Title: ISOLATION AND TARGETED SUPPRESSION OF LIGNIN BIOSYNTHETIC GENES FROM SUGARCANE

Abstract: The subject invention concerns materials and methods for modulating lignin biosynthesis in sugarcane plants. In one embodiment, lignin biosynthesis is down-regulated. Genes and the proteins encoded thereby that can be targeted for achieving down-regulation of lignin in sugarcane include, for example, 4-coumarate-CoA ligase (4CL). In one embodiment, the 4CL gene is 4CL-M, 4CL-N, or 4CL-L. The subject invention also concerns a sugarcane plant, specific plant tissue, and plant cells having modulated (e.g., down-regulated) lignin biosynthesis. The subject invention also concerns methods for producing a sugarcane plant having modulated (e.g., decreased or down-regulated) biosynthesis of lignin.
DESCRIPTION

ISOLATION AND TARGETED SUPPRESSION OF LIGNIN BIOSYNTHETIC GENES FROM SUGARCANE

CROSS-REFERENCE TO RELATED APPLICATION

The present application claims the benefit of U.S. Provisional Application Serial No. 61/217,950, filed June 5, 2009, which is hereby incorporated by reference herein in its entirety, including any figures, tables, nucleic acid sequences, amino acid sequences, and drawings.

GOVERNMENT SUPPORT

The subject matter of this application has been supported by a research grant from the USDA-CSREES under grant number 00075788. Accordingly, the government has certain rights in this invention.

BACKGROUND OF THE INVENTION

Sugarcane is the highest yielding biomass producer. Typically, farmers reduce the sugarcane post-harvest leaf residue by open air burning, which negatively impacts air quality. Fuel grade ethanol can be made from sugarcane leaf litter residue following acid hydrolysis pre-treatments to remove lignin which acts as a physical barrier to enzyme hydrolysis. Thus, down-regulation of lignin biosynthesis pathway enzymes is a promising strategy to increase the efficiency of bio-ethanol production from hemicellulosic sugarcane residues. In the lignin pathway, 4-coumarate-CoA ligase (4CL) is a key enzyme that catalyze the formation of CoA thiol esters of 4-coumarate and other hydroxycinnamates. However, sugarcane has a complex polyploid genome and these genes belong to a large gene family. Their broad substrate specificities have made it difficult to identify orthologs that are specifically involved in lignin biosynthesis. Thus, there remains a need in the art for means for inhibiting lignin biosynthesis in sugarcane.
BRIEF SUMMARY OF THE INVENTION

The subject invention concerns materials and methods for modulating lignin biosynthesis in sugarcane plants. In one embodiment, lignin biosynthesis is down-regulated. Genes and the proteins encoded thereby that can be targeted for achieving down-regulation of lignin in sugarcane include, for example, 4-coumarate-CoA ligase (4CL). In a specific embodiment, the 4CL gene is 4CL-M, 4CL-N, or 4CL-L. In another embodiment, lignin biosynthesis is decreased or down-regulated in stem tissue of a sugarcane plant. Expression of one or more target genes can be inhibited or down-regulated using standard methods known in the art. In a specific embodiment, expression of the 4CL-L gene is suppressed or down-regulated.

The subject invention also concerns a sugarcane plant, plant tissue, and plant cells wherein lignin biosynthesis has been down-regulated. In a specific embodiment, expression of one or more 4CL genes is inhibited or down-regulated in the sugarcane plant.

The subject invention also concerns methods for producing a sugarcane plant having decreased or down-regulated biosynthesis of lignin. In one embodiment, lignin biosynthesis is decreased or down-regulated in leaf tissue of a sugarcane plant. In another embodiment, lignin biosynthesis is decreased or down-regulated in stem tissue of a sugarcane plant. In one embodiment, a method of the invention comprises suppressing or inhibiting the expression of one or more 4CL genes. In one embodiment, the gene is inhibited using antisense nucleic acid or RNA interference.

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO:1 is a nucleotide sequence of a 4CL-L gene of the present invention.
SEQ ID NO:2 is a nucleotide sequence of a 4CL-M gene of the present invention.
SEQ ID NO:3 is a nucleotide sequence of a 4CL-N gene of the present invention.
SEQ ID NO:4 is a nucleotide sequence of a Sc4CL-Li RNAi construct of the present invention.
SEQ ID NO:5 is a nucleotide sequence of a Sc4CL-Mi RNAi construct of the present invention.
SEQ ID NO:6 is an amino acid sequence encoded by SEQ ID NO:1.
SEQ ID NO:7 is an amino acid sequence encoded by SEQ ID NO:2.
SEQ ID NO:8 is an amino acid sequence encoded by SEQ ID NO:3.
SEQ ID NO:9 is an amino acid sequence of *Arabidopsis thaliana* 4CL1.
SEQ ID NO:10 is an amino acid sequence of *Arabidopsis thaliana* 4CL2.
SEQ ID NO:11 is an amino acid sequence of *Arabidopsis thaliana* 4CL3.
SEQ ID NO:12 is an amino acid sequence of *Arabidopsis thaliana* 4CL4.
SEQ ID NO:13 is an amino acid sequence of *Poplar* 4CL1.
SEQ ID NO:14 is an amino acid sequence of *Poplar* 4CL2.
SEQ ID NO:15 is an amino acid sequence of *Poplar* 4CL3.
SEQ ID NO:16 is an amino acid sequence of *Poplar* 4CL4.
SEQ ID NO:17 is a gene specific primer based on the partial genomic DNA sequence of 4CL-L.
SEQ ID NO:18 is a gene specific primer based on the partial genomic DNA sequence of 4CL-L.
SEQ ID NO:19 is a forward primer for 4CL-N.
SEQ ID NO:20 is a reverse primer for 4CL-N.
SEQ ID NO:21 is a forward primer for 4CL-M and 4CL-L RNAi constructs.
SEQ ID NO:22 is a reverse primer for 4CL-M and 4CL-L RNAi constructs.
SEQ ID NO:23 is an amino acid sequence of a 4CL polypeptide of *Sorghum bicolor* 04g005210 (XP_002451647).
SEQ ID NO:24 is an amino acid sequence of a 4CL polypeptide of *Sorghum bicolor* 10g026130 (XP_002438783).
SEQ ID NO:25 is an amino acid sequence of a 4CL polypeptide of *Sorghum bicolor* 04g031010 (XP_002452704).
SEQ ID NO:26 is an amino acid sequence of a 4CL polypeptide of *Zea mays* LOC542166 (NPJ)O1 105258.
SEQ ID NO:27 is an amino acid sequence of a 4CL polypeptide of *Lolium perenne* 4CL3 (AAF37734).
SEQ ID NO:28 is an amino acid sequence of a 4CL polypeptide of *Lolium perenne* 4CL2 (AAF37733).
SEQ ID NO:29 is an amino acid sequence of a 4CL polypeptide of *Lolium perenne* 4CL1 (AAF37732).
SEQ ID NO:30 is an amino acid sequence of a 4CL polypeptide of *Oryza sativa* 4CL3 (NPJ)Ol 046069.

SEQ ID NO:31 is an amino acid sequence of a 4CL polypeptide of *Oryza sativa* 4CL4 (NP_001058252).

SEQ ID NO:32 is an amino acid sequence of a 4CL polypeptide of *Oryza sativa* 4CL1 (NP_001061353).

SEQ ID NO:33 is an amino acid sequence of a 4CL polypeptide of *Oryza sativa* 4CL2 (NPJ)01047819).

SEQ ID NO:34 shows the conserved AMP-binding motif of *Arabidopsis thaliana* 4CL1.

SEQ ID NO:35 shows the conserved AMP-binding motif of *Arabidopsis thaliana* 4CL2.

SEQ ID NO:36 shows the conserved AMP-binding motif of *Arabidopsis thaliana* 4CL3.

SEQ ID NO:37 shows the conserved AMP-binding motif of *Arabidopsis thaliana* 4CL4.

SEQ ID NO:38 shows the conserved AMP-binding motif of Poplar 4CL1.

SEQ ID NO:39 shows the conserved AMP-binding motif of Poplar 4CL2.

SEQ ID NO:40 shows the conserved AMP-binding motif of Poplar 4CL3.

SEQ ID NO:41 shows the conserved AMP-binding motif of Poplar 4CL4.

SEQ ID NO:42 shows the conserved AMP-binding motif of Sugarcane 4CL1.

SEQ ID NO:43 shows the conserved AMP-binding motif of Sugarcane 4CLM.

SEQ ID NO:44 shows a signature motif of *Arabidopsis thaliana* 4CL1.

SEQ ID NO:45 shows a signature motif of *Arabidopsis thaliana* 4CL2.

SEQ ID NO:46 shows a signature motif of *Arabidopsis thaliana* 4CL3.

SEQ ID NO:47 shows a signature motif of *Arabidopsis thaliana* ACLA.

SEQ ID NO:48 shows a signature motif of Poplar 4CL1.

SEQ ID NO:49 shows a signature motif of Poplar 4CL2.

SEQ ID NO:50 shows a signature motif of Poplar 4CL3.

SEQ ID NO:51 shows a signature motif of Poplar 4CL4.

SEQ ID NO:52 shows a signature motif of a Sugarcane 4CL1.

SEQ ID NO:53 shows a signature motif of a Sugarcane 4CLM.
SEQ ID NO:54 shows a common signature motif of 4CL genes.

DETAILED DESCRIPTION OF THE INVENTION

The subject invention concerns materials and methods for modulating lignin biosynthesis in plants, and in particular, sugarcane plants. In one embodiment, lignin biosynthesis is down-regulated in the plant. The subject invention contemplates the use of any method that can be used to inhibit or decrease expression of a gene (including at the transcriptional, post-transcriptional, and translational levels) and/or function or activity of a protein encoded by the gene. Genes, and the proteins encoded thereby, that can be targeted for achieving down-regulation of lignin biosynthesis in sugarcane include, but are not limited to, 4-coumarate-CoA ligase (4CL). In one embodiment, a 4CL gene encodes a 4CL polypeptide comprising an AMP-binding motif sequence (e.g., SEQ ID NO:34) and/or the signature motif sequence of SEQ ID NO:54. In one embodiment, the 4CL gene is 4CL-M, 4CL-L, or 4CL-N. In one embodiment, a 4CL-L gene encodes a polypeptide having the amino acid sequence shown in SEQ ID NO:6, or a fragment or variant thereof. In a further embodiment, a 4CL-M gene encodes a polypeptide having the amino acid sequence shown in SEQ ID NO:7, or a fragment or variant thereof. In another embodiment, a 4CL-N gene encodes a polypeptide having the amino acid sequence shown in SEQ ID NO:8, or a fragment or variant thereof. In a specific embodiment, the 4CL-L gene comprises all or a part of the nucleotide sequence shown in SEQ ID NO:1 and the 4CL-M gene comprises all or a part of the nucleotide sequence shown in SEQ ID NO:2. In a specific embodiment, the 4CL-N gene comprises all or a part of the nucleotide sequence shown in SEQ ID NO:3.

Expression of one or more target genes can be inhibited or down-regulated in a sugarcane plant using standard methods known in the art. In one embodiment, lignin biosynthesis is selectively down-regulated in leaf cells and/or tissue. In a specific embodiment, expression of one or more 4CL genes and/or translation or function of a protein encoded by a 4CL gene is suppressed or down-regulated. In one embodiment, a 4CL gene encodes a 4CL polypeptide comprising an AMP-binding motif sequence (e.g., SEQ ID NO:34) and/or the signature motif sequence of SEQ ID NO:54. In a more specific embodiment, expression of the 4CL-M, 4CL-L, and/or 4CL-N gene is suppressed or down-regulated. In one embodiment, expression of a target gene is down-regulated
using antisense technology. In another embodiment, cosuppression technology can be used to inhibit or down-regulate expression of a target gene. In still another embodiment, expression of a target gene is down-regulated using RNA interference (RNAi) technology, including, for example, the use of short interfering RNA (siRNA). In a still further embodiment, mutations in a target gene, such as "knockout" mutations, can be provided in a sugarcane plant of the invention. Expression and/or activity (e.g., enzymatic activity) of a protein encoded by a target gene can also be inhibited, for example, by contacting the protein with an antibody or an aptamer that binds to and blocks functional activity of the protein.

Antisense technology can be used to inhibit expression of a target gene involved in lignin biosynthesis in sugarcane. In antisense methodologies, a nucleic acid that hybridizes with a nucleotide sequence of an mRNA of a target gene is provided in a plant cell. Nucleic acid constructs that when expressed provide the nucleic acid that hybridizes with the mRNA can be incorporated (e.g., stably) in the genome of a sugarcane plant. The antisense nucleic acid can hybridize to an entire coding strand of a target sequence, or to a portion thereof, or to a non-coding portion of a target sequence or to both a coding and non-coding portion of a target sequence. Antisense constructs can have, for example, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 96%, 97%, 98%, or 99% sequence identity, or up to 100% sequence identity to the portion of the mRNA that the antisense nucleic acid hybridizes with. Antisense nucleic acids can comprise any suitable number of nucleotides. For example, an antisense nucleic acid construct of the invention can comprise at least 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40 or more nucleotides. In one embodiment, the antisense nucleic acid comprises at least about 40, or at least about 50, or at least about 60, or at least about 70, or at least about 80, or at least about 90, or at least about 100, or at least about 150, or at least about 200, or at least about 250, or at least about 300, or at least about 350, or at least about 400, or at least about 450, or at least about 500, or at least about 550, or at least about 600 or more nucleotides, in one embodiment, the antisense construct is selectively expressed in leaf cells and/or tissue of the plant, e.g., by use of a leaf-specific promoter. Antisense methods for down-regulating or inhibiting expression of a target gene are known in the art. Plants comprising and
expressing the antisense nucleic acid constructs can be grown from cells transformed with and/or incorporating the nucleic acid construct.

