



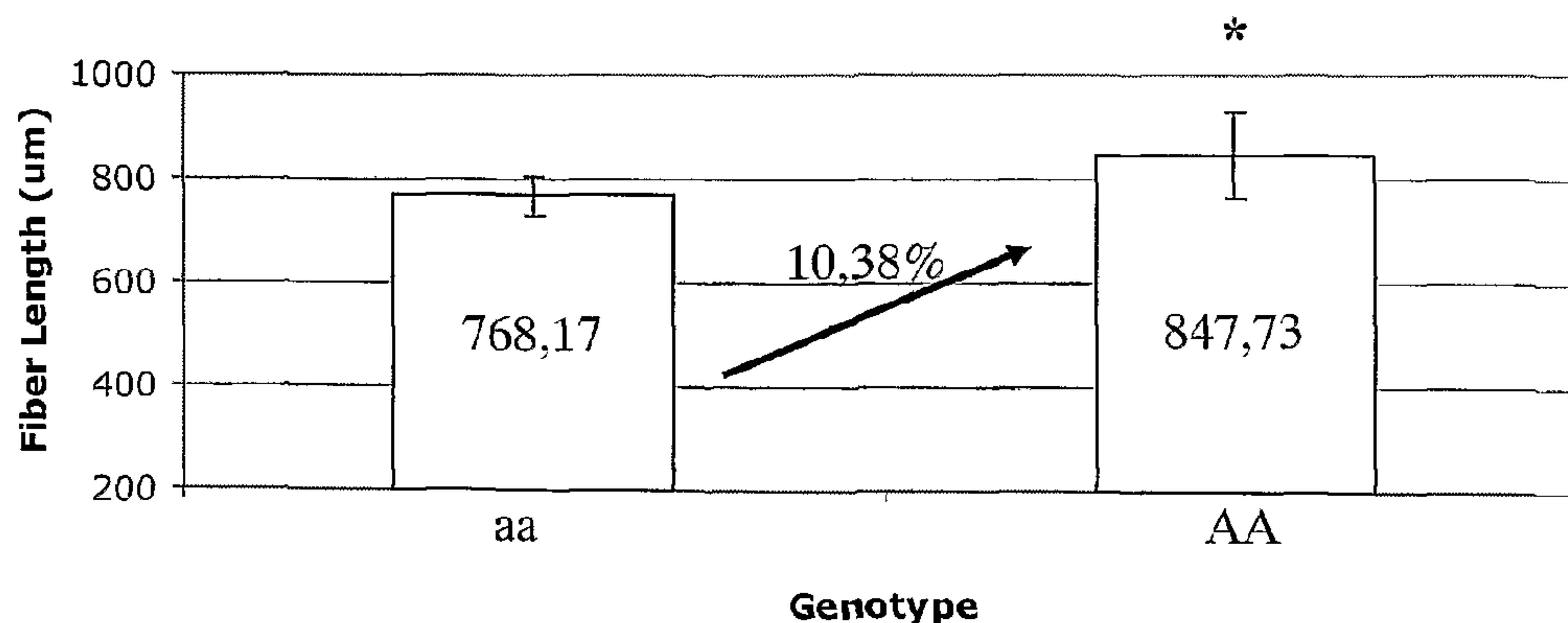
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(54) **Titre : CONSTRUCTION D'ACIDES NUCLEIQUES ET PROCEDES DESTINES A MODIFIER LA LONGUEUR DES FIBRES VEGETALES ET/OU LA HAUTEUR DE LA VARIETE**

(54) **Title: NUCLEIC ACID CONSTRUCTS AND METHODS FOR ALTERING PLANT FIBER LENGTH AND/OR PLANT HEIGHT**

### Fiber Length of WAK4-51B T1 Plants



(57) **Abrégé/Abstract:**

Nucleic acid constructs and methods are disclosed for modifying fiber length, plant height, and/or plant biomass in plant tissues. Plants are genetically engineered with constructs encoding an Arabidopsis thaliana wall-associated kinase gene, which alters fiber length and/or plant height when overexpressed under the control of a cambium/xylem preferred promoter. Plant transformants harboring a wall-associated kinase gene show increased fiber length, a trait that is thought to improve woody trees for pulping and papermaking.



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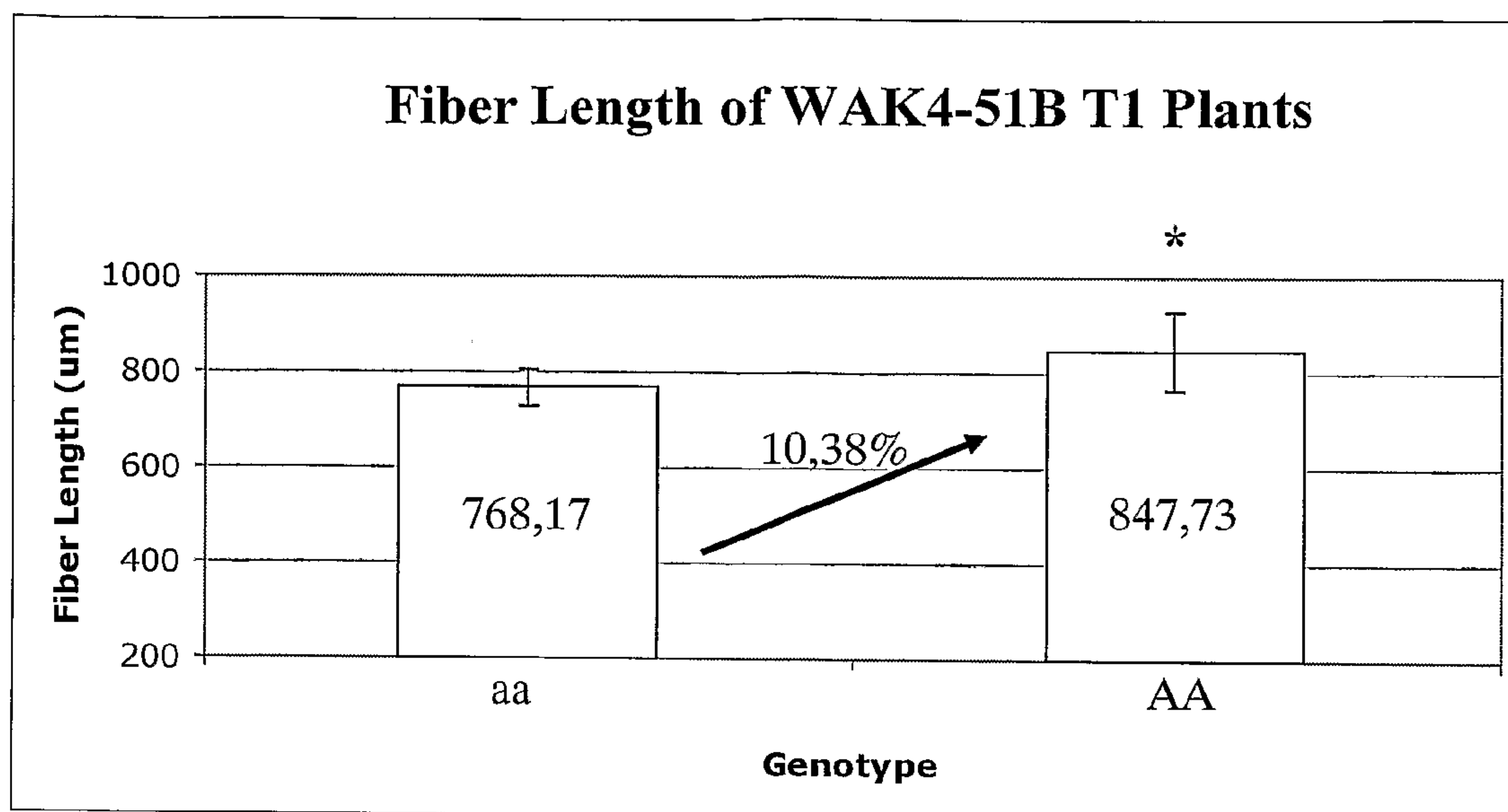
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(54) Title: NUCLEIC ACID CONSTRUCTS AND METHODS FOR ALTERING PLANT FIBER LENGTH AND/OR PLANT HEIGHT



(57) Abstract: Nucleic acid constructs and methods are disclosed for modifying fiber length, plant height, and/or plant biomass in plant tissues. Plants are genetically engineered with constructs encoding an Arabidopsis thaliana wall-associated kinase gene, which alters fiber length and/or plant height when overexpressed under the control of a cambium/xylem preferred promoter. Plant transformants harboring a wall-associated kinase gene show increased fiber length, a trait that is thought to improve woody trees for pulping and papermaking.

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Fiber length is controlled by endogenous regulation of cell elongation, a process which results from the interaction between internal turgor pressure and the mechanical strength of the cell wall, but its mechanism and genes involved have not been yet totally discerned.

5 Xylem fiber cells develop from already much-elongated fusiform initials located within the vascular cambium. They increase in diameter by extension of their radial walls, and, in addition, developing fiber cells elongate by intrusive tip growth, which results in up to a severalfold increase in cell length. Gray-Mitsumune *et al.*, 2004, *Plant Physiol.* 135: 1552-1564.

10 In tip-growing cells, expansion occurs over a small area of the cell surface, which results in tubular, elongated cells. For example, poplar fibers elongate intrusively in the radial-expansion zone in the xylem, reaching 150% of their initial cell length at the average when fully differentiated. Hussey *et al.*, 2006, *Annu. Rev. Plant Biol.* 57: 109-125; Mellerowicz *et al.*, 2001, *Plant Mol. Biol.* 47: 239-274.

15 The rapid expansion of fiber cells may be achieved by concerted action of pushing against the cell wall exerted by turgor and loosening of the cell wall. In cotton fibers, the phase of cell elongation follows a significant rise of turgor, resulted from the observed accumulation of malate, sugars, and  $K^+$ , the major osmoticum, hence the influx of water and the generation of high turgor in the fiber cells. Ruan *et al.*, 2004, *Plant Physiol.* 136: 4104-4113.

20 Vacuolar invertases can play an importante role in turgor maintenance and cell wall expansion. Recent work in *Arabidopsis thaliana* has shown that a wall-associated kinase (WAK) can regulate a vacuolar invertase thus establishing a cross-compartmental link between WAK and vacuolar invertase(s). Kohorn *et al.*, 2006, *Plant J.* 46: 307-316.

