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Genetic engineering of wood color in plants

Vincent Lee C. Chiang

Chung-Jui Tsai

Michigan Technological University, chtsai@mtu.edu

Gopi K. Podila


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United States Patent [19]

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[54] **GENETIC ENGINEERING OF WOOD
COLOR IN PLANTS**

[75] Inventors: **Vincent Lee C. Chiang; Chung Jui
Tsai**, both of Hancock; **Gopi K. Podila**,
Houghton, all of Mich.

[73] Assignee: **Board of Control of Michigan
Technological University**, Houghton,
Mich.

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[51] **Int. Cl.**⁶ **C12N 15/29**; C12N 15/82;
A01H 5/00; A01H 4/00

[52] **U.S. Cl.** **800/205**; 800/DIG. 48;
435/172.3; 435/320.1; 435/419; 536/23.6;
536/24.1

[58] **Field of Search** 800/205, DIG. 8;
435/172.3, 320.1, 419; 536/23.6, 24.1

[56] **References Cited**

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5,451,514 9/1995 Boudet et al. 435/172.3

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2005597 6/1990 Canada C12N 15/00
WO 93/05160 3/1993 WIPO C12N 15/54

Primary Examiner—Douglas W. Robinson

Assistant Examiner—Thomas Haas

Attorney, Agent, or Firm—Michael Best & Friedrich LLP

[57] **ABSTRACT**

The invention relates to genetically engineering the wood color of woody plants by incorporation of the lignin pathway gene O-methyltransferase into the genome of the plants.

30 Claims, 1 Drawing Sheet

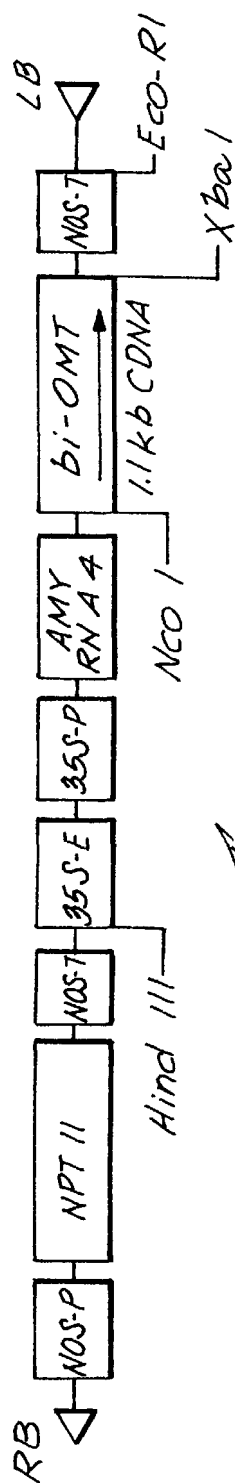


Fig. 1

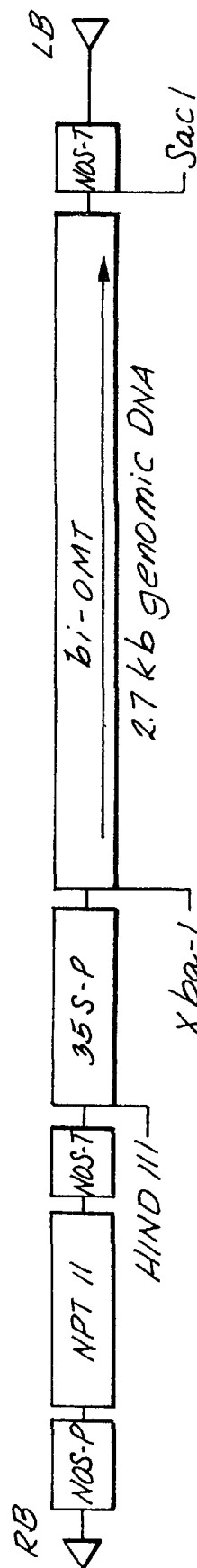


Fig. 2

GENETIC ENGINEERING OF WOOD COLOR IN PLANTS

RELATED APPLICATION

This application claims the benefit of prior filed, copending provisional application Ser. No. 60/007727 filed Nov. 30, 1995 entitled "GENETIC MODIFICATION OF ANGIOSPERM PULPWOOD SPECIES".

FIELD OF THE INVENTION

The invention relates to genetically modifying the wood color of woody plants, and more particularly, to genetically modifying the wood color of woody plants through the genetic manipulation of a lignin pathway gene such as O-methyltransferase.

BACKGROUND OF THE INVENTION

Genetic engineering of forest tree species to conform to desired traits has shifted the emphasis in forest tree improvement away from the traditional breeding programs during the past decade. Although research on genetic engineering of forest trees has been vigorous, the progress has been slow due.