Cosuppression or post-transcriptional gene silencing (PTGS) technology can also be used to inhibit expression of a target gene involved in lignin biosynthesis in sugarcane. Generally, a nucleic acid sequence corresponding to and having sequence homology with a target gene sequence is provided in a plant cell in a sense orientation and in a construct suitable for expression of the nucleic acid (e.g., a construct comprising the nucleic acid operably linked to a promoter sequence capable of driving transcription in a plant cell). The nucleic acid can have, for example, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or at least about 96%, 97%, 98%, or 99% sequence identity, or up to 100% sequence identity to the target gene sequence. In one embodiment, the nucleic acid construct is selectively expressed in leaf cells and/or tissue of the plant, e.g., by use of a leaf-specific promoter. Plants comprising and expressing the nucleic acid constructs can be grown from cells transformed with and/or incorporating the nucleic acid construct.

RNA interference (RNAi) technologies can also be used to inhibit expression of a target gene involved in lignin biosynthesis in a sugarcane plant. In RNAi, a double-stranded RNA molecule that is complementary to all or a portion of an expressed RNA of a target gene is provided in a plant cell. The double-stranded RNA molecule is processed into smaller RNA molecules which are then processed into a silencing complex which results in inhibition of expression of the target gene, such as by cleavage of target gene mRNA. Generally, the RNAi molecule has 100 or more nucleotides, and more typically has 200 or more nucleotides. RNAi molecules can be provided by introduction and expression in a cell of a nucleic acid construct that results in transcription and production of the RNAi molecule. In one embodiment, RNA interference via expression of a nucleic acid that provides for micro RNA (miRNA) is contemplated within the scope of the invention. miRNAs are generally 19 to 23 nucleotide RNAs that have been processed from a longer precursor RNA comprising hairpin structures. In another embodiment, RNA interference via expression of a nucleic acid that provides for short interfering RNA (siRNA) is contemplated with the scope of the invention. siRNAs are generally 20 to 25 nucleotide RNAs having 3' overhangs and that have been processed from a longer precursor double-stranded RNA. Plants comprising and expressing RNAi molecules,
including miRNAs and siRNAi can be grown from cells transformed with and/or incorporating polynucleotide molecules that provide for the RNAi molecules. Methods and materials for RNA interference have been described, for example, in U.S. Patent Nos. 7,056,704; 7,078,196; 7,365,058; 7,232,086; 6,506,559; 7,282,564; and 7,538,095 and reviewed in Milhavet et al. (2003); Agrawal et al. (2003); Kusaba (2004); and Doran and Helliwell (2009). In one embodiment, an RNAi construct of the invention for inhibiting 4CL gene expression in a plant comprises all or a part of the nucleotide sequence of SEQ ID NO:4 or SEQ ID NO:5. In a specific embodiment, the RNAi molecules are selectively expressed in leaf cells and/or tissue of the plant, e.g., by use of a leaf-specific promoter.

Ribozyme technology can also be used to inhibit expression of a target gene involved in lignin biosynthesis in sugarcane. Ribozymes are a type of RNA that can be engineered to enzymatically cleave and inactivate other RNA targets in a specific, sequence-dependent fashion. By cleaving the target RNA, ribozymes inhibit translation, thus preventing the expression of the target gene. Ribozymes can be chemically synthesized in the laboratory and structurally modified to increase their stability and catalytic activity using methods known in the art. Ribozyme encoding nucleotide sequences can be introduced into plant cells and incorporated into the plant genome through gene-delivery mechanisms known in the art. Plants comprising and expressing the ribozyme encoding sequences can be grown from cells transformed with and/or incorporating the ribozyme encoding sequences. A ribozyme having specificity for 4CL can include one or more sequences complementary to the nucleotide sequence of at least a portion of one or more 4CL mRNA, and a sequence having known catalytic sequence responsible for mRNA cleavage (see U.S. Patent No. 5,093,246 or Haselhoff et al. 1988). For example, a derivative of a Tetrahyymena L-19 IVS RNA can be constructed in which the nucleotide sequence of the active site is complementary to the nucleotide sequence to be cleaved in the 4CL mRNA (see, e.g., U.S. Patent No. 4,987,071; and U.S. Patent No. 5,116,742). Alternatively, 4CL mRNA encoding a 4CL protein can be used to select a catalytic RNA having a specific ribonuclease activity from a pool of RNA molecules (see, e.g., Bartel et al. 1993). In one embodiment, the ribozymes are selectively expressed in leaf cells and/or tissue of the plant, e.g., by use of a leaf-specific promoter.

In addition to inhibition of target genes involved in lignin biosynthesis in sugarcane, the subject invention also contemplates mutations in target genes, or wherein
mutant genes can be provided for in a plant cell wherein target gene expression or gene product levels or activity is decreased or inhibited. In one embodiment, a mutant 4CL gene is incorporated into the genome of a sugarcane plant wherein the mutant 4CL gene exhibits decreased or no expression of gene transcripts or translation thereof. In one embodiment, a mutation is introduced into a 4CL gene of a plant that results in decreased transcription of the 4CL gene, or decreased translation of mRNA, and/or that results in a protein exhibiting decreased enzymatic activity. In a specific embodiment, one or more mutations are introduced in the protein coding region of a 4CL gene. In another embodiment, a mutation is introduced in a 4CL gene upstream of the transcription start site and/or downstream of the transcription start site. In one embodiment, a mutation is introduced into or near a regulatory sequence of a 4CL gene, e.g., in a promoter sequence. The mutation may block or inhibit transcription of the 4CL gene sequence, e.g., by blocking or inhibiting binding of transcription factors or polymerase to the 4CL nucleic acid sequence. In one embodiment, a mutation in the 4CL gene is selectively introduced into leaf cells and/or leaf tissue of the plant. Mutations can also include one or more nucleotide or amino acid insertions, deletions, and/or substitutions that inhibit or decrease functional activity (e.g., enzymatic) of a 4CL polypeptide. Methods for creating and introducing mutations are known in the art. In one embodiment, the mutation is introduced into one or more wild-type 4CL genes in a plant. In another embodiment, a mutant 4CL gene replaces one or more wild-type 4CL genes in a plant. In one embodiment, mutant 4CL genes are selectively expressed in leaf cells and/or tissue of the plant.

In addition to inhibition or suppression of target genes involved in lignin biosynthesis, the activity (e.g., enzymatic) of proteins encoded by the target genes of the invention can also be inhibited. In one embodiment, a nucleic acid encoding an antibody, or an antigen binding fragment thereof, that binds to and inhibits activity (e.g., enzymatic activity) of a protein can be incorporated and expressed in a cell of a sugarcane plant. A plant comprising and expressing a nucleic acid encoding an antibody, or an antigen binding fragment thereof, can be grown from cells transformed with and/or incorporating the nucleic acid. Methods for preparing an antibody that binds to and inhibits a specific target protein and for obtaining the nucleic acid that encodes the antibody are well known in the art. In one embodiment, the antibody is a monoclonal antibody, or an antigen
binding fragment thereof. Antigen binding fragments include, but are not limited to, F(ab')₂, FaV, Fab, and Fv, and can be prepared using standard methods known in the art. The antibody can be derived from any animal capable of producing antibodies to a target protein epitope, and include, for example, human, primate, mouse, rat, goat, sheep, pig, and cow. In a specific embodiment, the antibody binds to a 4CL protein. In one embodiment, a 4CL gene encodes a 4CL polypeptide comprising an AMP-binding motif sequence (e.g., SEQ ID NO:34) and/or the signature motif sequence of SEQ ID NO:54. In a more specific embodiment, the 4CL protein is encoded by a 4CL-M gene, a 4CL-L gene, or a 4CL-N gene. In a specific embodiment, the 4CL-L gene comprises all or a part of the nucleotide sequence shown in SEQ ID NO:1, the 4CL-M gene comprises all or a part of the nucleotide sequence shown in SEQ ID NO:2, and the 4CL-N gene comprises all or a part of the nucleotide sequence shown in SEQ ID NO:3. In one embodiment, the 4CL-M, 4CL-L, and 4CL-N genes encode a polypeptide having the amino acid sequence shown in SEQ ID NOs:6, 7, and 8, respectively, or a fragment or variant thereof, hi one embodiment, the antibody binds to a 4CL protein comprising the amino acid sequence of SEQ ID NO:6, 7, or 8, or a fragment or epitope thereof. In a specific embodiment, the nucleic acid encoding the antibody is selectively expressed in leaf tissue of the plant, e.g., by using a leaf specific promoter.

The activity (e.g., enzymatic) of proteins encoded by target genes involved in lignin biosynthesis can also be inhibited by expressing and/or contacting the target protein with an aptamer that binds to a specific target protein. Aptamers are oligonucleotides or peptides that can be selected for binding to a target molecule (see, for example, Ellington and Szostak (1990) and Hoppe-Seyler and Butz (2000) and U.S. Patent Nos. 5,582,981; 5,270,163; 5,595,877; 5,817,785; 6,344,318; 6,933,116; 7,368,236; and 7,700,759). In one embodiment, a nucleic acid encoding an aptamer that binds to a protein involved in lignin biosynthesis is incorporated and expressed in a cell of a plant. A plant comprising and expressing a nucleic acid encoding an aptamer can be grown from cells transformed with and/or incorporating the nucleic acid. In one embodiment, the aptamer binds to and inhibits a 4CL protein. In one embodiment, a 4CL gene encodes a 4CL polypeptide comprising an AMP-binding motif sequence (e.g., SEQ ID NO:34) and/or the signature motif sequence of SEQ ID NO:54. In a specific embodiment, the 4CL protein is encoded by a 4CL-L, 4CL-M, or a 4CL-N gene of the invention. In a specific embodiment, the
4CL-L gene comprises all or a part of the nucleotide sequence shown in SEQ ID NO:1, the 4CL-M gene comprises all or a part of the nucleotide sequence shown in SEQ ID NO:2, and the 4CL-N gene comprises all or a part of the nucleotide sequence shown in SEQ ID NO:3. In one embodiment, the 4CL-M, 4CL-L, and 4CL-N genes encode a polypeptide having the amino acid sequence shown in SEQ ID NOs:6, 7, and 8, respectively, or a fragment or variant thereof. In one embodiment, the aptamer binds to a 4CL protein comprising the amino acid sequence of SEQ ID NO:6, 7, or 8, or a fragment or epitope thereof. In a specific embodiment, the nucleic acid encoding the aptamer is selectively expressed in leaf tissue of the plant, e.g., by using a leaf specific promoter.

The subject invention also concerns a sugarcane plant wherein lignin biosynthesis has been modulated (e.g., down-regulated). In one embodiment, the lignin biosynthesis is selectively down-regulated in leaf cells and/or tissue. In one embodiment, expression of one or more 4CL genes and/or translation or activity of a protein encoded by a 4CL gene is inhibited or down-regulated in the sugarcane plant. In one embodiment, a 4CL gene encodes a 4CL polypeptide comprising an AMP-binding motif sequence (e.g., SEQ ID NO:34) and/or the signature motif sequence of SEQ ID NO:54. In one embodiment, the 4CL gene inhibited is 4CL-L, 4CL-M, or 4CL-N. In one embodiment, the 4CL-M, 4CL-L, and 4CL-N genes encode a polypeptide having the amino acid sequence shown in SEQ ID NO:6, 7, and 8, respectively, or a fragment or variant thereof. In a specific embodiment, the 4CL-L gene comprises all or a part of the nucleotide sequence shown in SEQ ID NO:1, the 4CL-M gene comprises all or a part of the nucleotide sequence shown in SEQ ID NO:2, and the 4CL-N gene comprises all or a part of the nucleotide sequence shown in SEQ ID NO:3. Sugarcane plants of the invention can have antisense, cosuppression, RNAi, or ribozyme nucleic acids that target one or more 4CL genes (e.g., 4CL-M, 4CL-N, and/or 4CL-L) incorporated into their genome. Sugarcane plants of the invention can have mutant 4CL genes in their genome wherein 4CL gene expression is inhibiting and/or wherein 4CL polypeptide has a mutation that inhibits or decreased functional activity (e.g., enzymatic) of the 4CL polypeptide. Sugarcane plants of the invention can also have incorporated into their genome nucleic acids that encode one or more antibodies (or antigen binding fragments thereof) and/or aptamers that bind to and inhibit enzymatic activity of a 4CL protein.
Optionally, the plants disclosed herein may further exhibit one or more agronomic traits that primarily are of benefit to a seed company, a grower, or a grain processor, for example, herbicide resistance, virus resistance, bacterial pathogen resistance, insect resistance, nematode resistance, and fungal resistance. See, e.g., U.S. Patent Nos. 5,569,823; 5,304,730; 5,495,071; 6,329,504; and 6,337,431. Such trait may also be one that increases plant vigor or yield (including traits that allow a plant to grow at different temperatures, soil conditions and levels of sunlight and precipitation), or one that allows identification of a plant exhibiting a trait of interest (e.g., selectable marker gene, seed coat color, etc.). Various traits of interest, as well as methods for introducing these traits into a plant, are described, for example, in U.S. Patent Nos. 5,569,823; 5,304,730; 5,495,071; 6,329,504; 6,337,431; 5,767,366; 5,928,937; 4,761,373; 5,013,659; 4,975,374; 5,162,602; 4,940,835; 4,769,061; 5,554,798; 5,879,903; 5,276,268; 5,561,236; 4,810,648; and 6,084,155; in European application No. 0242246; in U.S. Patent Application No. 20010016956; and on the worldwide web at www.lifesci.sussex.ac.uk/home/Neil_Crickmore/Bt/.