In *Arabidopsis* WAKs are encoded by five tightly linked and highly similar genes, and are expressed in leaves, meristems, and cells undergoing expansion. Wagner and Kohorn, 2001, *Plant Cell* 13: 303-318.

25 Mutant seedlings of *Arabidopsis thaliana* presenting a T-DNA insertion in the *WAK2* gene were significantly shorter than wild-type plants, with the roots more affected than the hypocotyls. Kohorn *et al.*, 2006, *Plant J.* 46: 307-316.

30 These mutant plants showed a reduced vacuolar invertase activity by 62%, and the authors proposed that *WAK2* regulates the transcription of vacuolar invertase as one constituent of a mechanism modulating solute concentrations and turgor regulation, thus providing a possible mechanism for WAK to regulate cell expansion.

The expression of an inducible antisense *WAK2* in *Arabidopsis* led to a 50% reduction in WAK protein levels, with a subsequent loss of cell elongation, and hence dwarf plants. Similar

results have been reported when an antisense *WAK4* gene was used to reduce total WAK protein levels. Wagner and Kohorn, 2001, *Plant Cell* 13: 303-318; Lally *et al.*, 2001, *Plant Cell* 13: 1317-1331.

It is also known that the wall-associated kinases contain extracellular domains that can be  
5 linked to pectin molecules of the cell wall, span the plasma membrane and have a cytoplasmic serine/threonine kinase domain. He *et al.*, 1999, *Plant Mol. Biol.* 39: 1189-1196.

When fibers undergo significant elongation at both ends (intrusive tip growth), the properties of the middle lamella limit this type of cell growth. Middle lamellae of developing wood cells are rich in pectins, and intrusive tip growth requires the dissolution of the middle  
10 lamella. See Berthold *et al.*, WO 2006/068603.

By their pectin attachment, it is possible that WAKs may sense a change in the cell wall environment, thus providing a molecular mechanism linking cell wall sensing to regulation of solute metabolism, which in turn is known to be involved in turgor maintenance and cell expansion in growing cells. Such information could be invaluable to adjustment of cell  
15 expansion or turgor. Huang *et al.*, 2007, *Functional Plant Biology*, 34: 499-507.

Fiber characteristics are controlled by a complex set of genetic factors and are not easily amenable to classical breeding methods. Through traditional forest tree breeding it is possible to achieve some modification of fiber characteristics. For example, interspecific triploid hybrids of poplar have been developed which have longer fibers than the parental species. Aziz *et al.*,  
20 1996, Wood and pulp properties of aspen and its hybrids. *TAPPI Proc. Pulping Conference*. p. 437-443. Yet, considering the disadvantage of traditional forest tree breeding, such as the slow progress due to their long generation periods and the difficulty of producing a plant with a desirable trait, the developments in gene technology can reduce significantly the time required to produce a new variety of plant and allow closer targeting of traits considered desirable by the  
25 forest and pulp industries in specific trees species.

### SUMMARY OF THE INVENTION

In one aspect, the invention provides a nucleic acid construct comprising a WAK polynucleotide sequence operably linked to a xylem-preferred promoter that causes  
30 overexpression of said WAK polynucleotide sequence. In an embodiment, the xylem-preferred promoter is selected from the group consisting of TUB gene promoter, SuSy gene promoter, COMT gene promoter and C4H gene promoter. In another embodiment, a transgenic plant comprises the nucleic acid construct and the plant has an increase in fiber length and/or height

compared to a non-transgenic plant of the same species. In further embodiments the plant is a dicotyledon, monocotyledon, gymnosperm, or hardwood tree. The invention further contemplates the progeny of the transgenic plant, as well as wood pulp and wood fiber produced from the transgenic plant.

5 In accordance with one embodiment of the present invention, there is provided a nucleic acid construct defined by SEQ ID NO:1 and comprising a polynucleotide sequence encoding a functional wall-associated kinase 4 (WAK4) operably linked to a xylem-preferred promoter that causes overexpression of the polynucleotide sequence.

10 In another aspect, the invention provides a method for increasing fiber length and/or plant height, comprising: (a) introducing into a plant cell a nucleic acid construct comprising a WAK polynucleotide sequence operably linked to a xylem-preferred promoter that causes overexpression of said WAK polynucleotide sequence; (b) culturing said plant cell under conditions that promote growth of a plant; and (c) selecting a transgenic plant that has increased fiber length and/or plant height compared to a non-transgenic plant of the same species.

15 In accordance with another embodiment of the present invention, there is provided a method for increasing fiber length and/or plant height, comprising: (a) introducing into a plant cell a nucleic acid construct defined by SEQ ID NO:1 and comprising a polynucleotide sequence encoding a functional wall-associated kinase 4 (WAK4) operably linked to a xylem-preferred promoter that causes overexpression of the polynucleotide sequence; (b) culturing the plant cell under conditions that promote growth of a plant; and (c) selecting a transgenic plant that has increased fiber length and/or plant height compared to a non-transgenic plant of the same species.

20

#### BRIEF DESCRIPTION OF THE DRAWINGS

**FIGURE 1** schematically illustrates the plant expression plasmidial vector pALELLYX-WAK of the invention comprising a cambium/xylem preferred promoter driving the expression of a wall-associated kinase nucleotide sequence of the invention.

25 **FIGURE 2** shows the fiber length of several transgenic lines transformed with the plant expression plasmidial vector pALELLYX-WAK of the invention and respective control non-transgenic plants. Asterisk denotes statistically significant higher mean fiber length values ( $P < 0.05$ , t-test).

30 **FIGURE 3** shows the fiber length of two genotypes of a T1 transgenic plant (line 51B) transformed with the plant expression plasmidial vector pALELLYX-WAK of the invention. Asterisk denotes statistically significant higher mean fiber length values ( $P < 0.05$ , t-test).

4a

**FIGURE 4** shows the fiber length of two genotypes of a T1 transgenic plant (line 47B) transformed with the plant expression plasmidial vector pALELLYX-WAK of the invention. Asterisk denotes statistically significant higher mean fiber length values ( $P < 0.05$ , t-test).

**FIGURE 5** shows the plant height of the three genotypes of a T1 transgenic line (line 51B) transformed with the plant expression plasmidial vector pALELLYX-WAK of the invention. Asterisk denotes statistically significant higher mean plant height values ( $P < 0.05$ , t-test).

#### **DETAILED DESCRIPTION OF THE INVENTION**

The present invention relates to processes for genetic manipulation of fiber length in plants and/or an increase in plant height.

The plant cell wall is a strong fibrillar network that gives each cell its stable shape. To enlarge, cells selectively loose this network, enabling it to yield to the expansive forces generated by cell turgor pressure. As a cell expands, there is increased need for a compensatory adjustment in turgor, which is dependent on cell solute metabolism.

5 A wall-associated kinase (WAK) may sense cell wall expansion by its attachment to pectin, thereby providing a mechanism for transducing these signals to systems regulating solute changes, as outlined above. The previous work on WAKs, however, did not presage that the overexpression of a WAK gene in plant, in a tissue-specific manner, results in significant changes in fiber length, as well as significant changes in plant height. The result opens the way  
10 to modifying traits that are extremely important for the plant fiber, forest, pulp, and paper industries.

According to an aspect of the present invention, therefore, a method is provided for modifying the fiber length in plant tissues, such as fiber cells of woody angiosperm xylem, tracheid cells of gymnosperm xylem, and fiber cells of cotton seeds, by controlling the activity of  
15 a wall-associated kinase. Pursuant to this aspect of the invention, plant cells or whole plants are genetically engineered with a wall-associated kinase coding sequence, which, when expressed in xylary fiber cells of angiosperms, xylary tracheids of gymnosperms, or fiber cells of cotton seeds, causes an increase in cell length.

All technical terms used herein are terms commonly used in biochemistry, molecular  
20 biology and agriculture, and can be understood by one of ordinary skill in the art to which this invention belongs. Those technical terms can be found in: MOLECULAR CLONING: A LABORATORY MANUAL, 3rd ed., vol. 1-3, ed. Sambrook and Russel, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001; CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, ed. Ausubel *et al.*, Greene Publishing Associates and Wiley-Interscience, New York,  
25 1988 (with periodic updates); SHORT PROTOCOLS IN MOLECULAR BIOLOGY: A COMPENDIUM OF METHODS FROM CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, 5<sup>th</sup> ed., vol. 1-2, ed. Ausubel *et al.*, John Wiley & Sons, Inc., 2002; GENOME ANALYSIS: A LABORATORY MANUAL, vol. 1-2, ed. Green *et al.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1997. Methodology involving plant biology techniques is described herein and is described in detail in  
30 treatises such as METHODS IN PLANT MOLECULAR BIOLOGY: A LABORATORY COURSE MANUAL, ed. Maliga *et al.*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1995. Various techniques using PCR are described, e.g., in Innis *et al.*, PCR PROTOCOLS: A GUIDE TO METHODS AND APPLICATIONS, Academic Press, San Diego, 1990 and in Dieffenbach and

Dveksler, PCR PRIMER: A LABORATORY MANUAL, 2<sup>nd</sup> ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2003. PCR-primer pairs can be derived from known sequences by known techniques such as using computer programs intended for that purpose, e.g., Primer, Version 0.5, 1991, Whitehead Institute for Biomedical Research, Cambridge, MA. Methods for chemical synthesis of nucleic acids are discussed, for example, in Beaucage and Caruthers, 1981, *Tetra. Letts.* 22: 1859-1862, and Matteucci and Caruthers, 1981, *J. Am. Chem. Soc.* 103: 3185.

Restriction enzyme digestions, phosphorylations, ligations and transformations were done as described in Sambrook *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL, 2<sup>nd</sup> ed. (1989), Cold Spring Harbor Laboratory Press. All reagents and materials used for the growth and maintenance of bacterial cells were obtained from Aldrich Chemicals (Milwaukee, WI), DIFCO Laboratories (Detroit, MI), Invitrogen (Gaithersburg, MD), or Sigma Chemical Company (St. Louis, MO) unless otherwise specified.

