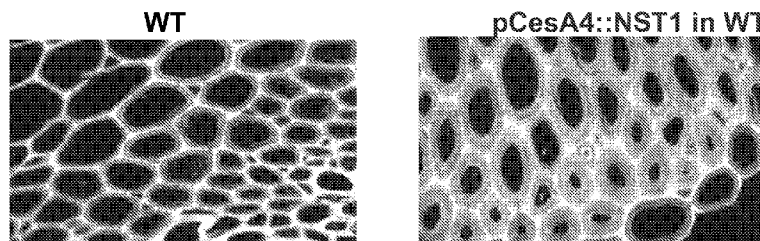




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(54) **Title:** SPATIALLY MODIFIED GENE EXPRESSION IN PLANTS

Fig. 32A. Density loop pCesA4::NST1 in wild type Arabidopsis (dicot)



Cell wall densification strategy in Arabidopsis wild type plants (dicotyledon)
UV images of stem cross sections from wildtype and wildtype containing the pCesA4::NST1 DNA construct. The creation of a positive feedback loop with the secondary cell wall cellulose promoter (pCesA4) and the secondary cell wall transcription factor (NST1) enhances secondary cell wall deposition in fiber cells.

(57) **Abstract:** The invention provides methods of engineering plants having lignin deposition or xylan deposition that is substantially localized to the vessels of xylem tissue in the plant. The invention also provides methods of engineering plants to increase production of a desired biosynthetic product, e.g., to have increased secondary cell wall deposition or increased wax/cutin accumulation. The engineered plants of the present invention have use in bioenergy production, e.g., by improving the density and the digestibility of biomass derived from the plant and to improve water usage requirements.



SPATIALLY MODIFIED GENE EXPRESSION IN PLANTS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims benefit of U.S. provisional application no. 61/437,569, filed
5 January 28, 2011, which is herein incorporated by reference for all purposes.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

[0002] This invention was made with government support under Contract No. DE-AC02-
05CH11231 awarded by the U.S. Department of Energy. The government has certain rights
10 in this invention.

BACKGROUND OF THE INVENTION

[0003] Plant cell wall is the only source of cellulose for the paper industry and is a
promising source of sugar for lignocellulosic biofuels. The utilization of plants to convert
solar energy into transportable and storable energy will have a positive impact on the
15 environment, since using plants can help to drastically reduce the utilization of fossil-derived
fuels, can reduce carbon emission into the atmosphere, and even can contribute to carbon
sequestration. However, even if lignocellulosic biofuels will be beneficial for the
environment, the cost to produce them is still not cost-effective, mainly due to the expensive
raw sugar derived from plant cell wall. The low density, recalcitrance to enzymatic
20 hydrolysis, and medium content in cellulose are the main contributors to the sugar cost
because they impact transportation cost and require high amount of energy and chemicals.
Therefore, improving the density and the digestibility of the raw biomass will have an
important beneficial impact on the cost of lignocellulosic biofuels production.

[0004] Cell wall recalcitrance is mainly caused by the presence of lignin, which embeds the
25 polysaccharide polymers and reduces their extractability and accessibility to hydrolytic
enzymes. Lignin content and saccharification efficiency of plant cell wall usually are highly
negatively correlated (Vinzant et al., 1997; Chen et al., 2007; Jorgensen et al., 2007).
Unfortunately, most attempts at reduction of plant lignin content resulted in severe biomass
yield reduction (Voelker et al., 2010; Shadle et al., 2007; Franke et al., 2002) and therefore,
30 crops with significant lignin reduction are not readily available. This cell wall-growth relation

is not unique to lignin; it is commonly observed and correlated with vessel collapse and occurs most of the time when secondary cell wall genes involved in hemicellulose or cellulose biosynthesis are defective (Voelker et al., 2010; Anterola and Lewis, 2002; Brown et al., 2005). These vessels are essential to feed above-ground tissues with water and nutrients absorbed by the root system (Gomez et al., 2008, Boyce et al., 2004). Hence, silencing strategies, which compromise between the level of the enzymatic step inhibition and biomass yield, are used to reduce lignin in plants.

[0005] In woody tissues, a new cell wall, so-called secondary cell wall, is produced and is the main component contributing to biomass density when water is removed. Optimizing cell wall deposition would increase biomass density and therefore energy density. This improvement would be beneficial in reducing the transportation cost of biomass, a significant component in the price of the biomass delivered at the gate of the biorefinery (Searcy et al., 2007; Aden et al., 2002; Kumar et al., 2005). Therefore, developing strategies allowing the thickening of cell wall of woody tissues or pith without altering plant growth can increase biomass and energetic density and would be favorable to the cost-effectiveness of lignocellulosic bioenergy production.

[0006] There is an additional need to engineer various biosynthetic pathways in path in a manner such that the production of biosynthetic product can be targeted in a tissue of interest.

[0007] This invention addresses these needs.

20

BRIEF SUMMARY OF THE INVENTION

[0008] Various biological processes exist in organisms from prokaryotes to eukaryotes that are regulated by a small number of transcription factors. In one aspect, this invention provides a positive feedback loop to increase expression of desired products in an organism, *e.g.*, a plant. An artificial positive feedback loop (AFPL) in accordance with the invention employs a transcription factor/promoter construct, typically where the transcription factor is a “master” transcription factor that modulates expression of all or most of the components of a targeted biosynthetic pathway. A promoter from a gene that is downstream in the pathway, where the transcription factor induces or increases expression of the gene, is operably linked to a nucleic acid encoding the transcription factor such that increased expression of the transcription factor results. An AFPL can be used in any biosynthetic process in plants, *e.g.*, to control cell wall deposition, wax/cutin accumulation, or lipid accumulation, and the like.

[0009] In one aspect, the invention provides a method of engineering a plant to increase the production of a biosynthetic product in a desired tissue, the method comprising: introducing an expression cassette into the plant, wherein the expression cassette comprises a polynucleotide encoding a transcription factor that regulates production of the biosynthetic product operably linked to a heterologous promoter, wherein the heterologous promoter is a promoter that induces gene expression of a gene that is a downstream target of the transcription factor in the desired tissue; and culturing the plant under conditions in which the transcription factor is expressed. The method may be applied to any plant, including monocots and dicots. In some embodiments, the plant is *Arabidopsis*, poplar, eucalyptus, rice, corn, switchgrass, sorghum, millet, miscanthus, sugarcane, pine, alfalfa, wheat, soy, barley, turfgrass, tobacco, hemp, poppy, bamboo, rape, sunflower, willow, or Brachypodium.

[0010] In some embodiments, the promoter is a tissue-specific secondary wall promoter and the transcription factor induces expression of secondary wall biosynthetic products. For example, the transcription factor may be NAC secondary wall-thickening promoting factor 1 (NST1), NST2, NST3, secondary wall-associated NAC domain protein 2 (SND2), SND3, MYB domain protein 103 (MYB103), MBY85, MYB46, MYB83, MYB58, or MYB63. In some embodiments, the tissue-specific secondary wall promoter is an IRX1, IRX3, IRX5, IRX8, IRX9, IRX14, IRX7, IRX10, GAUT13, GAUT14, or CESA4 promoter.

[0011] In some embodiments, of the methods of engineering a plant to increase production of a biosynthetic product in a desired tissue, the transcription factor induces expression of wax and/or cutin. In some embodiments, the transcription factor is a shine (SHN) transcription factor selected from SHN1 (also known as WIN1), SHN2, SHN3, SHN4, or SHN5; or MYB 96. In some embodiments, the promoter is a CER1, CER2, CER3, CER4, CER5, CER6, CER10, WSD1, Mah1, WBC11, KCS1, KCS2, FATB, LACS1, LACS2, CYP864A, CYP86A7, CYP86A5, KCS10, or KCS5 promoter.

[0012] In a further aspect, the invention provides a plant comprising an expression cassette that comprises a polynucleotide encoding a transcription factor that regulates production of a biosynthetic product operably linked to a heterologous promoter, wherein the heterologous promoter is a promoter that induces gene expression of a gene that is a downstream target of the transcription factor in the desired tissue; and culturing the plant under conditions in which the transcription factor is expressed. The plant may be any plant, including monocots and dicots. In some embodiments, the plant is *Arabidopsis*, poplar, eucalyptus, rice, corn, switchgrass, sorghum, millet, miscanthus, sugarcane, pine, alfalfa, wheat, soy, barley, turfgrass, tobacco, hemp, poppy, bamboo, rape, sunflower, willow, or Brachypodium.

[0013] In some embodiments, the plant comprises an expression construct in which the promoter is a tissue-specific secondary wall promoter and the transcription factor encoded by the construct induces expression of secondary wall biosynthetic products. For example, the transcription factor may be NAC secondary wall-thickening promoting factor 1 (NST1), NST2, NST3, secondary wall-associated NAC domain protein 2 (SND2), SND3, MYB domain protein 103 (MYB103), MBY85, MYB46, MYB83, MYB58, or MYB63. In some embodiments, the tissue-specific secondary wall promoter is an IRX1, IRX3, IRX5, IRX8, IRX9, IRX14, IRX7, IRX10, GAUT13, GAUT14, or CESA4 promoter.

[0014] In some embodiments, the transcription factor encoded by the expression construct induces expression of wax and/or cutin. In some embodiments, the transcription factor is a shine (SHN) transcription factor selected from SHN1 (also known as WIN1), SHN2, SHN3, SHN4, or SHN5; or MYB 96. In some embodiments, the promoter is a CER1, CER2, CER3, CER4, CER5, CER6, CER10, WSD1, Mah1, WBC11, KCS1, KCS2, FATB, LACS1, LACS2, CYP864A, CYP86A7, CYP86A5, KCS10, or KCS5 promoter.

[0015] In one aspect, the present invention provides methods of engineering a plant having lignin deposition that is substantially localized to the vessels of xylem tissue of the plant. In some embodiments, the method comprises:

introducing an expression cassette into the plant, wherein the plant is modified to have a reduced level of expression of a lignin biosynthesis enzyme; and further, wherein the expression cassette comprises a polynucleotide encoding the lignin biosynthesis enzyme operably linked to a heterologous vessel-specific promoter; and

culturing the plant under conditions in which the lignin biosynthesis enzyme is expressed.

[0016] In some embodiments, the lignin biosynthesis enzyme is PAL, C4H, 4CL, HCT, C3H, or CCR1. In some embodiments, the lignin biosynthesis enzyme is C4H.

[0017] In some embodiments, the promoter is a VND1, VND2, VND3, VND4, VND5, VND6, VND7, VNI2, REF4 or RFR1, *e.g.*, a promoter substantially identical to a VND1, VND2, VND3, VND4, VND5, VND6, VND7, VNI2, REF4 or RFR1 promoter; or a native VND1, VND2, VND3, VND4, VND5, VND6, VND7, VNI2, REF4 or RFR1 promoter.

[0018] In some embodiments, the level of activity of the lignin biosynthesis enzyme in the modified plant is reduced by contacting the plant with an antisense oligonucleotide that silences expression of the gene encoding the lignin biosynthesis enzyme. In some embodiments, the modified plant in which the polynucleotide operably linked to the

heterologous promoter is expressed has a mutation in the gene encoding the lignin synthesis enzyme that decreases expression of the enzyme.

[0019] In some embodiments, the plant is selected from the group consisting of Arabidopsis, poplar, eucalyptus, rice, corn, switchgrass, sorghum, millet, miscanthus, sugarcane, pine, alfalfa, wheat, soy, barley, turfgrass, tobacco, hemp, bamboo, rape, sunflower, willow, and Brachypodium.

[0020] In some embodiments, the present invention provides plants, plant cells, seeds, flowers, leave, fruit, or biomass comprising plant tissue engineered to have lignin deposition that is substantially localized to the vessels of xylem tissue of the plant.

[0021] In another aspect, the present invention provides methods of obtaining an increased amount of soluble sugars from a plant in a saccharification reaction. In some embodiments, the method comprises subjecting a plant engineered to have lignin deposition that is substantially localized to the vessels of xylem tissue of the plant to a saccharification reaction, thereby increasing the amount of soluble sugars that can be obtained from the plant as compared to a wild-type plant.

[0022] In still another aspect, the present invention provides methods of engineering a plant having increased secondary cell wall deposition. In some embodiments, the method comprises:

introducing an expression cassette into the plant, wherein the expression cassette comprises a polynucleotide encoding a transcription factor that regulates the production of secondary cell wall in woody tissue operably linked to a heterologous promoter, wherein the promoter is substantially identical to the native promoter of a gene that is a downstream target of the transcription factor; and

culturing the plant under conditions in which the transcription factor is expressed. In some embodiments, the promoter and the transcription factor, or either the promoter or the transcription factor are from a different plant species than the host cell in which the artificial positive feedback loop is created. In further embodiments, the transcription factor and the promoter are from different plant species.

[0023] In some embodiments, the transcription factor is NST1, NST2, NST3, MYB103, MYB85, MYB46, MYB83, MYB58, or MYB63. In some embodiments, the transcription factor is NST1.

[0024] In some embodiments, the promoter is an IRX1, IRX3, IRX5, IRX8, IRX9, IRX14, IRX7, or IRX10 promoter. In some embodiments, the promoter is a native IRX1, IRX3, IRX5, IRX8, IRX9, IRX14, IRX7, or IRX10 promoter.

5 [0025] In some embodiments, the plant in which the polynucleotide operably linked to the heterologous promoter is expressed is a wild-type plant. In some embodiments, the plant in which the polynucleotide operably linked to the heterologous promoter is expressed is an engineered plant having lignin deposition that is substantially localized to the vessels of xylem tissue of the plant.

10 [0026] In some embodiments, the plant is selected from the group consisting of Arabidopsis, poplar, eucalyptus, rice, corn, switchgrass, sorghum, millet, miscanthus, sugarcane, pine, alfalfa, wheat, soy, barley, turfgrass, tobacco, hemp, bamboo, rape, sunflower, willow, and Brachypodium.

15 [0027] In some embodiments, the present invention provides plants, plant cells, seeds, flowers, leave, fruit, or biomass comprising plant tissue engineered to have increased secondary cell wall deposition.

20 [0028] In yet another aspect, the present invention provides methods of increasing bioenergy production from biomass derived from a plant. In some embodiments, the method comprises harvesting biomass from a plant engineered to have increased secondary cell wall deposition; and subjecting the biomass to a conversion reaction, thereby increasing bioenergy production as compared to a wild-type plant.

25 [0029] Ina further aspect, the present invention provides methods of increasing stem/straw/timber strength, which can reduce lodging, and increase wood density from a plant. Thus, the invention provides a method of increasing stem, straw or timber strength from plants during growth, the method comprising: cultivating plants engineered to have increased secondary cell wall deposition, thereby improving resistance lodging as compared to a wild type plants. Plants having increased secondary wall deposition may also be cultivated to provide plants, or biomass from such plants that have increased resistance to mechanical stress compared to a wildtype plant.

30 [0030] In yet another aspect, present invention provides methods of engineering a plant having xylan deposition that is substantially localized to the vessels of xylem tissue of the plant. In some embodiments, the method comprises:

introducing an expression cassette into the plant, wherein the plant is modified to have a reduced level of activity of a xylan biosynthesis enzyme; and further, wherein the expression cassette comprises a polynucleotide encoding the xylan biosynthesis enzyme operably linked to a heterologous vessel-specific promoter; and

5 culturing the plant under conditions in which the xylan biosynthesis enzyme is expressed. In some embodiments, the plant into which the expression cassette is introduced is modified to have a reduced level of expression of a xylan biosynthesis enzyme.

[0031] In some embodiments, the xylan biosynthesis enzyme is irregular xylem 8 (IRX8), IRX14, IRX14-like, IRX9, IRX9-like, IRX7, IRX10, IRX10-like, IRX15, IRX15-like, F8H,
10 or PARVUS.

[0032] In some embodiments, the promoter is a VND1, VND2, VND3, VND4, VND5, VND6, VND7, VNI2, REF4 or RFR1, *e.g.*, a promoter substantially identical to a VND1, VND2, VND3, VND4, VND5, VND6, VND7, VNI2, REF4 or RFR1 promoter; or a native VND1, VND2, VND3, VND4, VND5, VND6, VND7, VNI2, REF4 or RFR1 promoter.

15 [0033] In some embodiments, the level of activity of the xylan biosynthesis enzyme in the modified plant is reduced by contacting the plant with an antisense oligonucleotide that silences expression of the gene encoding the xylan biosynthesis enzyme. In some embodiments, the modified plant in which the polynucleotide operably linked to the heterologous promoter is expressed has a mutation in the gene encoding the xylan synthesis
20 enzyme that decreases expression of the enzyme. In some embodiments, the activity of the xylan biosynthesis enzyme in the modified plant is reduced by contacting the plant with a mutated xylan biosynthesis gene that encodes a protein with a dominant negative mutation and causes a decrease in xylan biosynthesis.

[0034] In some embodiments, the plant is selected from the group consisting of
25 Arabidopsis, poplar, eucalyptus, rice, corn, switchgrass, sorghum, millet, miscanthus, sugarcane, pine, alfalfa, wheat, soy, barley, turfgrass, tobacco, hemp, bamboo, rape, sunflower, willow, and Brachypodium.

[0035] In some embodiments, the present invention provides plants, plant cells, seeds, flowers, leave, fruit, or biomass comprising plant tissue engineered to have xylan deposition
30 that is substantially localized to the vessels of xylem tissue of the plant.

[0036] In yet another aspect, the present invention provides methods of obtaining an increased amount of soluble sugars from a plant in a saccharification reaction. In some embodiments, the method comprises subjecting a plant engineered to have xylan deposition

that is substantially localized to the vessels of xylem tissue of the plant to a saccharification reaction, thereby increasing the amount of soluble sugars that can be obtained from the plant as compared to a wild-type plant.

[0037] In still another aspect, the present invention provides methods of engineering a plant
5 having xylan *O*-acetylation that is substantially localized to the vessels of xylem tissue of the plant. In some embodiments, the method comprises:

introducing an expression cassette into the plant, wherein the plant is modified
to have a reduced level of expression of an enzyme responsible for xylan *O*-acetylation; and
further, wherein the expression cassette comprises a polynucleotide encoding the xylan *O*-
10 acetylation enzyme operably linked to a heterologous vessel-specific promoter; and
culturing the plant under conditions in which the xylan *O*-acetylation enzyme
is expressed.

[0038] In some embodiments, the xylan *O*-acetylation enzyme is an RWA protein.

[0039] In some embodiments, the xylan *O*-acetylation enzyme is a member of the
15 Trichome Birefringence Like family of proteins (PF03005 family also known as Domain of
Unknown Function 231).

[0040] In some embodiments, the promoter is a VND1, VND2, VND3, VND4, VND5,
VND6, VND7, VNI2, REF4 or RFR1, *e.g.*, a promoter substantially identical to a VND1,
VND2, VND3, VND4, VND5, VND6, VND7, VNI2, REF4 or RFR1 promoter; or a native
20 VND1, VND2, VND3, VND4, VND5, VND6, VND7, VNI2, REF4 or RFR1 promoter.

[0041] In some embodiments, the level of expression of the xylan *O*-acetylation enzyme in
the modified plant is reduced by contacting the plant with an antisense oligonucleotide that
silences expression of the gene encoding the xylan *O*-acetylation enzyme. In some
embodiments, the modified plant in which the polynucleotide operably linked to the
25 heterologous promoter is expressed has a mutation in the gene encoding the xylan *O*-
acetylation enzyme that decreases expression of the enzyme.

[0042] In some embodiments, the plant is selected from the group consisting of
Arabidopsis, poplar, eucalyptus, rice, corn, switchgrass, sorghum, millet, miscanthus,
sugarcane, pine, alfalfa, wheat, soy, barley, turfgrass, tobacco, hemp, bamboo, rape,
30 sunflower, willow, and Brachypodium.

[0043] In some embodiments, the present invention provides plants, plant cells, seeds, flowers, leave, fruit, or biomass comprising plant tissue engineered to have xylan deposition that is substantially localized to the vessels of xylem tissue of the plant.

[0044] In yet another aspect, the present invention provides methods of obtaining an increased amount of soluble sugars from a plant in a saccharification reaction. In some embodiments, the method comprises subjecting a plant engineered to have xylan *O*-acetylation that is substantially localized to the vessels of xylem tissue of the plant to a saccharification reaction, thereby increasing the amount of soluble sugars that can be obtained from the plant as compared to a wild-type plant.

10

BRIEF DESCRIPTION OF THE DRAWINGS

[0045] **Figure 1. Phenylalanine ammonia-lyase (PAL) alignment.** The protein sequences for PAL from *Arabidopsis thaliana* ("AtPAL1"), *Physcomitrella patens* (moss) ("PpPAL3"), *Oryza sativa* (rice) ("OsPAL"), *Zea mays* (maize) ("ZmPAL"), *Sorghum bicolor* (sorghum) ("SbPAL"), *Pinus massoniana* (pine) ("PIPAL"), *Medicago sativa* (alfalfa) ("MsPAL"), *Triticum aestivum* (wheat) ("TaPAL"), *Glycine max* (soybean) ("GmPAL2"), *Beta vulgaris* (sugar beet) ("BvPAL"), *Nicotiniana tabacum* (tobacco) ("NtPAL1"), *Solanum tuberosum* (potato) ("StPAL1"), *Bambusa oldhamii* (bamboo) ("BoPAL"), *Brassica rapa* ("BnPAL1"), *Helianthus annuus* (sunflower) ("HaPAL"), *Ricinus communis* ("RcPAL"), *Vitis vinifera* (grape) ("VvPAL"), *Jatropha curcas* ("JcPAL"), *Euphorbia pulcherrima* (poinsettia) ("EpPAL"), *Trifolium pratense* (clover) ("TpPAL"), *Lotus japonicus* ("LjPAL5"), and *Selaginella moellendorffii* (spike moss) ("SmPAL") were aligned using ClustalW.

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[0046] **Figure 2. Cinnamate 4-hydroxylase (C4H) alignment.** The protein sequences for C4H from *Arabidopsis thaliana* ("AtC4H"), *Pinus taeda* (pine) ("PtC4H"), *Oryza sativa* (rice) ("OsC4H"), *Zea mays* (maize) ("ZmC4H"), *Sorghum bicolor* (sorghum) ("SbC4H"), *Medicago truncatula* ("MtC4H"), *Triticum aestivum* (wheat) ("TaC4H"), *Glycine max* (soybean) ("GmC4H"), *Nicotiniana tabacum* (tobacco) ("NtC4H"), ("StC4H"), *Solanum tuberosum* (potato) ("StC4H"), *Bambusa oldhamii* (bamboo) ("BoC4H"), *Brassica napus* ("BnC4H1"), *Helianthus annuus* (sunflower) ("HaC4H"), *Ricinus communis* ("RcC4H"), *Vitis vinifera* (grape) ("VvC4H"), *Euphorbia pulcherrima* (poinsettia) ("EpC4H"), *Trifolium pratense* (clover) ("TpC4H"), and *Selaginella moellendorffii* (spike moss) ("SmC4H") were aligned using ClustalW.

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[0047] **Figure 3. 4-coumarate-CoA ligase (4CL) alignment.** The protein sequences for 4CL from *Arabidopsis thaliana* ("At4CL2" and "At4CL1"), *Nicotiniana tabacum* (tobacco) ("Nt4CL1" and "Nt4CL2"), *Eucalyptus camaldulensis* ("Ec4CL", "Ec4CL1", and "Ec4CL2"), *Pinus taeda* (pine) ("Pt4CL" and "Pt4CL1"), *Glycine max* (soybean) ("Gm4CL1"), *Oryza sativa* (rice) ("Os4CL3" and "Os4CL4"), *Sorghum bicolor* (sorghum) ("Sb4CL"), *Zea mays* (maize) ("Zm4CL"), *Panicum virgatum* (switchgrass) ("Pv4CL"), *Lolium perenne* (ryegrass) ("Lp4CL3"), *Selaginella moellendorffii* (spike moss) ("Sm4CL1"), and *Physcomitrella patens* (moss) ("Pp4CL1") were aligned using ClustalW.

[0048] **Figure 4. Hydroxycinnamoyl CoA: shikimate hydroxycinnamoyl transferase (HCT) alignment.** The protein sequences for HCT from *Arabidopsis thaliana* ("AtHCT"), *Arabidopsis lyrata* ("AlHCT"), *Pinus taeda* (pine) ("PtHCT"), *Ricinus communis* ("RcHCT"), *Coffea canephora* ("CcHCT"), *Vitis vinifera* (grape) ("VvHCT"), *Nicotiniana tabacum* (tobacco) ("NtHCT"), *Trifolium pratense* (clover) ("TpHCT"), *Oryza sativa* (rice) ("OsHCT" and "OsHCT3"), *Sorghum bicolor* (sorghum) ("SbHCT"), *Zea mays* (maize) ("ZmHCT" and "ZmHCT2"), *Avena sativa* (oat) ("AsHCT"), and *Selaginella moellendorffii* (spike moss) ("SmHCT1" and "SmHCT2") were aligned using ClustalW.

[0049] **Figure 5. Coumaroyl shikimate 3-hydroxylase (C3H) alignment.** The protein sequences for C3H from *Arabidopsis thaliana* ("AtC3H"), *Eucalyptus globulus* ("EgC3H"), *Ricinus communis* ("RcC3H"), *Vitis vinifera* (grape) ("VvC3H"), *Glycine max* (soybean) ("GmC3H"), *Trifolium pratense* (clover) ("TpC3H"), *Medicago truncatula* ("MtC3H"), *Coffea canephora* ("CcC3H"), *Osimum basilicum* (basil) ("ObC3H"), *Pinus taeda* (pine) ("PtC3H"), *Nicotiniana tabacum* (tobacco) ("NtC3H"), *Ginkgo biloba* ("Gbc3H"), ("SbC3H"), *Zea mays* (maize) ("ZmC3H"), *Oryza sativa* (rice) ("OsC3H"), *Triticum aestivum* (wheat) ("TaC3H"), *Selaginella moellendorffii* (spike moss) ("SmC3H"), and *Physcomitrella patens* (moss) ("FpC3H") were aligned using ClustalW.

[0050] **Figure 6. Cinnamoyl-CoA reductase (CCR) alignment.** The protein sequences for CCR from *Arabidopsis thaliana* ("AtCCR1"), *Solanum lycopersicum* (tomato) ("SlCCR"), *Euphorbia pulcherrima* (poinsettia) ("EpCCR"), *Solanum tuberosum* (potato) ("StCCR"), *Eucalyptus gunnii* ("EgCCR"), *Vitis vinifera* (grape) ("VvCCR"), *Ricinus communis* ("RcCCR"), *Pinus taeda* (pine) ("PtCCR"), *Glycine max* (soybean) ("GmCCR"), *Picea abies* (spruce) ("PaCCR"), *Pinus massoniana* (pine) ("PmCCR"), *Oryza sativa* (rice) ("OsCCR"), *Lolium perenne* (ryegrass) ("LpCCR"), *Panicum virgatum* (switchgrass) ("PvCCR"), *Sorghum bicolor* (sorghum) ("SbCCR"), *Saccharum officinarum* (sugarcane) ("SoCCR"),

Hordeum vulgare (barley) ("HvCCR"), *Zea mays* (maize) ("ZmCCR"), and *Selaginella moellendorffii* (spike moss) ("SmCCR") were aligned using ClustalW.

[0051] **Figure 7. IRX8 sequence alignment.** Alignment of amino acid sequences of *Arabidopsis* IRX8 (GAUT12) and homologous proteins. The alignment was made with
 5 COBALT (Papadopoulos JS and Agarwala R (2007) COBALT: constraint-based alignment tool for multiple protein sequences, *Bioinformatics* 23:1073-79). Proteins are identified by their GenBank protein IDs. gi15239707: IRX8 from *Arabidopsis thaliana*; gi2241262287: homolog from *Populus trichocarpa*, gi224117396: homolog from *P. trichocarpa*, gi224141469: homolog from *P. trichocarpa*, gi224077712: homolog from *P. trichocarpa*;
 10 gi302803855: homolog from *Selaginella moellendorffii*; gi30678270: GAUT13 from *A. thaliana*; gi30685369: GAUT14 from *A. thaliana*; gi115489272: homolog from *Oryza sativa*; gi224131384: homolog from *P. trichocarpa*; gi22331857: GAUT15 from *A. thaliana*.

[0052] **Figure 8. IRX14 alignment.** Alignment of amino acid sequences of *Arabidopsis* IRX14 and homologous proteins. The alignment was made with COBALT (Papadopoulos JS
 15 and Agarwala R (2007) COBALT: constraint-based alignment tool for multiple protein sequences, *Bioinformatics* 23:1073-79). Proteins are identified by their GenBank protein IDs. gi|30690793: IRX14 from *A. thaliana*; gi|15240245: IRX14-like from *A. thaliana*; gi|224096716 and gi|224081752: homologs from *P. trichocarpa*; gi|302797519: homolog from *S. moellendorffii*; gi|115469624: homolog from *O. sativa*.

20 [0053] **Figure 9. IRX9 alignment.** Alignment of amino acid sequences of *Arabidopsis* IRX9 and homologous proteins. The alignment was made with COBALT (Papadopoulos JS and Agarwala R (2007) COBALT: constraint-based alignment tool for multiple protein sequences, *Bioinformatics* 23:1073-79). Proteins are identified by their GenBank protein IDs.
 25 gi|15228084: IRX9 from *A. thaliana*; gi|224140167 and gi|224069352: homologs from *P. trichocarpa*; gi|297600755 and gi|115461821: homologs from *O. sativa*; gi|224092304: homolog from *P. trichocarpa*; gi|302759368: Homolog from *S. moellendorffii*; gi|42571663: IRX9-like from *A. thaliana*; gi|224063335: homolog from *P. trichocarpa*; gi|115439133, gi|115474279, gi|115465403 451, gi|115481434 and gi|115456794: homologs from *O. sativa*.

[0054] **Figure 10. IRX7 alignment.** Alignment of amino acid sequences of *Arabidopsis*
 30 IRX7 (FRA8) and homologous proteins. The alignment was made with COBALT (Papadopoulos JS and Agarwala R (2007) COBALT: constraint-based alignment tool for multiple protein sequences, *Bioinformatics* 23:1073-79). Proteins are identified by their GenBank protein IDs. gi|42570324: IRX7 from *A. thaliana*; gi|224106838: homolog from *P.*

trichocarpa; gi|42568020: IRX7-like (F8H) from *A. thaliana*; gi|115450193: homolog from *O. sativa*; gi|302786830 and gi|302826405: homologs from *S. moellendorffii*.

[0055] **Figure 11. IRX10 alignment.** Alignment of amino acid sequences of Arabidopsis IRX10 and homologous proteins. The alignment was made with COBALT (Papadopoulos JS and Agarwala R (2007) COBALT: constraint-based alignment tool for multiple protein sequences, *Bioinformatics* 23:1073-79). Proteins are identified by their GenBank protein IDs. gi|18424516: IRX10-like (GUT1) from *A. thaliana*; gi|224119858: homolog from *P. trichocarpa*; gi|15223522: IRX10 (GUT2) from *A. thaliana*; gi|224053575 and gi|224075447: homologs from *P. trichocarpa*; gi|115441967: Os01g0926600 from *O. sativa*; gi|302783378: GT47D1 from *S. moellendorffii*; gi|115458146: Os04g0398600 from *O. sativa*; gi|115441965: Os01g0926400 from *O. sativa*; gi|115481310: Os10g0180000 from *O. sativa*; gi|224106838: homolog from *P. trichocarpa*.

[0056] **Figure 12. Parvus sequence alignment.** Alignment of amino acid sequences of Arabidopsis PARVUS (GATL1) and homologous proteins. The alignment was made with COBALT (Papadopoulos JS and Agarwala R (2007) COBALT: constraint-based alignment tool for multiple protein sequences, *Bioinformatics* 23:1073-79). Proteins are identified by their GenBank protein IDs. gi|18394719: PARVUS from *A. thaliana*. The other proteins are some of the homologs from *A. thaliana*, *P. trichocarpa*, and *O. sativa*, and the single homolog from *S. moellendorffii* (gi|302807664).

[0057] **Figure 13. NAC secondary wall-thickening promoting factor (NST) alignment.** The protein sequences for NST from *Arabidopsis thaliana* ("AtNST1", "AtNST2", and "SND"), *Pinus taeda* (pine) ("PtNAC023", "PtNAC065", and "PtNAC"), *Medicago truncatula* ("MtNAC1"), *Glycine max* (soybean) ("GmNAM1"), *Vitis vinifera* (grape) ("VvNST"), *Ricinus communis* ("RcNST"), *Eucalyptus gunnii* ("EgNST"), *Zea mays* (maize) ("ZmNST"), *Sorghum bicolor* (sorghum) ("SbNST"), *Oryza sativa* (rice) ("OsNAC7" and "OsNST"), *Picea sitchensis* (spruce) ("PsNST"), apple ("AppleT"), and *Selaginella moellendorffii* (spike moss) ("SmNST1") were aligned using ClustalW.

[0058] **Figure 14. Transcriptional network regulating secondary cell wall biosynthesis.** Major transcription factors regulating secondary cell wall deposition in tracheary elements and fibers are presented as well as several of the downstream target genes that are induced during secondary cell wall biosynthesis. The transcription factors presented are able to induce expression of genes involved in cellulose, hemicellulose and/or lignin biosynthesis. The drawing is adapted from Zhong *et al.*, 2007.

[0059] **Figure 15. Lignin analysis of cell wall of engineered plant lines.** A. Lignin quantification using the acetyl bromide method on senesced stems from wild-type (W) and engineered ("Eng Lig I") (*ref3-2+pVND6:C4H*) plants. B. Bright-light images of stem cross-sections stained with phloroglucinol of same-age wild-type (W) and two engineered Eng Lig I plants from left to right respectively.

[0060] **Figure 16. Analysis of the Eng Lig I line.** A. Plant growth phenotype of Eng Lig I compared at two different growth stages. The top panel represents the vegetative stage and the bottom panel represents the adult stage (bolting stage). Wild-type plants are shown on the left and the engineered Eng Lig I plants are shown on the right in A-D. B. Sugar released from dry stems pretreated with NaOH and incubated with a cellulase cocktail for 0, 24, or 48 hrs. C. Sugar released from dry stems pretreated with hot water and incubated with a cellulase cocktail for 0, 24, or 48 hrs. D. Sugar released from dry stems pretreated with dilute acid and incubated with a cellulase cocktail for 0, 24, or 48 hrs.

[0061] **Figure 17. Analysis of the Eng Lig II line.** A. Plant growth phenotype of Eng Lig II (*ref3-2+pVND6:C4H+pIRX8:NST1*) compared at two different growth stages. The top panel represents the vegetative stage and the bottom panel represents the adult stage (bolting stage). Wild-type plants are shown on the left and the engineered Eng Lig II plants are shown on the right. B. Bright-light images of stem cross-sections stained with phloroglucinol of same-age wild-type (W), *ref3-2* mutant, and the engineered Eng Lig II plants from left to right respectively. C. Lignin quantification using the acetyl bromide method on senesced stems from wild-type (W), engineered Eng Lig I, and engineered Eng Lig II plants.

[0062] **Figure 18.** Transmission electron micrographs of cross-sections through wild-type (A, C) and engineered (*ref3-2+pVND6:C4H+pIRX8:NST1*) (B, D) plants. A-B. Xylem tissues of the plants. C-D. Interfascicular tissues of the plants. "Ve," "Xf," and "If" stand for vessels, xylery fibers, and interfascicular fibers, respectively.

[0063] **Figure 19. Saccharification efficiency of the Eng Lig I and Eng Lig II lines.** A. Sugar released from dry stems pretreated with hot water and incubated with a cellulase cocktail for 0 to 144 hrs. Stems are from wild-type (wt; blue) plant, engineered Eng Lig I (orange) plants, or Eng Lig II (red) plants. B. Sugar released from dry stems pretreated with NaOH and incubated with a cellulase cocktail for 0 to 144 hrs. Stems are from wild-type (wt; blue) plant, engineered Eng Lig I (orange) plants, or Eng Lig II (red) plants.

[0064] **Figure 20. Promoter activity characterization.** A. Bright-field image of stem cross-section from the base of 5-10 cm stems from wild-type (WT), *cadc/d* mutant, *cadc/d*

mutant transformed with pVND6:CADc, and *cadc/d* mutant transformed with pC4H:CADc, from left to right respectively. The redness is generated by the lack of CAD activity. B. Bright-field image from Maule stained stem cross-section from the base of 5-10 cm stems from wild-type (WT), *f5h* mutant, *f5h* mutant transformed with pVND6:F5H, and *f5h* mutant transformed with pC4H:F5H, from left to right respectively. The redness is generated by the presence of Sinapyl alcohol and is representative of the amount of Sinapyl alcohol in the lignin that reacts during the Maule staining reaction. The production of Sinapyl alcohol is restored in the *f5h* mutant by the expression of the native F5H gene.

[0065] Figure 21. Xylem collapse. A. Same-age adult *ref3-2* mutant (homozygote *c4h* mutant) and wild-type plants (wt) (right and left, respectively). B. Same vegetative age *ref3-2* mutant (homozygote *c4h* mutant) and wild-type plants (right and left, respectively). C. Top and bottom panels represent a bright-field image of phloroglucinol-stained stem cross-sections, magnified 20 and 40X fold respectively, from wild-type and *ref3-2* (left and right respectively) sampled at the same age as presented on A. The yellow arrows point to some collapse vessels in the *ref3-2* mutant.

[0066] Figure 22. Expression analysis of NST1. NST1 expression was analyzed by semi-quantitative RT-PCR. pIRX8:NST1: specific NST1 primers were used to verify the expression of NST1 driven by pIRX8 promoter. NST1: specific NST1 primers were used to verify the expression of both NST1 genes each driven by pIRX8 and pNST1 promoters. pVND6:C4H: specific C4H primers were used to verify the expression of the C4H genes driven by pVND6. C4H: specific C4H primers were used to verify the expression of the C4H genes driven by pVND6 or pC4H promoters (wild-type and *ref3-2* mutant alleles). Tubulin: specific tubulin primers was used to verify the quality and quantity of the RNA used for the RT-PCR. Lanes 1 to 4 show independent Eng Lig II (*ref3-2*+pVND6:C4H+pIRX8:NST1) plants; lane 4 shows a wild-type plant; lanes 5 and 6 show independent Eng Lig I (*ref3-2*+pVND6:C4H) plants; and lane 7 shows a *ref3-2* mutant plant.

[0067] Figure 23. Cell wall thickness. A-D. Cell wall thickness and cell diameters were measured on 20 independent fiber cells from the intrafascicular regions in Col0 (WT) (A), *ref3-2* (*c4h* mutant) (B), Eng Lig I (C), and Eng Lig II (D) plants. Cell wall ratio was measured by the sum of the cell wall thickness (μm) divided by the cell diameter (μm). E. Cell wall thickness and cell diameter measurement method. The green bar (a) and yellow bar (b) each represent cell wall thickness measurements and the pink bar represents the cell diameter. Cell wall ratio was measured by the sum of the cell wall thickness (μm) divided by the cell diameter (μm), $(a+b)/\text{cell diameter}$.

[0068] Figure 24. Sugar release from cell wall after chemical hydrolysis. A-B.

Hemicellulose composition after TFA hydrolysis. A. Quantification (mg of sugar/mg dry cell wall) of the major sugar released). B. Percentage of each sugar in the total released. C. Total sugar released after H₂SO₄ hydrolysis.

- 5 **[0069] Figure 25. Alignment of SHN protein sequences.** The protein sequences for SHN polypeptides from *Arabidopsis thaliana* ("At"), *Populus trichocarpa* ("Pt"), *Medicago truncatula* ("Mt"), *Oryza sativa* ("Os"), *Brachypodium distachyon* ("Bd"), *Zea mays* ("Zm"), *Sorghum bicolor* ("Sb"), *Hordeum vulgare* ("Hv"), *Picea sitchensis* ("Ps"), *Selaginella moellendorffi* ("Sm"), and *Physcomitrella patens* ("Pp) were aligned using ClustalW.
- 10 **[0070] Figure 26. Alignment of Myb96 protein sequences.** The protein sequences for Myb96 polypeptides from *Arabidopsis thaliana* ("At"), *Thellungiella halophila* ("Th), *Medicago truncatula* ("Mt"), *Populus trichocarpa* ("Pt"), *Vitis vinifera* ("Vv"), *Citrus macrophylla* ("Cm"), *Brachypodium distachyon* ("Bd"), *Triticum aestivum* ("Ta"), *Oryza sativa* ("Os"), and *Zea Mays* ("Zm") were aligned using ClustalW.
- 15 **[0071] Figure 27. Representation of cell wall artificial positive feed back loop.** Figure 27 depicts an illustrative cell wall densification strategy.
- [0072] Figure 28. Induction of wax biosynthetic pathways in target tissues.** Figure 28 depicts an illustrative artificial positive feed back loop to induce a wax biosynthetic pathway in target tissues.
- 20 **[0073] Figure 29. Plant growth phenotype of engineered cell wall plant lines.** Growth comparison of wildtype, *c4h* mutant plants and engineered plant lines in which the *ref3-2* mutation is complemented with either pREF4::C4H (A) or pRFR1::C4H (B) DNA construct.
- [0074] Figure 30. Lignin distribution and content of engineered cell wall plant lines.** Lignin distribution is shown in the upper panel. Lignin quantification is shown in the lower panel.
- 25 **[0075] Figure 31. Saccharificaton efficiency of lignin engineered plant lines.** Panels A and B show sugar released form dry stems using hot-water (Panel A) or alkali (Panel B) pretreatment follow by incubation was a cellulase cocktail. Panel C provides a summary of the saccharification results.
- 30 **[0076] Figure 32. Cell wall densification feed back loop.** Panel A illustrates cell wall densification in *Arabidopsis* wildtype plants containing a DNA construct pCesA4::NST1. Panel B shows cell wall densification in *Brachypodium* wildtype plants using

pAtIRX8::AtNST1 DNA construct where the promoter and transcription factor are both from *Arabidopsis*.

[0077] **Figure 33. Examples of xylan engineering.** Comparison of growth in wildtype, mutant, and mutant plants complemented with the wildtype version of the mutated *IRX7*,
5 *IRX8*, or *IRX9* gene drive by *pVND6* or *pVND7*.

[0078] **Figure 34. Growth of offspring of transformants.** Growth of offspring of four individual transformants made by transforming *irx7* mutant with a *pVND7::IRX7* expression construct.

[0079] **Figure 35. Growth of offspring of transformants.** Growth of offspring of two
10 individual transformants made by transforming *irx9* mutant with a *pVND7::IRX9* expression construct.

[0080] **Figure 36 Non-cellulosic monosaccharide composition prepared from transformants.** Non-cellulosic monosaccharide composition of cell walls prepared from four individual transformants made by transforming *irx7* mutant with a *pVND7::IRX7*
15 expression construct.

[0081] **Figure 37 Non-cellulosic monosaccharide composition prepared from transformants.** Non-cellulosic monosaccharide composition of cell walls prepared from four individual transformants made by transforming *irx8* mutant with a *pVND6::IRX8* expression construct.

20 [0082] **Figure 38. Noncellulosic monosaccharide composition of stem cell walls prepared from individual transformants.** Non-cellulosic monosaccharide composition of stem cell wall prepared from offspring of four individual transformants made by transforming *irx9* mutant with a *pVND7::IRX9* expression construct.

[0083] **Figure 39. Saccharification analysis of cells walls.** Saccharification analysis of
25 cell walls prepared from offspring of two individual transformants made by transforming *irx9* mutant with a *pVND6::IRX9* expression construct.

[0084] **Figure 40. Wax deposition in plants transformed to create an artificial positive feedback loop.** Visual analysis of the *Arabidopsis* plant transformed with the different constructs showed increased shininess of the leaves compared with control plants.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

[0085] As used herein, the term "lignin biosynthesis enzyme" refers to a protein that regulates the synthesis of lignin monomers (p-coumaryl (4-hydroxycinnamyl) alcohol, coniferyl (3-methoxy 4-hydroxycinnamyl) alcohol, and sinapyl (3,5-dimethoxy 4-hydroxycinnamyl) alcohol) in plants. The term includes polymorphic variants, alleles, mutants, and interspecies homologs to the specific enzymes described herein. A nucleic acid that encodes a lignin biosynthesis enzyme refers to a gene, pre-mRNA, mRNA, and the like, including nucleic acids encoding polymorphic variants, alleles, mutants, and interspecies homologs of the particular sequences described herein. Thus, in some embodiments a lignin biosynthesis nucleic acid (1) has a nucleic acid sequence that has greater than about 50% nucleotide sequence identity, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, preferably 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or higher nucleotide sequence identity, preferably over a region of at least about 10, 15, 20, 25, 50, 100, 200, 500 or more nucleotides or over the length of the entire polynucleotide, to a nucleic acid sequence of any of SEQ ID NOs:1, 3, 5, 7, 9, or 11; or (2) encodes a polypeptide having an amino acid sequence that has greater than about 50% amino acid sequence identity, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, preferably 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or greater amino acid sequence identity, preferably over a region of at least about 25, 50, 100, 200 or more amino acids or over the length of the entire polypeptide, to a polypeptide encoded by a nucleic acid sequence of any of SEQ ID NOs:1, 3, 5, 7, 9, or 11 or to an amino acid sequence of any of SEQ ID NOs:2, 4, 6, 8, 10, or 12 or to any one of the sequences shown in any of Figures 1-6. In some embodiments, a lignin biosynthesis enzyme, or a lignin biosynthesis polypeptide has an amino acid sequence having greater than about 50% amino acid sequence identity, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, preferably 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or greater amino acid sequence identity, preferably over a region of at least about 25, 50, 100, 200 or more amino acids or over the length of the entire polypeptide, to an amino acid sequence of any of SEQ ID NOs:2, 4, 6, 8, 10, or 12 or to any one of the amino acid sequences shown in any of Figures 1-6.