Very little progress has been reported regarding the genetic engineering of color in plant species. The ability to genetically alter the color of plants would be of great value to industries such as the furniture industry to make furniture from genetically modified wood or to the paper industry. Accordingly, there exists a need for such genetic color modification of plant species.

Further, there is a need for improving the efficiency of pulping of wood. Considerable monetary and environmental costs are incurred by the paper industry in removing lignin from cellulose during the production of wood pulp and paper.

SUMMARY OF THE INVENTION

The invention provides a method to genetically alter the wood color of woody plants using the lignin pathway gene O-methyltransferase. The genetically altered color creates unique grain patterns in wood. Due to the genetic modification using a lignin pathway gene, the genetically altered woody plant also has an altered lignin structure making processing such as pulping easier and more energy efficient.

It is one object of the present invention to provide a method to genetically alter the wood color of woody plants.

It is another object of the present invention to provide a method to genetically alter the natural color of wood through the manipulation of a lignin pathway gene.

It is another object of the present invention to provide a method to both genetically alter the color of wood as well as genetically alter the structure of the lignin in that wood.

It is another object of the present invention to genetically alter the color of the wood of plants from the genus *Populus*.

Other features and advantages of the invention will become apparent to those of ordinary skill in the art upon review of the following detailed description, claims, and drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a diagram of construct pFOMT1 which contains a 1.1 kb bi-OMT cDNA sense fragment with the whole coding region between 35S enhancer-promoter/AMV RNA4 and NOS terminator; and

FIG. 2 is a diagram of construct pFOMT2 which contains a 2.7 kb genomic bi-OMT full-length DNA in the sense orientation between 35S promoter and NOS terminator.

Before one embodiment of the invention is explained in detail, 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 or illustrated in the drawings. The invention is capable of other embodiments and of being practiced or being carried out in various ways. 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.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

The color of woody plant species can be modified by transformation with a lignin pathway gene and specifically the lignin pathway gene that codes for the enzyme O-methyltransferase (OMT).

The O-methyltransferase enzyme of gymnosperms and angiosperms differ in substrate specificity for caffeic acid, with gymnosperms being monospecific for caffeic acid and angiosperms being bispecific, catalyzing the methylation of both caffeic acid and 5-hydroxyferulic acid. Gymnosperm lignin also termed guaiacyl lignin is composed mainly of one precursor (coniferyl alcohol) whereas angiosperm lignin also termed guaiacyl-syringyl lignin is formed from the polymerization of two main precursors (coniferyl alcohol and sinapyl alcohol). The ratio of syringyl to guaiacyl units is directly related to the efficiency of kraft delignification, with higher syringyl quantities improving the efficiency. Softwoods largely synthesize coniferyl alcohol and form a lignin which is virtually completely made up of guaiacyl units. Hardwoods synthesize both coniferyl and sinapyl alcohols forming less condensed lignin of guaiacyl/syringyl mixtures in various proportions. The ratio of syringyl to guaiacyl units is directly related to the efficiency of kraft pulping, as the lignin found in angiospermous trees is less condensed than the lignin in gymnospermous trees, and is therefore more easily separated from the wood's cellulose in the pulping process. The sinapyl alcohol precursor of syringyl lignin is absent in softwoods, due to a deficiency of two key enzymes in the phenylpropanoid pathway; bi-specific O-methyltransferase and ferulic acid 5-hydroxylase.

The OMT enzyme has been studied in many plants some of which include Japanese black pine, shoots of bamboo, ginkgo, poplar, tobacco, spinach beet, soybean, parley, alfalfa root nodules, eucalyptus and aspen.

Generally, the wood color of woody plant species can be altered by genetic transformation with a homologous OMT gene in the sense orientation. The description of the invention below refers to aspen (*Populus tremuloides*) when necessary for the sake of example. However, it should be noted that the invention is not limited to the modification of the wood color in aspen. The method of the present invention is capable of being practiced for other woody plant species using an homologous OMT gene.

A. OMT Gene

The present invention utilizes a homologous OMT gene to genetically alter the wood color of woody plants. The invention as described below utilizes a cDNA clone of the OMT gene. However, it should be noted that genomic DNA can also be utilized in the present invention.

Purified and isolated OMT DNA can be obtained using a cDNA cloning method such as set forth below and in Bugos et al., Plant Mol. Bio. 17:1203-1215 (1991) which is incorporated herein by reference. A cDNA clone encoding OMT is isolated by immunological screening of a λ gt11 expression library prepared from poly(A)⁺ RNA of developing secondary xylem as follows.