The terms "encoding" and "coding" refer to the process by which a gene, through the mechanisms of transcription and translation, provides information to a cell from which a series of amino acids can be assembled into a specific amino acid sequence to produce an active enzyme. Because of the degeneracy of the genetic code, certain base changes in DNA sequence do not change the amino acid sequence of a protein. It is therefore understood that modifications in the DNA sequence encoding wall-associated kinase which do not substantially affect the functional properties of the protein are contemplated.

In this description, "expression" denotes the production of the protein product encoded by a gene. Alternatively or additionally, "expression" denotes the combination of intracellular processes, including transcription and translation, undergone by a coding DNA molecule such as a structural gene to produce a polypeptide. "Overexpression" refers to the expression of a particular gene sequence in which the production of mRNA or polypeptide in a transgenic organism exceeds the levels of production in non-transgenic organism.

The term "heterologous nucleic acid" refers to a nucleic acid, DNA or RNA, which has been introduced into a cell (or the cell's ancestor) through the efforts of humans. Such exogenous nucleic acid may be a copy of a sequence which is naturally found in the cell into which it was introduced, or fragments thereof.

In contrast, the term "endogenous nucleic acid" refers to a nucleic acid, gene, polynucleotide, DNA, RNA, mRNA, or cDNA molecule that is present in a plant or organism that is to be genetically engineered. An endogenous sequence is "native" to, i.e., indigenous to, the plant or organism that is to be genetically engineered.

The term “homologous sequences” refers to polynucleotide or polypeptide sequences that are similar due to common ancestry and sequence conservation.

The term “functional homolog” refers to a polynucleotide or polypeptide sequences that are similar due to common ancestry and sequence conservation and have identical or similar  
5 function at the catalytic, cellular, or organismal levels.

### Wall-Associated Kinase Sequences

In this description, the term “wall-associated kinase polynucleotide sequence” denotes any nucleic acid, gene, polynucleotide, DNA, RNA, mRNA, or cDNA molecule that encodes a  
10 wall-associated kinase polypeptide whose overexpression alters fiber length and/or plant height. The DNA or RNA may be double-stranded or single-stranded. Single-stranded DNA may be the coding strand, also known as the sense strand, or it may be the non-coding strand, also called the anti-sense strand. Illustrative of this category are polynucleotide molecules that comprise SEQ ID NOs: 1, 3, 5, 7 and 9, identified from *Arabidopsis thaliana* and that can be employed to  
15 enhance fiber length and/or plant height.

A wall-associated kinase polynucleotide sequence suitable for the present invention may be identified from a myriad of organisms characterized by the presence of a WAK gene. Although the aforementioned nucleotide sequences are disclosed herein, they are not to be taken as limitations on the present invention. Thus, a WAK sequence can be identified and  
20 functionally annotated by sequence comparison. The skilled person can readily identify a functionally related WAK sequence in a suitable database, such as GenBank, using publicly available sequence-analysis programs and parameters. Alternatively, screening cDNA libraries or genomic libraries employing suitable hybridization probes or primers based on DNA or protein sequences disclosed herein should lead to the identification of functionally related WAK  
25 sequences (functional homolog). It is appreciated in the field as well that sequences with reduced levels of identity also can be isolated with the aid of degenerate oligonucleotides and PCR-based methodology. While the polynucleotides of the inventions are isolated from *Arabidopsis thaliana*, functional homologs from other plants can be employed to produce plants with enhanced fiber length and/or plant height. Examples of plant species from which WAK  
30 genes may be isolated include dicotyledons, such as Cucurbitaceae, Solanaceae, Brassicaceae, Papilionaceae such as alfalfa and *Vigna unguiculata*, Malvaceae, Asteraceae, Malpighiaceae

such as *Populus*, Myrtaceae such as *Eucalyptus*, and monocotyledons, such as gramineae, including rice, wheat, sugarcane, barley, and corn.

In this description, the terms “wall-associated kinase polynucleotide sequence,” “WAK polynucleotide sequence” and “WAK DNA sequence” also refer to any nucleic acid molecule with a nucleotide sequence capable of hybridizing under stringent conditions with any of the sequences disclosed herein, and coding for a polypeptide with WAK activity equivalent to the proteins having amino acid sequences disclosed herein under SEQ ID NOs: 2, 4, 6, 8, or 10. The terms also include sequences which cross-hybridize with SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7 or SEQ ID NO: 9, preferably having at least 65% homology or identity with one or more of SEQ ID NO: 1, 3, 5, 7 or 9. The nucleotide sequences of the invention may encode a protein which is homologous to the predicted gene product disclosed herein under any of SEQ ID NOs: 2, 4, 6, 8, or 10. Further, the nucleotide sequences of the invention include those sequences that encode a WAK polypeptide having an amino acid sequence which has at least 55%, preferably at least 60%, more preferably at least 70%, more preferably at least 80%, more preferably at least 90% and most preferably at least 95% sequence identity to an amino acid sequence disclosed herein under any of SEQ ID NOs: 2, 4, 6, 8 and 10. The degeneracy of the genetic code enables major variations in the nucleotide sequence of a polynucleotide while maintaining the amino acid sequence of the encoded protein.

The phrase “stringent conditions” here connotes parameters with which the art is familiar. Single-stranded polynucleotides hybridize when they associate based on a variety of well-characterized physicochemical forces, such as hydrogen bonding, solvent exclusion, and base stacking. The stringency of a hybridization reflects the degree of sequence identity of the nucleic acids involved, such that the higher the stringency, the more similar are the two polynucleotide strands. Stringency is influenced by a variety of factors, including temperature, salt concentration and composition, organic and non-organic additives, solvents, etc. present in both the hybridization and wash solutions and incubations (and number). One with ordinary skill in the art can readily select such conditions by varying the temperature during the hybridization reaction and washing process, the salt concentration during the hybridization reaction and washing process, and so forth.

For hybridization of complementary nucleic acids which have more than 100 complementary residues, on a filter in a Southern or Northern blot, “stringent” hybridization conditions are exemplified by a temperature that is about 5°C to 20°C lower than the thermal melting point ( $T_m$ ) for the specific sequence, at a defined ionic strength and pH. The  $T_m$  is the

temperature, under defined ionic strength and pH, at which 50% of the target sequence hybridizes to a perfectly matched probe. Nucleic acid molecules that hybridize under stringent conditions typically will hybridize to a probe based on either the entire cDNA or selected portions. More preferably, "stringent conditions" here refers to parameters with which the art is familiar, such as hybridization in 3.5 x SSC, 1 x Denhardt's solution, 25 mM sodium phosphate buffer (pH 7.0), 0.5% SDS, and 2mM EDTA for 18 hours at 65°C, followed by four washes of the filter, at 65°C for 20 minutes, in 2 x SSC and 0.1% SDS, and a final wash for up to 20 minutes in 0.5 x SSC and 0.1% SDS or 0.3 x SSC and 0.1% SDS for greater stringency, and 0.1x SSC and 0.1% SDS for even greater stringency. Other conditions may be substituted, as long as the degree of stringency is equal to that provided herein, using a 0.5xSSC final wash. For identification of less closely related homologues washes can be performed at a lower temperature, e.g., 50°C. In general, stringency is increased by raising the wash temperature and/or decreasing the concentration of SSC.

Additionally, the category of suitable wall-associated kinase sequences includes a nucleic acid molecule comprised of a variant of SEQ ID NOs: 1 or 3 or 5 or 7 or 9 with one or more bases deleted, substituted, inserted, or added, which variant codes for a polypeptide when overexpressed results in alteration in fiber length and/or plant height. The "base sequences with one or more bases deleted, substituted, inserted, or added" referred to here are widely known by those having ordinary skill in the art to retain physiological activity even when the amino acid sequence of a protein generally having that physiological activity has one or more amino acids substituted, deleted, inserted, or added. For example, the poly A tail or 5' or 3' end nontranslation regions may be deleted, and bases may be deleted to the extent that amino acids are deleted. Bases may also be substituted, as long as no frame shift results. Bases also may be "added" to the extent that amino acids are added. It is essential, however, that any such modification does not result in the loss of physiological activity. A modified DNA in this context can be obtained by modifying the DNA base sequences of the invention so that amino acids at specific sites are substituted, deleted, inserted, or added by site-specific mutagenesis, for example. Zoller & Smith, 1982, *Nucleic Acid Res.* 10: 6487-6500. Accordingly, the term "variant" is a nucleotide or amino acid sequence that deviates from the standard, or given, nucleotide or amino acid sequence of a particular gene or protein. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties, e.g., replacement of leucine with isoleucine. A variant may have "nonconservative" changes, e.g., replacement of a glycine with a tryptophan. Analogous minor variations may also

include amino acid deletions or insertions, or both. Guidance in determining which amino acid residues may be substituted, inserted, or deleted may be found using computer programs well known in the art such as Vector NTI Suite (InforMax, MD) software. "Variant" may also refer to a "shuffled gene," as described, for example, in U.S. patents No. 6,506,603, No. 6,132,970, 5 No. 6,165,793 and No. 6,117,679.

A further way of obtaining a WAK DNA sequence is to synthesize it *ab initio* from the appropriate bases, for example, by using the appropriate cDNA sequence as a template.

### **Nucleic Acid Constructs**

10 The present invention includes recombinant constructs comprising one or more of the nucleic acid sequences herein. The constructs typically comprise a vector, such as a plasmid, a cosmid, a phage, a virus (e.g., a plant virus), a bacterial artificial chromosome (BAC), a yeast artificial chromosome (YAC), or the like, into which a nucleic acid sequence has been inserted, in a forward or reverse orientation. Large numbers of suitable vectors are known and commercially available and need not be reiterated here.

15 Recombinant nucleic acid constructs may be made using standard techniques. For example, a nucleotide sequence for transcription may be obtained by treating a vector containing said sequence with restriction enzymes to cut out the appropriate segment. The nucleotide sequence for transcription may also be generated by annealing and ligating synthetic oligonucleotides or by using synthetic oligonucleotides in a polymerase chain reaction (PCR) to 20 give suitable restriction sites at each end. The nucleotide sequence then is cloned into a vector containing suitable regulatory elements, such as upstream promoter and downstream terminator sequences. Typically, plant transformation vectors include one or more cloned plant coding sequence (genomic or cDNA) under the transcriptional control of 5' and 3' regulatory sequences and a selectable marker. Such plant transformation vectors typically also contain a promoter, a 25 transcription initiation start site, an RNA processing signal (such as splicing signal sequences), a transcription termination site, and/or a polyadenylation signal. Enhancers and targeting sequences may also be present.

