcctgt acccgtaaaa caatccgggt cacctccgaa acccccgaag 480
 ctttacaggg gtgtgaggca aaggcactgg ggaaaatggg tcgccgagat ccgtttaccc 540
 aagagccgga cccggctttg gttgggtaca ttcgactctg cggaggaagc ggcgttggca 600
 tatgataaag ccgctataaa gctccgtgga gactacgcc ggctcaactt tccgcagctt 660
 cggcaaaacg gctcttatgt caccgacttt aagccgttgc attcttctgt cgtcgccaag 720
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 ggttcacggc ggtcatcggg gaagacagca acagcaactg tttgtcgca agaggctgtg 840
 aaggtggaag gttgtgagag cgaagggtac gccggatcgg gaaactcatc gccgtcgtct 900
 gatctgacgt tcccggagtt cacggaggac gacaatgctt ggtcggagaa tttctcactg 960
 gagaagtatc cgtcgtatga gatcgactgg gggtctatct ag 1002

<210> 35
 <211> 195
 <212> PRT
 <213> *Populus*

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

Leu Thr Phe Pro Asp Phe Glu Glu Ala Pro Leu Asp Phe Glu Ser Gly
 165 170 175

Asn Phe Met Leu Gln Lys Tyr Pro Ser Tyr Glu Ile Asp Trp Ala Ser
 180 185 190

Ile Leu Ser
 195

<210> 36

<211> 588

<212> DNA

<213> Populus

<400> 36

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 ggcacattcg acacagctga ggaggcagct ttggcttatg acagagcagc ttataaacta 180
 agaggcgact ttgcaagact gaacttccca aacttaatcc accaaggggtc ctacatcggc 240
 gaatacaagc ctctccattc ctcagtggat gcgaaactta aagctatttg taaaagcttg 300
 gagaactctt cgcagcagaa acaaggaggg aaaaacgaaga ggcaaagtaa ctcgacgaag 360
 aagaaaagcca acttggcagt ggtgaccag gaggaaggagc aagtggttgt taaggctgag 420
 acacagtctc cggcattgac cgagagtact gggtcgggtg gatcttcgcc tttgtcggat 480
 ctgacgtttc cggattttga ggaagaccg ttggattttg aatcggggaa tttcatgttg 540
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<210> 37

<211> 232

<212> PRT

<213> Sorghum bicolor

<400> 37

Met Ala Ala Ala Ile Asp Met Tyr Lys Tyr Tyr Asn Ala His Gln Ile
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Ala Ser Ser Ser Pro Ser Asp Glu Glu Leu Ala Lys Ala Leu Glu Pro
 20 25 30

