



**Michigan  
Technological  
University**

Michigan Technological University  
**Digital Commons @ Michigan Tech**

---

Michigan Tech Patents

Vice President for Research Office

---

12-11-2012

## Manipulation of plants by transformation with sequences promoting cell division

Victor B. Busov

*Michigan Technological University, vbusov@mtu.edu*

Steven H. Strauss

Follow this and additional works at: <https://digitalcommons.mtu.edu/patents>


 Part of the [Engineering Commons](#)

---

### Recommended Citation

Busov, Victor B. and Strauss, Steven H., "Manipulation of plants by transformation with sequences promoting cell division" (2012). *Michigan Tech Patents*. 117.  
<https://digitalcommons.mtu.edu/patents/117>

Follow this and additional works at: <https://digitalcommons.mtu.edu/patents>

 Part of the [Engineering Commons](#)



US008329992B2

(12) **United States Patent**  
**Busov et al.**

(10) **Patent No.:** **US 8,329,992 B2**  
(45) **Date of Patent:** **Dec. 11, 2012**

(54) **MANIPULATION OF PLANTS BY TRANSFORMATION WITH SEQUENCES PROMOTING CELL DIVISION**

(75) Inventors: **Victor B. Busov**, Houghton, MI (US);  
**Steven H. Strauss**, Corvallis, OR (US)

(73) Assignees: **The State of Oregon Acting by and Through The State Board of Higher Education on Behalf of Oregon State University**, Corvallis, OR (US);  
**Michigan Technological University**, Houghton, MI (US)

(\*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 299 days.

(21) Appl. No.: **12/663,063**

(22) PCT Filed: **Jun. 9, 2008**

(86) PCT No.: **PCT/US2008/066293**

§ 371 (c)(1),

(2), (4) Date: **Dec. 4, 2009**

(87) PCT Pub. No.: **WO2008/154479**

PCT Pub. Date: **Dec. 18, 2008**

(65) **Prior Publication Data**

US 2010/0175152 A1 Jul. 8, 2010

**Related U.S. Application Data**

(60) Provisional application No. 60/933,646, filed on Jun. 7, 2007.

(51) **Int. Cl.**

**C12N 15/82** (2006.01)

**C12N 15/29** (2006.01)

**C12N 5/10** (2006.01)

**C07K 14/00** (2006.01)

(52) **U.S. Cl.** ..... **800/290; 800/286; 800/303; 800/319; 435/320.1; 435/419; 435/468; 536/23.6; 530/350; 530/379**

(58) **Field of Classification Search** ..... None  
See application file for complete search history.

(56) **References Cited**

**U.S. PATENT DOCUMENTS**

6,395,892 B1 \* 5/2002 Strauss et al. .... 536/24.1  
6,696,623 B1 \* 2/2004 Doerner et al. .... 800/298  
7,196,245 B2 \* 3/2007 Jiang et al. .... 800/278  
2005/0198711 A1 9/2005 Evans  
2006/0070142 A1 3/2006 Yanofsky

**FOREIGN PATENT DOCUMENTS**

WO 2011038332 3/2011

**OTHER PUBLICATIONS**

Jiang et al. 2007a. Alignment of sequence encoding SEQ ID No. 2 with SEQ ID No. 499 of US Patent 7,196,245.\*

Jiang et al. 2007b. Alignment of SEQ ID No. 1 with SEQ ID No. 499 of US Patent 7,196,245.\*

Altshul, S.F. et al., "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs," Nucl. Acids Res. (1997) 25(17):3389-3402.

Bonke, M. et al., "APL regulates vascular tissue identity in *Arabidopsis*," Nature (2003) 426(6963):181-186.

Brunner et al., "Poplar genome sequence: functional genomics in an ecologically dominant plant species," Trends in Plant Science (2004) 9:49-56.

Byzova et al., "*Arabidopsis* sterile apetala, a multifunctional gene regulating inflorescence, flower and ovule development," Genes & Development (1999) 13:1002-1014.

Chen, H. et al., "Enhanced recovery of transformants of *Agrobacterium tumefaciens* after freeze-thaw transformation and drug selection," Biotechniques (1994) 16:664-668.

Gleave, A., "A versatile binary vector system with a T-DNA organisational structure conducive to efficient integration of cloned DNA into the plant genome," Plant Mol. Biol. (1992) 20:1203-1207.

Han, K.H. et al., "An *Agrobacterium tumefaciens* transformation protocol effective on a variety of cottonwood hybrids (genus *Populus*)," Plant Cell Rep. (2000) 19:315-320.

Kumar, M. et al., "An update on the nomenclature for the cellulose synthase genes in *Populus*," Trends in Plant Sci. (2009) 14:248-254.

Liu, Y.G. et al., "Efficient isolation and mapping of *Arabidopsis thaliana* T-DNA insert junctions by thermal asymmetric interlaced PCR," Plant J. (1995) 8:457-463.

Matsumura, Y. et al., "Characterization of genes in the asymmetric leaves2/lateral organ boundaries (AS2/LOB) family in *Arabidopsis thaliana*, and functional and molecular comparisons between AS2 and other family members," Plant J. (2009) 58(3):525-537.

Shuai, B. et al., "The lateral organ boundaries gene defines a novel, plant-specific gene family," Plant Physiol. (2002) 129(2):747-761.

Sterky et al., "A *Populus* EST resource for plant functional genomics," Proc. Natl. Acad. Sci. USA (2004) 101:13951-13956.

Tan, G.H. et al., "SiteFinding-PCR: a simple and efficient PCR method for chromosome walking," Nucl. Acids Res. (2005) 33(13):e122.

Tuskan et al., "The genome of black cottonwood, *Populus trichocarpa* (Torr. & Gray)," Science (2006) 313:1596-1604.

UniProtKB/TrEMBL Direct Submission Accession B9HU24, Jun. 16, 2009, retrieved from internet (Nov. 12, 2010) <http://www.uniprot.org/uniprot/B9HU24.txt?version=3>.

Wilkins, O. et al., "Expansion and diversification of the *Populus* R2R3-Myb family of transcription factors," Plant Physiol. (2009) 149(2):981-993.

Zhao, C. et al., "The xylem and phloem transcriptomes from secondary tissues of the *Arabidopsis* root-hypocotyl," Plant Physiol. (2005) 138(2):803-818.

Zhu, Q.H. et al., "DPTF: a database of poplar transcription factors," Bioinformatics (2007) 23(10):1307-1308.

International Search Report and Written Opinion for Application No. PCT/US2008/066293 dated Oct. 15, 2008 (6 pages).

International Search Report and Written Opinion for Application No. PCT/US10/050371 dated Feb. 2, 2011 (11 pages).

Invitation to Pay Additional Fees for Application No. PCT/US2010/050371 dated Nov. 23, 2010 (2 pages).

\* cited by examiner

*Primary Examiner* — David T Fox

(74) *Attorney, Agent, or Firm* — Michael Best & Friedrich LLP

(57) **ABSTRACT**

Polynucleotides encode polypeptides for increasing the rate of growth of plants. Introduction of the polynucleotides into plants produces plants having altered characteristics, such as increased growth, increased leaf area and reduced fertility. Expression of polypeptides in plants or plant cells promotes cell division. Expression of the polynucleotides in plants in the antisense orientation produces plants that are sterile or have smaller leaves.