The invention provides nucleic acid molecules likely to cause altered fiber length and plant height in a transformed plant. An important aspect of the present invention is the use of 30 nucleic acid constructs wherein a wall-associated kinase-encoding nucleotide sequence is operably linked to one or more promoters, which drive expression of the wall-associated kinase-encoding sequence in a constitutive manner or in certain cell types, organs, or tissues so as to

alter the fiber length of a transformed plant compared to the fiber length of a non-transgenic plant.

Suitable constitutive plant promoters which can be useful for expressing the wall-associated kinase sequences suitable for the present invention include but are not limited to the cauliflower mosaic virus (CaMV) 35S promoter, the maize and the *Populus* polyubiquitin promoters, which confer constitutive, high-level expression in most plant tissues (see, e.g., WO 2007/00611, U.S. patent No. 5,510,474; Odell *et al.*, *Nature*, 1985, 313: 810-812); the nopaline synthase promoter (An *et al.*, 1988, *Plant Physiol.* 88: 547-552); the FMV promoter from figwort mosaic virus (U.S. patent No. 5,378,619) and the octopine synthase promoter (Fromm *et al.*, 1989, *Plant Cell* 1: 977-984).

The promoter can also be chosen so that the expression occurs at a determined time point in the plant's development, or at a time point determined by outside influences, or in a tissue-specific or tissue-preferred manner. For example, it may ensure specific or preferred expression in fibers cells (cotton fiber-, xylem fiber-, or extra xylary fiber-specific or -preferred promoters).

Exemplary cotton fiber-specific or -preferred promoters include, for example, the cotton CFACT1 gene promoter (U.S. patent No. 6,995,256); the E6 gene promoter (U.S. patent No. 6,096,950, John *et al.*, 1996, *Plant Mol. Biol.* 30: 297-306; John *et al.*, 1996, *Proc. Natl. Acad. Sci.* 93: 12768-12773); H6 gene promoter (John *et al.*, 1995, *Plant Physiol.* 108: 669-676); GhTUB1 gene promoter (Li *et al.*, 2002, *Plant Physiol.* 130: 666-674) and FbL2A (Rinehart *et al.*, 1996, *Plant Physiol.* 112: 1331-1341 and John *et al.*, 1996, *Proc. Natl. Acad. Sci. USA* 93: 12768-12773).

Vascular system-preferred or -specific promoters, such as xylem-preferred promoters, may be useful for effecting expression of nucleic acid molecules within the invention, specifically in vascular tissue, especially xylem tissue. Thus, "xylem-preferred" means that the nucleic acid molecules of the current invention are more active in the xylem than in any other plant tissue. The selected promoter should cause the overexpression of the wall-associated kinase, pursuant to the invention, thereby to modify the length of the cell xylem, to modify the height of the host plant, or both.

Suitable promoters are illustrated by but are not limited to the xylem-preferred tubulin (TUB) gene promoter, the caffeic acid 3-O-methyltransferase gene promoter (COMT), the sucrose synthase gene promoter (SuSy), and the xylem-preferred coumarate-4-hydroxylase (C4H) gene promoter. Other suitable xylem-preferred promoters are disclosed in international patent application WO 2005/096805.

Synthetic promoters including specific nucleotide regions conferring tissue-specific or tissue-preferred expression may also be used, as exemplified by identification of regulatory elements within larger promoters conferring xylem-preferred expression. Seguin *et al.*, 1997, *Plant Mol. Biol.* 35: 281-291; Torres-Schumann *et al.*, 1996, *Plant J.* 9: 283-296; and Leyva *et al.*, 1992, *Plant Cell* 4: 263-271.

Although the gene expression rate is mainly modulated by the promoter, improvement in expression may also be achieved by the identification and use of enhancer sequences, such as intronic portions of genes, which elevate the expression level of the nearby located genes in an independent manner orientation. In plants, the inclusion of some introns in gene constructs in a position between the promoter and the gene coding sequence leads to increases in mRNA and protein accumulation. Introns known to elevate expression in plants have been identified in maize genes, for example, hsp70, tubA1, Adh1, Sh1, UbH (Brown and Santino, U.S. patent Nos. 5,424,412 and 5,859,347; Jeon *et al.*, 2000, *Plant Physiol.* 123: 1005-1014; Callis *et al.*, 1987, *Genes Dev.* 1 :1183-1200; Vasil *et al.*, 1989, *Plant Physiol.* 91 :1575-1579), and in dicotyledonous plant genes such as rbcS from petunia (Dean *et al.*, 1989, *Plant Cell* 1: 201-208); ST-LS1 from potato (Leon *et al.*, 1991, *Plant Physiol.* 95: 968-972) and UBQ3 (Norris *et al.*, 1993, *Plant Mol. Biol.* 21: 895-906) and PAT1 from *Arabidopsis thaliana* (Rose and Last, 1997, *Plant J.* 11: 455-464).

In accordance with one aspect of the invention, a wall-associated kinase sequence is incorporated into a nucleic acid construct that is suitable for plant transformation. Accordingly, nucleic acid constructs are provided comprising a wall-associated kinase sequence, under the control of a transcriptional initiation region operative in a plant, so that the construct can generate RNA in a host plant cell. Preferably, the transcriptional initiation region is part of a vascular or xylem-preferred promoter, such as any of those mentioned above. Such a nucleic acid construct can be used to modify wall-associated kinase gene expression in plants, as described above.

Expression vectors may also contain a selection marker by which transformed cells can be identified in culture. The marker may be associated with the heterologous nucleic acid molecule, i.e., the gene operably linked to a promoter. As used herein, the term "marker" refers to a gene encoding a trait or a phenotype that permits the selection of, or the screening for, a plant or cell containing the marker. In plants, for example, the marker gene will encode antibiotic or herbicide resistance. This allows for selection of transformed cells from among cells that are not transformed or transfected.

Examples of suitable selectable markers include adenosine deaminase, dihydrofolate reductase, hygromycin-B-phosphotransferase, thymidine kinase, xanthine-guanine phosphoribosyltransferase, glyphosate and glufosinate resistance, and amino-glycoside 3'-O-phosphotransferase (kanamycin, neomycin and G418 resistance). These markers may include  
5 resistance to G418, hygromycin, bleomycin, kanamycin, and gentamicin. The construct also may contain the selectable marker gene *Bar*, which confers resistance to herbicidal phosphinothricin analogs like ammonium glufosinate. Thompson *et al.*, *EMBO J.* 6: 2519-23 (1987). Other suitable selection markers are known as well.

Visible markers such as green fluorescent protein (GFP) may be used. Methods for  
10 identifying or selecting transformed plants based on the control of cell division have also been described. See John and Van Mellaert, WO 2000/052168, and Fabijansk *et al.*, WO 2001/059086.

Replication sequences, of bacterial or viral origin, may also be included to allow the vector to be cloned in a bacterial or phage host. Preferably, a broad host range prokaryotic origin  
15 of replication is used. A selectable marker for bacteria may be included to allow selection of bacterial cells bearing the desired construct. Suitable prokaryotic selectable markers also include resistance to antibiotics such as kanamycin or tetracycline.

Other DNA sequences encoding additional functions may also be present in the vector, as is known in the art. For instance, when *Agrobacterium* is the host, T-DNA sequences may be  
20 included to facilitate the subsequent transfer to and incorporation into plant chromosomes.

### **Plants for Genetic Engineering**

The present invention comprehends the genetic manipulation of plants, especially hardwood trees, to overexpress a wall-associated kinase in vascular tissues via introducing a wall-associated gene, preferably under the control of a xylem-preferred or xylem-specific  
25 promoter. The result is enhanced fiber length and plant height.

In this description, the term "plant" denotes any fiber-containing plant material that can be genetically manipulated, including but not limited to differentiated or undifferentiated plant cells, protoplasts, whole plants, plant tissues, or plant organs, or any component of a plant such as a leaf, stem, root, bud, tuber, fruit, rhizome, or the like.

30 Plants that can be engineered in accordance with the invention include but are not limited to trees, such as *Eucalyptus* species (*E. alba*, *E. albens*, *E. amygdalina*, *E. aromaphloia*, *E. baileyana*, *E. balladoniensis*, *E. bicostata*, *E. botryoides*, *E. brachyandra*, *E. brassiana*, *E.*

