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Manipulation of plants by transformation with sequences promoting cell division

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

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

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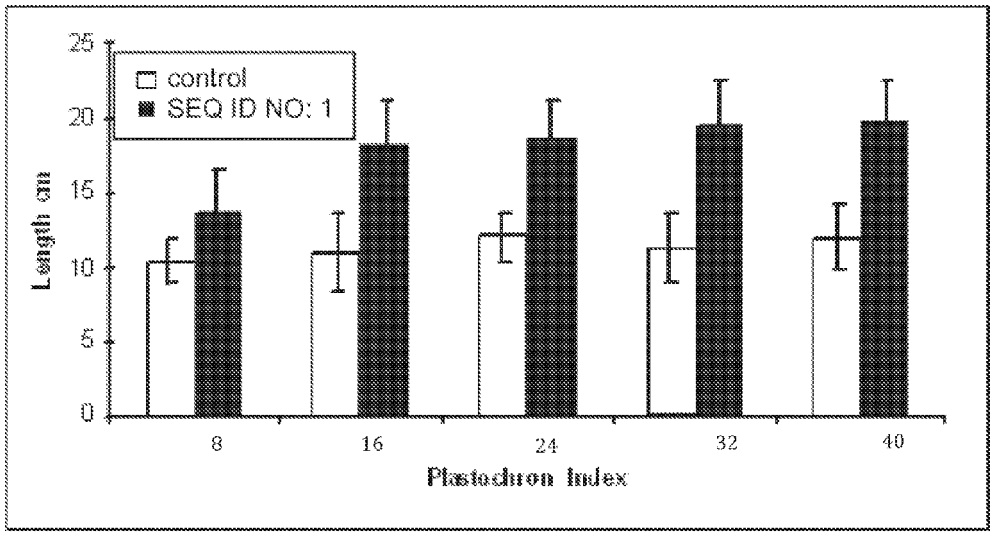
(57) **ABSTRACT**

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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Plastochron Index