[0086] Lignin biosynthesis enzymes can be identified by name (*e.g.*, cinnamate 4-hydroxylase); gene symbol (*e.g.*, C4H); or accession number (*e.g.*, NM_128601 for nucleic acid or NP_180607 for protein). It is understood that all of these identifiers reference the same biomarker and thus are equivalent. In some embodiments, the lignin biosynthesis enzyme is phenylalanine ammonia lyase (PAL) (accession number NM_129260 or

NP_181241), cinnamate 4-hydroxylase (C4H) (accession number NM_128601 or NP_180607), 4-coumarate-CoA ligase (4CL) (accession number NM_113019 or NP_188761), hydroxycinnamoyl CoA:shikimate hydroxycinnamoyl transferase (HCT) (accession number NM_124270 or NP_199704), coumaryol shikimate 3-hydroxylase (C3H) (accession number NM_119566 or NP_850337), or cinnamoyl-CoA reductase 1 (CCR1) (accession number NM_101463 or NP_173047).

[0087] As used herein, the term "xylan biosynthesis enzyme" refers an enzyme that is involved in xylan synthesis. The term as used herein can also relate to an enzyme that modifies xylan, *e.g.*, enzymes that acetylate xylan. The term encompasses polymorphic variants, alleles, mutants, and interspecies homologs to the specific polypeptides described herein. A nucleic acid that encodes a xylan biosynthesis enzyme refers to a gene, pre-mRNA, mRNA, and the like, including nucleic acids encoding polymorphic variants, alleles, mutants, and interspecies homologs of the particular amino acid sequences described herein. Thus, in some embodiments, a xylan biosynthesis enzyme encodes a polypeptide having an amino acid sequence that has greater than about 50% amino acid sequence identity, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, preferably 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or greater amino acid sequence identity, preferably over a region of at least about 25, 50, 100, 200 or more amino acids or over the length of the entire polypeptide, to any one of the amino acid sequences shown in any of Figures 7-12. Nucleic acid sequence of examples of xylan biosynthesis enzymes are available under the accession numbers provided in Figures 7-12. In some embodiments, a xylan biosynthesis enzyme has an amino acid sequence having greater than about 50% amino acid sequence identity, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, preferably 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or greater amino acid sequence identity, preferably over a region of at least about 25, 50, 100, 200 or more amino acids or over the length of the entire polypeptide, to any one of the amino acid sequences shown in any of Figures 7-12. In some embodiments, the xylan biosynthesis enzyme is irregular xylem 8 (IRX8), IRX14, IRX14-like, IRX9, IRX9-like, IRX7, IRX10, IRX10-like, F8H, PARVUS, or RWA1, RWA2, RWA3, or RWA4.

[0088] The term "substantially localized," when used in the context of describing a plant having lignin deposition and/or xylan deposition that is substantially localized to a particular tissue, refers to lignin deposition and/or xylan deposition that is produced in substantially higher amounts in the particular cell type of interest as compared to other cell types that normally have a high content of lignin and/or xylan, such as interfascicular fibers or phloem fibers. In some embodiments, lignin deposition and/or xylan deposition is substantially

localized to a particular cell type of interest when the amount of lignin deposition and/or xylan deposition in the particular cell type of interest is at least 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold higher or more as compared to the amount of lignin deposition and/or xylan deposition in other cell types that normally have a high content of

5 lignin and/or xylan. In some embodiments, lignin deposition and/or xylan deposition is substantially localized to a particular cell type of interest when the amount of lignin deposition and/or xylan deposition in the particular cell type of interest is at least 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold higher or more as compared to the amount of lignin deposition and/or xylan deposition in interfascicular fibers or phloem fibers.

10 In some embodiments, lignin deposition and/or xylan deposition is substantially localized to a particular cell type of interest when there is no detectable lignin deposition and/or xylan deposition in cell types other than the particular cell type of interest. In some embodiments, xylan *O*-acetylation is similarly substantially localized to specific cell types, while the content of xylan in general is not necessarily substantially localized in a way different from

15 the natural (*i.e.*, wild-type) situation. Lignin deposition and/or xylan deposition can be assessed using any method known in the art, including but not limited to spectrophotometry using acetyl-bromide reagent, histochemical staining (*e.g.*, with phloroglucinol), and immunohistochemistry (*e.g.*, with LM10 monoclonal antibody). Xylan *O*-acetylation can be assessed using immunohistochemistry (*e.g.*, with LM23 monoclonal antibody), with

20 biochemical assays for acetyl esters, or by determining the effect of hydrolytic enzymes.

[0089] As used herein, the term “transcription factor that regulates the production of components of a biosynthetic pathway” or “master transcription factor” refers to a transcription factor that regulates expression of one or of multiple genes in a biosynthetic pathway.

25 [0090] As used herein, the term “transcription factor that regulates the production of secondary cell wall” refers to a polypeptide, and variants, mutants, and homologs of the polypeptide, that regulates the expression of one or more genes involved in lignin biosynthesis and/or polysaccharide (cellulose and hemicellulose) biosynthesis by modulating transcription. In some embodiments, nucleic acids that encodes such a transcription factor:

30 (1) have a nucleic acid sequence that has greater than about 50% nucleotide sequence identity, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, preferably 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or higher nucleotide sequence identity, preferably over a region of at least about 10, 15, 20, 25, 50, 100, 200, 500 or more nucleotides or over the length of the entire polynucleotide, to a nucleic acid sequence of any of SEQ ID NOs:13, 15,

17, 19, 21, 23, 25, 27, 29, 31, or 33; (2) encode a polypeptide having an amino acid sequence that has greater than about 50% amino acid sequence identity, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, preferably 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or greater amino acid sequence identity, preferably over a region of at least about 25, 50, 100, 200 or
5 more amino acids or over the length of the entire polypeptide, to a polypeptide encoded by a nucleic acid sequence of any of SEQ ID NOs:13, 15, 17, 19, 21, 23, 25, 27, 29, 31, or 33 or an amino acid sequence of any of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34 or to any one of the amino acid sequences shown in Figure 13. In some embodiments, a transcription factor polypeptide that regulates the production of secondary cell wall: (1) has
10 an amino acid sequence having greater than about 50% amino acid sequence identity, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, preferably 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or greater amino acid sequence identity, preferably over a region of at least about 25, 50, 100, 200 or more amino acids or over the length of the entire polypeptide, to an amino acid sequence of any of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34 or to
15 any one of the amino acid sequences shown in Figure 13.

[0091] In some embodiments, the transcription factor is NAC secondary wall-thickening promoting factor 1 (NST1) (ANAC043; accession number NM_130243 or NP_182200), NST2 (ANAC066; accession number NM_116056 or NP_191750), NST3 (SND1/ANAC012; accession number NM_103011 or NP_174554), secondary wall-associated NAC domain protein 2 (SND2) (ANAC073; accession number NM_118992 or NP_194579), SND3 (ANAC010; accession number NM_102615 or NP_564309), MYB domain protein 103 (MYB103) (accession number NM_105065 or NP_176575), MYB85 (accession number NM_118394 or NP_567664), MYB46 (accession number NM_121290 or NP_196791), MYB83 (accession number NM_111685 or NP_187463), MYB58 (accession
20 number NM_101514 or NP_173098), or MYB63 (accession number NM_106569 or NP_178039).

[0092] The term "downstream target," when used in the context of a downstream target of a transcription factor that regulates a component of a biosynthetic pathway of interest refers to a gene or protein whose expression is directly or indirectly regulated by the transcription
30 factor. In some embodiments, the downstream target is a gene or protein that is directly or indirectly upregulated by the transcription factor. In some embodiments, the downstream target is a gene or protein that is directly or indirectly downregulated by the transcription factor.

[0093] In the context of secondary wall production, a downstream target can be, for example, IRX1, IRX3, IRX5, IRX8, IRX9, IRX14, IRX14-L, IRX7, or IRX10. See, for example, Figures 7-12 for examples of accession numbers and sequences for downstream targets. Downstream target genes are also described in the art; *see*, for example, Oikawa et al., 2010, PLoS ONE 5(11):e15481. As understood in the art, and further explained hereinbelow, some of the downstream targets (*e.g.*, IRX9-Like and RWA2) may not be expressed in secondary wall tissue *per se*, but can be linked to a secondary wall-specific promoter or a vessel-specific promoter that is regulated by a transcription factor that regulates secondary wall production and can then serve to substantially localize xylan or xylan acetylation to the secondary wall.

[0094] As used herein, the term transcription factor that regulates the production "wax and/or cutin" components (*e.g.*, wax ester, alkane, fatty alcohol and fatty esters) refers to a polypeptide, and variants, mutants, and homologs of the polypeptide, that regulates the expression of one or more genes involved in wax and/or cutin biosynthesis by modulating transcription. In some embodiments, nucleic acids that encodes such a transcription factor: encode a polypeptide having an amino acid sequence that has greater than about 50% amino acid sequence identity, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, preferably 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% or greater amino acid sequence identity, preferably over a region of at least about 25, 50, 100, 200 or more amino acids or over the length of the entire polypeptide, to a polypeptide encoded by a nucleic acid sequence of any one of SEQ ID NOs:80-93, or an amino acid sequence of any of any one of SEQ ID NOs:80-93.

[0095] When used in the context of a transcription factor that regulates wax/cutin production, "downstream target" refers to a non-coding RNA, gene, or protein involved in wax/cutin production whose expression is directly or indirectly regulated by the transcription factor. In some embodiments, the downstream target is a non-coding RNA, gene, or protein that is directly or indirectly upregulated by the transcription factor. In some embodiments, the downstream target is a non-coding RNA, gene, or protein that is directly or indirectly downregulated by the transcription factor. Examples of such genes include the following (synonyms for the gene are listed in parenthesis): CER1, aldehyde decarbonylase; CER2 (VC2), BAHD-type acyl-transferase; CER3 (WAX2), sterol desaturase; CER4 (FAR3), fatty acyl-CoA reductase; CER5 (WBC12), ABC transporter; CER6 (CUT1), very long chain fatty acid condensing enzyme; CER10 (ECR), enoyl-CoA reductase; WSD1, wax ester synthase; MAH1, mid-chain alkane hydrolase; WBC11 (ABCG11, DSO, COF1), ABC transporter; KCS1, very long chain fatty acid condensing enzyme; KCS2 (DAISY), very long chain fatty

acid condensing enzyme; FATB, acyl carrier; LACS1, long chain acyl-CoA synthase; LACS2, long chain acyl-CoA synthase; CYP86A4, cytochrome P450-dependent fatty acid hydroxylase; CYP86A7, cytochrome P450-dependent fatty acid hydroxylase; LCR (CYP86A5), cytochrome P450-dependent fatty acid hydroxylase; KCS10 (FDH), very long chain fatty acid condensing enzyme; and CER60 (KCS5), very long chain fatty acid condensing enzyme. Examples of accession numbers are provided in the Listing of Illustrative Wax/Cutin genes.

[0096] The terms "reduced level of activity," "reduced activity" and "decreased activity" refer interchangeably to a reduction in the amount of activity of a protein, *e.g.*, a cell wall

10 biosynthesis enzyme of interest or a xylan biosynthesis enzyme gene or protein of interest in an engineered plant as compared to the amount of activity in a wild-type (*i.e.*, naturally occurring) plant. In some embodiments, reduced activity results from reduces expression levels. A reduced level of activity or a reduces level of expression can be a reduction in the amount of activity or expression of a protein, *e.g.*, a cell wall biosynthesis enzyme gene or

15 protein or a xylan biosynthesis enzyme gene or protein, of at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, or 90% or greater. In some embodiments, the reduced level of activity or reduced level of expression is a reduction in the amount of activity or expression of the enzyme, *e.g.*, a cell wall biosynthesis enzyme gene or protein of interest or a xylan

20 biosynthesis enzyme gene or protein of interest, throughout all the tissues of the engineered plant. In some embodiments, the reduction in the amount of activity or expression of the protein or gene, *e.g.*, a cell wall biosynthesis enzyme gene or protein of interest or a xylan

25 biosynthesis enzyme gene or protein of interest, is localized to one or more tissues of the engineered plant. In some embodiments, the biosynthetic enzyme is not reduced in amount but is modified in amino acid sequence so that the enzymatic activity is reduced directly or

indirectly (*e.g.*, expression of inhibitory protein). Reduction in the amount of expression of a gene or protein can be assessed by measuring decreases in the level of RNA encoded by the gene of interest and/or decreases in the level of protein expression or activity for the protein of interest.

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[0097] The terms "polynucleotide" and "nucleic acid" are used interchangeably and refer to a single or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. A nucleic acid of the present invention will generally contain phosphodiester bonds, although in some cases, nucleic acid analogs may be used that may have alternate backbones, comprising, *e.g.*, phosphoramidate, phosphorothioate, phosphorodithioate, or O-methylphosphoroamidite linkages (see Eckstein, Oligonucleotides

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and Analogues: A Practical Approach, Oxford University Press); positive backbones; non-ionic backbones, and non-ribose backbones. Thus, nucleic acids or polynucleotides may also include modified nucleotides that permit correct read-through by a polymerase.

"Polynucleotide sequence" or "nucleic acid sequence" includes both the sense and antisense strands of a nucleic acid as either individual single strands or in a duplex. As will be appreciated by those in the art, the depiction of a single strand also defines the sequence of the complementary strand; thus the sequences described herein also provide the complement of the sequence. Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses variants thereof (*e.g.*, degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. The nucleic acid may be DNA, both genomic and cDNA, RNA or a hybrid, where the nucleic acid may contain combinations of deoxyribo- and ribo-nucleotides, and combinations of bases, including uracil, adenine, thymine, cytosine, guanine, inosine, xanthine hypoxanthine, isocytosine, isoguanine, etc.

[0098] The term "substantially identical," used in the context of two nucleic acids or polypeptides, refers to a sequence that has at least 50% sequence identity with a reference sequence. Percent identity can be any integer from 50% to 100%. Some embodiments include at least: 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, compared to a reference sequence using the programs described herein; preferably BLAST using standard parameters, as described below. For example, a polynucleotide encoding a lignin biosynthesis enzyme may have a sequence that is at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to a sequence of SEQ ID NO:1, SEQ ID NO:3, SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, or SEQ ID NO:11.

[0099] Two nucleic acid sequences or polypeptide sequences are said to be "identical" if the sequence of nucleotides or amino acid residues, respectively, in the two sequences is the same when aligned for maximum correspondence as described below. The terms "identical" or percent "identity," in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence over a comparison window, as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. When percentage of sequence identity is used in reference to proteins or peptides, it is recognized that residue positions that are not identical often differ by conservative amino acid

substitutions, where amino acids residues are substituted for other amino acid residues with similar chemical properties (*e.g.*, charge or hydrophobicity) and therefore do not change the functional properties of the molecule. Where sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated according to, *e.g.*, the algorithm of Meyers & Miller, *Computer Applic. Biol. Sci.* 4:11-17 (1988) *e.g.*, as implemented in the program PC/GENE (Intelligenetics, Mountain View, California, USA).

[0100] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0101] A "comparison window," as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well-known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by manual alignment and visual inspection.

[0102] Algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.* (1990) *J. Mol. Biol.* 215: 403-410 and Altschul *et al.* (1977) *Nucleic Acids Res.* 25: 3389-

3402, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (NCBI) web site. The algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold (Altschul *et al, supra*). These initial neighborhood word hits acts as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity X from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a word size (W) of 28, an expectation (E) of 10, M=1, N=-2, and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a word size (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (*see Henikoff & Henikoff, Proc. Natl. Acad. Sci. USA 89:10915 (1989)*).

[0103] The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (*see, e.g., Karlin & Altschul, Proc. Nat'l. Acad. Sci. USA 90:5873-5787 (1993)*). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.01, more preferably less than about 10^{-5} , and most preferably less than about 10^{-20} .

[0104] Nucleic acid or protein sequences that are substantially identical to a reference sequence include "conservatively modified variants." With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially identical sequences. Because of the

degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded

5 polypeptide. Such nucleic acid variations are "silent variations," which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine) can be modified to yield a functionally identical molecule.

10 Accordingly, each silent variation of a nucleic acid which encodes a polypeptide is implicit in each described sequence.

[0105] As to amino acid sequences, one of skill will recognize that individual substitutions, in a nucleic acid, peptide, polypeptide, or protein sequence which alters a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" where the alteration results in the substitution of an amino acid with a chemically

15 similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art.

[0106] The following six groups each contain amino acids that are conservative substitutions for one another:

- 20 1) Alanine (A), Serine (S), Threonine (T);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
25 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).
(see, e.g., Creighton, *Proteins* (1984)).

[0107] Another indication that nucleotide sequences are substantially identical is if two molecules hybridize to each other, or a third nucleic acid, under stringent conditions. Stringent conditions are sequence dependent and will be different in different circumstances.

30 Generally, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Typically, stringent conditions will be those in which the salt concentration is about 0.02 molar at pH 7 and the temperature is at least about

60°C. For example, stringent conditions for hybridization, such as RNA-DNA hybridizations in a blotting technique are those which include at least one wash in 0.2X SSC at 55°C for 20 minutes, or equivalent conditions.

5 [0108] The term "promoter," as used herein, refers to a polynucleotide sequence capable of driving transcription of a DNA sequence in a cell. Thus, promoters used in the polynucleotide constructs of the invention include cis- and trans- acting transcriptional control elements and regulatory sequences that are involved in regulating or modulating the timing and/or rate of transcription of a gene. For example, a promoter can be a cis- acting transcriptional control element, including an enhancer, a promoter, a transcription terminator, 10 an origin of replication, a chromosomal integration sequence, 5' and 3' untranslated regions, or an intronic sequence, which are involved in transcriptional regulation. These cis-acting sequences typically interact with proteins or other biomolecules to carry out (turn on/off, regulate, modulate, *etc.*) gene transcription. Promoters are located 5' to the transcribed gene, and as used herein, include the sequence 5' from the translation start codon (i.e., including the 15 5' untranslated region of the mRNA, typically comprising 100-200 bp). Most often the core promoter sequences lie within 1-2 kb of the translation start site, more often within 1 kbp and often within 500 bp of the translation start site. By convention, the promoter sequence is usually provided as the sequence on the coding strand of the gene it controls. In the context of this application, a promoter is typically referred to by the name of the gene for which it 20 naturally regulates expression. A promoter used in an expression construct of the invention is referred to by the name of the gene. Reference to a promoter by name includes a wildtype, native promoter as well as variants of the promoter that retain the ability to induce expression. Reference to a promoter by name is not restricted to a particular plants species, but also encompasses a promoter from a corresponding gene in other plant species.

25 [0109] A "constitutive promoter" in the context of this invention refers to a promoter that is capable of initiating transcription in nearly all cell types, whereas a "cell type-specific promoter" or "tissue-specific promoter" initiates transcription only in one or a few particular cell types or groups of cells forming a tissue. In some embodiments, a promoter is tissue - specific if the transcription levels initiated by the promoter in a particular cell-type or tissue 30 are at least 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 50-fold, 100-fold, 500-fold, 1000-fold higher or more as compared to the transcription levels initiated by the promoter in non-vessel tissues. In some embodiments, the promoter is vessel-specific. As used herein, a "vessel-specific" promoter refers to a promoter that initiates substantially higher levels of transcription in vessels as compared to other non-vessel cells of the plant. As

used herein, the term "vessel" refers to xylem vessels, a conductive component of the vascular tissues in plants that function in the transport of water, nutrients, and signaling molecules throughout the plant. In some embodiments, a promoter is vessel-specific if the transcription levels initiated by the promoter in vessel tissues are at least 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 50-fold, 100-fold, 500-fold, 1000-fold higher or more as compared to the transcription levels initiated by the promoter in non-vessel tissues. Non-limiting examples of vessel-specific promoters include the native promoter of any of the genes encoding Vascular-Related NAC-Domain Protein 1 (VND1), VND2, VND3, VND4, VND5, VND6, VND7. *See, e.g., Kubo et al., Genes Dev. 19:1855-1860 (2005),* which is incorporated by reference herein. Another example of a vessel-specific promoter includes the native promoter of REF4 and RFR1 (*see, e.g., Bonawitz et al., "The REF4 and RFR1 subunits of the eukaryotic transcriptional coregulatory complex Mediator are required for phenylpropanoid homeostasis in Arabidopsis."* doi:10.1074/jbc.M111.312298 (2012)).

[0110] In the context of an artificial positive feedback loop, an "induced" promoter from a downstream gene in a biosynthetic pathway of interest refers to a promoter where expression of the gene is enhanced, *i.e.*, expression may be directly or indirectly activated (turned on and/or increased) by the transcription factor employed in the artificial positive feedback loop. Thus, in when referring to a promoter employed in an artificial feedback loop construct, it is understood that the promoter is "induced" by the transcription factor regardless of whether it is explicitly stated that the promoter is an induced promoter.

[0111] A polynucleotide is "heterologous" to an organism or a second polynucleotide sequence if it originates from a foreign species, or, if from the same species, is modified from its original form. For example, when a polynucleotide encoding a polypeptide sequence is said to be operably linked to a heterologous promoter, it means that the polynucleotide coding sequence encoding the polypeptide is derived from one species whereas the promoter sequence is derived from another, different species; or, if both are derived from the same species, the coding sequence is not naturally associated with the promoter (*e.g.*, is a genetically engineered coding sequence, *e.g.*, from a different gene in the same species, or an allele from a different ecotype or variety).

[0112] The term "operably linked" refers to a functional relationship between two or more polynucleotide (*e.g.*, DNA) segments. Typically, it refers to the functional relationship of a transcriptional regulatory sequence to a transcribed sequence. For example, a promoter or enhancer sequence is operably linked to a DNA or RNA sequence if it stimulates or modulates the transcription of the DNA or RNA sequence in an appropriate host cell or other

expression system. Generally, promoter transcriptional regulatory sequences that are operably linked to a transcribed sequence are physically contiguous to the transcribed sequence, i.e., they are cis-acting. However, some transcriptional regulatory sequences, such as enhancers, need not be physically contiguous or located in close proximity to the coding sequences whose transcription they enhance.

[0113] The term "expression cassette" or "DNA construct" or "expression construct" refers to a nucleic acid construct that, when introduced into a host cell, results in transcription and/or translation of an RNA or polypeptide, respectively. Antisense or sense constructs that are not or cannot be translated are expressly included by this definition. In the case of both expression of transgenes and suppression of endogenous genes (*e.g.*, by antisense, RNAi, or sense suppression) one of skill will recognize that the inserted polynucleotide sequence need not be identical, but may be only substantially identical to a sequence of the gene from which it was derived. As explained herein, these substantially identical variants are specifically covered by reference to a specific nucleic acid sequence. One example of an expression cassette is a polynucleotide construct that comprises a transcription factor operably linked to a heterologous promoter that is a promoter from a gene that is regulated by the transcription factor.

[0114] The term "plant" as used herein can refer to a whole plant or part of a plant, *e.g.*, seeds, and includes plants of a variety of ploidy levels, including aneuploid, polyploid, diploid and haploid. The term "plant part," as used herein, refers to shoot vegetative organs and/or structures (*e.g.*, leaves, stems and tubers), branches, roots, flowers and floral organs (*e.g.*, bracts, sepals, petals, stamens, carpels, anthers), ovules (including egg and central cells), seed (including zygote, embryo, endosperm, and seed coat), fruit (*e.g.*, the mature ovary), seedlings, and plant tissue (*e.g.*, vascular tissue, ground tissue, and the like), as well as individual plant cells, groups of plant cells (*e.g.*, cultured plant cells), protoplasts, plant extracts, and seeds. The class of plants that can be used in the methods of the invention is generally as broad as the class of higher and lower plants amenable to transformation techniques, including angiosperms (monocotyledonous and dicotyledonous plants), gymnosperms, ferns, bryophytes, and multicellular algae.

[0115] The term "biomass," as used herein, refers to plant material that is processed to provide a product, *e.g.*, a biofuel such as ethanol, or livestock feed, or a cellulose for paper and pulp industry products. Such plant material can include whole plants, or parts of plants, *e.g.*, stems, leaves, branches, shoots, roots, tubers, and the like.

[0116] The term "increased secondary cell wall deposition" refers to an increased amount of secondary cell wall that is produced in an engineered plant of the present invention as compared to a wild-type (*i.e.*, naturally occurring) plant, e.g., an increased density or thickness and/or an increased ratio between the cell diameter and cell wall thicknesses.

5 "Secondary cell wall" is mainly composed of cellulose, hemicellulose, and lignin and is deposited in some, but not all, tissues of a plant, such woody tissue. Secondary cell wall deposition is said to be increased in an engineered plant as compared to a wild-type plant when the amount of one or more components of secondary cell wall (*e.g.*, cellulose, hemicellulose, or lignin) in the engineered plant, or the ratio between the cell diameter and
10 cell wall thickness, is increased by at least 10%, at least 20, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more relative to the amount of the one or more components of secondary cell wall in a wild-type plant. The amount of a component of secondary cell wall that is present can be assessed using any method known in the art, including but not limited to microscopy (*e.g.*, electron-microscopy, RAMAN-microscopy), histochemical staining (*e.g.*,
15 phloroglucinol) and enzymatic or chemical reaction (*e.g.*, polysaccharide hydrolysis or TFA hydrolysis).

[0117] The term "saccharification reaction" refers to a process of converting biomass, usually cellulosic or lignocellulosic biomass, into monomeric sugars, such as glucose and xylose.

20 [0118] The term "soluble sugar" refers to monomeric, dimeric, or trimeric sugar that is produced from the saccharification of biomass.

[0119] The term "increased amount," when referring to an amount of sugar or soluble sugar obtained from an engineered plant of the present invention, refers to an increase in the amount or yield of sugar that is obtained from saccharification of biomass per amount of
25 starting material, in comparison to corresponding biomass from a wild-type (*i.e.*, naturally occurring) plant. In the context of the present invention, "corresponding biomass from a wild-type plant" refers to plant material that is from the same part of the plant as the biomass from a plant having a reduced level of expression of a lignin biosynthesis enzyme and/or xylan biosynthesis enzyme. As understood in the art, increased amount or increased yield is based
30 upon comparisons of the same amount of corresponding plant material.

[0120] The term "conversion reaction," as used herein, refers to a reaction that converts biomass into a form of bioenergy. Examples of conversion reactions include, but are not

limited to, combustion (burning), gasification, pyrolysis, and polysaccharide hydrolysis (enzymatic or chemical).

[0121] The term "increased production," when referring to an amount of bioenergy production obtained from an engineered plant of the present invention, refers to an increased amount of bioenergy that is produced from subjecting biomass from an engineered plant to a conversion reaction (*e.g.*, combustion, gasification, pyrolysis, or polysaccharide hydrolysis) as compared to the amount of bioenergy that is produced from corresponding biomass from a wild-type (*i.e.*, naturally occurring) plant.

II. Introduction

[0122] In one aspect, the present invention relates to the discovery that an artificial positive feedback loop (APFL) can be created in plants to regulate gene expression in desired biosynthetic pathways, for example, to modulate gene expression in one or more desired tissues. Accordingly, the invention provides an APFL in plants wherein the APFL comprises a gene encoding a transcription factor that controls expression of a biosynthetic pathway of interest operably linked to a promoter from an induced downstream gene in the biosynthetic pathway where the expression of the downstream gene is controlled by the transcription factor. Examples of biosynthetic pathways that can be regulated by such a system include secondary cell wall deposition, wax/cutin biosynthesis, lipid biosynthesis, alkaloid biosynthesis and terpenoid biosynthesis. Thus, one example of an APFL in accordance with the invention relates to increasing cell wall deposition in specific tissues whereby a nucleic acid encoding a transcription factor as described herein that controls the biosynthesis of secondary cell wall is operably linked to a promoter from a downstream induced gene involved in secondary wall biosynthesis where expression of the downstream gene is induced by the transcription factor. A second example of an APFL of the invention comprises a nucleic acid encoding a transcription factor as described herein that controls expression of wax and/or cutin biosynthesis operably linked to a promoter from a downstream induced gene involved in wax and/or cutin biosynthesis where expression of the downstream gene is induced by the transcription factor. A further example of an APFL of the invention comprises a nucleic acid encoding a transcription factor as described herein that regulates lipid biosynthesis and, *e.g.*, accumulation in seed and other tissues, operably linked to a promoter from a downstream induced gene involved in lipid biosynthesis where expression of the downstream gene is induced by the transcription factor.

[0123] In various embodiments, the invention provides nucleic acids, expression constructions, and plants comprising AFPLs of the invention and methods of using such compositions.

[0124] In one aspect, the present invention is based, in part, on the discovery that focusing lignin deposition in the vessels of plants while reducing lignin and/or xylan content elsewhere in the plant overcomes problems typically associated with plants having reduced lignin or xylan content, specifically vessel collapse and stunting of plant development. Although cell wall components such as lignin and xylan are beneficial to plants for purposes such as providing structural support to the vessels which supply water and nutrients throughout the plant, these cell wall components (*e.g.*, lignin and xylan) also account for much of the recalcitrance of cell walls to enzymatic degradation and polysaccharide extractability. Therefore, specific localization of lignin and xylan in vessels represents a method by which the cell walls of plants can be made more susceptible to enzymatic degradation and polysaccharide extractability, thus improving saccharification and, *e.g.*, biofuel production from plants; and also providing for improved substrates for the paper and pulp industry. Accordingly, in one aspect the present invention provides methods of engineering a plant having lignin and/or xylan deposition and/or xylan *O*-acetylation that is substantially localized to the vessels of xylem tissue of the plant. Vessel-specific lignin and/or xylan deposition and/or xylan *O*-acetylation is accomplished by reducing a lignin and/or xylan biosynthesis enzyme and/or xylan *O*-acetylation enzyme and expressing a substantially identical enzyme (*e.g.*, an ortholog or a paralog of the enzyme reduced in the plant, or an enzyme that has the same biochemical function) under the control of a vessel-specific promoter that is not the native promoter of the lignin and/or xylan biosynthesis enzyme and/or xylan *O*-acetylation enzyme. Plants of the present invention or biomass comprising the plants of the present invention are suitable for use in a saccharification reaction to obtain an increased amount of soluble sugars than can be obtained from wild-type plants, or in the paper industry.

[0125] The present invention is also based, in part, on the discovery that increasing cell wall deposition specifically in woody tissues results in plants having cells that are filled with cell wall polymers. Increased cell wall deposition is beneficial because it increases the biomass density of the plant, which in turn can increase the amount of bioenergy production that can be obtained from the plant. Accordingly, in another aspect the present invention provides methods of engineering a plant having increased cell wall deposition using an AFPL. A transcription factor that regulates secondary cell wall production is expressed in a

plant under the control of a promoter from an induced gene that is a downstream target of the transcription factor. The expression of the transcription factor increases the expression driven from the downstream promoter, which in turn, because it is operably linked to a gene encoding the transcription factor, increases the expression of the transcription factor, thus
5 generating a positive feedback loop that enhances the expression of the downstream genes of the secondary cell wall pathway and consequently increases secondary cell wall deposition. The transcription factor and promoter may both be from a different plant species than the host plant, or either the transcription factor or promoter may be from a different plant species. Similarly, the transcription factor and promoter need not be from the same plant species.

10 Plants of the present invention or biomass comprising the plants of the present invention are suitable for use in a biomass conversion reaction to increase bioenergy production as compared to the bioenergy production of wild-type plants.

[0126] The methods of the present invention can further be used in combination with one another. Thus, in some embodiments, the present invention provides methods of making
15 plants having increased lignin deposition that is substantially localized to the vessels of xylem tissue of the plant and having increased secondary cell wall deposition. In some embodiments, the present invention provides methods of making plants having increased xylan deposition that is substantially localized to the vessels of xylem tissue of the plant and having increased secondary cell wall deposition. In some embodiments, the present invention
20 provides methods of making plants having increased xylan *O*-acetylation deposition that is substantially localized to the vessels of xylem tissue of the plant and having increased secondary cell wall deposition. In some embodiments, the present invention provides methods of making plants having increased lignin deposition that is substantially localized to the vessels of xylem tissue of the plant and having increased xylan deposition that is
25 substantially localized to the vessels of xylem tissue of the plant. In some embodiments, the present invention provides methods of making plants having lignin deposition that is substantially localized to the vessels of xylem tissue of the plant and having increased xylan *O*-acetylation deposition that is substantially localized to the vessels of xylem tissue of the plant.

30 [0127] In another aspect, the invention provides a method of increasing wax/cutin production in a desired tissue. A transcription factor that regulates wax/cuticle production is expressed in a plant under the control of a promoter from an induced gene that is a downstream target of the transcription factor. The expression of the transcription factor increases the expression driven by the downstream promoter, which in turn, because it is

operably linked to a gene encoding the transcription factor, increases the expression of the transcription factor, thus generating a positive feedback loop that increases wax/cutin production. The transcription factor and promoter, or the transcription factor or promoter, can be from a different species than the host plant cell in which the artificial positive
5 feedback loop is created. In some embodiments, the transcription factor and promoter are from different species. Plants generated in accordance with this aspect of the invention have increased drought tolerance and reduced water consumption.

III. Plants Having Spatially Modified Gene Expression

A. Modification of Expression of a Lignin or Xylan Biosynthesis Enzyme

10 [0128] In one aspect, the present invention provides methods of engineering a plant having lignin deposition that is substantially localized to the vessels of xylem tissue of the plant. In some embodiments, the method comprises:

introducing an expression cassette into the plant, wherein the plant is modified to have a reduced level of expression of a lignin biosynthesis enzyme; and wherein the
15 expression cassette comprises a polynucleotide encoding the lignin biosynthesis enzyme operably linked to a heterologous vessel-specific promoter; and

culturing the plant under conditions in which the lignin biosynthesis enzyme is expressed.

[0129] In another aspect, the present invention provides methods of engineering a plant
20 having xylan deposition that is substantially localized to the vessels of xylem tissue of the plant. In some embodiments, the method comprises:

introducing an expression cassette into the plant, wherein the plant is modified to have a reduced level of expression of a xylan biosynthesis enzyme; and wherein the
25 expression cassette comprises a polynucleotide encoding the xylan biosynthesis enzyme operably linked to a heterologous vessel-specific promoter; and

culturing the plant under conditions in which the xylan biosynthesis enzyme is expressed.

[0130] The expression cassette as described herein, when introduced into a plant that is modified to have a reduced level of expression of the lignin or xylan biosynthesis enzyme,
30 results in a plant having fine-tuned lignin or xylan deposition in which lignin is still expressed in vessel tissues, thus preventing vessel collapse, but in which lignin or xylan is not highly expressed in other tissues, thus reducing cell wall recalcitrance.

[0131] One of skill in the art will understand that the lignin biosynthesis enzyme and/or xylan biosynthesis enzyme that is introduced into the plant by an expression cassette does not have to be identical to the lignin biosynthesis enzyme and/or xylan biosynthesis enzyme that was modified in the plant before introduction of the expression cassette. In some
5 embodiments, the lignin biosynthesis enzyme and/or xylan biosynthesis enzyme that is introduced into the plant by an expression cassette is substantially identical (*e.g.*, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the lignin biosynthesis enzyme
10 and/or xylan biosynthesis enzyme that was modified in the plant before introduction of the expression cassette. In some embodiments, the lignin biosynthesis enzyme and/or xylan biosynthesis enzyme that is introduced into the plant by an expression cassette is a homolog (*e.g.*, a homolog as shown in any of the alignments of Figures 1-12 or an enzyme with the same biochemical function, *e.g.*, paralog) of the lignin biosynthesis enzyme and/or xylan
15 biosynthesis enzyme that was modified in the plant before introduction of the expression cassette.

1. Lignin Biosynthesis Enzymes

[0132] In some embodiments, the expression cassette comprises a polynucleotide encoding a lignin biosynthesis enzyme. A lignin biosynthesis enzyme may be selected for use in the
20 present invention on the basis that regulates the production of monolignols and therefore lignin biosynthesis. In some embodiments, the lignin biosynthesis enzyme is phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase (C4H), 4-coumarate-CoA ligase (4CL), hydroxycinnamoyl CoA:shikimate hydroxycinnamoyl transferase (HCT), coumaryol shikimate 3-hydroxylase (C3H), or cinnamoyl-CoA reductase 1 (CCR1).

[0133] The lignin biosynthesis enzymes PAL, C4H, 4CL, HCT, C3H, and CCR1 have been characterized in *Arabidopsis* and have been shown to mediate the synthesis of lignin monomers (monolignols) from phenylalanine. *See, e.g.*, Bonawitz and Chapple, *Annu. Rev. Genet.* 44:337-63 (2010). Thus, in some embodiments, the polynucleotide encoding a lignin biosynthesis enzyme is substantially identical to any of the polynucleotide sequences of SEQ
30 ID NOs:1, 3, 5, 7, 9, or 11. In some embodiments, the lignin biosynthesis enzyme is substantially identical to any of the polypeptide sequences of SEQ ID NOs:2, 4, 6, 8, 10, or 12. Additionally, many of the enzymes involved in lignin biosynthesis are conserved among species. Thus, in some embodiments, the polynucleotide encoding a lignin biosynthesis enzyme comprises a homolog of any of the polynucleotide sequences of SEQ ID NOs:1, 3, 5,

7, 9, or 11. In some embodiments, the lignin biosynthesis enzyme comprises a homolog of any of the polypeptide sequences of SEQ ID NOs:2, 4, 6, 8, 10, or 12 or any of the polypeptide sequences shown in any of Figures 1-6.

[0134] In some embodiments, the polynucleotide encoding a lignin biosynthesis enzyme
5 comprises a polynucleotide sequence that is substantially identical (*e.g.*, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to any of SEQ ID NOs:1, 3, 5, 7, 9, or 11. In some embodiments, the polynucleotide encoding a lignin biosynthesis enzyme comprises a
10 polynucleotide sequence that encodes a polypeptide sequence that is substantially identical (*e.g.*, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to any of SEQ ID NOs:2, 4, 6, 8, 10, or 12 or any of the polypeptide sequences shown in any of Figures 1-6. In
15 some embodiments, the lignin biosynthesis enzyme comprises an amino acid sequence that is substantially identical (*e.g.*, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to any of SEQ ID NOs:2, 4, 6, 8, 10, or 12 or any of the polypeptide sequences
20 shown in any of Figures 1-6.

[0135] Gene and protein sequences and/or accession numbers for PAL, C4H, 4CL, HCT, C3H, and CCR1 are described in the Sequence Listing herein. Amino acid sequence alignments for lignin biosynthesis enzymes showing the amino acid sequences for each of these proteins from multiple plant species are shown in Figures 1-6. Additionally, gene and
25 protein sequences for these proteins, and methods for obtaining the genes or proteins, are known and described in the art. See, for example, Schilmiller et al., 2009, *Plant J.*, doi: 10.1111/j.1365-313X.2009.03996.x. One of skill in the art will recognize that these gene or protein sequences known in the art and/or as described herein can be modified to make substantially identical lignin biosynthesis enzymes, *e.g.*, by making conservative substitutions
30 at one or more amino acid residues. One of skill will also recognize that the known sequences (*e.g.*, the alignments provided herein) provide guidance as to what amino acids may be varied to make a substantially identical lignin biosynthesis enzyme. For example, using any of the alignments shown in Figures 1-6, one of skill will recognize which amino acid residues are

not highly conserved and thus can likely be changed without resulting in a significant effect on the function of the lignin biosynthesis enzyme.

2. Xylan Biosynthesis Enzymes

[0136] The methods of the invention can also employ xylan biosynthesis enzymes. Several enzymes involved in xylan biosynthesis are known. Glycosyltransferases (GTs) belonging to the family GT43 (known as IRX9, IRX9-like, IRX14 and IRK14-like) have been demonstrated to be involved in xylan biosynthesis. The nomenclature for GT families used here is according to the CAZy database (www.cazy.org) (Cantarel et al., 2009). Other GTs in the GT47 family have also been shown to be involved in xylan biosynthesis: IRX10, IRX10-like, IRX7 and F8H. In addition GTs in GT8 have been shown to be involved in xylan biosynthesis: IRX8 (GAUT12) and PARVUS (GATL1). All the mentioned enzymes are known to be involved in xylan biosynthesis because plants where the genes have been mutated are deficient in xylan. (Brown, 2009; Wu et al., 2010) (Lee et al., 2009) (Pena et al., 2007; Persson et al., 2007; Liepman et al., 2010; Scheller and Ulvskov, 2010). Proteins belonging to the DUF579 family (also known as IRX15) are also involved in xylan biosynthesis although they do not appear to be GTs (Brown et al., 2011). The GTs responsible for adding glucuronic acid residues to the xylan backbone have been identified and are known as PGSIP or GUX, however, inactivation of these genes does not lead to xylan deficiency (Mortimer et al., 2010; Oikawa et al., 2010). GTs involved in adding arabinose residues to the xylan backbone have been identified in the literature as members of the GT61 family of enzymes (Anders et al. 2012). Proteins involved in *O*-acetylation of polysaccharides, including xylan, have been identified and designated as RWA proteins (Manabe et al., 2011), and proteins involved in *O*-acetylation of xyloglucan and mannan have been shown to be members of the DUF231 family (Gille et al. 2011). Most likely other members of the large DUF231 family are required for xylan *O*-acetylation.

[0137] Protein sequences and accession numbers for various IRX proteins and Parvus proteins are shown in Figures 7-12. Figures 7-12 provide amino acid sequence alignments of the indicated proteins. Additionally, gene and protein sequences for these proteins, and methods for obtaining the genes or proteins, are known and described in the art. One of skill in the art will recognize that these gene or protein sequences known in the art and/or as described herein can be modified to make substantially identical lignin biosynthesis enzymes, *e.g.*, by making conservative substitutions at one or more amino acid residues. One of skill will also recognize that the known sequences (*e.g.*, the alignments provided herein) provide guidance as to what amino acids may be varied to make a substantially identical lignin

biosynthesis enzyme. For example, using any of the alignments shown in Figures 7-12, one of skill will recognize which amino acid residues are not highly conserved and thus can likely be changed without resulting in a significant effect on the function of the lignin biosynthesis enzyme.

5 [0138] In addition to the xylan synthesis genes (*e.g.*, those listed hereinabove) a similar strategy may also be used to regulate polysaccharide *O*-acetylation expression patterns via RWA gene expression. RWA proteins function in acetylation in general, including in xylan *O*-acetylation. Thus, combining specific expression of RWA with the RWA knock-out/downregulation plants that have very low acetate content but still have excellent growth
10 properties can also be produced using the techniques described herein. In *Arabidopsis* there are 4 RWA genes and three (RWA1, RWA3 and RWA4) are predominantly expressed in tissues with secondary walls (Manabe et al., 2011;). Downregulation or inactivation of two or more of these RWA genes results in decreased xylan *O*-acetylation and impaired function of vascular tissues (Scheller et al., 2010; WO/2010/096488). Thus, RWA may be
15 downregulated in plants, *e.g.*, using methods and compositions described in WO2010/096488 and an RWA gene then reintroduced into the plant where the RWA gene is under the control of a promoter/transcription factor as described herein. Alternative to targeting RWA proteins, one or more DUF231 proteins involved in xylan *O*-acetylation can be targeted.

[0139] Although the genes and proteins used as illustrations above have been studied
20 primarily using *Arabidopsis thaliana*, orthologs are easily identified in other plant species. For example, for many genes, it has been demonstrated by complementation experiments, silencing, or RNAi that orthologs from other plants have the same function as the *A. thaliana* proteins (Zhou et al., 2006; Zhou et al., 2007; Lee et al., 2009).

[0140] Gene and protein sequences and/or accession numbers for IRX8, IRX14, IRX14-
25 like, IRX9, IRX9-like, IRX7, IRX10, IRX10-like, IRX15, IRX15-like, F8H, and PARVUS are described herein. Amino acid sequence alignments for xylan biosynthesis enzymes showing the amino acid sequences for each of these proteins from multiple plant species are also shown in Figures 7-12. Additionally, gene and protein sequences for these proteins, and methods for obtaining the genes or proteins, are known and described in the art as discussed
30 above. One of skill in the art will recognize that these gene or protein sequences known in the art and/or as described herein can be modified to make substantially identical xylan biosynthesis enzymes, *e.g.*, by making conservative substitutions at one or more amino acid residues. One of skill will also recognize that the known sequences (*e.g.*, the alignments provided herein) provide guidance as to what amino acids may be varied to make a

substantially identical xylan biosynthesis enzyme. For example, using any of the alignments shown in Figures 7-12, one of skill will recognize which amino acid residues are not highly conserved and thus can likely be changed without resulting in a significant effect on the function of the xylan biosynthesis enzyme.

5 3. Vessel-Specific Promoters

[0141] In some embodiments, the polynucleotide encoding the lignin biosynthesis enzyme or xylan biosynthesis enzyme is operably linked to a vessel-specific promoter. The vessel-specific promoter is heterologous to the polynucleotide encoding the lignin biosynthesis enzyme or xylan biosynthesis enzyme (*i.e.*, is not the native promoter associated with the
10 lignin biosynthesis enzyme or xylan biosynthesis enzyme). A promoter is suitable for use as a vessel-specific promoter if the promoter is expressed strongly in vessel cells of the plant but is expressed at lower levels in fiber cells of the plant as compared to the level of expression of the native promoter of the lignin biosynthesis enzyme or xylan biosynthesis enzyme whose expression is to be modified.

15 [0142] In some embodiments, the promoter is substantially identical (*e.g.*, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the native promoter of a gene encoding vascular-related NAC-domain 1 (VND1), VND2, VND3, VND4, VND5, VND6, VND7, or
20 VND-interacting 2 (VNI2). In some embodiments, the promoter is substantially identical (*e.g.*, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the native promoter of a gene encoding REF4 or RFR1.