The subject invention also concerns sugarcane plant tissue and plant parts, including, but not limited to, plant cells, plant protoplasts, plant cell tissue cultures from which plants can be regenerated, plant calli, plant clumps, and plant cells that are intact in plants or parts of plants such as branches, kernels, ears, cobs, husks, root tips, anthers, seeds, roots, embryos, hypocotyls, cotyledons, pollen, ovules, anthers, shoots, stalks, stems, leaves, fruits, and flowers, from a sugarcane plant of the invention having modulated (e.g., down-regulated) lignin biosynthesis. In one embodiment, expression of one or more 4CL genes, or the gene product thereof, is inhibited or down-regulated in the plant tissue or plant cell. In one embodiment, the 4CL gene inhibited is 4CL-M, 4CL-L, or 4CL-N. In one embodiment, the 4CL-M, 4CL-L, and 4CL-N genes encode a polypeptide having the amino acid sequence shown in SEQ ID NO:6, 7, and 8, respectively, or a fragment or variant thereof. In a specific embodiment, the 4CL-L gene comprises all of a part of the nucleotide sequence shown in SEQ ID NO:1, the 4CL-M gene comprises all of a part of the nucleotide sequence shown in SEQ ID NO:2, and the 4CL-N gene comprises all of a part of the nucleotide sequence shown in SEQ ID NO:3.

The subject invention also concerns sugarcane cells or protoplasts having modulated or down-regulated lignin biosynthesis. In one embodiment, expression of one
or more 4CL genes or translation or activity of a protein encoded by a 4CL gene is inhibited or down-regulated in the sugarcane cell or protoplast. In one embodiment, the 4CL gene inhibited is 4CL-M, 4CL-L, or 4CL-N. In one embodiment, the 4CL-M, 4CL-L, and 4CL-N genes encode a polypeptide having the amino acid sequence shown in SEQ ID NO:6, 7, and 8, respectively, or a fragment or variant thereof. In a specific embodiment, the 4CL-L gene comprises all of a part of the nucleotide sequence shown in SEQ ID NO:1, the 4CL-M gene comprises all of a part of the nucleotide sequence shown in SEQ ID NO:2, and the 4CL-N gene comprises all of a part of the nucleotide sequence shown in SEQ ID NO:3.

The subject invention also concerns methods for producing a sugarcane plant having decreased or down-regulated biosynthesis of lignin. In one embodiment, lignin biosynthesis is decreased or down-regulated in leaf cells and/or tissue of a sugarcane plant. In another embodiment, lignin biosynthesis is decreased or down-regulated in stem tissue of a sugarcane plant. In one embodiment, a method of the invention comprises suppressing or inhibiting the expression of one or more 4CL genes or inhibiting the translation or activity (e.g., enzymatic) of a protein encoded by a 4CL gene. In one embodiment, a 4CL gene encodes a 4CL polypeptide comprising an AMP-binding motif sequence (e.g., SEQ ID NO:34) and/or the signature motif sequence of SEQ ID NO:54. In one embodiment, the 4CL gene inhibited is 4CL-M, 4CL-L, or 4CL-N. In one embodiment, the 4CL-M, 4CL-L, and 4CL-N genes encode a polypeptide having the amino acid sequence shown in SEQ ID NO:6, 7, and 8, respectively, or a fragment or variant thereof. In a specific embodiment, the 4CL-L gene comprises all of a part of the nucleotide sequence shown in SEQ ID NO:1, the 4CL-M gene comprises all of a part of the nucleotide sequence shown in SEQ ID NO:2, and the 4CL-N gene comprises all of a part of the nucleotide sequence shown in SEQ ID NO:3. In one embodiment, the target gene expression is inhibited using antisense nucleic acid, cosuppression, RNA interference, or ribozymes. In another embodiment, the expression of the target gene is inhibited by mutation of the gene. In a still further embodiment, the activity of the protein encoded by a target gene is inhibited in the plant by expression of an antibody, or an antigen binding fragment thereof, and/or an aptamer that binds to the protein, or by providing mutations in the gene that inhibit translation of the mRNA of the gene into protein or that disrupt or inhibit function of the encoded protein (e.g., via changes in
amino acid sequence). Nucleic acid constructs that provide for inhibition of target gene expression can be introduced into a plant genome, and transformed and transgenic plants prepared therefrom using standard methods and materials known in the art.

Polynucleotides useful in the present invention can be provided in an expression construct. Expression constructs of the invention generally include regulatory elements that are functional in the intended host cell in which the expression construct is to be expressed. Thus, a person of ordinary skill in the art can select regulatory elements for use in bacterial host cells, yeast host cells, plant host cells, insect host cells, mammalian host cells, and human host cells. Regulatory elements include promoters, transcription termination sequences, translation termination sequences, enhancers, and polyadenylation elements. As used herein, the term "expression construct" refers to a combination of nucleic acid sequences that provides for transcription of an operably linked nucleic acid sequence. As used herein, the term "operably linked" refers to a juxtaposition of the components described wherein the components are in a relationship that permits them to function in their intended manner. In general, operably linked components are in contiguous relation.

An expression construct of the invention can comprise a promoter sequence operably linked to a polynucleotide sequence of the invention. Promoters can be incorporated into a polynucleotide using standard techniques known in the art. Multiple copies of promoters or multiple promoters can be used in an expression construct of the invention. As a preferred embodiment, a promoter can be positioned about the same distance from the transcription start site in the expression construct as it is from the transcription start site in its natural genetic environment. Some variation in this distance is permitted without substantial decrease in promoter activity. A transcription start site is typically included in the expression construct.

If the expression construct is to be provided in or introduced into a plant cell, then plant viral promoters, such as, for example, a cauliflower mosaic virus (CaMV) 35S (including the enhanced CaMV 35S promoter (see, for example U.S. Patent No. 5,106,739)) or a CaMV 19S promoter or a cassava vein mosaic can be used. Other promoters that can be used for expression constructs in plants include, for example, prolifera promoter, Ap3 promoter, heat shock promoters, T-DNA 1' or 2'-promoter of A. tumefaciens, polygalacturonase promoter, chalcone synthase A (CHS-A) promoter from
petunia, tobacco PR-1a promoter, ubiquitin promoter, actin promoter, alcA gene promoter, pin2 promoter (Xu et al., 1993), maize Wipl promoter, maize, trpA gene promoter (U.S. Patent No. 5,625,136), maize CDPK gene promoter, and RUBISCO SSU promoter (U.S. Patent No. 5,034,322) can also be used. Tissue-specific promoters, for example fruit-specific promoters, such as the E8 promoter of tomato (accession number: AF515784; Good et al. (1994)) can be used with the invention. Leaf-specific promoters that can be used in a nucleic acid construct of the invention include Cabl promoter (Brusslan and Tobin, 1992), Cabl 9 promoter (Bassett et al., 2007), PPDK promoter (Matsuoka et al., 1993), and ribulose biphosphate carboxylase (RBCS) promoter (Matsuoka et al. (1994) and U.S. Patent No. 7,723,575). Other plant leaf-specific promoters that can be used with an expression construct of the invention include, but are not limited to, the Actl promoter (U.S. Published Application No. 20090031441), AS-I promoter (U.S. Patent No. 5,256,558), RBC-3A promoter (U.S. Patent No. 5,023,179), the CaMV 35S promoter (Odell et al., 1985), the enhanced CaMV 35S promoter, the Figwort Mosaic Virus (FMV) promoter (Richins et al., 1987), the mannopine synthase (mas) promoter, the octopine synthase (ocs) promoter, or others such as the promoters from CaMV 19S (Lawton et al., 1987), nos (Ebert et al., 1987), Adh (Walker et al., 1987), sucrose synthase (Yang et al., 1990), α-tubulin, ubiquitin, actin (Wang et al., 1992), cab (Sullivan et al., 1989), PEPCase (Hudspeth et al., 1989) or those associated with the R gene complex (Chandler et al., 1989). See also published U.S. application 2007/006346 and Yamamoto et al. (1997); Kwon et al. (1994); Yamamoto et al. Fruit-specific promoters such as flower organ-specific promoters can be used with an expression construct of the present invention for expressing a polynucleotide of the invention in the flower organ of a plant. Examples of flower organ-specific promoters include any of the promoter sequences described in U.S. Patent Nos. 6,462,185; 5,639,948; and 5,589,610. Seed-specific promoters such as the promoter from a β-phaseolin gene (for example, of kidney bean) or a glycinin gene (for example, of soybean), and others, can also be used. Root-specific promoters, such as any of the promoter sequences described in U.S. Patent No. 6,455,760 or U.S. Patent No. 6,696,623, or in published U.S. patent application Nos. 20040078841; 20040067506; 20040019934; 20030177536; 20030084486; or 20040123349, can be used with an expression construct of the invention. Xylem-specific promoters include the cinnamate-4-hydroxylase (C4H)
of rice. Constitutive promoters (such as the CaMV, ubiquitin, actin, or NOS promoter), developmentally-regulated promoters, and inducible promoters (such as those promoters than can be induced by heat, light, hormones, or chemicals) are also contemplated for use with polynucleotide expression constructs of the invention.

Methods for identifying and characterizing promoter regions in plant genomic DNA are known in the art and include, for example, those described in the following references: Jordano et al. (1989); Bustos et al. (1989); Green et al. (1988); Meier et al. (1991); and Zhang et al. (1996). Published U.S. application 2009/0199307 also describes methods for identifying tissue-specific promoters using differential display (see, e.g., U.S. Patent No. 5,599,672). In differential display, mRNAs are compared from different tissue types. By identifying mRNA species which are present in only a particular tissue type, or set of tissue types, corresponding genes can be identified which are expressed in a tissue specific manner. RNA can be transcribed by reverse transcriptase to produce a cDNA, and the cDNA can be used to isolate clones containing the full-length genes. The cDNA can also be used to isolate homeologous or homologous promoters, enhancers or terminators from the respective gene using, for example, suppression PCR. See also U.S. Patent No. 5,723,763.

Expression constructs of the invention may also optionally contain a transcription termination sequence, a translation termination sequence, a sequence encoding a signal peptide, and/or enhancer elements. Transcription termination regions can typically be obtained from the 3' untranslated region of a eukaryotic or viral gene sequence. Transcription termination sequences can be positioned downstream of a coding sequence to provide for efficient termination. A signal peptide sequence is a short amino acid sequence typically present at the amino terminus of a protein that is responsible for the relocation of an operably linked mature polypeptide to a wide range of post-translational cellular destinations, ranging from a specific organelle compartment to sites of protein action and the extracellular environment. Targeting gene products to an intended cellular and/or extracellular destination through the use of an operably linked signal peptide sequence is contemplated for use with the polypeptides of the invention. Classical enhancers are cis-acting elements that increase gene transcription and can also be included in the expression construct. Classical enhancer elements are known in the art, and include, but are not limited to, the CaMV 35S enhancer element, cytomegalovirus
(CMV) early promoter enhancer element, and the SV40 enhancer element. Intron-mediated enhancer elements that enhance gene expression are also known in the art. These elements must be present within the transcribed region and are orientation dependent. Examples include the maize shrunken-1 enhancer element (Clancy and Hannah, 2002).

DNA sequences which direct polyadenylation of mRNA transcribed from the expression construct can also be included in the expression construct, and include, but are not limited to, a CaMV 35S, octopine synthase, or nopaline synthase signal. The expression constructs of the invention can also include a polynucleotide sequence that directs transposition of other genes, i.e., a transposon.

Expression constructs can also include one or more dominant selectable marker genes, including, for example, genes encoding antibiotic resistance for selecting transformed cells. Antibiotic-resistance genes can provide for resistance to one or more of the following antibiotics: hygromycin, kanamycin, bleomycin, G418, streptomycin, paromomycin, neomycin, and spectinomycin. Kanamycin resistance can be provided by neomycin phosphotransferase (NPT II). Other markers used for cell transformation screening include genes encoding β-glucuronidase (GUS), β-galactosidase, luciferase, nopaline synthase, chloramphenicol acetyltransferase (CAT), green fluorescence protein (GFP), or enhanced GFP (Yang et al. (1996)).