*brevistylis*, *E. brockwayi*, *E. camaldulensis*, *E. ceracea*, *E. cloeziana*, *E. coccifera*, *E. cordata*, *E. cornuta*, *E. corticosa*, *E. crebra*, *E. croajingolensis*, *E. curtisii*, *E. dalrympleana*, *E. deglupta*, *E. delegatensis*, *E. delicata*, *E. diversicolor*, *E. diversifolia*, *E. dives*, *E. dolichocarpa*, *E. dundasii*,  
5 *E. dunnii*, *E. elata*, *E. erythrocorys*, *E. erythrophloia*, *E. eudesmoides*, *E. falcata*, *E. gamophylla*,  
*E. glaucina*, *E. globulus*, *E. globulus* subsp. *bicostata*, *E. globulus* subsp. *globulus*, *E. gongylocarpa*, *E. grandis*, *E. grandis x urophylla*, *E. guilfoylei*, *E. gunnii*, *E. hallii*, *E. houseana*,  
*E. jacksonii*, *E. lansdowneana*, *E. latisinensis*, *E. leucophloia*, *E. leucoxyton*, *E. lockyeri*, *E. lucasii*, *E. maidenii*, *E. marginata*, *E. megacarpa*, *E. melliodora*, *E. michaeliana*, *E. microcorys*,  
10 *E. microtheca*, *E. muelleriana*, *E. nitens*, *E. nitida*, *E. obliqua*, *E. obtusiflora*, *E. occidentalis*, *E. optima*, *E. ovata*, *E. pachyphylla*, *E. pauciflora*, *E. pellita*, *E. perriniana*, *E. petiolaris*, *E. pilularis*, *E. piperita*, *E. platyphylla*, *E. polyanthemos*, *E. populnea*, *E. preissiana*, *E. pseudoglobulus*, *E. pulchella*, *E. radiata*, *E. radiata* subsp. *radiata*, *E. regnans*, *E. risdonii*, *E. robertsonii*, *E. rodwayi*, *E. rubida*, *E. rubiginosa*, *E. saligna*, *E. salmonophloia*, *E. scoparia*, *E. sieberi*, *E. spathulata*, *E. staeri*, *E. stoatei*, *E. tenuipes*, *E. tenuiramis*, *E. tereticornis*, *E.*  
15 *tetragona*, *E. tetradonta*, *E. tindaliae*, *E. torquata*, *E. umbra*, *E. urophylla*, *E. vernicosa*, *E. viminalis*, *E. wandoo*, *E. wetarensis*, *E. willisii*, *E. willisii* subsp. *falciformis*, *E. willisii* subsp. *willisii*, *E. woodwardii*), *Populus* species (*P. alba*, *P. alba x P. grandidentata*, *P. alba x P. tremula*, *P. alba x P. tremula* var. *glandulosa*, *P. alba x P. tremuloides*, *P. balsamifera*, *P. balsamifera* subsp. *trichocarpa*, *P. balsamifera* subsp. *trichocarpa x P. deltoides*, *P. ciliata*, *P.*  
20 *deltoides*, *P. euphratica*, *P. euramericana*, *P. kitakamiensis*, *P. lasiocarpa*, *P. laurifolia*, *P. maximowiczii*, *P. maximowiczii x P. balsamifera* subsp. *trichocarpa*, *P. nigra*, *P. sieboldii x P. grandidentata*, *P. suaveolens*, *P. szechuanica*, *P. tomentosa*, *P. tremula*, *P. tremula x P. tremuloides*, *P. tremuloides*, *P. wilsonii*, *P. canadensis*, *P. yunnanensis*), Conifers such as  
loblolly pine (*Pinus taeda*), slash pine (*Pinus elliotii*), ponderosa pine (*Pinus ponderosa*),  
25 lodgepole pine (*Pinus contorta*), and Monterey pine (*Pinus radiata*); Douglas-fir (*Pseudotsuga menziesii*); Western hemlock (*Tsuga canadensis*); Sitka spruce (*Picea glauca*); redwood (*Sequoia sempervirens*); true firs such as silver fir (*Abies amabilis*) and balsam fir (*Abies balsamea*); and cedars such as Western red cedar (*Thuja plicata*) and Alaska yellow-cedar (*Chamaecyparis nootkatensis*).

30 Fiber-producing plants also are included in this context. Illustrative crops are cotton (*Gossypium* spp.), flax (*Linum usitatissimum*), stinging nettle (*Urtica dioica*), hop (*Humulus lupulus*), lime trees (*Tilia cordata*, *T. x. europaea* and *T. platyphyllus*), spanish broom (*Spartium junceum*), ramie (*Boehmeria nivea*), paper mulberry (*Broussonetya papyrifera*), New Zealand

flax (*Phormium tenax*), dogbane (*Apocynum cannabinum*), *Iris* species (*I. douglasiana*, *I. macrosiphon* and *I. purdyi*), milkweeds (*Asclepia species*), pineapple, banana and others. Also contemplated are forage crops, such as alfalfa, lolium, festuca and clover.

In the present description, "transgenic plant" refers to a plant that has incorporated a nucleic acid sequence, including but not limited to genes that are not normally present in a host plant genome, nucleic acid sequences not normally transcribed into RNA or translated into a protein, or any other genes or nucleic acid sequences that one desires to introduce into the wild-type plant, such as genes that normally may be present in the wild-type plant but that one desires either to genetically engineer or to have altered expression. The "transgenic plant" category includes both a primary transformant and a plant that includes a transformant in its lineage, e.g., by way of standard introgression or another breeding procedure.

A "hybrid plant" refers to a plant or a part thereof resulting from a cross between two parent plants, wherein one parent is a genetically engineered plant of the invention. Such cross can occur naturally by, for example, sexual reproduction, or artificially by, for example, in vitro nuclear fusion. Methods of plant breeding are well-known and within the level of one of ordinary skill in the art of plant biology.

In contrast, a plant that is not genetically manipulated is a control plant and is referred to as a "non-transgenic" or "control" plant. Non-transgenic plant can be a plant which genome is neither modified by the introduction of a construct comprising the polynucleotide sequences or fragment thereof of the present invention. It can also be a plant regenerated from cultured cells or tissues without prior modification by the introduction of a construct comprising the polynucleotide sequence of the invention, or may comprise a homozygote recessive progeny (i.e., do not have any copy of the transgene) resulting from self-fertilization of a transgenic plant.

It is contemplated that, in some instances, the genome of an inventive transgenic plant will have been augmented through the stable introduction of a transgene. In other instances, however, the introduced gene will replace an endogenous sequence. A preferred gene in the regard, pursuant to the present invention, is a wall-associated kinase DNA sequence, for example, one obtained from *Arabidopsis thaliana*.

### Methods for Genetic Engineering

Constructs according to the invention may be introduced into any plant cell, using a suitable technique. Both monocotyledonous and dicotyledonous angiosperm or gymnosperm plant cells may be genetically engineered in various ways known to the art. For example, see

Klein *et al.*, 1993, *Biotechnology* 4: 583-590; Bechtold *et al.*, 1993, *C. R. Acad. Sci. Paris* 316: 1194-1199; Koncz and Schell, 1986, *Mol. Gen. Genet.* 204: 383-396; Paszkowski *et al.*, 1984, *EMBO J.* 3: 2717-2722; Sagi *et al.*, 1994, *Plant Cell Rep.* 13: 262-266.

5 *Agrobacterium* species such as *A. tumefaciens* and *A. rhizogenes* can be used, for example, in accordance with Nagel *et al.*, 1990, *Microbiol Lett* 67: 325. In brief, *Agrobacterium* may be used with a plant expression vector via, e.g., electroporation, after which the *Agrobacterium* is introduced to plant cells via, e.g., the well known leaf-disk method.

10 Additional methods for accomplishing this include, but are not limited to, transformation by *Rhizobium*, *Sinorhizobium* or *Mesorhizobium* (Broothaerts *et al.*, 2005, *Nature* 433: 629-633), electroporation, particle gun bombardment, calcium phosphate precipitation, and polyethylene glycol fusion, transfer into germinating pollen grains, direct transformation (Lorz *et al.*, 1985, *Mol. Genet.* 199: 179-182), and other methods known to the art. If a selection marker, such as kanamycin resistance, is employed, it makes it easier to determine which cells have been successfully transformed.

15 The *Agrobacterium* transformation methods discussed above are known to be useful for transforming dicots. Additionally, de la Pena *et al.*, 1987, *Nature* 325: 274-276; Rhodes *et al.*, 1988, *Science* 240: 204-207; and Shimamoto *et al.*, 1989, *Nature* 328: 274-276, all of which are incorporated by reference, have transformed cereal monocots using *Agrobacterium*. Also see Bechtold and Pelletier, 1998, *Methods Mol. Biol.* 82: 259-266, showing the use of vacuum  
20 infiltration for *Agrobacterium*-mediated transformation.

The presence of a protein, polypeptide, or nucleic acid molecule in a particular cell can be measured to determine if, for example, a cell has been successfully transformed or transfected. The ability to carry out such assay is well known and need not be reiterated here.

### **Quantifying Fiber Length and Plant Height**

25 The word "fiber" is often used to unify a diverse group of plant cell types that share in common the features of having an elongated shape and abundant cellulose in thick cell walls, usually, but not always, described as secondary walls. Such walls may or may not be lignified, and the protoplast of such cells may or may not remain alive at maturity. In some industries, the term "fiber" is usually inclusive of thick-walled conducting cells such as vessels and tracheids  
30 and to fibrillar aggregates of many individual fiber cells. For the purposes of the present invention, the term "fiber" includes: (a) conducting and non-conducting cells of the xylem; (b)

fibers of extraxylary origin, including those from phloem, bark, ground tissue, and epidermis; and (c) fibers from stems, leaves, roots, seeds, and flowers or inflorescences.

Transgenic plants of the invention are characterized by increased fiber length and preferably increased height as well. Increased fiber length in the genetically engineered plant is preferably achieved via WAK overexpression in the plant tissues wherein cell expansion occurs. In describing a plant of the invention, "increased fiber length" refers to a quantitative augmentation in the length of fiber cells in the plant when compared to the length of fiber cells in a wild-type plant". A quantitative increase of fiber length can be measured by several techniques, such as digitizing, the Kajaani procedure, and the Fiber Quality Analyzer. Han *et al.*, 1999, In: Kenaf Properties, Processing and Products, Mississippi State University, Ag & Bio Engineering, pp 149-167.

The fiber length in the engineered plant of the invention is at least from 5 to 15% longer, preferably at least 10-30% and most preferably at least from 20-50% longer than the fiber length of the wild-type plant.

Because increased fiber length can be followed by an increase in plant height, transgenic plants of the invention may have increase fiber length and height. In this description, therefore, the phrase "increased plant height" connote a quantitative increase in plant height, when compared to the height of a wild-type plant. The height in the engineered plant of the invention can be increased to levels of about 5% to about 90%, preferably about 10% to about 75%, even more preferably about 15% to about 65% of the height of the wild-type plant.

\*\*\*\*\*

Specific examples are presented below of methods for obtaining wall-associated kinase genes, as well as for introducing the target gene, via *Agrobacterium*, to produce plant transformants. They are meant to be exemplary and not as limitations on the present invention.

### Example 1

#### Isolation of a wall-associated kinase DNA sequence from *Arabidopsis thaliana*

##### (a) RNA preparation from *Arabidopsis thaliana* stem and cDNA synthesis

Stem cuttings of three-months-old *Arabidopsis thaliana* plants were cut in small pieces, frozen in liquid nitrogen, and used for RNA extraction via the cetyltrimethyl-ammonium bromide (CTAB) extraction method. Aldrich and Cullis, 1993, *Plant Mol. Biol. Report*, 11:128-141. A cDNA pool was used in RT-PCR experiments in which the isolated total RNA was used

as template, and Superscript II reverse transcriptase (Invitrogen) and oligo(dT) primer were used to synthesize the first-strand cDNA. Double-stranded cDNA was obtained by the subsequent polymerase reaction, using gene-specific primers, as described below.