**24 Claims, 2 Drawing Sheets**

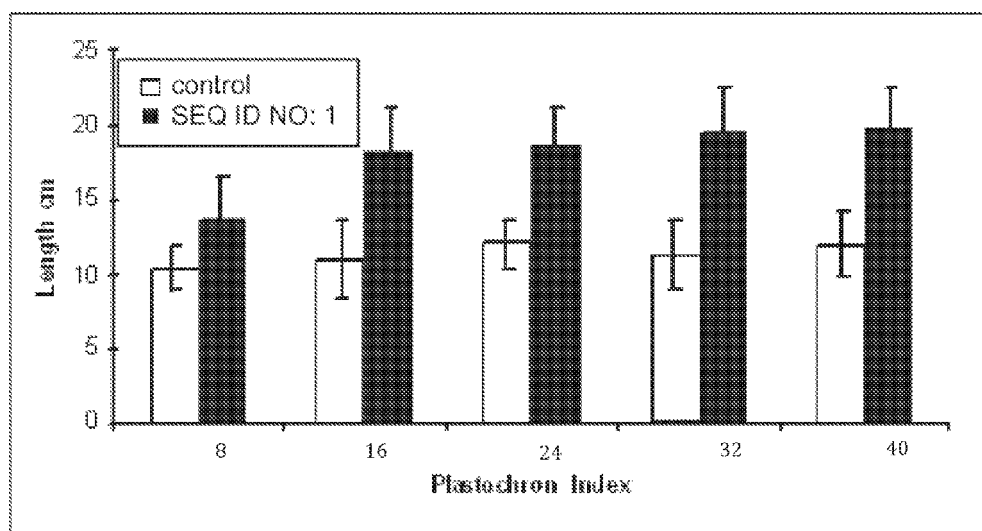
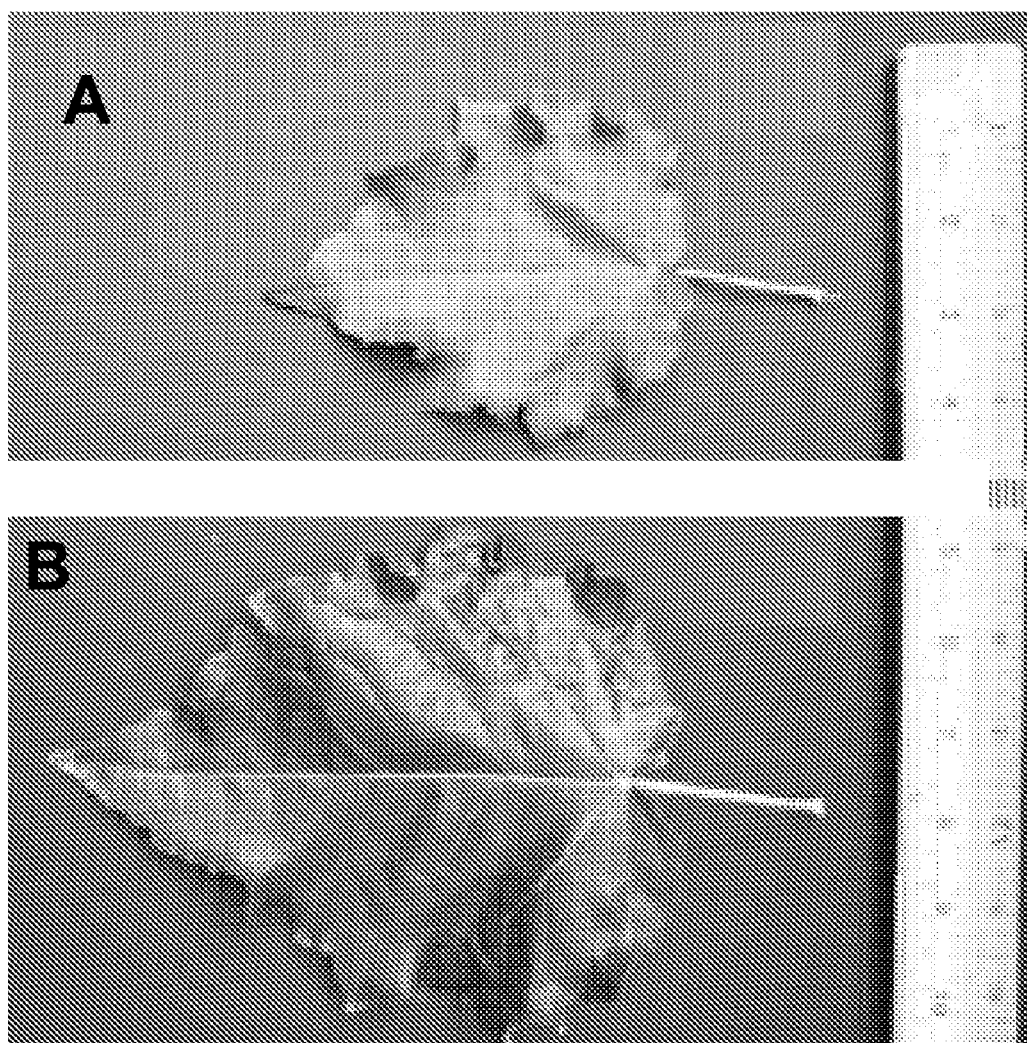


FIG. 1



**FIG. 2**

1

# MANIPULATION OF PLANTS BY TRANSFORMATION WITH SEQUENCES PROMOTING CELL DIVISION

## CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a national stage filing under 35 U.S.C. 371 of International Application No. PCT/US2008/066293, filed on Jun. 9, 2008, which claims the benefit of priority to U.S. provisional application 60/933,646, filed on Jun. 7, 2007, which is incorporated herein by reference in their entireties.

## STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

This invention was made with United States government support awarded by the following agencies: CREES/USDA, Grant No. 2004-35300-14687. The United States government has certain rights in this invention.

## INTRODUCTION

Modified plants having altered characteristics such as increased leaf size may increase the supply of leafy vegetables for food consumption, plant-derived pharmaceutical or industrial products, biomass supply for the generation of biofuels or contribute to carbon remediation programs. Increasing the leaf size of a plant may increase the overall photosynthetic capacity of the plant, which may result in an increased yield of plant material in leaves and other tissues.

## SUMMARY

The invention provides an isolated polynucleotide comprising a contiguous coding sequence encoding a polypeptide having at least 95% identity with SEQ ID NO: 2, and plants and plant cells containing such polynucleotides. In one aspect, the plant containing the isolated polynucleotide exhibits increased expression of the polypeptide, relative to a control plant, and may exhibit increased growth and/or reduced fertility.

In another aspect, the invention provides an isolated polypeptide comprising a sequence having at least 95% identity with SEQ ID NO: 2.

In another aspect, the invention provides methods of producing transgenic plants by introducing into a plant cell a polynucleotide encoding a polypeptide comprising an amino acid sequence having at least 95% identity with SEQ ID NO: 2, and regenerating the transformed cell to produce a transgenic plant. In one aspect, the transformed plant exhibits increased growth and/or reduced fertility.

In another aspect, the invention provides methods of producing transgenic plants by introducing into a plant cell a polynucleotide encoding a polypeptide comprising an amino acid sequence having at least 95% identity with the reverse complement (antisense) of SEQ ID NO: 1 and regenerating the transformed cell to produce a transgenic plant. The polynucleotide is suitably operably linked to a promoter. In one aspect, the plant containing the antisense sequence exhibits decreased growth and/or sterility.

## BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 is a graph showing the correlation of plastocron index and leaf length for control plants and poplar hybrid plants overexpressing the polynucleotides of the invention.

2

FIG. 2 is a photograph showing a sample leaf from (A) a control poplar plant and (B) a poplar plant over-expressing a SAP polypeptide (SEQ ID NO. 2).

## DETAILED DESCRIPTION

The present invention relates to novel polynucleotides and polypeptides and use of the polynucleotides and polypeptides for modifying the phenotype of plants or plant cells. The invention further provides modified plants or plant cells comprising the polynucleotides of the invention. Suitably, the modified plants or plant cells exhibit increased growth or cell division compared with control plants or plant cells. The polynucleotides and polypeptides are of the present invention are termed SAP polynucleotides and SAP polypeptides because they show some similarity to STERILE APETALA (SAP) sequences from *Arabidopsis thaliana*.

It was surprisingly discovered that increasing the expression of a SAP polypeptide in plants (for example, by introducing SEQ ID NO: 2 into the plant) results in plants that exhibit increased growth, larger leaves, and/or show reduced fertility or are sterile, relative to plants in which expression of the SAP polypeptide has not been increased.

SAP polynucleotides useful in the invention include SEQ ID NO: 1, which is derived from the hybrid poplar clone resulting from a cross of *Populus alba* and *Populus tremula*. One of skill in the art will appreciate that, given the degeneracy of the genetic code, many other suitable polynucleotides are encompassed within the invention. SEQ ID NO: 1 encodes the polypeptide shown in SEQ ID NO: 2. SEQ ID NO: 2 has 58% amino acid identity to the SAP polypeptide sequence from *Arabidopsis thaliana* (SEQ ID NO: 5). The cDNA for the SAP polypeptide from *Arabidopsis thaliana* is shown in SEQ ID NO: 4, from position 82 to position 1422 (including the stop codon). SEQ ID NO: 2 has 61% amino acid identity to a polypeptide sequence from *Vitis vinifera* (SEQ ID NO: 7). The polynucleotide encoding SEQ ID NO: 7 from *Vitis vinifera* is shown in SEQ ID NO: 6. The genomic structure of the polynucleotide containing the SAP polynucleotide coding sequence from *Poplar* hybrid (SEQ ID NO: 1) is shown in SEQ ID NO: 8. Position 1 of SEQ ID NO: 8 corresponds to position 9250675 of the sequenced genome and position 5197 of SEQ ID NO: 8 at corresponds to position 9245479 of the sequenced genome. SEQ ID NO: 8 contains the 5' UTR from position 1 to position 200, exon 1 from position 201 to position 443, an intron from position 444 to position 3860, exon 2 from position 3861 to position 4997 including the stop codon (to position 4994 excluding the stop codon), and the 3' UTR from position 4998 to position 5197.

Other suitable SAP polynucleotides of the invention encode a polypeptide comprising a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% identity with SEQ ID NO: 2, and include SEQ ID NO: 1. Percent identity may be determined using the algorithm of Altschul et al., *Nucleic Acids Res.* 25:3389-3402 (1997). Such algorithm is incorporated into the BLASTP program, which may be used to obtain amino acid sequences homologous to a reference polypeptide, as is known in the art. Suitably, the polynucleotide is an isolated polynucleotide, a recombinant polynucleotide or a synthetic polynucleotide and encodes SEQ ID NO: 2, and/or is a contiguous coding sequence encoding a polynucleotide having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% identity to SEQ ID NO: 2. As used herein,

3

“contiguous” with respect to a coding sequence means that the nucleotides of the coding sequence are connected in an unbroken sequence.

Polynucleotides of the invention may be isolated or recombinant and may comprise a contiguous coding sequence encoding a polypeptide corresponding to the sequence from position 1 to position 81 of SEQ ID NO. 2 and/or from position 82 to position 459 of SEQ ID NO. 2, which are encoded by exon 1 and exon 2 of SEQ ID NO. 1. With reference to SEQ ID NO: 1, exon 1 begins at position 1 and ends at position 243 and exon 2 begins at position 244 and ends at position 1380 (or 1377 excluding the stop codon). Suitably, the polynucleotide is an isolated or recombinant polynucleotide and/or a contiguous coding sequence. Suitably, the polynucleotide is a contiguous coding sequence encoding a polynucleotide having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% identity to the sequence from position 1 to position 81 of SEQ ID NO. 2 and/or to the sequence from position 82 to position 459 of SEQ ID NO. 2. As will be appreciated, the invention also encompasses polypeptides including conservative amino acid substitutions, and polynucleotides encoding such polypeptides.