25 [0143] In some embodiments, the vessel-specific promoter comprises SEQ ID NO:36, 94, or 95. In some embodiments, the vessel-specific promoter comprises a subsequence of SEQ ID NO:36, 94, or 95 or a variant thereof. In some embodiments, the vessel-specific promoter comprises a subsequence of SEQ ID NO:36, 94, or 95 comprising about 50 to about 1000 or more contiguous nucleotides of the sequences. In some embodiments, the vessel-specific
30 promoter comprises a subsequence of SEQ ID NO:36, 94, or 95 comprising 50 to 1000, 50 to 900, 50 to 800, 50 to 700, 50 to 600, 50 to 500, 50 to 400, 50 to 300, 50 to 200, 50 to 100; 75 to 1000, 75 to 900, 75 to 800, 75 to 700, 75 to 600, 75 to 500, 75 to 400, 75 to 300, 75 to 200;

100 to 1000, 100 to 900, 100 to 800, 100 to 700, 100 to 600, 100 to 500, 100 to 400, 100 to 300, or 100 to 200 contiguous nucleotides of the sequence.

[0144] Vessel-specific promoters are also described in the art. See, for example, Yamaguchi et al., 2010, *Plant Cell*; Kubo et al., 2009, *Genes Dev.*; and Yamaguchi et al., 2008, *Plant J.*; each of which is incorporated by reference herein in its entirety.

[0145] It will be appreciated by one of skill in the art that a promoter region can tolerate considerable variation without diminution of activity. Thus, in some embodiments, the vessel-specific promoter is substantially identical (*e.g.*, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to SEQ ID NO:36, SEQ ID NO:94, or SEQ ID NO:95.

4. Genetic Background of Plants

[0146] In some embodiments, a plant in which an expression cassette comprising a lignin or xylan biosynthesis enzyme is to be introduced has a genetic background that is modified to have a reduced level of activity of the lignin or xylan biosynthesis enzyme. In some embodiments, the plant is modified to have a level of activity of the lignin or xylan biosynthesis enzyme that is reduced throughout the entire plant. In some embodiments, the plant is modified to have a level of activity of the lignin or xylan biosynthesis enzyme that is reduced only in a subset of cells or tissues of the plant. The genetic background of the plant can be modified according to any method known in the art, such as antisense, siRNA, microRNA, dsRNA, sense suppression, mutagenesis, or use of a dominant negative inhibition strategy. In some embodiments, the level of expression of the protein is reduced. In some embodiments, the modified plant having the reduced level of activity, or expression, of a lignin and/or xylan biosynthesis enzyme is then used to express an expression cassette expressing that same lignin and/or xylan biosynthesis enzyme, but under the control of a vessel-specific promoter rather than its native promoter. In some embodiments, the lignin and/or xylan biosynthesis enzyme that is introduced into the plant by expression cassette is substantially identical, but not completely identical, to the lignin and/or xylan biosynthesis enzyme that is reduced in the plant, in order to avoid silencing of the lignin and/or xylan biosynthesis enzyme that is introduced by the expression cassette (*e.g.*, silent nucleotide changes can be made in the lignin and/or xylan biosynthesis enzyme that is introduced by the expression cassette such that the amino acid sequence, but not the nucleotide sequence, is identical to the lignin and/or xylan biosynthesis enzyme being reduced in the plant).

a) Gene silencing techniques

[0147] In some embodiments, expression of the lignin or xylan biosynthesis enzyme is inhibited by an antisense oligonucleotide. In antisense technology, a nucleic acid segment from the desired gene is cloned and operably linked to a promoter such that the antisense strand of RNA will be transcribed. The expression cassette is then transformed into plants and the antisense strand of RNA is produced. In plant cells, it has been suggested that antisense RNA inhibits gene expression by preventing the accumulation of mRNA which encodes the enzyme of interest, see, e.g., Sheehy *et al.*, *Proc. Nat. Acad. Sci. USA*, 85:8805-8809 (1988); Pnueli *et al.*, *The Plant Cell* 6:175-186 (1994); and Hiatt *et al.*, U.S. Patent No. 4,801,340.

[0148] The antisense nucleic acid sequence transformed into plants will be substantially identical to at least a portion of the endogenous gene or genes to be repressed. The sequence, however, does not have to be perfectly identical to inhibit expression. Thus, an antisense or sense nucleic acid molecule encoding only a portion of the lignin or xylan biosynthesis enzyme-encoding sequence can be useful for producing a plant in which expression of the lignin or xylan biosynthesis enzyme is inhibited. For antisense suppression, the introduced sequence also need not be full length relative to either the primary transcription product or fully processed mRNA. Generally, higher homology can be used to compensate for the use of a shorter sequence. Furthermore, the introduced sequence need not have the same intron or exon pattern, and homology of non-coding segments may be equally effective. In some embodiments, a sequence of at least, e.g., 20, 25, 30, 50, 100, 200, or more continuous nucleotides (up to mRNA full length) substantially identical to an endogenous lignin or xylan biosynthesis enzyme mRNA, or a complement thereof, can be used.

[0149] Catalytic RNA molecules or ribozymes can also be used to inhibit expression of a gene encoding a lignin or xylan biosynthesis enzyme. It is possible to design ribozymes that specifically pair with virtually any target RNA and cleave the phosphodiester backbone at a specific location, thereby functionally inactivating the target RNA. In carrying out this cleavage, the ribozyme is not itself altered, and is thus capable of recycling and cleaving other molecules, making it a true enzyme. The inclusion of ribozyme sequences within antisense RNAs confers RNA-cleaving activity upon them, thereby increasing the activity of the constructs.

[0150] A number of classes of ribozymes have been identified. One class of ribozymes is derived from a number of small circular RNAs that are capable of self-cleavage and

replication in plants. The RNAs replicate either alone (viroid RNAs) or with a helper virus (satellite RNAs). Examples include RNAs from avocado sunblotch viroid and the satellite RNAs from tobacco ringspot virus, lucerne transient streak virus, velvet tobacco mottle virus, solanum nodiflorum mottle virus and subterranean clover mottle virus. The design and use of target RNA-specific ribozymes is described in Haseloff *et al. Nature*, 334:585-591 (1988).

[0151] Another method by which expression of a gene encoding a lignin or xylan biosynthesis enzyme can be inhibited is by sense suppression (also known as co-suppression). Introduction of expression cassettes in which a nucleic acid is configured in the sense orientation with respect to the promoter has been shown to be an effective means by which to block the transcription of target genes. For an example of the use of this method to modulate expression of endogenous genes, see Napoli *et al., The Plant Cell* 2:279-289 (1990); Flavell, *Proc. Natl. Acad. Sci., USA* 91:3490-3496 (1994); Kooter and Mol, *Current Opin. Biol.* 4:166-171 (1993); and U.S. Patents Nos. 5,034,323, 5,231,020, and 5,283,184.

[0152] Generally, where inhibition of expression is desired, some transcription of the introduced sequence occurs. The effect may occur where the introduced sequence contains no coding sequence *per se*, but only intron or untranslated sequences homologous to sequences present in the primary transcript of the endogenous sequence. The introduced sequence generally will be substantially identical to the endogenous sequence intended to be repressed. This minimal identity will typically be greater than about 65%, but a higher identity can exert a more effective repression of expression of the endogenous sequences. In some embodiments, sequences with substantially greater identity are used, *e.g.*, at least about 80%, at least about 95%, or 100% identity are used. As with antisense regulation, further discussed below, the effect can be designed and tested to apply to any other proteins within a similar family of genes exhibiting homology or substantial homology.

[0153] For sense suppression, the introduced sequence in the expression cassette, needing less than absolute identity, also need not be full length, relative to either the primary transcription product or fully processed mRNA. Furthermore, the introduced sequence need not have the same intron or exon pattern, and identity of non-coding segments will be equally effective. In some embodiments, a sequence of the size ranges noted above for antisense regulation is used, *i.e.*, 30-40, or at least about 20, 50, 100, 200, 500 or more nucleotides.

[0154] Endogenous gene expression may also be suppressed by means of RNA interference (RNAi) (and indeed co-suppression can be considered a type of RNAi), which uses a double-stranded RNA having a sequence identical or similar to the sequence of the target gene.

RNAi is the phenomenon in which when a double-stranded RNA having a sequence identical or similar to that of the target gene is introduced into a cell, the expressions of both the inserted exogenous gene and target endogenous gene are suppressed. The double-stranded RNA may be formed from two separate complementary RNAs or may be a single RNA with internally complementary sequences that form a double-stranded RNA. Although complete details of the mechanism of RNAi are still unknown, it is considered that the introduced double-stranded RNA is initially cleaved into small fragments, which then serve as indexes of the target gene in some manner, thereby degrading the target gene. RNAi is known to be also effective in plants (see, e.g., Chuang, C. F. & Meyerowitz, E. M., *Proc. Natl. Acad. Sci. USA* 97: 4985 (2000); Waterhouse *et al.*, *Proc. Natl. Acad. Sci. USA* 95:13959-13964 (1998); Tabara *et al.* *Science* 282:430-431 (1998); Matthew, *Comp Funct Genom* 5: 240-244 (2004); Lu, *et al.*, *Nucleic Acids Res.* 32(21):e171 (2004)).

[0155] Thus, in some embodiments, inhibition of a gene encoding a lignin or xylan biosynthesis enzyme is accomplished using RNAi techniques. For example, to achieve suppression of the expression of a DNA encoding a protein using RNAi, a double-stranded RNA having the sequence of a DNA encoding the protein, or a substantially similar sequence thereof (including those engineered not to translate the protein) or fragment thereof, is introduced into a plant of interest. As used herein, RNAi and dsRNA both refer to gene-specific silencing that is induced by the introduction of a double-stranded RNA molecule, see e.g., U.S. Pat. Nos. 6,506,559 and 6,573,099, and includes reference to a molecule that has a region that is double-stranded, e.g., a short hairpin RNA molecule. The resulting plants may then be screened for a phenotype associated with the target protein, for example, screening for an increase in the extractability of sugar from the plants as compared to wild-type plants, and/or by monitoring steady-state RNA levels for transcripts encoding the protein. Although the genes used for RNAi need not be completely identical to the target gene, they may be at least 70%, 80%, 90%, 95% or more identical to the target gene sequence. See, e.g., U.S. Patent Publication No. 2004/0029283. The constructs encoding an RNA molecule with a stem-loop structure that is unrelated to the target gene and that is positioned distally to a sequence specific for the gene of interest may also be used to inhibit target gene expression. See, e.g., U.S. Patent Publication No. 2003/0221211.

[0156] The RNAi polynucleotides may encompass the full-length target RNA or may correspond to a fragment of the target RNA. In some cases, the fragment will have fewer than 100, 200, 300, 400, 500 600, 700, 800, 900 or 1,000 nucleotides corresponding to the target sequence. In addition, in some embodiments, these fragments are at least, e.g., 50, 100,

150, 200, or more nucleotides in length. In some cases, fragments for use in RNAi will be at least substantially similar to regions of a target protein that do not occur in other proteins in the organism or may be selected to have as little similarity to other organism transcripts as possible, *e.g.*, selected by comparison to sequences in analyzing publicly-available sequence
5 databases.

[0157] Expression vectors that continually express siRNA in transiently- and stably-transfected have been engineered to express small hairpin RNAs, which get processed *in vivo* into siRNAs molecules capable of carrying out gene-specific silencing (Brummelkamp *et al.*, *Science* 296:550-553 (2002), and Paddison, *et al.*, *Genes & Dev.* 16:948-958 (2002)). Post-transcriptional gene silencing by double-stranded RNA is discussed in further detail by
10 Hammond *et al.* *Nature Rev Gen* 2: 110-119 (2001), Fire *et al.* *Nature* 391: 806-811 (1998) and Timmons and Fire *Nature* 395: 854 (1998).

[0158] Yet another way to suppress expression of an endogenous plant gene is by recombinant expression of a microRNA that suppresses a target (*e.g.*, a gene encoding a
15 lignin or xylan biosynthesis enzyme). Artificial microRNAs are single-stranded RNAs (*e.g.*, between 18-25-mers, generally 21-mers), that are not normally found in plants and that are processed from endogenous miRNA precursors. Their sequences are designed according to the determinants of plant miRNA target selection, such that the artificial microRNA specifically silences its intended target gene(s) and are generally described in Schwab *et al.*,
20 *The Plant Cell* 18:1121-1133 (2006) as well as the internet-based methods of designing such microRNAs as described therein. *See also*, US Patent Publication No. 2008/0313773.

[0159] Another example of a method to reduce levels of a gene expression product of a gene or gene of interest employ riboswitch techniques (see, *e.g.*, U.S. Patent Application Publication Nos. US20100286082, and US20110245326).

[0160] Methods of inhibiting plant gene expression for one or more lignin and/or xylan biosynthesis enzymes, including plants that have inhibited RWA expression, have been described in the art. See, for example, Coleman *et al.*, *Plant Physiol.* 148:1229-37 (2008) (C3'H RNAi in poplar); Kitin *et al.*, *Plant Physiol.* 154:887-98 (2010) (4CL antisense in poplar); Coleman *et al.*, *Proc. Acad. Natl. Sci. USA* 105:4501-06 (2008) (C3'H RNAi in
30 poplar); and Voelker *et al.*, *Plant Physiol.* 154:874-86 (2010) (4CL antisense in poplar), and WO2010/096488 (*RWA* inhibition), each of which is incorporated by reference herein in its entirety.

[0161] As appreciated by one of skill in the art, the isoforms that are highly expressed in xylem and fibers are targeted. For example, using *Arabidopsis* for illustration purposes, IRX7, IRX8, IRX9, PARVUS, IRX15 are highly expressed in xylem and fibers and would therefore be targeted. For IRX10 and IRX14, both isoforms (*Arabidopsis* has 2 isoforms) would be typically targeted since they both have expression in xylem and fibers. Similarly, for making plants that are inhibited in Rwa expression, the isoforms that are expressed in xylem and fibers are targeted. For example, again using *Arabidopsis* for illustration, one of, typically two or more of, RWA1, RWA3 and RWA4 are targeted (RWA2 is not expressed in xylem and fibers).

[0162] As further understood in the art, in the steps of the methods of the invention in which the activity is introduced back into the xylan-deficient or lignin-deficient plant using a vessel specific promoter (e.g. VND6), it is not necessary to express the same isoform as the one that was targeted for inhibition. For example, an *irx9* mutant plant may be employed that has very little xylan, but it is not necessary to express the tissue specific IRX9 isoform in the plant, rather a IRX9 homolog that is not normally expressed in those tissues may also be readily employed. Many plants, including *Arabidopsis*, have a second IRX9-like gene which is mostly expressed in tissues apart other than xylem and fibers. Similar relationships are true for IRX7/F8H, IRX14/IRX14-like, and IRX15/IRX15-like. Likewise, RWA1/RWA3/RWA4 mutants can be engineered to express *Rwa2* under control of the vessel-specific promoter, e.g., a VND6 promoter.

b) Plants having mutant backgrounds

[0163] In some embodiments, the level of expression of the lignin or xylan biosynthesis enzyme is reduced by generating a plant that has a mutation in a gene encoding the lignin or xylan biosynthesis enzyme. One method for abolishing or decreasing the expression of a gene encoding a lignin or xylan biosynthesis enzyme is by insertion mutagenesis using the T-DNA of *Agrobacterium tumefaciens*. After generating the insertion mutants, the mutants can be screened to identify those containing the insertion in the gene of interest. Mutants containing a single mutation event at the desired gene may be crossed to generate homozygous plants for the mutation (Koncz et al. (1992) *Methods in Arabidopsis Research*. World Scientific).

[0164] Alternatively, random mutagenesis approaches may be used to generate new alleles that will generate truncated or defective (non-functional or poorly active) enzymes or unstable RNA, or to disrupt or "knock-out" the expression of a gene encoding a lignin or

xylan biosynthesis enzyme using either chemical or insertional mutagenesis or irradiation. One method of mutagenesis and mutant identification is known as TILLING (for targeting induced local lesions in genomes). In this method, mutations are induced in the seed of a plant of interest, for example, using EMS treatment. The resulting plants are grown and self-fertilized, and the progeny are assessed. For example, the plants may be assessed using PCR to identify whether a mutated plant has a mutation in the gene of interest, or by evaluating whether the plant has reduced lignin content in a part of the plant that expressed the gene of interest. TILLING can identify mutations that may alter the expression of specific genes or the activity of proteins encoded by these genes (see Colbert et al (2001) *Plant Physiol* 126:480-484; McCallum et al (2000) *Nature Biotechnology* 18:455-457).

[0165] Methods of making plants having a mutant background for one or more lignin and/or xylan biosynthesis enzymes have been described in the art. See, for example, Schillmiller *et al.*, *Plant J.* 60:771-82 (2009) (*Arabidopsis* mutant for C4H); and Weng *et al.*, *Plant Cell* 22:1033-45 (2010) (*Selaginella* mutant for F5H), each of which is incorporated by reference herein in its entirety. Methods of making plants that have an *RWA* mutant background are described, *e.g.*, in WO2010/096488.

[0166] In some embodiments, where expression cassettes comprising a lignin biosynthesis enzyme and a xylan biosynthesis enzyme are to be introduced into a plant, the plant has a genetic background that is modified to have reduced levels of expression of both the lignin biosynthesis enzyme and the xylan biosynthesis enzyme. Such plants can be generated using known methods as described herein sections of the application describing modification of plants to suppress or reduce expression of a desired product.

B. Modification of Expression Using a Transcription Factor that Regulates the Production of Secondary Cell Wall

[0167] In another aspect, the present invention provides methods of engineering a plant having increased secondary cell wall deposition. In some embodiments, the method comprises:

introducing an expression cassette into the plant, wherein the expression cassette comprises a polynucleotide encoding a transcription factor that regulates the production of secondary cell wall in woody tissue operably linked to an induced heterologous promoter, wherein the promoter is substantially identical to the native promoter of a gene that is a downstream target of the transcription factor in the biosynthetic pathway; and

culturing the plant under conditions in which the transcription factor is expressed. The downstream target may be a direct or indirect target of the transcription factor.

[0168] The expression cassette as described herein, when introduced into a plant, generates a positive feedback loop that allows the maintenance of expression or the overexpression of genes involved in secondary cell wall biosynthesis, due to the transcription factor directly or indirectly inducing expression of the promoter from the downstream target gene, which in turn is operably linked to the polynucleotide encoding the transcription factor, resulting in increased expression of the transcription factor. This positive feedback loop results in the continued production or overproduction of secondary cell walls components such as cellulose, hemicellulose, and lignin.

1. Transcription Factors that Regulate the Production of Secondary Cell Wall

[0169] In some embodiments, the expression cassette comprises a polynucleotide encoding a transcription factor that regulates the production of secondary cell wall. A transcription factor may be selected for use in the present invention on the basis that it induces one or more genes involved in lignin biosynthesis and/or polysaccharide (cellulose and hemicellulose) biosynthesis. Alternatively or additionally, the transcription factor may be selected for use on the basis of an overexpression or loss-of-function phenotype in a plant (*e.g.*, a plant overexpressing that transcription factor that exhibits a phenotype of increased cell wall thickening or secondary cell wall deposition, or a plant having a dominant repression or loss-of-function mutation of that transcription factor that exhibits a phenotype of decreased cell wall thickening or secondary cell wall deposition). In some embodiments, the transcription factor is NAC secondary wall-thickening promoting factor 1 (NST1), NST2, NST3, secondary wall-associated NAC domain protein 2 (SND2), SND3, MYB domain protein 103 (MYB103), MBY85, MYB46, MYB83, MYB58, or MYB63.

[0170] The transcription factors NST1, NST2, NST3, SND2, SND3, MYB103, MBY85, MYB46, MYB83, MYB58, and MYB63 have been characterized in *Arabidopsis* and have been shown to regulate secondary cell wall production in that species. *See, e.g.*, Mitsuda *et al.*, *Plant Cell* 17:2993-3006 (2005); Mitsuda *et al.*, *Plant Cell* 19:270-80 (2007); Ohashi-Ito *et al.*, *Plant Cell* 22:3461-73 (2010); Zhong *et al.*, *Plant Cell* 20:2763-82 (2008); Zhong *et al.*, *Plant Cell* 19:2776-92 (2007); Ko *et al.*, *Plant J.* 60:649-65 (2009); and McCarthy *et al.*, *Plant Cell Physiol.* 50:1950-64 (2009). Thus, in some embodiments, the polynucleotide

encoding a transcription factor that regulates the production of secondary cell wall is substantially identical to any of the polynucleotide sequences of SEQ ID NOs:13, 15, 17, 19, 21, 23, 25, 27, 29, 31, or 33. Additionally, these transcription factors have been identified in a variety of other plants, including rice, sorghum, poplar, grape, moss, maize, and switchgrass. Furthermore, the general mechanism of secondary cell wall biosynthesis is conserved not only between monocots and dicots, but also within these groups. Thus, in some embodiments, the polynucleotide encoding a transcription factor that regulates the production of secondary cell wall comprises a homolog of any of the polynucleotide sequences of SEQ ID NOs:13, 15, 17, 19, 21, 23, 25, 27, 29, 31, or 33 or any of the amino acid sequences of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34 or any of the amino acid sequences of Figure 13.

[0171] In some embodiments, the polynucleotide encoding a transcription factor that regulates the production of secondary cell wall in woody tissue comprises a polynucleotide sequence that is substantially identical (*e.g.*, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to any of SEQ ID NOs:13, 15, 17, 19, 21, 23, 25, 27, 29, 31, or 33. In some embodiments, the polynucleotide encoding a transcription factor that regulates the production of secondary cell wall in woody tissue comprises a polynucleotide sequence that encodes a polypeptide sequence that is substantially identical (*e.g.*, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to any of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34. In some embodiments, the transcription factor that regulates the production of secondary cell wall in woody comprises an amino acid sequence that is substantially identical (*e.g.*, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to any of SEQ ID NOs:14, 16, 18, 20, 22, 24, 26, 28, 30, 32, or 34 or to any of the amino acid sequences of Figure 13.

[0172] Gene and protein sequences and/or accession numbers for NST1, NST2, NST3, SND2, SND3, MYB103, MYB85, MYB46, MYB83, MYB58, and MYB63 are described in the Sequence Listing herein. Additionally, amino acid sequence alignments for the transcription factors, showing the amino acid sequences for each of these proteins from

multiple plant species, are shown in Figures 1-6. Gene and protein sequences for these proteins, and methods for obtaining the genes or proteins, are also known and described in the art. See, for example, Goicoechea et al., 2005, *Plant J.* 43:553-67; McCarthy et al., 2009, *Plant Cell Physiol.* 50:1950-64; Shen et al., 2009, *Bioenerg. Res.* 2:217-32; and Zhong et al., 2010, *Trends in Plant Sciences*, <http://dx.doi.org/10.1016/j.tplants.2010.08.007>. One of skill in the art will recognize that these gene or protein sequences known in the art and/or as described herein can be modified to make substantially identical transcription factors, *e.g.*, by making conservative substitutions at one or more amino acid residues. One of skill will also recognize that the known sequences (*e.g.*, the alignments provided herein) provide guidance as to what amino acids may be varied to make a substantially identical transcription factor. For example, using any of the alignments shown in Figures 1-6, one of skill will recognize which amino acid residues are not highly conserved and thus can likely be changed without resulting in a significant effect on the function of the transcription factor.

2. Promoters from Downstream Targets of the Transcription Factors that Regulate the Production of Secondary Cell Wall

[0173] In some embodiments, the polynucleotide encoding the transcription factor that regulates secondary cell wall production is operably linked to a promoter that is a downstream target of the transcription factor. The promoter is heterologous to the polynucleotide encoding the transcription factor that regulates secondary cell wall production (*i.e.*, is not the native promoter associated with the transcription factor that regulates secondary cell wall production). A promoter is suitable for use with the transcription factor that regulates secondary cell wall production if expression of the promoter is induced, directly or indirectly, by the transcription factor to be expressed, and if the promoter is expressed in the desired location, *e.g.*, the stem of the plant but not strongly expressed in leaves of the plant.

[0174] In some embodiments, the promoter is substantially identical (*e.g.*, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the native promoter of a gene that is a downstream target of the transcription factor. In some embodiments, the promoter is substantially identical to the native promoter of IRX1, IRX3, IRX5, IRX8, IRX9, IRX14, IRX7, or IRX10. In some embodiments, the transcription factor is selected from NST1, NST2, NST3, SND2, SND3, MYB103, MYB85, MYB46, MYB83, MYB58, and MYB63 and the promoter is substantially identical to a native promoter selected from IRX1, IRX3,

IRX5, IRX8, IRX9, IRX14, IRX7, IRX10, GAUT13, or GAUT14. See Figure 14.

Alternative promoters may also be used. For example, alternative promoters can be identified by coexpression analysis, e.g., using Atted II database and known promoters as bait; or by identifying functional motifs of interest in the promoters of candidate genes.

5 Promoters from other genes that are regulated by the transcription factor may also be used.

[0175] In some embodiments, the promoter comprises a subsequence of SEQ ID NO:35 or a variant thereof. In some embodiments, the promoter comprises a subsequence of SEQ ID NO:35 comprising about 50 to about 1000 or more contiguous nucleotides of SEQ ID NO:35.

10 In some embodiments, the promoter comprises a subsequence of SEQ ID NO:35 comprising 50 to 1000, 50 to 900, 50 to 800, 50 to 700, 50 to 600, 50 to 500, 50 to 400, 50 to 300, 50 to 200, 50 to 100; 75 to 1000, 75 to 900, 75 to 800, 75 to 700, 75 to 600, 75 to 500, 75 to 400, 75 to 300, 75 to 200; 100 to 1000, 100 to 900, 100 to 800, 100 to 700, 100 to 600, 100 to 500, 100 to 400, 100 to 300, or 100 to 200 contiguous nucleotides of SEQ ID NO:35.

15 [0176] Promoters that are downstream targets of the transcription factors described herein are also described in the art. See, for example, Oikawa et al, 2010, *PLoS ONE*; Taylor et al., 2000, *Plant Cell*; Betancur et al., 2010, *J. Integrative Plant Biol.*; Persson et al., 2007, *Plant Physiol.*; Wu et al., 2010, *Plant Physiol.*; Zhong et al., 2005, *Plant Cell*; and Wu et al., 2009, *Plant J.*; each of which is incorporated by reference herein in its entirety.

20 [0177] It will be appreciated by one of skill in the art that a promoter region can tolerate considerable variation without diminution of activity. Thus, in some embodiments, the promoter is substantially identical (e.g., at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to SEQ ID NO:35.

25 C. Modification of Expression Using a Transcription Factor that Regulates Wax/Cutin Production

[0178] Improving plant water use efficiency is an important priority to reduce water consumption by plant per ton of CO₂ fixed and improve plant drought stress tolerance. It would improve or maintain biomass yields under water limiting conditions by reducing cellular oxidative stresses, which also cause a reduction of photosynthesis efficiency. Developing strategies that can reduce water losses by plants without reducing biomass yield reduces water needs, improves drought stress tolerance and is compatible with drought stress tolerance technologies already developed. Part of the water that is lost by plants occurs by

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water evaporation through the cuticle on the surface of leaf epidermis, also called epicuticle. Transcription factors to control wax/cutin biosynthesis have been identified. Although overexpression in plants of some of these in plants improved resistance to drought-stress and reduced water losses, the expression strategies used to increase the expression of these

5 transcription factors also caused deposition of wax or/and cutin in sensitive tissues generating undesired effects on plant growth and development (Aharoni *et al.*, *The Plant Cell* 16:2463-2480, 2004; Zhang *et al.*, *Plant J.* 42:689-797, 2005). Beyond water use efficiency, modifying epicuticular wax composition and content has several other potential advantages since the epicuticle is the first barrier for many pathogens, insects and chemicals. The
10 invention thus provides an artificial positive feedback loop system to increase wax and/or cutin deposition on the epidermis of plants in order to improve plant water use efficiency and drought-stress tolerance.

[0179] Thus, in another aspect, the present invention provides methods of engineering a plant having modified, *e.g.*, increased, wax and/or cutin production. In some embodiments,
15 the method comprises:

introducing an expression cassette into the plant, wherein the expression cassette comprises a polynucleotide encoding a transcription factor that regulates the production of wax/cutin components linked to a heterologous induced promoter, wherein the promoter is substantially identical to the native promoter of a gene that is a downstream
20 target of the transcription factor; and

culturing the plant under conditions in which the transcription factor is expressed. The downstream target may be a direct or indirect target of the transcription factor.

[0180] The expression cassette as described herein, when introduced into a plant, generates
25 a positive feedback loop that allows the maintenance of expression or the overexpression of genes involved in wax and/or cutinbiosynthesis, due to the transcription factor directly or indirectly inducing expression driven by the promoter from the downstream target gene, which in turn is operably linked to the polynucleotide encoding the transcription factor, resulting in increased expression of the transcription factor. This positive feedback loop
30 results in the continued production or overproduction of wax and/or cutin.

1. Transcription Factors that Regulate the Production of Wax/Cutin

[0181] In some embodiments, the expression cassette comprises a polynucleotide encoding a transcription factor that regulates the production of wax and/or cutin components for the

production of wax (and/or cutin). A transcription factor may be selected for use in the present invention on the basis that it induces one or more genes, typically multiple genes, involved in the wax biosynthetic pathway. Alternatively or additionally, the transcription factor may be selected for use on the basis of an overexpression or loss-of-function

5 phenotype in a plant (*e.g.*, a plant overexpressing that transcription factor that exhibits a phenotype of increased wax production, or a plant having a dominant repression or loss-of-function mutation of that transcription factor that exhibits a phenotype of decreased wax production). In some embodiments, the transcription factor is an *shine* (SHN) transcription factor, such as SHN1 (also known as WIN1), SHN2, SHN3, SHN4, SHN5, or MYB 96.

10 **[0182]** The transcription factors SHN1, SHN2, SHN3, SHN4, SHN5, and MYB96 have been characterized in *Arabidopsis* and have been shown to regulate wax and/or cutin biosynthesis in *Arabidopsis* and other plant species. *See, e.g.*, Shi *et al.*, *PLoS Genet.* 7, e1001388 (2011); Seo *et al.*, *Plant Cell* 23:1138-1152 (2011); Kannangara *et al.*, *Plant Cell* 19:1278-1294 (2007); Zhang *et al.*, *Plant J.* 42:689-707 (2005), Aharoni *et al.*, *Plant Cell* 15 16:2463-2480 (2004); Broun *et al.*, *Proc. Natl. Acad Sci USA* 101:4706-4711 (2004); and Suh *et al.*, *Plant Physiol.* 139:1649-1665 (2005). Additionally, SHN transcription factor sequences have been identified in a variety of other plants, including, including poplar, Medicago, rice, grasses *e.g.*, Brachypodium, corn, sorghum, barley, spruce, spikemoss, and bryophytes. Similarly, Myb96 transcription factor sequences have been identified in various

20 other plants including *Thellungiella*, Medicago, poplar, grape vine, citrus, brachypodium, wheat, barley, rice, and sorghum. Furthermore, the general mechanism of wax/cutin biosynthesis is conserved not only between monocots and dicots, but also within these groups.

[0183] In some embodiments, the polynucleotide encoding a transcription factor that

25 regulates the production of wax/cutin encodes a SHN transcription factor. In some embodiments, the polynucleotide encodes an SHN transcription factor of any one of SEQ ID NOs:37-59, or a variant thereof. Thus, in some embodiments, the polynucleotide encoding a transcription factor that regulates the production of wax/cutin synthesis encodes a protein that is substantially identical to any one of SEQ ID NOS:37-59.

30 **[0184]** In some embodiments, the polynucleotide encoding a transcription factor that regulates the production of wax cutin synthesis comprises a polynucleotide sequence encodes an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least

93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to any of SEQ ID NOS:37-59.

[0185] In some embodiments, the polynucleotide encoding a transcription factor that regulates the production of wax/cutin encodes a Myb96 transcription factor. In some
5 embodiments, the polynucleotide encodes a Myb96 transcription factor of any one of SEQ ID NOS:80-93, or a variant thereof. Thus, in some embodiments, the polynucleotide encoding a transcription factor that regulates the production of wax/cutin synthesis encodes a protein that is substantially identical to any one of SEQ ID NOS:80-93.

[0186] In some embodiments, the polynucleotide encoding a transcription factor that
10 regulates the production of wax cutin synthesis comprises a polynucleotide sequence that encodes an amino acid sequence that is at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical to any of SEQ ID NOS:80-93.

[0187] Illustrative protein sequences and/or accession numbers for SHN1, SHN2, SHN3, SHN4, SHN5, or MYB 96 are provided herein. Additionally, amino acid sequence alignments for the transcription factors, showing the amino acid sequences for each of these proteins from multiple plant species, are shown in Figures 25 and 26. Gene and protein sequences for these proteins, and methods for obtaining the genes or proteins, are also known
20 and described in the art (see, *e.g.*, references cited hereinabove). One of skill in the art will recognize that these gene or protein sequences known in the art and/or as described herein can be modified to make variant transcription factors, *e.g.*, by making conservative substitutions at one or more amino acid residues. One of skill will also recognize that the known sequences (*e.g.*, the alignments provided herein) provide guidance as to which amino
25 acids may be varied to make a substantially identical transcription factor. For example, using the alignment provided in Figures 25 and 26, one of skill will recognize which amino acid residues are not highly conserved and thus can likely be changed without resulting in a significant effect on the function of the transcription factor. Similarly, one of skill can identify highly conserved domains that are conserved in all or almost all of the transcription
30 factors and use this information in identifying variants for use in the invention.

2. Promoters from Downstream Targets of the Transcription Factors that Regulate Wax and/or Cutin Production

[0188] In some embodiments, the polynucleotide encoding the transcription factor that regulates wax and/or cutin production is operably linked to a promoter that is a downstream target of the transcription factor. The promoter is heterologous to the polynucleotide encoding the transcription factor that regulates wax and/or cutin production (*i.e.*, is not the native promoter associated with the transcription factor). A promoter is suitable for use with the transcription factor if expression of the promoter is induced, directly or indirectly, by the transcription factor to be expressed, and if the promoter is expressed in the plant at the desired location, *e.g.*, in the leaf of the plant.

[0189] In some embodiments, the promoter is substantially identical (*e.g.*, at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% identical) to the native promoter of a gene that is a downstream target of the transcription factor. In some embodiments, the promoter is a CER1, CER2, CER3, CER4, CER5, CER6, CER10, WSD1, Mah1, WBC11, KCS1, KCS2, FATB, LACS1, LACS2, CYP864A, CYP86A7, CYP86A5, KCS10, or KCS5 promoter, or a variant thereof that is substantially identical to a native promoter. In some embodiments, the transcription factor is selected from SHN1, SHN2, SHN3, SHN4, SHN5, or MYB 96 and the promoter is substantially identical to a native promoter selected from CER1, CER2, CER3, CER4, CER5, CER6, CER10, WSD1, Mah1, WBC11, KCS1, KCS2, FATB, LACS1, LACS2, CYP864A, CYP86A7, CYP86A5, KCS10, or KCS5. Alternative promoters may also be used. For example, alternative promoters can be identified by coexpression analysis, *e.g.*, using Atted II database and known promoters as bait; or by identifying functional motifs of interest in the promoters of candidate genes. Promoters from other genes that are induced by the transcription factor may also be used.

[0190] In some embodiments, the promoter comprises a subsequence of any one of SEQ ID NOs:60-79, *e.g.*, the sequence form WBC11 or CER1, or a variant thereof. In some embodiments, the promoter comprises a subsequence comprising about 50 to about 1000 or more contiguous nucleotides of any one of SEQ ID NOs:60-79. In some embodiments, the promoter comprises a subsequence of any one of SEQ ID NOs:60-79 comprising 50 to 1000, 50 to 900, 50 to 800, 50 to 700, 50 to 600, 50 to 500, 50 to 400, 50 to 300, 50 to 200, 50 to 100; 75 to 1000, 75 to 900, 75 to 800, 75 to 700, 75 to 600, 75 to 500, 75 to 400, 75 to 300,

75 to 200; 100 to 1000, 100 to 900, 100 to 800, 100 to 700, 100 to 600, 100 to 500, 100 to 400, 100 to 300, or 100 to 200 contiguous nucleotides of the sequence.

[0191] Promoters that are downstream targets of the transcription factors described herein are also described in the art. See, for example, review of wax biosynthesis in plants and
5 references cited therein (Schreiber, *Trends Plant Sci.*, 2010; Kunst & Samuels, *Curr. Opin. Plant Biol.* 12:721-727, 2009; Samuels *et al.*, *Annu. Rev. Plant Biol.* 59:683-707, 2008; Nawrath, *Plant Physiol* 139:281-287, 2006; Kunst & Samuels, *Progress in Lipid Res.* 42:51-80, 2003; Lemieux, *Trends in Plant Sci.* 1:312, 1996). References describing wax mutants analyzed in
10 *Arabidopsis* include Bourdenx *et al.*, *Plant Physiol* 156, 29–45 (2011); Panikashvili *et al.* *Mol Plant* 3, 563–575 (2010); Weng, *et al.*, *Planta* 231, 1089–1100 (2010); Lee *et al.* *Plant J* 60, 462–475 (2009); Li *et al.*, *Plant Physiol* 148, 97–107 (2008); Greer *et al.*, *Plant Physiol* 145, 653–667 (2007); Rowland *et al.*, *FEBS Lett* 581, 3538–3544 (2007); Rowland *et al.*, *Plant Physiol* 142, 866–877 (2006); Costaglioli *et al.*, *Biochim Biophys Acta* 1734, 247–258 (2005); Sturaro *et al.*, *Plant Physiol* 138, 478–489 (2005); Schnurr *et al.*, *Plant Cell* 16, 629–
15 642 (2004); Pighin *et al.*, *Science* 306, 702–704 (2004); Bonaventure *et al.*, *Plant Cell* 15, 1020–1033 (2003); Chen *et al.*, *Plant Cell* 15, 1170–1185 (2003); Fiebig *et al.*, *Plant Cell* 12, 2001–2008 (2000); and Millar *et al.*, *Plant Cell* 11, 825–838 (1999). Wax biosynthetic pathways are also conserved among plants species (see, *e.g.*, Wang *et al.*, *Plant Mol Biol* 78, 275–288 (2011); Mao *et al.*, *Planta* 235, 39–52 (2012); Yu *et al.*, *Planta* 228, 675–685
20 (2008); Tacke *et al.*, *Plant J* 8, 907–917 (1995); Islam *et al.*, *Plant Mol Biol* 70, 443–456 (2009); Post-Beittenmiller *Plant Physiol Bioch* 36, 157–166 (1998); and Park *et al.*, *Plant Mol Biol* 74, 91–103 (2010)).

D. Artificial Positive Feedback Loops

25 [0192] In a further aspect, the invention provides artificial positive feedback loops for regulating gene expression in plants. An APFL over-induces or increases lifetime expression of a particular transcription factor and its downstream pathway. Examples of such systems are described above for secondary wall deposition in fiber stems and for wax deposition. Illustrative examples for cell wall densification and wax deposition of the principle
30 underlying this strategy are shown in Figures 27 and 28. A transcription factor suitable for use in an APFL typically plays a role in controlling expression of multiple components of a pathway of interest. A cell type-specific promoter where expression is driven by the transcription factor is used as the promoter in the APFL construct. The APFL is created by

introducing an expression construct into a plant cell where the construct comprises a polynucleotide encoding a transcription factor of interest operably linked to the desired promoter. Upon expression of the native transcription factor, expression of downstream gene is induced along with expression of the introduced transcription factor encoded by the APFL construct.

[0193] Additional examples of biosynthetic pathways that can employ an APFL include lipid biosynthetic pathways. For example, it is known that lipid biosynthesis and accumulation in seeds and other tissues occurs in specific cell types and is regulated by transcription factors such as WRL1 (WRINKLED; At3g54320), LEC1 (At1g21970), or LEC2 (At1g28300). These transcription factors can thus be used to create an APFL to increase the accumulation of lipids in a desired tissue such as seed. Other transcription factors and appropriate promoters for use in an APFL can also be identified for other biosynthetic pathways. Lipid biosynthesis pathways are discussed, e.g., in Ohlrogge & Browse, *Plant Cell* 7:957, 1995; Hildebrand, *et al.*, *Plant Lipids: Biology, Utilisation and Manipulation*, 67-102 (2005); and Dyer & Mullen, *Seed Sci. Res.* 15:255-267 (2005).

[0194] Other biosynthetic pathways that may be engineered to create an APFL include the terpenoid pathway. For example, an APFL may be created to increase terpenoid indole alkaloid biosynthesis. Transcription factors that may be used for such an APFL include CrMYC2, ORCA2 or ORCA3. A nucleic acid encoding the transcription factor may be operably linked to an induced promoter such as pSTR, which controls the expression of the strictosidine synthase from *catharanthus roseus*. The terpenoid indole alkaloid pathway is known (see, e.g., Peebles, *et al.*, *Metab Eng* 11: 76-86 (2009); Liu, *et al.*, *J Integr Plant Biol* 49:961-974 (2007); Menke, *et al.*, *EMBO J* 18:4455-4463 (1999), each of which references is incorporated by reference).

[0195] A further example of an APFL is one that is employed to increase artemisinin biosynthesis (sesquiterpene). An illustrative transcription factor that may be used for such an APFL is AaWRK1 (from *Artemisia annua*). A nucleic acid encoding the transcription factor may be operably linked to an induced promoter such as pADS, which controls the expression of the amorpha-4,11-diene synthase from *Artemisia annua*. This biosynthetic pathway is known (see, e.g., Ma, *et al.*, *Plant Cell Physiol* 50:2146-2161 (2009), which is incorporated by reference).

[0196] Yet another example of an APFL is one that is employed to increase berberine (an alkaloid) biosynthesis. An illustrative transcription factor that may be used for such an APFL

is CjWRK1 (from *Coptis japonica*). A nucleic acid encoding the transcription factor may be operably linked to an induced promoter such as pCYP719A1, which controls the expression of the canadine synthase from *Coptis japonica*. This biosynthetic pathway is known (see, e.g., Kato, *et al.*, *Plant Cell Physiol* 488–18 (2007), which is incorporated by reference).

5

E. Genetic Background of Plants in which An Artificial Feedback Loop is introduced

[0197] In some embodiments, the plant in which the polynucleotide encoding a transcription factor, linked to a promoter from a downstream gene where expression is driven
10 by the transcription factor, as described herein is expressed is a wild-type (*i.e.*, naturally occurring) plant. In some embodiments, the plant in which the polynucleotide encoding a transcription factor as described herein is expressed is a mutant plant. As used herein, a "mutant plant" includes a plant having any loss-of-function or gain-of-function mutation of any gene or genes of interest as well as a plant in which endogenous expression of any gene
15 or genes of interest is suppressed or decreased using known methodology (*e.g.*, by antisense, siRNA, microRNA, dsRNA, or sense suppression). For example, in some embodiments, levels of a gene expression product of a gene or gene of interest can be reduced using known technologies such as riboswitch techniques (see, *e.g.*, U.S. Patent Application Publication Nos. US20100286082, and US20110245326.)

20 [0198] In some embodiments, the plant in which the polynucleotide encoding a transcription factor as described herein is expressed is a plant having spatially modified gene expression of a lignin biosynthesis enzyme and/or a xylan biosynthesis enzyme, as described above. In some embodiments, the plant has been modified to have a reduced level of
25 expression of a lignin biosynthesis enzyme and/or a xylan biosynthesis enzyme at least in tissues other than xylem tissue, and further comprises an expression cassette comprising a polynucleotide encoding the lignin biosynthesis enzyme (*e.g.*, PAL, C4H, 4CL, HCT, C3'H, or CCR1) and/or a xylan biosynthesis enzyme (*e.g.*, IRX8, IRX14, IRX9, IRX7, IRX10, F8H, PARVUS, RWA1, RWA2, RWA3 or RWA4) operably linked to a heterologous vessel-specific promoter (*e.g.*, pVND1, pVND2, pVND3, pVND4, pVND5, pVND6, pVND7,
30 pVNI2, pREF4, or pRFR1).

F. Preparation of Recombinant Expression Vectors

[0199] Once the promoter sequence and the coding sequence for the gene of interest (*e.g.*, lignin biosynthesis enzyme, xylan biosynthesis enzyme, or transcription factor regulating the production of secondary cell wall) are obtained, the sequences can be used to prepare an expression cassette for expressing the gene of interest in a transgenic plant. Typically, plant transformation vectors include one or more cloned plant coding sequences (genomic or cDNA) encoding a protein of interest, such as a transcription factor, under the transcriptional control of 5' and 3' regulatory sequences. Vectors also typically comprise a dominant selectable marker. In typical embodiments, such plant transformation vectors also contain a promoter of interest (*e.g.*, a vessel-specific promoter as described herein or a promoter whose expression is regulated by a transcription factor regulating the production of secondary cell wall), a transcription initiation start site, an RNA processing signal (such as intron splice sites), a transcription termination site, and/or a polyadenylation signal.

[0200] The plant expression vectors may include RNA processing signals that may be positioned within, upstream or downstream of the coding sequence. In addition, the expression vectors may include regulatory sequences from the 3'-untranslated region of plant genes, *e.g.*, a 3' terminator region to increase mRNA stability of the mRNA, such as the PI-II terminator region of potato or the octopine or nopaline synthase 3' terminator regions.

[0201] Plant expression vectors routinely also include dominant selectable marker genes to allow for the ready selection of transformants. Such genes include those encoding antibiotic resistance genes (*e.g.*, resistance to hygromycin, kanamycin, bleomycin, G418, streptomycin or spectinomycin), herbicide resistance genes (*e.g.*, phosphinothricin acetyltransferase), and genes encoding positive selection enzymes (*e.g.* mannose isomerase).

[0202] Once an expression cassette comprising a polynucleotide encoding the lignin biosynthesis enzyme, xylan biosynthesis enzyme, or transcription factor regulating the production of secondary cell wall and operably linked to a promoter as described herein has been constructed, standard techniques may be used to introduce the polynucleotide into a plant in order to modify gene expression. *See, e.g.*, protocols described in Ammirato et al. (1984) Handbook of Plant Cell Culture--Crop Species. Macmillan Publ. Co. Shimamoto et al. (1989) Nature 338:274-276; Fromm et al. (1990) Bio/Technology 8:833-839; and Vasil et al. (1990) Bio/Technology 8:429-434.

[0203] Transformation and regeneration of plants is known in the art, and the selection of the most appropriate transformation technique will be determined by the practitioner.