Phe Ile Thr Ser Ala Ser Ser Ser Ser Pro Tyr His Arg Tyr Ser Ser
 35 40 45

Ser Pro Ser Met Ser Gln Asp Ser Tyr Met Pro Thr Pro Ser Tyr Thr
 50 55 60

Ser Phe Ala Thr Ser Pro Leu Pro Thr Pro Ala Ala Thr Ser Ser Ser
 65 70 75 80

Ser Ser Pro Phe Ser Gln Leu Pro Pro Leu Tyr Ser Ser Pro Tyr Ala
 85 90 95

Ala Pro Ser Met Val Gly Gln Met Gly Leu Asn Gln Leu Gly Pro Ala
 100 105 110

Gln Ile Gln Gln Ile Gln Ala Gln Phe Met Phe Gln Gln Gln Gln
 115 120 125

Gln Gln Arg Gly Leu His Ala Ala Phe Leu Gly Pro Arg Ala Gln Pro
 130 135 140

Met Lys Gln Ser Gly Ser Pro Pro Leu Ala Pro Ala Gln Ser Lys Leu
 145 150 155 160

Tyr Arg Gly Val Arg Gln Arg His Trp Gly Lys Trp Val Ala Glu Ile
 165 170 175

Arg Leu Pro Lys Asn Arg Thr Arg Leu Trp Leu Gly Thr Phe Asp Thr
 180 185 190

Ala Glu Asp Ala Ala Leu Ala Tyr Asp Lys Ala Ala Phe Arg Leu Arg
 195 200 205

Gly Asp Met Ala Arg Leu Asn Phe Pro Ala Leu Arg Arg Asp Gly Ala
 210 215 220

His Leu Ala Gly Pro Leu His Ala
 225 230

<210> 38

<211> 698

<212> DNA

<213> Sorghum bicolor

<400> 38

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 ccctcggatg aggagctcgc gaaagcactc gagccttcta taacgagtgc ttctctctcc 120
 tctccctacc atcgctactc gtctttctoca tccatgtccc aagattctta catgcctaca 180
 ccctcctaca ccagcttcgc cacctcgcct ctccccaccc ccgccgccac ctctctctcc 240
 tcctcgcctt tctcgcagct tccgccactc tactcgtcgc cttacgcggc gccgagcatg 300
 gttgggcaga tgggcctgaa ccagctcggc ccggcccgaga tccagcagat ccaggcccag 360
 ttcatgttcc agcagcagca gcagcagcag aggggtctgc acgcggcggt cctggggccc 420
 cgggcgcagc cgatgaagca gtccggatcg ccgccgctgg cgccggcgca gtcgaagctg 480
 taccgcggcg tccgccagcg cactggggc aagtgggtgg cagagatccg cctccccaag 540
 aaccgcacc ggctgtggct cggcaccttc gacaccgcc aggcgcggc gctcgcctac 600
 gacaaggccg ccttcgcct ccgccgcgac atggcgcgcc tcaacttccc ggccctccgc 660
 cgcgacggcg cgcacctcgc cggcccgcct cacgcc tc 698

<210> 39

<211> 361

<212> PRT

<213> Solanum tuberosum

<400> 39

Met Ala Ala Met Asp Phe Trp Asn Ser Thr Ser Ser Val Asp Phe Gln
 1 5 10 15

Ser Ser Ser Glu Pro Val Thr Ser Gly Gly Glu Leu Met Glu Ala Leu
 20 25 30

Glu Pro Phe Met Lys Ser Ala Ser Ser Ser Pro Ser Pro Pro Pro Thr
 35 40 45

Thr Phe Pro Pro Val Phe Pro Ser Ser Ser Asp Phe Gln Ser Phe Pro
 50 55 60

Ser Phe Pro Pro Pro Thr Pro Thr Ile Ser Tyr Pro Tyr Thr Ser Ser
 65 70 75 80

Phe Tyr Pro Ser Ser Gln Ser Thr Met Ser Ser Glu Val Cys Ser Thr

85										90					95				
Ser	Thr	Glu	Met	Asn	Ser	Gln	Ile	Phe	Ser	Thr	Arg	Phe	Ser	Gly	Tyr				
			100					105					110						
Gly	Met	Glu	Gln	Gln	Gly	Ser	Ile	Gly	Leu	Asn	Gln	Leu	Thr	Pro	Ile				
			115				120					125							
Gln	Ile	Gln	Gln	Ile	Gln	Ala	Gln	Ile	Asn	Phe	Gln	Asn	Gln	Gln	Gln				
			130			135					140								
Gln	Gln	Gln	Met	Met	Leu	Gln	Thr	Ala	His	His	Ala	Ser	Thr	Met	Asn				
145					150					155					160				
Phe	Leu	Ala	Pro	Lys	Pro	Val	Pro	Met	Lys	Gln	Ser	Gly	Ser	Pro	Pro				
				165					170					175					
Lys	Pro	Ser	Lys	Leu	Tyr	Arg	Gly	Val	Arg	Gln	Arg	His	Trp	Gly	Lys				
			180					185					190						
Trp	Val	Ala	Glu	Ile	Arg	Leu	Pro	Lys	Asn	Arg	Thr	Arg	Leu	Trp	Leu				
		195					200					205							
Gly	Thr	Phe	Asp	Thr	Ala	Glu	Glu	Ala	Ala	Leu	Ala	Tyr	Asp	Lys	Ala				
		210				215					220								
Ala	Tyr	Met	Leu	Arg	Gly	Asp	Phe	Ala	Arg	Leu	Asn	Phe	Pro	Gln	Leu				
225					230					235					240				
Arg	His	Asn	Gly	Asn	Leu	Ile	Gly	Gly	Asp	Phe	Gly	Glu	Tyr	Asn	Pro				
				245					250					255					
Leu	His	Ser	Ser	Val	Asp	Ala	Lys	Leu	Lys	Asp	Ile	Cys	Gln	Ser	Leu				
			260					265					270						
Ala	Gln	Gly	Lys	Ser	Ile	Asp	Ser	Lys	Lys	Lys	Lys	Thr	Lys	Gly	Ser				
		275					280					285							
Ser	Ala	Glu	Lys	Ala	Ala	Val	Val	Lys	Met	Glu	Glu	Glu	Glu	Ser	Lys				
		290				295					300								
Thr	Val	Glu	Val	Gly	Ser	Glu	Ser	Asp	Gly	Ser	Gly	Ser	Gly	Ser	Gly				
305					310					315					320				
Gly	Ser	Ser	Pro	Val	Thr	Glu	Leu	Ile	Phe	Pro	Glu	Phe	Thr	Glu	Glu				
				325					330					335					
Glu	Thr	Thr	Trp	Asp	Met	Ser	Glu	Asn	Phe	Leu	Leu	Gln	Lys	Tyr	Pro				
			340					345					350						
Ser	His	Glu	Ile	Asp	Trp	Ala	Ser	Leu											
		355					360												