(b) Primer design

5 A cDNA sequence representing the wall-associated kinase 4 mRNA from *Arabidopsis thaliana* has been determined and deposited in the GenBank under accession number NM101974. Based on this sequence, DNA oligomers were synthesized as primers for PCR, including either the region around the first codon ATG or around the termination codon of the main ORF encoding the wall-associated kinase 4.

10 Primers were designed to amplify the entire coding region of the wall-associated kinase 4 ORF, i.e., from the ATG through the translation stop codon. The sequences of the primers are given below:

15 WAK\_NDE Length: 23 SEQ ID NO: 11  
CATATGAAAGTGCAGCGTCTGTT

WAK\_XBA Length: 23 SEQ ID NO: 12  
TCTAGATCAGCGGCCTGCTTCAA

20 (c) PCR Amplification

The cDNA sample obtained in (a) was used as template, and the primers designed in (b) were used for PCR. The PCR steps involved 40 cycles of 1 minute at 94°C, 1 minute at 50°C, and 2 minutes at 72°C followed by an extra step of elongation at 72°C for 7 minutes. The PCR products were isolated by gel electrophoresis on 1.0% agarose followed by ethidium bromide staining of the electrophoresed gel and detection of amplified bands on a UV transilluminator. The detected amplified band was verified and cut out of the agarose gel with a razor. The pieces of gel were transferred to 1.5 mL microtubes, and the DNA fragments were isolated and purified using a GFX PCR clean-up and gel band purification kit (Amersham). The recovered DNA fragments were subcloned to the pGEM-T cloning vector (Promega), transformed into *E. coli*, and then used to prepare plasmid DNA in the usual manner, which was then sequenced by the dideoxy method (Messing, 1983, *Methods in Enzymol.* 101: 20-78), using BigDye chemistry (Applied Biosystems), to yield the DNA sequence disclosed here as SEQ ID NO: 1, for use pursuant to the present invention.

## Example 2

### Preparation of Transgenic *Nicotiana tabacum* Plants

The wall-associated kinase gene obtained in Example 1 above was introduced into a plant host to produce transgenic *Nicotiana tabacum* plants.

(a) Preparation of constructs and transformation of *Agrobacterium*

5 Expression constructs were prepared by cleaving the wall-associated kinase gene obtained in Example 1 above with suitable restriction enzymes so as to include all of the open reading frame and inserting the gene into the plant transformation vector pALELLYX-WAK (FIGURE. 1) together with an appropriate promoter. For example, the wall-associated kinase gene obtained in Example 1 was cloned into the aforementioned expression vector downstream  
10 to a xylem-preferred tubulin gene (TUB) promoter from *Populus deltoides*, as set forth in international application WO 2005/096805. The resulting expression construct was amplified in *E. coli*, and then transformed by freeze thawing into *Agrobacterium tumefaciens* LBA4404 strain.

(b) *Agrobacterium*-mediated transformation of *Nicotiana tabacum*

15 Transformation of *Nicotiana* sp. was accomplished using the leaf disk method of Horsch *et al.*, 1985, *Science* 227:1229, using a nucleic acid construct comprising the wall-associated kinase gene obtained in (a) operably linked to the TUB promoter of a xylem-preferred gene. The transformants were selected on Murashige and Skoog medium (Sigma, St. Louis, MO) containing 100 milligrams/liter of kanamycin and 500 mg/L carbenicillin (Sigma). The  
20 transformed tobacco shoots were allowed to root on the Murashige and Skoog medium, and were subsequently transferred to soil and grown in the greenhouse.

(c) PCR verification of foreign gene insertion into the host plant genome

PCR can be used to verify the integration of the gene construct in the genome of transgenic plants. The PCR reaction mixture contained 100 ng genomic DNA of transformed  
25 plant, and 0.2  $\mu$ M of each primer described above, 100  $\mu$ M of each deoxyribonucleotide triphosphate, 5 $\mu$ L PCR buffer and 2.5 Units of AmpliTaq<sup>®</sup> DNA polymerase (Applied Biosystems) in a total volume of 50  $\mu$ L. The cycling parameters were as follows: 94°C for 1 minute, 50°C for 1 minute and 72°C for 3 minutes, for 40 cycles, with 5 minutes at 72°C extension. The PCR products were electrophoresized on a 1% agarose gel.

30 (d) Determination of transgene expression level in transgenic plants

Semi-quantitative RT-PCR was used to detect the accumulation of wall-associated kinase transcripts in stem tissue of the transgenic plants. Total RNA was isolated from stem cuts of 3-

months old transgenic *Nicotiana* T0 and T1 plants using the CTAB method described by Aldrich and Cullis, 1993, *Plant Mol. Biol. Report.* 11:128-141.

cDNA was synthesized from 500 ng of total RNA using Superscript II RNase H- RT (Invitrogen, USA). The primers described above were used along with primers for the constitutive gene encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as an internal control to normalize the quantity of total RNA used in each sample. PCR was done with a 12.5-fold dilution of the first-strand cDNA under the following conditions: 94°C for 3 minutes and 27 cycles of 94°C for 1 minute, 52 to 60°C for 45 seconds, and 72°C for 1 minute and 30 seconds.

### Example 3

#### Increase in Fiber Length in Tobacco Transgenic Plants Overexpressing Wall-Associated Kinase Gene in Vascular Tissues

Stem regions corresponding to 50% height of transgenic and control plants of 5 months old were macerated in acetic acid-peroxide solution at 70°C for 48 hours or until single cells were obtained. Cells were stained with safranine and examined under a microscope (Leica DMIL) fitted with a camera (Sony) linked to a personal computer. Cells (about 100 per line) were measured directly on the screen, using the "Image Tool" software.

Three of the transgenic events, known to express the transgene according to procedure detailed in Example 2, showed a statistically significant increase in fiber length (FIGURE 2). Transgenic event 43B exhibits an increase of 21% in fiber length as compared to the control plants ( $P < 0.05$ , t-test). Transgenic event 47B exhibits an increase of 19% in fiber length when compared to the control plants (FIGURE 2;  $P < 0.05$ , t-test). Additionally, transgenic event 43B exhibit an increase of 15% in fiber length as compared to the control plants (FIG. 2  $P < 0.05$ , t-test).

It is important to mention that another strategy to increase fiber length by the overexpression of a pectin methyl esterase gene (Berthold *et al.*, WO 2006/068603) has achieved an increase of only 5% on fiber length of transgenic plants when compared to control plants.

After grown to maturity, the T0 events were selfed to generate T1 lines. Plants that are homozygote dominant present a significant increase of 10% in fiber length ( $P < 0.05$ , t-test), when compared to homozygote recessive plants. These results were observed in two different lines (FIGURE 3 and FIGURE 4).

### Example 4

## Increase in Plant Height in Tobacco Transgenic Plants Overexpressing Wall-Associated Kinase Gene in Vascular Tissues

5 T<sub>1</sub> progeny resulting from self-fertilization of transgenic plants was individually potted 3 weeks after sowing. Growth was measured periodically until the first flower was formed (plants were about 5 months old), and was recorded as total height.

10 The results presented are an example of the increase in plant height observed in the homozygote dominant plants of different lines. Plant height of the three genotypes from the event 51B was compared. Plants that are homozygote dominant are 12% higher than the homozygote recessive plants. Plants that are hemizygote are 9% higher than the homozygote recessive plants (P<0.05, t-test) (FIGURE 5).

### Example 5

15

#### Preparation of Transgenic *Populus* Plants

The gene obtained in Example 1 above was introduced into a plant host to produce transgenic *Populus* plants.

20 (a) Preparation of constructs and transformation of *Agrobacterium*

Expression constructs can be prepared by cleaving the wall-associated kinase gene obtained in Example 1 above with suitable restriction enzymes so as to include the entire open reading frame and inserting the gene into the plant transformation vector pALELLYX-WAK (FIG. 1) together with an appropriate promoter. For example, the wall-associated kinase gene 25 obtained in Example 1 was cloned into the aforementioned expression vector downstream to a xylem-preferred tubulin gene (TUB) promoter from *Populus deltoides*, as set forth in international application WO 2005/096805. The resulting expression construct was amplified in *E. coli*, and then transformed by freeze thawing into *Agrobacterium tumefaciens* LBA4404 strain.

30 (b) *Agrobacterium*-mediated transformation of *Populus*

Wild-type aspen was transformed with *Agrobacterium tumefaciens* carrying a construct comprising an *Arabidopsis thaliana* wall-associated kinase gene obtained in Example 1 operably linked to the promoter of a xylem-preferred gene (TUB). Petioles and internodal stem segments from in vitro micropropagated plants were used as explants. Transformed shoots are selected on

regeneration medium containing 100mg/L of kanamycin and allowed to root on the Murashige and Skoog medium. Selected plants are subsequently transferred to soil and grown in the greenhouse.