The subject invention also concerns polynucleotide vectors comprising a polynucleotide sequence of the invention that encodes a desired protein that is to be provided to a cell or cells provided with the bioreactor device of the invention. Unique restriction enzyme sites can be included at the 5’ and 3’ ends of an expression construct or polynucleotide of the invention to allow for insertion into a polynucleotide vector. As used herein, the term "vector" refers to any genetic element, including for example, plasmids, cosmids, chromosomes, phage, virus, and the like, which is capable of replication when associated with proper control elements and which can transfer polynucleotide sequences between cells. Vectors contain a nucleotide sequence that permits the vector to replicate in a selected host cell. A number of vectors are available for expression and/or cloning, and include, but are not limited to, pBR322, pUC series, M13 series, and pBLUESCRIPT vectors (Stratagene, La Jolla, CA).
Polynucleotides of the present invention can be composed of either RNA or DNA. Preferably, the polynucleotides are composed of DNA. The subject invention also encompasses those polynucleotides that are complementary in sequence to the polynucleotides disclosed herein.

Techniques for transforming plant cells with a gene are known in the art and include, for example, *Agrobacterium* infection, biolistic methods, electroporation, calcium chloride treatment, PEG-mediated transformation, etc. See, for example, U.S. Patent Nos. 5,036,006; 5,591,616; 5,100,792; published U.S. Application No. 2006/026001; and published PCT Application No. WO 93/07278 and WO 93/21335. U.S. Patent No. 5,661,017 teaches methods and materials for transforming an algal cell with a heterologous polynucleotide. Transformed cells can be selected, redifferentiated, and grown into plants that contain and express a polynucleotide of the invention using standard methods known in the art. The seeds and other plant tissue and progeny of any transformed or transgenic plant cells or plants of the invention are also included within the scope of the present invention.

Polynucleotides and polypeptides of the subject invention can also be defined in terms of more particular identity and/or similarity ranges with those sequences exemplified herein. The sequence identity will typically be greater than 60%, preferably greater than 75%, more preferably greater than 80%, even more preferably greater than 90%, and can be greater than 95%. The identity and/or similarity of a sequence can be 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% as compared to a sequence exemplified herein. Unless otherwise specified, as used herein percent sequence identity and/or similarity of two sequences can be determined using the algorithm of Karlin and Altschul (1990), modified as in Karlin and Altschul (1993). Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul *et al.* (1990). BLAST searches can be performed with the NBLAST program, score = 100, wordlength = 12, to obtain sequences with the desired percent sequence identity. To obtain gapped alignments for comparison purposes, Gapped BLAST can be used as described in Altschul *et al.* (1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (NBLAST and XBLAST) can be used. See NCBI/NIH website.
The subject invention also contemplates those polynucleotide molecules of the invention (and those encoding polypeptides of the invention) having sequences which are sufficiently homologous with the polynucleotide sequences encoding a polypeptide of the invention so as to permit hybridization with that sequence under standard stringent conditions and standard methods (Maniatis, T. et al, 1982). As used herein, "stringent" conditions for hybridization refers to conditions wherein hybridization is typically carried out overnight at 20-25°C below the melting temperature (Tm) of the DNA hybrid in 6x SSPE, 5x Denhardt's solution, 0.1% SDS, 0.1 mg/ml denatured DNA. The melting temperature is described by the following formula (Beltz, G.A. et al, 1983):

\[
Tm=81.5 \times C+16.6 \times \log[Na^+] + Q.41 \times (\% G+C)-0.61 \times (\% \text{ formamide})-600/\text{length of duplex in base pairs}.
\]

Washes are typically carried out as follows:

1. Twice at room temperature for 15 minutes in 1x SSPE, 0.1% SDS (low stringency wash).
2. Once at Tm-20°C for 15 minutes in 0.2x SSPE, 0.1% SDS (moderate stringency wash).

As used herein, the terms "nucleic acid" and "polynucleotide sequence" refer to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, would encompass known analogs of natural nucleotides that can function in a similar manner as naturally-occurring nucleotides. The polynucleotide sequences include both the DNA strand sequence that is transcribed into RNA and the RNA sequence that is translated into protein. The polynucleotide sequences include both full-length sequences as well as shorter sequences derived from the full-length sequences. It is understood that a particular polynucleotide sequence includes the degenerate codons of the native sequence or sequences which may be introduced to provide codon preference in a specific host cell. The polynucleotide sequences falling within the scope of the subject invention further include sequences which specifically hybridize with the sequences coding for a polypeptide of the invention. The polynucleotide includes both the sense and antisense strands as either individual strands or in the duplex.

Polypeptides having substitution of amino acids other than those specifically exemplified in the subject polypeptides are also contemplated within the scope of the present invention. For example, non-natural amino acids can be substituted for the amino
acids of a polypeptide of the invention, so long as the polypeptide having substituted amino acids retains substantially the same activity as the polypeptide in which amino acids have not been substituted. Examples of non-natural amino acids include, but are not limited to, ornithine, citrulline, hydroxyproline, homoserine, phenylglycine, taurine, iodotyrosine, 2,4-diaminobutyric acid, a-amino isobutyric acid, 4-aminobutyric acid, 2-amino butyric acid, γ-amino butyric acid, e-amino hexanoic acid, 6-amino hexanoic acid, 2-amino isobutyric acid, 3-amino propionic acid, norleucine, norvaline, sarcosine, homocitrulline, cysteic acid, t-butyrglycine, t-butyrlalanine, phenylglycine, cyclohexylalanine, β-alanine, fluoro-amino acids, designer amino acids such as β-methyl amino acids, C-methyl amino acids, N-methyl amino acids, and amino acid analogues in general. Non-natural amino acids also include amino acids having derivatized side groups. Furthermore, any of the amino acids in the protein can be of the D (dextrorotary) form or L (levorotary) form.

Amino acids can be generally categorized in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby a polypeptide having an amino acid of one class is replaced with another amino acid of the same class fall within the scope of the subject invention so long as the polypeptide having the substitution still retains substantially the same biological activity as a polypeptide that does not have the substitution. Nonpolar amino acids include Ala, Val, Leu, lie, Pro, Met, Phe, and Trp. Uncharged polar amino acids include Gly, Ser, Thr, Cys, Tyr, Asn, and Gln. Acidic amino acids include Asp and Glu. Basic amino acids include Lys, Arg, and His.

Once a nucleic acid sequence of the present invention has been incorporated into an expression system, it can be transformed into a plant cell. The word "plant" refers to any plant, particularly to seed plant, and "plant cell" is a structural and physiological unit of the plant, which comprises a cell wall but may also refer to a protoplast. The plant cell maybe in form of an isolated single cell or a cultured cell, or as a part of higher organized unit such as, for example, a plant tissue, or a plant organ. The term "transformation" refers to the transfer of a nucleic acid fragment into the genome of a host cell, resulting in genetically stable inheritance. Host cells containing the transformed nucleic acid fragments are referred to as "transgenic" cells, and organisms comprising transgenic cells are referred to as "transgenic organisms."
Examples of methods of transformation of plants and plant cells include Agrobacterium-mediated transformation (Deblaere et al. (1987)) and particle bombardment technology (Klein et al. (1987); U.S. Patent No. 4,945,050). Whole plants may be regenerated from transgenic cells by methods well known to the skilled artisan (see, for example, Fromm et al. (1990)).

The expression constructs of the present invention can be introduced into the plant cell in a number of art-recognized ways. The term "introducing" in the context of a polynucleotide, for example, a nucleotide encoding an enzyme disclosed herein, is intended to mean presenting to the plant the polynucleotide in such a manner that the polynucleotide gains access to the interior of a cell of the plant. Where more than one polynucleotide is to be introduced, these polynucleotides can be assembled as part of a single nucleotide construct, or as separate nucleotide constructs, and can be located on the same or different transformation vectors.

Accordingly, these polynucleotides can be introduced into the host cell of interest in a single transformation event, in separate transformation events, or, for example, in plants, as part of a breeding protocol. The methods of the invention do not depend on a particular method for introducing one or more polynucleotides into a plant, only that the polynucleotide(s) gains access to the interior of at least one cell of the plant. Methods for introducing polynucleotides into plants are known in the art and include, but are not limited to, transient transformation methods, stable transformation methods, and virus-mediated methods.

"Transient transformation" in the context of a polynucleotide is intended to mean that a polynucleotide is introduced into the plant and does not integrate into the genome of the plant.

By "stably introducing" or "stably introduced" in the context of a polynucleotde introduced into a plant is intended to mean that the introduced polynucleotide is stably incorporated into the plant genome, and thus the plant is stably transformed with the polynucleotide.

"Stable transformation" or "stably transformed" is intended to mean that a polynucleotide, for example, a nucleotide construct described herein, introduced into a plant integrates into the genome of the plant and is capable of being inherited by the progeny thereof, more particularly, by the progeny of multiple successive generations.
Numerous transformation vectors available for plant transformation are known to those of ordinary skill in the plant transformation arts, and the genes pertinent to this invention can be used in conjunction with any such vectors. The selection of vector will depend upon the preferred transformation technique and the target species for transformation. For certain target species, different antibiotic or herbicide selection markers may be preferred.

Methods for regeneration of transformed plants are well known in the art. For example, Ti plasmid vectors have been utilized for the delivery of foreign DNA, as well as direct DNA uptake, liposomes, electroporation, microinjection, and microprojectiles. In addition, bacteria from the genus Agrobacterium can be utilized to transform plant cells. Below are descriptions of representative techniques for transforming both dicotyledonous and monocotyledonous plants, as well as a representative plastid transformation technique.

Many vectors are available for transformation using Agrobacterium tumefaciens. These typically carry at least one T-DNA border sequence and include vectors such as pBIN19 (Bevan (1984)). For the construction of vectors useful in Agrobacterium transformation, see, for example, U.S. Patent Application Publication No. 2006/026001 1.

Transformation without the use of Agrobacterium tumefaciens circumvents the requirement for T-DNA sequences in the chosen transformation vector and consequently vectors lacking these sequences can also be utilized. Transformation techniques that do not rely on Agrobacterium include, but are not limited to, transformation via particle bombardment, protoplast uptake (e.g., PEG and electroporation) and microinjection. The choice of vector depends largely on the preferred selection for the species being transformed. For the construction of such vectors, see, for example, U.S. Published Application No. 2006/026001 1.

Transformation techniques for dicotyledons are well known in the art and include Agrobacterium-based techniques and techniques that do not require Agrobacterium. Non-Agrobacterium techniques involve the uptake of exogenous genetic material directly by protoplasts or cells and can be accomplished, for example, by PEG or electroporation mediated uptake, particle bombardment-mediated delivery, or microinjection. Examples of these techniques are described by Paszkowski et al. (1984), Potrykus et al. (1985),
Reich et al. (1986), and Klein et al. (1987). In each case the transformed cells are regen-erated to whole plants using standard techniques known in the art.

Agrobacterium-mediated transformation is a preferred technique for transformation of dicotyledons because of its high efficiency of transformation and its broad utility with many different species. Agrobacterium transformation typically involves the transfer of the binary vector carrying the foreign DNA of interest to an appropriate Agrobacterium strain which may depend on the complement of vir genes carried by the host Agrobacterium strain either on a co-resident Ti plasmid or chromosomally (Uknes et al. (1993)). The transfer of the recombinant binary vector to Agrobacterium is accomplished by a triparental mating procedure using E. coli carrying the recombinant binary vector, a helper E. coli strain which carries a plasmid that is able to mobilize the recombinant binary vector to the target Agrobacterium strain. Alternatively, the recombinant binary vector can be transferred to Agrobacterium by DNA transformation (Hofgen and Willmitzer (1988)).

Transformation of the target plant species by recombinant Agrobacterium usually involves co-cultivation of the Agrobacterium with explants from the plant and follows protocols well known in the art. Transformed tissue is regenerated on selectable medium carrying the antibiotic or herbicide resistance marker present between the binary plasmid T-DNA borders.

Another approach to transforming plant cells with a gene involves propelling inert or biologically active particles at plant tissues and cells. This technique is disclosed in U.S. Patent Nos. 4,945,050, 5,036,006, and 5,100,792. Generally, this procedure involves propelling inert or biologically active particles at the cells under conditions effective to penetrate the outer surface of the cell and afford incorporation within the interior thereof. When inert particles are utilized, the vector can be introduced into the cell by coating the particles with the vector containing the desired gene. Alternatively, the target cell can be surrounded by the vector so that the vector is carried into the cell by the wake of the particle. Biologically active particles (e.g., dried yeast cells, dried bacterium or a bacteriophage, each containing DNA sought to be introduced) can also be propelled into plant cell tissue.