<210> 40

<211> 1086

<212> DNA

<213> Solanum tuberosum

<400> 40

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tcttctcctt ctctctcccc tactactttt cctcctgttt tcccttcttc ttcagatttc 180
caatcttttc cttcatttcc tctctcaact cctaccattt catatcccta cacttcttct 240
ttttacccat cttctcaatc tactatgagc tctgaggttt gttccacttc cacagagatg 300
aattcccaaa tcttttcaac tcggttttct gggtatggaa tggagcaaca ggggttcaatt 360
gggttgaatc agttaacccc aatccagatc cagcaaattc aagcccaaatt caacttccaa 420
aaccaacaac aacagcagca gatgatgtta cagactgcgc atcatgcttc caccatgaat 480
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aagaatcgaa cccggctttg gcttgggtaca tttgacaccg ctgaagaagc tgccttggct 660
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aagaagaaga aaaccaaagg gtcgtcggcg gagaaagcgg cggtggtgaa gatggaggaa 900
gaggagagca aaacggtaga agttggatcc gaaagtgacg ggtcgggttc cgggtccggt 960
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ctataa 1086

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<210> 41

<211> 299

<212> PRT

<213> Solanum tuberosum

<400> 41

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Met Phe Ser Gln Gly Tyr Ser Cys Phe Asn Asn Met Gly Val Ala Glu
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Thr Gly Ser Ile Gly Leu Asn His Leu Thr Pro Ser Gln Ile Leu Gln
      20                      25                      30

Ile Gln Ala Gln Ile Gln Phe Gln Asn Gln Gln Gln Leu Leu Gln Leu
      35                      40                      45

His Gln Gln Gln Gln Ser Leu Gly Leu Leu Thr Pro Thr Ser Thr Tyr
      50                      55                      60

Ser Lys Asn Leu Asn Ser Thr Asn Phe Leu Gly Met Lys Pro Val Pro
      65                      70                      75                      80

Met Lys Gln Thr Gly Gly Ala Ser Ser Gln Lys Ala Thr Lys Leu Tyr
      85                      90                      95

Arg Gly Val Arg Gln Arg His Trp Gly Lys Trp Val Ala Glu Ile Arg
      100                      105                      110

Leu Pro Lys Asn Arg Thr Arg Leu Trp Leu Gly Thr Phe Asp Thr Ala
      115                      120                      125

Glu Glu Ala Ala Leu Ala Tyr Asp Lys Ala Ala Tyr Lys Leu Arg Gly
      130                      135                      140

Glu Phe Ala Arg Leu Asn Phe Pro His Leu Arg His Gln Leu Asn Asn
      145                      150                      155                      160

Glu Phe Ser Pro Leu His Ser Ser Val Asp Ala Lys Leu Gln Ala Ile
      165                      170                      175

Cys Gln Ser Leu Ala Asn Pro Lys Ser Asp Asp Leu Cys Ser Lys Ser
      180                      185                      190

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Asn Ser Lys Pro Arg Lys Ser Lys Thr Ala Ala Ala Val Pro Val
 195 200 205
 Asp Ser Asn Ser Ala Gln Glu Ser Ser Ser Lys Ser Glu Ile Thr Ile
 210 215 220
 Asp Asp Ser Leu Lys Gly Glu Phe Ser Tyr Pro Glu Asn Ala Asn Ile
 225 230 235 240
 Lys Ile Glu Ala Ser Ser Ser Ser Pro Ala Pro Ser Glu Glu Ser Ser
 245 250 255
 Ser Ser Pro Glu Ser Asp Ile Thr Phe Leu Asp Phe Ala Glu Pro Pro
 260 265 270
 Ser Phe Asp Glu Ser Glu Asn Phe Phe Leu Pro Lys Tyr Pro Ser Val
 275 280 285
 Glu Ile Asp Trp Ala Ala Leu Cys Asn Leu Leu
 290 295