The differentiating xylem of the species is obtained. Total RNA is extracted from the developing secondary xylem. See for example Logemann et al., *Anal. Biochem* 163:16–20 (1987). 5M guanidine hydrochloride is used in order to reduce starch gelatinization. The RNA is further purified by precipitation with 2.5M LiCl. See for example Okita et al., *Plant Physiol.* 69:834–839 (1982). Poly(A)⁺ RNA is isolated using Hybond-mAP paper. From the poly(A)⁺ RNA, double-stranded cDNA is prepared using a library construction system from Invitrogen Corporation, San Diego, Calif. See for example Sambrook et al., *Molecular Cloning*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1989). The double stranded cDNA is ligated to linkers and then cloned into a vector, for example to EcoR1-Not1 linkers and then into the EcoR1 site of λ gt11 (Stratagene Cloning Systems, La Jolla, Calif.).

The vector is then used to transform or transfect a host cell. With λ gt11, the insert-containing lambda vectors are packaged with lambda proteins and infected into *Escherichia coli* such as strain Y1090. See for example Mierendorf et al., *Methods In Enzymol.* 152:458–569 (1987).

The cDNA library thus prepared can be screened in any suitable manner. In a preferred embodiment, the host cells are transformed or transfected in a manner allowing the host cell to express the polypeptide of the DNA inserted into the vector. This can be done by utilizing a vector having DNA sequences flanking the insertion area with one or more codons preferred for expression in *E. coli* cells. In such a case, the host cells themselves, or extracts of the host cells, can be screened with antibodies against the OMT enzyme.

The OMT enzyme used to prepare antibodies is purified from xylem using a combination of purification techniques as set forth in Bugos et al., *Phytochemistry* 31:5:1495–1498 (1992). The OMT enzyme is isolated from differentiating xylem and is then purified such as 180-fold by a process using DEAE-cellulose chromatography, HPLC gel filtration and affinity chromatography on S-adenosyl-L-homocysteine agarose. Using denaturing polyacrylamide gel electrophoresis (SDS-PAGE), one protein band with a molecular weight of 45,000 daltons is observed. The purified OMT enzyme catalyzes the methylation of both 5-hydroxyferulic acid and caffeic acid, with an activity ratio of 3.1:1. S-adenosyl-L-homocysteine is an effective inhibitor of the enzyme.

Using the purified enzyme, rabbit antibodies to the OMT enzyme can be produced in a conventional manner. Bugos et al., *Phytochemistry* 31:5:1495–1498 (1992). The cDNA can then be screened with antibodies against the OMT enzyme. Clones can be detected by the antibodies as expressing OMT polypeptides. The DNA from the clones can then be isolated. The clones have an insert DNA of about 1.5 bp. After the putative, positive λ gt11 clones are plaque purified. Insert DNA of a clone is excised with Not1 and sub-cloned into Bluescript II. See for example Sambrook et al., *Molecular Cloning*, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor (1989).

The nucleotide sequence can then be determined by the dideoxynucleotide method, for example Sanger et al., *Proc. Natl. Acad. Sci.* 74:5463–5467 (1977), using a T7 Sequencing Kit for the entire length of the clone in both directions. Subclones are prepared by excision with EcoR1, BamH1, XhoI and HincII and sequenced using primers complementary to sequences bordering the multiple cloning site of Bluescript II (Stratagene Cloning Systems, La Jolla, Calif.). Synthetic oligonucleotide primers are used to verify overlap regions of restriction sites. An open-reading frame of 1095 bp encodes a polypeptide of 365 amino acid residues with a

predicted molecular weight of 39,802 daltons which corresponds well with the size of the bispecific OMT subunit.

The nucleotide sequence of bispecific aspen OMT cDNA clone is set forth as SEQ ID NO:1. The nucleotide sequence of SEQ ID NO:1 is numbered beginning with the 5' end of the cDNA clone. SEQ ID NO:1 includes the amino acid sequence, in standard three letter designations, directly beneath the corresponding codons.

The deduced amino-acid sequence for aspen bispecific OMT is set forth as SEQ ID NO:2. The first methionine of the open reading frame of the amino acid sequence is designated as the first amino acid of the putative polypeptide.

Three internal peptides of purified aspen bispecific OMT sequenced by automated Edman degradation are identical to portions of SEQ ID NO:1. Since the amino terminus of aspen bispecific OMT is blocked, as determined by the automated Edman degradation, these three internal peptides were deduced by digesting the purified enzyme with *Staphylococcus aureus* endoproteinase Glu-C. The peptides were isolated by reverse-phase HPLC, and were sequenced by automated Edman degradation.