Suitable methods may include, but are not limited to: electroporation of plant protoplasts; liposome-mediated transformation; polyethylene glycol (PEG) mediated transformation; transformation using viruses; micro-injection of plant cells; micro-projectile bombardment of plant cells; vacuum infiltration; and *Agrobacterium tumefaciens* mediated transformation.

5 Transformation means introducing a nucleotide sequence in a plant in a manner to cause stable or transient expression of the sequence. Examples of these methods in various plants include: U.S. Pat. Nos. 5,571,706; 5,677,175; 5,510,471; 5,750,386; 5,597,945; 5,589,615; 5,750,871; 5,268,526; 5,780,708; 5,538,880; 5,773,269; 5,736,369 and 5,610,042.

[0204] Following transformation, plants can be selected using a dominant selectable marker
10 incorporated into the transformation vector. Typically, such a marker will confer antibiotic or herbicide resistance on the transformed plants or the ability to grow on a specific substrate, and selection of transformants can be accomplished by exposing the plants to appropriate concentrations of the antibiotic, herbicide, or substrate.

[0205] The polynucleotides coding for a lignin biosynthesis enzyme, xylan biosynthesis
15 enzyme, or transcription factor regulating the production of secondary cell wall, as well as the polynucleotides comprising promoter sequences for vessel-specific promoters or promoters from downstream targets of a transcription factor regulating the production of secondary cell wall, can be obtained according to any method known in the art. Such methods can involve amplification reactions such as PCR and other hybridization-based reactions or can be
20 directly synthesized.

G. Plants in Which Gene Expression Can Be Modified

[0206] An expression cassette comprising a polynucleotide encoding the lignin
biosynthesis enzyme, xylan biosynthesis enzyme, or transcription factor regulating the
production of secondary cell wall and operably linked to a promoter, as described herein, can
25 be expressed in various kinds of plants. The plant may be a monocotyledonous plant or a dicotyledonous plant. In some embodiments of the invention, the plant is a green field plant. In some embodiments, the plant is a gymnosperm or conifer.

[0207] In some embodiments, the plant is a plant that is suitable for generating biomass. Examples of suitable plants include, but are not limited to, Arabidopsis, poplar, eucalyptus,
30 rice, corn, switchgrass, sorghum, millet, miscanthus, sugarcane, pine, alfalfa, wheat, soy, barley, turfgrass, tobacco, hemp, bamboo, rape, sunflower, willow, Jatropha, and Brachypodium.

[0208] In some embodiments, the plant into which the expression cassette is introduced is the same species of plant as the promoter and/or as the polynucleotide encoding lignin biosynthesis enzyme, xylan biosynthesis enzyme, or transcription factor (*e.g.*, a vessel-specific promoter, lignin biosynthesis enzyme, xylan biosynthesis enzyme, and/or transcription factor from *Arabidopsis* is expressed in an *Arabidopsis* plant). In some
5 embodiments, the plant into which the expression cassette is introduced is a different species of plant than the promoter and/or than the polynucleotide encoding lignin biosynthesis enzyme, xylan biosynthesis enzyme, or transcription factor (*e.g.*, a vessel-specific promoter, lignin biosynthesis enzyme, xylan biosynthesis enzyme, and/or transcription factor from
10 *Arabidopsis* is expressed in a poplar plant). *See, e.g., McCarthy et al., Plant Cell Physiol.* 51:1084-90 (2010); and *Zhong et al., Plant Physiol.* 152:1044-55 (2010).

H. Screening for Plants Having Modified Gene Expression

[0209] After transformed plants are selected, the plants or parts of the plants may be evaluated to determine whether the expression patterns of the gene or genes of interest have
15 been modified, *e.g.*, by evaluating the level of RNA or protein, by evaluating the lignin content, xylan content, and/or amount of secondary cell wall deposition in the plant or part of the plant, or by determining the amounts of soluble sugars that can be extracted from the plants. These analyses can be performed using any number of methods known in the art.

[0210] In some embodiments, plants are screened by evaluating the level of RNA or
20 protein. Methods of measuring RNA expression are known in the art and include, for example, PCR, northern analysis, reverse-transcriptase polymerase chain reaction (RT-PCR), and microarrays. Methods of measuring protein levels are also known in the art and include, for example, mass spectroscopy or antibody-based techniques such as ELISA, Western blotting, flow cytometry, immunofluorescence, and immunohistochemistry.

[0211] In some embodiments, plants are screened by evaluating lignin content, xylan
25 content, and/or amount of secondary cell wall deposition. Lignin content can be assessed, for example, by spectrophotometry, microscopy, klason lignin assays, acetyl-bromide reagent or by histochemical staining (*e.g.*, with phloroglucinol). Xylan content can be assessed, for example, by immunohistochemistry (*e.g.*, with LM10 monoclonal antibody). The amount of
30 secondary cell wall deposition can be assessed, for example, by histochemical staining (*e.g.*, phloroglucinol or Maule reagent) or enzymatic or chemical reaction (*e.g.*, polysaccharide hydrolysis or TFA hydrolysis).

IV. Methods of Using Plants Having Spatially Modified Gene Expression

[0212] Plants, parts of plants, or plant biomass material from plants having spatially modified gene expression of one of more of a lignin biosynthesis enzyme, xylan biosynthesis enzyme, and/or transcription factor that regulates secondary cell wall production can be used for a variety of methods. In some embodiments, the plants, parts of plants, or plant biomass material are used in a conversion reaction to generate an increased amount of bioenergy as compared to wild-type plants. For example, the plants, parts of plants, or plant biomass material can be used in a combustion reaction, gasification, pyrolysis, or polysaccharide hydrolysis (enzymatic or chemical). In some embodiments, the plants, parts of plants, or plant biomass material are used in a saccharification reaction, *e.g.*, enzymatic saccharification, to generate an increased amount of soluble sugars as compared to wild-type plants. In some embodiments, the plants, parts of plants, or plant biomass material are used to increase biomass yield or simplify downstream processing for wood industries (such as paper, pulping, and construction) as compared to wild-type plants. In some embodiments, the plants, parts of plants, or plant biomass material are used to increase the quality of wood for construction purposes.

[0213] In some embodiments, the modification of cell wall (composition or content) are used to increase stem/stalk strength to reduce lodging of cereals (wheat, barley, corn...) and seed loss.

[0214] Methods of conversion, for example biomass gasification, are known in the art. Briefly, in gasification plants or plant biomass material (*e.g.*, leaves and stems) are ground into small particles and enter the gasifier along with a controlled amount of air or oxygen and steam. The heat and pressure of the reaction break apart the chemical bonds of the biomass, forming syngas, which is subsequently cleaned to remove impurities such as sulfur, mercury, particulates, and trace materials. Syngas can then be converted to products such as ethanol or other biofuels.

[0215] Methods of enzymatic saccharification are also known in the art. Briefly, plants or plant biomass material (*e.g.*, leaves and stems) are optionally pre-treated with hot water or dilute acid, followed by enzymatic saccharification using a mixture of cellulose and beta-glucosidase in buffer and incubation of the plants or plant biomass material with the enzymatic mixture. Following incubation, the yield of the saccharification reaction can be readily determined by measuring the amount of reducing sugar released, using a standard method for sugar detection, *e.g.* the dinitrosalicylic acid method well known to those skilled

in the art. Plants engineered in accordance with the invention provide a higher sugar yield as compared to wild-type plants.

EXAMPLES

[0216] The following examples are provided to illustrate, but not limit the claimed invention.

Example 1: Reengineering secondary cell wall deposition in plants

[0217] This study pooled two approaches for overcoming cell wall recalcitrance and filling up fiber cells with cell wall polymers without altering plant development. The first approach allowed the reduction of lignin except in the vessels, while the second approach increased cell wall deposition specifically in woody tissues. This strategy of combining approaches uses synthetic biology to fine-tune lignin biosynthesis and to create new feedback loops to reengineer the control of secondary cell wall deposition.

Materials and Methods

Construction of plasmids

[0218] The protein-coding regions of the C4H (ref3) gene (AT2G30490), F5H (At4g36220), and CADc gene (AT3G19450) were amplified from *Arabidopsis thaliana* cDNA, and the 5' upstream region of 2756bp, which is from the initial site of translation for VND6 gene (At5g62380), was amplified as pVND6 from genomic DNA with appropriate primers (see Table 1).

Table 1. Primers used for plasmid construction and genotyping

pVND6-F3-KpnI	5'- <u>cccgggtacc</u> TCCTTTACGATGTTGTTATGGGTTA-3'
pVND6-R3-SpeI	5'- <u>cccgactagt</u> GTGTGCGAGACTTTGGATTTGATCTTTTTAATTTTA-3'
FY100908-C4h-GW-F	5'-ggggacaagtttgtaaaaaagcaggcttcATGGACCTCCTCTTGCTGGA-3'
FY100908-C4h-GW-R	5'-ggggaccactttgtacaagaaagctgggtcACAGTTCCTTGGTTTCATAACG-3'
DL-F5G3-At3g19450-GW	5'- ggggacaagtttgtaaaaaagcaggcttcATGGGAAGTGTAGAAGCAGGAGAA- 3'
DL-R5G3-At3g19450-	5'-ggggaccactttgtacaagaaagctgggtcGTTTGTAGTTGTTGCAGCCTCCTC-3'

GW	
FY081508-F5h-1-GW-F	5'- ggggacaagtttgtacaaaaaagcaggcttcATGGAGTCTT CTATATCACA A -3'
FY081508-F5h-1-GW-R	5'-ggggaccactttgtacaagaaagctgggtcAAGAGCACAGATGAGGCGCGT -3'
ref3-2F1	5'-TTC CGT ATC ATG TTC GAT AG-3'
ref3-2R1	5'-AAT GTC AAT TTC CCA AAA TC-3'
pcr-pVND6F1	5'-CAAATTGCCACATTGCAGAA-3'
pcr-REF3-R1	5'-CGACGAGATTACGGTGGTTGA-3'

[0219] The gateway fragment (Invitrogen) was introduced into pCAMBIA1390 and the VND6 promoter was cloned using KpnI-SpeI/AvrII sites, then the C4H and CADc genes were introduced into the expression vector through a gateway system to get the final expression vectors pCAMBIA1390-pVND6:C4H, pCAMBIA1390-pVND6:F5H, and pCAMBIA1390-pVND6:CADc.

Plant growth and transformation

[0220] Arabidopsis plants were grown in soil at 22°C with 8 hr of light daily (short-day condition) for 4-5 weeks and 16 hr of light daily (long-day condition) for 4-5 weeks.

10 The expression vector pCAMBIA1390-pVND6:C4H, pCAMBIA1390-pVND6:F5H, or pCAMBIA1390-pVND6:CADc was introduced into *Agrobacterium tumefaciens* strain GV3101 by electroporation, and was used to transfer Arabidopsis *f5h*, *cadc/d* homozygote *ref3-2* (*c4h* mutant) heterozygote, *f5h* homozygote and *cadc/d* homozygote mutant plants, respectively, using the floral dip method (Clough and Bent, 1998).

15 *Analysis of genotype of Arabidopsis plants*

[0221] Seeds of *ref3-2* heterozygote mutants were sowed, genomic DNA of the plants was extracted through the CTAB method, and genotypes were analyzed through PCR with primers ref3-2F1 and ref3-2R1 (see Table 1). PCR products were digested with HinfI. The expected PCR products are 188bp and 106 bp fragments for wild type plants, and a 294 bp fragment for the *ref3-2* homozygotes.

[0222] Transformants of pVND6:C4H were identified through PCR with primers pcr-pVND6F1 and pcr-REF3-R1. The PCR product is 238bp for the transformants. The PCR reactions above were carried out by using DyNAzyme DNA polymerase (Finnzymes, USA).

RNA isolation and cDNA synthesis

[0223] Total RNA was isolated using an RNeasy Plant Mini Kit (Qiagen, Valencia, CA) from the leaves of Arabidopsis plants under short day condition for 4 weeks. cDNA was synthesized using the Transcriptor High Fidelity cDNA Synthesis Kit (Roche Applied Science, Indianapolis, IN).

Microscopy analysis

[0224] To investigate the lignin content and anatomy of the cells of the stem, transverse sections were prepared from the base of the stems of mutant, wild-type and transgenic lines (when the plants were 30-35 cm high for healthy plants, 15-20 cm for mutant plants). The stem base of mature plants was embedded in 7% agarose before sectioning to a thickness of 100 μ m using a vibratome (Leica VT1000S). Sections were mounted in water and examined under bright field. Lignified cell walls were also visualized under UV illumination. Lignin is a UV absorber so lignified cell walls emitted blue autofluorescence under UV illumination. A 2% (w/v) solution of Phloroglucinol dissolved in a 2:1 mixture of ethanol and concentrated HCl was applied to the stem sections directly to detect all lignin (Adler, 1977). Stem sections were also stained with calcofluor, a specific dye for β -glucans such as cellulose to determine the general anatomy of the cells (Mori, 1996). Fresh sections were immersed in 0.5% calcofluor for 5 minutes, followed by 2 water washes of 5 minutes each to remove any excess of unbound calcofluor. Sections were immediately observed using a fluorescent microscope (Leica DM4000B). Images were registered using a Leica DC500 camera.

Preparation of alcohol insoluble residue (AIR)

[0225] Stems of plants were collected, dried and grinded into powder, then alcohol insoluble residue (AIR) was prepared according to Goubet et al. (2009). Grinded powder of stem was mixed with 1 mL 95% Ethanol and incubated at 100°C for 30 min. After centrifugation, the supernatant was removed and the pellets were washed with 1 mL 70% Ethanol for 2~3 times and dried completely.

Lignin measurement

[0226] 5 mg of AIR samples were analyzed for lignin assay through acetyl bromide methods (Fukushima, 2004). The AIR samples were mixed with 200 μ L acetone bromide solution (25% v/v acetyl bromide in glacial acetic acid) in 2 mL Eppendorf tube with screw lids, shaking at 600 rpm in 50°C for 2 hrs, then diluted to total volume of 1 mL with acetic acid. After centrifugation, 100 μ L of supernatant was transferred to a new tube and mixed

with 500 uL acetic acid, 300 uL 0.3M sodium hydroxide, and 100 uL hydroxylamine hydrochloride, respectively, then diluted to total volume of 2 mL with acetic acid. 360 uL of the solution was transferred to UV specific 96-well plates (Greiner, Monroe, NC) and absorbance at 280nm was read. The percentage of acetyl bromide soluble lignin (%ABSL) was calculated based on published extinction coefficients (Fukushima, 2004; Foster, 2010).

Saccharification and DNS assay

[0227] 5 mg of AIR samples was pretreated with 170 uL of water, diluted alkaline (1% NaOH, 30 min at 30°C, 30 min at 100°C) or diluted acid (1.2% H₂SO₄, 30 min at 30°C, 1 hr at 120°C). HCl or NaOH was added for neutralization for the last pretreatments, then the samples were added with 8 uL 5mg/mL tetracycline, 25 uL 1M citrate buffer pH 6.2, 2 uL of diluted enzyme mix (Novozyme enzymes NS50013 (cellulase) and NS50010 (beta-glucosidase), 1:10 and 1:100 dilutions in 0.1M citrate buffer pH 5.0, respectively), and diluted to a final volume of 500 ul with water. The samples were shaken at 850 rpm in 50°C for 24 hr. After saccharification, sugar amounts were analyzed through DNS assay. Glucose of 0, 0.125, 0.25, 0.5, 0.75, 1 and 2 mg/mL in citrate buffer pH 5.0 were used as standards. DNS reagent was added to samples and standards, incubated in 95°C for 10 min, then absorbance at 540 nm was read for the assay.

Analysis of the hemicellulose compositions

[0228] Approximately 5 mg of AIR was hydrolyzed in 1 ml of 2 M TFA for 1 h at 120 C. TFA was removed by drying under vacuum. Monosaccharide composition was subsequently determined by HPAEC-PAD of hydrolyzed material using a PA20 column (Dionex, Sunnyvale, CA) as described previously (Obro, 2004; Christensen, 2010). Monosaccharide standards included L-Fuc, L-Rha, L-Ara, D-Gal, D-Glc, D-Xyl, D-GalA and D-GlcA, and were obtained from Sigma. For verification of the response factors, a standard calibration was performed before analysis of each batch of samples.

Results

Characterization of vessel specific promoter pVND6

[0229] Integrity of the vessels is required for good development of plants due to the importance of the vessel tissue in transporting water and nutrients to photosynthetic organs. The VND-type transcription factors have been characterized as master regulators for vessel formation, suggesting they would have a vessel restricted expression pattern (Kubo et al., 2005). In order to correlate the spatiotemporal expression of these transcription factors with

lignin biosynthesis, the promoter pVND6 was used to complement CAD mutants (described in Sibout et al., 2005) (Fig. 20A). The redness disappearance of xylem and the restoration of the vessel integrity were the acceptance criteria to use this promoter.

[0230] In order to compare the strength of the promoter pVND6 to promoter pC4H, both promoters were used to complement an *f5h* mutant (Meyer et al., 1998). The activity of the promoters was compared by measuring the amount of sinapyl alcohol unit incorporated into the lignin using Maule staining as a readout (Fig. 20B). Cross-sections of stems from the lines expressing the F5H gene under the VND6 promoter showed a much lower redness after Maule staining than the one with pC4H. These results indicate that the accumulation of sinapyl alcohol in the lignin in pVND6::F5H lines was due to a lower and more restricted F5H activity as compared to pC4H::F5H lines, a finding which is in agreement with the *cadc/d* complementation described above (Fig. 20A).

Restriction of lignin biosynthesis

[0231] The lignin biosynthetic pathway is well characterized and loss of function of any of several genes of the lignin biosynthesis pathway results to deleterious growth effect and sterility. Therefore, controlling the expression of one of these genes should give the opportunity to control the production of monolignols. We selected the C4H gene, an early gene in the lignin biosynthesis pathway, as a target gene to control the flux of the pathway to produce the monolignols. In order to control the expression of C4H, we used the *ref3-2* mutant (Schillmiller et al., 2009) and transformed the heterozygote line, due to the sterility, with a binary vector containing the pVND6::C4H gene construct. Transformants were selected and genotyped for the homozygosity of the *ref3-2* allele. Interestingly, *ref3-2* homozygotes harboring the pVND6::C4H fragment, called "EngSCW1g" (engineered secondary cell wall 1st generation), did not show a growth difference as compared to Col0 wild-type plants grown at the same time. These transformed plants were able to generate a large rosette and a tall stem and were fertile (Fig. 16A). However, leaves from the transformed plants were purpled due to anthocyanin accumulation only in the vessel, in contrast to wild-type leaves that turned completely purpled under high light. This result demonstrates the restricted activity of the pVND6 promoter as compared to pC4H.

[0232] Analysis of lignin content of EngSCW1g plants via an acetyl-bromide method showed that lignin content in senesced stem approached approximately 2/3 of the lignin content of Col0 stem plants grown at the same time under the same conditions. In order to verify the lignin distribution in the stem, cross-sections of approximates 15-20 cm old stems

were analyzed using phloroglucinol and Maule staining methods. Cross-sections of the engineered lines showed a reduction of lignin staining in the interfascicular fibers as compared to wild-type plants expressing the C4H gene under the control of its native C4H promoter. In contrast to the homozygote *ref3-2* mutant, xylem tissues of the EngCW1g plants exhibited strong phloroglucinol staining and no vessel collapse, similar to the wild-type plants (Fig. 15B and Fig. 21).

Increase of cell wall deposition

[0233] The transcriptional network controlling secondary cell wall deposition in vessels and fibers has already been well investigated. Secondary cell wall deposition is controlled by two independent networks, although these two networks lead to the activation of the same groups of downstream secondary wall biosynthetic genes to regulate the synthesis of cellulose, hemicellulose and lignin. Several groups have showed that overexpressing a secondary cell wall transcription factor with the constitutively active 35S promoter in *Arabidopsis* generates ectopic secondary cell wall and lignification everywhere, including in elongating cells and photosynthetic tissues, which as a result inhibits plant growth (Zhong et al., 2008; Mitsuda et al., 2005; Goicoechea et al., 2005). Interestingly, even with restrained development, the plants exhibited enhanced secondary cell wall thickness in fiber cells (Zhong et al., 2008), suggesting that increasing the expression of a secondary cell wall transcription factor could be a route to increase cell wall deposition (and therefore increase biomass density).

[0234] Accordingly, we overexpressed NST1 cDNA in the EngCW1g plant with an IRX8 promoter. Because IRX8 is a gene that is downstream of (*i.e.*, under the control of) the NST1 transcription factor (Mitsuda et al., 2005; Zhong et al., 2010), this pIRX8::NST1 construct creates a positive feedback loop for overexpressing NST1 cDNA only in secondary cell wall tissues. EngCW1g plants were chosen for the transformation because the VND6 promoter is not a downstream target of NST1, and therefore the lignin biosynthesis under the control of pVND6 in EngCW1g should be disconnected from NST1 regulation. The generated plants, which were called "EngSCW2g" (engineered secondary cell wall 2nd generation), did not exhibit a growth difference when compared to Col0 and EngSCW1g plants grown at the same time. The EngSCW2g plants were able to generate a large rosette and tall stem and were fertile (Fig. 17A). Like EngSCW1g plants, leaves from the EngSCW2g lines were purpled due to anthocyanin accumulation only in the vessel, in contrast to wild-type leaves that turned completely purpled under high light. The verification of the expression of both NST1 genes (native and cDNA) was verified by semi-quantitative PCR and revealed that the native NST1

was expressed at the same level in wild-type, EngSCW1g, and EngSCW2g lines. However, only in the EngSCW2g lines was the expression of the new NST1 copy detected, resulting in a higher general expression level of the NST1 gene (native and cDNA) in the stem (Fig. 22).

[0235] In order to verify the effect of NST1 overexpression on cell wall deposition in stems, lignin distribution in the stem cross-sections of old stems was analyzed using a phloroglucinol staining method. Cross-sections of the EngSCW2g lines still showed a reduction of lignin staining in the interfascicular fibers as compared to wild-type, while xylem tissues exhibited strong phloroglucinol staining and no vessel collapse, similar to wild-type and EngSCW1g lines (Figs. 15B and 17B). Cell wall thickening was analyzed via transmission electron microscopy (TEM) on cross-sections from the base of xxx cm old stems. Intense thickening of cell wall in EngSCW2g lines compared to wild-type was observed in fiber cells from interfascicular fibers and xylem but not in vessel (Figs. 18 and 23), which is in agreement with the overexpression of the NST transcription factors (Zhong et al., 2008). In wild-type stem cross-sections, the usual 4 distinct layers (S1, S2 and S3 and the middle lamella) were observed, in contrast to EngSCW2g lines where additional layers with different intensity were observed, which almost fill up the entire cell space.

Fine-tuning secondary cell wall deposition for bioenergy

[0236] Analyses of cell wall cross-sections from EngSCW2g plants with gold-labeled CBM revealed that the extra cell wall layers contained cellulose, suggesting that the amount of cellulose had been increased. In order to verify an enhancement of cellulose, a complete polysaccharide hydrolysis was performed using H₂SO₄ (Suilter et al 2008, Technical report NREL/TP-510-4218) on senesced stems from EngSCW2g. The amount of glucose and other sugar released from the stem cell wall was similar among wild type, EngSCW1g, and EngSCW2g lines. The amount of xylose and glucuronic acid was also increased, suggesting that hemicellulose deposition was also enhanced in these plants. The composition analysis of hemicellulose by trifluoroacetic acid (TFA) hydrolysis of mature stems from the EngSCW1g and EngSCW2g lines did not exhibit major differences as compared to wild-type plants grown at the same time (Fig. 24).

[0237] To analyze the saccharification efficiency of the EngSCW2g lines, 5 mg of ball-milled stems from EngSCW2g lines were subjected to two different mild pre-treatments, hot-water and dilute alkaline, followed by a saccharification kinetic. After each of the pre-treatments, glucose was released from the stem in presence of a cellulase cocktail much faster, and 2 to 3 times higher for EngSCW2g plants than for the control plants, when

alkaline and hot-water pretreatments respectively were performed prior to a 120 hr saccharification (Fig. 19A-B).

[0238] Saccharification improvement was also observed with the EngSCW1g lines; for those plants, sugar hydrolyzed in the presence of the same amount of cellulase after hot water or dilute alkaline pre-treatments was 2.3 and 1.5 fold better than a control plant after hot water or dilute alkaline pre-treatment, respectively. The overexpression of the NST1 transcription factor in EngSCW2g lines increased cell wall deposition but did not reduce saccharification efficiency, which translated into an higher amount of glucose released by this line due to the increased polysaccharide content as compared to the parental EngSCW1g line.

10 *Analysis of additional ref3-2 mutant plants that are modified to express C4H*

[0239] *Ref3-2* mutant plants were also engineered to express C4H using either promoter pREF4 or pRFR1. Mutant plants were modified to contain either pREF4::C4H or pRFR1::C4H to express C4H. Plant growth and phenotype of engineered cell wall plant lines were analyzed. Figure 29 shows photographs of the plants. Growth was restored in mutant plants transformed with either construct. Lignin distribution in the plants is shown in Figure 30. The results show that lignin was produced in the vessels, but reduced in fibers in the engineered plants, which resulted in >35% reduction of the total lignin compared to wild type plants without affecting plant growth. Figure 31 provides data showing the saccharification efficiency of the engineered lines. These results show that the reduction of lignin in fibers greatly improved saccharification efficiency. Therefore, these results demonstrate that both promoters pREF4 and pRFR1 can be used to engineer plants with low lignin similar to the "EngSCW1g" plants (*ref3-2* complemented with pVND6::C4H construct) and be used as genetic background for the secondary cell wall positive feed back loop.

25 **Example 2. Positive feedback loops engineered in Arabidopsis (dicot) and Brachypodium (monocot)**

[0240] Figure 27 illustrates a cell wall deposition positive feed back loop. Cell wall densification is based on the creation of an artificial positive feedback loop to enhance the expression of fiber-specific transcription factor. It is created by the expression of a new copy of a fiber specific transcription factor (e.g., NST1) under the control of a downstream-induced promoter from xylan or cellulose biosynthesis. This approach is compatible with xylan and lignin engineering strategies.

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[0241] Figure 31A shows UV images of stem cross-sections from wildtype *Arabidopsis* (dicotyledon) and wiltype *Arabidopsis* genetically modified to contain a pCesA4::NST1 expression construct. The creation of a positive feedback loop with the secondary cell wall cellulose promoter (pCesA4) and the secondary cell wall transcription factor (NST1) enhanced secondary cell wall deposition in fiber cells.

[0242] Figure 31B shows UV images of stem cross-sections from wildtype *Brachypodium* (monocotyledon) and wiltype *Brachypodium* genetically modified to contain a pAtIRX8::AtNST1 expression construct. The creation of a positive feedback loop with the secondary cell wall cellulose promoter (pAtIRX8) and the secondary cell wall transcription factor (AtNST1) enhanced secondary cell wall deposition in Brachypodium.

[0243] This example demonstrated that this pathway is conserved in both monocots and dicots and that positive feedback loop could be generated to enhance secondary cell wall deposition.

Example 3. Engineering a xylan biosynthesis enzyme

[0244] *Arabidopsis* mutants *irx7-1* (At2g28110, salk_120296), *irx8-1* (At5g54690, salk_008642), *irx9-1* (At2g37090, salk_058238), *irx9-2* (salk_057033C), *parvus* (At1g19300, CS16279) were obtained from *Arabidopsis* Biological Resource Center. The wild-type *IRX7*, *IRX8*, *IRX9*, and *PARVUS* genes were cloned into Gateway entry clones and recombined into Gateway destination vectors with the pVND6 or pVND7 promoters as described above for the lignin biosynthesis genes.

[0245] The expression vector pCAMBIA1390-pVND6:IRX7, pCAMBIA1390-pVND7:IRX7, pCAMBIA1390-pVND6:IRX8, pCAMBIA1390-pVND7:IRX8, pCAMBIA1390-pVND6:IRX9, pCAMBIA1390-pVND7:IRX9, pCAMBIA1390-pVND6:PARVUS, pCAMBIA1390-pVND7:PARVUS were introduced into *Agrobacterium tumefaciens* strain GV3101 by electroporation. Constructs expressing IRX7, IRX8, IRX9, and PARVUS were used to transform *Arabidopsis* heterozygote mutant plants (*irx7-1*, *irx8-1*, *irx9-1* and *parvus*, respectively) using the floral dip method (Clough and Bent, 1998). Constructs expressing IRX9 were also used to transform homozygous mutants of *irx9-2*.

[0246] Seeds of the transformed *irx7*, *irx8*, *parvus*, *irx9-1*, and *irx9-2* plants were planted on growth medium supplemented with hygromycin. Hygromycin resistant plants were

recovered and transferred to soil. The plants showed a healthy growth phenotype unlike the untransformed homozygous mutants, which were clearly affected in growth.

[0247] Transformed *irx7*, *irx8*, *irx9-2*, *parvus*, and *irx9-1* mutants were selected. The recovered, transformed mutants were characterized by PCR to ensure their homozygous phenotype with respect to the original mutations, and to ensure the presence of the pVND6 or pVND7 driven transgenes. The growth of the plants was compared with that of wild type and homozygous mutants, and their content of xylan determined by sugar composition analysis of inflorescence stems. Lignin was determined by acetyl bromide method. The localization of xylan deposition was determined by immunofluorescence microscopy using LM10 antibody and deposition of lignin by microscopy and determination of autofluorescence under UV illumination and Phloroglucinol staining. Saccharification was determined as described above.

[0248] Figure 33 provides data demonstrating that mutants in the *IRX7*, *IRX8* or *IRX9* genes exhibited strong growth reduction. Transformation of the mutants with constructs where the wild type version of the mutated gene was driven by *pVND6* or *pVND7* promoter restored the growth. Similar results were obtained with *pVND6::IRX9* and *pVND7::IRX7*.

[0249] Figure 34 provides data showing growth of offspring of four individual transformants made by transforming *irx7* mutant with the *pVND7::IRX7* construct. Growth was quantified by measuring rosette diameter. Two of the plant lines grew identically to wild type (Col0), while one plant line grew slightly better than the wildtype plant and for one plant, growth was only partially restored.

[0250] Figure 35 provides data showing growth of offspring of two individual transformants made by transforming *irx9* mutant with the *pVND7::IRX9* construct. Growth was quantified by measuring rosette diameter. The transformed plant lines grew identically to wild type (Col0). Similar results were obtained with plants transformed with *pVND6::IRX9*.

[0251] Figure 36 provides data showing an analysis of non-cellulosic monosaccharide composition of cell walls prepared from four individual transformants made by transforming *irx7* mutant with the *pVND7::IRX7* construct. All the transformants still exhibited the low xylan content of the original *irx7* mutant in spite of the restored growth.

[0252] Figure 37 provides data showing an analysis of non-cellulosic monosaccharide composition of cell walls prepared from offspring of four individual transformants made by

transforming *irx8* mutant with the *pVND6::IRX8* construct. All the transformants still exhibited the low xylan content of the original *irx8* mutant in spite of the restored growth.

[0253] Figure 38 provides data showing an analysis of non-cellulosic monosaccharide composition of stem cell walls prepared from offspring of four individual transformants made by transforming *irx9* mutant with the *pVND7::IRX9* construct and two individual transformants with the *pVND6::IRX9* construct. All the transformants still exhibited the low xylan content of the original *irx9* mutant in spite of the restored growth.

[0254] Figure 39 provides data showing a saccharification analysis of cell walls prepared from offspring of two individual transformants made by transforming *irx9* mutant with the *pVND6::IRX9* construct and three individual transformants made by transforming *irx9* mutant with the *pVND7::IRX9* construct. All the transformants exhibited improved saccharification similar to the original *irx9* mutant in spite of the restored growth.

Example 4. Generation of wax-APFL in epidemic cells and conservation across species.

[0255] Waxes are highly energetic and contain large amounts of long chain alkanes and fatty acids that have potential fuel applications. Therefore, using the wax-APFL to generate plants capable to produce and accumulate large amount of waxes in non-essential tissues such as pith and fiber in stems offer new opportunities generate bioenergy crops with high energy density that are also water use efficient.

[0256] Figure 28 illustrates an artificial positive feed back loop for wax deposition.

[0257] This example employed *Arabidopsis* as a model plant to develop the wax-APFL to increase wax biosynthesis and accumulation in epidermis cells. Eight DNA constructs were designed to create a wax AFPL in epidermal cells, which produce some wax. These constructs were generated by using pAtCER1 or pAtWBC11 as promoters to express AtSHN1(NP_172988) from *Arabidopsis* and selected homologs OsSHN1(NP_001046226), BdSHN1 (XP_003563662) or SmSHN1(XP_002969836) from Rice, Brachypodium and Selaginella respectively. All constructs were transferred individually to wildtype *Arabidopsis* using *Agrobacterium* transformation. For each wax-APFL, several transgenic plants were recovered.

[0258] In *Arabidopsis*, as in many plant species, wax biosynthesis occurs principally in epidermic cells from leaves and stems. It has also been reported by several studies that plants over-expressing SHN genes using constitutive or chemically induced -promoters resulted in

shiny phenotype of the leaves or/and stem surfaces, which was attributed to modifications of wax deposition or/and composition (McNevin et al 1993; Broun et al 2004; Kannangara et al 2007; Shi et al. 2011). Visual analysis of the *Arabidopsis* plant transformed with the different constructs showed increased shininess of the leaves (Figure 40).

- 5 [0259] Additional analyses are performed on homozygous lines, including composition analysis of leaf and stem epidermal waxes. Plant development, additional assessment of shininess of leaf epidermis, chlorophyll leaching assays, wax accumulation and composition analysis, gene expression analysis and biological impact on drought stress and water losses are the primary criteria used to characterize the wax-APFL in plants. The chlorophyll
- 10 leaching assays is a general assay to indentify modification of the cuticle permeability to ethanol and is performed by monitoring the chlorophyll extraction on intact leaves in presence of ethanol (Aharoni *et al.*, *Plant Cell* 2004 *supra*; Seo *et al*, *Plant Cell* 2011, *supra*). Epicuticular wax accumulation and composition are analyzed after being extracted by short immersing of whole leaf or stem into chloroform containing some n-triacontane as standard.
- 15 The general composition the extracts are pre-analyzed by TLC plates using hexane:ethyl-ether:acetic-acid at 90:7.5:1 solvent system and derivatized with N,Obis (trimethylsilyl)trifluoroacetamide):trimethylchlorosilane at 99:1 for GC/MS analysis (Aharoni *et al. Plant Cell*, 2004, *supra*; Kannangara *et al.*, *Plant Cell*, 2007, *supra*). In order to evaluate the impact of enhanced wax deposition on plant water use efficiency, water loss
- 20 assays are performed on detached leaves by monitoring weight losses. Finally, the impact of wax deposition modification on plant drought stress tolerance are performed by plant survival counts of 5-6 weeks old plants after 7-15 days dehydration period followed by 1 week of watering recovery period.

Discussion

- 25 [0260] Modifying lignin content has always been a challenge in crops or trees, since the more severe the reduction is, the more biomass yields are affected. This reduction is also often associated with a loss of integrity of the vessel tissues that are responsible for water and nutrient transport and distribution from the root into the aboveground organs. Lignin is one of the main inhibitory factors for efficient enzymatic hydrolysis of plant cell wall
- 30 polysaccharides. Therefore, our strategy focused on reducing lignin in most tissues except vessels (in order to maintain vessel integrity) and on the disconnection of lignin biosynthesis from key secondary cell wall transcription factor switches in order to manipulate the expression of the transcription factors without affecting lignin deposition.

[0261] Our strategy to reengineer secondary cell wall biosynthesis demonstrated that we can reduce the lignin content and increase cell wall thickening in woody tissues without altering plant growth. Replacing the promoter of a gene controlling an essential step in the lignin biosynthesis by another one with a more restricted spatiotemporal expression profile gives better control of the lignin deposition than silencing approaches alone. This fine-tuning avoids the reduction of lignin deposition in every tissue and allows keeping it in essential tissues such as vessels, in contrast to silencing approaches that affect every tissue and therefore limit the power of such a strategy. The use of the pVND6 promoter to control the activity of C4H allowed a partial disconnection of the lignin biosynthesis from the general transcription factor network controlling secondary cell wall deposition in fiber cells and permitted for the first time to increase polysaccharide deposition without over-lignification. In order to increase secondary cell wall deposition only in woody tissue with a self-induction, we generated an artificial positive feedback loop using the pIRX8 promoter to express a second copy of the master transcription factor NST1. This promoter is specifically active in tissue producing secondary cell wall and is already under the control of the NST1 transcription factor in fiber cells. Therefore, such a chimeric gene allowed the over-expression of NST1 by self-induction, increasing as well the expression of downstream target genes involved in polysaccharide biosynthesis. In addition, using a downstream promoter of NST1 to express a new copy of itself may have increased the time-dependent expression of the NST1 transcription factor, therefore increasing the time of secondary cell wall deposition in fiber cells, which consequently increases cell wall thickness.

[0262] To our knowledge, only one artificial negative feedback loop has been generated in plants to regulate a developmental process, and it corresponds to the delay of senescence (Gan and Amasino, *Science* 1995). This strategy corresponds to the expression of the IPT gene encoding for an isopentenyltransferase at the beginning of the senescencing process using an early senescence induced promoter (pSAG12) in order to produce cytokinin specifically at that stage. This hormone is known to repress senescencing processes and keep the plants photosynthetically active much longer (Gan and Amasino, *Science* 1995). Due to the conservation of the regulatory mechanism and gene network of the senescence processes across species, and in particular the delay of senescencing processes by the hormone cytokinin, this synthetic construct was transferred into various crops (grasses and dicots) and could improve biomass yields due to an increase life time of the plant (McCabe et al., 2001; Lin et al., *Acta Botanica Sinica* 2002, 44:1333-1338; Robson et al., 2004; Li et al., 2004;

Swartzberg et al., 2006; Calderini et al., 2007; Li et al., *Plant Physiology* 2010; and Chen et al., *Molecular Breeding* 2001).

[0263] Secondary cell wall biosynthesis falls in the same category of conserved regulatory networks, since this biological process is well conserved within vascular plants (Zhong et al., 5 2010). For example, transcriptional networks and genes involved in secondary cell wall biosynthesis are well conserved. The conservation of this network allowed us the utilization of the model plant *Arabidopsis*, allowing rapid testing and robustness of this approach.

Because increased polysaccharide content has multiple applications from bioenergy to the paper industry, including forage crops, the transferability of this strategy need to be versatile.

10 The approach described herein should be compatible and rapidly transferable from model species to bioenergy crops (dicots and monocots). It has previously been shown that overexpressing secondary cell wall transcription factors across species results in similar phenotypes and functions, suggesting that promoter regulatory elements are also well conserved. See, e.g., Shen et al., 2009 *Bioenerg. Res* 2:217-232; Zhong et al., 2010 *Plant* 15 *Physiol* 152:1044-1055; Goicoechea et al 2005 *Plant J* 43:553-567; Franke et al., 2000, *Plant J.* 22:223-234. Therefore, the genome sequence of the target crop should not be required and the cassette promoter (e.g., pIRX5) and the transcription factor (e.g., NST1) from another species, such as *Arabidopsis* or a crop-related species, could be used to transform the target plant.

20 [0264] In contrast to yeast, *E. coli*, *Physcomitrella*, and a few other species, promoter replacement by *in vivo* recombination in plants still has to be developed; therefore, in order to manipulate tissue specific lignin deposition, mutants are required. Natural loss of function mutants in essential genes in the lignin biosynthetic pathway are poorly available in crops due to the deleterious effects of mutations. In addition, tissue/cell specific gene expression 25 inhibition has not yet been developed in plants. Therefore, general silencing strategies are regularly used to modify gene expression in order to reduce enzymatic activity in crops, which at least requires EST sequences of genes involved in the targeted biosynthesis pathway. One concern with the lignin biosynthesis pathway is that compromises between the gene repression level, plant health, and desired phenotype are often conflicting. For example, 30 the improvement in saccharification by the repression of genes involved in the monolignol biosynthesis very often affects vessel integrity, therefore affecting water and nutrient transport and consequently plant growth. In order to transfer the presented technology to crops, the degeneracy of the genetic code (flexibility of the codon usage) could be used to generate silent resistant lignin genes that would be expressed with a vessel specific promoter

from *Arabidopsis* or related species of the target crop together with a silencing construct to reduce or eliminate the expression of the corresponding native gene. For example, expressing in poplar a different 4CL encoding sequence with a vessel specific promoter such as VND6 would restore the growth and biomass yield of a 4CL antisense lines (Kitin et al., 2010; 5 Voelker et al., 2010) and retain good saccharification efficiency. Alternatively, strategies that could bypass the defective enzymatic steps could be exploited. For example, the SmF5H gene from *Selaginella* could be expressed with a vessel specific promoter in a C3H RNAi-expressing poplar to restore the integrity of vessel and normal plant growth (Coleman et al., 2008a, 2008b). This SmF5H gene was recently shown in *Arabidopsis* to be able to restore the 10 growth of HCT and C3H deficient mutants (Li et al., 2010 *Plant Cell* 22:1620-1632) and lignin mutants lacking the ability to produce p-coumaroyl shikimate and to meta-hydroxylate the p-coumaroyl shikimate respectively, which are essential steps in the lignin biosynthesis (Weng et al 2010). Similarly to this SmF5H strategy, both enzymatic steps converting phenylalanine into p-coumaric acid could be bypassed by using a tyrosine ammonia lyase 15 (TAL) gene that converts tyrosine into p-hydroxycoumaric acid.

[0265] In summary, we have demonstrated that two approaches, one to increase cell biomass density and one to restrict lignin biosynthesis into essential tissue containing the vessels, were compatible and allowed the generation of healthy plants with a large amount of non-recalcitrant cell wall, allowing efficient enzymatic conversion into fermentable sugar 20 without severe pre-treatments. These approaches open new doors for crop optimization and should benefit the lignocellulosic biofuel, paper and forage industries.

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[0284] It is understood that the examples and embodiments described herein are for
20 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 scope of the appended claims. All publications, patents, accession
numbers, and patent applications cited herein are hereby incorporated by reference in their
entirety for all purposes.