Transformation of most monocotyledon species has now also become routine. Preferred techniques include direct gene transfer into protoplasts using PEG or
electroporation techniques, and particle bombardment into callus tissue. Transformations
can be undertaken with a single DNA species or multiple DNA species (i.e.,
cotransformation) and both of these techniques are suitable for use with this invention. Co-
transformation may have the advantage of avoiding complete vector construction and of
generating transgenic plants with unlinked loci for the gene of interest and the selectable
marker, enabling the removal of the selectable marker in subsequent generations, should
this be regarded desirable.

techniques for the preparation of callus and protoplasts from an elite inbred line of maize,
transformation of protoplasts using PEG or electroporation, and the regeneration of maize
plants from transformed protoplasts. Gordon-Kamm et al. (1990) and Fromm et al.
(1990) have published techniques for transformation of A188-derived maize line using
particle bombardment. Furthermore, WO 93/07278 and Koziel et al. (1993) describe
techniques for the transformation of elite inbred lines of maize by particle bombardment.
This technique utilizes immature maize embryos of 1.5-2.5 mm length excised from a
maize ear 14-15 days after pollination and a PDS-1000He Biolistics device for
bombardment.

The plants obtained via transformation with a nucleic acid sequence of the present
invention can be any of a wide variety of plant species, including those of monocots and
dicots. The expression of a gene of the present invention in combination with other
characteristics important for production and quality can be incorporated into plant lines
through breeding. The polynucleotides of the invention disclosed herein may also be
incorporated into or maintained in plant lines through breeding or through common
genetic engineering technologies. Breeding approaches and techniques are known in the
art. See, for example, Welsh (1981); Wood (1983); Mayo (1987); Singh (1986); and
Wricke and Weber (1986).

The genetic properties engineered into the transgenic seeds and plants described
above are passed on by sexual reproduction or vegetative growth and can thus be
maintained and propagated in progeny plants. Generally, maintenance and propagation
make use of known agricultural methods developed to fit specific purposes such as tilling,
sowing or harvesting.
The relevant techniques are well known in the art and include but are not limited to hybridization, inbreeding, backcross breeding, multi-line breeding, dihaploid inbreeding, variety blend, interspecific hybridization, aneuploid techniques, *etc.* Hybridization techniques also include the sterilization of plants to yield male or female sterile plants by mechanical, genetic (including transgenic), chemical, or biochemical means.

For the purposes of the present invention, "sugarcane" will refer to any Saccharum plant or hybrid. Sugarcane plants included within the scope of the invention include, for example, *Saccharum arundinaceum, Saccharum bengalense, Saccharum edule, Saccharum officinarum, Saccharum procerum, Saccharum ravennae, Saccharum robustum, Saccharum sinense,* and *Saccharum spontaneum.* Sugarcane plants of the invention can be inbred lines or hybrids. Hybrid plants include those generated by the traditional *Saccharum spontaneum* by *Saccharum officinarum* hybrid material that makes up all current commercial sugarcane and energycane germplasm, and any other hybrids that are produced by crossing sugarcane with closely or distantly related species. Examples of other species that sugarcane can be crossed with to generate hybrid plants or new varieties of sugarcane include Miscanthus, Erianthus, and Sorghum.

"Isolated" means altered "by the hand of man" from its natural state, *i.e.,* if it occurs in nature, it has been changed or removed from its original environment, or both. For example, a naturally occurring polynucleotide or a polypeptide naturally present in a living animal in its natural state is not "isolated," but the same polynucleotide or polypeptide separated from the coexisting materials of its natural state is "isolated," as the term is employed herein. For example, with respect to polynucleotides, the term isolated means that it is separated from the chromosome and cell in which it naturally occurs. A sequence is also isolated if separated from the chromosome and cell in which it naturally occurs in but inserted into a genetic context, chromosome, or cell in which it does not naturally occur.

As used herein the term "transgenic" refers to plants that include an exogenous polynucleotide (*e.g.,* gene) that is stably maintained in the transformed plant and is stably inherited by progeny in successive generations. The term "transgenic plant" can refer either to the initially transformed plant or to the progeny of the initially transformed plant. Techniques for transforming plants, plant cells or plant tissues can include, but are not
limited to, transformation with DNA employing *A. tumefaciens* or *A. rhizogenes* as the
transforming agent, electroporation, DNA injection, microprojectile bombardment, and
particle acceleration. See, for example, EP 295959 and EP 138341. As used herein, the
terms "plant material" or "plant part" includes plant cells, plant protoplasts, plant cell
tissue cultures from which plants can be regenerated, plant calli, plant clumps, and plant
cells that are intact in plants or parts of plants such as embryos, pollen, ovules, seeds,
leaves, flowers, branches, fruit, kernels, ears, cobs, husks, stalks, roots, root tips, anthers,
tubers, rhizomes and the like.

<table>
<thead>
<tr>
<th>Letter Symbol</th>
<th>Amino Acid</th>
<th>Letter Symbol</th>
<th>Amino Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Alanine</td>
<td>M</td>
<td>Methionine</td>
</tr>
<tr>
<td>B</td>
<td>Asparagine or aspartic acid</td>
<td>N</td>
<td>Asparagine</td>
</tr>
<tr>
<td>C</td>
<td>Cysteine</td>
<td>P</td>
<td>Proline</td>
</tr>
<tr>
<td>D</td>
<td>Aspartic Acid</td>
<td>Q</td>
<td>Glutamine</td>
</tr>
<tr>
<td>E</td>
<td>Glutamic Acid</td>
<td>R</td>
<td>Arginine</td>
</tr>
<tr>
<td>F</td>
<td>Phenylalanine</td>
<td>S</td>
<td>Serine</td>
</tr>
<tr>
<td>G</td>
<td>Glycine</td>
<td>T</td>
<td>Threonine</td>
</tr>
<tr>
<td>H</td>
<td>Histidine</td>
<td>V</td>
<td>Valine</td>
</tr>
<tr>
<td>I</td>
<td>Isoleucine</td>
<td>W</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>K</td>
<td>Lysine</td>
<td>Y</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>L</td>
<td>Leucine</td>
<td>Z</td>
<td>Glutamine or glutamic acid</td>
</tr>
</tbody>
</table>

**MATERIALS AND METHODS**

**Plant materials.** Field grown mature sugarcane (*Saccharum* spp. Hybrid) var.
CP88-1762 in addition to green-house grown immature CP88-1762 and L 79-1002 were
used in expression analysis.

**RNA extraction, isolation of genes, and RT-PCR.** Total RNA was isolated using
TRIZol regent (Invitrogen) from leaves, stems, nodes, and roots. First-strand cDNAs were
synthesized from lug of total RNA using a cDNA synthesis kit (Bio-Rad).

Isolation of 4CL from sugarcane

**Sc4CL-L.**

Partial 4CL-L was obtained by RACE (Rapid Amplification of cDNA Ends) technique. RACE was performed using SMART RACE kit (Clontech) according to the
manufacturer's instructions. 5'- and 3'-RACE ready cDNA pools were synthesized from 2 µg of total RNA, and these pools were used as the PCR template. Primary (LPF: 5'CGTTGCCTGTGAAAGTCGGC-3' (SEQ ID NO:17)) and nested (LNF: 5'CCACGGGCAAGACCATCGACTCG-3' (SEQ ID NO:18)) gene specific primer designed based on the partial genomic DNA sequence of 4CL-L. Primary PCR was performed with the LPF and manufacturer provided Universal Primer Mix (UPM). The PCR conditions consisted of 25 cycles of 94°C for 30 sec, 68°C for 60 sec and 72°C for 180 sec. The primary PCR products were diluted from one to 50 and used as templates for the secondary PCR with the LNF and manufacturer provided Nested Universal Primer (NUP). The second PCR was performed under 20 cycles of the same conditions as the first PCR. The product of 3'-RACE PCR product were cloned into the pCR2.1 TOPO vector (Invitrogen) and sequenced.

_Sc4CL-M_

4CL-M was isolated by cDNA library screening. Leaf, internode, node and immature leaf roll of sugarcane (Saccharum spp. Hybrid) var. CP88-1 762 were harvested from field grown plants (Belle Grade and Citra, FL). Root and emerging shoot were collected from hydroponic solution grown plants. Total RNA was extracted from each tissue using Trizol (Invitrogen) and Total RNA from each sample was mixed in the same proportion. mRNA was purified from mixed total RNA using Oligotex mRNA Mini Kit (Qiagen). cDNA was synthesized form 5.9 µg of mRNA and ligated to the Uni-ZAP XR vector using cDNA Synthesis Kit and ZAP-cDNA Synthesis Kit (Stratagene). Packing and amplification were performed using ZAP-cDNA Gigapack III Gold Cloning Kit according to the manufacturer's instructions (Stratagene). For screening, 447 bp partial 4CLM specific probe was generated by PCR and labeled with 32P-dCTP, using a random primer kit (Promega). Approximately 2.0 x 10^5 of recombinant phages were screened, and one positive phages was isolated. To obtain the cDNA containing pBluscript phagemid, _in vivo_ excision was performed, and the isolate was sequence.

_Sc4CL-N_

4CL-N was PCR-amplified from cDNA with gene-specific primer deduced from the sugarcane EST sequences. '4-coumarate coenzyme A ligase' were used as subjects for
keyword search against the DFCI Saccharum officinarum Gene Index (SoGI; http://compbio.dfci.harvard.edu/cgi-bin/tgi/gireport.pl?gudb=s_officinarum). One Tentative Consensus (TC) sequences, TC88322, which had the complete open reading frame, was detected and used for primer design. Forward primer (4CL1F: 5'-ATGGGTTCCGTGGACACGGCGGTCGCG-S' (SEQ ID NO:19)) and reverse primer (4CL1R: S'-TCAGTGAACACCGGCGGAGCCTGG-S' (SEQ ID NO:20)) were designed from start and stop codon regions, respectively. Total RNA was isolated form leaf, internode, node, and shoot using Trizol according to the manufacturer's instructions (Invitrogen), and cDNA synthesis was performed using the iScript cDNA synthesis kit (Bio-rad). 200 ng of cDNA mixture from each tissue was used as the PCR template. PCR was carried out using TaKaRa LA taq polymerase (Takara BIO Inc.), and the PCR conditions consisted of 35 cycles of 95°C for 45 sec, 56°C for 45 sec and 72°C for 120 sec. PCR product of two independent amplifications were cloned into pCR2.1 TOPO vector (Invitrogen) and sequenced.

Construction of the Sc4CL RNAi suppression constructs

Based on the sequencing information for Sc4CL-L (SEQ ID NO:1) and Sc4CL-M (SEQ ID NO:2), specific primers were designed to amplify 200 bp region named exon 2 and exon 1, respectively. Sequence correspondent to two restriction enzymes, EcoRI and Xbal, were added to the forward primer and for the reverse primer sequence specific to EcoRV restriction enzyme was added to facilitate subcloning. The plasmid pWF BgH4CL_RNAi consist of two inverted repeats separated by Bg4CL native intron and the transcription terminator CaMV35SpolyA was used for the construction Sc4CL interference constructs. In two separate and sequential subloning steps the inverted repeats in the plasmid pWF BgH4CL_RNAi were replaced by Sc4CL specific sequences. Then the rice C4H promoter was subcloned and the two for Sc4CL-Li and Sc4CL-Mi were generated (SEQ ID NO:4 and SEQ ID NO:5).

Generation of 4CLI sugarcane lines

Transverse sections of immature leaf rolls of sugarcane (Saccharum spp. Hybrid) var. CP88-1762, were used to induce callus on modified MS basal medium (CI-3), supplemented with 20 g/L sucrose and 13.6 uM 2,4-D, pH adjusted to 5.8 (Chengalrayan
and Gallo-Meagher, 2001). After callus induction, biolistic gene transfer was carried out using the PDS-1000/He biolistic particle delivery system. (Bio-Rad) as described previously (James et al., 2008). Selection was performed with geneticin as described (Chengalrayan and Gallo-Meagher, 2001) with minor modification where further selection was conducted for the regenerated plants as they subcultured at MS basal medium containing paromomycin (30 mg/L) at the rooting stage for 4 biweekly subcultures. Selected plants that developed healthy roots were transferred to the soil and were transferred to the greenhouse.

Characterization of transgenic lines

Transgenic sugarcane plants were confirmed by NPTII-ELISA and PCR following selection and regeneration of plants. Total protein extraction and NPTII-ELISA were performed using Pathoscreen nptll ELISA kit (Agdia) according to the manufacturer's instruction. Genomic DNA was extracted from the expanding leaf of each regenerated plant using DNeasy Plant Mini kit (Qiagen). 75 ng of genomic DNA was used as the PCR template. To detect each expression construct, primers were designed from each gene and promoter regions as follows: For the Sc4CL-Mi and Sc4CL-Li RNAi constructs, 4CL SF (5'-CATCAAGGGTCACGGATGC-S' (SEQ ID NO:21)) and OSPRO SR (5'-GTAGCCTGCTAGTTCTCTCATT-3'(SEQ ID NO:22)). PCR was performed using iTaq polymerase (Bio-Rad) as following conditions: 35 cycles of 95°C for 30 sec, 58°C for 30 sec, and 72°C for 60 sec. For Northern blot analysis, total RNA was extracted from the 3rd leaf of the plant for the 4CL-Li lines and from a side-tiller around 25 cm long for the 4CL-Mi lines (Sambrook et al., 1989). Samples from the wild type plants growing under the same conditions as the transgenic plants were collected at the same developmental stage. Northern hybridization was carried out with a radio-labeled probe from the open reading frame of the targeted 4CL gene following electrophoresis and transfer of 20μg total RNA.