As used herein, “polynucleotide” includes reference to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form. The use of the terms “polynucleotide constructs” or “nucleotide constructs” herein is not intended to limit the present invention to nucleotide constructs comprising DNA. Polynucleotide constructs and oligonucleotides composed of ribonucleotides and combinations of ribonucleotides and deoxyribonucleotides, may also be employed in the methods disclosed herein. The nucleotide constructs, nucleic acids, and nucleotide sequences of the invention additionally encompass all complementary forms of such constructs, molecules, and sequences.

It is envisaged the invention encompasses the production of transgenic plants or plant cells by the introduction into a plant or plant cell of polynucleotides encoding a polypeptide comprising a sequence having at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% identity to SEQ ID NO: 2, to the sequence from position 1 to position 81 of SEQ ID NO. 2, or to the sequence from position 82 to position 459 of SEQ ID NO. 2. Suitably, the polynucleotide is provided as a construct in which a promoter is operably linked to the polynucleotide.

It is envisaged that a plant produced by the introduction of such polynucleotides exhibits altered or modified characteristics. The modified characteristics include, but are not limited to, increased growth, reduced fertility, increased leaf area, increased leaf length, increased leaf width, increased leaf number, increased plant height, increased plant diameter, and increased branch length relative to a control or wild-type plant. For example, plants modified according to the present invention may display altered characteristics wherein the leaf area, leaf length, leaf width, leaf number, plant height, plant diameter, and/or branch length is at least about 20%, at least about 30%, at least about 40%, at least about 50%, or at least about 60% greater than a leaf of a control plant.

Plants modified according to the present invention may suitably show reduced fertility or be sterile. For example, plants may show at least a 10%, at least a 20%, at least a 30%, at least a 40%, at least a 50%, at least a 60%, at least a 60%, at least a 60%, at least a 90%, at least a 95%, at least a 98%, at least a 99% reduction in the number of seeds produced compared with a control plant.

As used herein, a “control plant” is a plant that is substantially equivalent to a test plant or modified plant in all param-

4

eters with the exception of the test parameters. For example, when referring to a plant into which a polynucleotide according to the present invention has been introduced, a control plant is an equivalent plant into which no such polynucleotide has been introduced. As used herein, “sterile” means that a plant is unable to reproduce naturally.

The polynucleotides of the present invention may be introduced into a plant cell to produce a transgenic plant. As used herein, “introduced into a plant” with respect to polynucleotides encompasses the delivery of a polynucleotide into a plant, plant tissue, or plant cell using any suitable polynucleotide delivery method. Methods suitable for introducing polynucleotides into a plant useful in the practice of the present invention include, but are not limited to, freeze-thaw method, microparticle bombardment, direct DNA uptake, whisker-mediated transformation, electroporation, sonication, microinjection, plant virus-mediated, and *Agrobacterium*-mediated transfer to the plant. Any suitable *Agrobacterium* strain, vector, or vector system for transforming the plant may be employed according to the present invention.

In some embodiments, a plant may be regenerated or grown from the plant, plant tissue or plant cell. Any suitable methods for regenerating or growing a plant from a plant cell or plant tissue may be used, such as, without limitation, tissue culture or regeneration from protoplasts. Suitably, plants may be regenerated by growing transformed plant cells on callus induction media, shoot induction media and/or root induction media.

Suitably, the polynucleotides to be introduced into the plant are operably linked to a promoter sequence and may be provided as a construct. As used herein, a polynucleotide is “operably linked” when it is placed into a functional relationship with a second polynucleotide sequence. For instance, a promoter is operably linked to a coding sequence if the promoter is connected to the coding sequence such that it may effect transcription of the coding sequence. Suitably, the polynucleotides may be operably linked to at least one, at least two, at least three, at least four, at least five, or at least ten promoters.

Promoters useful in the practice of the present invention include, but are not limited to, constitutive, inducible, temporally-regulated, developmentally regulated, chemically regulated, tissue-preferred and tissue-specific promoters. Suitably, the promoter causes sufficient expression in the plant to produce the phenotypes described herein. Suitable promoters include, without limitation, the 35S promoter of the cauliflower mosaic virus, ubiquitin, tCUP cryptic constitutive promoter, the Rsyn7 promoter, pathogen-inducible promoters, the maize In2-2 promoter, the tobacco PR-1a promoter, glucocorticoid-inducible promoters, and tetracycline-inducible and tetracycline-repressible promoters.

Polynucleotides may also be provided in a vector. Suitable vectors include plasmids and virus-derived vectors. Vectors known in the art that are suitable for transformation into plants, cloning, and protein expression may be used.

It is envisaged that the invention encompasses isolated polypeptides comprising SEQ ID NO: 2, which is the amino acid sequence of the protein product of the poplar SAP, or polypeptides comprising from position 1 to position 81 of SEQ ID NO. 2, and/or from position 82 to position 459 of SEQ ID NO. 2. Suitable SAP polypeptides according to the present invention may have at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% identity with SEQ ID NO: 2, with polypeptides from position 1 to position 81 of SEQ ID NO. 2 and/or from position 82 to position 459 of SEQ ID NO. 2. Polypeptides of the present invention suitably promote divi-

5

sion of cells, such as plant cells, and accordingly, the invention provides methods for increasing the rate or amount of cell division in cells and/or maintaining cells in a stage or phase where cell division occurs, for example, in a meristematic stage. Suitably, polypeptides of the invention may be synthesized and contacted with cells.

Accordingly, the invention further provides for methods of increasing the rate of growth and/or rate of cell division in plants, cells or tissue cultures by contacting one or more plant cells with one or more isolated SAP polypeptides, or otherwise effecting an increase in the amount or concentration of the SAP polypeptide in the plant cell, such as by introducing a polynucleotide encoding the SAP polypeptide into the cell. The polynucleotide may be introduced in a vector or construct and may be expressed transiently. Plant cells may also be transformed with polynucleotide sequences encoding polypeptides of the invention, such that the polynucleotide stably integrates into the genome or chromosomes of a plant cell.

The polynucleotides may be introduced into the plant or plant cell, either alone or in combination with other polynucleotides. It is envisaged that the expression of the SAP polynucleotides in plant cells, suitably under the control of an inducible promoter, may assist in regenerating plants transformed with other polynucleotides of interest. For example, the expression of the SAP polypeptides may promote cellular regeneration, and may be particularly useful in transformation of plants which are difficult to culture or to regenerate from culture. Without being limited by any theory, it is believed that the SAP polypeptides of the invention may be involved with or stimulate cell division, may promote cells to divide continuously, and/or may promote the retention of cells in a meristematic phase. The invention further provides plant or plant cells produced by the expression of polypeptides of the present invention exhibits altered phenotypes described above.

A variety of plants are suitable for use with methods, polynucleotides and polypeptides of the present invention. For example, as described in the examples below, both poplar and *Arabidopsis* plants transformed with the poplar sequence shown in SEQ ID NO: 1 displayed a phenotype evidenced by larger leaves and exhibited increased growth. The transformed *Arabidopsis* plants also may have reduced fertility as they produced thin siliques, which appeared to be sterile. It is envisaged that the transformed poplar plants will also show reduced fertility or will be sterile.

The plants that can be used in the methods of the invention include any amenable to transformation techniques, including both monocotyledonous and dicotyledonous plants. Suitable plants include, but are not limited to, woody plants and crop plants. Crop plants may include, for example, alfalfa, cotton, maize, rice, tobacco, grapevine, wheat, barley, rye, oat, soybean, lettuce, cabbage, beets, broccoli, cauliflower, squash, potato and tomato. Woody plants may include shrubs, vines, or trees such as aspen, fir, maple, acacia, box elder, horse chestnut, buckthorn, buckeye, mimosa, alder, birch, hornbeam, hickory, chestnut, cedar, red bud, cypress, buck wheat, dogwood, hawthorn, persimmon, olive, eucalyptus, rubber, euonymus, beech, ash, witch-hazel, holly, juniper, myrtle, larch, sweet gum, poplar, oak, magnolia crabapple, redwood, spruce (Norway spruce, dragon spruce, white spruce, black spruce, Colorado blue spruce, red spruce, Himalayan spruce), pine (bristle cone pine, weston white pine, longleaf pine, ponderosa pine, scotch pine, loblolly pine), sycamore, plane, cottonwood, poplar, plum, cherry, laurel, peach, Douglas fir, sumac, willow, elderberry, mountain ash, bladdernut, yew, linden, hemlock, and elm.

6

As used herein, the term "plant" includes reference to whole plants, plant organs (e.g., leaves, stems, roots, etc.), seeds, plant cells, and progeny of same. Parts of transgenic plants are to be understood within the scope of the invention to comprise, for example, plant cells, protoplasts, tissues, callus, embryos as well as flowers, ovules, stems, fruits, leaves, roots originating in transgenic plants or their progeny previously transformed with a DNA molecule of the invention and therefore consisting at least in part of transgenic cells, are also an object of the present invention. As used herein, the term "plant cell" includes, without limitation, protoplasts and cells of seeds, suspension cultures, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen, and microspores.

The invention further provides methods of producing a transgenic plant by introducing in to a plant or plant cell the antisense (reverse complement) of SEQ ID NO.1, or a sequence showing at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 98%, or at least about 99% identity with the reverse complement of SEQ ID NO. 1. Suitably, a transgenic plant may be regenerated from the transformed plant or plant cell. The antisense sequence is suitably operably linked to a promoter functional in the plant. Suitably, the plant transformed with the antisense sequence shows sterility or reduced fertility, lower growth and/or smaller leaves.

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 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 similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention.