25

**Illustrative genes involved in wax/cutin biosynthesis: including accession numbers and
synonymous gene designations.**

AtCER1: At1g02205: Aldehyde decarbonylase

AtCER2: VC2: At4g24510: BAHD-type acyl-transferase

30 AtCER3: WAX2: At5g57800: Sterol desaturase

AtCER4: FAR3: At4g33790: Fatty acyl-CoA reductase

AtCER5: WBC12: ABCG12: At1g51500: ABC transporter

AtCER6: CUT1: KCS6: At1g68530: Very long chain fatty acid condensing enzyme

AtCER10: ECR: At3g55360: Enoyl-CoA reductase

5 AtWSD1: At5g37300: Wax ester synthase

AtMAH1: CYP96A15: At1g57750: Mid Chain alkane hydrolase

AtWBC11: ABCG11: DSO: COF1: At1g17840: ABC transporter

AtKCS1: At1g01120: Very long chain fatty acid condensing enzyme

AtKCS2: DAISY: At1g04220: Very long chain fatty acid condensing enzyme

10 AtFATB: At1g08510: Acyl Carrier

AtLACS1: At2g47240: Long chain acyl-CoA synthase

AtLACS2: At1g49430: Long chain acyl-CoA synthase

AtCYP86A4: At1g01600: Cytochrome P450-dependent fatty acid hydroxylase

AtCYP86A7: At1g63710: Cytochrome P450-dependent fatty acid hydroxylase

15 AtLCR: CYP86A5: At2g45970: Cytochrome P450-dependent fatty acid hydroxylase

AtKCS10: FDH: At2g26250: Very long chain fatty acid condensing enzyme

AtCER60: KCS5: At1g25450: Very long chain fatty acid condensing enzyme

ILLUSTRATIVE SEQUENCES

20 **SEQ ID NO:1**

Arabidopsis thaliana PAL1 nucleic acid (At2g37040) NM_129260

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 15 tccattccaatatgtaa

SEQ ID NO:2

Arabidopsis thaliana PAL1 protein (At2g37040) NP_181241

MEINGAHKSNNGGVDAMLCGGDIKTKNMVINAEDPLNWGAAAEQMKGSHLDEVK
 20 RMVAEFRKPVVNLGGETLTIGQVAAISTIGNSVKVELSETARAGVNASSDWVMESM
 NKGTDSYGVTTGFGATSHRRTKNGVALQKELIRFLNAGIFGSKETSHTLPHSATRA
 AMLVRINTLLQGFSGIRFEILEAITSFLNINNITPSLPLRGTITASGDLVPLSYIAGLLTGR
 PNSKATGPNGEALTAEEAFKLAGISSGFFDLQPKEGLALVNGTAVGSGMASMVLFT
 NVLSVLAEILSAVFAEVMMSGKPEFTDHLTHRLKHHPGQIEAAIMEHILDGSSYMKL
 25 AQKLHEMDPLQKPKQDRYALRTSPQWLGPQIEVIRYATKSIEREINSVNDNPLIDVSR
 NKAIHGGNFQGTPIGVSMNTRLAIAAIGKLMFAQFSELVNDFYNNGLPSNLTASRN
 PSLDYGFKGAEIAMASYCSELQYLANPVTSHVQSAEQHNQDVNSLGLISSRKTSEAV
 DILKLMSTTFLVAICQAVDLRHLEENLRQTVKNTVSQVAKKVLTTGVNGELHPSRFC
 EKDLLKVVREQVYTYADDPCSATYPLIQKLRQVIVDHALINGESEKNAVTSIFHKIG
 30 AFEEELKAVLPKEVEAARAAYDNGTSAIPNRIKECRSYPLYRFVREELGTELLTGEKV
 TSPGEEFDKVFTAICEGKIIDPMMECLNEWNGAPIPIC

SEQ ID NO:3

Arabidopsis thaliana C4H nucleic acid (At2g30490) NM_128601

35 atggacctccttctgctggagaagctttaatcgccgctctcgtggcgggtgattctcgcacgggtgattcaagctccgcggcaagaat
 tgaagctacctccaggtcctataccaattccgatctcggaaactggctcaagtcggagatgatctcaaccaccgtaatctcgtcgatta
 cgctaagaaatcggcgatctctcctcctccgatgggtcagcgaacctaagtcgctcctcaccggatcaacaaagggaagtgt
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 tacggcgagcattggaggaagatgagaagaatcatgacgggtccttctcaccacaagttgtcaacagaatcgtaaggtggga
 40 gtttgaagcagctagtgttgaagatgtaagaagaatccagattctgctacgaaaggaatcgtgttgaggaaacgtttcaattgatg
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 gaagtcgattagctcagagctttgagtataactatggagatttcattcctatccttagaccattcctcagaggctattgaaagattgtcaag
 atgtgaaagatcgaagaatcgtctttcaagaagtaactttgtgatgagaggaagcaaatcgcgagttcaagcctacaggtagtgaa
 gattgaaatgtgccattgatcacatccttgaagctgagcagaaggagaaatcaacgaggacaatgttctttacatcgtcgagaacatc
 45 aatgtcgccgctgattgagacaacattgtggtctatcgagtggggaattgcagagctagtgaaccatcctgaaatccagagtaagctaag
 gaacgaactgcacagttctggaccgggtgtgcaagtcaccgagcctgatctcacaacttcataccttcaagctgtggttaagg
 agactctcgtctgagaatggcgattctcctcctcgtgctccatgaacctccatgatgcgaagctcgtggctacgatacccagcag
 aaagcaaaatccttgaatgcttgggtgctagcaacaacccaacagctggaagaagcctgaagagtttagaccagagaggttctt
 gaagaagaatcgcagctggaagctaaccgtaatgacttcaggtatgtccatttgggtgtggacgtcgaagctgtcccgggattatattg
 50 gcattgctattttggggatcaccattggtaggtggtccagaactcagcttctcctcctccaggacagctaaagtggatactagt
 agaaggtggacaatcagcttcacatccttaaccactccataatcgttatgaaaccaaggaactgtaa

SEQ ID NO:4

Arabidopsis thaliana C4H protein (At2g30490) NP_180607

MDLLLLLEKSLIAVFVAVILATVISKLRGKKLKLPPIPIPIFGNWLQVGDDLNHRNLV
 DYAKKFGDLFLLRMGQRNLVVSPPDLTKEVLLTQGVVEFGSRTRNVVFDIFTGKGQ
 5 DMVFTVYGEHWRKMRRIMTVPFFTNKVVQQNREGWEFEAASVVEDVKKNPDSAT
 KGIVLRKRLQLMMYNNMFRIMFDRRFESDDPLFLRLKALNGERSRLAQSFEYNYG
 DFIPILRPFLRGYLKICQDVKDRRIALFKKYFVDERKQIASSKPTGSEGLKCAIDHILEA
 EQKGEINEDNVLYIVENINVAAIETTLWSIEWGIAELVNHPEIQSKLRNELD TVLGPV
 QVTEPDLHKLPLYLQAVVKETLRLRMAIPLLPHMNLHDAKLAGYDIPAESKILVNA
 10 WWLANNPNSWKKPEEFRPERFFEEESHVEANGNDFRYVVPFGVGRRSCPGIILALPILG
 ITIGRMVQNFELLPPPQSKVDTSEKGGQFSLHILNHSIIVMKPRNC

SEQ ID NO:5

Arabidopsis thaliana 4CL2 nucleic acid (At3g21240) NM_113019

15 atgacgacacaagatgtgatagtcfaatgatcagaatgatcagaaacagtgtagtaatgacgtcatttccgatcgagattgcctgatata
 acatccctaaccactcccactcccagactacatcttcgaaaatactcagagttccgctgaagccatgcttgatcaacggctccaccg
 gcgagatatacctacgccgatgtccacgtaacatctcggaaactcggcggcttcataacctcggcgtgaagcaacacgacgt
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 20 aacctcaaaaacgacggcgtttgatcgtcaccaccgactccgacgccatccccgaaaactgcctccgtttctccgagttaactcagtc
 cgaagaaccacgagtgactcaataccggagaagattcggcagaagacgtcgtggcgttctttctcatccggcacgacgggtctc
 cccaaaggagtgtatgtaacacacaaaggtctagtcacgagcgtggcgcagcaagtcgacggcgagaatccgaatcttacttcaac
 agagacgacgtgatcctctgtgcttgcctatgttccatatatacgtctcaactccatcatgctctgtagctcagagttggtgccacgatc
 ttgataatgcctaagttcgaatcactctctttagagcagatacaaaaggtgtaaagtcacgggtggctatggctggtccaccgatcgttt
 25 agctatcgcaagtcgcccggagacggagaagtagctgagctcggtaggatggtaagtcgagcagctcctcttggtaaggag
 cttgaagatgctattagtgtaagttcctaacgcaagcttggcagggctatgggatgacagaagcaggtccgggtgctagcaatgct
 gttagggttctaaagagccgtttccagtgaaagtcaggagcatgtggtacgggtggtgaggaacgccgagatgaagataactgatcca
 gacacaggagattcttgcctaggaacaaaccggcgaatatgcatccgtggcaaccaaatcatgaaaggctatctcaatgaccctt
 ggccacggcatcgacgatcgataaagatggttggctcacactggagacgtcggattatcgatgatgacgacgagcttttcattgtgg
 30 atagattgaaagaactcatcaagtacaaaggattcaagtggtccagctgagctagagctctcctcataggtcatccagaaatcaatg
 atgttgctgctgcgccatgaaggaagaagatgctggtgaggtcctgttgcgtttgtggtgagatcgaagattcaaatatccgaag
 atgaaatcaagcaattcgtgtcaaacaggtgtgtttataagagaatcaacaaggttctcactgactctattcctaaagctccatcag
 ggaagatattgaggaaggatctaagagcaagactagcaaatggattaatgaactag

35 SEQ ID NO:6

Arabidopsis thaliana 4CL2 protein (At3g21240) NP_188761

MTTQDVIVNDQNDQKQCSNDVIFRSRLPDIYIPNHLPLHDYIFENISEFAAKPCLINGP
 TGEVYTYADVHVTSRKLAAAGLHNLGVKQHDVVMILLPNSPEVVLTFLLAASFIAIT
 SANPFFTPAEISKQAKASAAKLIVTQSRVYVDKIKNLQNDGVLIVTTDSDAIPENCLRFS
 40 ELTQSEEPVDSIPEKISPEDVVALPFSSGTTGLPKGVMLTHKGLVTSVAQQVDGENP
 NLYFNRRDDVILCVLPMFHIALNSIMLCSLRVGATILIMPKFEITLLLEQIQRCKVTVA
 MVVPPIVLAIKSPETEKYDLSSVRMVKSGAAPLGKELEDAISAKFPNAKLGGQYGM
 TEAGPVLAMSLGFAKEPFPVKSGACGTVVRNAEMKILDPDTGDSLPRNKPGEICIRG
 NQIMKGYLNDPLATASTIDKDGWLHTGDVGFIDDDDELFIVDRLKELIKYKGFQVAP
 45 AELESLLIGHPEINDVAVVAMKEEDAGEVPVAFVVRSKDSNISEDEIKQFVSKQVVVFY
 KRINKVFFTD SIPKAPSGKILRKDLRANGLMN

SEQ ID NO:7

Arabidopsis thaliana HCT nucleic acid (At5g48930) NM_124270

50 atgaaaattaacatcagagattccaccatggctccggcctgccaccgagacaccaatcactaatcttggactccaacgtcgacctgtc
 atccccagattccatacccctagtgtctacttctacagaccaccggcgcttccaatttcttgaccctcaggtcatgaaggaagctcttc
 caaagcccttgcctctttaccctatggctggtcgttgaagagagacgatgatggtcgtattgagatcgattgtaacggctgctggttc

tcttcgttggtgctgatactccttctgttatcgcgatgatttgggtatttgcctacccttaactcctcgcagcttattcccgaagttgatcactc
 cgctggcattcactcttcccgtctcgtttgcaggtgactttcttaaatgtgggggagcttcacttgggggtgggatgcaacatcacgc
 ggagatggttctctggtcttcttcaacacatggtctgatggctcgtggtcttgacctaacctccaccttccattgatcgaaca
 ctctccgagctagggaccgccacagcctgctttcatcatggtgaatcagcctgcaccaagtatgaagatacctcttgatccgtcta
 5 aatcaggacctgagaatacactgtctctatattcaaaataacacagaccagcttgttgccttaaggcgaaatccaaggaggatggga
 acactgtcagctacagctcatacagagatgttggcagggcatgtgtggagatcagtggaagggcgagggctccaacgaccaa
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 acaccattggctgttgcaggggatttctatctaaagcaacatggtatgtcgcaggacagattcatgatttctggttcgatggatgataa
 ctatctgaggtcagctcttgactacctggagatgcagcctgatctgcagccctgtccgcggtgcacatacctacaagtgcccaatttg
 10 ggaatcacaagctgggttagattacctattatgatgcagacttgggtggggctgcctctatctttatgggacctggtggaattccatacga
 gggtttcttttgcctaccaagtctactaatgatggcagcttatccgttgcctcctcaatctgaacacatgaaactggttgagaa
 gttttggttgatgatga

SEQ ID NO:8

15 **Arabidopsis thaliana HCT protein (At5g48930) NP_199704**
 MKINIRDSTMVRPATETPITNLWNSNVDLVIPRFHTPSVYFYRPTGASNFFDPQVMKE
 ALSKALVPFYPMAGRLKRDDDGRIEIDCNGAGVLFVADTPSVIDDFGDFAPTLNLR
 QLIPEVDHSAGIHSFLLVLQVTFFKCGGASLGVGMQHHAADGFSGLHFINTWSDMA
 RGLDLTIPPFIDRTLRLRARDPPQPAFHVVEYQPAPSMKIPLDPSKSGPENTTVSIFKLTR
 20 DQLVALKAKSKEDGNTVSYSSEMLAGHVWRSVGKARGLPNDQETKLYIATDGRS
 RLRPQLPPGYFGNVIFTATPLAVAGDLLSKPTWYAAGQIHDFLVRMDDNYLRSALD
 YLEMQPDLALVRGAHTYKCPNLGITSWVRLPIYDADFGWGRPIFMGPGGIPYEGLS
 FVLPSPTNDGSLVAIALQSEHMKLFEKFLFEI

SEQ ID NO:9

25 **Arabidopsis thaliana C3H nucleic acid (At4g34050) NM_119566**
 atggcgacgacaacaacagaagcaacgaagacatcatcgaccaatggagaagatcagaagcagctcagaatcttcgacatcaaga
 agttggtcacaagagtctcttacagagcgtgatctctaccagatatactggagacaagtgtgatcctagagaaccagaatcaatgaa
 ggaactcagggagtgacagcaaaacatccatggaacataatgaccacatcagctgatgaaggacagttcttaacatgcttatcaag
 30 ctctgtaacgccaagaacacaatggagatcggagtttactggtactctctctcgcaccgctcttgcctcctgaagacggcaaa
 attctggctatggatgtcaacagagagaattacgaattgggttaccgatcattgagaaagccggcgttgcacaaagatcgactcagg
 gaaggccctgctctcccgttcttgatgaaatcgttgcagcagagaagaacatggaacatagactttatattcgttgatcgtgacaag
 acaactacatcaactaccacaagcgtttgatcgtcttgaaaattggaggatgattggctacgacaacactctgtggaatggtctgt
 cgtggctcctcctgatgcaccaatgaggaagtacgttcttactacagagacttgttcttgagcttaacaaggctcttgcctgacccctc
 35 ggatcgagatctgatgctcctggtggtggaatcactatctgccctcggatcagttga

SEQ ID NO:10

Arabidopsis thaliana C3H protein (At4g34050) NP_850337
 MSWFLIAVATIAAVVSYKLIQRLRYKFPSPKPIVGNLYDIKPVRFRCYYEWAQSY
 40 GPIISVWIGSILNVVSSAELAKEVLKEHDQKLADHRNRSTEAFSRNGQDLIWADY
 GPHYVKVRKVCTLELFTPKRLESRLPIREDEVTAMVESVFRDCNLPENRAKGLQLRK
 YLGAVAFNNITRLAFGKRFMNAEGVVDEQGLEFKAIVSNGLKLGASLSIAEHIPWLR
 WMFPADEKAFAEHGARRDRLTRAIMEEHTLARQKSSGAKQHFVDALLTKDQYDL
 SEDTIIGLLWDMITAGMDTTAITAEWAMAEMIKNPRVQQKVQEEFDRVVGLDRILTE
 45 ADFSRLPYLQC VVKESFRLHPPTPLMLPHRSNADV KIGGYDIPKGSNVHVNWAVA
 RDPAVWKNPFEFRPERFLEEDVDMKGHDFRLLPFGAGRRVCPGAQLGINLVTSMMS
 HLLHFFVWTPPQGTKPEEIDMSENPLVTYMRTPVQAVATPRLPSDLYKRVPYDM

SEQ ID NO:11

50 **Arabidopsis thaliana CCR1 nucleic acid (At1g15950) NM_101463**
 atgccagtcgacgttagcctcaccggccggaaaaaccgtctgcgtcaccggagctggtggatacatcgcttcttgattgtaagatact
 tctcgagagaggttacacagtc aaaggaaccgtacggaatccagatgatccgaagaacacacatttgagagaactagaaggaggaa

aggagagactgattctgtgcaaagcagatcttcaggactacgaggctctaaggcggcgattgatggtgacgagcggcgtttcacacg
gcttctctgtcaccgacgatccggaacaaatggtggagccggcgtgaatggagccaagtttgaattaatgctgaggcgtgaggcca
aggtcaagcgcgtggtcatcacctcctcattggtgcccgtctacatggaccgaaccgtgaccctgaggctgctggtgacgaaagtgtg
5 tggagtgatcttgacttctgcaaaaacaccaagaattggtattgttacggcaagatggtggcggaacaagcggcgtgggagacagca
aaggagaaaggtggtgacttgggtggtgtaatccggctgctggttcttgaccgcccgttacagccgacgatcaacgccagctttacca
cgtcctcaaatatctaaccggctcggctaagacttatgctaatttgactcaagcttatggtgatgctgcatgctgctgctcatgttct
10 ggtctatgaggcaccctcggcctccggacgttatctctagccgagagtgtcgcaccgcggggaagtgtgagattctggctaag
ctattcccggagtatccttccgaccaagtgaaggacgagaagaacctagagccaagccatacaaatcactaaccagaagatta
aggacttaggcttagagttcactccaccaagcaaagcctctacgacacagtaagagcttacaagaaagggccatcttctcctcct
cctcctcctcctcagcatcgcaagaatccgtggaaaatggcattaagatcgggtcttga

SEQ ID NO:12

Arabidopsis thaliana CCR1 protein (At1g15950) NP_173047

MPVDVASPAGKTVCVTGAGGYIASWIVKILLERGYTVKGTVRNPDDPKNTHLRELE
15 GGKERLILCKADLQDYEALKAAIDGCDGVFHTASPVTDDPEQMVPEPAVNGAKFVIN
AAAEAKVKRVVITSSIGAVYMDPNRDPEAVVDESCWSDLDFCKNTKNWYCYGKMV
AEQAAWETAKEKGVLDLVLNPLVLGPPLOPTINASLYHVLKYL TGS AKTYANLTQ
AYVDVRDVALAHVLVYEAPSASGRYLLAESARHRGEVVEILAKLFP EYPLPTKCKDE
20 KNPRAKPYKFTNQKIKDLGLEFTSTKQSLYDTVKSLQEKGHLAPPPPPSASQESVEN
GIKIGS

SEQ ID NO:13

Arabidopsis thaliana NST1 (At2g46770) nucleic acid NM_130243

atgatgtcaaaatctatgagcatatcagtgaaacggaatctcaagtgcctcctgggttaggttcatccgaccgaggaagagctgtg
25 cagtattatctccggaagaaagtaatagcatcgagatcgatcttgatgtcattcgcgacgttgatctcaacaagctcgagccttgggaca
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ccccgaggatgtcaccgttcatgaggtcgtgagtattataggggaagcatcaacaagacgaaggatgggtggtgtgctgtatttcaag
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cccaaacctgaaagccctaacagtcaggcaatcaacaactgccacgtaagctctccgacactaatcataatccacgtcagcaac
gtggtcgacactagctttgttactagctgggcccgttttagaccgctcgtggcctcgcagcttaacggaccacatcatattcaattaca
gccgtcaatgagagccacgtgggcatgatcatctcgtttgccttcgctcgcagctcctgacccccagcctaaaccggctccgttcgtac
35 cacgccggttaacacaggaatatacaccggagatggagctatggaatacagacgacgctcgtctctatcgtcatcgctggccattttgt
cacgtgtcgaatggtagtggataa

SEQ ID NO:14

Arabidopsis thaliana NST1 (At2g46770) protein NP_182200

MMSKSMSISVNGQSQVPPGFRFHPTEEELLQYYLRKKVNSIEIDLVDVIRVDLNLKLEP
40 WDIQEMCKIGTTPQNDWYFFSHKDKKYPTGTRTNRATAAGFWKATGRDKIISNGR
RIGMRKTLVFKGRAPHGQKSDWIMHEYRLDDNIISPEDVTVHEVVSIIGEASQDEG
WVVCRIKFKKLNHLKTLNSPVGGASLSGGDTPKTTSSQIFNEDTLDQFLELMGRSCK
EELNLDPFMKLPNLESPNSQAINNCHVSSPDTNHNHVSINVVDTSFVTSWAALDRLV
45 ASQLNGPYSYSITAVNESHVGHDLALPSVRSPYPSLNRASASYHAGLTQEYTPEMEL
WNTTSSLSPPGFCHVSNVSGS

SEQ ID NO:15

Arabidopsis thaliana NST2 (At3g61910) nucleic acid NM_116056

atgaacatcatcagtaaacggacagtcacaagtacctcctggttttaggtttacccaaccgaggaagagctcttgaagtattacctccg
50 aagaaaatctctaactcaagatcgatctcgtatgtattctgacattgatctcaacaagctcgagccttgggatattcaagagatgtgtaa
gattggaacgacgccgcaaacgattggtactttatagccataaggacaagaagatcccaccgggactagaaccaacagagccac

cacggctcggattttggaaagcgacgggacgtgacaagaccatataaccaatggatgataagaatcgggatgcgaaagacgcttgtctt
 tacaaggctcagcccctcatggtcagaaatccgattggatcatgcacgaatatagactcgacgagagtgtattaatctctcgtgtggc
 gatcatgacgtcaacgtagaacggtgatgtcataggaagtacgaaggatgggtggtgtctgctgtttcaagaaaaataacctttgc
 5 aaaaacatgattagtagtagcccgaggtcggtgaaaacgccgtcgttcaatgaggagactatcgagcaactctcgaagtatggg
 gcaatctttaaaggagagatagtttagaccctttctaaactccctaacctcgaatgccataacaacaccaccatcaggttatcagt
 ggtaatcgacgaccaagtcaacaactgccacgtcagcaaagtatggatcccagcttcatcactagctgggccgcttggatcggctc
 gttgcctcacagttaaattgggcccactcgtattcaataaccagccgtaatgagactcacaatcaccgtatcatggactgaaccggtcc
 ggttgaataaccggttaacaccagattactatataccggagattgattatggaacgaggcagatttcgcgagaacgacatgccacttgt
 tgaacggtagtgataa

10

SEQ ID NO:16

Arabidopsis thaliana NST2 (At3g61910) protein NP_191750

MNISVNGQSQVPPGFRFHPTEEELLKYLRKKISNIKIDLDVIPDIDLNKLEPWDIQEM
 CKIGTTPQNDWYFYSHKDKKYPTGTRTNRATTVGFWKATGRDKTIYTNDRIGMRK
 15 TLVFKYKGRAPHGQKSDWIMHEYRLDESVLISSCGDHDVNVETCDVIGSDEGWVVC
 VFKKNLCKNMISSPASSVKTPSFNEETIEQLLEVMGQSKGEIVLDPFLKLPNLECH
 NNTTITSYQWLIDDQVNNCHVSKVMDPSFITSWAALDRLVASQLNGPNSYSIPAVNE
 TSQSPYHGLNRS GCNTGLTPDYIPEIDLWNEADFARTTCHLLNGSG

20

SEQ ID NO:17

Arabidopsis thaliana NST3/SND1 (At1g32770) nucleic acid NM_103011

atggctgataataaggtaactttcgattaatggacaatcaaaagtgcctccaggttcagattccatcccaccgaagaagaacttctcc
 attactatctccgtaagaaagtaactctcaaaagatcgatcttgatgctcctcgaagtgatctaaacaagcttgagccttgggatattc
 aagaggaatgtagaatcgggtcaacgccacaaaacgactggacttcttcagccacaaggacaagaagtatccaaccgggaccagg
 25 acgaaccgggcaacagtcgctggattctggaagctaccggacgtgacaaaatcatctgcagttgtgtccggagaattggactgagg
 aagacactcgtgttctacaaaggaagagctcctcacggtcagaaatccgactggatcatgcatgagatcgctcgcgatactccaat
 gtctaattggctatgctgatgtgttacagaagatccaatgagctataacgaagaaggttgggtggtatgctcgagtgttcaggaagaagaa
 ctatcaaaagattgacgattgtcctaaaactctctatcttctttacctgatgacacggaggaagagaagggggccaccttcacaacact
 caaaacgttaccggttagaccatgttctctctacatggaccgtaccgggttaacatttgcacccgagagccaaacaacgactcaa
 30 catcaagatgatgtcttattcatgcaactcccaagtcttgagacacctaataccgagagcccggctcgaccaaagtctcctgactccaagc
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 attgaacaatggctcatataatccgtgtcatcgtaagatgttgatgaagaagaagaaatggtgatactatgatgcagcagatgggatctt
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35

SEQ ID NO:18

Arabidopsis thaliana NST3/SND1 (At1g32770) protein NP_174554

MADNKVNL SINGQSKVPPGFRFHPTEEELLHYLRKKVNSQKIDLDVIREVDLNKLE
 PWDIQEERIGSTPQNDWYFFSHKDKKYPTGTRTNRATVAGFWKATGRDKIICSVR
 RIGLRKTLVFKYKGRAPHGQKSDWIMHEYRLDDTPMSNGYADVVTEDPMSYNEEGW
 40 VVCRVFRKKNYQKIDDCPKITLSSLPDDTEEEKGPTFHNTQNV TGLDHVLLYMDRTG
 SNICMPESQTTTQHQQDDVLFMQLPSLETPKSESPVDQSFLTSPKLD FSPVQEKITERPV
 CSNWASLDRLVAWQLNNGHHNPCRKSFDEEEENGDTMMQRWDLHWNNDDNVD
 LWSSFTESSSSLDPLLHLSV

45

SEQ ID NO:19

Arabidopsis thaliana SND2 (At4g28500) nucleic acid NM_118992

atgacttgggtcaatgaccgtagcgatgttcagaccgtgaaagaatattccctccccggggcggtgagtcccccgtagcctcact
 tccggctctctgtcacaaaacttgcccttctgtggccataactcaagtttcacgaacaggctgggatccatgacttgccgggacttctctg
 ctggagtaaaattgatccgacggatcaagaggtcttgagcatcttgaaaggcaaggtaagagatgacgcaaaaaagcttcatcctctc
 50 attgatgagttatccgtaccatcgatggtgaaaacggcatttgtataccatctgaaaaattgccaggagtgaacaaggacgggacg
 gtgcgtcatttctccaccgaccgtcgaaggcatacacgacgggaacaagaagcagcgtaaagtccacactgattctgacgtcgggt
 gagagacacgggtggcacaagacaggaacacggccagttctgtcggaggaagagtgagaggctacaagaaaaatcctagtgtc

ctacacaaactacggcaacaaaaaaacccgagaagactaattgggtaatgcatcaatatcatcttggcactagcgaggaagagaa
 agaaggtgagctcgtcgtcctcaaagtctttaccagactcaaccacgtcaatgcggggctccggtgctgctgcagccaccgtaagg
 accgaccttacctccacggcctcggtggaggtggggccgaccttcattaccatcttcatataacaacggtaacggtaagagcaa
 cggcagtggggggaaccgccggagccggtgagtagtattatcacaatattccggctattatctcgttcaatcagaccgggatacagaaccac
 ttggttcatgactcacaacctttatcccttaa

SEQ ID NO:20

Arabidopsis thaliana SND2 (At4g28500) protein NP_194579

MTWCNDRSDVQTVRIIPSPGAAESPVASLPVSCHKTCPSCGHNFKFHEQAGIHDLPG
 LPAGVKFDPDQEVLEHLEGKVRDDAKKLHPLIDEFIRTIDGENGICYTHPEKLPGVN
 KDGTVRHFFHRPSKAYTTGTRKRRKVHTSDVGGETRWHKTGKTRPVLGGRVRG
 YKKILVLYTNYGKQKPEKTNWVMHQYHLGTSEEEKEGELVSKVFYQTQPRQCG
 GSVAAAATAKDRPYLHGLGGGGGRHLHYHLHHNNGNGKSNGSGGTAGAGEYYHN
 IPAISFNQTGIQNHLVHDSQPFIP

SEQ ID NO:21

Arabidopsis thaliana SND3 (At1g28470) nucleic acid NM_102615

atgagttggtgatggttcagatgataactacgatcttaattctgaaagagtatcgaactgatcatccatcggttcaactcaaagacca
 atctcaatcatgttaacgagccgtccagattccaagattagcgtgaaactccatcacgacttgctcttctgaggacacaagctccat
 catcaccaagacgaccaggttgtagcatcaaagatttaccagcttaccggcaggagtcaaattcgatccgctcggataaagagatcc
 ttatgcatttggaggcgaaggtatcatccgataaagcgaaaactcatccggttgattgatgaattatactacgcttgaaggagagaatgg
 aatttgtatagcctcctgagaaactcctggagtaagcaaggacgggcaagtacggcacttctccaccggccatcaaaggcttatac
 gaccggaacacgaaaacgaagaaaagtgagcacagatgaggaaggccatgaaacaagggtggcacaaaacaggcaagactcgac
 ctgtttgtctcaatcaggagaaaaccggttcaagaagatcctagtgcctacaccaactatggtcgcagagaagaagcctgagaagacg
 aattgggtgatgcatcagatcattaggtagcagcaggacgaaaaagacgggtgaaccagtcctctctaaagtctctaccaaacaca
 gcctaggcaatgcggttcgatggaacctaaaccgaaaaatctcgtaaacctaaaccggttagttatgaaatattcaggccggttcgg
 gtatgagcatggtgtaaaagtgaagagacgacgcaggtgattcgagagttgtagttcgtgaaggcgatgggtcatgttcgtttctta
 gtttacttggatgcaagtaagggtaaagaagcttcatgaagaatcaatag

SEQ ID NO:22

Arabidopsis thaliana SND3 (At1g28470) protein NP_564309

MSWCDGSDDNLDLNLERSVNTDHPVQLKQSQSCVTSRPSKISAETPITTCPSCG
 HKLHHHQDDQVGSIKDLPSLPAGVKFDPSPDKEILMHLEAKVSSDKRKLHPLIDEFIPT
 LEGENGICYTHPEKLPGVSKDGQVRHFFHRPSKAYTTGTRKRRKVSTDEEGHETRW
 HKTGKTRPVLSQSGETGFKKILVLYTNYGRQKPEKTNWVMHQYHLGSSEDEKDG
 PVLSKVFYQTQPRQCGSMEPKPKNLVNLNRFSYENIQAGFGYEHGGKSEETTQVIRE
 LVVREGDGSCSFLSFTCDASKGKESFMKNQ

SEQ ID NO:23

Arabidopsis thaliana MYB103 (At1g63910) nucleic acid NM_105065

atgggtcatcactcatgctgcaaccagcaaaaggtgaagagagggccttgggtcaccggaagaatgagaagcttattagatatatca
 caactcatggctatggatgttgagtgaaagtcctgaaaaagcaggcctcaaatgtggaaaaagttgtagattcgcgatggataaac
 tatcttcgacctgatcaggagaggaaggtctctccagaagaagagaaattgatcataagccttcatggagttgtgggaaacaggtg
 ggctcatatagctagtcaattaccgggaagaacagataaacgagattaaaaactattggaattcatggattaaagaaaaagatacga
 cgcacatcattacagtcgtcatcaaccgtcagtaactactgtgacattgaatgcggacactacatcgattgccactaccatcgaggcct
 ctaccaccacaacatcgactatcgataacttacatttgcaggttactgattctcctaaccaattaaattcaccaatgatcaagaacta
 atataaagattcaagaactttttctccataaacctcctcttctcatgtagacacaacacttctatcctagaaggaatgttctctgaaa
 catcatcacaacaataacaagaacaatgatcatgatgacacgcaagaggaggaagagaaaaatgttgtgaacaagcatttctaaca
 actaacacggaagaatgggatgaatcttctcagcaagagccgttcaagtctactggtcgtcactgtgttcaacaactcttcca
 attcaaatattgacaggttataagttataatctaccggcctaataaggggaaatgctgataacatcgccataatgaaaacagcaatgt
 ccaagatggagaaatggcgtccacattcgaatgtttaaagaggcaagaactaagctatgatcaatgggacgattcacaacaatgctccta

acttttctttgggacaaccttaataaacgtggaaggttcatctcttggaaaccaagaccatcaatgaattgggatcatctgcctt
atcttcttcttcccttctcgttttaa

SEQ ID NO:24

5 **Arabidopsis thaliana MYB103 (At1g63910) protein NP_176575**
MGHHSCCNQQKVKRGLWSPEEDEKLIRYITTHGYGCWSEVPEKAGLQRCGKSCRLR
WNYLRPDIRRGRFSPEEEKLIISLHG VVGNRWAHIA SHLPGR TDNEIKNYWNSWIKK
KIRKPHHHYSRHQPSVTTVTLNADTTTSIATTIEASTTTTSTIDNLHFDGFTDSPNQLNF
TNDQETNIKIQETFFSHKPLFMVDTTLPILGFMFSENIITNNKNNDHDDTQRGGRE
10 NVCEQAFLLTNTTEWDMNLRQQEPFQVPTLASHVFNSSNSNIDTVISYNLPALIEGN
VDNIVHNENSNVQDGEMASTFECLKRQELSYDQWDDSQQCSNFFFWDNLNINVEGS
SLVGNQDPSMNLGSSALSSSFSSF

SEQ ID NO:25

15 **Arabidopsis thaliana MYB85 (At4g22680) nucleic acid NM_118394**
atgggggagacagccatgctgtgacaagctaggggtgaagaaagggccgtggacggtggagggaagataagaagcttataaacttcat
actaaccaatggccattgttctgctggcgtgctttgccgaagctggccggtctccgctgctgtggaaagagctccgcctccggtggacta
actatctccggcctgacttaaacgaggccttctctcgcgatgatgaagaacaactgtcatagatcttcctgtaatactcggcaataagtg
gtctaagatagcttcaagattacctggaagaacagataacgaaataaaaaaccattggaatactcatatcaagaagaacttctaagat
20 gggaatcgatectatgacctcaaccctaaatcaagaaccttctaataatcgataattccaaaaccattccgccaatccagacgatgct
tcagtgaaccaaagacaactaacacgaaatacgtggagataaggtcacgacaacagaagaagaagtagtagcacggttactgat
caaaacagttcgtggataatgaaatcatctaattgacaacattatgatgatgaattgtttagttacttatggtccgacgaaactact
aaagatgaggcctctggagtgatagtaactttggtgtggggaacattatgaccacaatatctccggcggcggatgcagattttccga
tatggtcaccggaaagaatcaatgacgagaagatgttttggattattgtcaagactttggtgttcatgattttgggtttga

SEQ ID NO:26

25 **Arabidopsis thaliana MYB85 (At4g22680) protein NP_567664**
MGRQPCCDKLGVKKG PWTVEEDKKLINFILTN GHCCWRALPKLAGLRRCGKSCRLR
WTNYLRPDLKRGLLSHDEEQLVIDLHANLGNKWSKIASRLPGR TDNEIKNHWNTHI
30 KKKLLKMGIDPMTHQPLNQEPSNIDNSKTIPSNPDDVSVEPKTTNTKYVEISVTTTEE
ESSSTVTDQNSSMDNENHLIDNIYDDDELFSYLWSEDTTKDEASWSDSNFGVGGTLY
DHNISGADADFP I WSPERINDEKMFLDYCQDFGVHDFGF

SEQ ID NO:27

35 **Arabidopsis thaliana MYB46 (At5g12870) nucleic acid NM_121290**
atgaggaagccagaggttagcattgcagctagtactaccaagtaaagaagatgaagaagggactttggtctctgaggaagactca
aagctgatgcaatacatgttaagcaatggacaaggatgttgagtgatgttgcgaaaaacgcaggacttcaagatgtggcaaaagct
gcegtctctgttgatcaactatctctgctcctgacctcaagcgtggcgctttctctcctcaagaagaggatctcatcttcgcttccattccat
cctcggcaacaggtggtctcagattgcagcacgattgcctggtcggaccgataacgagatcaagaattctggaactcaacaataaag
40 aaaaggctaaagaagatgtccgatacctcaacttaatacaaacctcatcctcataccaacacagcaagcgattcctcttctaattcc
gcatctctttggatattaaagacattataggaagcttcatgtccttacaagaacaaggtctcgtcaacccttcttgaccacatacaaac
caacaatccattccaacgggaaacatgatcagccaccggtgcaatgacgattttacccttatgtagatggtatctatggagtaaagc
aggggtacaaggggaacttacttcccactttggaatgtgaagaaggtgattggtacaatgcaatataaacaaccacttagacgagt
tgaactaatggatccggaacgcacctgagggatgatgagaccagtggagaattttgggaccttgaccagttgatgaactgaggt
45 tccttcgtttacttcaactcaacaaagcatatga

SEQ ID NO:28

50 **Arabidopsis thaliana MYB46 (At5g12870) protein NP_196791**
MRKPEVAIAASTHQVKKMKKGLWSPEEDSKLMQYMLSNGQGCWSDVAKNAGLQR
CGKSCRLRWNYLRPDLKRGA FSPQEEDLIIRFHSILGNRWSQIAARLPGR TDNEIKNF
WNSTIKKRLKKMSDTSNLI NSSSSPNTASDSSSNSASSLDIKDIIGSFMSLQEQGFVN

PSLTHIQTNPFPTGNMISHPCNDDFTPYVDGIYGVNAGVQGELYFPPLECEEGDWY
NANINNHLDLNTNGSGNAPEGMRPVVEEFWDLQMLNTEVPSFYFNFKQSI

SEQ ID NO:29

5 **Arabidopsis thaliana MYB83 (At3g08500) nucleic acid NM_111685**
 atgatgatgaggaaaccggacattactacgatcagagacaaaggcaagccaaatcatgcatgtggtggaataacaacaaccgaag
 ctaagaaaaggactttggtcgcctgatgaagatgagaagctgataagatacatgttgactaatggacaaggatgtggagtgcacgcg
 tagaaatgctggtctttacgttggtgtaaaagtgtcgccttcgctggatcaattactgaggcctgatctaaacgtggatcctctctct
 caggaggaggatctcatctccattgcattccattctggtaacaggtggtctcaaatagctactcggctccaggtagaacagacaacg
 10 agatcaaaaacttttggaaactcgacattgaagaagcggcctaagaacaacagcaacaacaatacttcatcaggatcatcacctaacaat
 agtaatagtaattcctggaccecaagagatcaacatgtggatattggaggcaactcaactcattgatggatgactatcatcatgatgaaa
 acatgatgacagtggggaacacatgcgcatggactcttctccccattcaatgttgaccaatggtaataatgtgggcttaaaccaac
 ttatgatcccttgatgatatcagtcgggataacggatatacacaatgggaaacacagtgaatgtgttcagcgtaatggttaggaga
 ttatggaaacacaattctgatccaattagcaagagatcagtagaaggtgatgattggttcattccccctcggagaataccaacgct
 15 attgctttagtagacaagcaacaacctaaactacaggccctgatccttctcaatagcaaaaactttgtcattcagaaagctcaaggta
 gggaatgtgtgggatagagaatggtcttgggaaatagaaaaccctaaaatcggagattgggattggatggtctcatcgataaca
 ctctcttttccctccttgattccaagtcgattga

SEQ ID NO:30

20 **Arabidopsis thaliana MYB83 (At3g08500) protein NP_187463**
 MMRKPDITTIRDKKGPNHACGNNNKPKLRKGLWSPDEDEKLIRYMLTNGQGCW
 SDIARNAGLLRCGKSCRLRWINYLRPDLKRGSFSPQEEDLIFHLHSILGNRWSQIATRL
 PGRTDNEIKNFWNSTLKKRLKNNNSNNTSSGSSPNSNSNSLDRDQHVDMGGNSTS
 25 LMDDYHHDENMMTVGNTMRMDSSSPFNVGPMVNSVGLNQLYDPLMISVPDNGYH
 QMGNTVNVFVSNGLGDYGNITLDPISKRVSVEGDDWFIPSENTNVIACSTSNLNL
 QALDPCFNSKNLCHSESEFKVGNVLGIENGSWEIENPKIGDWDLGLIDNNSFPFLDF
 QVD

SEQ ID NO:31

30 **Arabidopsis thaliana MYB58 (At1g16490) nucleic acid NM_101514**
 atgggcaaaggaagagcaccatgtgtgacaaaaccaaagtgaagagaggaccatggagccatgatgaagactgaaactcatctct
 ttcattcacaagaatggtcatgagaattggagatctctccaaagcaagctggattgtgaggtgtggcaagagtgtcgtctgcgatgg
 attaattacctcagacctgatgtgaaacgtggcaatttcagtcgagaggaagaagacaccatcatcaactcaccagagctttggtaac
 aagtgtcgaagattgcttcaagctgcctggaagaacagacaatgatgaagaatgtgtggcatacacatctcaagaaaagattga
 35 gctcggaaactaaccttaatgccgatgaagcgggttcaaaaggtcttgaatgaagaagagaactctcaagagtcattcctcaaatgctt
 caatgtctttgctggtccaacattcaagcaagacgatgatgcacagataagtcaaatgttgagcacattctaactatagcgagtta
 cggggatgttacaagaggtagacaaccagagctgctggagatgccttttgatttagatcctgacattggagttcatagatggttcaga
 ctattccaacaaccagagaacagagctctcaagagctgaagaagatgaagttgataaatggttaagcacctggaaagcgaactc
 gggtagaagaaaacgataaccaacaacaacaacagcataaacagggaacagaaatgaacattcatcactcttgagaggt
 40 tacgagctctcatacattaa

SEQ ID NO:32

Arabidopsis thaliana MYB58 (At1g16490) protein NP_173098
 MGKGRAPCCDKTKVKRGPWSHDEDLKLISFIHKNGHENWRSLPKQAGLLRCGKSCR
 45 LRWINYLRPDVKRGNFSAEEDTIKHLHQSFGNKWSKIASKLPGRDNEIKNVWHTH
 LKKRLSSETNLNADEAGSKGSLNEEENSQESSPNASMSFAGSNISSKDDDAQISQMFE
 HILTYSEFTGMLQEVDKPELLEMPFDLDPDIWSFIDGSDSFQQPENRALQESEDEVD
 KWFKHLESELGLEENDNQQQQQHKQGTEDHSSSLLESYELLIH

50 **SEQ ID NO:33**

Arabidopsis thaliana MYB63 (At1g79180) nucleic acid NM_106569

atggggaagggaagagcaccttgtgtgacaagaccaaagtgaagagaggtccatggagcccagaagaagacattaaactcatctct
 tcattcaaaagtttggatcatgagaactggagatctctcccaacaatctgggctattgaggtgtgggaagagttgtcgtctaaggtgga
 ttaactatcttaggccagatctgaagcgtggcaacttcacttcagaggaggaagaacaatcattaagcttcaccacaactatgggaac
 aagtggtcgaaaaatcgcttctcaactccaggtagaacagataacgagatcaagaatgtgtggcacactcatctaaagaaaagactgg
 5 ctcagagctcaggaactgcagatgaaccggcctcgccttgttcgagtgattctgtttctcgtgggaagatgataagtcacacgtag
 aagattcttgaacagagagactaatcataggaatgagttgtctacatctatgtcttctgggggtccaaccaacaagatgatccaaagat
 agacgaactcaggttgagtatatagaagaagcttatagcgagtttaacgacattattattcaagaggtagacaaaccgatctgctgga
 gatacatttgattcagatcctgacattggagtttcttagatactcaactcattcaacaatccactgcaaatgagaacagctcaggctc
 aagagcaacaacagaagaagagctgatgaggatgaggttaagaaatgggtcaagcacctagaagcgaactcgggttagaagaag
 10 acgataatcaacaacaatacaagaagaagaatcatcatcatcactctgaagaactacgagctcatgatacattga

SEQ ID NO:34**Arabidopsis thaliana MYB63 (At1g79180) protein NP_178039**

MGKGRAPCCDKTKVKRGPWSPEEDIKLISFIQKFGHENWRSPLKQSGLLRCGKSCRL
 15 RWINYLRPDLKRGNFTSEEEETIIKLHHNYGNKWSKIASQLPGRTDNEIKNVWHTHL
 KKRLAQSSGTADEPASPSSDSVSRGKDDKSSHVEDSLNRETNHRNELSTSMSSGGS
 NQQDDPKIDELRFEYIEEA YSEFNDIIIQEVDKPDLEIPFSDPDIWSFLDTSNSFQQST
 ANENSSGSRATTEEESDEDEVKKWFKHLESELGLEEDDNQQQYKEEESSSSLLKNY
 ELMIH

20

SEQ ID NO:35**Promoter pIRX8**

ACGAGCTGACTTGTACCGATGAGCTGGCTCTTCTGGGCGAGCTGGCTGATCTTGA
 CGAGCAGACTTCTCCCGACGAGCTGACTTGTGTGCGATGAGCTGGCTCTTCTGGGC
 25 GAGTTGGCTGATCTTGACGAGCAGACTTCTCCCGACGAGCTGACTTGTGTGCGATG
 AGCTGGCTCTTCTGGGCGAACTGGCTGATCTTGACGAGCAGACTTCTCCCGACGA
 GCTGACTTGTGCTATCCTTTCTCCAGGTCTCGAAAAAGTCCCCTTTCCCGAGACTT
 TCTATTCTTATTTATACCCGTCCGTATAGTAGGGTACGCAAGGTGAATTCTCGA
 GAGTGCCCTTTTCTACGCAGCCGAACTCACATCCTGACCAGGCCGGGCTTCGGC
 30 CTGGTGGGCCGGCTCGAGTTCTAAAGTGATGGTTCGGGGCTGGGTTCGTTATTCCTT
 GAAATGGGCCGGTTGATCACTGAGGCCCAATTGATGTATCAACATGTGGTTTTTA
 TAAAAAGAGTCGTGAGAAGAGTTTTCTCTAAAAATCCCTTGTGTTTGGTAATCAA
 ACTTCATTCAACCAACGAATTCCAAAAAACAATAAATTGTTTCGGGTATATAAA
 ATGATTGGTAATGATATATCCCATAGAGGCCGTAGACATAGGCCCAAAAAGTTTC
 35 CATAACTAGCAGAAATTGAACTTGCAAGTTGCAAATATTATTACACTGGAAAG
 GCAACAAGTCTTGAAGTACAACTACAAAGACTTCTTGTGTTTGGATGGGGACGACT
 GACGAGTTTGAATAACTTAAGAGAAAAGGGTCGCAATCGAAATTAGACAAGAAA
 TAGTCCTCAAAAAGTAAATTCTGAAGTTGAAGCTCCAATGTCTTTGTTCAAAGA
 CTTTATTTAGATGTAAAGTTATGTCTTGTAAACCACCAACAGCTCCTTTTCATCTA
 40 CACTCCCAATTTTTTTAACATCTATGTTTTGCATTGCCTTTGACTTGTCTTTCTCTC
 TCCAACTTCTCTCCTTCAACATAAAGCCAAATCCTAAATCCAAATCCCTTAAACC
 GAACCGAATTAACCGAAGCTGTTGAACTATCGCAAAATTCAGATCTTACTAAT
 CATAACATGTGACGTTTAATTCATTTAAGAGTTTCATGATTTGCACTGAATGGT
 ATTCCGAGTCCACCGGAAAAAACTTTTCTACAAGTAGAAAAAGGATAACCCC
 45 ATAAATCCAAATAACCTAACCGATCAAACATATAACCAATATAAACCAAAAACAAG
 ATTCAGATTCATCGGTTTAGTAATCGAAGTAATGTACTAATGTGTAATATTGATT
 CCACCACCAGCTTAGAGATTTCGAACCAAAAACCGAATAGCGCATAACCGAGAAA
 ACCCAAAGCTTCCTAACAAATACATAAAACCGTGGTGTCTTCTAATTCTAACCAAC
 ACACGTTTCCTTTTTATTCAACAAGAACATCAGAGTTATGATCTGCCATTAATAA
 50 CCTAAACACAAAGCAAGGTTAGGTAAATGATATGGACCCCTAATGAATAATCAT
 ACAATACATAACAACGTAAGATCCAGTTTCCCTCTTCG