The polynucleotide code for the OMT enzyme was expressed as a protein in *E. coli*, as the Bluescript II vector has codons preferred for expression in *E. coli* cells. The OMT expressed from the Bluescript II vector in *E. coli* was found to have bispecific activities in approximately the same ratio as that of the natural enzyme. This expressed protein was also recognized by the antibodies for bispecific OMT enzyme.

The antibody for aspen bispecific OMT was also used to select an OMT clone from an alfalfa cDNA library, which was prepared from RNAs induced by a fungal elicitor, and 85% of the alfalfa OMT's predicted amino acid residues were found to be identical to that of the aspen bispecific OMT. This demonstrates a substantial amount of duplication in amino acid sequences encoding plant O-methyltransferases from diverse plant species.

The OMT gene has been isolated and sequenced in the following plant species: aspen, hybrid aspen, hybrid poplar, alfalfa, tobacco, prunus, zinnia and eucalyptus.

Comparisons have been made between the nucleotide level between the nucleotide sequence of aspen OMT cDNA and that of other plant species. The percentage identity of OMT of various plant species is set forth below with accession numbers referring to the Gene Bank:

Hybrid poplar (*P.trichocarpa* x *deltoides*) OMT cDNA, accession #M73431, 97% identity;
 Hybrid aspen (*P.Kitakamiensis*) OMT genomic DNA, accession #D49710, 97% identity (exons);
 Hybrid aspen (*P.Kitakamiensis*) OMT genomic DNA, accession #D49711, 89% identity (exons);
 Prunus (*Prunus amygdalus*) OMT cDNA, accession #X83217, 81% identity;
 Eucalyptus (*Eucalyptus gunnii*) OMT cDNA, accession #X74814, 74% identity;
 Alfalfa (*Medicago sativa*) OMT cDNA, accession #M63853, 77% identity;
 Tobacco (*N.tabacum*) OMT1a cDNA, accession #X74452, 74% identity;
 Tobacco (*N.tabacum*) OMT1b cDNA, accession #X74453, 75% identity;
 Zinnia (*Zinnia elegans*) OMT cDNA, accession #U19911, 71% identity; and
 Chrysosplenium americanum OMT cDNA, accession #U16793, 75% identity.

B. Transformation and Regeneration

Several methods for gene transformation of plant species with the OMT gene are available such as the use of a transformation vector, agroinfection, electroinjection, particle bombardment with a gene gun or microinjection. Preferably, a binary vector construct such as those set forth in FIGS. 1 and 2 is mobilized into a strain of *Agrobacterium* species. Preferably, *Agrobacterium* such as *tumefaciens* strain C58 is used as the DNA delivery system due to its efficiency and low cost. See Koncz, C. et al., Mol. Gen. Genet. 204:383–396 (1986). The vectors are mobilized in *Agrobacterium tumefaciens* using the freeze-thaw method of Holstein et al., Mol. Gen. Genet. 163:181–187 (1978). The vectors are described in Tsai et al., Plant Cell Reports 14:94–97 (1994) which is hereby incorporated by reference. The constructs pFOMT1 and pFOMT2 are also available from Michigan Technological University, Houghton, Mich.

Explants of young leaves from cuttings of aspen are obtained by cutting leaf disks from the young leaves along the midrib of the leaves using a corkborer that is 7 mm in diameter. The explants are surface sterilized in 20% commercial bleach for 10 minutes followed by rinsing three times with sterile double-distilled water.

All of the culture media used in this method includes the basal medium of woody plant medium (WPM) as described in Lloyd et al., Proc. Int. Plant Prop. Soc. 30:421–437 (1980) and supplemented with 2% sucrose. 650 mg/L calcium gluconate and 500 mg/L MES are added as pH buffers as described in De Block, Plant Physiol. 93:1110–1116 (1990). All culture media is adjusted to pH 5.5 prior to the addition of 0.075% Difco Bacto Agar and then autoclaved at 121° C. and 15 psi for 20 minutes. Filter sterilized antibiotics are added to all culture media after autoclaving. All culture media are maintained at 23±1° C. in a growth chamber with 16 hour photoperiods (160 $\mu\text{E}\times\text{m}^{-2}\times\text{S}^{-1}$) except for callus induction (as will be described later) which is maintained in the dark.

The sterilized explants are then inoculated with the mobilized vector with an overnight-grown agrobacterial suspension containing 20 μM acetosyringone. After cocultivation, the explants are washed in sterile distilled water containing 300 mg/L cefotaxime to decontaminate. The explants are blotted dry with sterile Whatman No. 1 filter paper and transferred onto callus induction medium containing 40 mg/L kanamycin for selection of transformed cells. The callus induction medium is the basal medium with the addition of 6-benzyladenine (BA) and 2,4-dichlorophenoxyacetic acid (2,4-D) at concentrations of 0.5 mg/L and 1 mg/L, respectively, to induce callus. Cefotaxime at 300 mg/L is added to kill *Agrobacterium*.