FIG. 1

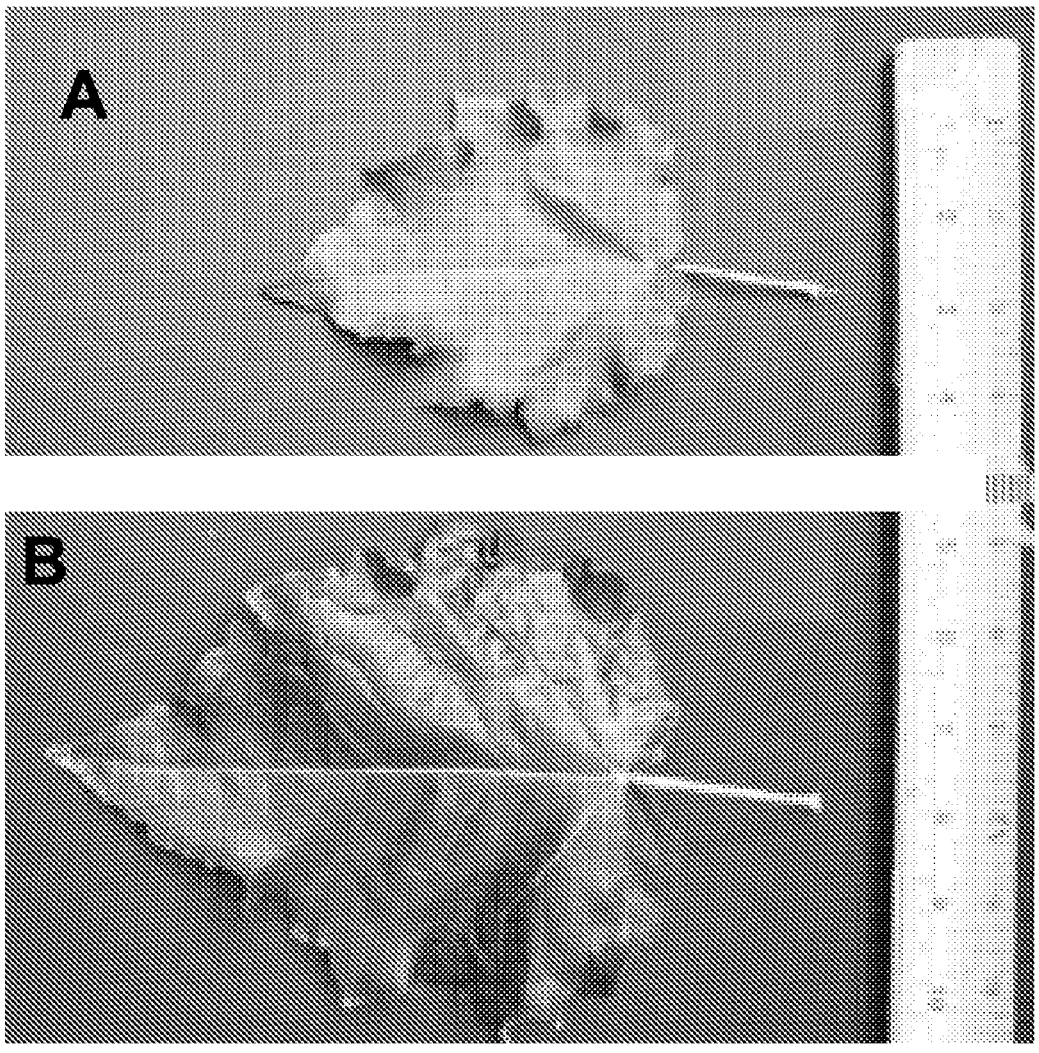

FIG. 2

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 are 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 polynucletide 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.

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,

"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 parameters 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, ubiquitine, 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-

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.

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 μM 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 μM benzyladenine, 0.5 μM zeatin, 5 μM naphthalene acetic acid, 5 μM 2,4-Dichlorophenoxyacetic acid, 0.3% gelling agent [such as Phytagar™ from Gibco BRL], 0.1% gelling agent [such as Phytagel™ 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 μM benzyladenine, 10 μM zeatin, 1 μM N-acetylaspartate, 0.3% Phytagar (Gibco BRL), 0.1% Phytagel (Sigma), 1.28 mM 2-(N-morpholino)ethanesulfonic acid, pH 5.8) for several weeks to months, and explants were subcultured every 2-4 weeks. Regenerated shoots were further screened for kanamycin resistance by rooting in medium supplemented with 0.5 μM 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 Tm, and a shorter arbitrary degenerate (AD) primer on the other side with lower Tm, 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 fgenesh1_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.

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 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

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<212> TYPE: DNA
<213> ORGANISM: Poplar hybrid

<400> SEQUENCE: 1

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agagccatta acgaggtttg gcctgagcct tttctggaag ctcttgctgc ccaagtcgcc 180

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gaggtgttga acgacggggc attagtggac ttcacgggcc gtgggcgatg gtgggtgggc 780

ctatacgctg ggcttccggg ccgggcctat cgtgtctggg atggtaacac cgaagaacct 840

ctctttgaag gcggggcgtt gactgaccca gaggctgtgt tgggttggca cacgttgaca 900

gaagtgactg agtttgtggg ccgagtcagg gtcacgagtc aggaatcggt cgtggcatgc 960

acgagttcga gacttgttat ttttgatttg ggggaccttg gggctgtact aagggaggag 1020

gactacacta acaggagagg catcttggtg ggttccttcg acgtatgcaa tgaggcgtat 1080

gtgattgtag atggcagggg gaatgctagc gtgcgcaggg cggacacctc ggaggagatg 1140

tgtggcttta ccgtgaggcc tccaagggga gtgcttgggt gcatgaatgg tgggtacgtg 1200

ctaacgtgcg cgggtggtgt agtaagggta tggcagatag agcagcctgg tcgccaagag 1260

tatttgtata gcttcaggga gaggataggc gaggtaaacg ctctcgttgc agatgagagg 1320

cacgtggcag cagcatcaag tgacacgaat atacacctgt gggattttgg ggcacagtag 1380

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<211> LENGTH: 459
<212> TYPE: PRT
<213> ORGANISM: Poplar hybrid

<400> SEQUENCE: 2

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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

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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
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<212> TYPE: DNA
<213> ORGANISM: Poplar hybrid

<400> SEQUENCE: 3

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ggtttcccag ccctgcaaag acggcgggga gatatagtta ggttcagttt caggttcatg 180

tgagaaactg ctggttcttc tatttagcca aggtgtgctc aggtgctttt gtacacgtac 240

cactctttgg accaactttc ttttgtactc tcttgtccat ttccaagtga aaactttatc 300

gacgagcttg ctttagtttt tattctttgt atagtcaagg attttcaaat atagtacatg 360

aacactaacg ggaaatacag aggtagctta gtaaaatttt gtttctgcgg gggcacctcc 420

catcatcttt agttcactca cctgaacctg ctctcactac aagctcactg cgagctccct 480

gcatctacag tttcgctgag aaatcttacc acatcaacat tttaaagtga aaaacgaaac 540

ctaaagaaaa cgaagaaaga aatcacgaca tcccacgaag aatcttaact agacaaatct 600

aaccattttt atttttttg aattgctaat gaaaattcca tctgatatgt cagcaatgtt 660

tatggaaatg ctaatataat ttttttatca tatcatgctt tcgtccgaaa ttattggcag 720

aaattcttca tcatattttc tttcggtaaa tcaatgataa aatatttatc catgcttttg 780

gttaaaaatg tataaaaatg atgacaggaa gtcaaagcag cgaaggggaa tggagaataa 840

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agaattcaaa ttagaaattc ttaaagtttt tcctgcccgt ggctatgcgg acactaaagc 960

caaagaaata tcatcatcag tctttcccaa ccaattacca actaccaagc atgcatgacc 1020

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<212> TYPE: DNA
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 4