SEQ ID NO:36

Promoter pVND6

CGCCAAAAAGATGTTATGATGTGATGCATTCTTTAATATAGATTAAACTATTGGT
GATTTGTTTTTCTATAGTTAATCACTAGCAAGAACATTTTTCTTTTATGTTTACAG
5 TTTTATAGATTATAAAAAAATGGTGTAAATAAGAACATGTCAAATCAAATGTATTTA
ATTTGTTAATATAGATTTTGTGTATAAACAATTGGTAGTTTTGATAATTCAATTTTT
CAGCAATCATCATAATAATACTTTACAAGAAAAGCTAAATCATCTGAAAATTTTA
TATGAATACGTACGGTTTAATTCCCAATCTACAACCTTTTTTAGTTGGATTATTAT
AACCGTTTTTCTAAAACAAAACACATTAATAATTTATAAGTGAAGATCCAATGGGT
10 TCAAACTTTTTAATTCTCAATAAATATACATAGATTCTCGAAGATATCCTATCAA
CTTGTAAGGTTGTTAATCAATCTTTTGTGTTGATGAAATCTTGTTCAACTGTTGA
TTTGGTTAAGTTTTATAGCTGAAATGTGTATAAGTGTCTGAACTTTTTAATTA
CTGCTAAATCAATTTATGTCTTACAACTTGCCGATGTATCATGTATGTTTATTAG
GGGTGTCAAATGAGCCAGCTCGCTCAGCTCAGCTCATGATGAACTTAAATCTTT
15 TATGAGCCAGCTCAGCTCAACTCATTATTATATGAGCTTCAAATAACAACTCG
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AATATATTTCTATTTTTATAGATTATATAAATATTTATGTTTTGATTGTTGACTTTT
TGTGTTTATTACTAATATTTATTTTCAGAAATTATTATGTAACCTTATATATTTTCTA
20 AAATATATTTCTATTTTATAGAAAAATATATATACTTTGATTGTTAGTTTTT
TGTATATATTACTTCAAAAAAGCCAACTCATGAGCTAGCTCATGTTTCAATTAAG
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AATGATCCTAAAAAATAGAATTCGCGCTCATGAATAACTGAGTCGAGTTGAGCC
AGCTCATGAGCTATCAGCTCATTGACACCATTAATGTTTATATAATAATCGTA
25 ATCCATCATGACCAATTAGGCAATTAAGACATACTATAACAAAACATTTTTTTTT
TTTTCGTCAAACATTGTTTTGTTAAAGGTTTCAAAAAGAACATGCGTATCAATTTT
ACCAAACGAATCTAATAAATAGCACGACCATCGGATATTATATTGCTATTTGACG
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TAGTGTTATTTAGACTTCGAGTAACATCAACGCGGGAGAAGAAACGCATGGGAT
30 GAATGTGTAAAGTGGTAACTTTCAACAATGTTTCTTAATTGCTAAGATGTTTAA
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AGTACACTACCTTATTAGGGCTCCATTTCTTTTTCTATCTAGGCCTAGGTCGATCA
GTACTGTGTATGTTACACATATGATATTAATAAAAAAATTGATTCTATAATAATC
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35 AGAGATAGTTCTAAAAGATCACATTACTTCTCTATCACACAGAGTGTAGAAAAGT
GAAAACCTTATGCAACACTTCAGGTAGAAAGAGAAAAGACAGTGATAGCTTAGTT
ATAATTAAGACCCCCAAAATCCAAATAGAATCTTCTCTTAAATAAACTATTGAA
AAAATATTCACAAAAAATAAAAAGCACATTTCCTTTTGCTTGCATCACGAGAGC
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40 TCGGAGTACTCGTTGTCTTTTATGATCTCAACATAAACAAGACAAAACGCTTTTG
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GATATTTTAGAAGTCTTGCATAAATAAGAAGAAGGTTTCAAGTAAGTTTCTTTCA
GTACATAGAGAAACCATGTATATGATTTATGATTGTCAAGATGTTACACAGTCGT
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45 ACTTTTGTTTTTGATTTTGTAAATTGAGTAGTTTTTGTTTTTATTATCATTTTTATG
TGTTTATAGTTGGTTCAGCCGAGATATTATGAGTAACCAAACGTAACCTTTTTTCAT
AATGAAACGGATCAAATATACTTTAATCTTTTTCTACATATGCTTAGTTACTTGA
AACTTGATTTACATTACTTCTATGCATATCTTTTCTATGTACCGCGCGATGATA
AAGTATGTGTTACAAATTGCCACATTGCAGAAAATATAAAATTA AAAAAGATCAA
50 ATGGAAAGTCTCGCACAC

Illustrative SHN1 Protein Sequences and accession numbers

Legend: At: *Arabidopsis thaliana*, Pt: *Populus trichocarpa*, Mt: *Medicago truncatula*, Os:
Oryza sativa, Bd: *Brachypodium distachyon*, Zm: *Zea mays*, Sb: *Sorghum bicolor*, Hv:
Hordeum vulgare, Ps: *Picea sitchensis*, Sm: *Selaginella moellendorffi*, Pp: *Physcomitrella*
 5 *patens*

SEQ ID NO: 37 AtSHN1_At1g15360_NP_172988

MVQTKKFRGVRQRHWGSWVAEIRHPLLKRRVWLGTFFETAEEAARAYDEAAVLMMSG
 RNAKTNFPLNNNTGETSEGKTDISASSTMSSTSSSSLSILSAKLRKCKKSPSPSLTC
 10 LRLDTASSHIGVWQKRAGSKSDSSWVMTVELGPASSSQETTSKASQDAILAPTTEVEI
 GGSREEVLDEEEKVALQMIEELLNTN

SEQ ID NO:38 AtSHN2_At5g11190_NP_196680

MVHSRKFRGVRQRQWGSWVSEIRHPLLKRRVWLGTFFETAEEAARAYDQAALLMN
 15 GQNAKTNFPVVKSEEGSDHVKDVNSPLMSPKSLSELLNAKLRKCKDLTPSLTCLRL
 DTSSSHIGVWQKRAGSKTSPTWVMRLELGNVNESAVDLGLTTMKNQNVEKEEEE
 EEAIISDEDQLAMEMIEELLNWS

SEQ ID NO:39 AtSHN3_At5g25390_NP_197921

MVHSSKFRGVRQRQWGSWVSEIRHPLLKRRVWLGTFFETAETAARAYDQAAVLMN
 20 GQSAKTNFPVIKSNNGSNSLEINSALRSPKSLSELLNAKLRKNCKDQTPYLTCLRLDND
 SSHIGVWQKRAGSKTSPNWVVKLVELGDKVNARPGDIETNKMKVRNEDVQEDDQ
 MAMQMIEELLNWTCPGSGSIAQV

SEQ ID NO:40 PtSHN1_XP_002324652

MVQSKKFRGVRQRHWGSWVSEIRHPLLKRRVWLGTFFETAEEAARAYDQAAILMSG
 RNAKTNFPIPQTSNEEDPKSSDEASLPTPPNGLSEILHAKLRKCKSKAPSPSMTCLRLDT
 ENSLIGVWQKRAGERSDSNWVMRVQLGQRESQVSESTLPLPQSSGGVSEPELRAEM
 GEDERIALQMIEELLNRNCPSPSFGVQDHGDGSLFL

SEQ ID NO:41 PtSHN2_XP_002308080

MVPSKKFRGVRQRRWGSWVSEIRHPLVKRRVWLGTFFETAEEAARAYDQAAILMSG
 RNAKTNFPMPQTSNEDDPKSSDHQPSLTPPNGLSQILHAKLRKCSKAPSPSMTCLRL
 5 DAENSIGVWQQRAGQRSNWSNVMQVQLGKRDESQVSEALPLPDQSPGGISGPEWR
 EEMDKKEERVALQMVEELLNRNCPSPFFGVQDHDDDSFFL

SEQ ID NO:42 PtSHN3_XP_002327422

MVQSKKFRGVRQRHWGSWVSEIRHPLLKRRVWLGTFFDTAEEAARAYDEAAILMSG
 10 RNAKTNFPVVANQTRNGQNSPSSSSALSALRKYCRSPYPSLTCLRLDAENCHIGVW
 QKRAGPRSVSNWIMTVELGKKDGRQAPEQKILISDTSDMAGQEGGSDDGPDDEERV
 ALQMIEELLNR

SEQ ID NO:43 PtSHN4_XP_002324859

15 MVQSKKFRGVRQRQWGSWVSEIRHPLLKRRVWLGTFFETAEEAARAYDQAAILMNG
 QNAKTNFPDTHLDQDTNLGKDNNSPKALAEELLNSKLRKCCGKDPSPSLTCLRLD
 NDNSHIGVWQKAGSRSSSNWVMKVELGNYNKKTESSPTVEIEPENGTEEEDRIAM
 QMIEELLNRN

20 **SEQ ID NO:44** PtSHN5_XP_002309625

MVQSKKFRGVRQRQWGSWVSEIRHPLLKRRVWLGTFFETAEEAARAYDQAAILMNG
 QNAKTNFPASHLDQDTKLGKDNNSPKALAEELLYSKLRKCCGKDPSPSLTCLRLD
 NDNSHIGVWQKAGSCSSSNWVMRVELGNSNRKSTQVMEELRPSLSSESSSRVEIEP
 EINGTDEEDKIAMQMIDELLNCN

25

SEQ ID NO:45 MtSHN1_XP_003609337

MVQSKKFRGVRQRHWGSWVSEIRHPLLKRRVWLGTFFETAEEAARAYDQAAILMSG
 RNAKTNFPITQTSEGDPKSITSNENKPSTSKDLEEILHAKLRKCSKVSPSMTCLRLDT

ENSHIGVWQKRAGKCESENWVMTVQLGKKMSVTQDSGSSSSSVAPSSAVATEEEIV
RGEIDEEDRIALQMIEELLNDKNCPSINNIKQGDDIDNSFFL

SEQ ID NO:46 MtSHN2_XP_003597892

5 MVHKKFRGVRQRHWGSWVSEIRHPLLKRRVWLGTFFETAEEAAKAYDEAAILMSG
RNAKTNFPINVENQTNSISSSTSSKAFSAVLSAKLRKCKFPSPSLTCLRLDAENSHIG
VWQKGAGPRSESNWIMMVELERKKSASVPEKAKPEELSKNGLDDEQKIALQMIEEL
LNRN

10 **SEQ ID NO:47** MtSHN3_XP_003604418

MVKSkkFRGVRQRHWGSWVSEIRHPLLKRRVWLGTFFETAEEAARAYDEAAILMTN
SNNKTFATSSSTSTKPNTSLSAILSAKLRKCKSPSPSLTCLRLDTENSHFGVWQKRA
GPRSDSSWIMMVELERKKKEQEESEVLPNSDSETLASVVDNEDSEKAVKPENEDEE
GNDKNKGLDEEQRIALQMIEELLNRN

15

SEQ ID NO:48 MtSHN4_XP_003603408

MVQQTKKFRGVRQRQWGSWVSEIRHPLLKRRVWLGTFFETAEEAARAYDQAAILMN
GQSAKTNFPVTKNQGEEVASDTPYNGGGGDDSFLSPKALSELLSTKLRKYCKDPSPS
LTCLRLDNDNSHIGVWQKRAGPHSDSNWVMRVELGGKKKTIESEEIGSKQHTIDGG

20

NNSNADNENRVVVEEERVALQMIEELLNWNYPGSGTSSN

SEQ ID NO:49 MtSHN5_XP_003588762

MVQRNKFRGVRQRQWGSWVSEIRHPLLKRRVWLGTFFETAEEAARAYDQAAILMN
GKNAKTNFPIPKDQTEDANSLTPNCDDNNSFHTSNALSHLLKQKLTCCQKQSQSL
25 TCLRLDADNSHIGVWQKGAGSHSDSNWILRVELGKKHEDSHESNYVSSSEKSAPNN
STIVGDCAEKNGIEHEEDIVTMQMIEELLN

SEQ ID NO:50 OsSHN1_NP_001046226

MVQPKKKFRGVRQRHWGSWVSEIRHPLLKRRVWLGTFFETAEEAARAYDEAAVLMS
GRNAKTNFPVQRNSTGDLATAADQDARSNGGSRNSSAGNLSQILSAKLRKCKKAPSP
SLTCLRLDPEKSHIGVWQKRAGARADSNWVMTVELNKEVEPTEPAAQPTSTATASQ
VTMDDEEKIALQMIEELLSRSSPASPSHGEGEGSFVI

5

SEQ ID NO:51 BdSHN1_XP_003563662

MVQSKKKFRGVRQRHWGSWVSEIRHPLLKRRVWLGTFFETAEEAARAYDEAAILMS
GRNAKTNFPVPRSATGEIIVAPAAARDSRGGGLGSSSGAGSLSQILSAKLRKCKKTPS
PSLTCLRLDTEKSHIGVWQKRAGTRADSSWVMTVELNKEPAAAATTTTLLSDSVAPT
10 TPSTSSTSASTAGSPPVGMDDDEERIALQMIEELGGSSPNPSHGGLLQGEEGSFVI

SEQ ID NO:52 BdSHN2_XP_003571428

MVQPKKKFRGVRQRHWGSWVSEIRHPLLKRRVWLGTFFETAEEAARAYDEAAVLMS
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15 SPSLTCLRLDPEKSHIGVWQKRAGARADSNWVMTVELNKGVGLPSDVEAQSTISTA
TTSSSVSTMDDEEKLTLMIEELLSRSGPVSPSHGEDEGDFVV

SEQ ID NO:53 ZmSHN1_NP_001148685

MVQPKKFRGVRQRHWGSWVSEIRHPLLKRRVWLGTFFETAEEAARAYDEAAVLMSG
20 RNAKTNFPPIQRSSTGEPTPAAGRDRARSNFSSGSSTTNLSQILSAKLRKCKKAPSPSLTC
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SEQ ID NO:54 SbSHN1_XP_002451740

MVQPKKFRGVRQRHWGSWVSEIRHPLLKRRVWLGTFFETAEEAARAYDEAAVLMSG
25 RNAKTNFPVQRSSTGEPTPAAGRDAHSNAGSGSSTANLSQILSAKLRKCKKAPSPSLT
CLRLDPEKSHIGVWQKRAGARADSNWVMTVELNKGAASTDAASQSTSATTAPPATP
MDDEERIALQMIEELSSSSPASPSHGDDQGRFII

SEQ ID NO:55 SbSHN2_XP_002438651

MVQSKKKFRGVRQRHWGSWVSEIRHPLLKRRVWLGTFETAEEAARAYDEAAVLMS
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 CCKTPSPSLTCLRLDPEKSHIGVWQKRAGARADSSWVMTVQLNKDVPPPASSSGEEP
 5 VPSDGGAAATPTSTSTSSSTVTTTGSPPPAMMMDDDEERIALQMIEELLGSSSHSHGMFQ
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SEQ ID NO:56 HvSHN1_BAG12386

MVQSKKKFRGVRQRHWGSWVSEIRHPLLKRRVWLGTFETAEEAARAYDEAAILMS
 10 GRNAKTNFPVPRSANGEIIVAPAAAARDIRGGVGSSSSSGAAGASSLSQILSAKLRKCC
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 S

SEQ ID NOP:57 PsSHN1_ABK22668

15 MARPQRYRGVRQRHWGSWVSEIRHPLLKTRIWLGTFETAEDAARAYDEAARMCG
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20 SEQ ID NO:58 SmSHN1_Sm92334_XP002969836

MGRPQRYRGVRQRHWGSWVSEIRHPLLKTRVWLGTFETAEDAARAYDEAARLMG
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 CLRLDPEQSNLGIWQKKSGRQPESNWVMKVHFGSQGGGGVSSDIVLPTDNPAPPQPI
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25

SEQ ID NO:59 PpSHN1_XP_001762992

MGRPQRYRGVRQRHWGSWVSEIRHPLLKTRVWLGTFETAEDAAHAYDEAARLMC
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 30 SEEDKFAAEMIEELLGYSPGQFSNFGSPAMSDSSSCSSSCSAVTTAFE

Illustrative promoter sequences of wax cutin genes that can be used to drive transcription factor expression in wax/cutin APFL:

SEQ ID NO:60 pAtCER1_At1g02205

5 TGGGATCCTCTCCATCGTTTCCATCATGGGATACATTACTTACATTGACTTCATGAACAACATGGGACATTGCAA
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 10 TTGTGGTCTCGACCCCATGGTACCTCACATGCTTCATGTGGCCCTTCACTCTCCTCTGCTCATTTGCCCTCACT
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 30 AGGAGCATGACTGCGGCAACACTTGCACGTCCTCCGTCTCCACGCCATATGGGAAGCTGCTCTTCCGCATGATT
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SEQ ID NO:61 pAtCER2_VC2_At4g24510

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SEQ ID NO:62 pAtCER3_WAX2_At5g57800

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 25 CTTGTGAATAATATCAACTAATTAAGAGTCGGCCGATACCTCGTATCTTTGATCACTTTCACCCATATCTTGAAA
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SEQ ID NOP63 pAtCER4_FAR3_At4g33790

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35 **SEQ ID NO:69** pAtWBC11_ABCG11_DSO_COF1_At1g17840

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SEQ ID NO:70 pAtKCS1_At1g01120

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SEQ ID NO:71 pAtKCS2_DAISSY_At1g04220

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30 SEQ ID NO:79 pAtCER60_KCS5_At1g25450

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Illustrative Myb 96 protein sequences and accession numbers:

Legend: At: *Arabidopsis thaliana*; Th: *Thellungiella halophila*; Mt: *Medicago truncatula*; Pt:
 5 *Populus trichocarpa*; Vv: *Vitis vinifera*; Cm: *Citrus macrophylla*; Bd: *Brachypodium*
distachyon; Ta: *Triticum aestivum*; Os: *Oryza sativa*; Zm: *Zea mays*

SEQ ID NO:80 Myb96_At5g62470_NP_201053

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20 **SEQ ID NO:85** MtMyb_MTR4g108430_XP_003609059

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SEQ ID NO:86 MtMyb_MTR_3g039990_XP_003599668

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SEQ ID NO:87 CmMyb60_ABK59039

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15 **SEQ ID NO:88** BdMyb_XP_003574549

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 DALSLDTTAPAPPKAPMERSSSKGAVYASSAENIARLLEGWMPGEGKASSGGSGSG
 20 SRSSASVVAEGASASHSGTAPTPEGSTVTSKTKDEVAVAAPPAFMLENWLFDGDM
 GMGHNGIGDVGLDDVPLGDPSEFF

SEQ ID NO:89 BdMyb_XP_00356188

MGRPPCCDKDGVKKGPWTPEEDIILVSYVQDHGPGNWRAVPPNTGLMRC SKSCRLR
 25 WTNYL R PGIRGNFSEQEEKHIVQLQALLGNRWAAIASYLPDRTDNDIKNYWNTHL
 KKKLLHRTSTATPAPPTTHKDQNNNKGQWERRLQTDIHLARQALREALSLDTAST
 SATPGPAAAYALSAQNVSRMLDDWAVAADSASSEVTECSGGSTASNGTLWSSLLGRE
 STGAAAAGVEDPAALSAIESWLLLDDGTDRQPPEQE QSGGQLLP

30 **SEQ ID NO:90** TaMyb_AEV91147

MGRPPCCDKEGVKKGPWTPEEDLVLVSYVQEHGPGNWRAVPTRTGLMRC SKSCRL
 RWTNYLRPGIKRGNFTDQEEKLIVHLQALLGNRWAAIASYLPERTDNDIKNYWNTH
 LKRNLQAGGDAAAKPAAQRPASSSKGQWERRLQTDINMARRALREALTTLDDIKRQ

QPDAADGVNPGAAAGADSGSPAASSSSAASLSQCSPSAAGPYVLTTANISRMLDGW
ASKGRSAVPAADSPSGSSASEVSYGSGAAARALGSAFEYDRKPAVLAPDQTQLNAIE
TWLFADDNSNNDHHGHGGGGSGLLGVPATLGYPF

5 **SEQ ID NO:91** OSMyb_Os09g0414300_NP_001063167
MGRPPCCDKVGVKKGWPTPEEDLMLVSYIQEHGAGNWRAVPTNTGLMRCSSKSCRL
RWTNYLRPGIKRGNFTEQEEKLIVHLQALLGNRWAAIASYLPERTDNDIKNYWNTH
LKKKLKMKQAAGGGEDSGAASEGGGGRGDGDGGGKSVKAAAPKGQWERRLQTDI
HTARQALRDALSLDHPDPSATAAAAATPAGSSAAYASSADNIARLLQGWMRPGGG
10 GGGNGKGPEASGSTSTATTQQQPQCSGEGAASASASASQSGAAAAATAQTPECSTE
TSKMATGGGAGGPAPAFSMLESWLLDDGGMGLMDVVPLGDPSF

SEQ ID NO:92 OSMyb_Os08g0437200_NP_001175597
MGRPPCCVKAEVKKGWPTPEEDLMLVAYVQEHGPGNWRAVPTNTGLMRCSSKSCR
15 LRWTNYLRPGIKRGNFTDQEEKLIVHLQALLGNRWAAIASYLPERTDNDIKNYWNT
HLKKKLKMSATGGGGDDGEGGGAGEVKTRAAAPKGQWERRLQTDIHTARQALR
DALSLDPSPPAKPLDSSSGATAPPSSQAATSYASSAENIARLLEGWMRPGGGGGKTTT
TPSSGSRSSAASVLSGEASHSGGATAPTPDGSTVTSKTKDEETAGAPPPPPPAFSMLE
SWLLDDGMGHGEVGLMDVVVPLGDPSEFF

20 **SEQ ID NO:93** ZmMyb_NP_001132068
MGRPPCCEKAGLKKGPWSPEEDLLLVSIVQEHGPGNWRAVPCSTGLMRCSSKSCRLR
WTNYLRPGIKRGSFSDQEEKLIIHLQELLGNKWSAIASYLPERTDNDIKNYWNTHLK
KKLAKTGARESGASAKTTKKS DRAAAPKGQWERRLQTNIHTARQALREALSMDDT
25 APPAIKPEPLPLPLGQLPAPASQAMYASSIDNIARLLEGWMRPSVSGNASAESMSSFS
AFSGGGDGASASHIGTAHTPEGFTGTRKEEGAGPGPASLPMFENWLLDDGMGNGDA
SLICVPLADPCEFF

SEQ ID NO:94 Illustrative REF4 promoter At2g48110; NP_566125 (encoded protein
30 sequene)
TTTCGCAGGCCCTAATTAAGACATTCAAGAAAACAAGAAGAAGCATAAGAAGAGGCCTAAATGCCAGAGAATT
AAACAATGGGCCCTTTAAACAATATTTTAAACAACACTGAACAATAGATGAGATCTCAACTTCGAAAAGCTAAAGC
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GCCGTCTCCTCCGCTCTATCCATATCCAAAACAGCTATAAAGATAAACTTCCAGAGCTTGGTGAAGGAGCAGCAA
35 CCCTAGTTTTCAATCCCTAAAGGTGAAGTTTTTGTCTTCTTTTTCTTTGATTCTACTCTTTTTTCGTACAATA
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 TGAAGAATCATTCTAATGCTGATGTTTATTTATGACAATTTTATGTCTTACTTCGAATTTAGCTAAGATAATAA
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 5 TTGATTAAGAATGTACATACTATTACAAATGTTATCGATTAACAAACGTCATTTTCAGATATTAGTTCTCCAGCGA
 GTTGACACAAAAACCGATTACGTTTCATCCGGCGACTCGCTTTGATATCCATGGATTCTCAGTTGAATCCTTCC
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 TTCAATAGACGAGAACACCTCGTTGAGCACATGAAGATTTCCACCCTCACTTCACCAGCCTCGCTGTGGGGTT
 10 TGCTCAAGCACTGTAAATCCTTCGAATCCGTGAGGGAACACCTTAACGGTATGTTTGTATTTGTATTGTTTTT
 CACCACACATCGTATGAATATGGTGGTTGTTGATGTTTGTGTTGATTCCGCTTTTCCAACATTTTCAGTTCCAGAC
 CATCTTTCCAAAGGAAAAGTCAAAAGCCATTTTCACTAAACGAGGCTGTACTCTCTGTCTTCAAATCTTTGAGGAG
 GCCTTTGCTCTCGCCGAGCATAAAAAAAGTGTACCTCTCCCCACCTCGTCTCTTGTAAAGTTTTGTTGGGAAT
 TATTTAGATAATGTGGACTATATATGCTCTGCCGCTCCAATATCCCAGTCTATTCTTTCTGATTATTGAAAT
 15 ATCAGCAGTTTTCCCTTAAATGATCTGATTAGTGTCTTATTCATATCAGGGAACATCTACCCAAAGGAATCCTT
 CTAGTTCACTTGCTGGTTCACGTCTCAAGGCTATGGCACTTGACTGTGAAATGGTTGGTGGTGGTGTGATGGGA
 CTATTGATCAGTGCATCGGTTTGCCTGGTTGACGATGACGAGAATGTGATCTTCTCCACTCACGTTCAACCAC
 TGCTCCCTGTACCGATTACAGGTTCTGCTTGTGGACCATTTGTGCTTGTGTTTGTGTTTATAATTCTTCTTTTAA
 ATCTCACCCCGCTCATGTTCAAGGCACGAGATAACTGGATTGACTAAGGAAGATTTGAAGGATGGTATGCCACTT
 20 GAGCATGTACGAGAGAGAGTTTTTTCGTTCTTGTGCGGTGGACAGAATGATGGGGCTGGAAGGCTTCTTCTTGT
 GGTGATGACCTTAGGCATGATATGAGTTGCTTGAAGCTTGAATACCCTAGCCATTTGTTGAGGTAACATAACTGAC
 CCGTTTTTGTGACTCTTTGCTTGAATCTAATGTAATTGCTATGCTTCACCTCAGAGACACAGCAAAATACGTG
 CCGTTGATGAAAGCAAACTTTGTAAGCCAATCGCTCAAGTACCTCACAAAGTCATATCTCGGGTAAGTTATGCTT
 GGGCTTTGATTTGTTGACATTGGATTATGAAAACCTTGAACATGAGATAGAAACTGGTTTTGTTTGTATGTGTAG
 25 ATACAAGATCCAGTGCGGGAAGCACGAGGTTTACGAGGACTGTGTATCTGCGATGAGACTGTACAAGAGAATGCG
 GGATCAAGAGCATGTTTGTAGTGGAAAGGCAGAAGGGAATGGTCTGAACTCGCGGAAACAGAGCGATCTAGAGAA
 GATGAATGCGGAAGAGCTGTACCAAAAATCAACGTGAGGTACCGGTGCTGGTGCCTTGACCGACTCAGCAATCC
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 30 CAACAACCTCTCCCTTTGGGAACTCGTATAAAAAGTTACTACTTAAGCTTCAAATCTGTGTAACATAAAATGGATAA
 AGTCTGATGCATCTGAGGCTTGGAACTCTGTTGCTCATAGTTGTGGATAACCAGCAACATCAAACATTATTACTTG
 TTTACCAAAACCACTCTCAGCTTCCATTTTATCAGTTTGAAGTCAAGTGGATAAAAAGACTGTCTACATAACTCA
 AATACTGTAGTATTATATTCACATATAAATGAGAAATTGACATTTCTCTAGAAAAAGAAAAGCAATCGTGTGAG
 AAGAAAACAAGTAAAAGGCTGAGGAAGAAGACGACTATTAGTCCCCGTCGAACCTGTTTTCTCCCGGTCGTCCAACA
 35 A

SEQ ID NO:95 Illustrative RFR1 promoter At3g23590; NP_189001 (encoded protein sequence)

AAATGATTTGTTTTGTGAATAGTTGATTCAGCTAATGTGGTGAAGACAATCATCTTACGTTTGGTGATTGTAT
 40 CAACTACATTGATGAAGAAGGGAACTTAGTAGGACAAAGAGGGGAATAAGCAGATGATAGGATTTGCTTCAAAGGA
 TGCATGGCGACATAATTTGCAGAAGGATTAGACATAATAACATGAAGATACTGTCAAGTATGAGCTCCTTCATGA
 ACTTAAGTACGTTTATAATTCACCTTGGATCCACAACCTTTGGGCTATTGGTATAAAAAGCTAACCTTTATCTAGATT

ACATGCTACATCTCAATTCAAGATGTCCATTTATTAAAGCAAAGGCATGACTTTATCTTCGACTAGAAACTGGTG
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 TTCTCTCAACACTATCCTATTTTTAGGTGATTTAGGGAAAAGAGAAATACTACACTTTGATGTAATTTTTTTTT
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 5 AAATGATGACGTTTTCTCAAGTTACTTATCGTAGTCTAATCGGTTATTGACAAAAATGATCTAGACGTTTTGAAA
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 AATCTAGAGATGATCTTGTCTCATTTGTTTATAGACTAAAGAGTATATAAAAGCTGGATTCATGTGGGTCAATCCA
 TCATTAATCGTGTCTTCAATTCACCAACTTCGTAGGTTCCGCATCTTTTGTACTTTCTTTCTATTTTTAATCTT
 GCAATATCAAAAAATAATATATACACCTTTTAAAAAAATCTGAATATGCATATGGTAATGGTATCATCAGAAAA
 10 CATTGAATATTGAATCGTCTATCTACCAATCTCTTCAAGTTGTGAATTTTTATTTATCAAAAAAAAAAAAAAAAAATC
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 15 ATAATATATAGCTTTGACAAATATCCCATCAGAATTCATAAATCGCCACATGAATCTTATAATTGTCAACTATGT
 GCAGTCCAATATTAATATCATGGAAAACCTAGATTACTAGCATTTAATTTCTTAATTGTATTTAGGTTATAGACTT
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 20 ATTTATTAATAAATGATAGTTTTTTTTAAGAAAGTTATTTGTATTTATTTATTTAAATTTGGATGCAAAAAATTACA
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 AGATCCATAGAACGTGGTAATGTTTTTTTTTTTTTTTTTTTTCTGGCAAGAAATGGTAATGTTAATCAATTAGACTCA
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 TTCATGCAAAAATGCTTCCAAAAGTCATACTTCGAAATCATGGCTTTGTAACCTACCCTTGCCACCATCTATCTA
 25 AACTTATTATTGTTTAAAACAAAAATGATTTTTCAAAGAAAAAAAAAATAAACTCCCACCGGTCAATAACTTTTT
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 GAGTTTCGAATTTTCTCACAACGATTTTAACCATTTTCGTCACTCGCAAAATTTTTAGTGGATAACAAAAAAAAA
 30 AAAAGTTGAAATTTAATGGAAATTTAGGAGATTTGTCCAATTTTTAATTTTCCCGATTAATTTAAAAAATCTACT
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 TTATAAACTAAATTTTGTAGAACCATCAGAAGAAATTTGTACTTTTAACTTTAAAATCAATTAATAAATTTCAA
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 GATTTGTGAAATGTGAACTCTTTGACGTCTTTCTCATATTTTCGTCTCGGGACAATTCACACGCACTGAATCATC
 35 TCTCTCACGAGGACTATTCAGCCATTTGCAAACGCACACACAAACGCACACGCGTTATTTTTTTTTTTCGCCAAAT
 CAAATCTGAAGAGTTCCTGTATCTTTTAAACCGCTCTTCTTCTTCTTTCAGAGAGCTTCGTTGATTGAACGGAAAA

WHAT IS CLAIMED IS:

- 1 1. A method of engineering a plant to increase the production of a
2 biosynthetic product in a desired tissue, the method comprising:
3 introducing an expression cassette into the plant, wherein the expression
4 cassette comprises a polynucleotide encoding a transcription factor that regulates production
5 of the biosynthetic product operably linked to a heterologous promoter, wherein the
6 heterologous promoter is a promoter that induces gene expression of a gene that is a
7 downstream target of the transcription factor in the desired tissue; and
8 culturing the plant under conditions in which the transcription factor is
9 expressed.
- 1 2. The method of 1, wherein the promoter is a tissue-specific secondary
2 wall promoter and the transcription factor induces expression of secondary wall biosynthetic
3 products.
- 1 3. The method of claim 2, wherein the transcription factor is NAC
2 secondary wall-thickening promoting factor 1 (NST1), NST2, NST3, secondary wall-
3 associated NAC domain protein 2 (SND2), SND3, MYB domain protein 103 (MYB103),
4 MBY85, MYB46, MYB83, MYB58, or MYB63.
- 1 4. The method of claim 2 or claim 3, wherein the tissue-specific
2 secondary wall promoter is an IRX1, IRX3, IRX5, IRX8, IRX9, IRX14, IRX7, IRX10,
3 GAUT13, GAUT14, or CESA4 promoter.
- 1 5. The method of 1, wherein the transcription factor induces expression
2 of wax and/or cutin.
- 1 6. The method of claim 5, wherein the transcription factor is a *shine*
2 (SHN) transcription factor selected from SHN1 (also known as WIN1), SHN2, SHN3, SHN4,
3 or SHN5; or MYB 96.
- 1 7. The method of claim 5 or claim 6, wherein the promoter is a CER1,
2 CER2, CER3, CER4, CER5, CER6, CER10, WSD1, Mah1, WBC11, KCS1, KCS2, FATB,
3 LACS1, LACS2, CYP864A, CYP86A7, CYP86A5, KCS10, or KCS5 promoter.
- 1 8. The method of claim 1, wherein the plant is Arabidopsis, poplar,
2 eucalyptus, rice, corn, switchgrass, sorghum, millet, miscanthus, sugarcane, pine, alfalfa,

3 wheat, soy, barley, turfgrass, tobacco, hemp, bamboo, rape, sunflower, willow, or
4 Brachypodium.

1 9. A method of engineering a plant having lignin deposition that is
2 substantially localized to the vessels of xylem tissue of the plant, the method comprising:
3 introducing an expression cassette into the plant, wherein the plant is modified
4 to have a reduced level of expression of a lignin biosynthesis enzyme; and further, wherein
5 the expression cassette comprises a polynucleotide encoding the lignin biosynthesis enzyme
6 operably linked to a heterologous vessel-specific promoter; and
7 culturing the plant under conditions in which the lignin biosynthesis enzyme is
8 expressed.

1 10. The method of claim 9, wherein the lignin biosynthesis enzyme is
2 PAL, C4H, 4CL, HCT, C3H, or CCR1.

1 11. The method of claim 10, wherein the lignin biosynthesis enzyme is
2 C4H.

1 12. The method of claim 9, wherein the promoter is a VND1, VND2,
2 VND3, VND4, VND5, VND6, VND7, VNI2, REF4, or RFR1 promoter.

1 13. The method of claim 9, wherein the level of expression of the lignin
2 biosynthesis enzyme in the modified plant is reduced by contacting the plant with an
3 antisense oligonucleotide that silences expression of the gene encoding the lignin
4 biosynthesis enzyme.

1 14. The method of claim 9, wherein the modified plant in which the
2 polynucleotide operably linked to the heterologous promoter is expressed has a mutation in
3 the gene encoding the lignin synthesis enzyme that decreases expression of the enzyme.

1 15. The method of claim 9, wherein the plant is selected from the group
2 consisting of Arabidopsis, poplar, eucalyptus, rice, corn, switchgrass, sorghum, millet,
3 miscanthus, sugarcane, pine, alfalfa, wheat, soy, barley, turfgrass, tobacco, hemp, bamboo,
4 rape, sunflower, willow, and Brachypodium.

1 16. A plant engineered by the method of any of claims 1-15, or a progeny
2 of the plant.

1 17. A plant cell from the plant of claim 16.

- 1 18. Seed from the plant of claim 16.
- 1 19. A plant cell comprising a polynucleotide encoding a lignin
2 biosynthesis enzyme operably linked to a heterologous vessel-specific promoter.
- 1 20. The plant cell of claim 19, wherein the lignin biosynthesis enzyme is
2 PAL, C4H, 4CL, HCT, C3H, or CCR1.
- 1 21. The plant cell of claim 19 or claim 20, wherein the promoter is a
2 VND1, VND2, VND3, VND4, VND5, VND6, VND7, VNI2, REF4, or RFR1 promoter.
- 1 22. A plant comprising the plant cell of claim 19, wherein the plant has
2 lignin deposition that is substantially localized to the vessels of xylem tissue of the plant.
- 1 23. Biomass comprising plant tissue from the plant or part of the plant of
2 claim 16 or claim 22.
- 1 24. A method of obtaining an increased amount of soluble sugars from a
2 plant in a saccharification reaction, the method comprising:
3 subjecting the plant of claim 16 or claim 22 to a saccharification reaction,
4 thereby increasing the amount of soluble sugars that can be obtained from the plant as
5 compared to a wild-type plant.
- 1 25. A method of engineering a plant having increased secondary cell wall
2 deposition, the method comprising:
3 introducing an expression cassette into the plant, wherein the expression
4 cassette comprises a polynucleotide encoding a transcription factor that regulates the
5 production of secondary cell wall in woody tissue operably linked to a heterologous
6 promoter, wherein the promoter enhances expression of a gene that is a downstream target of
7 the transcription factor; and
8 culturing the plant under conditions in which the transcription factor is
9 expressed.
- 10 26. The method of claim 25, wherein the transcription factor is NST1,
11 NST2, NST3, MYB103, MYB85, MYB46, MYB83, MYB58, or MYB63.
- 1 27. The method of claim 26, wherein the transcription factor is NST1.

1 28. The method of claim 25 or 26, wherein the promoter is an IRX1, IRX3,
2 IRX5, IRX8, IRX9, IRX14, IRX7, or IRX10 promoter.

1 29. The method of claim 28, wherein the promoter is the native promoter
2 of an IRX1, IRX3, IRX5, IRX8, IRX9, IRX14, IRX7, or IRX10 gene.

1 30. The method of claim 25, wherein the plant in which the polynucleotide
2 operably linked to the heterologous promoter is expressed is a wild-type plant.

1 31. The method of claim 25, wherein the plant in which the polynucleotide
2 operably linked to the heterologous promoter is expressed is an engineered plant having
3 lignin deposition that is substantially localized to the vessels of xylem tissue of the plant.

1 32. The method of claim 25, wherein the plant is selected from the group
2 consisting of Arabidopsis, poplar, eucalyptus, rice, corn, switchgrass, sorghum, millet,
3 miscanthus, sugarcane, pine, alfalfa, wheat, soy, barley, turfgrass, tobacco, hemp, bamboo,
4 rape, sunflower, willow, and Brachypodium.

1 33. A plant engineered by the method of any of claims 25-32, or a progeny
2 of the plant.

1 34. A plant cell from the plant of claim 33.

1 35. Seed from the plant of claim 33.

1 36. A plant cell comprising an expression cassette that comprises a
2 polynucleotide encoding a transcription factor that regulates the production of secondary cell
3 wall in woody tissue operably linked to a heterologous promoter, wherein the promoter is a
4 promoter that induces expression of a gene that is a downstream target of the transcription
5 factor.

1 37. The plant cell of claim 36, wherein the transcription factor is NST1,
2 NST2, NST3, MYB103, MYB85, MYB46, MYB83, MYB58, or MYB63.

1 38. The plant cell of claim 36, wherein the promoter is an IRX1, IRX3,
2 IRX5, IRX8, IRX9, IRX14, IRX7, or IRX10 promoter.

1 39. A plant comprising the plant cell of claim 36, wherein the plant has
2 increased secondary wall deposition.

1 40. A plant comprising the plant cell of claim 36, wherein the plant has
2 lignin deposition that is substantially localized to the vessels of xylem tissue of the plant..

1 41. Biomass comprising plant tissue from the plant or part of the plant of
2 claim 33, claim 39, or claim 40.

1 42. A method of increasing bioenergy production from biomass derived
2 from a plant, the method comprising:
3 harvesting biomass from the plant of claim 33, claim 39, or claim 40; and
4 subjecting the biomass to a conversion reaction, thereby increasing bioenergy
5 production as compared to a wild-type plant.

1 43. A method of increasing stem, straw or timber strength from plants
2 during growth, the method comprising
3 cultivating the plant of claim 33 or claim 39 to produce seed, wherein
4 resistance lodging is improved as compared to a wild type plant.

1 44. A method of obtaining a plant having improved resistance to
2 mechanical stress, the method comprising:
3 cultivating the plant of claim 33 or claim 39, thereby obtaining a plant having
4 improved resistance to mechanical stress compared to a wildtype plant that has not been
5 genetically modified to increase secondary cell wall production.

1 45. A method of engineering a plant having xylan deposition that is
2 substantially localized to the vessels of xylem tissue of the plant, the method comprising:
3 introducing an expression cassette into the plant, wherein the plant is modified
4 to have a reduced level of expression of a xylan biosynthesis enzyme; and further, wherein
5 the expression cassette comprises a polynucleotide encoding the xylan biosynthesis enzyme
6 operably linked to a heterologous vessel-specific promoter; and
7 culturing the plant under conditions in which the xylan biosynthesis enzyme is
8 expressed.

1 46. The method of claim 45, wherein the xylan biosynthesis enzyme is
2 irregular xylem 8 (IRX8), IRX14, IRX14-like, IRX9, IRX9-like, IRX7, IRX10, IRX10-like,
3 IRX15, IRX15-like, F8H, or PARVUS.

- 1 47. The method of claim 45, wherein the promoter is a VND1, VND2,
2 VND3, VND4, VND5, VND6, VND7, VNI2, REF4, or RFR1 promoter.
- 1 48. The method of claim 45, wherein the level of expression of the xylan
2 biosynthesis enzyme in the modified plant is reduced by contacting the plant with an
3 antisense oligonucleotide that silences expression of the gene encoding the xylan biosynthesis
4 enzyme.
- 1 49. The method of claim 45, wherein the modified plant in which the
2 polynucleotide operably linked to the heterologous promoter is expressed has a mutation in
3 the gene encoding the xylan synthesis enzyme that decreases expression of the enzyme.
- 1 50. The method of claim 45, wherein the plant is selected from the group
2 consisting of Arabidopsis, poplar, eucalyptus, rice, corn, switchgrass, sorghum, millet,
3 miscanthus, sugarcane, pine, alfalfa, wheat, soy, barley, turfgrass, tobacco, hemp, bamboo,
4 rape, sunflower, willow, and Brachypodium.
- 1 51. A plant engineered by the method of any of claims 45-50, or a progeny
2 of the plant.
- 1 52. A plant cell from the plant of claim 51.
- 1 53. Seed from the plant of claim 51.
- 1 54. A plant cell comprising an expression cassette that comprises a
2 polynucleotide encoding a xylan biosynthesis enzyme operably linked to a heterologous
3 vessel-specific promoter.
- 1 55. The plant cell of claim 54, wherein the xylan biosynthesis enzyme is
2 .irregular xylem 8 (IRX8), IRX14, IRX14-like, IRX9, IRX9-like, IRX7, IRX10, IRX10-like,
3 IRX15, IRX15-like, F8H, or PARVUS.
- 1 56. The plant cell of claim 54, wherein the promoter is a VND1, VND2,
2 VND3, VND4, VND5, VND6, VND7, VNI2, REF4, or RFR1 promoter.
- 1 57. A plant comprising the plant cell of claim 54, wherein the plant has
2 xylan deposition that is substantially localized to the vessels of xylem tissue of the plant

1 58. Biomass comprising plant tissue from the plant or part of the plant of
2 claim 51 or claim 57.

1 59. A method of obtaining an increased amount of soluble sugars from a
2 plant in a saccharification reaction, the method comprising:

3 subjecting the plant of claim 51 or claim 57 to a saccharification reaction,
4 thereby increasing the amount of soluble sugars that can be obtained from the plant as
5 compared to a wild-type plant.

1 60. A method of obtaining an increased C6/C5 sugars ratio from a plant
2 after a hydrolysis reaction, the method comprising:

3 subjecting the plant of claim 51 or claim 57 to chemical or enzymatic
4 hydrolysis, thereby increasing the amount of C6 sugars compared to C5 sugars that can be
5 obtained from the plant as compared to a wild-type plant

1 61. A method of engineering a plant having xylan *O*-acetylation that is
2 substantially localized to the vessels of xylem tissue of the plant, the method comprising:

3 introducing an expression cassette into the plant, wherein the plant is modified
4 to have a reduced level of expression of an enzyme responsible for xylan *O*-acetylation; and
5 further, wherein the expression cassette comprises a polynucleotide encoding the xylan *O*-
6 acetylation enzyme operably linked to a heterologous vessel-specific promoter; and

7 culturing the plant under conditions in which the xylan *O*-acetylation enzyme
8 is expressed.

1 62. The method of claim 61, wherein the xylan *O*-acetylation enzyme is an
2 RWA protein.

1 63. The method of claim 61, wherein the promoter is a VND1, VND2,
2 VND3, VND4, VND5, VND6, VND7, VNI2, REF4, or RFR1 promoter.

1 64. The method of claim 61, wherein the level of expression of the xylan
2 *O*-acetylation enzyme in the modified plant is reduced by contacting the plant with an
3 antisense oligonucleotide that silences expression of the gene encoding the xylan *O*-
4 acetylation enzyme.

1 65. The method of claim 61, wherein the modified plant in which the
2 polynucleotide operably linked to the heterologous promoter is expressed has a mutation in
3 the gene encoding the xylan *O*-acetylation enzyme that decreases expression of the enzyme.

1 66. The method of claim 61, wherein the plant is selected from the group
2 consisting of Arabidopsis, poplar, eucalyptus, rice, corn, switchgrass, sorghum, millet,
3 miscanthus, sugarcane, pine, alfalfa, wheat, soy, barley, turfgrass, tobacco, hemp, bamboo,
4 rape, sunflower, willow, and Brachypodium.

1 67. A plant engineered by the method of any of claims 61-66, or a progeny
2 of the plant.

1 68. A plant cell from the plant of claim 67.

1 69. Seed from the plant of claim 67.

1 70. A plant cell comprising an expression cassette that comprises a
2 polynucleotide encoding the xylan *O*-acetylation enzyme operably linked to a heterologous
3 vessel-specific promoter.

1 71. The plant cell of claim 70, wherein the xylan *O*-acetylation enzyme is
2 an RWA protein.

1 72. The plant cell of claim 70 or claim 71, wherein the promoter is a
2 VND1, VND2, VND3, VND4, VND5, VND6, VND7, VNI2, REF4, or RFR1 promoter.

1 73. A plant comprising the plant cell of claim 70, wherein the plant has
2 xylan *O*-acetylation that is substantially localized to the vessels of xylem tissue of the plant

1 74. Biomass comprising plant tissue from the plant or part of the plant of
2 claim 67 or claim 70.

1 75. A method of obtaining an increased amount of soluble sugars from a
2 plant in a saccharification reaction, the method comprising:
3 subjecting the plant of claim 67 or 73, or claim to a saccharification reaction,
4 thereby increasing the amount of soluble sugars that can be obtained from the plant as
5 compared to a wild-type plant.