Total lignin was quantified in transgenic sugarcane and non-transgenic (wild type) sugarcane plants using Klason procedure form senescent leaves and internodes of 4CL-Li lines and 4CL-Mi lines, respectively as described by Browning (1967) with minor modifications (Yoshihara et al., 1984). Briefly, after grinding the dried samples (0.5- to 1-mm screen), samples were extracted with 50% warm ethanol to remove soluble sugars
and dried. Then 0.1 g dry cell wall samples were subjected to hydrolysis using 12M 
$\text{H}_2\text{SO}_4$ at $30^\circ\text{C}$ for 2 h. The contents were diluted with distilled water and autoclaved for 1 h. After autoclaving the insoluble materials (lignin and ash) were collected by filtrations and weighed. Then the lignin was burned at $500^\circ\text{C}$ for 5 h. Following this step the ash was weighed and the lignin was calculated as the difference in the weight before and after burning.

AU patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

Following are examples that illustrate procedures for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

**Example 1—Isolation of Sc4CL genes**

The sequences of two full-length and one partial sugarcane 4CL genes were isolated and characterized in this study. The cDNA sequences of Sc4CL-N and Sc4CL-M have an open reading frame of 1665 and 1728 nucleotides encoding a 555 and a 576 amino acid protein, respectively. The partial Sc4CL-L cDNA sequence is 616 bp long that includes the 3'UTR, and 141 amino acid residue of the open reading frame. A pairwise comparison between the Sc4CL-N and Sc4CL-M showed 59% similarity. Sc4CL-N is the most closely related to previously identified 4CLs, showing 96% and 86% similarities with Sb4CL-like 1 from *Sorghum bicolor* and Os4CL3 from *Oryza sativa*, respectively, whereas Sc4CL-M shares lower similarities with Sb4CL-like 1 and Os4CL3, but it shows higher similarities with Sb4CL-like 2 and Os4CL3 (96% and 83%, respectively). A comparison of the deduced amino acid sequences between the Sc4CLs and the At4CLs from *Arabidopsis thaliana* showed similarities ranging from 60% to 63% (Table 2).

Alignment (performed by CLUSTALW (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=/NPSA/npsa_clustalw.html) using the default parameters) between Sc4CLs and other 4CLs, Ptd4CLs from Poplar and At4CLs from *Arabidopsis*

Table 2. The percentage similarities between 4CL amino acid sequences

<table>
<thead>
<tr>
<th></th>
<th>Sc4CL1</th>
<th>Sc4CL2</th>
<th>Sb4CL-like 1</th>
<th>Sb4CL-like 2</th>
<th>Os4CL2</th>
<th>Os4CL3</th>
<th>At4CL1</th>
<th>At4CL2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sc4CL1</td>
<td>59</td>
<td>96</td>
<td>60</td>
<td>59</td>
<td>86</td>
<td>61</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td>Sc4CL2</td>
<td></td>
<td>58</td>
<td>96</td>
<td>83</td>
<td>59</td>
<td>61</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>Sb4CL-like 1</td>
<td></td>
<td>60</td>
<td>59</td>
<td>87</td>
<td>62</td>
<td>61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sb4CL-like 2</td>
<td></td>
<td></td>
<td>85</td>
<td>61</td>
<td>61</td>
<td>63</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Os4CL2</td>
<td></td>
<td></td>
<td></td>
<td>59</td>
<td>60</td>
<td>60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Os4CL3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>62</td>
<td>62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At4CL1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At4CL2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sc: Sugarcane, Sb: *Sorghum bicolor*, Os: *Oryza sativa*, At: *Arabidopsis thaliana*

**Example 2—Expression profile of the 4CL genes**

Sugarcane 4CL-M was predominantly expressed in stems, while Sc4CL-L was predominantly expressed in leaves (Table 3). This suggests that expression of different 4CL genes can be regulated in a tissue specific manner and offers the opportunity to suppress lignin in specific tissues.
Table 3. Tissue and cultivar-specific expression of Sc4CL genes

<table>
<thead>
<tr>
<th>Sugarcane cultivar</th>
<th>L79-1002 Leaf</th>
<th>L79-1002 Stem</th>
<th>L79-1002 Node</th>
<th>CP88-1762 Leaf</th>
<th>CP88-1762 Stem</th>
<th>CP88-1762 Node</th>
</tr>
</thead>
<tbody>
<tr>
<td>4CL-M</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>4CL-L</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>+++</td>
<td>++</td>
<td>-</td>
</tr>
</tbody>
</table>

- L, S, and N indicate leaves, stems, and nodes; respectively.

Example 3—Generation of 4CL down-regulated sugarcane

RNAi is a powerful tool for crop improvement and to study gene function. Thus, to investigate physiological roles of individual 4CL gene products in lignin biosynthesis,

4CL down-regulated sugarcane was generated utilizing the sequence information for Sc4CL, two RNA suppression constructs were generated (SEQ ID NO:4 and SEQ ID NO:5), targeting different regions of Sc4CL-L, and Sc4CL-M genes, under the control of a xylem specific promoter, the rice *cinnamaie*-4-hydroxylase (*C4H*) promoter (Fouad and Altpeter, unpublished) and CaMV 35S polyA signal. The generated suppression cassettes were co-introduced, individually or together, into embryogenic sugarcane callus with selectable *nptII* gene under regulatory control of the strong constitutive maize ubiquitin promoter with first intron (pLJbi) and 35S 3'UTR using biolistic gene transfer. The selection of transgenic events was conducted using the nptII/geneticin and paromomycin selection system where several transgenic lines were generated.

A total of 88 bombardments were conducted utilizing the two generated suppression cassettes and 160 plants were regenerated following callus selection. 152 independent plants showed NPTII expression using Pathoscreen nptII ELISA kit (Table 4). The presence of the 4CL-RNAi suppression cassette in the genomic DNA of the transgenic plants was confirmed using PCR (Table 4).

The expression analysis for sugarcane 4CL in the transgenic sugarcane plants indicated suppression of the Sc4CL-L (Table 5) and Sc4CL-M gene (Table 6) in several transgenic sugarcane plants compared to non-trangenic (WT) sugarcane.
Table 4. Summary of transgenic 4CLi sugarcane

<table>
<thead>
<tr>
<th>Construct(s)</th>
<th>Plants regenerated following selection</th>
<th>NPT II ELISA (positive/tested)</th>
<th>PCR for 4CL (positive/tested)</th>
<th>4CL Northern Suppressed/tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4H-4CL-L</td>
<td>13</td>
<td>11/13</td>
<td>10/13</td>
<td>8/9</td>
</tr>
<tr>
<td>C4H-4CL-M</td>
<td>66</td>
<td>65/66</td>
<td>NA</td>
<td>11/20</td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td>76/79</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NA = Not analyzed

Table 5. Expression levels of 4CL-L gene and lignin content in senescent leaves of sugarcane 4CL-Li transgenic plants

<table>
<thead>
<tr>
<th>Lines</th>
<th>4CL-L expression(^1) (percentage)</th>
<th>Klason Lignin (percentage DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>100</td>
<td>24.225(^2)</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>23.9</td>
</tr>
<tr>
<td>6</td>
<td>0-10</td>
<td>23.25(^2)</td>
</tr>
<tr>
<td>9</td>
<td>0-10</td>
<td>24.3</td>
</tr>
<tr>
<td>14-2A</td>
<td>0-10</td>
<td>21.267(^2)</td>
</tr>
</tbody>
</table>

\(^1\) Expression based on RNA blot analysis relative to the WT expression (100%)
\(^2\) Mean of two to three biological replicates, generated by analyzing two to three individual plants of the same genotype.
Table 6. Expression levels of 4CL-M gene and lignin content in immature internodes of sugarcane 4CL-Mi transgenic plants

<table>
<thead>
<tr>
<th>Lines</th>
<th>4CL-M expression (percentage)</th>
<th>Kason Lignin (percentage DW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>100</td>
<td>15.73^2</td>
</tr>
<tr>
<td>5b-A1</td>
<td>10</td>
<td>14.45</td>
</tr>
<tr>
<td>4a</td>
<td>5</td>
<td>14.45^2</td>
</tr>
<tr>
<td>7c-B2</td>
<td>30</td>
<td>15.25</td>
</tr>
</tbody>
</table>

1- Expression based on RNA blot analysis relative to the WT expression (100%)
2- Mean of two biological replicates, generated by analyzing two individual plants of the same genotype.

Example 4—Generation sugarcane with reduced lignin

Total lignin was quantified in senescent leaves of 4CL-Li transgenic plants and non-transgenic sugarcane using the Klason procedure form lines following the standard protocol. As shown in Table 5, Klason lignin was about 23.6% in senescent leaves of the non-transgenic sugarcane plants. In contrast, transgenic line 14-2A with suppression of the 4CL-L gene had an average of 21% Klason lignin in senescent leaves (Table 5), indicating 11% reduction in total lignin through 4CL-L suppression. Lignin was also analyzed in the immature internodes of 4CL-Mi lines (Table 6) and wild type plants. Line 4a exhibited about 8% reductions in total lignin compared to wild type plants. Lines 5b-A1 also showed a similar level of lignin reduction (Table 6). Greater lignin reduction in these transgenic 4CL-Mi plants is expected in mature internodes, where lignin content of non-transgenic plants increase to more than 20%.

Example 5—Quantitative PCR analysis of 4CLM expression

Quantitative real-time RT-PCR analysis confirmed 4CLM suppression in several transgenic lines. Table 7 shows the relative expression ratio of 4CLM gene to the reference gene (Sugarcane GAPDH). Table 7 shows strong suppression of the 4CLM transcript in transgenic sugarcane lines, including lines 4A and 5BA1. Developmentally matching side tillers were harvested and total RNA was extracted from the first internode.
4CLM gene specific primers were designed from 3′UTR. All reactions were performed in parallel and each reaction was carried out in triplicate. Standard errors were calculated using Q-gene software.

Table 7. Quantitative PCR analysis of 4CLM expression

<table>
<thead>
<tr>
<th>Plant line</th>
<th>Mean normalized expression of 4CLM</th>
<th>standard error</th>
</tr>
</thead>
<tbody>
<tr>
<td>wild-type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WT1</td>
<td>0.158183469</td>
<td>0.030398171</td>
</tr>
<tr>
<td>WT2</td>
<td>0.085389181</td>
<td>0.023563549</td>
</tr>
<tr>
<td>WT3</td>
<td>0.068677827</td>
<td>0.008813278</td>
</tr>
<tr>
<td>WT4</td>
<td>0.095779515</td>
<td>0.0036248</td>
</tr>
<tr>
<td>4CLM RNAi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>transgenic lines</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4A</td>
<td>0.002506449</td>
<td>0.000235822</td>
</tr>
<tr>
<td>5BA1</td>
<td>0.00765043</td>
<td>0.001561502</td>
</tr>
<tr>
<td>2D301</td>
<td>0.030256393</td>
<td>0.006236083</td>
</tr>
<tr>
<td>D303</td>
<td>0.032826712</td>
<td>0.007700117</td>
</tr>
<tr>
<td>3D101</td>
<td>0.042700512</td>
<td>0.000739096</td>
</tr>
<tr>
<td>2E301</td>
<td>0.051611828</td>
<td>0.013784677</td>
</tr>
<tr>
<td>2F302</td>
<td>0.067672462</td>
<td>0.013071891</td>
</tr>
<tr>
<td>F302</td>
<td>0.106257424</td>
<td>0.001041556</td>
</tr>
<tr>
<td>2D101</td>
<td>0.10903124</td>
<td>0.022234267</td>
</tr>
<tr>
<td>2E201</td>
<td>0.147694218</td>
<td>0.022925399</td>
</tr>
<tr>
<td>2E401</td>
<td>0.171012655</td>
<td>0.009865807</td>
</tr>
</tbody>
</table>

Example 6—Estimates of evolutionary divergence among plant 4CL genes

The number of amino acid substitutions per site from analysis between Sugarcane 4CLs and other plant 4CLs is shown in Table 8. All results are based on the pairwise analysis of amino acid sequences. Analyses were conducted using the Poisson correction method in MEGA4 (Zuckerkandl and Pauling (1965); Tamura et al (2007)). AU positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 513 positions in the final dataset.
Table 8. Estimates of evolutionary divergence among plant 4CLs.