It is to be understood that the invention is not limited in its application to the details of construction and the arrangement of components set forth in the following description. Also, it is to be understood that the phraseology and terminology used herein is for the purpose of description and should not be regarded as limiting. The use of "including," "comprising," or "having" and variations thereof herein is meant to encompass the items listed thereafter and equivalents thereof as well as additional items.

It also is understood that any numerical range recited herein includes all values from the lower value to the upper value. For example, if a concentration range is stated as 1% to 50%, it is intended that values such as 2% to 40%, 10% to 30%, or 1% to 3%, etc., are expressly enumerated in this specification. These are only examples of what is specifically intended, and all possible combinations of numerical values between and including the lowest value and the highest value enumerated are to be considered to be expressly stated in this application.

The following non-limiting examples are purely illustrative.

## EXAMPLES

### Example 1

#### Identification of the SAP Sequence

Hybrid poplar clone INRA 717 (*P. alba* × *P. tremula*) was transformed with activation tagging vector pSKI074 using

*Agrobacterium*-mediated transformation. Briefly, *Agrobacterium* cells carrying the binary vector (such as pV-LEGT02) were grown in luria broth, collected by centrifugation, resuspended in induction medium (MS salts, vitamins, 10  $\mu$ M AS, 10 mM galactose, 1.28 mM 2-(N-morpholino)ethanesulfonic acid [MES], pH 5.0), and induced at room temperature. Explants were soaked for 10-20 minutes in the bacterial suspension under 0.6-bar vacuum and shaken (50 rpm) at room temperature. The inoculated explants were co-cultivated in dark for 2-3 days at 19-25° C. in callus induction medium (CIM) (MS salts, 0.5  $\mu$ M benzyladenine, 0.5  $\mu$ M zeatin, 5  $\mu$ M naphthalene acetic acid, 5  $\mu$ M 2,4-Dichlorophenoxyacetic acid, 0.3% gelling agent [such as Phytagar™ from Gibco BRL], 0.1% gelling agent [such as Phytigel™ from Sigma], 1.28 mM 2-(N-morpholino)ethanesulfonic acid, pH 5.8). Explants were cultured for 10-30 days in the dark on CIM with 500 mg/L cefotaxime and 50 mg/L kanamycin. Shoot regeneration was induced on shoot induction medium (SIM) (MS salts, 10  $\mu$ M benzyladenine, 10  $\mu$ M zeatin, 1  $\mu$ M N-acetylaspargate, 0.3% Phytagar (Gibco BRL), 0.1% Phytigel (Sigma), 1.28 mM 2-(N-morpholino)ethanesulfonic acid, pH 5.8) for several weeks to months, and explants were sub-cultured every 2-4 weeks. Regenerated shoots were further screened for kanamycin resistance by rooting in medium supplemented with 0.5  $\mu$ M indole-3-butyric acid and 25 mg/L kanamycin.

Transgenic lines were recovered after the transformation, and the presence of the activation tagging vector was verified by PCR-amplification using primers specific for the activation tagging vector. Transgenic lines were grown, and in the second year of growth under a field trial, plants displaying big leaves were identified. Leaves were approximately 50% larger than control plants. FIG. 2 shows the difference in size between a leaf from a control plant (WT) and a comparable leaf from a plant overexpressing SEQ ID NO. 2. This big leaf phenotype was consistently displayed in 4 ramets, i.e., clones of the same line.

To identify the DNA sequence responsible for the phenotype, thermal asymmetric interlaced (TAIL)-PCR was used. Briefly, three PCT reactions were carried out sequentially to amplify target sequences using nested primers specific for the activation tagging vector on one side with higher T<sub>m</sub>, and a shorter arbitrary degenerate (AD) primer on the other side with lower T<sub>m</sub>, so high temperature annealing favored the specific primer. The first reaction included about 5 high stringency cycles, about 1 low stringency cycle, and about 15 super cycles. Each super cycle included 2 high stringency cycles and 1 reduced stringency cycle. The second reaction included about 12 super cycles of 2 high stringency cycles and 1 reduced stringency cycle. The third reaction included about 20 cycles of reduced stringency.

A genomic DNA sequence flanking the left border of the activation tagging vector in the transgenic plants was identified and sequenced. The sequence was used in a BLASTn search of the poplar genome sequence, and the insertion was determined to be located on LG\_X at position 9249978. Inspection of the genome regions showed that the vector was inserted in a putative intron sequence of a predicted model fgenes1\_pm.C\_LG\_XIV000424, consisting of two exons and one intron. RT-PCR was used with primers to amplify the first and second exons as well as the whole cDNA. Primers used to target the exon 1 sequence showed a hyperactivation of this region. In contrast, both full cDNA and exon 2 targeting primers showed down regulation of the whole transcript and the part of the gene downstream of the insertion.

## Example 2

### Introduction of a Vector Comprising the SAP Sequence into a *Poplar* Hybrid

The coding region of SAP cDNA was PCR-amplified from the transgenic plants. The PCR product was cloned downstream of the CamV35S promoter and upstream of the OCS terminator. The construct was inserted into the NotI site of the pART27 binary vector and transformed into the *Agrobacterium* strain C58 using a freeze thaw method. *Poplar* clone INRA 717 (*P. tremula*×*P. alba*) was transformed using *Agrobacterium*-mediated transformation as described in Example 1. Approximately 40 independent lines were recovered. All transgenic plants were PCR-verified for the presence of the transgene prior to morphological characterization.

Plants were acclimated to greenhouse environment and grown under standard greenhouse conditions. On average, with a leaf plastochron index (LPI) of 45, transgenic plants displayed 58.5% increase in leaf length relative to control with the increase being most pronounced in the older leaves (FIGS. 1 and 2).

## Example 3

### Transformation of *Arabidopsis thaliana* With a Vector Comprising the SAP Sequence

The vector comprising the SAP sequence of the poplar hybrid (*P. tremula*×*P. alba*), as described in Example 2, was introduced into *Arabidopsis thaliana* using *Agrobacterium*-mediated transformation as described in Example 1. The transformed *Arabidopsis thaliana* displayed leaves that were larger than control *Arabidopsis thaliana*. The transformed *Arabidopsis thaliana* also produced thin siliques and appeared to be sterile.

## Example 4

### Transformation of *Eucalyptus* and *Pinus* With a Vector Comprising the SAP Sequence

The vector comprising the SAP sequence of the poplar hybrid (*P. tremula*×*P. alba*), as described in Example 2, will be transformed into plants from the genus *Eucalyptus* and the genus *Pinus* using *Agrobacterium*-mediated transformation as described in Example 1. It is expected that the transformed plants will display leaves 20-70% larger than control plants and will be sterile or show reduced fertility.

## Example 5

### In vitro Production of the SAP Protein and Introduction into Plant Cells

The SAP protein from the poplar hybrid (*P. tremula*×*P. alba*), SEQ ID NO: 2, will be expressed in vitro and isolated. Briefly, SEQ ID NO: 1 will be inserted into the multiple cloning site of vector pET21, the vector will be introduced into an *Escherichia coli* expression strain, and the transformed *E. coli* cells will be grown in luria broth and induced to overexpress protein. The *E. coli* cells will be harvested by centrifugation and lysed by sonication. The SAP protein will be further isolated using ion exchange chromatography. Isolated SAP protein will be added to plant cells in culture. It is expected that the SAP protein will promote cellular regeneration.

## 9

## Example 6

Expression of Antisense SAP Polynucleotide in a  
*Poplar* Hybrid

The antisense reverse complement of SEQ ID NO: 1 will be inserted into the NotI site of the pART27 binary vector and transformed into the *Agrobacterium* strain C58 using a freeze thaw method. A *Poplar* clone (*P. tremula*×*P. alba*) will be

## 10

transformed using *Agrobacterium*-mediated transformation as described in Example 1. Multiple independent lines will be recovered, and all transgenic plants will be PCR-verified for the presence of the transgene prior to morphological characterization. Plants will be acclimated to greenhouse environment and grown under standard greenhouse conditions. It is expected that the transgenic plants will be sterile and have smaller leaves than control plants not comprising the antisense sequence.

## SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 8

<210> SEQ ID NO 1

<211> LENGTH: 1380

<212> TYPE: DNA

<213> ORGANISM: Poplar hybrid

<400> SEQUENCE: 1

```

atgtcttcct cctcctcctc ctcatcatca tctagcagtg gcaatggcaa tggcagcggt    60
gggtggttaatt acggtgcaag aagagccggg gagtacgaag ggccatcaag gtctcgtcca    120
agagccatta acgaggtttg gcctgagcct tttctggaag ctcttgctgc ccaagtcgcc    180
attgatgctt ctgcgcttgt cggccggctg gtcgcggcac aggcacttgc caatgtattt    240
cagggtgtgtt caacgtggcg agcagttctg cgttcggatc ctctttggca ccgtctcact    300
cgcggtatct ggggccgcac caaccttttc catgacactt ggcgagagga gtatatctac    360
cgccacccaaa cggcccaaaa ctccgatcc ggaagagctg tccatttcgc tctccacttt    420
gatccagctg atgtggatga cccaacgac cctgatgctc taatatgccg ctgtctcgct    480
ttctccgacg gctaccttgc atgcgggttt gctgacggcg ctgtccgcct ctttgatctc    540
accacgcgcc ttcctgcacg cactttccgc cctgaacacc atgaccgctt gggcaggttc    600
tctcgtgcgg tctcgggcat cgtcatcacg gccacacgct tgggtgtttgc cacgttagat    660
gggtgatatcc acgtggcggg ggtaaatagc aatgccaatc cacggagggc cgccttaggt    720
gagggtgttg acgacggggc attagtggac ttcacgggcc gtgggcgatg gtgggtgggc    780
ctatacgtg ggcctccggg ccgggcctat cgtgtctggg atggtaacac cgaagaacct    840
ctctttgaag cggggcgctt gactgaccca gaggtgtgtg tgggttgcca cacgttgaca    900
gaagtgactg agtttgtggg ccgagtcagg gtcacgagtc aggaatcggt cgtggcatgc    960
acgagttcga gacttgttat ttttgatttg ggggaccttg gggtgtact aaggaggag    1020
gactacacta acaggagagg catcttggtg gggtccttcg acgtatgcaa tgaggcgat    1080
gtgattgtag atggcagggg gaatgctagc gtgcgcaggg cggacacctc ggaggagatg    1140
tgtggcttta ccgtgaggcc tccaagggga gtgcttgggt gcatgaatgg tgggtacgtg    1200
ctaacgtgcg cgggtggtgt agtaagggta tggcagatag agcagcctgg tcgccaagag    1260
tatttgtata gcttcaggga gaggatagc gaggtaaacg ctctcgttgc agatgagagg    1320
cacgtggcag cagcatcaag tgacacgaat atacacctgt gggatttttg ggcacagtag    1380