Majority ---MXAEAGXGHAN-----GSX--XCX-----SXADPLNWGVAAEXMKGSHLDEVKRMVAEYR---KPVVKL

10 20 30 40 50 60 70 80

AtPAL1 ---M EINGAHKSNG-----GGVDAMLCGGDIKTKNMVINAEDPLNWGAAAEQMKGSHLDEVKRMVAEFR---KPVVNL 67
PpPAL3 ---M ETITKNGYQN-----GSSESLCT-----QRDPLSWGVAAEAMKGSHLDEVKRMVAEYR---KPVVNL 55
OsPAL ---M ECENGHVAAA-----ANG--SSLC---V-----AKPRADPLNWGKAAEELSGSHLDAVKRMVAEYR---RPVVTI 58
ZmPAL ---M ECENGHVAAAS-----GNGGVCLA---T-----PAPRADPLNWAKAAEDLAGSHLDAVKRMVAEYR---RPLVKI 59
SbPAL ---M AGNG-----ATIVESDPLNWGAAAEELSGSHLDEVKRMVAQAR---QPVVKI 44
PlPAL ---M VAAAEMTQAN-----EVQVKSTGLCT-----DFGSSGSDPLNWVRAAKAMEGSHFEVVKAMVDSYLG-VKEIF-I 64
MsPAL METISAAITKNNAN---ESFCLIHAKNNNN---MKV-----NEADPLNWGVAAEAMKGSHLDEVKRMVAEYR---KPVVRL 67
TaPAL ---M ACAWR-----SRFRADPLNWGKAAEELSGSHLEAVKRMVAEYR---KPVVTM 45
GmPAL2 ---M ASEANAANTN---FCVNVS---NN---GYI-----SANDPLNWGAAEAMAGSHLDEVKRMVAEYR---RPVVKL 59
BvPAL ---M ECENAHVAAN-----GDG--LCV---A-----QPARADPLNWGKAAEELSGSHLDAVKRMVAEYR---KPVVTM 57
NtPAL1 ---M ASNGFVN-----GGENFELCKK-----S---ADPLNWEAAEELRGSHLDEVKRMVAEYR---KPVVKL 54
StPAL1 ---M APSIAQNGFVN-----GEVEEVLWKK-----STHDPLNWEAVDSL RGSHLDEVKRMVAEYR---KPIVKL 59
BoPAL ---M PREDCHVAAN-----GNG---LC---M-----AAPRADPLNWGKAAEELMGSHLDEVKRMVAEYR---QPVVKI 56
BnPAL1 ---M EVNG---LSHG-----GEVDAMLCGGEIK-KNATVVGADPLNWGAAAEQMKGSHLDEVKRMVAEFR---RPVVNL 64
HaPAL ---M MENGTHVN-----GSANGFCIK-----DPLNWGVAAEALTGSHLDEVKRMVAEFR---KPVVKL 51
RcPAL ---M AAMAENGSKN-----DSLESFCN-----MGRDPLSWGAAEELMKGSHLDEVKRMVAEYR---KPFVKL 56
VvPAL ---M DATNCHGSKN-----VESFC-----VSDPLNWGAAEELMKGSHLDEVKRMVAEYR---KPVVRL 52
JcPAL ---M ATITNGHQN-----GSLEGLC-----ITRDPLSWGVAAEELMKGSHLDEVKRMVAEYR---KPLVKL 55
EpPAL ---M ESIHQNGFKN-----GSLNSLCTDSESI-----RSHDPLSWGAAEELMKGSHLDEVKRMVAEYR---KPVVKL 61
TpPAL MEAVAAAITKNNINDYDSFCLTHA-NANN---MKV-----NEADPLNWGVAAEAMKGSHLDEVKRMVAEYR---KPVVRL 68
LjPAL5 ---M APTTNSNHES-----LNS-IFCT---AAK-----AGSDPLSWGVAADSMKGSHLDEVKRMVAEYR---KPVVKL 59
SmPAL -----MGGSHLEEVREVMHTVYGAAKPSFPI 26

Majority GGETLTIAQVAAIAAXDD-G--VKVELS-ESARAGVKASSDWVMSMNGTDSYGVTTFGATSHRRTKGGALQKELIR

90 100 110 120 130 140 150 160

AtPAL1 GGETLTIGQVAAIISTIGN-S--VKVELS-ETARAGVNASDWMVMSMNGTDSYGVTTFGATSHRRTKGGALQKELIR 143
PpPAL3 AGOTLTIAQVASTAGHDA-SN-VKVELS-ESARPRVKASSDWVMSMDKGTDSYGVTTFGATSHRRTKGGALQKELIR 132
OsPAL EGASLTIAQVAAVASAGAAAR---VELD-ESARGRVKASSDWVMSMNGTDSYGVTTFGATSHRRTKGGALQKELIR 133
ZmPAL EGASLTIAQVAAVASAGAAAR---VELD-ESARGRVKASSDWVMSMNGTDSYGVTTFGATSHRRTKGGALQKELIR 134
SbPAL EGSTLRVQVAAVASAGAAAR---VELD-ESARPRVKASSEITLDCIAHGCDTYGVTTFGATSHRRTKGGALQKELIR 122
PlPAL EGKSLTISDVAAVARR---SQVKVLDAAAKSRVEESSNWWLTQMNKGTDTYGVTTFGATSHRRTKGGALQKELIR 140
MsPAL GGETLTISQVAAIAAH-DH-G--VKVELS-ESARDGVKASSEWVMSMNGTDSYGVTTFGATSHRRTKGGALQKELIR 143
TaPAL EGATLTIAQVAAVAAAGDTR---VELD-ESARGRVKASSDWVMSMNGTDSYGVTTFGATSHRRTKGGALQKELIR 119
GmPAL2 GGETLTISQVAAIAAH-DQ-G--VKVELS-ESSRAGVKASSDWVMSMNGTDSYGVTTFGATSHRRTKGGALQKELIR 135
BvPAL EGASLTIAQVAAVAAAGDTR---VELD-ESARGRVKASSDWVMSMNGTDSYGVTTFGATSHRRTKGGALQKELIR 132
NtPAL1 GGESLTIAQVAAIAAVRDKSANGVKVELS-ETARAGVKASSDWVMSMNGTDSYGVTTFGATSHRRTKGGALQKELIR 133
StPAL1 WGETLTIAQVAAIANADNKTSGFVKVELS-ESARAGVKASSDWVMSMNGTDSYGVTTFGATSHRRTKGGALQKELIR 138
BoPAL EGASLTIAQVAAVAVACDAK---VELD-ESARERVKASSDWVMSMNGTDSYGVTTFGATSHRRTKGGALQKELIR 131
BnPAL1 GGETLTIGQVAAIISTLGN-G--VKVELS-ETARAGVKASSDWVMSMNGTDSYGVTTFGATSHRRTKGGALQKELIR 140
HaPAL GGETLTISQVAAIASHDC-G--VKVELS-ESARAGVKASSDWVMSMNGTDSYGVTTFGATSHRRTKGGALQKELIR 129
RcPAL GGETLTIAQVAAIAASHDC-G--VKVELS-ESARAGVKASSDWVMSMNGTDSYGVTTFGATSHRRTKGGALQKELIR 132
VvPAL GGETLTISQVAAIAGREG-D--VKVELS-ETARAGVNASDWMVMSMNGTDSYGVTTFGATSHRRTKGGALQKELIR 128
JcPAL GGETLTIAQVAAIASHDA-G--VKVELS-ESARAGVKASSDWVMSMNGTDSYGVTTFGATSHRRTKGGALQKELIR 131
EpPAL GGETLTIAQVAAIAASNG-SENIRVELA-ESARAGVKASSDWVMSMNGTDSYGVTTFGATSHRRTKGGALQKELIR 139
TpPAL GGETLTISQVAAIAAH-D--G--ATVELS-ESARAGVKASSDWVMSMNGTDSYGVTTFGATSHRRTKGGALQKELIR 143
LjPAL5 GGETLTIAQVAAIAANDQ-G--VSVELC-ESARAGVKASSDWVMSMNGTDSYGVTTFGATSHRRTKGGALQKELIR 135
SmPAL ECTLTIAQVAAVAKRG---AAAVRLDSAAAKKRVDESSNWWLDNAMKGTDTYGVTTFGATSHRRTKGGALQKELIR 103

Fig. 1 (Cont'd)

Majority FLNAGIFGNGTE-XHTLPHSATRAAMLVRINTLLQGYSGIRFEILEAITKLLNNNITPCLPLRGTITASGDLVPLSYIAG
 170 180 190 200 210 220 230 240

AtPAL1	FLNAGIFG ST KETSHTLPHSATRAAMLVRINTLLQ G YSGIRFEILEAIT S FLNNNITP S LPLRGTITASGDLVPLSYIAG	223
PpPAL3	FLNAGIFGNGTET C HTLPHSATRAAMLVRINTLLQGYSGIRFEILEAITKLLNNNITPCLPLRGTITASGDLVPLSYIAG	212
OsPAL	FLNAGIFGNGDD-GH V LPA A ATRAAMLVRINTLLQGYSGIRFEILEIT A TLLNAN V TPCLPLRGTITASGDLVPLSYIAG	212
ZmPAL	FLNAGIFG T GDD-GH V LPA A ATRAAMLVRINTLLQGYSGIRFEILEIT A VLLNAN V TPCLPLRGTITASGDLVPLSYIAG	213
SbPAL	HLNAGIFG T GSD-GHTL P SE V RAAMLVRINTLLQGYSGIRFEILEAITKLL N TGVSPCL P LRGTITASGDLVPLSYIAG	201
PlPAL	FLNAG V L G -K C P-EN V SE D ITRAAMLVR I NTLLQGYSGIR W ILE T VE K LLNAGL T P K LPLRGTITASGDLVPLSYIAG	218
MsPAL	FLNAGIFGNGTES N H L PK T ATRAAMLVRINTLLQGYSG I D F EILEAIT K PL N K V TPCLPLRGTITASGDLVPLSYIAG	223
TaPAL	FLNAGIFG T G D -GH V LPA A ATRAAMLVR V NTLLQGYSGIRFEILEIT A TLLNAN V TPCLPLRGTITASGDLVPLSYIAG	198
GmPAL2	FLNAGIFGNGTES N C T LPHSATRAAMLVRINTLLQGYSGIRFEILEIT K L L NNNITPCLPLRGTITASGDLVPLSYIAG	215
BvPAL	FLNAGIFG T G D -GH V LPA A ATRAAMLVR V NTLLQGYSGIRFEILEIT A TLLNAN V TPCLPLRGTITASGDLVPLSYIAG	211
NtPAL1	FLNAGIFGNGTETSHTLPHSATRAAMLVRINTLLQGYSGIRFEILEAITKLL N SNITPCLPLRGTITASGDLVPLSYIAG	213
StPAL1	FLNAGIFGNGTES H T L PHSATRAAMLVRINTLLQGYSGIRFEILEAITKLL N SNITPCLPLRGTITASGDLVPLSYIAG	218
BoPAL	FLNAGIFG T G C D-GH V LPA E ATRAAMLVRINTLLQGYSGIRFEILEAITKLLNAN V TPCLPLRGTITASGDLVPLSYIAG	210
BnPAL1	FLNAGIFG ST KETSHTLPHSATRAAMLVRINTLLQGYSGIRFEILEAIT S FLNNNITP S LPLRGTITASGDLVPLSYIAG	220
HaPAL	FLNAGIFGNGTES S H T LPHSATRAAMLVRINTLLQGYSGIRFEILEIT K F L NNNITPCLPLRGTITASGDLVPLSYIAG	209
RcPAL	FLNAGIFGNGTES C H T LPHSATRAAMLVRINTLLQGYSGIRFEILEITKLL N F N ITPCLPLRGTITASGDLVPLSYIAG	212
VvPAL	FLNAGIFGNGTES C H T LPHSATRAAMLVRINTLLQGYSGIRFEILEAITKLL N F N ITPCLPLRGTITASGDLVPLSYIAG	208
JcPAL	FLNAGIFGNGTET C H T LPHSATRAAMLVRINTLLQGYSGIRFEILEAITKLL N F N ITPCLPLRGTITASGDLVPLSYIAG	211
EpPAL	FLNAGIFGNGTETS H LPHSAT K A A M L VRINTLLQGYSGIRFEILEAITKLL N F N ITPCLPLRGTITASGDLVPLSYIAG	219
TpPAL	FLNAGIFGNGTES N H L PHSATRAAMLVRINTLLQGYSGIRFEILEAITKLLNNNITPCLPLRGTITASGDLVPLSYIAG	223
LjPAL5	FLNAGIFGNGTETSHTL P Q P ATRAAMLVRINTLLQGYSGIRFEILEAITKLLNNNITPCLPLRGTITASGDLVPLSYIAG	215
SmPAL	FLNAGIF S EDDS-T N V L L P L A F A RAAMLVR I NTLLQGYSGIR E IL S A M E K L V NS G I V A R I P L R GTITASGDLVPLSYIAG	182

Majority LLTGRPN**S**KAV**G**PD**G**E**K**L**N**A**E**A**F**K**L**A**G**I**E**S**G**F**F**E**L**Q**P**K**E**G**L**A**L**V**N**G**T**A**V**G**S**G**L**A**S**M**V**L**F**E**A**N**V**L**A**V**L**S**E**V**L**S**A**I**F**A**E**V**M**
 250 260 270 280 290 300 310 320

AtPAL1	LLTGRPN S KAT G P N G E A L T A E E A F K L A G I S S G F F D L Q P K E G L A L V N G T A V G S G V A S M V L F E T N V L S V L A E I L S A V F A E V M	303
PpPAL3	LLTGRPN S KAT G P N G E V L D A V E A F K A A G I D S G F F E L Q P K E G L A L V N G T A V G S G L A S M V L F E T N V L A V L S E L S A I F A E V M	292
OsPAL	L V TGRPN S V A T P D G R K V D A A E A F K I A G I O H G F F E L Q P K E G L A V N G T A V G S G L A S M V L F E A N V L S V L A E V L S A V F C E V M	292
ZmPAL	L V TGRPN S T A V A P D G R K V G A A E A F E I A G I O H G F F E L Q P K E G L A V N G T A V G S G L A S M V L F E A N V L A V L A E V M S A V F C E V M	293
SbPAL	L I TGRPN S Q A T T V D G R K V D A A E A F K I A G I E G G F F K L N P K E G L A I V N G T S V G S A L A A T M Y D A N V L A V L S E V L S A I F C E V M	281
PlPAL	L L TGRPN S R V R S R D G E M S G A E L K K V G L E K -P F E L Q P K E G L A I V N G T S V G A L A S I V C F D A N V L A L S E V L S A M F C E V M	297
MsPAL	L L TGRPN S K A H G P S G E V L N A K E A F N L A G I N A E F F E L Q P K E G L A L V N G T A V G S G L A S I V L F E A N I L A V L S E V L S A I F A E V M	303
TaPAL	L V TGRPN S M A T A P D G S K V N A A E A F K I A G I O H G F F E L Q P K E G L A V N G T A V G S G L A S M V L F E A N V L S L L A E V L S A V F C E V M	278
GmPAL2	L L TGRPN S K A V G P S G E L N A K E A F E L A N I G A E F F E L Q P K E G L A L V N G T A V G S G L A S I V L F E A N I L A V L S E V L S A I F A E V M	295
BvPAL	L V TGRPN S V A T A P D G T K V N A A E A F K I A G I O H G F F E L Q P K E G L A V N G T A V G S G L A S M V L F E A N I L S L L A E V L S A V F C E V M	291
NtPAL1	L L TGRPN S K A V G P N G E T L N A E E A F R V A V S G F F E L Q P K E G L A L V N G T A V G S G V A S M V L F D S N I L A V M S E V L S A I F A E V M	293
StPAL1	L L TGRPN S K A V G P S G S K L D A D E A F R V A V S G F F E L Q P K E G L A L V N G T A V G S G V A S I V L Y D S N I L A V M F E V L S A I F A E V M	298
BoPAL	L V TGR E N S V A V A P D G R K V N A A E A F K I A G I O G F F E L Q P K E G L A V N G T A V G S G L A S I V L F E A N I L A T L A E V L S A V F C E V M	290
BnPAL1	L L TGRPN S K A T G P N G E A L N A E E A F K V A G V T S G F F D L Q P K E G L A L V N G T A V G S G V A S M V L F E A N V L S V L A E V L S A V F A E V M	300
HaPAL	L L TGRPN S K A V G P A G E V L N A E S A F A Q A G V E G F F E L Q P K E G L A L V N G T A V G S G V A S M V L F E A N V L A L S E V L S A I F A E V M	289
RcPAL	L L TGRPN S K A T G P N G S M D A L E A F R L A G I E S G F F E L Q P K E G L A L V N G T A V G S G L A S M V L F E A N I L A V L S E L S A I F A E V M	292
VvPAL	L L TGRPN S K A V G P S G E V V N A E E A F K V A G I E S G F F E L Q P K E G L A L V N G T A V G S G L A S M V L F E T N V L A V L S E V L S A I F A E V M	288
JcPAL	L L TGRPN S K A T G P S G E S L D A V E A F R L A D I D S G F F E L Q P K E G L A L V N G T A V G S G L A S M V L F E A N V L A V L S E L S A I F A E V M	291
EpPAL	L L TGR S N S K A T G P N G S L D A Q C A F S E A G I D S G L F E L Q P K E G L A L V N G T A V G S G L A S M V L F D A N I L A V L S E V L S A I F A E V M	299
TpPAL	L L TGRPN S K A H G P S G E L N A K E A F Q L A G I N A D F F E L Q P K E G L A L V N G T A V G S G L A S I V L F E A N I L A V L S E V L S A I F A E V M	303
LjPAL5	L L TGR Q N S K A V G P S G E V V N A K E A F Q L A G I D T G F F E L Q P K E G L A L V N G T A V G S G L A S I V L F D A N V L A T L A E V L S A I F A E V M	295
SmPAL	L L TGR S N A R A V L P D G K V V T S A E A L K L V G V E Q -P F E L Q P K E G L A I V N G T A V G A V A S I A C F D A N V L A L L A E I L S A M F C E A M	261

Fig. 1 (Cont'd)

Majority NGKPEFTDHLTHLKHHPGQIEAAAIMEHILDGSSYMKAAKKLHEMDPLQKPKQDRYALRTSPQWLGPOIEVIRAATKSI
 330 340 350 360 370 380 390 400

AtPAL1 SGKPEFTDHLTHRLKHHPGQIEAAAIMEHILDGSSYMKLAQKLHEMDPLQKPKQDRYALRTSPQWLGPOIEVIRYATKSI 383
 PpPAL3 NGKPEFTDHLTHLKHHPGQIEAAAIMEHILDGSA^YYMKAAKKLHEMDPLQKPKQDRYALRTSPQWLGPOIEVIRFSTKSI 372
 OsPAL NGKPEYTDHLTHLKHHPGQIEAAAIMEHILEGSSYMLAKKLGELDPLMKPKQDRYALRTSPQWLGPOIEVIRAATKSI 372
 ZmPAL NGKPEYTDHLTHLKHHPGQIE^SAAIMEHILEGSSYMLAKKLGELDPLMKPKQDRYALRTSPQWLGPOIEVIRAATKSI 373
 SbPAL NGKPEYTDHLTHLKHHPG^SIEAAAIMEHILDGSA^FFMKHA^KKNELDPLMKPKQDRYALRTSPQWLGPOIEVIRAATKSI 361
 PlPAL NGKPEFTD^PLTHLKHHPGQIEAAAIME^YLDGSSYMKLA^AKLHEMDPLQKPKQDRYALRTSPQWLGPOIEVIR^SATHMI 377
 MsPAL QGKPEFTDHLTHLKHHPGQIEAAAIMEHILDGSSY^VKAAKKLHEIDPLQKPKQDRYALRTSPQWLG^LVEVIRFSTKSI 383
 TaPAL NGKPEFTDHLTHLKHHPGQIEAAAIMEHILEGSSYMLAKKLGELDPLMKPKQDRYALRTSPQWLGPOIEVIRAATKSI 358
 GmPAL2 QGKPEFTDHLTHLKHHPGQIEAAAIMEHILEGSSY^VKAAKKLHEIDPLQKPKQDRYALRTSPQWGL^LIEVIRFSTKSI 375
 BvPAL NGKPEYTDHLTHLKHHPGQIEAAAIMEHILEGSSYMLAKKLGELDPLMKPKQDRYALRTSPQWLGPOIEVIRAATKSI 371
 NtPAL1 NGKPEFTDHLTHLKHHPGQIEAAAIMEHILDGSSY^VKAQKLHEMDPLQKPKQDRYALRTSPQWLGPOIEVIRAATKMI 373
 StPAL1 NGKPEFTD^YLTHLKHHPGQIEAAAIMEHILDGSSY^VKAQKLHEMDPLQKPKQDRYALRTSPQWLGPOIEVIRAATKMI 378
 BoPAL NGKPEYTDHLTHLKHHPGQIEAAAIMEHILEGSSYMKLAKKLG^DLPLMKPKQDRYALRTSPQWLGPOIEVIRAATKSI 370
 BrPAL1 SGKPEFTDHLTHRLKHHPGQIEAAAIMEHILDGSSYMKLAQKLHEMDPLQKPKQDRYALRTSPQWLGPOIEVIRYATKSI 380
 HaPAL QGKPEFTDHLTHLKHHPGQIEAAAIME^YLDGSD^VYKAQ^VHEMDPLQKPKQDRYALRTSPQWLGPOIEVIRSATKMI 369
 RcPAL NGKPEFTDHLTHLKHHPGQIEAAAIMEHILDGSSY^VKAQKLHEMDPLQKPKQDRYALRTSPQWLGPOIEVIRFSTKSI 372
 VvPAL QGKPEFTDHLTHLKHHPGQIEAAAIMEHILDGSSY^VKAQKLHEMDPLQKPKQDRYALRTSPQWLG^PIEVIRASTKSI 368
 JcPAL NGKPEFTDHLTHLKHHPGQIEAAAIMEHILDGSSY^VKAQKLHEIDPLQKPKQDRYALRTSPQWLGPOIEVIRFSTKSI 371
 EpPAL NGKPEFTDHLTHLKHHPGQIEAAAIMEHILDGSSY^VKAQKLHEMDPLQKPKQDRYALRTSPQWLGPOIEVIRFSTKSI 379
 TpPAL QGKPEFTDHLTHLKHHPGQIEAAAIMEHILDGSA^YYMKAAKKLHEIDPLQKPKQDRYALRTSPQWGL^LIEVIRFSTKSI 383
 LjPAL5 QGKPEFTDHLTHLKHHPGQIEAAAIMEHILDGSSYMKAAKKLHEMDPLQKPKQDRYALRTSPQWGL^LIEVIRFSTKSI 375
 SmPAL QGKPEFTD^PLTHLKHHPGQIEAAAIME^VLDGSSYMKLA^AKLHEIDPLMKPKQDRYALRTSPQWLGPOIEVIRHATKSI 341

Majority EREINSVNDNPLIDVSRNKALHGGNFQGTPIGVSMNTRLATAAIGKLMFAQFSELVNDFYNNGLPSNLSXGRNPSLDYG
 410 420 430 440 450 460 470 480

AtPAL1 EREINSVNDNPLIDVSRNKALHGGNFQGTPIGVSMNTRLATAAIGKLMFAQFSELVNDFYNNGLPSNLTA^SRNPSLDYG 463
 PpPAL3 EREINSVNDNPLIDVSRNKALHGGNFQGTPIGVSMN^VRLATA^SIGKLL^LFAQFSELVNDFYNNGLPSNLTA^SRNPSLDYG 452
 OsPAL EREINSVNDNPLIDVSR^GKALHGGNFQGTPIGVSMNTRLATAAIGKLMFAQFSELVNDFYNNGLPSNLS^GGRNPSLDYG 452
 ZmPAL EREINSVNDNPLIDVSR^GKALHGGNFQGTPIGVSMNTRLATAAIGKLMFAQFSELVNDFYNNGLPSNL^SGGRNPSLDYG 453
 SbPAL EREINSVNDN^VIDV^RRGKALHGGNFQGTPIGVSMN^RRLATA^NIGKLMFAQFSELVND^VFYNNGL^LPSNL^SAGRNPSLDYG 441
 PlPAL EREINSVNDN^VIDV^RADKALHGGNFQGTPIGVSMN^LRL^STAIGKLMFAQFSELVND^YYNGGLPSNL^SCGRNPSLDYG 457
 MsPAL EREINSVNDNPLIDVSRNKALHGGNFQGTPIGVSMNTRLATAAIGKLMFAQFSELVNDFYNNGLPSNLSA^SRNPSLDYG 463
 TaPAL EREINSVNDNPLIDVSR^GKALHGGNFQGTPIGVSMNTRLATAAIGKLMFAQFSELVNDFYNNGLPSNLS^GGRNPSLDYG 438
 GmPAL2 EREINSVNDNPLIDVSRNKALHGGNFQGTPIGVSMNTRLATAAIGKLMFAQFSELVND^YYNNGLPSNLTA^SRNPSLDYG 455
 BvPAL EREINSVNDNPLIDVSR^GKALHGGNFQGTPIGVSMNTRLATAAIGKLMFAQFSELVNDFYNNGLPSNLS^GGRNPSLDYG 451
 NtPAL1 EREINSVNDNPLIDVSRNKALHGGNFQGTPIGVSMN^RRLATA^SIGKLMFAQFSELVND^YYNNGLPSNLTA^SRNPSLDYG 453
 StPAL1 EREINSVNDNPLIDVSRNKALHGGNFQGTPIGVSMNTRLATAAIGKLMFAQFSELVND^YYNNGLPSNLTA^SGRNPSLDYG 458
 BoPAL EREINSVNDNPLIDVSRNKALHGGNFQGTPIGVSMNTRLATAAIGKLMFAQFSELVNDFYNNGLPSNLS^GGRNPSLDYG 450
 BrPAL1 EREINSVNDNPLIDVSRNKALHGGNFQGTPIGVSMNTRLATAAIGKLMFAQFSELVND^YYNNGLPSNLTA^SRNPSLDYG 460
 HaPAL EREINSVNDNPLIDVSRNKALHGGNFQGTPIGVSMNTRLATAAIG^VTTIAQFSELVNDFYNNGLPS^HL^SGGRNPSLD^SG 449
 RcPAL EREINSVNDNPLIDVSRNKALHGGNFQGTPIGVSMN^RRLATA^SIGKLMFAQFSELVNDFYNNGLPSNLTA^SGRNPSLDYG 452
 VvPAL EREINSVNDNPLIDVSRNKALHGGNFQGTPIGVSMNTRLATAAIGKLMFAQFSELVNDFYNNGLPSNLS^GGRNPSLDYG 448
 JcPAL EREINSVNDNPLIDVSRNKALHGGNFQGTPIGVSMN^RRLATA^SIGKLMFAQFSELVNDFYNNGLPSNLS^GGRNPSLDYG 451
 EpPAL EREINSVNDNPLIDVSRNKALHGGNFQGTPIGVSMN^RRLATA^SIGKLMFAQFSELVNDFYNNGLPSNLTA^SGRNPSLDYG 459
 TpPAL EREINSVNDNPLIDVSRNKALHGGNFQGTPIGVSMNTRLATAAIGKLMFAQFSELVNDFYNNGLPSNLSA^SRNPSLDYG 463
 LjPAL5 EREINSVNDNPLIDVSRNKALHGGNFQGTPIGVSMNTRLATAAIGKLMFAQ^FTEL^VDD^HYNNGLPSNLTA^SRNPSLDYG 455
 SmPAL QREINSVNDN^VIDV^RADKALHGGNFQGTPIGVSMN^LRLATAAIGKLMFAQFSELVNDFYNNGLPSNLS^GGRNPSLDYG 421

Fig. 1 (Cont'd)

Majority FKGAEIAMASYCSELQYLANPVTNHVQSAEQHNQDVNSLGLISSRKTAEAIDILKLMSSSTFLVALCQAIIDLRLHEENLKS

	490	500	510	520	530	540	550	560	
AtPAL1	FKGAEIAMASYCSELQYLANPVTSHVQSAEQHNQDVNSLGLISSRKTAEAIDILKLMSSSTFLVALCQAVDLRHL	ENLRS	RO	543					
PpPAL3	FKGAEIAMASYCSELQYLANPVTSHVQSAEQHNQDVNSLGLISSRKTAESVDILKLMSTIFLVALCQAIIDLRL	HEENLRS	532						
OsPAL	FKGAEIAMASYCSELQFLANPVTNHVQSAEQHNQDVNSLGLISSRKTAEAIDVILKLMSSSTFLIALCQAIIDL	RHL	ENLRS	532					
ZmPAL	FKGAEIAMASYCSELQFLANPVTNHVQSAEQHNQDVNSLGLISSRKTAEAIDVILKLMSSSTFLVALCQAIIDL	RHL	ENLRS	533					
SbPAL	FKGTEIAMASYCSELQFLGNPTLNHVQSAEQHNQDVNSLGLVSSARKTAEATDILKLMSSSTYIVALCQAIIDL	RHL	ENLRS	521					
PlPAL	LKGAEIAMASYTSELQYLANPVTSHVQSAEQHNQDVNSLGLVSSARKSAEAIDILKLMSTYLTALCQAVDLRHL	ENMLA	537						
MsPAL	FKGAEIAMASYCSELQYLANPVTTHVQSAEQHNQDVNSLGLISSARKTAEATDILKLMSSSTFLIALCQAIIDL	RHL	ENLKN	543					
TaPAL	FKGAEIAMASYCSELQFLGNPVTNHVQSAEQHNQDVNSLGLISSRKTAEAIDILKLMSSSTFLVALCQAIIDL	RHL	ENLKN	518					
GmPAL2	FKGAEIAMASYCSELQYLANPVTSHVQSAEQHNQDVNSLGLISSRKTHAEATDILKLMSSSTFLVALCQAIIDL	RHL	ENLKN	535					
BvPAL	FKGAEIAMASYCSELQFLGNPVTNHVQSAEQHNQDVNSLGLISSRKTAEAIDILKLMSSSTFLVALCQAIIDL	RHL	ENLKN	531					
NtPAL1	FKGAEIAMASYCSELQFLANPVTNHVQSAEQHNQDVNSLGLISSARKTAEAVDILKLMSSSTYIVALCQAIIDL	RHL	ENLKN	533					
StPAL1	FKGAEIAMASYCSELQFLANPVTNHVQSAEQHNQDVNSLGLISSARKTAEAVDILKLMSSSTYIVALCQAIIDL	RHL	ENLKS	538					
BoPAL	FKGAEIAMASYCSELQFLGNPVTNHVQSAEQHNQDVNSLGLISSRKTAEAIDILKLMSSSTFLVALCQAIIDL	RHL	ENLKS	530					
BnPAL1	FKGAEIAMASYCSELQYLANPVTSHVQSAEQHNQDVNSLGLISSRKTAEAIDILKLMSTIFLVGLCQAIIDL	RHL	ENLKO	540					
HaPAL	FKGTEIAMASYCSELQFLANPVTNHVQSAEQHNQDVNSLGLISSARKTAEAVDILKLMSSSTYIVALCQAIIDL	RHL	ENLKS	529					
RcPAL	FKGAEIAMASYCSELQYLANPVTSHVQSAEQHNQDVNSLGLISSRKTAEAIDILKLMSTYIVALCQAIIDL	RHL	ENLKO	532					
VvPAL	FKGAEIAMASYCSELQFLANPVTNHVQSAEQHNQDVNSLGLISSRKTAEAIDILKLMSTYIVALCQAIIDL	RHL	ENLKS	528					
JcPAL	FKGAEIAMASYCSELQFLANPVTSHVQSAEQHNQDVNSLGLISSRKTEAIDILKLMSSSTFLVALCQAIIDL	RHL	ENLKH	531					
EpPAL	FKGAEIAMASYCSELQYLANPVTSHVQSAEQHNQDVNSLGLISSRKTEAIDILKLMSTIFLVALCQAIIDL	RHL	ENLRO	539					
TpPAL	FKGTEIAMASYCSELQYLANPVTTHVQSAEQHNQDVNSLGLISSRKTEAIDILKLMSSSTFLIALCQAIIDL	RHL	ENLKN	543					
LjPAL5	LKGAEIAMASYCSELQYLANPVTTHVQSAEQHNQDVNSLGLISSRKTEAIDILKLMSSSTFLIALCQAIIDL	RHL	ENLKH	535					
SmPAL	FKGAEIAMASYTSELQYLANPVTTHVQSAEQHNQDVNSLGLVSSARKTAEALDILKLMSSSTYIVALCQAIIDL	RHL	ENLQA	501					

Majority AVKNTVSQVAKKTLTTGANGELHPSRFCEKDLLKVVVDREYVFAYADDPCSATYPLMQKLRQVLVXHALANGENEKNANTS

	570	580	590	600	610	620	630	640	
AtPAL1	TVKNTVSQVAKKTLTTGANGELHPSRFCEKDLLKVVVDREYVFAYADDPCSATYPLIQKLRQVLVDHALNGE	SEKN	AVTS	623					
PpPAL3	AVKNTVSHVSKRVLTTGANGELHPSRFCEKDLLKVVVDREYVFAYADDPCSATYPLMQKLRQVLVDHALANG	ENEKNT	STS	612					
OsPAL	AVKGVTTVARKTLSTGATGLHARFCEKDLLQAIIDREAVFAYADDPCSATYPLMQKLRVLTVEHALANG	AE	ERNVDS	612					
ZmPAL	AVKRCVTAVARKTLSTGATGLHARFCEKDLLTAVDREAVFAYADDPCSATYPLMQKLRVLTVEHALANG	AE	ERDPDS	613					
SbPAL	SVKNTVIQVAKKVLTMNPSGDLSSARFSEKELITADREYVFAYADDPCSATYPLMQKLRVLTVEHALANG	AE	ERDPDS	599					
PlPAL	TVKQIVSQVAKKTLSTGLNGELHPSRFCEKDLLKVVVDREYVFAYADDPCSATYPLIQKLRVLTVEHALNG	E	GEKDPNTS	617					
MsPAL	SVKNTVSQVAKKTLTVGANGELHPSRFCEKDLLKVVVDREYVFAYADDPCSATYPLSQKLRQVLVDHALNG	E	SEKNFNTS	623					
TaPAL	AVKSCVKTIVARKTLSTIDNGELHARFCEKDLLTIDREAVFAYADDPCSATYPLMQKLRVLTVEHALANG	AE	AHVETS	598					
GmPAL2	TVKNVVSQVAKRLLTTGANGELHPSRFCEKDLLKVVVDREYVFAYADDPCSATYPLMQKLRQVLVDHAL	ANGENEKNT	STS	615					
BvPAL	AVKSCVKTIVARKTLSTIDNGELHARFCEKDLLTIDREAVFAYADDPCSATYPLMQKLRVLTVEHALANG	AE	AHDVETS	611					
NtPAL1	AVKNTVSQVAKRLLTTGANGELHPSRFCEKDLLKVVVDREYVFAYADDPCSATYPLMQKLRQVLVDHAL	NGE	SEKNVNTS	613					
StPAL1	VVKNTVSQVAKRLLTTGANGELHPSRFCEKDLLKVVVDREYVFAYADDPCSATYPLMQKLRQVLVDHAL	NGE	SEKNVNTS	618					
BoPAL	AVKSCVMTIVAKKTPSTNSTGDLHVARFCEKDLLKIDREAVFAYADDPCSATYPLMQKLRVLTVEHALANG	MAE	FNAETS	610					
BnPAL1	TVKNTVSQVAKKVLTTGANGELHPSRFCEKDLLKVVVDREYVFAYADDPCSATYPLIQKLRQVLVDHAL	NGE	SEKNVNTS	620					
HaPAL	TVKNTVSQVAKKVLTTGANGELHPSRFCEKDLLKVVVDREYVFAYADDPCSATYPLMQKLRQVLVDHAL	NGE	TEKNANTS	609					
RcPAL	AVKNTVSQVAKRVLTTGANGELHPSRFCEKDLLKVVVDREYVFAYADDPCSATYPLMQKLRQVLVDHAL	ANGENEKNA	CTS	612					
VvPAL	TVKNTVSQVAKKTLTTGANGELHPSRFCEKDLLKVVVDREYVFAYADDPCSATYPLMQKLRQVLVDHAL	ANGENEKNG	STS	608					
JcPAL	AVKNTVIQVAKRVLTTGANGELHPSRFCEKDLLKVVVDREYVFAYADDPCSATYPLMQKLRQVLVDHAL	ANGENEKNA	STS	611					
EpPAL	TVKNTVSQVAKRVLTTGANGELHPSRFCEKDLLKVVVDREYVFAYADDPCSATYPLMQKLRQVLVDHAL	NGE	NEKNANTS	619					
TpPAL	SVKNTVSQVAKKTLTVGANGELHPSRFCEKDLLKVVVDREYVFAYADDPCSATYPLSQKLRQVLVDHAL	NGE	SEKNVNTS	623					
LjPAL5	SVKNTVSQVAKRLLTTGANGELHPSRFCEKDLLKVVVDREYVFAYADDPCSATYPLMQKLRQVLVDHAL	NGENEKNS	KTS	615					
SmPAL	TVKQTVSHAVKKTLLTTGANGELHPSRFCEKDLLKVVVDREYVFAYADDPCSATYPLMQKLRQVLVDHAL	KNIGDE	KETSS	581					

Fig. 1 (Cont'd)

Majority IFQKIAAFEELKAXLPKEVEXARAAYESGXAAIPNRIKECRSYPLYRFVREELGTPELLTGEKVRSPGEEFDKVFAMCO

	650	660	670	680	690	700	710	720	
AtPAL1	IFHKT	GAFEELKAVLP	KEVEAARAAYDN	GTS	AIPNRIKECRSY	PLYRFVREEL	GTPELLTGEK	VITSPGEE	FDKVFATICE 703
PpPAL3	VFQKIT	AFEELKALLP	KEVESARAAYD	SGNSATENK	IKECRSYPLYK	FVREELGTCLL	TGEKVRSPG	EEDKVFAMCO 692	
OsPAL	VFQKIT	AFEELRVALP	REVEAARAAVEN	GTA	AKANRITTECRSY	PLYRFVREEL	GTEYLTGEK	TRSPGEEVNVKVFAMN 692	
ZmPAL	VFQKIT	AFEELRAALP	REVDAARAAVEN	GTA	AKNRITTECRSY	PLYRFVREEL	GTEYLTGEK	ARSPEGEEVDKVFAMNL 693	
SbPAL	VFSKIT	KFEELRAVLP	REVEAARVAE	GTAPVANRIAD	SRSFPLYRFVREEL	GCVFLTGEK	LKSPGEECTKVFNGIN 679		
PlPAL	IFNKI	PLFEELKAQL	ELQVSLARESY	DKGTSP	LPNRIQECRSY	PLYRFVFNQ	LGTKLLSG	TRTISPGEVIEVYDAISE 697	
MsPAL	IFQKIT	AFEELKTL	LPKEVESART	AYESGNPTIP	NKINGCRSY	PLYKRVREEL	GTCLLTGEN	VITSPGEECDKILFSAMCO 703	
TaPAL	VFQKIT	AFEELRAVLP	KEVEAARS	AVENGTAA	QNRITTECRSY	PLYRFVREL	GTTEYLTGEK	TRSPGEEVDKVFAMN 678	
GmPAL2	IFQKIT	AFEELKTL	LPKEVEGAR	VAYENDQCAIP	NKIKECRSY	PLYKRVREEL	GTALLTGER	VITSPGEECDKVFALCO 695	
BvPAL	VFQKIT	AFEELRAVLP	TEVEAARS	AVENGTAA	QNRITTECRSY	PLYRFVREL	GTTEYLTGEK	TRSPGEEVDKVFAMN 691	
NtPAL1	IFQKIT	GAFEDLKAVLP	KEVESARAAL	ESGNPAIP	NRIITECRSY	PLYRFVREL	GTPELLTGEK	VRSPGEECDKVFAMCN 693	
StPAL1	IFQKIT	GAFEDLNAVLP	KEVESARAL	LES	GNPSTIPNRI	TECRSYPLYR	VRC	ELGTPELLTGEKVRSPGEEITKVFAMCN 698	
BoPAL	IFARVAL	FEELRAALP	RAVEAARAS	VENGTAA	APNRIITECRSY	PLYRFVREEL	GTEYLTGEK	TRSPGEEELNVLLATIN 690	
BnPAL1	IFHKT	GAFEELKAVLP	KEVEAARAAYDN	GTA	AIPNRIKECRSY	PLYRFVREEL	GTPELLTGEK	ATSPGEEFDKVFATICE 700	
HaPAL	IFQKIT	AFEELKAILP	KEVESVRVAFEN	GTMS	IPNRIKACRSY	PLYRFVREEL	GGAT	667	
RcPAL	VFQKIT	AFEELKTL	LPKEVESVRI	AYESGNPAT	ANRIKECRSY	PLYKRVREEL	GTCLLTGD	KVMSPEGEEFDKVFAMCO 692	
VvPAL	IFQKIT	AFEELKAVLP	KEVESARDG	VESGNPSTIP	NRIKECRSY	PLYKRVREEL	GTCLLTGEK	VRSPGEEFDKVFAMCO 688	
JcPAL	VFQKIT	AFEELKTL	LPKEVESARE	AYESGSAATGN	KIKECRSY	PLYKRVREEL	GSGLLTGEK	VRSPGEEFDKVFAMCO 691	
EpPAL	IFQKIT	SAFEELKTL	LPKEVEAARA	AYESGNAP	IPNRIE	CRSYPLYKRVREEL	GTGLLTGD	KVRSPGEEFDKVFAMCO 699	
TpPAL	IFQKIT	AFEELKTL	LPKEVESART	AYESGNSTIAN	KINGCRSY	PLYKRVREEL	GTSLLTGER	VITSPGEECDKILFTAMCO 703	
LjPAL5	IFQKIT	AFEELKSL	LPKEVESARA	AYESGNPTIP	NKINECRSY	PLYKRVREEL	GTPELLTGEK	TRSPGEECDKILFTATICE 695	
SmPAL	VLHKT	IGLFEELKAA	LSVEVPAARE	AYESGNAVL	PNRIFDCA	SAPLYE	FVRKGA	GTALLMGTKSGTPGEDFTKVDATICE 661	

Majority GKIIDPLLECLKEWNGAPLPIC---

	730	740	
AtPAL1	GKIIDP	MMECLN	EWNGAPLPIC 725
PpPAL3	GKIIDP	MLECLG	EWNGAPLPIC 714
OsPAL	GKHID	ALLECLKE	WNGEPLPIC 714
ZmPAL	GKHID	AVLECLKE	WNGEPLPIC 715
SbPAL	GKLVDP	MLECLKE	WDGKPLPINVVN 704
PlPAL	DKVIV	PLFKCLD	GWKCTPGPF 718
MsPAL	GKIIDP	LLECLG	EWNGAPLPIC 725
TaPAL	GKHID	ALLECLKE	WNGEPLPIC 700
GmPAL2	GKIIDP	LLECLG	EWNGAPLPIC 717
BvPAL	GKHID	ALLECLKE	WNGEPLPIC 713
NtPAL1	GCITDP	VLECLK	SWNGAPLPIC 715
StPAL1	GCINDP	LLECLK	SWNGAPLPIC 720
BoPAL	GKHID	P	LLECLKEWNGEPLPIC 712
BnPAL1	GKIIG	P	LMECLDEWNGAPLPIC 722
HaPAL			667
RcPAL	GKIIDP	MMDCL	KEWNGAPLPIC 714
VvPAL	GKIIDP	LLDCL	SAWNGAPLPIC 710
JcPAL	GKIIDP	MMECL	KEWNGAPLPIC 713
EpPAL	GKIIDP	LMDC	LKEWNGAPLPIC 721
TpPAL	GKIIDP	LLECLG	EWNGSPLPIC 725
LjPAL5	GKIIDP	LLECLG	EWNGAPLPIC 717
SmPAL	GKLV	TPLKCLD	GWSTP-SF 681

Decoration 'Decoration #1': Shade (with solid black) residues that match the Consensus exactly.