<table>
<thead>
<tr>
<th></th>
<th>Sc4CLI</th>
<th>Sc4CLM</th>
<th>Sb</th>
<th>Sb</th>
<th>Sb</th>
<th>Zm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>04g005210</td>
<td>04g031010</td>
<td>10g026130</td>
<td>LOC542166</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sc4CLI</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sc4CLM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sb 04g005210</td>
<td>0.03</td>
<td>0.47</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sb 04g031010</td>
<td>0.45</td>
<td>0.03</td>
<td>0.45</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sb 10g026130</td>
<td>0.16</td>
<td>0.44</td>
<td>0.15</td>
<td>0.43</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zm LOC542166</td>
<td>0.07</td>
<td>0.46</td>
<td>0.06</td>
<td>0.44</td>
<td>0.18</td>
<td></td>
</tr>
<tr>
<td>At4CL1</td>
<td>0.42</td>
<td>0.41</td>
<td>0.42</td>
<td>0.41</td>
<td>0.38</td>
<td>0.43</td>
</tr>
<tr>
<td>At4CL2</td>
<td>0.43</td>
<td>0.40</td>
<td>0.43</td>
<td>0.39</td>
<td>0.39</td>
<td>0.43</td>
</tr>
<tr>
<td>At4CL3</td>
<td>0.46</td>
<td>0.37</td>
<td>0.46</td>
<td>0.35</td>
<td>0.47</td>
<td>0.46</td>
</tr>
<tr>
<td>At4CL4</td>
<td>0.55</td>
<td>0.53</td>
<td>0.54</td>
<td>0.52</td>
<td>0.52</td>
<td>0.55</td>
</tr>
<tr>
<td>Lp4CL1</td>
<td>0.48</td>
<td>0.16</td>
<td>0.48</td>
<td>0.14</td>
<td>0.45</td>
<td>0.47</td>
</tr>
<tr>
<td>Lp4CL2</td>
<td>0.18</td>
<td>0.45</td>
<td>0.17</td>
<td>0.44</td>
<td>0.13</td>
<td>0.18</td>
</tr>
<tr>
<td>Lp4CL3</td>
<td>0.16</td>
<td>0.49</td>
<td>0.15</td>
<td>0.47</td>
<td>0.19</td>
<td>0.18</td>
</tr>
<tr>
<td>Os4CL1</td>
<td>0.38</td>
<td>0.50</td>
<td>0.38</td>
<td>0.49</td>
<td>0.35</td>
<td>0.38</td>
</tr>
<tr>
<td>Os4CL2</td>
<td>0.46</td>
<td>0.14</td>
<td>0.47</td>
<td>0.13</td>
<td>0.44</td>
<td>0.46</td>
</tr>
<tr>
<td>Os4CL3</td>
<td>0.10</td>
<td>0.46</td>
<td>0.09</td>
<td>0.44</td>
<td>0.14</td>
<td>0.10</td>
</tr>
<tr>
<td>Os4CL4</td>
<td>0.15</td>
<td>0.44</td>
<td>0.14</td>
<td>0.43</td>
<td>0.13</td>
<td>0.14</td>
</tr>
<tr>
<td>Po4CL1</td>
<td>0.40</td>
<td>0.41</td>
<td>0.39</td>
<td>0.41</td>
<td>0.37</td>
<td>0.39</td>
</tr>
<tr>
<td>Po4CL2</td>
<td>0.41</td>
<td>0.44</td>
<td>0.40</td>
<td>0.43</td>
<td>0.39</td>
<td>0.41</td>
</tr>
<tr>
<td>Po4CL3</td>
<td>0.46</td>
<td>0.49</td>
<td>0.46</td>
<td>0.48</td>
<td>0.45</td>
<td>0.46</td>
</tr>
<tr>
<td>Po4CL4</td>
<td>0.44</td>
<td>0.32</td>
<td>0.44</td>
<td>0.31</td>
<td>0.44</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Table 8 continued

<table>
<thead>
<tr>
<th></th>
<th>At4CL1</th>
<th>At4CL2</th>
<th>At4CL3</th>
<th>At4CL4</th>
<th>Lp4CL1</th>
<th>Lp4CL2</th>
<th>Lp4CL3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sc4CL1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sc4CLM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sb_04g0G5210</td>
<td>0.16</td>
<td>0.44</td>
<td>0.37</td>
<td>0.41</td>
<td>0.44</td>
<td>0.40</td>
<td>0.43</td>
</tr>
<tr>
<td>Sb_04g031010</td>
<td>0.44</td>
<td>0.43</td>
<td>0.44</td>
<td>0.43</td>
<td>0.44</td>
<td>0.46</td>
<td>0.44</td>
</tr>
<tr>
<td>Sb_10g026130</td>
<td>0.39</td>
<td>0.41</td>
<td>0.45</td>
<td>0.51</td>
<td>0.45</td>
<td>0.47</td>
<td>0.44</td>
</tr>
<tr>
<td>Zm_LOC542166</td>
<td>0.44</td>
<td>0.40</td>
<td>0.54</td>
<td>0.50</td>
<td>0.47</td>
<td>0.47</td>
<td>0.49</td>
</tr>
<tr>
<td>At4CL1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At4CL2</td>
<td>0.44</td>
<td>0.43</td>
<td>0.50</td>
<td>0.54</td>
<td>0.50</td>
<td>0.20</td>
<td>0.34</td>
</tr>
<tr>
<td>At4CL3</td>
<td>0.39</td>
<td>0.45</td>
<td>0.51</td>
<td>0.48</td>
<td>0.45</td>
<td>0.42</td>
<td>0.40</td>
</tr>
<tr>
<td>At4CL4</td>
<td>0.42</td>
<td>0.44</td>
<td>0.53</td>
<td>0.48</td>
<td>0.34</td>
<td>0.45</td>
<td>0.44</td>
</tr>
<tr>
<td>Lp4CL1</td>
<td>0.42</td>
<td>0.41</td>
<td>0.46</td>
<td>0.52</td>
<td>0.47</td>
<td>0.47</td>
<td>0.49</td>
</tr>
<tr>
<td>Lp4CL2</td>
<td>0.41</td>
<td>0.41</td>
<td>0.46</td>
<td>0.53</td>
<td>0.46</td>
<td>0.16</td>
<td>0.19</td>
</tr>
<tr>
<td>Lp4CL3</td>
<td>0.41</td>
<td>0.41</td>
<td>0.46</td>
<td>0.53</td>
<td>0.46</td>
<td>0.16</td>
<td>0.19</td>
</tr>
<tr>
<td>Os4CL1</td>
<td>0.30</td>
<td>0.29</td>
<td>0.38</td>
<td>0.45</td>
<td>0.42</td>
<td>0.40</td>
<td>0.44</td>
</tr>
<tr>
<td>Os4CL2</td>
<td>0.31</td>
<td>0.31</td>
<td>0.39</td>
<td>0.44</td>
<td>0.45</td>
<td>0.41</td>
<td>0.46</td>
</tr>
<tr>
<td>Os4CL3</td>
<td>0.31</td>
<td>0.33</td>
<td>0.44</td>
<td>0.45</td>
<td>0.48</td>
<td>0.47</td>
<td>0.49</td>
</tr>
<tr>
<td>Os4CL4</td>
<td>0.43</td>
<td>0.42</td>
<td>0.26</td>
<td>0.53</td>
<td>0.31</td>
<td>0.45</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Sc: Sugarcane, Sb: Sorghum bicolor, Zm: Zea Mays, At: Arabidopsis Thaliana, Lp: Lolium perenne, Os: Oryza Sativa, Po: Poplar Hybrid (Populus trichocarpa x Populus deltoids)
<table>
<thead>
<tr>
<th></th>
<th>Os4CL1</th>
<th>Os4CL2</th>
<th>Os4CL3</th>
<th>Os4CL4</th>
<th>Po4CL1</th>
<th>Po4CL2</th>
<th>Po4CL3</th>
<th>Po4CL4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sc4CL1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sc4CLM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sb_04g005210</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sb_04g031010</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sb_10g026130</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zm_LOC542166</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A14CL1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At4CL2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At4CL3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>At4CL4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lp4CL1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lp4CL2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lp4CL3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Os4CL1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Os4CL2</td>
<td>0.49</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Os4CL3</td>
<td></td>
<td>0.36</td>
<td>0.45</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Os4CL4</td>
<td>0.36</td>
<td>0.44</td>
<td>0.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Po4CL1</td>
<td>0.47</td>
<td>0.44</td>
<td>0.38</td>
<td>0.39</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Po4CL2</td>
<td>0.47</td>
<td>0.47</td>
<td>0.41</td>
<td>0.41</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Po4CL3</td>
<td>0.47</td>
<td>0.49</td>
<td>0.46</td>
<td>0.47</td>
<td>0.27</td>
<td>0.27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Po4CL4</td>
<td>0.50</td>
<td>0.31</td>
<td>0.45</td>
<td>0.43</td>
<td>0.37</td>
<td>0.40</td>
<td>0.44</td>
<td>0.46</td>
</tr>
</tbody>
</table>

It should be understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims. In addition, any elements or limitations of any invention or embodiment thereof disclosed herein can be combined with any and/or all other elements or limitations (individually or in any combination) or any other invention or embodiment thereof disclosed herein, and all such combinations are contemplated with the scope of the invention without limitation thereto.
REFERENCES

U.S. Patent No. 4,761,373
U.S. Patent No. 4,769,061

5 U.S. Patent No. 4,810,648
U.S. Patent No. 4,940,835
U.S. Patent No. 4,945,050
U.S. Patent No. 4,975,374
U.S. Patent No. 4,987,071

10 U.S. Patent No. 5,013,659
U.S. Patent No. 5,023,179
U.S. Patent No. 5,034,322
U.S. Patent No. 5,036,006
U.S. Patent No. 5,093,246

15 U.S. Patent No. 5,100,792
U.S. Patent No. 5,106,739
U.S. Patent No. 5,106,742
U.S. Patent No. 5,162,602
U.S. Patent No. 5,256,558

20 U.S. Patent No. 5,270,163
U.S. Patent No. 5,276,268
U.S. Patent No. 5,304,730
U.S. Patent No. 5,495,071
U.S. Patent No. 5,554,798

25 U.S. Patent No. 5,561,236
U.S. Patent No. 5,569,823
U.S. Patent No. 5,582,981
U.S. Patent No. 5,589,610
U.S. Patent No. 5,591,616

30 U.S. Patent No. 5,595,877
U.S. Patent No. 5,599,672
U.S. Patent No. 5,625,136
U.S. Patent No. 5,639,948
U.S. Patent No. 5,661,017
U.S. Patent No. 5,723,763
U.S. Patent No. 5,767,366
U.S. Patent No. 5,817,785
U.S. Patent No. 5,879,903
U.S. Patent No. 5,928,937
U.S. Patent No. 6,084,155
U.S. Patent No. 6,329,504
U.S. Patent No. 6,337,431
U.S. Patent No. 6,344,318
U.S. Patent No. 6,455,760
U.S. Patent No. 6,462,185
U.S. Patent No. 6,506,559
U.S. Patent No. 6,696,623
U.S. Patent No. 6,933,116
U.S. Patent No. 7,056,704
U.S. Patent No. 7,078,196
U.S. Patent No. 7,232,086
U.S. Patent No. 7,282,564
U.S. Patent No. 7,365,058
U.S. Patent No. 7,368,236
U.S. Patent No. 7,538,095
U.S. Patent No. 7,700,759
U.S. Patent No. 7,723,575
U.S. Published Application No. 20010016956
U.S. Published Application No. 20030084486
U.S. Published Application No. 20030177536
U.S. Published Application No. 20040019934
U.S. Published Application No. 20040067506
U.S. Published Application No. 20040078841
U.S. Published Application No. 20040123349
U.S. Published Application No. 20060260011
42

U.S. Published Application No. 2007006346
U.S. Published Application No. 20090031441
U.S. Published Application No. 20090199307
PCT Published Application No. WO 93/07278

5
PCT Published Application No. WO 93/21335
EP 0242246
EP 0292435
EP 0392225
EP 138341
EP 295959


Doran, T. and Helliwell, C. (2009) RNA interference: Methods for plants and animals; Publisher: CABI, Wallingford, UK.


Good, X. et al. (1994) "Reduced ethylene synthesis by transgenic tomatoes expressing S-adenosylmethionine hydrolase" *Plant Molec. Biol.* 26:781-790.


Reich et al. (1986) Biotechnology 4:1001-1004.


We claim:

1. A method for modulating lignin biosynthesis in a sugarcane plant, comprising targeting the expression of a gene and/or function of an enzyme involved in the lignin biosynthesis pathway in the plant.

2. The method according to claim 1, wherein lignin biosynthesis is downregulated in the plant.

3. The method according to claim 2, wherein expression of one or more 4-coumarate-CoA ligase (4CL) genes and/or one or more 4CL enzymes is suppressed or downregulated.

4. The method according to claim 2 or 3, wherein lignin biosynthesis is selectively downregulated in leaf tissue or a leaf cell.

5. The method according to claim 2, 3, or 4, wherein down-regulation is achieved by introducing in a cell of said sugarcane plant a polynucleotide sequence that provides for an antisense, cosuppression, RNA interference (RNAi), short interfering RNA (siRNA), or ribozyme sequence that when expressed in a cell of said plant suppresses or down-regulates expression of a gene involved in lignin biosynthesis.

6. The method according to claim 2, 3, or 4, wherein down-regulation is achieved by introducing in a cell of said sugarcane plant a knockout mutation in one or more genes encoding an enzyme involved in the lignin biosynthesis pathway.

7. The method according to claim 2, 3, or 4, wherein modulation or down regulation is achieved by contacting said enzyme involved in the lignin biosynthesis pathway with an antibody, or an antigen binding fragment thereof, or an aptamer that can bind to and block or inhibit function of said enzyme.
8. The method according to claim 3, wherein said 4CL gene is 4CL-L, 4CL-M, or 4CL-N.