The kanamycin-resistant explants are then subcultured on fresh callus induction media every two weeks. Callus formation occurs after approximately four weeks. Formed callus are separated from the explant and subcultured periodically for further proliferation.

When the callus clumps reach approximately 3 mm in diameter, the callus clumps are transferred to shoot regeneration medium. The shoot regeneration medium is the basal medium containing 100 mg/L kanamycin, 0.5 mg/L thidi-

azuron (TDZ) as a plant growth regulator and cefotaxime at 300 mg/L to kill *Agrobacterium*. Shoots were regenerated about four weeks after callus is transferred to regeneration medium.

Accordingly, as soon as the shoots are regenerated, they are immediately transferred to hormone-free elongation medium containing 100 mg/L kanamycin and, whenever necessary, cefotaxime (300 mg/L), to promote elongation. Green and healthy shoots elongated to 2–3 cm in length are excised and planted separately in a hormone-free rooting medium containing 100 mg/L kanamycin. The efficient uptake of kanamycin by shoots during their rooting stage provides the most effective selection for positive transformants.

Transgenic plants are then transplanted into soil medium of vermiculite:peatmoss:perlite at 1:1:1 and grown in the greenhouse.

The above described transformation and regeneration protocol is readily adaptable to other woody species. Other published transformation and regeneration protocols for tree species include Danekar et al., Bio/Technology 5:587–590 (1987); McGranahan et al., Bio/Technology 6:800–804 (1988); McGranahan et al., Plant Cell Reports 8:512–616 (1990); Chen, PhD Thesis, North Carolina State University, Raleigh, N.C. (1991); Sullivan et al., Plant Cell Reports 12:303–306 (1993); Huang et al., In Vitro Cell Dev. Bio. 4:201–207 (1991); Wilde et al., Plant Physiol. 98:114–120 (1992); Minocha et al., 1986 Proc. TAPPI Research and Development Conference, TAPPI Press, Atlanta, pp. 89–91 (1986); Parsons et al., Bio/Technology 4:533–536 (1986); Fillatti et al., Mol. Gen. Genet 206:192–199 (1987); Pythoud et al., Bio/Technology 5:1323–1327 (1987); De Block, Plant Physiol. 93:1110–1116 (1990); Brasileiro et al., Plant Mol. Bio. 17:441–452 (1991); Brasileiro et al., Transgenic Res. 1:133–141 (1992); Howe et al., Woody Plant Biotech., Plenum Press, New York, pp.283–294 (1991); Klopfenstein et al., Can. J. For. Res. 21:1321–1328 (1991); Leple et al., Plant Cell Reports 11:137–141 (1992); and Nilsson et al., Transgenic Res. 1:209–220 (1992).

C. Color Alteration and Lignin Structure

The results of the transformation can be confirmed with conventional PCR and Southern analysis.

The present invention alters the natural wood color of woody plants. In aspen, the natural white/yellow color of the wood is altered to a brownish-red. The appearance of the wood color in aspen is achievable in both a solid and spotted appearance and is stable over time. Furthermore, the altered color of the wood appears in plants that are vegetatively propagated from the original transgenic plant. It should also be noted that with the present invention, the alteration of the natural color in the woody plants is not linked to any threshold increase or decrease in OMT activity.

The transformation of woody plants with a homologous OMT gene also alters the structure of lignin since the OMT gene is a part of the lignin synthesis pathway. For example, in aspen, due to cosuppression, the syringyl units decrease thus altering the structure of the lignin. The altered lignin will aid in the more efficient pulping of the wood of the transgenic plants.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i i i) NUMBER OF SEQUENCES: 2

(2) INFORMATION FOR SEQ ID NO:1:

-continued

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 1503 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(i i) MOLECULE TYPE: cDNA to mRNA

(i i i) HYPOTHETICAL: no

(i v) ANTI-SENSE: no

(v i) ORIGINAL SOURCE:

(A) ORGANISM: Populus Tremuloides
 (D) DEVELOPMENTAL STAGE: four year old sapling
 undergoing lignification in summer
 (F) TISSUE TYPE: secondary xylem

(v i i) IMMEDIATE SOURCE:

(A) LIBRARY: cDNA to total mRNA
 (B) CLONE: PtOMT1

(x i) SEQUENCE DESCRIPTION:SEQ ID NO:1:

TCAC	TTCT	CCTT	TCCT	TACACC	TTCT	TCAACC	TTTT	GTTT	TCC	TTGT	AGAATT	CAAT	CTCG	GAT	60	
CAAG	ATG	GGT	TCA	ACA	GGT	GAA	ACT	CAG	ATG	ACT	CCA	ACT	CAG	GTA	106	
	Met	Gly	Ser	Thr	Gly	Glu	Thr	Gln	Met	Thr	Pro	Thr	Gln	Val		
	1				5					10						
TCA	GAT	GAA	GAG	GCA	CAC	CTC	TTT	GCC	ATG	CAA	CTA	GCC	AGT	GCT	TCA	154
Ser	Asp	Glu	Glu	Ala	His	Leu	Phe	Ala	Met	Gln	Leu	Ala	Ser	Ala	Ser	
	15				20					25					30	
GTT	CTA	CCA	ATG	ATC	CTC	AAA	ACA	GCC	ATT	GAA	CTC	GAC	CTT	CTT	GAA	202
Val	Leu	Pro	Met	Ile	Leu	Lys	Thr	Ala	Ile	Glu	Leu	Asp	Leu	Leu	Glu	
				35					40					45		
ATC	ATG	GCT	AAA	GCT	GGC	CCT	GGT	GCT	TTC	TTG	TCC	ACA	TCT	GAG	ATA	250
Ile	Met	Ala	Lys	Ala	Gly	Pro	Gly	Ala	Phe	Leu	Ser	Thr	Ser	Glu	Ile	
			50					55					60			
GCT	TCT	CAC	CTC	CCT	ACC	AAA	AAc	CCT	GAT	GCG	CCT	GTC	ATG	TTA	GAC	298
Ala	Ser	Mis	Leu	Pro	Thr	Lys	Asn	Pro	Asp	Ala	Pro	Val	Met	Leu	Asp	
		65					70					75				
CGT	ATC	CTG	CGC	CTC	CTG	GCT	AGC	TAC	TCC	ATT	CTT	ACC	TGC	TCT	CTG	346
Arg	Ile	Leu	Arg	Leu	Leu	Ala	Ser	Tyr	Ser	Ile	Leu	Thr	Cys	Ser	Leu	
	80					85					90					
AAA	GAT	CTT	CCT	GAT	GGG	AAG	GTT	GAG	AGA	CTG	TAT	GGC	CTC	GCT	CCT	394
Lys	Asp	Ileu	Pro	Asp	Gly	Lys	Val	Glu	Arg	Leu	Tyr	Gly	Leu	Ala	Pro	
	95				100				105					110		
GTT	TGT	AAA	TTC	TTG	ACC	AAG	AAC	GAG	GAC	GGT	GTC	TCT	GTC	AGC	CCT	442
Val	Cys	Lys	Phe	Leu	Thr	Lys	Asn	Glu	Asp	Gly	Val	Ser	Val	Ser	Pro	
				115					120					125		
CTC	TGT	CTC	ATG	AAC	CAG	GAC	AAG	GTC	CTC	ATG	GAA	AGC	TGG	TAT	TAT	490
Leu	Cys	Leu	Met	Asn	Gln	Asp	Lys	Val	Leu	Met	Glu	Ser	Trp	Tyr	Tyr	
			130					135					140			
TTG	AAA	GAT	GCA	ATT	CTT	GAT	GGA	GGA	ATT	CCA	TTT	AAC	AAG	GCC	TAT	538
Leu	Lys	Asp	Ala	Ile	Leu	Asp	Gly	Gly	Ile	Pro	Phe	Asn	Lys	Ala	Tyr	
		145					150					155				
GGG	ATG	ACT	GCA	TTT	GAA	TAT	CAT	GGC	ACG	GAT	CCA	AGA	TTC	AAC	AAG	586
Gly	Met	Thr	Ala	Phe	Glu	Tyr	His	Gly	Thr	Asp	Pro	Arg	Phe	Asn	Lys	
	160					165					170					
GTC	TTC	AAC	AAG	GGA	ATG	TCT	GAC	CAC	TCT	ACC	ATT	ACC	ATG	AAG	AAG	634
Val	Phe	Asn	Lys	Gly	Met	Ser	Asp	His	Ser	Thr	Ile	Thr	Met	Lys	Lys	
	175				180					185					190	
ATT	CTT	GAG	ACC	TAC	AAA	GGC	TTT	GAA	GGC	CTC	ACG	TCC	TTG	GTG	GAT	682
Ile	Leu	Glu	Thr	Tyr	Lys	Gly	Phe	Glu	Gly	Leu	Thr	Ser	Leu	Val	Asp	
				195					200					205		