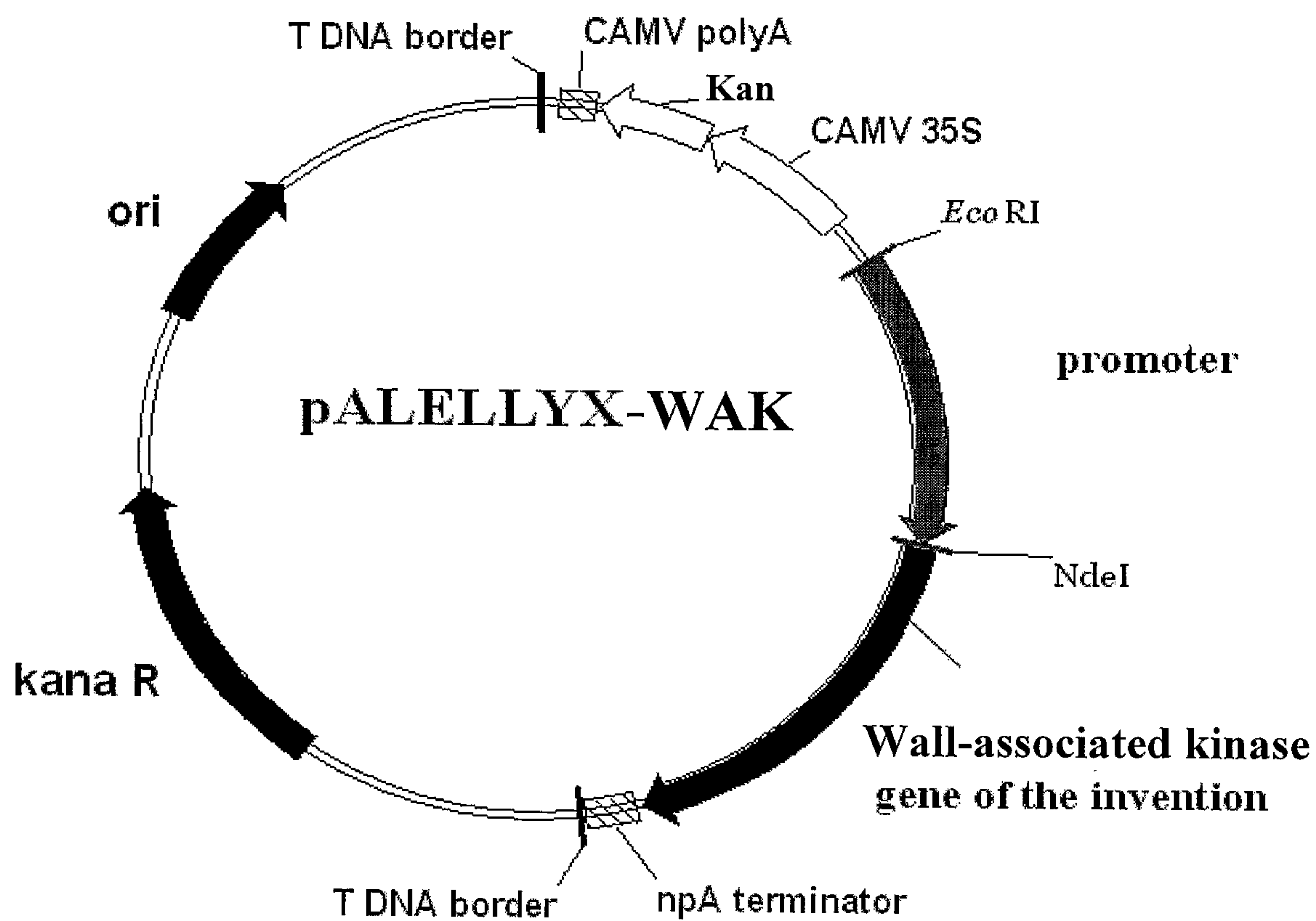
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The embodiments of the present invention for which an exclusive property or privilege is claimed are defined as follows:

1. A nucleic acid construct defined by SEQ ID NO:1 and comprising a polynucleotide sequence encoding a functional wall-associated kinase 4 (WAK4) operably linked to a xylem-preferred promoter that causes overexpression of said polynucleotide sequence.
2. The nucleic acid construct of claim 1, wherein said xylem-preferred promoter is selected from the group consisting of TUB gene promoter, SuSy gene promoter, COMT gene promoter and C4H gene promoter.
3. A transgenic plant cell comprising the nucleic acid construct of claim 1, wherein said plant cell has an increase in cell length compared to a non-transgenic plant cell of the same species.
4. The transgenic plant cell of claim 3, wherein the xylem-preferred promoter is selected from the group consisting of TUB gene promoter, SuSy gene promoter, COMT gene promoter, and C4H gene promoter.
5. The transgenic plant cell of claim 3, wherein said plant cell is a dicotyledon plant cell.
6. The transgenic plant cell of claim 3, wherein said plant cell is a monocotyledon plant cell.
7. The transgenic plant cell of claim 3, wherein said plant cell is a gymnosperm plant cell.
8. The transgenic plant cell of claim 3, wherein said plant cell is a hardwood tree plant cell.
9. The transgenic plant cell of claim 8, wherein said hardwood tree plant cell is an *Eucalyptus* tree plant cell.
10. The transgenic plant cell of claim 8, wherein said hardwood tree plant cell is a *Populus* tree plant cell.
11. The transgenic plant cell of claim 7, wherein said gymnosperm plant cell is a *Pinus* tree plant cell.

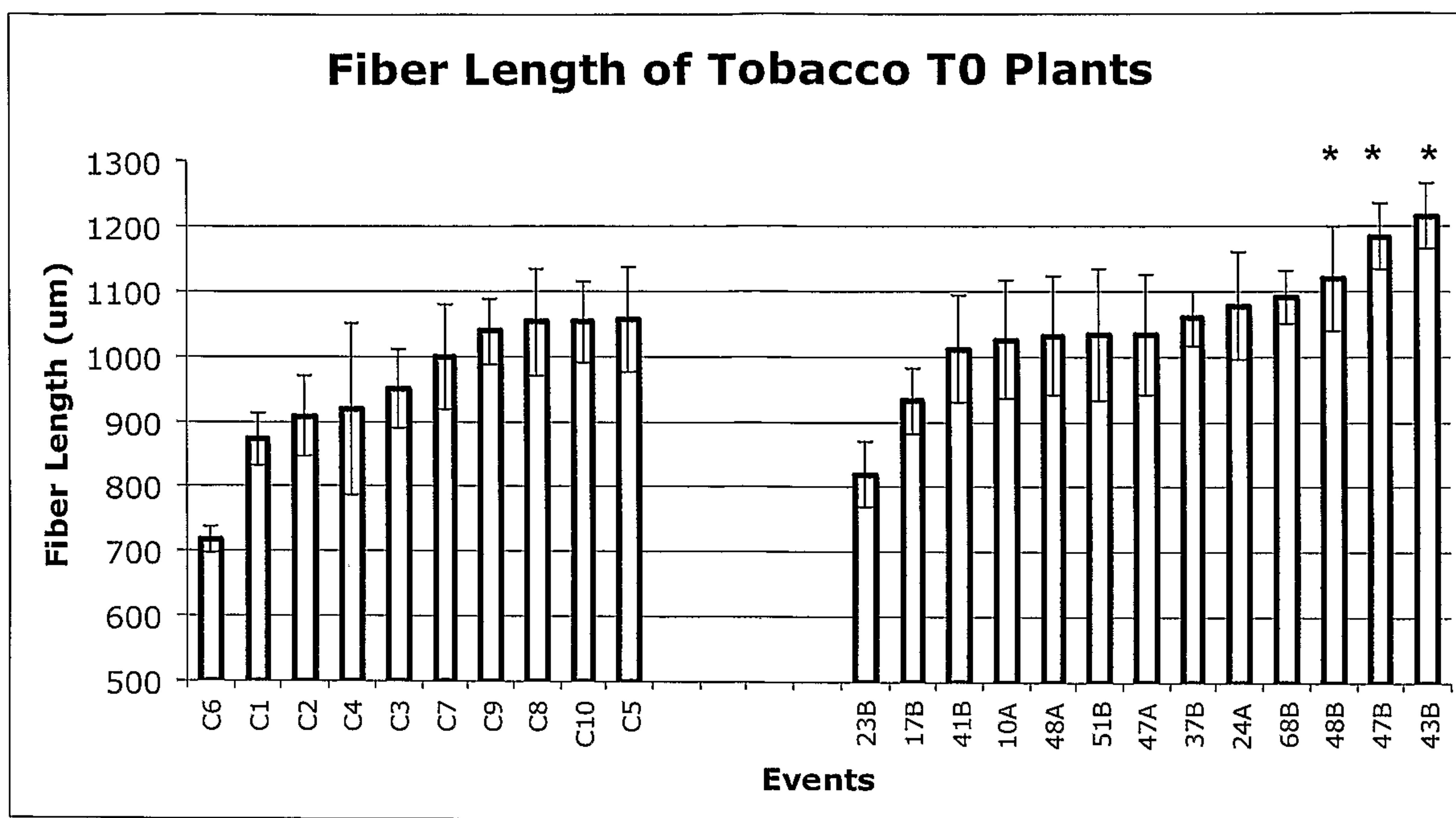
12. A method for increasing fiber length and/or plant height, comprising:
  - (a) introducing into a plant cell a nucleic acid construct defined by SEQ ID NO:1 and comprising a polynucleotide sequence encoding a functional wall-associated kinase 4 (WAK4) operably linked to a xylem-preferred promoter that causes overexpression of said polynucleotide sequence;
  - (b) culturing said plant cell under conditions that promote growth of a plant; and
  - (c) selecting a transgenic plant that has increased fiber length and/or plant height compared to a non-transgenic plant of the same species.
13. The method of claim 12, wherein said xylem-preferred promoter is selected from the group consisting of TUB gene promoter, SuSy gene promoter, COMT gene promoter, and C4H gene promoter.
14. A wood pulp obtained from transgenic plant cells of claim 3, wherein said plant cells are fiber cells having an increase in cell length compared to wood pulp obtained from non-transgenic fiber cells of the same species.
15. A wood fiber obtained from transgenic plant cells of claim 3, wherein said plant cells are fiber cells having an increase in cell length compared to wood pulp obtained from non-transgenic fiber cells of the same species.
16. A recombinant plasmid comprising the nucleic acid construct as defined in claim 1.
17. Use of the transgenic plant cell of any one of claims 3 to 11 to produce a transgenic plant.