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cctaggtgta tatgtagggt ttctgattat aaattaaatt gggaaagtga cggagggaag 1560

atggtgacag gagggtgaaa tttacaaggg aaattgattc tttctacaaa gtttgaccaa 1620

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<211> LENGTH: 446
<212> TYPE: PRT
<213> ORGANISM: Arabidopsis thaliana

<400> SEQUENCE: 5

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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

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Cys Thr Arg Trp Arg Ile Met Val Ile Asp Leu Arg Asn Gln Gly Val
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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
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Thr Ala Gln Ser Ile Ile His Leu Trp Asp Phe Gly Ala Leu
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<212> TYPE: DNA
<213> ORGANISM: Vitis vinifera

<400> SEQUENCE: 6

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ggaaatgggg tttggccgga gccttttgtg gaggctctgg 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 ttttgaccca    420

gcttacgtca gctccggcac agccacagct ctctcctgcc gctgcctggc actctccgac    480

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ctccacgtca gcacctgcct ccctcatcac aatgttctta tgggtccctt ctcccgcgcc    600

gtctctggca tcatcctctc tcgcgcccgc ctcgtcttcg cttccctcca cggcgacgtc    660

aacgtcgcgg ctctcgacat cgttgcccct ctcgatcccg ttatccccac tcgccacggc    720

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tgcatcggga gtgtgataag agcgtgggag gtggaacgtg gggtgtatct gtacaacttg   1260

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<212> TYPE: PRT
<213> ORGANISM: Vitis vinifera

<400> SEQUENCE: 7

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20 25 30

Pro Ser Ser Ser Arg Arg Arg Ser Gly Asn Gly Val Trp Pro Glu Pro
35 40 45

Phe Val Glu Ala Leu Ala Ser Gln Val Ala Ile Asp Ala Ser Arg Ser
50 55 60

Ile Gly Arg Leu Ala Ala Ala Pro Ala Leu Ala Asn Val Phe Gln Val
65 70 75 80

Cys Ser Thr Trp Arg Ala Val Ser Arg Ser Asp Leu Leu Trp Arg Asn
85 90 95

Leu Cys Arg Arg Ile Trp Gly Arg Ala Arg Arg Leu His Asp Thr Trp
100 105 110

Arg Glu Glu Phe Ile Tyr Arg His Arg Thr Ala Met Asn Phe Arg Asn
115 120 125

Arg Arg Tyr Asn Tyr Thr Thr Leu Asp Phe Asp Pro Ala Tyr Val Ser
130 135 140

Ser Gly Thr Ala Thr Ala Leu Ser Cys Arg Cys Leu Ala Leu Ser Asp
145 150 155 160

Phe His Leu Ala Val Gly Phe Glu Asp Gly Thr Val Arg Leu Phe Asp
165 170 175

Leu Ala Thr Arg Leu His Val Ser Thr Cys Leu Pro His His Asn Val
180 185 190

Leu Met Gly Pro Phe Ser Arg Ala Val Ser Gly Ile Ile Leu Ser Arg
195 200 205

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

Leu Asp Ile Val Ala Pro Leu Asp Pro Val Ile Pro Thr Arg His Gly
225 230 235 240

Ala Ser Asp Gly Ala Leu Val Asp Phe Ala Gly Cys Ser Arg Trp Trp
245 250 255

Val Gly Leu Tyr Ala Gly Ala Pro Gly Gln Ala Phe Arg Ile Trp Asp
260 265 270

Gly Glu Thr Glu Glu Leu Val Phe Val Gly Gly Thr Leu Thr Asp Pro
275 280 285

Glu Ala Val Met Gly Trp His Met Phe Thr Glu Leu Ala Asp Phe Val
290 295 300

Gly Arg Val Arg Val Thr Ser Gln Glu Ser Ala Val Ala Cys Thr Ser
305 310 315 320

Arg Arg Val Ile Val Phe Asn Leu Arg Asn Gly Gly Asn Val His Gly
325 330 335

Glu Gln Glu Phe Ala Pro Gly Phe Ile Val Gly Ser Leu Asp Ala Asn
340 345 350

Asn Glu Ala Tyr Val Ile Val Asp Gly Arg Gly Val Ala Ser Val Arg
355 360 365

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

<400> SEQUENCE: 8

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tatatattgt ttattaatta atgtcttcct cctcctcctc ctcatcatca tctagcagtg    240

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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 that 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.

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