```

<210> SEQ ID NO 2

<211> LENGTH: 459

<212> TYPE: PRT

<213> ORGANISM: Poplar hybrid

<400> SEQUENCE: 2

```

Met Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Ser Gly Asn Gly
1           5           10           15

```

-continued

---

Asn Gly Ser Gly Gly Gly Asn Tyr Gly Ala Arg Arg Ala Gly Glu Tyr  
                   20                                  25                                  30  
 Glu Gly Pro Ser Arg Ser Arg Pro Arg Ala Ile Asn Glu Val Trp Pro  
                   35                                  40                                  45  
 Glu Pro Phe Leu Glu Ala Leu Ala Ala Gln Val Ala Ile Asp Ala Ser  
                   50                                  55                                  60  
 Arg Leu Val Gly Arg Leu Val Ala Ala Gln Ala Leu Ala Asn Val Phe  
                   65                                  70                                  75                                  80  
 Gln Val Cys Ser Thr Trp Arg Ala Val Ser Arg Ser Asp Pro Leu Trp  
                   85                                  90                                  95  
 His Arg Leu Thr Arg Gly Ile Trp Gly Arg Thr Asn Leu Phe His Asp  
                   100                                  105                                  110  
 Thr Trp Arg Glu Glu Tyr Ile Tyr Arg His Gln Thr Ala Gln Asn Phe  
                   115                                  120                                  125  
 Arg Ser Gly Arg Ala Val His Phe Ala Leu His Phe Asp Pro Ala Asp  
                   130                                  135                                  140  
 Val Asp Asp Pro Asn Asp Pro Asp Ala Leu Ile Cys Arg Cys Leu Ala  
                   145                                  150                                  155                                  160  
 Phe Ser Asp Arg Tyr Leu Ala Cys Gly Phe Ala Asp Gly Ala Val Arg  
                   165                                  170                                  175  
 Leu Phe Asp Leu Thr Thr Arg Leu His Ala Arg Thr Phe Arg Pro Glu  
                   180                                  185                                  190  
 His His Asp Arg Leu Gly Arg Phe Ser Arg Ala Val Ser Gly Ile Val  
                   195                                  200                                  205  
 Ile Thr Ala Thr Arg Leu Val Phe Ala Thr Leu Asp Gly Asp Ile His  
                   210                                  215                                  220  
 Val Ala Ala Val Asn Ser Asn Ala Asn Pro Arg Arg Ala Arg Leu Gly  
                   225                                  230                                  235                                  240  
 Glu Val Leu Asn Asp Gly Ala Leu Val Asp Phe Thr Gly Arg Gly Arg  
                   245                                  250                                  255  
 Trp Trp Val Gly Leu Tyr Ala Gly Leu Pro Gly Arg Ala Tyr Arg Val  
                   260                                  265                                  270  
 Trp Asp Gly Asn Thr Glu Glu Pro Leu Phe Glu Gly Gly Ala Leu Thr  
                   275                                  280                                  285  
 Asp Pro Glu Ala Val Leu Gly Trp His Thr Leu Thr Glu Val Thr Glu  
                   290                                  295                                  300  
 Phe Val Gly Arg Val Arg Val Thr Ser Gln Glu Ser Val Val Ala Cys  
                   305                                  310                                  315                                  320  
 Thr Ser Ser Arg Leu Val Ile Phe Asp Leu Gly Asp Leu Gly Ala Val  
                   325                                  330                                  335  
 Leu Arg Glu Glu Asp Tyr Thr Asn Arg Arg Gly Ile Leu Val Gly Ser  
                   340                                  345                                  350  
 Phe Asp Val Cys Asn Glu Ala Tyr Val Ile Val Asp Gly Arg Gly Asn  
                   355                                  360                                  365  
 Ala Ser Val Arg Arg Ala Asp Thr Ser Glu Glu Met Cys Gly Phe Thr  
                   370                                  375                                  380  
 Val Arg Pro Pro Arg Gly Val Leu Gly Cys Met Asn Gly Gly Tyr Val  
                   385                                  390                                  395                                  400  
 Leu Thr Cys Ala Gly Gly Val Val Arg Val Trp Gln Ile Glu Gln Pro  
                   405                                  410                                  415  
 Gly Arg Gln Glu Tyr Leu Tyr Ser Phe Arg Glu Arg Ile Gly Glu Val  
                   420                                  425                                  430  
 Asn Ala Leu Val Ala Asp Glu Arg His Val Ala Ala Ala Ser Ser Asp

-continued

435	440	445
Thr Asn Ile His Leu Trp Asp Phe Gly Ala Gln		
450	455	
<210> SEQ ID NO 3		
<211> LENGTH: 2820		
<212> TYPE: DNA		
<213> ORGANISM: Poplar hybrid		
<400> SEQUENCE: 3		
tagtttatac acgtctttac agtacatggt gtggatagat tttcctttga tattgtgggc		60
aatttgacac gaattctgta gcatttcttt cttttctcga gaggctgggg tgttcttgga		120
ggtttcccag ccctgcaaag acggcgggga gatatagtta gggtcagttt cagggtcatg		180
tgagaaactg ctggttcttc tatttagcca aggtgtgctc aggtgctttt gtacacgtac		240
cactctttgg accaactttc ttttgtaact tctgttccat ttccaagtga aaactttatc		300
gacgagcttg ctttagtttt tattctttgt atagtcaagg attttcaaat atagtacatg		360
aacactaacg ggaataacag aggtagctta gtaaaatttt gtttctgcgg gggcacctcc		420
catcatcttt agttcactca cctgaacctg ctctcactac aagctcactg cgagctccct		480
gcatctacag tttcgtgag aaactctacc acatcaacat tttaaagtga aaaacgaaac		540
ctaaagaaaa cgaagaaaga aatcacgaca tcccacgaag aatcttaact agacaaatct		600
aaccattttt attttttttg aattgctaata gaaaattcca tctgatattg cagcaatggt		660
tatggaaatg ctaataataat ttttttatca tatcatgctt tcgtccgaaa ttattggcag		720
aaattcttca tcatattttc tttcggtaaa tcaatgataa aatatttatt catgcttttg		780
gttaaaaaatg tataaaaaatg atgacaggaa gtcaaagcag cgaaggggaa tggagaataa		840
aaaatgtttt ttcttagcgg ggaacggat aacaatgacg acgaggccat ctttaaatat		900
agaattcaaa ttagaatttc ttaaagtgtt tcttgccctg ggctatgcgg aactaaagc		960
caaagaaata tcatcatcag tctttcccaa ccaattacca actaccaagc atgcatgacc		1020
attgactggg atcgaatgct atgagccttt ttatttattt atttatttgg tattgttatt		1080
ttcttaatta aaggcttatt aaaataatat ctttattatt tttaatatta tcacgttaaa		1140
attgtaagaa aaaacaggta aaaaacagtt taataaatgc tttagcaggc actcatttca		1200
tcttttgtgc tggtaattc tctgggaacg tatgagggtc gggttcacat ctgagcgttg		1260
atagtataaa gtgatagatt tacatggatt tggggagtga ttttttatta aaatattttt		1320
aagaaatgaa ttacaatgaa aaataaactg ggaattagat tctatatttg gttttgtaag		1380
gagaaatata tagaaaataa tattttttat tcttttaaat attgaaaatt gaaagggttt		1440
ttcccaagtt taaaggagga aaacactact ttcccctcgt aaaatgagtt gtatgctttt		1500
ctttgagttg aaaacacttc atgctactta tttttcaaat gggttatcaa ataattaatt		1560
tcttataaaa caaacgggtc tctttttatt tttcatagag ttatcaaaga aaaaaagat		1620
gcacgtaatt gtgaaggcta ctgtagagta tataacgtag agatttttta tttttatttt		1680
agttttgatg aaagaaacca tcttattgca gtgctcctac atatatggac atgtaattcc		1740
actgcctggt aaaccaggca caagctgggt acttcgaggg tgcaggacac aacaaaaagc		1800
acttgaggtg gcatgaagaa atgaccaaata ccaaagcata tgatttgtga tgataaaaag		1860
gttgagtttt ctccaaaccg tgataaaaac tagaaaatca agcgtgaact tgctgtccct		1920
catgtctgtg ccccggtggc cacagcatat gatttgtgat gattattagc tatgtacaat		1980
ttgttctata gaattctatga aaaggtaact cgggcagtga attgcagtaa tgatttgcct		2040