-continued

GTT	GGT	GGT	GGG	ACT	GGA	CCC	GTC	GTT	AAC	ACC	ATC	GTC	TCT	AAA	TAC	730
Val	Gly	Gly	Gly	Thr	Gly	Ala	Val	Val	Asn	Thr	Ile	Val	Ser	Lys	Tyr	
			210					215					220			
CCT	TCA	ATC	AAG	GGC	ATT	AAC	TTC	GAT	CTG	CCC	CAC	GTC	ATT	GAG	GAT	778
Pro	Ser	Ile	Lys	Gly	Ile	Asn	Phe	Asp	Leu	Pro	His	Val	Ile	Glu	Asp	
		225					230					235				
GCC	CCA	TCT	TAT	CCC	GGA	GTG	GAG	CAT	GTT	GGT	GGC	GAC	ATG	TTT	GTT	826
Ala	Pro	Ser	Tyr	Pro	Gly	Val	Glu	His	Val	Gly	Gly	Asp	Met	Phe	Val	
	240					245					250					
AGT	GTG	CCC	AAA	GCA	GAT	GCC	GTT	TTC	ATG	AAG	TGG	ATA	TGC	CAT	GAT	874
Ser	Val	Pro	Lys	Ala	Asp	Ala	Val	Phe	Met	Lys	Trp	Ile	Cys	His	Asp	
255					260					265					270	
TGG	AGC	GAC	GCC	CAC	TGC	TTA	AAA	TTC	TTG	AAG	AAT	TGC	TAT	GAC	GCG	922
Trp	Ser	Asp	Ala	His	Cys	Leu	Lys	Phe	Leu	Lys	Asn	Cys	Tyr	Asp	Ala	
			275					280						285		
TTG	CCG	GAA	AAC	GGC	AAG	GTG	ATA	CTT	GTT	GAG	TGC	ATT	CTT	CCC	GTG	970
Leu	Pro	Glu	Asn	Gly	Lys	Val	Ile	Leu	Val	Glu	Cys	Ile	Leu	Pro	Val	
			290					295					300			
GCT	CCT	GAC	ACA	AGC	CTT	GCC	ACC	AAG	GGA	GTC	GTG	CAC	GTT	GAT	GTC	1018
Ala	Pro	Asp	Thr	Ser	Leu	Ala	Thr	Lys	Gly	Val	Val	His	Val	Asp	Val	
		305					310					315				
ATC	ATG	CTG	GCG	CAC	AAC	CCC	GGT	GGG	AAA	GAG	AGG	ACC	GAG	AAG	GAA	1066
Ile	Met	Leu	Ala	His	Asn	Pro	Gly	Gly	Lys	Glu	Arg	Thr	Glu	Lys	Glu	
	320					325					330					
TTT	GAG	GGC	TTA	GCT	AAG	GGA	GCT	GGC	TTC	CAA	GGT	TTT	GAA	GTA	ATG	1114
Phe	Glu	Gly	Leu	Ala	Lys	Gly	Ala	Gly	Phe	Gln	Gly	Phe	Glu	Val	Met	
335					340					345					350	
TGC	TGT	GCA	TTC	AAC	ACA	CAT	GTC	ATT	GAA	TTC	CGC	AAG	AAG	GCC		1159
Cys	Cys	Ala	Phe	Asn	Thr	His	Val	Ile	Glu	Phe	Arg	Lys	Lys	Ala		
				355					360					365		
TAAGGCCCAT GTCCAAGCTC CAAGTTACTT GGGGTTTTGC AGACAACGTT GCTGCTGTCT																1219
CTGCGTTTGA TGTCTTGAT TGCTTTTTTTT TATACGAGGA GTAGCTATCT CTTATGAAAC																1279
ATGTAAGGAT AAGATTGCGT TTTGTATGCC TGATTTTCTC AAATAACTTC ACTGCCTCCC																1339
TCAAAATTCT TAATACATGT GAAAAGATTT CCTATTGGCC TTCTGCTTCA AACAGTAAAG																1399
ACTTCTGTAA CGGAAAAGAA AGCAATTCAT GATGTATGTA TCTTGCAAGA TTATGAGTAT																1459
TGTTCTAAGC ATTAAGTGAT TGTTCAAAAA AAAAAAAAAA AAAAA																1503

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 365 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(i i) MOLECULE TYPE:

(A) DESCRIPTION: protein

(i i i) HYPOTHETICAL: no

(x i) SEQUENCE DESCRIPTION:SEQ ID NO:2:

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

-continued

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

We claim:

1. A method for altering the wood color of a woody plant comprising incorporating into the genome of the woody plant a nucleotide sequence encoding the endogenous full-length enzyme O-methyltransferase in the sense orientation such that when the nucleotide sequence is expressed in the woody plant, the wood color of the woody plant is altered from the natural color.
2. A method for altering the natural wood color of a woody plant as set forth in claim 1 wherein the color of the altered wood is reddish-brown.
3. A method for altering the natural wood color of a woody plant as set forth in claim 1 wherein the nucleotide sequence is incorporated in the genome of the woody plant by transformation.
4. A method for altering the natural wood color of a woody plant as set forth in claim 3 wherein the transformation includes the use of an Agrobacterium transfer vector.
5. A method for altering the natural wood color of a woody plant as set forth in claim 1 wherein the nucleotide

sequence is a cloned cDNA sequence of O-methyltransferase.

6. A method for altering the natural wood color of a woody plant as set forth in claim 1 wherein the nucleotide sequence includes a gene promoter sequence.

7. A method for altering the natural wood color of a woody plant as set forth in claim 6 wherein the gene promoter sequence includes CaMV35S.

8. A method for altering the natural wood color of a woody plant as set forth in claim 1 wherein when the nucleotide sequence is expressed in the woody plant, the structure of the lignin of the woody plant is altered.

9. A method for altering the natural wood color of a woody plant as set forth in claim 1 wherein the woody plant is of the genus Populus.

10. A woody plant having the color of its wood altered through the incorporation into the genome of the woody plant a nucleotide sequence encoding the endogenous full-length enzyme O-methyltransferase in the sense orientation.

11. A woody plant as set forth in claim 10 wherein the color of the altered wood is reddish-brown.

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12. A woody plant as set forth in claim 10 wherein the nucleotide sequence is incorporated in the genome of the woody plant by transformation.

13. A woody plant as set forth in claim 12 wherein the transformation includes the use of an Agrobacterium transfer vector.

14. A woody plant as set forth in claim 10 wherein the nucleotide sequence is derived from cloned cDNA of O-methyltransferase.

15. A woody plant as set forth in claim 10 wherein the nucleotide sequence includes a gene promoter sequence.

16. A woody plant as set forth in claim 15 wherein the gene promoter sequence includes CaMV35S.

17. A woody plant as set forth in claim 10 wherein when the nucleotide sequence is expressed in the woody plant, the structure of the lignin of the woody plant is altered.

18. A woody plant as set forth in claim 10 wherein the woody plant is of the genus Populus.

19. A recombinant DNA comprising a gene promoter sequence, a gene terminator, and an interposed region comprising a nucleotide sequence encoding the endogenous full-length enzyme O-methyltransferase in the sense orientation such that when the nucleotide sequence is expressed in the woody plant, the wood color of the woody plant is altered from its natural color.

20. A recombinant DNA as set forth in claim 19 wherein the gene promoter sequence includes CaMV35S.

21. A recombinant DNA as set forth in claim 19 and further when the nucleotide sequence is expressed in the woody plant, the structure of the lignin of the woody plant is altered.

22. A method for altering the wood color of a plant of the genus Populus comprising incorporating into the genome of the plant through transformation a nucleotide sequence encoding the endogenous full-length enzyme

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O-methyltransferase in the sense orientation such that when the nucleotide sequence is expressed in the plant, the wood color of the plant is altered from its natural color.

23. A method for altering the wood color of a plant of the genus Populus as set forth in claim 22 wherein the color of the altered wood is reddish-brown.

24. A method for altering the wood color of a plant of the genus Populus as set forth in claim 22 wherein the transformation includes the use of an Agrobacterium transfer vector.

25. A method for altering the wood color of a plant of the genus Populus as set forth in claim 22 wherein the nucleotide sequence includes a cloned cDNA sequence of O-methyltransferase.

26. A method for altering the wood color of a plant of the genus Populus as set forth in claim 22 wherein the nucleotide sequence includes a gene promoter sequence.

27. A method for altering the wood color of a plant of the genus Populus as set forth in claim 26 wherein the gene promoter sequence includes CaMV35S.

28. A method for altering the wood color of a plant of the genus Populus as set forth in claim 22 wherein when the nucleotide sequence is expressed in the woody plant, the structure of the lignin of the plant is altered.

29. A woody plant of the genus Populus having the natural color of its wood altered through the incorporation into the genome of the woody plant a nucleotide sequence encoding the endogenous full-length enzyme O-methyltransferase in the sense orientation.

30. A woody plant of the genus Populus as set forth in claim 29 wherein the nucleotide sequence includes a CaMV35S gene promoter sequence.

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