9. The method according to claim 8, wherein said 4CL-L gene encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:6.

10. The method according to claim 8, wherein said 4CL-M gene encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:7.

11. The method according to claim 8, wherein said 4CL-N gene encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:8.

12. The method according to claim 8, wherein said 4CL-L gene comprises the nucleotide of SEQ ID NO:1.

13. The method according to claim 8, wherein said 4CL-M gene comprises the nucleotide of SEQ ID NO:2.

14. The method according to claim 8, wherein said 4CL-N gene comprises the nucleotide of SEQ ID NO:3.

15. The method according to claim 5, wherein down-regulation is achieved using RNAi to down-regulate expression of one or more 4CL genes.

16. The method according to claim 15, wherein said 4CL gene is 4CL-L, 4CL-M, or 4CL-N.

17. The method according to claim 16, wherein expression of said 4CL-L gene is down-regulated by expression in said sugarcane plant of an RNAi construct comprising the nucleotide sequence of SEQ ID NO:4.
18. The method according to claim 16, wherein expression of said 4CL-M gene is down-regulated by expression in said sugarcane plant of RNAi construct comprising the nucleotide sequence of SEQ ID NO:5.

19. A transformed or transgenic sugarcane plant, plant tissue, or plant cell having modulated lignin biosynthesis, wherein expression and/or function of an enzyme involved in a lignin biosynthesis pathway is modulated.

20. The sugarcane plant, plant tissue, or plant cell according to claim 19, wherein lignin biosynthesis is down-regulated in the plant.

21. The sugarcane plant, plant tissue, or plant cell according to claim 20, wherein expression of one or more 4-coumarate-CoA ligase (4CL) genes and/or one or more 4CL enzymes is suppressed or down-regulated.

22. The sugarcane plant, plant tissue, or plant cell according to claim 20 or 21, wherein lignin biosynthesis is selectively down-regulated in leaf tissue.

23. The sugarcane plant, plant tissue, or plant cell according to claim 20, 21, or 22, wherein down-regulation is achieved by introducing in a cell of said sugarcane plant a polynucleotide sequence that provides for an antisense, cosuppression, RNA interference (RNAi), short interfering RNA (siRNA), or ribozyme sequence that when expressed in a cell of said plant suppresses or down-regulates expression of a gene involved in lignin biosynthesis.

24. The sugarcane plant, plant tissue, or plant cell according to claim 20, 21, or 22, wherein down-regulation is achieved by introducing in a cell of said sugarcane plant a knockout mutation in one or more genes encoding an enzyme involved in the lignin biosynthesis pathway.

25. The sugarcane plant, plant tissue, or plant cell according to claim 20, 21, or 22, wherein down regulation is achieved by contacting said enzyme involved in the lignin
biosynthesis pathway with an antibody, or an antigen binding fragment thereof, or an aptamer that can bind to and block or inhibit function of said enzyme.

26. The sugarcane plant, plant tissue, or plant cell according to claim 19, wherein said plant tissue is branches, kernels, ears, cobs, husks, root tips, anthers, seeds, roots, embryos, hypocotyls, cotyledons, pollen, ovules, anthers, shoots, stalks, stems, leaves, fruits, or flowers.

27. The sugarcane plant, plant tissue, or plant cell according to claim 19, wherein said sugarcane plant is *Saccharum arundinacewn*, *Saccharum bengalense*, *Saccharum edule*, *Saccharum officinarum*, *Saccharum procerum*, *Saccharum ravennae*, *Saccharum robustum*, *Saccharum sinense*, or *Saccharum spontaneum*.

28. The sugarcane plant, plant tissue, or plant cell according to claim 19, wherein said sugarcane plant is a hybrid or inbred line.

29. The sugarcane plant, plant tissue, or plant cell according to claim 19, wherein said sugarcane plant comprises one or more beneficial agronomic traits.

30. The sugarcane plant, plant tissue, or plant cell according to claim 29, wherein said agronomic trait is herbicide resistance, virus resistance, bacterial pathogen resistance, insect resistance, nematode resistance, fungal resistance, increased plant vigor, or increased plant yield.

31. An RNAi construct comprising a polynucleotide sequence that down regulates or suppresses expression of a 4CL gene of sugarcane.

32. The RNAi construct according to claim 31, wherein said 4CL gene is 4CL-L, 4CL-M, or 4CL-N.

33. The RNAi construct according to claim 32, wherein said 4CL-L gene comprises the nucleotide sequence of SEQ ID NO:1.
34. The RNAi construct according to claim 32, wherein said 4CL-M gene comprises the nucleotide sequence of SEQ ID NO:2.

35. The RNAi construct according to claim 32, wherein said 4CL-N gene comprises the nucleotide sequence of SEQ ID NO:3.

36. The RNAi construct according to claim 32, wherein said construct comprises the nucleotide sequence in SEQ ID NO:4 or SEQ ID NO:5.

37. A method for preparing a transformed or transgenic sugarcane plant, plant tissue, or plant cell having modulated lignin biosynthesis, wherein expression and/or function of an enzyme involved in a lignin biosynthesis pathway is modulated comprising: incorporating a polynucleotide in a cell of said plant, wherein expression of said polynucleotide modulates the expression of a gene and/or the function of an enzyme involved in lignin biosynthesis in said plant.

38. The method according to claim 37, wherein lignin biosynthesis is downregulated in the plant.

39. The method according to claim 38, wherein expression of one or more 4-coumarate-CoA ligase (4CL) genes and/or one or more 4CL enzymes is suppressed or downregulated.

40. The method according to claim 38 or 39, wherein lignin biosynthesis is selectively downregulated in leaf tissue or a leaf cell.

41. The method according to claim 38, 39, or 40, wherein down-regulation is achieved by introducing in a cell of said sugarcane plant a polynucleotide sequence that provides for an antisense, co-suppression, RNA interference (RNAi), short interfering RNA (siRNA), or ribozyme sequence that when expressed in a cell of said plant suppresses or down-regulates expression of a gene involved in lignin biosynthesis.
42. The method according to claim 38, 39, or 40, wherein down-regulation is achieved by introducing in a cell of said sugarcane plant a knockout mutation in one or more genes encoding an enzyme involved in the lignin biosynthesis pathway.

43. The method according to claim 38, 39, or 40, wherein modulation or down regulation is achieved by contacting said enzyme involved in the lignin biosynthesis pathway with an antibody, or an antigen binding fragment thereof, or an aptamer that can bind to and block or inhibit function of said enzyme.

44. The method according to claim 39, wherein said 4CL gene is 4CL-L, 4CL-M, or 4CL-N.

45. The method according to claim 44, wherein said 4CL-L gene encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:6.

46. The method according to claim 44, wherein said 4CL-M gene encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:7.

47. The method according to claim 44, wherein said 4CL-N gene encodes a polypeptide comprising the amino acid sequence of SEQ ID NO:8.

48. The method according to claim 44, wherein said 4CL-L gene comprises the nucleotide of SEQ ID NO:1.

49. The method according to claim 44, wherein said 4CL-M gene comprises the nucleotide of SEQ ID NO:2.

50. The method according to claim 44, wherein said 4CL-N gene comprises the nucleotide of SEQ ID NO:3.
51. The method according to claim 41, wherein expression of one or more 4CL genes is down-regulated using RNAi.

52. The method according to claim 51, wherein said 4CL gene is 4CL-L, 4CL-M, or 4CL-N.

53. The method according to claim 52, wherein expression of said 4CL-L gene is down-regulated by expression in said sugarcane plant of an RNAi construct comprising the nucleotide sequence of SEQ ID NO:4.

54. The method according to claim 52, wherein expression of said 4CL-M gene is down-regulated by expression in said sugarcane plant of RNAi construct comprising the nucleotide sequence of SEQ ID NO:5.

55. The method according to claim 1, wherein said sugarcane plant is Saccharum arundinaceum, Saccharum hengalense, Saccharum edule, Saccharum officinarum, Saccharum procerum, Saccharum ravennae, Saccharum robustum, Saccharum sinense, or Saccharum spontaneum.

56. The method according to claim 1, wherein said sugarcane plant is a hybrid or inbred line.

57. The method according to claim 37, wherein said sugarcane plant is Saccharum arundinaceum, Saccharum hengalense, Saccharum edule, Saccharum officinarum, Saccharum procerum, Saccharum ravennae, Saccharum robustum, Saccharum sinense, or Saccharum spontaneum.

58. The method according to claim 37, wherein said sugarcane plant is a hybrid or inbred line.

59. The sugarcane plant, plant tissue, or plant cell according to claim 28, wherein said sugarcane plant is a hybrid of Saccharum and Miscanthus or Erianthus, or Sorghum.
60. The sugarcane plant, plant tissue, or plant cell according to claim 56, wherein said sugarcane plant is a hybrid of Saccharum and Miscanthus or Erianthus, or Sorghum.

61. The sugarcane plant, plant tissue, or plant cell according to claim 58, wherein said sugarcane plant is a hybrid of Saccharum and Miscanthus or Erianthus, or Sorghum.

62. The method according to claim 1, wherein said sugarcane plant is transformed with a polynucleotide that is stably incorporated into the genome of said sugarcane plant, wherein expression of said polynucleotide inhibits or down-regulates lignin biosynthesis in said plant.

63. The transformed or transgenic sugarcane plant, plant tissue, or plant cell according to claim 19, wherein said sugarcane plant is transformed with a polynucleotide that is stably incorporated into the genome of said sugarcane plant, wherein expression of said polynucleotide inhibits or down-regulates lignin biosynthesis in said plant.

64. The method according to claim 37, wherein said sugarcane plant is transformed with a polynucleotide that is stably incorporated into the genome of said sugarcane plant, wherein expression of said polynucleotide inhibits or down-regulates lignin biosynthesis in said plant.

65. The method according to claim 5, wherein said polynucleotide is provided in an expression construct.

66. The sugarcane plant, plant tissue, or plant cell according to claim 23, wherein said polynucleotide is provided in an expression construct.

67. The RNAi construct according to claim 31, wherein said polynucleotide is provided in an expression construct.
68. The method according to claim 41, wherein said polynucleotide is provided in an expression construct.

69. The method according to claim 65, wherein said expression construct comprises a leaf-specific promoter.

70. The sugarcane plant, plant tissue, or plant cell according to claim 66, wherein said expression construct comprises a leaf-specific promoter.

71. The RNAi construct according to claim 67, wherein said expression construct comprises a leaf-specific promoter.

72. The method according to claim 68, wherein said expression construct comprises a leaf-specific promoter.

73. The method according to claim 69, wherein said leaf-specific promoter is Cabl promoter, Cabl9 promoter, PPDK promoter, ribulose biphosphate carboxylase (RBCS) promoter, Actl promoter, AS-I promoter, RBC-3A promoter, mannopine synthase (mas) promoter, octopine synthase (ocs) promoter, promoters from CaMV 19S, nos, Adh, sucrose synthase, a-tubulin, ubiquitin, actin, cab, PEPCase, or those associated with the R gene complex.

74. The sugarcane plant, plant tissue, or plant cell according to claim 70, wherein said leaf-specific promoter is Cabl promoter, Cabl9 promoter, PPDK promoter, ribulose biphosphate carboxylase (RBCS) promoter, Actl promoter, AS-I promoter, RBC-3A promoter, mannopine synthase (mas) promoter, octopine synthase (ocs) promoter, promoters from CaMV 19S, nos, Adh, sucrose synthase, a-tubulin, ubiquitin, actin, cab, PEPCase, or those associated with the R gene complex.

75. The RNAi construct according to claim 71, wherein said leaf-specific promoter is Cabl promoter, Cabl9 promoter, PPDK promoter, ribulose biphosphate carboxylase (RBCS) promoter, Actl promoter, AS-I promoter, RBC-3A promoter, mannopine synthase (mas)
promoter, octopine synthase (ocs) promoter, promoters from CaMV 19S, nos, Adh, sucrose synthase, a-tubulin, ubiquitin, actin, cab, PEPCase, or those associated with the R gene complex.

76. The method according to claim 72, wherein said leaf-specific promoter is Cabl promoter, Cab19 promoter, PPDK promoter, ribulose biphosphate carboxylase (RBCS) promoter, Actl promoter, AS-I promoter, RBC-3A promoter, mannopine synthase (mas) promoter, octopine synthase (ocs) promoter, promoters from CaMV 19S, nos, Adh, sucrose synthase, a-tubulin, ubiquitin, actin, cab, PEPCase, or those associated with the R gene complex.

77. A method for modulating lignin biosynthesis in a plant, comprising targeting the expression of a gene and/or function of an enzyme involved in the lignin biosynthesis pathway in the plant.

78. A transformed or transgenic plant, plant tissue, or plant cell having modulated lignin biosynthesis, wherein expression and/or function of an enzyme involved in a lignin biosynthesis pathway is modulated.

79. A method for preparing a transformed or transgenic plant, plant tissue, or plant cell having modulated lignin biosynthesis, wherein expression and/or function of an enzyme involved in a lignin biosynthesis pathway is modulated comprising: incorporating a polynucleotide in a cell of said plant, wherein expression of said polynucleotide modulates the expression of a gene and/or the function of an enzyme involved in lignin biosynthesis in said plant.