-continued

---

```

gtgtggtggc caggcaagcc gatggagccc ttggaacgcg aggctcgtca cttcaaggta 2100
cttgcgagca caaccatggg ctcatcaaag tatcaaacta aaacagcaca gttcagagag 2160
agagagagag agggagagag agggagagag agagcagttc atacttcata gcattagcag 2220
cgcaatggaa ccaaaactgcc gctaaaagga ttatcccaaa ttcgaacagt gcttgacctc 2280
gaggagcagc tccaccctgc cctcgtctctt caaattgaag cttattttgc tgttcataag 2340
tacttagatc ttttttttaa ttttgtatga tctgtcaata tgagacatgc aacatcgacg 2400
cacctgggag aatgctggga aaataatgtg tgtgaatgta gagcaatata gtagtttaat 2460
ttttttataa atcaaaataa tatcattttg gttaaaaaaa tagtcaacaa tttataaccg 2520
agtttttgac cgggtcttac caggccagat cagttggttc acctgagttt ttaatttttc 2580
ttattttttg aaattcagct tggttccagt tccaaatcaa tcgggtctca agtcaatctg 2640
ttaaaccaga ttaaatTTTA aaattataat aatatgtaca acagattgaa tattttcatg 2700
agactctatc ttaaacatag atttaataat ctttttagatt ttagatttta gatttgtttc 2760
tttattgtta tttggatacc gattccacca ttaaattata agtttataac acattaattg 2820

```

&lt;210&gt; SEQ ID NO 4

&lt;211&gt; LENGTH: 1806

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Arabidopsis thaliana

&lt;400&gt; SEQUENCE: 4

```

gtttctcttc tccccacca ttctttcttc tctctcgcgt tcttctcttc cccctgttcc 60
tcttataacc cctctcgtct catgtctacc tctctctctt ctcccgacaa cggagccggg 120
ggaagcggcg gcgttttcga ggccccatct ccatcccgcc ctgcgcggcg agccaacgat 180
gtttggccgg agccttttct tgaatctctc gccgttcaag ttgccgttaa cgttccaca 240
tccgcgggcc tctctcgcgc agctccggct cttgccaaag ttttccgggt ttgcaccacg 300
tggctagctg tctctcgcgc cgaccatcta tggcaactac tatctcgcca agtttgggca 360
agaacacatt tgatgcacga cacgtggcgg gacgagttca tctaccgtca tcggacggct 420
agaaaacttc ggacgcgtac tcacacctac ttcactctcc aatttgaccc gtctgatgtg 480
gacgagcctg atagtctctc ttgccgttgt ctcaccctct cagacctcta cttagccgga 540
gggttcgcgc acggaaccgt ccggcttttt cttctaaaca accgactcca cgtcaggacc 600
ttacggccac ctctacgtga ccgcttttgt agattctcac gagccgtctc aggcattgtt 660
atctccgact caaggctcac gttcgtctac atggacggag acatccacgt ggcggaata 720
gacggtgttg gtcacacacg cacggcttac gcaggagata tagttaacga cggtgcggtg 780
gtagatttca ctggctgtgg acgttggtgg gtcggtcttt tcgcgggtgt gccaggtcgt 840
gcctttcaca tatgggactg taacagcgaa gagacaacat tcgtcgggtg tacactcacc 900
gacctgaag ctgtcatggg atggcacacg ttaacagagc taacaacgtc ccttggccgt 960
ctcagaatct ccgtaacga gacggcggta gcatgcacga gatggagaat catggtgatc 1020
gatctaagaa accaaggagt gatcatcgga gaagacgaag agccacgtag aggactaata 1080
gtgacaggct tcgatgcaa cgacgaagcg tacgttagat tggacagtag aggaaacgct 1140
agcgtgcgaa ggggtaacac gcaacaaacg gtgtgtgagt tccgtgttag tggagcggcg 1200
cagagaagag taatgggttg tgttaataga ctgcacgcgc taatgtgcgc aggtgtgata 1260
atgcgcgtgt gggaggtaga gagaggagag tatctgtaca gtattaggga gagagtagga 1320
gaagttgacg ccattgttgc cgatgataga catgtggcgg ttgcgtcagc ttcacaaacg 1380

```

-continued

---

```

gctcagagta ttatacatct atgggatttc ggtgcactgt agcgtaccag attactaaaa 1440
tttttttttt taacttgaaa aaaaagaaac tttttttctc tgaaaaagcg aaaccctaaa 1500
cctaggtgta tatgtagggt ttctgattat aaattaaatt gggaaagtga cggagggaag 1560
atggtgacag gagggtgaaa ttacaagggt aaattgattc tttctacaaa gtttgaccaa 1620
aaaattaagt tactgttttt gtctaaaacc aaacaaagtg gactttttct tgttgttctt 1680
gtttttttcc ttgtgttttag ggctctctca attttgactt tgtacgaaat gtcttttttt 1740
ttttcttcta cttgtaactc ttcactttac tggagtttat tattttgtat aatggtecca 1800
aaaaaa 1806

```

&lt;210&gt; SEQ ID NO 5

&lt;211&gt; LENGTH: 446

&lt;212&gt; TYPE: PRT

&lt;213&gt; ORGANISM: Arabidopsis thaliana

&lt;400&gt; SEQUENCE: 5

```

Met Ser Thr Ser Ser Ser Ser Ser Asp Asn Gly Ala Gly Gly Ser Gly
1          5          10          15
Gly Val Phe Glu Ala Pro Ser Pro Ser Arg Pro Arg Arg Gly Ala Asn
20          25          30
Asp Val Trp Pro Glu Pro Phe Leu Glu Ser Leu Ala Val Gln Val Ala
35          40          45
Val Asn Ala Ser Thr Ser Ala Gly Leu Leu Ala Ala Ala Pro Ala Leu
50          55          60
Ala Asn Val Phe Arg Val Cys Thr Thr Trp Leu Ala Val Ser Arg Ser
65          70          75          80
Asp His Leu Trp Gln Leu Leu Ser Arg Gln Val Trp Ala Arg Thr His
85          90          95
Leu Met His Asp Thr Trp Arg Asp Glu Phe Ile Tyr Arg His Arg Thr
100         105         110
Ala Arg Asn Phe Arg Thr Arg Thr His Thr Tyr Phe Thr Leu Gln Phe
115         120         125
Asp Pro Ser Asp Val Asp Glu Pro Asp Ser Leu Ser Cys Arg Cys Leu
130         135         140
Thr Leu Ser Asp Leu Tyr Leu Ala Gly Gly Phe Ala Asp Gly Thr Val
145         150         155         160
Arg Leu Phe Leu Leu Asn Asn Arg Leu His Val Arg Thr Leu Arg Pro
165         170         175
Pro Leu Arg Asp Arg Phe Gly Arg Phe Ser Arg Ala Val Ser Gly Ile
180         185         190
Val Ile Ser Asp Ser Arg Leu Thr Phe Ala Thr Met Asp Gly Asp Ile
195         200         205
His Val Ala Glu Ile Asp Gly Val Gly His Thr Arg Thr Ala Tyr Ala
210         215         220
Gly Asp Ile Val Asn Asp Gly Ala Leu Val Asp Phe Thr Gly Cys Gly
225         230         235         240
Arg Trp Trp Val Gly Leu Phe Ala Gly Val Pro Gly Arg Ala Phe His
245         250         255
Ile Trp Asp Cys Asn Ser Glu Glu Thr Thr Phe Val Gly Gly Thr Leu
260         265         270
Thr Asp Pro Glu Ala Val Met Gly Trp His Thr Leu Thr Glu Leu Thr
275         280         285
Thr Ser Leu Gly Arg Leu Arg Ile Ser Gly Asn Glu Thr Ala Val Ala

```

-continued

290	295	300
Cys Thr Arg Trp Arg Ile Met Val Ile Asp Leu Arg Asn Gln Gly Val 305 310 315 320		
Ile Ile Gly Glu Asp Glu Glu Pro Arg Arg Gly Leu Ile Val Thr Gly 325 330 335		
Phe Asp Ala Asn Asp Glu Ala Tyr Val Arg Leu Asp Ser Arg Gly Asn 340 345 350		
Ala Ser Val Arg Arg Val Asn Thr Gln Gln Thr Val Cys Glu Phe Arg 355 360 365		
Val Ser Gly Ala Ala Gln Arg Arg Val Met Gly Cys Val Asn Arg Leu 370 375 380		
His Ala Leu Met Cys Ala Gly Gly Ile Met Arg Val Trp Glu Val Glu 385 390 395 400		
Arg Gly Glu Tyr Leu Tyr Ser Ile Arg Glu Arg Val Gly Glu Val Asp 405 410 415		
Ala Ile Val Ala Asp Asp Arg His Val Ala Val Ala Ser Ala Ser Ser 420 425 430		
Thr Ala Gln Ser Ile Ile His Leu Trp Asp Phe Gly Ala Leu 435 440 445		