<210> 42
 <211> 900
 <212> DNA
 <213> Solanum tuberosum

<400> 42
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 aatcaacagc aactactaca actgcatcaa caacaacaga gcttaggttt gttaacacct 180
 acttcaacat attctaagaa tctgaattct acgaactttc ttggtatgaa accagtcccg 240
 atgaagcaaa ctggtggtgc ttcttcacag aaggctacta agctttatcg tggagttaga 300
 caacgccatt ggggtaaatg ggttgctgaa attagacttc ctaagaacag aaccaggctt 360
 tgggtaggca cttttgatac agctgaagaa gctgctttgg cttatgacaa agctgcttat 420
 aagctaagag gagagtttgc tagacttaat tttccacatc taagacatca attaaataat 480
 gaattctcgc ctttacattc ctccgttgat gctaaacttc aagccatttg ccaaagcttg 540
 gctaattcca aatcagatga cttgtgttct aaatctaatt ccaagccaag aaagtccaaa 600
 accgcagcag cagcagttcc agtggattca aattcagctc aagaatcttc atcaaagtcc 660
 gaaatcacca tagatgattc attgaaagga gaattcagct atccagaaaa tgctaatatc 720
 aagattgagg catcatcatc atctcctgca ccatctgagg aatcatcatc ttcgcctgag 780
 tctgatatta ctttcttgga ttctcgtgaa ccaccatctt tcgatgaatc agaaaatttc 840
 ttcttaccba agtacccttc agtggagatt gattgggcag ctctttgtaa cttattgtag 900

<210> 43
 <211> 374
 <212> PRT
 <213> Vitis vinifera

<400> 43
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 Glu Arg Glu Glu Leu Met Lys Ala Leu Glu Pro Phe Met Lys Gly Ala
 20 25 30
 Ser Ser Thr Ser Ser Leu Thr Ser Pro Tyr Pro Tyr Pro Ser Thr Ser
 35 40 45

Ser Leu Ser Pro Pro Ser Thr Ser Ser Ser Ser Leu Pro Phe Phe Ser
 50 55 60
 Tyr Pro Pro Asp Ser Thr Gln Pro Asn Met Tyr Pro Asp Phe Cys Ser
 65 70 75 80
 Thr Ser Thr Thr His Met Phe Ser Gln Gly Phe Ser Gly Tyr Asp Gln
 85 90 95
 Met Gly Leu Glu Gln Thr Arg Ser Ile Gly Leu Asn His Ile Thr Pro
 100 105 110
 Ala Gln Ile Leu Gln Ile Gln Ala Gln Ile Gln Phe Gln Gln Gln Gln
 115 120 125
 Gln Gln Arg Met Ala Ala Val Ala Thr Ala Ser Ser Ile Gln Asn Gln
 130 135 140
 Arg Leu Ser Gln Trp Gln Gln Gln His Thr Leu Asn Phe Leu Gly Pro
 145 150 155 160
 Lys Ala Ile Pro Met Lys Gln Val Gly Thr Pro Pro Lys Pro Ala Lys
 165 170 175
 Leu Tyr Arg Gly Val Arg Gln Arg His Trp Gly Lys Trp Val Ala Glu
 180 185 190
 Ile Arg Leu Pro Lys Asn Arg Thr Arg Leu Trp Leu Gly Thr Phe Asp
 195 200 205
 Thr Ala Glu Glu Ala Ala Leu Ala Tyr Asp Lys Ala Ala Phe Lys Leu
 210 215 220
 Arg Gly Glu Phe Ala Arg Leu Asn Phe Pro Asn Leu Arg His Gln Gly
 225 230 235 240
 Ser Leu Val Ala Gly Glu Phe Gly Asp Tyr Lys Pro Leu His Ser Ser
 245 250 255
 Val Asp Ala Lys Leu Gln Ala Ile Cys Gln Asn Leu Ala Ile Ser Gln
 260 265 270
 Lys Gln Gly Asn Ser Gly Lys Pro Gly Leu Val Ala Asp Ala Lys Ile
 275 280 285
 Glu Ser Ser Thr His Gln Ala Glu Met Val Leu Asp Asn Ser Ser Asp
 290 295 300
 Pro Glu Ser Gly Phe Leu Gly Leu Glu Asp Cys Lys Val Glu Ile Ser
 305 310 315 320
 Ser Ser Ser Ser Ser Pro Ser Pro Ser Asp Glu Ser Ser Ala Gly Ser
 325 330 335
 Ser Ser Pro Glu Ser Asp Ile Ser Phe Leu Asp Phe Thr Asp Ser Gln
 340 345 350
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 Arg Thr Arg Leu Trp Leu Gly Thr Phe Asp Thr Ala Glu Glu Ala Ala
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 Leu Asn Phe Pro Asp Asn Ala Ala Ser Arg Gly Pro Leu His Ala Ser
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