**FIG. 1**

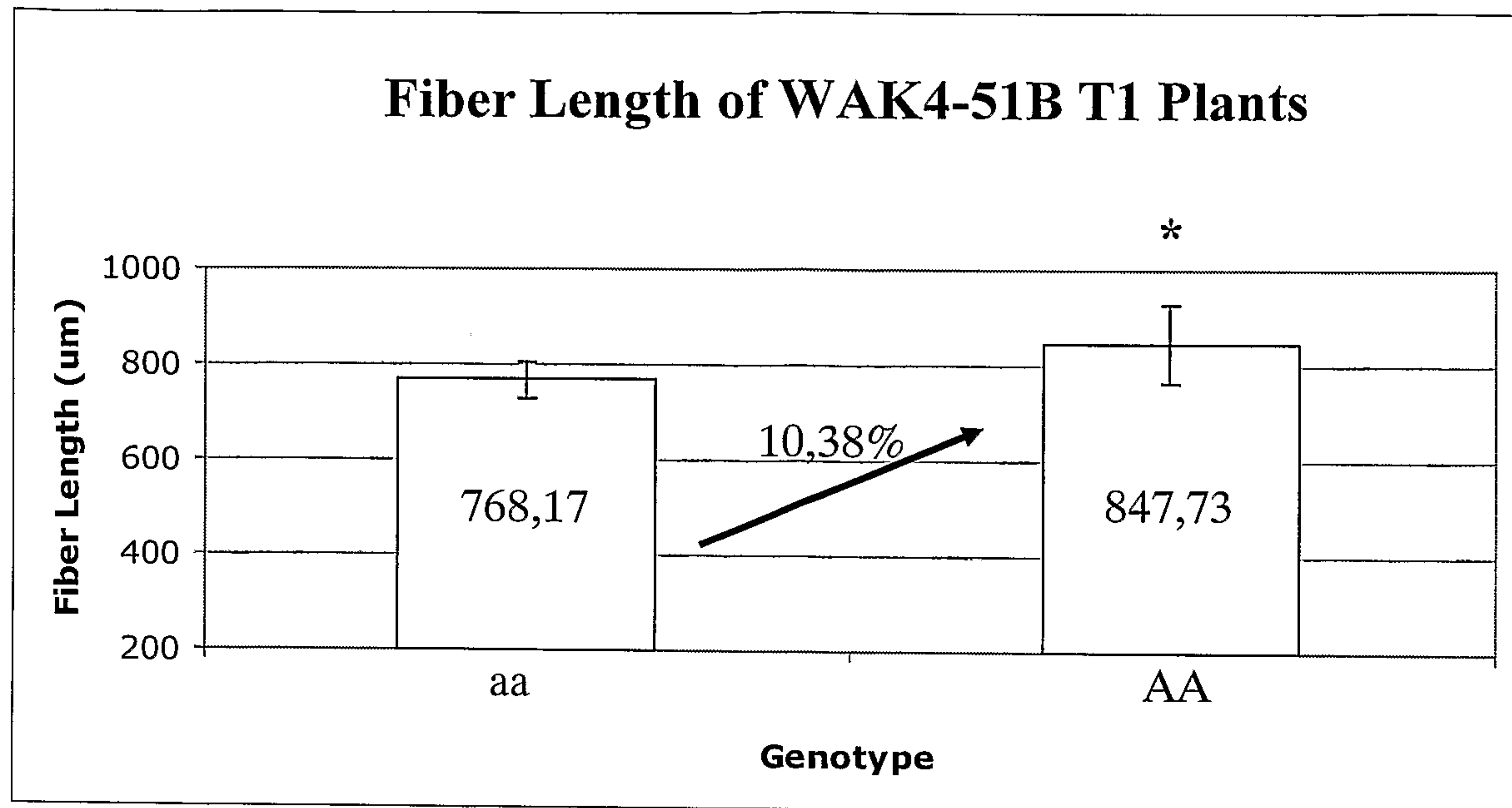


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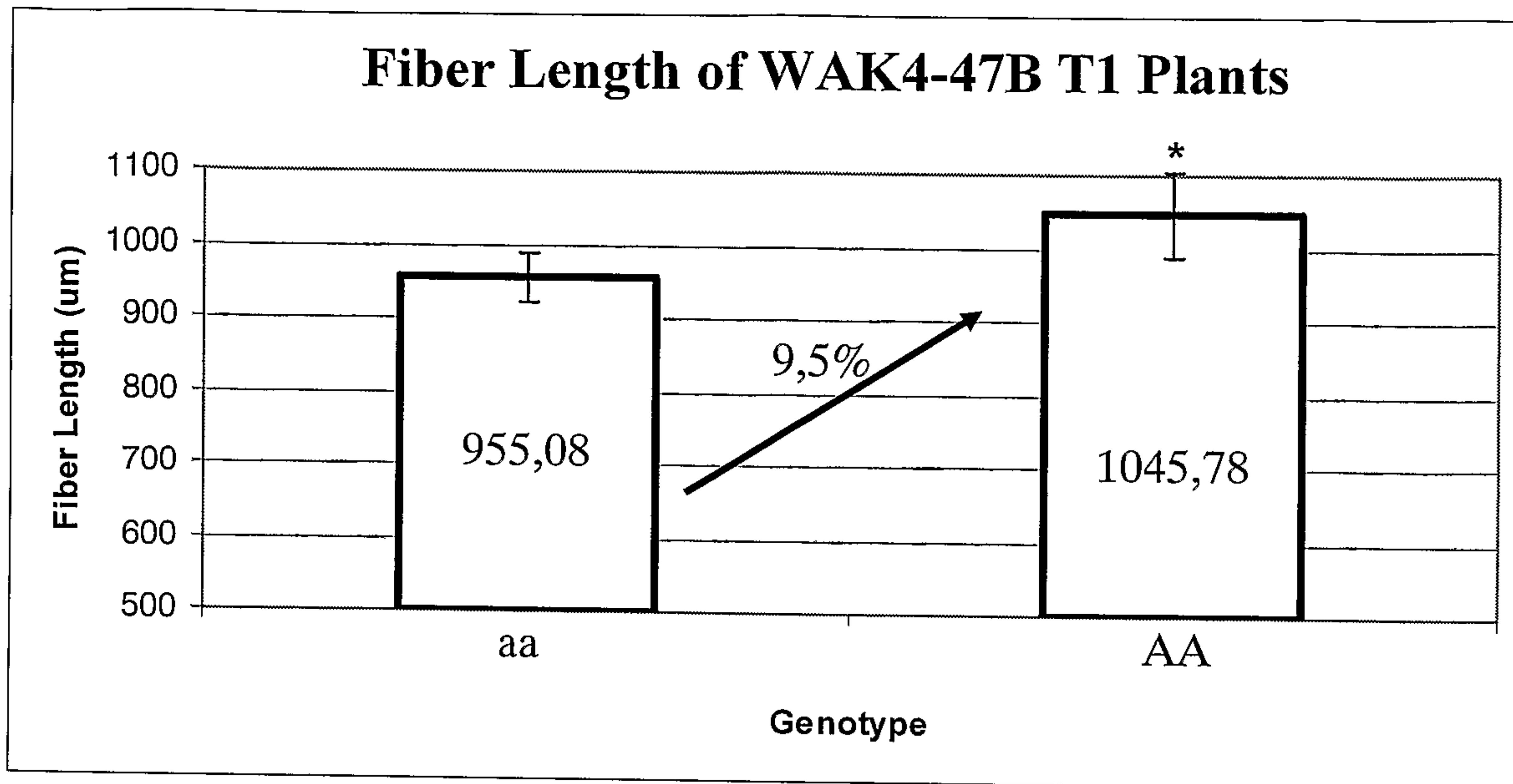
**FIG. 2**



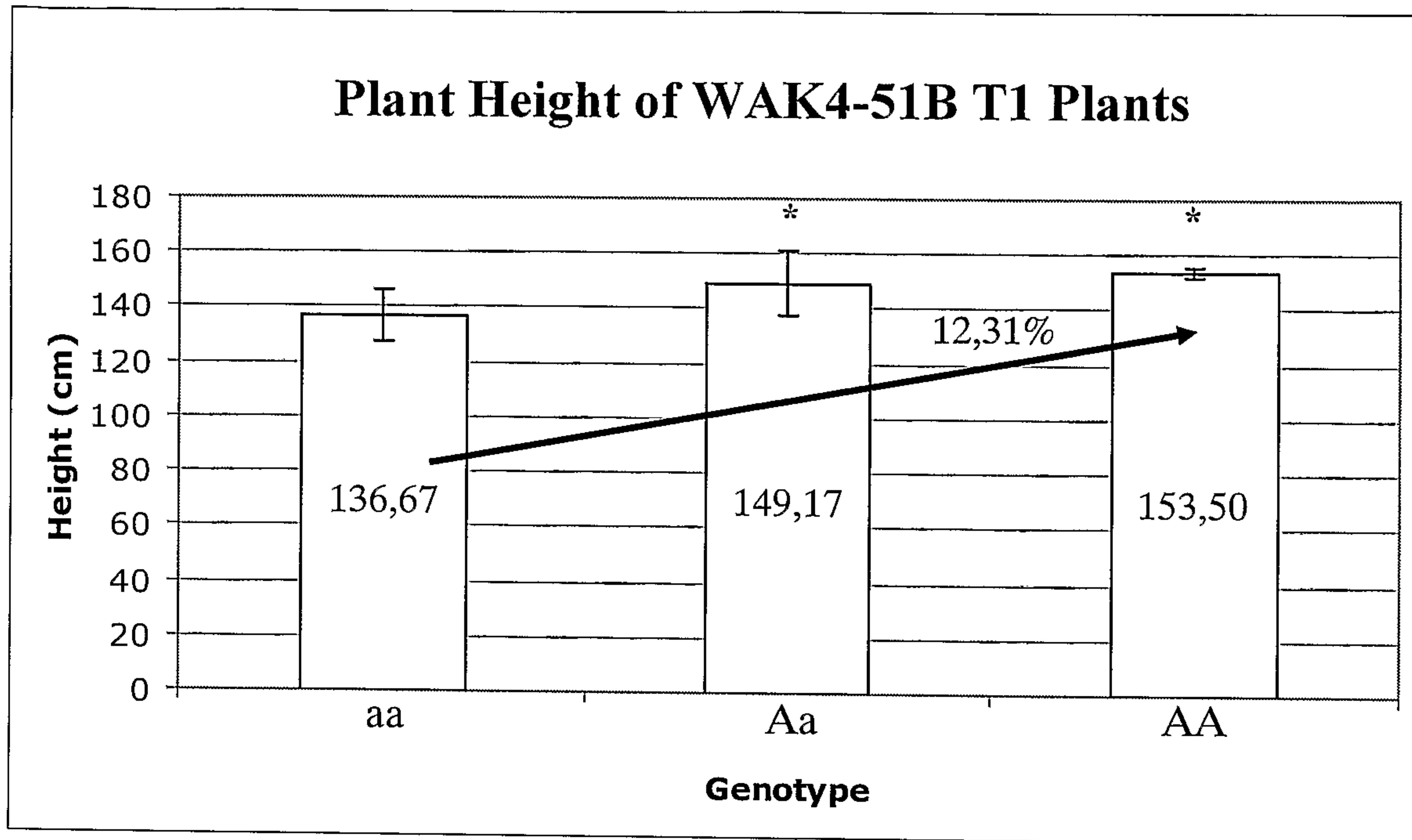
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**FIG. 3**

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**FIG. 4**

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**FIG. 5**

## Fiber Length of WAK4-51B T1 Plants