&lt;210&gt; SEQ ID NO 6

&lt;211&gt; LENGTH: 1368

&lt;212&gt; TYPE: DNA

&lt;213&gt; ORGANISM: Vitis vinifera

&lt;400&gt; SEQUENCE: 6

```

atgtcgtcgt cttcttcttc ttctctctcg tcttcttcat cttcacaaga tggcgaggtt    60
ggtaggtggag gtggtggggg tggtagtttc gagggctcct cctcttctcg gcgacgttcc    120
ggaaatgggg tttggcggga gccctttgtg gaggtctctg cttcccaagt ggccattgat    180
gcttctcgga gcattggtcg tctggccgct gctcctgccc tcgccaatgt cttccaggtg    240
tgctcaacat ggcgggcagt gtctcgatcg gaccttctat ggcgcaacct ctgccgccgt    300
atctggggcc gggcacggcg gcttcatgac acatggcggg aagagtttat ctaccgccac    360
cgcaccgcca tgaactttcg gaaccgccgg tataactata cgacactgga ttttgaacca    420
gcttacgtca gctcggcgac agccacagct ctctcctgcc gctgcctggc actctccgac    480
ttccacctcg ccgtgggttt cgaggatggc accgttcgcc tctttgacct cgccaccgac    540
ctccacgtca gcacctgect cctcatcac aatgttctta tgggtccctt ctcccgcgcc    600
gtctctggca tcactctctc tcgcgcccgc ctctctctcg cttccctcca cggcgacgtc    660
aacgtcgcgg ctctcgacat cgttgccctt ctcgatcccg ttatcccccac tcgccacggc    720
gccagcgacg gagccctagt cgacttcgcc ggctgcagcc gctggtaggt cggcctctac    780
gccggcgccc ctggccaagc cttccgcata tgggatggcg aaaccgaaga gctcgtcttc    840
gtcgtgggga cctcaccga cccagaagcc gtcattgggt ggcacatgtt cacagagtta    900
gccgatttct ttggccgagt gcgagtcacg agccaggaat cggccgtggc atgcacgagt    960
cgccgagtca tcgtcttcaa ccttagaaac ggaggcaatg tacacgggga acaagagttc   1020
gcgccagggt ttatcgtggg cagcctcgac gccacaatg aggcgtacgt tatcgtggac   1080
gggagggggc ttgctagcgt acgcagggcg gagaatctag aagaggtgtg cagggtttgt   1140
gtgggaggtt cctcgcagag gcgagttttg gggtagcatg acggggggta cgccctgatg   1200
tgcatcggga gtgtgataag agcgtgggag gtggaacgtg ggggtgtatc gtacaacttg   1260
agggaaagaa cgtaggggga tctggttgcc atggtggcag atgatagata cgtggcagca   1320

```

-continued

---

 tgcaacagtg acaccacgat tcacttgtgg gactttgggg ccgaataa 1368

<210> SEQ ID NO 7  
 <211> LENGTH: 455  
 <212> TYPE: PRT  
 <213> ORGANISM: Vitis vinifera

<400> SEQUENCE: 7

Met	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Ser	Gln
1				5					10					15
Asp	Gly	Glu	Val	Gly	Gly	Gly	Gly	Gly	Gly	Gly	Asp	Phe	Glu	Gly
			20					25				30		
Pro	Ser	Ser	Ser	Arg	Arg	Arg	Ser	Gly	Asn	Gly	Val	Trp	Pro	Glu
			35				40				45			
Phe	Val	Glu	Ala	Leu	Ala	Ser	Gln	Val	Ala	Ile	Asp	Ala	Ser	Arg
	50					55				60				
Ile	Gly	Arg	Leu	Ala	Ala	Ala	Pro	Ala	Leu	Ala	Asn	Val	Phe	Gln
65				70					75					80
Cys	Ser	Thr	Trp	Arg	Ala	Val	Ser	Arg	Ser	Asp	Leu	Leu	Trp	Arg
				85					90				95	
Leu	Cys	Arg	Arg	Ile	Trp	Gly	Arg	Ala	Arg	Arg	Leu	His	Asp	Thr
			100					105					110	
Arg	Glu	Glu	Phe	Ile	Tyr	Arg	His	Arg	Thr	Ala	Met	Asn	Phe	Arg
			115				120					125		
Arg	Arg	Tyr	Asn	Tyr	Thr	Thr	Leu	Asp	Phe	Asp	Pro	Ala	Tyr	Val
			130				135				140			
Ser	Gly	Thr	Ala	Thr	Ala	Leu	Ser	Cys	Arg	Cys	Leu	Ala	Leu	Ser
145					150					155				160
Phe	His	Leu	Ala	Val	Gly	Phe	Glu	Asp	Gly	Thr	Val	Arg	Leu	Phe
				165					170					175
Leu	Ala	Thr	Arg	Leu	His	Val	Ser	Thr	Cys	Leu	Pro	His	His	Asn
			180					185					190	
Leu	Met	Gly	Pro	Phe	Ser	Arg	Ala	Val	Ser	Gly	Ile	Ile	Leu	Ser
			195				200					205		
Ala	Arg	Leu	Val	Phe	Ala	Ser	Leu	His	Gly	Asp	Val	Asn	Val	Ala
			210				215				220			
Leu	Asp	Ile	Val	Ala	Pro	Leu	Asp	Pro	Val	Ile	Pro	Thr	Arg	His
225					230					235				240
Ala	Ser	Asp	Gly	Ala	Leu	Val	Asp	Phe	Ala	Gly	Cys	Ser	Arg	Trp
				245					250					255
Val	Gly	Leu	Tyr	Ala	Gly	Ala	Pro	Gly	Gln	Ala	Phe	Arg	Ile	Trp
			260					265					270	
Gly	Glu	Thr	Glu	Glu	Leu	Val	Phe	Val	Gly	Gly	Thr	Leu	Thr	Asp
			275				280					285		
Glu	Ala	Val	Met	Gly	Trp	His	Met	Phe	Thr	Glu	Leu	Ala	Asp	Phe
			290				295				300			
Gly	Arg	Val	Arg	Val	Thr	Ser	Gln	Glu	Ser	Ala	Val	Ala	Cys	Thr
305					310					315				320
Arg	Arg	Val	Ile	Val	Phe	Asn	Leu	Arg	Asn	Gly	Gly	Asn	Val	His
				325					330					335
Glu	Gln	Glu	Phe	Ala	Pro	Gly	Phe	Ile	Val	Gly	Ser	Leu	Asp	Ala
			340					345					350	
Asn	Glu	Ala	Tyr	Val	Ile	Val	Asp	Gly	Arg	Gly	Val	Ala	Ser	Val
			355				360					365		

-continued

---

Arg	Ala	Glu	Asn	Leu	Glu	Glu	Val	Cys	Arg	Phe	Val	Val	Gly	Gly	Thr
370						375					380				
Ser	Gln	Arg	Arg	Val	Leu	Gly	Cys	Met	Asn	Gly	Gly	Tyr	Ala	Leu	Met
385					390					395					400
Cys	Ile	Gly	Ser	Val	Ile	Arg	Ala	Trp	Glu	Val	Glu	Arg	Gly	Val	Tyr
				405					410					415	
Leu	Tyr	Asn	Leu	Arg	Glu	Arg	Thr	Leu	Gly	Asp	Leu	Val	Ala	Met	Val
			420					425					430		
Ala	Asp	Asp	Arg	Tyr	Val	Ala	Ala	Cys	Asn	Ser	Asp	Thr	Thr	Ile	His
	435						440					445			
Leu	Trp	Asp	Phe	Gly	Ala	Gln									
450						455									

<210> SEQ ID NO 8  
 <211> LENGTH: 5197  
 <212> TYPE: DNA  
 <213> ORGANISM: Poplar hybrid

<400> SEQUENCE: 8

acatgtatag cgtatcaata tacgcgtata tacatggaaa gactctaaat cgcaaatata	60
tatagagaga gagaggtgga ggagtctccc ttgggaccct cacattgcgt ctttatctag	120
acctttttgt ccttgtttct ttgtagccca tattttcctt tttttcatag cttogetcct	180
tatatattgt ttattaatta atgtcttctt cctctctctc ctcacatca tctagcagt	240
gcaatggcaa tggcagcggg ggtggtaatt acgggtgcaag aagagccggg gagtacgaag	300
ggccatcaag gtctcgtcca agagccatta acgaggtttg gcctgagcct tttctggaag	360
ctcttgctgc ccaagtgcgc attgatgctt ctgcgcttgt cggccggctg gtcgcggcac	420
aggcacttgc caatgtatct caggtaaatg tattctcttg agactcacga tggttttaat	480
cttactctgt gtatgcataa gaagtggagg ccattttgtc tgctgcacat atgggtttgt	540
cttgttgaga tgtgttagct gttttgagag gtaattatgc taatcagtta gcagccgggt	600
aggctgagag gtaggcgcga tagaataagag tcacttatca ccacccgggt gatatggtag	660
cgaaaatgga caaaaacttg gtctagtcaa agcactgttt ggaatctgtc agggagctaa	720
aatgatgtta tgaactccat tggttttgta tacaaaata gattctgaga ctcttctgtt	780
tttatttcaa tggaaactga agaaatgctg acacagggtg acagcagctt gggttggatg	840
tatacaagca tccaaggaga aaccatacct ttggaaggaa ctgcttttta atgggtccat	900
ttctaactaa atttatgtcc cttttggatg tagcagtttc atcttggcaa tttttagttg	960
cttcatcttt tgaatgctcc taaagaaaat atccattttt ggaccccggt tcatatcttg	1020
cggaaaaggga ctcttttgggt tttgaatttc tctttccctt tcctggcatg attttatatt	1080
agctctataa tcccttgctt ttttgactca actttcttac cttgctaaaa gatccattga	1140
aataactttt ctaatgctaa aatgtggata gagacgtttg aaacctactt ttaactcggc	1200
taatactttt agacttctgc ccagattcaa tttttcggga gcaacatttt ctttttgctg	1260
tggaaacaac catctccgct tggtttttag gtaaagccaa atttggggga ttgacttgtt	1320
tatgacagtg aatcgaacat tctagggttt tgttcaaact gaattttctc atcaaacaaa	1380
accaaatact tgggtatttt atacgaggag cagcagttct cattaatagc aaagagcttt	1440
gagaaagttc tcatcattac atgggtccaat gtagctcggg gatgggctga tttagatgga	1500
taggagctat gaagtgattg ttttactatt tttatatcaa acaatttagg cttctgtttt	1560
ggcaattaag aaggatatct cacaaattga ccagtcttgt gggatcagtc aatagtcagc	1620

-continued

atgtgcaacg	ccaaaagtac	atggagccca	atgctgggtgc	ctggtttcat	aaattgatgt	1680
cacttcagag	ataggggtctt	ctcttcgctc	aagccagagg	cagtcagtcg	gtgactcaac	1740
ttttattcct	tgttatatcc	tgtgttttta	ctacctccat	tttcatttct	atcactgtct	1800
tctttcgttc	ctctagtttt	agcacagtaa	atttcgtatc	tcctcgttgg	attctgaacc	1860
tttcatgaaa	aacacttggt	caatggcttt	agcttttggt	aactttcaag	tcctggagac	1920
ctcgagggat	tgttctagtg	agtaacccaa	tctcttgtaa	aatgtatatg	atccaaactc	1980
aaacgggctt	ttgtaaagct	tgtacctagg	aaagattcta	atttatagat	ctactatcat	2040
aactgccaat	aacttggcag	taagaatggg	caaagaatta	attaattatt	atcaaaactg	2100
gaaggagtaa	attattttaa	tcttaaaaa	acaaggagg	aagttagaat	tcattgattg	2160
aaaggggggg	gggggatatg	atgtagggtg	tgtgggaagt	agaagttagg	gggacaatcg	2220
aagaaaaagg	tagtcggaga	agatatggat	tcgcttcttt	taacgagaca	agcctttggc	2280
caactctttc	ttggtaatga	gagagagaga	gagagagaga	gagagagatg	ggaaagcacg	2340
cttcgagtta	taatcctttt	taacagtaaa	tctaactcca	tcttttatcc	ttgtaataaa	2400
aatctcatte	tgacacacac	aacacatgtg	tgtgtgtgtc	gtgtgtgtgt	gtctcttggc	2460
atgcgggtgg	ctttctctct	cttattttga	attatgtctt	gaaacctccc	atgtttgatt	2520
catttctgta	tagcgtttgt	gggtgtgtaa	actctctgct	agcgtctgtc	cttcagtgtt	2580
ctcaaagtaa	tttccctccc	tgtccaacca	caactaatgg	ttctttgacc	cgaagatttt	2640
gtctctccct	gcccgatgtc	atttagattt	cgccattgta	ctttgctgta	ccttatectg	2700
tatggcatag	aggatgaatg	aataagagta	attataataa	aaagttaaag	tttataataa	2760
caataaagag	agagaggggt	cgtttctctc	tgatagtagt	aggatactgt	tgatgtgggt	2820
taccacagca	tgtctaggct	agaagacaag	tctgttctgc	tttgaaaaga	aacactttat	2880
gtgagagctc	tgactgggtc	tgacgcatgg	aaccatttaa	aataacatgt	ctttctcttt	2940
tctctaacca	ccctctaact	cagctctcta	gtccctagtc	tctcttgcc	gctatttcct	3000
gtctggcatc	ggcatgtatg	aattgggcac	cagcttcaag	ccttctcaca	attaaaagct	3060
taaatgcgca	tgctttcaag	tgccctcgga	tactgacgtt	ttcccacact	ctatgtgatc	3120
agcatttgta	gatgacactg	acacgttttg	ctggatcggt	tcttgagatt	gtttaatcat	3180
ttaaaatgcc	ggcctcttta	gcttgggttg	tggtctttgt	tttttcacaa	tattattatt	3240
attattaaga	tgacctatgc	attgatatat	atatatatat	atatatatat	atatatatat	3300
atatatatat	atatatatat	atatatatat	atgttatgaa	accatttaa	attatggttt	3360
tttaagacat	taattattaa	tcaattttgt	gaggtcacgt	ctttcatcat	cacctctctg	3420
tttttgttgc	atccatgggtg	atcttcgtat	tcttgcccta	gctgggtgct	ggccatttat	3480
ataatggcat	cccatagtta	tgtaaattta	cttttcatgt	ggagtagttt	aaaaataaaa	3540
atcaatggcg	tctcatagat	ctccatctat	gaacattaat	tagttttctt	ctactgtggg	3600
tccgtataat	ccctaccac	cagctaacga	agccttaaac	atgggtgctc	tgctgaatca	3660
ctgaaccgta	actagcaact	tgtcttagct	agcttcgcat	aagttcaaat	gatggaataa	3720
aaaaaaatta	tggttgaaag	tttcattaca	tgtatcttct	gctagctgtt	gttgcatgat	3780
gcaattgcta	cgtgtgcatg	catagccgtc	gtctctgtat	ttatcttttt	atgctttaca	3840
tggtgtccac	tttgtttcag	gtgtgttcaa	cgtggcgagc	agtctcgcgt	tcggatcctc	3900
tttggcaccg	tctcactcgc	ggtatctggg	gccgcaccaa	ccttttccat	gacacttggc	3960
gagaggagta	tatctaccgc	caccaaaccg	cccaaaactt	ccgatccgga	agagctgtcc	4020

-continued

atttcgctct ccactttgat ccagctgatg tggatgaccc caacgacct gatgctctaa	4080
tatgcgctg tctcgtttc tccgatcgct accttgcatg cggttttgct gacggcgctg	4140
tccgcctctt tgatctcacc acgcgccttc atgcacgcac ttccgcctc gaacaccatg	4200
accgcctggg caggttctct cgtgcggtct cgggcacgt catcacggcc acacgcttgg	4260
tgtttgccac gttagatggt gatatccacg tggcgggcgt aaatagcaat gccaatccac	4320
ggagggcccg cttaggtgag gtgttgaacg acggggcatt agtggacttc acgggcccgtg	4380
ggcgatggtg ggtgggccta tacgctgggc ttccgggccc ggccatcgt gtctgggatg	4440
gtaacaccga agaacctctc ttgaaggcg gggcggtgac tgaccagag gctgtgttgg	4500
gttgccacac gttgacagaa gtgactgagt ttgtgggccc agtcagggtc acgagtcagg	4560
aatcggtcgt ggcatgcacg agttcgagac ttgttatttt tgatttgggg gaccttgggg	4620
ctgtactaag ggaggaggac tacactaaca ggagaggcat cttggtgggt tccttcgacg	4680
tatgcaatga ggcgtatgtg attgtagatg gcagggggaa tgctagcgtg cgcagggcgg	4740
acacctcgga ggagatgtgt ggctttaccg tgaggcctcc aaggggagtg cttgggtgca	4800
tgaatggttg gtacgtgcta acgtgcgcgg gtggtgtagt aagggtagtg cagatagagc	4860
agcctggtcg ccaagagtat ttgtatagct tcagggagag gataggcgag gtaaacgctc	4920
tcgttgacga tgagaggcac gtggcagcag catcaagtga cacgaatata cacctgtggg	4980
attttggggc acagtaggtg gagggcacgg ggggtggggg tagggtagg gttaggcgac	5040
ctgacctaga aagggaaata gacctaaagt aagatggagg ggggatgggt acaagagggg	5100
atgtaggctc tcggtagggt ttgatattag ttttagcacg aggaacctga tatctttgac	5160
ttgtatgaaa gtactttgtg atttttgata tggagag	5197

35

What is claimed is:

1. An isolated polynucleotide comprising: a contiguous coding sequence encoding a polypeptide having at least 95% identity with SEQ ID NO:2, or the complement thereof, or the reverse complement thereof.

2. A vector comprising the polynucleotide of claim 1.

3. A polynucleotide construct comprising a promoter operably linked to the polynucleotide of claim 1.

4. The construct of claim 3, wherein the promoter comprises a constitutive promoter.

5. A plant cell comprising the construct of claim 3.

6. A plant comprising the plant cell of claim 5.

7. The plant of claim 6, wherein the plant exhibits increased expression of the polypeptide, relative to a control plant lacking the construct.

8. The plant of claim 6, wherein the plant exhibits increased growth.

9. The plant of claim 6, wherein the increased growth includes at least one of increased leaf area, increased leaf length, increased leaf width, increased plant height, increased leaf number, increased branch length, and increased stem diameter, relative to a control plant lacking the construct.

10. The plant of claim 6, wherein the plant comprises leaves having an average length at least 20% greater than the average length of leaves of a control plant lacking the construct.

11. The plant of claim 6, wherein the plant has reduced fertility relative to a control plant lacking the construct.

12. The plant of claim 6, wherein the plant is sterile.

13. The plant of claim 6, wherein the plant is a tree.

14. The tree of claim 13, wherein the tree is a poplar, aspen, pine, eucalyptus or sweetgum tree.

15. An isolated polypeptide comprising a sequence having at least 95% identity with SEQ ID NO: 2.

16. A method of producing a transgenic plant comprising: (a) introducing into a plant cell a polynucleotide encoding a polypeptide comprising an amino acid sequence having at least 95% identity with SEQ ID. NO 2; and (b) regenerating the transformed cell to produce a transgenic plant.

17. The method of claim 16, wherein the polypeptide has activity that promotes cell division.

18. The method of claim 16, wherein the plant exhibits increased growth.

19. The method of claim 16, wherein the plant has reduced fertility relative to a control plant.

20. The method of claim 16, wherein the plant is a tree.

21. The method of claim 20, wherein the tree is a poplar, aspen, pine, eucalyptus or sweetgum tree.

22. A transgenic plant produced by the method of claim 16.

23. A method of producing a transgenic plant comprising: (a) introducing into a plant cell a polynucleotide having at least 95% identity to the reverse complement of SEQ ID. NO: 1 and operably linked to a promoter; and (b) regenerating the transformed cell to produce a transgenic plant.

24. The method of claim 23, wherein the plant exhibits decreased growth, sterility, or a combination thereof.

\* \* \* \* \*