6/67 Fig. 2

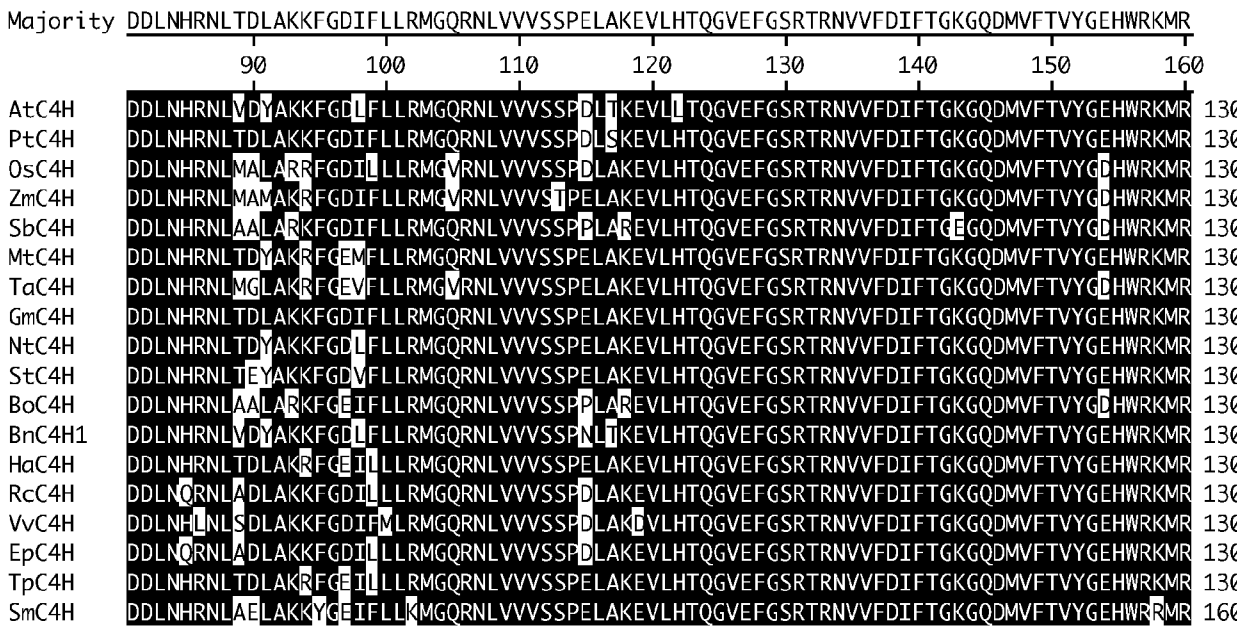
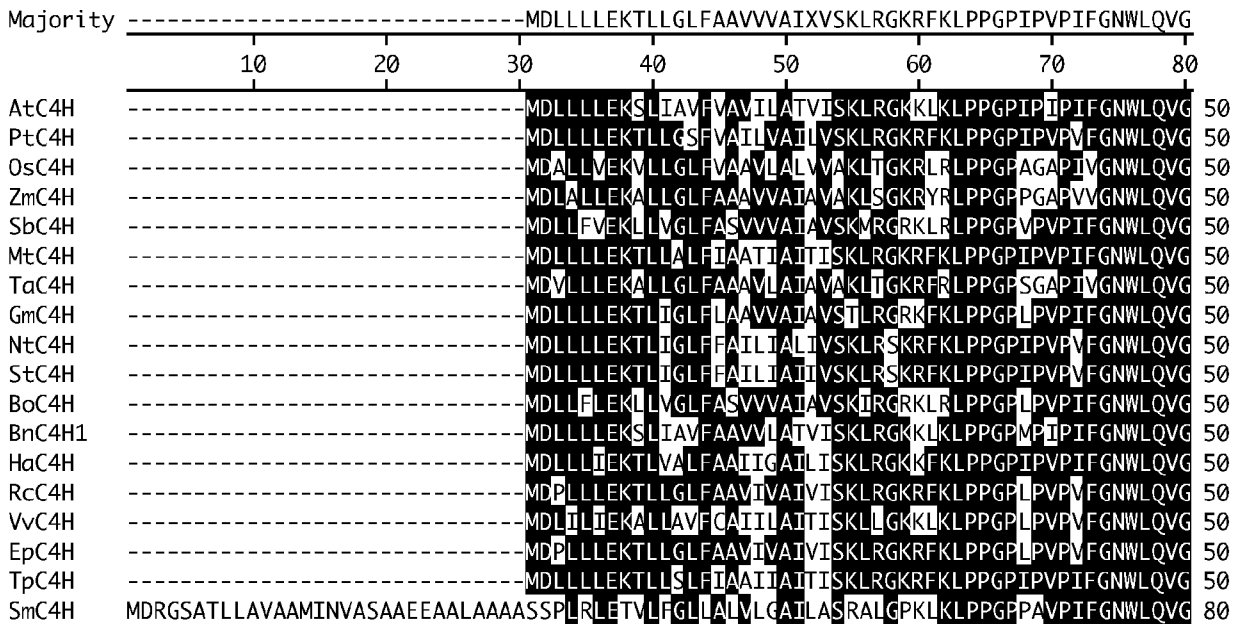


Fig. 2 (Cont'd)

Majority RIMTVPFFTNKVVQQYRYGWEXEAAASVVEDVKKNPEAATNGIVLRRRLQLMMYNNMYRIMFDRRFESDDPLFVKLKALN
 170 180 190 200 210 220 230 240

AtC4H RIMTVPFFTNKVVQQYRYGWEEAASVVEDVKKNPDSATKGIIVLRKRLQLMMYNNMYRIMFDRRFESDDPLFVKLKALN 210
 PtC4H RIMTVPFFTNKVVQQYRYGWEEAAQVVEDVKKNPEAATNGIVLRRRLQLMMYNNMYRIMFDRRFESDDPLFVKLKALN 210
 OsC4H RIMTVPFFTNKVVQQYRYGWEEEARLVVEDVRRDPAAATSGVIRRRRLQLMMYNNMYRIMFDRRFESDDPLFVKLKALN 210
 ZmC4H RIMTVPFFTNKVVQQYRYGWEEEARLVVEDVRRDPAAAGGVVIRRRRLQLMMYNNMYRIMFDRRFESDDPLFVKLKALN 210
 SbC4H RIMTVPFFTNKVVQQYRYGWEEAASVVEDVRRDPAAATEGVVIRRRRLQLMMYNNMYRIMFDRRFESDDPLFVKLKALN 210
 MtC4H RIMTVPFFTNKVVQQYRYGWEEAASVVEDVKKNPEASIGGIVIRKRLQLMMYNNMYRIMFDRRFESDDPLFVKLKALN 210
 TaC4H RIMTVPFFTNKVVQQYRYGWEEEARLVVEDLKADPAAATAGVIRRRRLQLMMYNNMYRIMFDRRFESDDPLFVKLKALN 210
 GmC4H RIMTVPFFTNKVVQQYRYGWEEAASVVEDVKKNPEAAVSGVIRRRRLQLMMYNNMYRIMFDRRFESDDPLFVKLKALN 210
 NtC4H RIMTVPFFTNKVVQQYRYGWEEAVSITIEDVKKNPEASATNGIVLRRRLQLMMYNNMYRIMFDRRFESDDPLFVKLKALN 210
 StC4H RIMTVPFFTNKVVQQYRYGWEEAASVVEDVKKNPEASATNGIVLRRRLQLMMYNNMYRIMFDRRFESDDPLFVKLKALN 210
 BoC4H RIMTVPFFTNKVVQQYRYGWEEAASVVEDVRRDPAAATEGVVIRRRRLQLMMYNNMYRIMFDRRFESDDPLFVKLKALN 210
 BrnC4H1 RIMTVPFFTNKVVQQYRYGWEEAASVVEDVKKNPDSATKGIIVLRKRLQLMMYNNMYRIMFDRRFESDDPLFVKLKALN 210
 HaC4H RIMTVPFFTNKVVQQYRYGWEEAASVVEDVKKNPEAATEGVVIRRRRLQLMMYNNMYRIMFDRRFESDDPLFVKLKALN 210
 RcC4H RIMTVPFFTNKVVQQYRYGWEEAASVVEDVKKNPEASATNGIVLRRRLQLMMYNNMYRIMFDRRFESDDPLFVKLKALN 210
 VvC4H RIMTVPFFTNKVVQQYRYGWEEAASVVEDVKKNPEASTNGIVLRRRLQLMMYNNMYRIMFDRRFESDDPLFVKLKALN 210
 EpC4H RIMTVPFFTNKVVQQYRYGWEEAASVVEDVKKNPEASATNGIVLRRRLQLMMYNNMYRIMFDRRFESDDPLFVKLKALN 210
 TpC4H RIMTVPFFTNKVVQQYRYGWEEAASVVEDVKKNPEASVGGIVVIRRRRLQLMMYNNMYRIMFDRRFESDDPLFVKLKALN 210
 SmC4H RIMTVPFFTNKVVQQYRYGWEEAASVVEDVKKNPEASVGGIVVIRRRRLQLMMYNNMYRIMFDRRFESDDPLFVKLKALN 240

Majority GERSRLAQSFEYNYGDFIPILRPFLRGYLKICKEVKERRLQFKDYFVDERKKLASTKSTDSN-GLKCAIDHILEAQQKG
 250 260 270 280 290 300 310 320

AtC4H GERSRLAQSFEYNYGDFIPILRPFLRGYLKICQDVKDRRRLALFKKVFVDERKQIASSKPTGSE-GLKCAIDHILEAQQKG 289
 PtC4H GERSRLAQSFEYNYGDFIPILRPFLRGYLKICQEVKERRLQFKDYFVDERKKLASTKMNNE-GLKCAIDHILEAQQKG 289
 OsC4H AERSRLAQSFEYNYGDFIPVLRPFLRRYLARCHQLKSRMKLFDHFDVQERKRVMEQTG-----EIRCAMDHILEAERKG 285
 ZmC4H AERSRLAQSFEYNYGDFIPVLRPFLRGYLNRCCHDLKTRRMKFFEDNFVQERKKVMAQTG-----EIRCAMDHILEAERKG 285
 SbC4H GERSRLAQSFEYNYGDFIPILRPFLRGYLRICKEVKEIRLKLFDHFDLEERKKLASTKAVDSN-GLKCAIDHILEAQQKG 289
 MtC4H GERSRLAQSFEYNYGDFIPILRPFLKGYLKVCKEVKDRRLQFKDYFVDERKKLESTKSTTSNDGLKCAIDHILEAQQKG 290
 TaC4H AERSRLAQSFEYNYGDFIPVLRPFLRRYLNRCNTNLKTKRMKVFEDHFDVQQRKEALEKTG-----EIRCAMDHILEAERKG 285
 GmC4H GERSRLAQSFEYNYGDFIPILRPFLKGYLKICKEVKEIRLKLFDYFVDERKKLGSTKSTNNNEGLKCAIDHILEAQQKG 290
 NtC4H GERSRLAQSFEYNYGDFIPILRPFLRGYLKICKEVKEIRLQFKDYFVDERKKLSNTKSSDSN-ALKCAIDHILEAQQKG 289
 StC4H GERSRLAQSFEYNYGDFIPILRPFLRGYLKICKEVKEIRLKLFDYFVDERKKLANTKSMDSN-ALKCAIDHILEAQQKG 289
 BoC4H GERSRLAQSFEYNYGDFIPILRPFLRGYLKICKEVKEIRLKLFDHFDLEERKKLASSKPMDS-GLKCAIDHILEAQQKG 289
 BrnC4H1 GERSRLAQSFEYNYGDFIPILRPFLRGYLKICQDVKDRRRLALFKKVFVDERKQIASSKPTGSE-GLKCAIDHILEAQQKG 289
 HaC4H GERSRLAQSFEYNYGDFIPILRPFLRYLKLCKEVKDRRLQFKDYFVDERKKLGSTKSMNNE-GLKCAIDHILEAQQKG 289
 RcC4H GERSRLAQSFEYNYGDFIPILRPFLRGYLKICKEVKEIRLQFKDYFVDERKKLGSTKSMNNE-GLKCAIDHILEAQQKG 289
 VvC4H GERSRLAQSFEYNYGDFIPILRPFLRGYLKICKEVKEIRLQFKDYFVDERKKLESTKSTTSNDGLKCAIDHILEAQQKG 289
 EpC4H GERSRLAQSFEYNYGDFIPILRPFLRGYLKICKEVKEIRLQFKDYFVDERKKLGSTKSMNNE-GLKCAIDHILEAQQKG 289
 TpC4H GERSRLAQSFEYNYGDFIPILRPFLKGYLKVCKEVKDRRLQFKDYFVDERKKLESTKSTTSNDGLKCAIDHILEAQQKG 290
 SmC4H GERSRLAQSFEYNYGDFIPILRPFLKRYLQMKDVKENRLGLFKKVFVDERKQLNAGKTGPD---KVAIDHILEAQQKG 317

Fig. 2 (Cont'd)

Majority EINEDNVLYIVENINVAAIETTLWSIEWGIAELVNHP EIQKLRXELDTVLGPGVQVTEPDLHKL PYLQAVIKETLRLRM
 330 340 350 360 370 380 390 400

AtC4H	EINEDNVLYIVENINVAAIETTLWSIEWGIAELVNHP EIQKLRXELDTVLGPGVQVTEPDLHKL PYLQAVIKETLRLRM	369
PtC4H	EINEDNVLYIVENINVAAIETTLWSIEWGIAELVNHP EIQKLRHELDLTLGPGHQITEPDTYKLPYL NAVIKETLRLRM	369
OsC4H	EINEDNVLYIVENINVAAIETTLWSIEWGIAELVNHP SIQSKVREEMASVLG-CAAVTEPDLERLPYLQAVIKETLRLRM	364
ZmC4H	EINEDNVLYIVENINVAAIETTLWSIEWGIAELVNHPAIOHKLREELASVLGAGVPVTEPDLERLPYLQAVIKETLRLRM	365
SbC4H	EINEDNVLYIVENINVAAIETTLWSIEWGIAELVNHP EIQKLRQELDTVLAPGQQITEPDTIHNLPYLQAVIKETLRLRM	369
MtC4H	EINEDNVLYIVENINVAAIETTLWSIEWGIAELVNHPGQIQNKVREEMDRVLGPGHQVTEPDLHKL PYLQAVIKETLRLRM	370
TaC4H	EINEDNVLYIVENINVAAIETTLWSIEWGIAELVNHP EIQKLRREETVAVLGAGVAVTEPDLERLPYLQAVIKETLRLRM	365
GmC4H	EINEDNVLYIVENINVAAIETTLWSIEWGIAELVNHP EIQKLRDEIDRVLGAGHQVTEPDTIQKLPYLQAVIKETLRLRM	370
NtC4H	EINEDNVLYIVENINVAAIETTLWSIEWGIAELVNHP HIQKLRDEIDTDLGPGVQVTEPDTIHLKLPYLQAVIKETLRLRM	369
StC4H	EINEDNVLYIVENINVAAIETTLWSIEWGIAELVNHP HIQKLRDEIDTDLGPGVQVTEPDMPKLPYLQAVIKETLRLRM	369
BoC4H	EINEDNVLYIVENINVAAIETTLWSIEWGIAELVNHP EIQKLRHELDVVLGPGHQITEPDTIHLKLPYLQAVIKETLRLRM	369
BnC4H1	EINEDNVLYIVENINVAAIETTLWSIEWGIAELVNHP EIQKLRNEIDTDLGPGVQVTEPDLHKL PYLQAVIKETLRLRM	369
HaC4H	EINEDNVLYIVENINVAAIETTLWSIEWGIAELVNHP EIQKLRHELDLTLGPGVQVTEPDTVQNL PYLQAVIKETLRLRM	369
RcC4H	EINEDNVLYIVENINVAAIETTLWSIEWGIAELVNHP EIQKLRDELDLTLGPGVQVTEPDTYKLPYLQAVIKETLRLRM	369
VvC4H	EINEDNVLYIVENINVAAIETTLWSIEWGIAELVNHP HIQKLRDELNTVLGPGVQVTEPDTIQKLPYLQAVIKETLRLRM	369
EpC4H	EINEDNVLYIVENINVAAIETTLWSIEWGIAELVNHP EIQKLRDELDLTLGPGVQVTEPDTYKLPYLQAVIKETLRLRM	369
TpC4H	EINEDNVLYIVENINVAAIETTLWSIEWGIAELVNHP EIQKLRREEMDRVLGPGHQVTEPDLQKLPYLQAVIKETLRLRM	370
SmC4H	EINEDNVLYIVENINVAAIETTLWSIEWGIAELVNHP NRDIQDKVREEMDRVLGPGVQVTEPDTIPKFTYLTAVIKETLRLRM	397

Majority AIPLLPHMNLHDAKLAGYDIPAESKILVNAWWLANNP AHWKKPEEFRPERFLEEEHXHVEANGNDFRYLPFGVGRRSCPG
 410 420 430 440 450 460 470 480

AtC4H	AIPLLPHMNLHDAKLAGYDIPAESKILVNAWWLANNP AHWKKPEEFRPERFLEEEHXHVEANGNDFRYLPFGVGRRSCPG	449
PtC4H	AIPLLPHMNLHDAKLAGYDIPAESKILVNAWWLANNP AHWKKPEEFRPERFLEEEAKVEANGNDFRYLPFGVGRRSCPG	449
OsC4H	AIPLLPHMNLHDAKLAGYDIPAESKILVNAWFLANDPKRWRPDEFRPERFLEEEKAVEAHGNDFRFVFPFGVGRRSCPG	444
ZmC4H	AIPLLPHMNLHDAKLAGYDIPAESKILVNAWFLANDPKRWRPDEFRPERFLEEEKAVEAHGNDFRFVFPFGVGRRSCPG	445
SbC4H	AIPLLPHMNLHDAKLAGYDIPAESKILVNAWYLANNPESWKRPEEFRPERFLEEEKHVEANGNDFRYLPFGVGRRSCPG	449
MtC4H	AIPLLPHMNLHDAKLAGYDIPAESKILVNAWWLANNP AHWKKPEEFRPERFLEEEHXHVEANGNDFRYLPFGVGRRSCPG	450
TaC4H	AIPLLPHMNLHDAKLAGYDIPAESKILVNAWFLANDPKRWRPDEFRPERFLEEEKAVEAHGNDFRFVFPFGVGRRSCPG	445
GmC4H	AIPLLPHMNLHDAKLAGYDIPAESKILVNAWWLANNP AHWKKPEEFRPERFLEEEHVEANGNDFRYLPFGVGRRSCPG	450
NtC4H	AIPLLPHMNLHDAKLAGYDIPAESKILVNAWWLANNP AHWKKPEEFRPERFLEEEKHVEANGNDFRYLPFGVGRRSCPG	449
StC4H	AIPLLPHMNLHDAKLAGYDIPAESKILVNAWWLANNP AHWKKPEEFRPERFLEEEKHVEANGNDFRYLPFGVGRRSCPG	449
BoC4H	AIPLLPHMNLHDAKLAGYDIPAESKILVNAWYLANNPDPQWRPEELRPERFLEEEKHVEANGNDFRYLPFGVGRRSCPG	449
BnC4H1	AIPLLPHMNLHDAKLAGYDIPAESKILVNAWWLANNPESWKKPEEFRPERFLEEEAHVEANGNDFRYLPFGVGRRSCPG	449
HaC4H	AIPLLPHMNLHDAKLAGYDIPAESKILVNAWWLANNPDPQWRPEEFRPERFLEEEAKVEANGNDFRYLPFGVGRRSCPG	449
RcC4H	AIPLLPHMNLHDAKLAGYDIPAESKILVNAWWLANNP ANWKKPEEFRPERFLEEEKVEANGNDFRYLPFGVGRRSCPG	449
VvC4H	AIPLLPHMNLHDAKLAGYDIPAESKILVNAWWLANNP DSSWKKPEEFRPERFLEEEKVEANGNDFRYLPFGVGRRSCPG	449
EpC4H	AIPLLPHMNLHDAKLAGYDIPAESKILVNAWWLANNP ANWKKPEEFRPERFLEEEKVEANGNDFRYLPFGVGRRSCPG	449
TpC4H	AIPLLPHMNLHDAKLAGYDIPAESKILVNAWWLANNP ALWKKPEEFRPERFLEEEHXHVEANGNDFRYLPFGVGRRSCPG	450
SmC4H	AIPLLPHMNLHDAKLAGYDIPAESKILVNAWWLANNP ELWKKPDVDFSRFLDG--KTEASGNDFRFVFPFGVGRRSCPG	475

Fig. 2 (Cont'd)

Majority	IILALPILGITIXGRLVQNFELLPPPGQSKIDTTEKGGQFSLHILKHSTIVAKPRSF--	
	490 500 510 520 530	
AtC4H	IILALPILGITIGRMVQNFELLPPPGQSKVDTSEKGGQFSLHILNHSIIVMKPRNC	505
PtC4H	IILALPILGITLGRVQNFELLPPPGQSKIDTAEKGGQFSLHILKHSTIVAKPRSF	505
OsC4H	IILALPITIGITLGRVQSFDLLPPPGMDKVDTTEKPGQFSNQLKHAIVVCKPIIDA	500
ZmC4H	IILALPITIGITLGRVQNFQLLPPPGLDKIDTTEKPGQFSNQLAKHAIVVCKPLEA	501
SbC4H	IILALPILGITIGRLVQNFELLPPPGQDKLDTTEKGGQFSLHILKHSTIVCKPRIF	505
MtC4H	IILALPILGITIGRLVQNFELLPPPGQSKIDTSEKGGQFSLHILKHSTIVAKPRSF	506
TaC4H	IILALPITIGITLGRVQNFQLLPPPGQDKIDTTEKPGQFTNQLKHAIVVCKPLEA	501
GmC4H	IILALPILGITLGRVQNFELLPPPGQSKIDTSEKGGQFSLHILKHSTIVAKPRSF	506
NtC4H	IILALPILGITLGRVQNFELLPPPGQSKLDTTEKGGQFSLHILKHSTIVLKPRSF	505
StC4H	IILALPILGITLGRVQNFELVPPPGQSKLDTSEKGGQFSLHILKHSTIVMKPRSF	505
BoC4H	IILALPILGITIGRLVQNFELLPPPGQDKLDTAEKGGQFSLHILKHSTIVAKPRALEL	507
BnC4H1	IILALPILGITIGRLVQNFELLPPPGQSKVDTSEKGGQFSLHILKHSTIVMKPRSF	505
HaC4H	IILALPILGITIGRLVQNFELLPPPGQSKIDTSEKGGQFSLHILKHSTIVAKPRSF	505
RcC4H	IILALPILGITLGRVQNFELLPPPGQSKLDTTEKGGQFSLHILKHSTIVAKPRSF	505
VvC4H	IILALPILGITIGRLVQNFELLPPPGQAKLDTTEKGGQFSLHILKHSTIVARPIEA	505
EpC4H	IILALPILGITLGRVQNFELLPPPGQSKLDTTEKGGQFSLHILKHSTIVAKPRSF	505
TpC4H	IILALPILGITIGRLVQNFELLPPPGQSKIDTSEKGGQFSLHILKHSTIVAKPRSF	506
SmC4H	IITAMPILHLVIGSLVAKFELLPPPGCDKIDVSEKGGQFSLHIAKHSTIVMKPRVL	531

Decoration 'Decoration #1': Shade (with solid black) residues that match the Consensus exactly.

Fig. 3

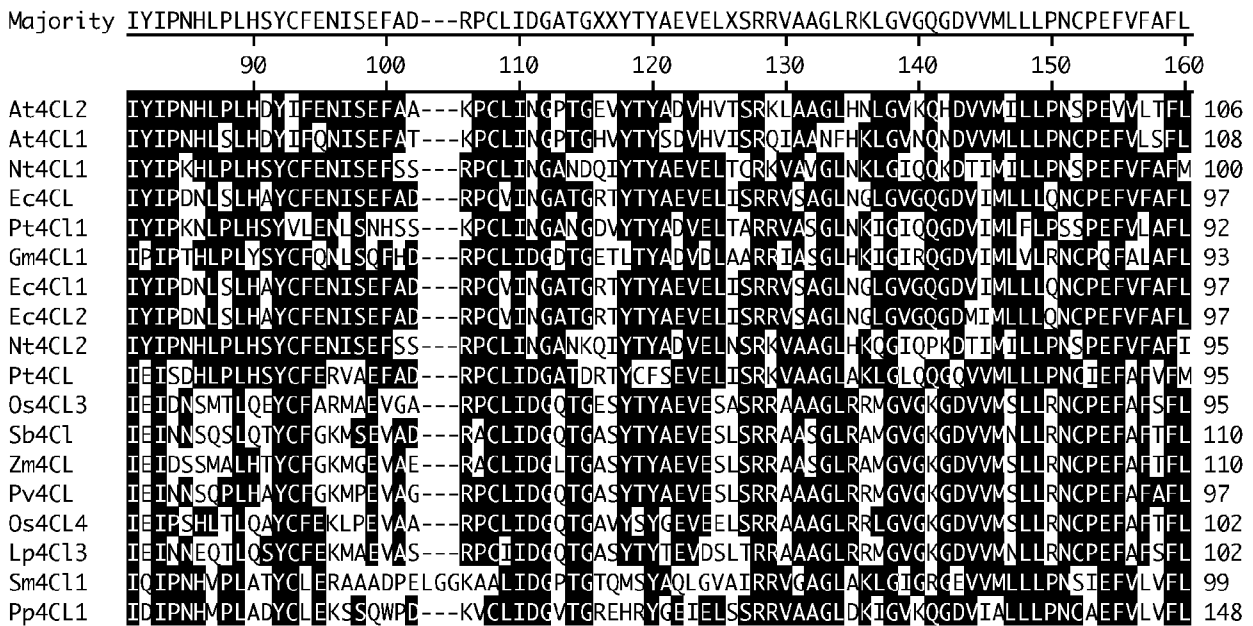
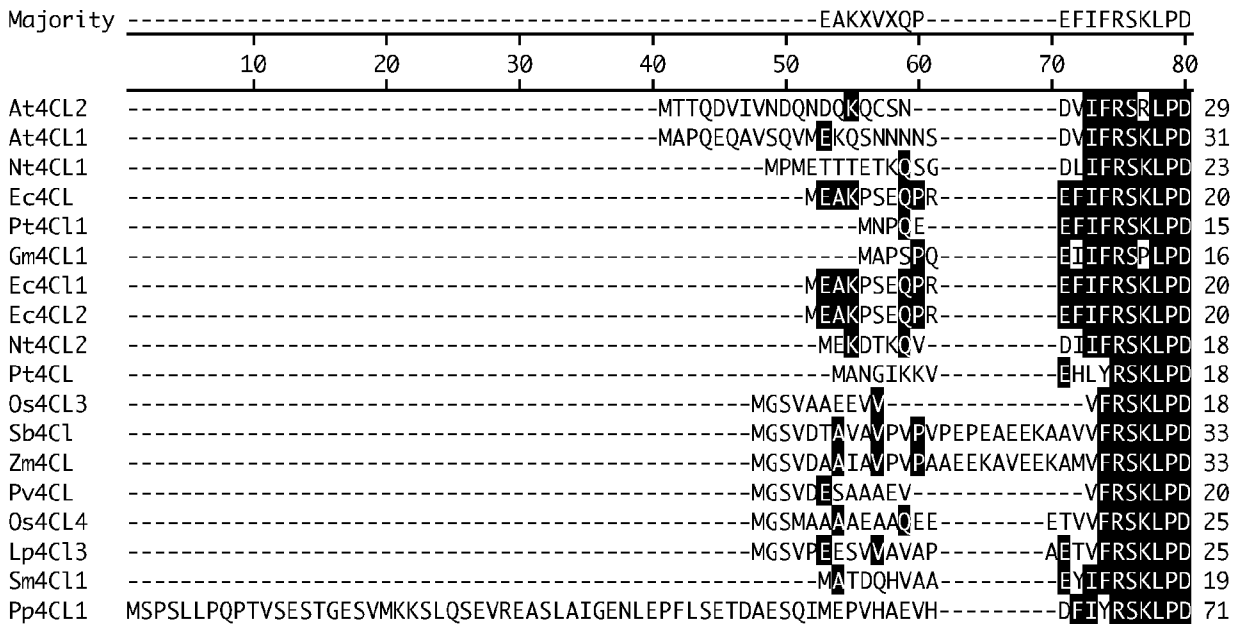


Fig. 3 (Cont'd)

Majority GASRRGAIITTTANPFYTPAEIAKQAKAAGAKLIVTQACYVEKVRFAAER-GVPVVXIDG-----PEGCLHFSSELTQAD

170 180 190 200 210 220 230 240

At4CL2 AASF~~IG~~AITTSANPFFTPAEISKQAKASAAKLIVTQSR~~Y~~VDKIKNLQND--GVLIVTTDSD---ATPENCLRFSELTQSE 181

At4CL1 AASF~~R~~GATA~~T~~AANPFFTPAEIAKQAKASNTKLIIT~~E~~ARYVDKIKPLONDD-GVIVICIDNESVPIPEGCLRF~~F~~ELTQST 187

Nt4CL1 GASYLGAISTMANPLFTPAEVVKQAKASSAKIIITQSGFVGVKDYASEN-DVKVICIDS-----APEGCLHFSSELTQSD 174

Ec4CL GASYRGAISTTANPFYTPGEIAKQASAAAKIVITQAAFADKVRPFAEEN-GVKVVCIDT-----APEGCLHFSSELTQAD 171

Pt4CL1 GAS~~H~~RGATIT~~T~~AANPFS~~T~~PAELAK~~H~~AKASRAKILLITQACYYEKVKDFARESDVKVMCVDS-----APD~~G~~CLHFSSELTQAD 166

Gm4CL1 GATHRGAVVTTANPFYTPAELAKQATATKTRLVITQAYVEKIKSFADSSDVMVICIDDDFS-YENDGVLHFS~~T~~LSNAD 172

Ec4CL1 GASYRGAISTTANPFYTPGEIAKQASAAAKIVITQAAAYADKVRPFAEEN-GLRVVCIDT-----APEGCLHFSSELTQAD 171

Ec4CL2 GASYRGAISTTANPFYTPGEIAKQASAAAKIVITQAAAYADKVRPFAEEN-GLSVVCIDT-----APEGCLHFSSELTQAD 171

Nt4CL2 GASYLGAISTMANPLFTPAEVVKQAKASSAKIIVTQACHV~~N~~KVDYAFEN-DVKIICIDS-----APEGCLHFSVLTQAN 169

Pt4CL GASVRGAVITTANPFYK~~P~~GEIAKQAKAAGARIIVT~~L~~AAYVEKLADLQSHD--VLVITIDDAP----KEGCG~~H~~TSVLTQAD 169

Os4CL3 GAARLGAATTTANPFYTPHEVHRQAEAAGARVIVTEACA~~V~~EKVRFAAER-GVPVVTVDGA-----FDGCV~~F~~EAEVLA~~E~~E 169

Sb4CL GAARLGAATTTANPFYTPHEIHRQAEAAGAKVIVTEACA~~V~~EKVRFAAGR-GVPVVTVDGR-----FDGCV~~F~~EAEVIA~~E~~E 184

Zm4CL GAARLGAATTTANPFYTPHEVHRQAEAAGARLIVTEACA~~V~~EKVRFAAER-GIPVVTVDGR-----FDGCV~~F~~EAE~~L~~IA~~E~~E 184

Pv4CL GAARLGAATTTANPFYTPHEIHRQAEAAGAKLIVTEACA~~V~~EKVRFAVAR-GIPVVTVDGR-----FDGCA~~F~~EAEVIA~~E~~E 171

Os4CL4 GAARLGAATTTANPFYTPHEIHRQAEAAGARVIVTEACA~~V~~EKVRGFAADR-GIPVAVDGD-----FDGCV~~F~~GEAMLDA 176

Lp4CL3 GAARLGAATTTANPFYTPHEIHRQAEAAGAKLIVTEACA~~V~~EKVL~~E~~FAAGR-GVPVVTVDGR-----RDGCV~~D~~F~~A~~E~~L~~IAGE 176

Sm4CL1 GCAVRGCVAITTANPFYTPPEIAKQAKASCTKLVITLSTYVDK~~V~~SGI-----AEVMSIDRE-----VEGCL~~H~~TSALTOAD 168

Pp4CL1 GAAKRGAVVTTANPFYTA~~A~~EEL~~E~~KQTEASGAGIVITQSSYVEKLAGLN-----VQIITVDQH-----VANCM~~H~~TSVLLNAC 218

Majority E----EAPAXDIHPDDVVALPYSSGTTGLPKGVM~~L~~THXGLVTSVAQQVDGENPNLYFHKEDVILCVLPLFHIYSLNSVLL

250 260 270 280 290 300 310 320

At4CL2 EPRVDSIP-EKTSPE~~D~~VVALP~~F~~SSGTTGLPKGVM~~L~~THKGLVTSVAQQVDGENPNLYFN~~R~~DDVILCVLPMFHIYALNSIML 260

At4CL1 TEASEVIDSVETSPDDVVALPYSSGTTGLPKGVM~~L~~THKGLVTSVAQQVDGENPNLYFHS~~D~~VILCVLPMFHIYALNSIML 267

Nt4CL1 E---H~~E~~IPEVKIQPDDVVALPYSSGTTGLPKGVM~~L~~THKGLVTSVAQQVDGENANLYMHS~~E~~DLMLCVLPLFHIYSLNSITLL 251

Ec4CL E---NAAPAADV~~K~~PDDV~~L~~ALPYSSGTTGLPKGVM~~L~~THRQVTSVAQQVDGDNPNLYFHKEDVIL~~C~~LPLFHIYSLNSVMF 248

Pt4CL1 E---NEAPQVDIS~~P~~DDVVALPYSSGTTGLPKGVM~~L~~THKGL~~I~~TSVAQQVDGDNPNLYFHS~~E~~DVILCVLPMFHIYALNSIML 243

Gm4CL1 E---TEAPAVKINPDELVALP~~F~~SSGTSGLPKGVM~~L~~SHKNLVT~~T~~IAQLVDGENPHQYTHS~~E~~DLMLCVLPMFHIYALNSITLL 249

Ec4CL1 E---NAAPAADV~~K~~PDDV~~L~~ALPYSSGTTGLPKGVM~~L~~THRQVTSVAQQVDGDNPNLYFHKEDVIL~~C~~LPLFHIYSLNSVMF 248

Ec4CL2 E---NAAPAADV~~K~~PDDV~~L~~ALPYSSGTTGLPKGVM~~L~~THRQVTSVAQQVDGDNPNLYFHKEDVIL~~C~~LPLFHIYSLNSVMF 248

Nt4CL2 E---HD~~I~~PEVEI~~Q~~PDDVVALPYSSGTTGLPKGVM~~L~~THKGLVTSVAQQVDGENPNLYIHS~~E~~DLMLCVLPLFHIYSLNSVLL 246

Pt4CL E---TQCPAVKIH~~P~~DDVVALPYSSGTTGLPKGVM~~L~~THKGLV~~S~~SVAQQVDGENPNLYFHS~~D~~DDVILCVLPLFHIYSLNSVLL 246

Os4CL3 EL----DADADVHPDDVVALPYSSGTTGLPKGVM~~L~~THRSLIT~~S~~VSAQQVDGENPNLYFSK~~D~~DDVIL~~C~~LPLFHIYSLNSVLL 245

Sb4CL EL----DADADVHPDDVVALPYSSGTTGLPKGVM~~L~~THRSLIT~~S~~VSAQQVDGENPNLYFSK~~D~~DDVIL~~C~~LPLFHIYSLNSVLL 260

Zm4CL EL----EADAD~~I~~HPDDVVALPYSSGTTGLPKGVM~~L~~THRSLIT~~S~~VSAQQVDGENPNLYFRK~~D~~DDVIL~~C~~LPLFHIYSLNSVLL 260

Pv4CL EL----EADAD~~I~~QPDVVALPYSSGTTGLPKGVM~~L~~THRSLIT~~S~~VSAQQVDGENPNLYFRK~~D~~DDVIL~~C~~LPLFHIYSLNSVLL 247

Os4CL4 SIE--PLDADEEVHPDDVVALPYSSGTTGLPKGVM~~L~~THRSLVTSVAQQVDGENPNLYFR~~R~~EDVIL~~C~~LPLFHIYSLNSVLL 255

Lp4CL3 ELP--EAEAGVLPDDVVALPYSSGTTGLPKGVM~~L~~THRSLVTSVAQ~~L~~VDGSNPNVCFNKDDALL~~C~~LPLFHIYSLHTVLL 254

Sm4CL1 E---GECPAVDIQPDDVVALP~~F~~SSGTTGLPKGVM~~L~~THKSLV~~S~~IAQQVDGDNPNLYMTPSDAVL~~C~~VLPMFHIYSLNSITLL 245

Pp4CL1 E---GECQVRIHPDDV~~V~~CLPYSSGTTGLPKGVM~~L~~THKSLV~~S~~VSQQVDGEAPNFNITV~~E~~DTLMCVLPMFHIYSLNSITLL 295

Fig. 3 (Cont'd)

Majority CGLRVGAAILIMQKFEIGALLELVQRYKVTIAPFVPPIVLATAKSPXVDAYDLSSIRMVMSGGAAPXGKELEDAXRAKXPN
 330 340 350 360 370 380 390 400

At4CL2	C	S	L	R	V	G	A	A	I	L	I	M	Q	K	F	E	I	G	A	L	L	E	L	V	Q	R	Y	K	V	T	I	A	P	F	V	P	P	I	V	L	A	T	A	K	S	P	X	V	D	A	Y	D	L	S	S	I	R	M	V	M	S	G	A	A	P	X	G	K	E	L	E	D	A	I	S	A	K	F	P	N	340
At4CL1	C	G	L	R	V	G	A	A	I	L	I	M	Q	K	F	E	I	N	L	L	E	L	I	Q	R	C	K	V	T	V	A	P	V	P	P	I	V	L	A	T	A	K	S	S	E	T	E	K	Y	D	L	S	S	I	R	V	M	S	G	A	A	P	L	G	K	E	L	E	D	A	V	N	A	K	F	P	N	347			
Nt4CL1	C	G	L	R	V	G	A	A	I	L	I	M	Q	K	F	E	I	A	P	F	L	E	L	I	Q	K	Y	K	V	T	I	A	P	F	V	P	P	I	V	L	A	T	A	K	S	P	I	V	D	S	Y	D	L	S	S	V	R	T	V	M	S	G	A	A	P	L	G	K	E	L	E	D	A	V	R	T	K	F	P	N	331
Ec4CL	C	A	L	R	V	G	A	A	I	L	I	M	Q	K	F	E	I	V	A	L	M	E	L	V	Q	R	Y	R	V	T	I	L	P	I	V	P	P	I	V	L	A	T	A	K	S	A	E	V	D	R	Y	D	L	S	S	I	R	T	I	M	S	G	A	A	P	V	G	K	E	L	E	D	T	V	R	A	K	L	P	N	328
Pt4CL1	C	G	L	R	V	G	A	P	T	I	L	I	M	Q	K	F	E	T	G	S	L	L	G	L	I	E	K	Y	K	V	T	A	P	V	V	P	P	I	V	L	A	T	A	K	S	P	D	L	K	H	D	L	S	S	L	R	M	I	K	S	G	G	A	P	L	G	K	E	L	E	D	T	V	R	A	K	F	P	Q	323	
Gm4CL1	C	G	L	R	S	G	A	A	V	L	I	L	Q	K	F	E	I	T	L	L	E	L	I	E	K	Y	K	V	T	V	A	S	F	V	P	P	I	V	L	A	L	V	K	S	G	E	T	H	R	Y	D	L	S	S	I	R	A	V	T	G	A	A	P	L	G	G	E	L	E	D	A	V	K	A	R	L	P	H	329		
Ec4CL1	C	A	L	R	V	G	A	A	I	L	I	M	Q	K	F	E	I	M	A	L	M	E	L	V	Q	R	Y	R	V	T	I	L	P	I	V	P	P	I	V	L	A	T	A	K	S	A	E	V	D	R	Y	D	L	S	S	I	R	T	I	M	S	G	A	A	P	V	G	K	E	L	E	D	T	V	R	A	K	L	P	N	328
Ec4CL2	C	A	L	R	V	G	A	A	I	L	I	M	Q	K	F	E	I	M	A	L	M	E	L	V	Q	R	Y	R	V	T	I	L	P	I	V	P	P	I	V	L	A	T	A	K	S	A	E	V	D	R	Y	D	L	S	S	I	R	T	I	M	S	G	A	A	P	V	G	K	E	L	E	D	T	V	R	A	K	L	P	N	328
Nt4CL2	C	G	L	R	V	G	A	A	I	L	I	M	Q	K	F	E	I	V	S	F	L	E	L	I	Q	R	Y	K	V	T	I	A	P	F	V	P	P	I	V	L	A	T	A	K	S	P	V	D	D	Y	D	L	S	S	V	R	T	V	M	S	G	A	A	P	L	G	K	E	L	E	D	T	V	R	A	K	F	P	N	326	
Pt4CL	C	A	L	R	A	G	A	A	T	L	I	M	Q	K	F	N	L	T	T	C	L	E	L	I	Q	K	Y	K	V	T	V	A	P	I	V	P	P	I	V	L	A	T	A	K	S	P	I	V	S	Q	Y	D	V	S	S	V	R	T	I	M	S	G	A	A	P	L	G	K	E	L	E	D	A	L	R	E	R	F	P	K	326
Os4CL3	A	G	L	R	A	G	S	T	I	V	I	M	R	K	F	D	L	G	A	L	V	D	L	V	R	K	H	N	I	T	A	P	F	V	P	P	I	V	E	I	A	K	S	P	R	V	T	A	E	D	L	S	I	R	M	V	M	S	G	A	A	P	V	G	K	D	L	C	D	A	F	M	A	K	I	P	N	325			
Sb4CL	A	G	L	R	A	G	S	T	I	V	I	M	R	K	F	D	L	G	A	L	V	D	L	V	R	K	H	G	I	T	A	P	F	V	P	P	I	V	E	I	A	K	S	P	R	V	T	A	D	D	L	S	I	R	M	V	M	S	G	A	A	P	V	G	K	E	L	C	D	A	F	M	T	K	I	P	N	340			
Zm4CL	A	G	L	R	A	G	S	T	I	V	I	M	R	K	F	D	L	G	A	L	V	D	L	V	R	R	Y	V	I	T	A	P	F	V	P	P	I	V	E	I	A	K	S	P	R	V	T	A	C	D	L	S	I	R	M	V	M	S	G	A	A	P	V	G	K	E	L	C	D	A	F	M	A	K	I	P	N	340			
Pv4CL	A	G	L	R	A	G	C	A	I	V	I	M	R	K	F	E	T	G	A	L	V	E	L	V	R	A	H	G	V	T	V	A	P	F	V	P	P	I	V	E	I	A	K	S	P	R	V	G	A	A	D	L	S	I	R	M	V	M	S	G	A	A	P	V	G	K	D	L	C	D	A	F	M	A	K	I	P	N	327		
Os4CL4	A	G	L	R	A	G	S	A	I	V	I	M	R	K	F	D	L	G	A	L	V	D	L	T	R	R	H	G	V	T	V	A	P	F	V	P	P	I	V	E	I	A	K	S	P	R	V	T	A	D	D	L	S	I	R	M	V	M	S	G	A	A	P	V	G	K	D	L	C	D	A	F	M	A	K	I	P	N	335		
Lp4CL3	A	G	L	R	V	G	A	A	I	V	I	M	R	K	F	D	V	G	A	L	V	D	L	V	R	A	H	R	I	T	A	P	F	V	P	P	I	V	E	I	A	K	S	D	R	V	G	A	D	D	L	S	I	R	M	V	L	S	G	A	A	P	V	G	K	D	L	C	D	A	F	M	A	K	I	P	N	334			
Sm4CL1	C	S	L	R	T	A	S	T	I	V	I	M	P	K	F	D	L	T	C	L	L	E	L	V	T	R	Y	S	I	S	T	A	P	I	V	P	P	I	V	L	A	L	A	K	N	P	A	V	L	A	Y	D	L	S	S	I	R	M	V	C	S	G	A	A	P	L	G	K	E	L	E	D	A	F	R	A	R	L	P	R	325
Pp4CL1	C	G	L	R	V	G	A	T	L	I	M	P	K	F	E	L	P	K	L	L	D	L	I	Q	R	H	K	V	T	M	G	P	L	V	P	P	I	V	L	A	T	A	K	N	P	I	V	E	N	Y	D	L	S	S	M	R	M	V	M	S	G	A	A	P	L	G	K	E	L	E	D	A	F	R	A	R	L	P	N	375	

Majority AXLGQGYGMTEAGPVLAMCLAFAKEPFEVKSXGCGTVVRNAEMKIVDPDTGASLPRNQGEICIRGEQIMKGYLNDPEAT
 410 420 430 440 450 460 470 480

At4CL2	A	K	L	G	Q	G	Y	G	M	T	E	A	G	P	V	L	A	M	S	L	G	F	A	K	E	P	F	F	V	K	S	G	A	C	G	T	V	V	R	N	A	E	M	K	I	V	D	P	D	T	G	S	L	P	R	N	K	P	G	E	I	C	I	R	G	E	Q	I	M	K	G	Y	L	N	D	P	L	A	T	420		
At4CL1	A	K	L	G	Q	G	Y	G	M	T	E	A	G	P	V	L	A	M	S	L	G	F	A	K	E	P	F	F	V	K	S	G	A	C	G	T	V	V	R	N	A	E	M	K	I	V	D	P	D	T	G	S	L	P	R	N	Q	P	G	E	I	C	I	R	G	E	Q	I	M	K	G	Y	L	N	D	P	A	T	427			
Nt4CL1	A	K	L	G	Q	G	Y	G	M	T	E	A	G	P	V	L	A	M	C	L	A	F	A	K	E	P	F	D	I	K	S	G	A	C	G	T	V	V	R	N	A	E	M	K	I	V	D	P	D	T	G	C	S	L	P	R	N	Q	P	G	E	I	C	I	R	G	E	Q	I	M	K	G	Y	L	N	D	P	E	A	T	411	
Ec4CL	A	K	L	G	Q	G	Y	G	M	T	E	A	G	P	V	L	A	M	C	P	A	F	A	K	E	P	F	E	I	K	S	G	A	C	G	T	V	V	R	N	A	E	M	K	I	V	D	P	E	T	G	A	S	L	A	R	N	Q	A	G	E	I	C	I	R	G	E	Q	I	M	K	G	Y	L	N	D	A	E	A	T	408	
Pt4CL1	A	R	L	G	Q	G	Y	G	M	T	E	A	G	P	V	L	A	M	C	L	A	F	A	K	E	P	F	D	I	K	P	G	A	C	G	T	V	V	R	N	A	E	M	K	I	V	D	P	E	T	G	A	S	L	P	R	N	Q	P	G	E	I	C	I	R	G	E	Q	I	M	K	G	Y	L	N	D	P	E	A	T	403	
Gm4CL1	A	T	F	G	Q	G	Y	G	M	T	E	A	G	P	-	L	A	T	S	M	A	F	A	K	V	P	S	K	I	K	P	G	A	C	G	T	V	V	R	N	A	E	M	K	I	V	D	T	E	T	G	D	S	L	P	R	N	K	H	G	E	I	C	I	T	I	G	T	K	V	M	K	G	Y	L	N	D	P	E	A	T	408
Ec4CL1	A	K	L	G	Q	G	Y	G	M	T	E	A	G	P	V	L	A	M	C	L	A	F	A	K	E	P	F	E	I	K	S	G	A	C	G	T	V	V	R	N	A	E	M	K	I	V	D	P	E	T	G	A	S	L	P	R	N	Q	A	G	E	I	C	I	R	G	E	Q	I	M	K	G	Y	L	N	D	P	E	A	T	408	
Ec4CL2	A	K	L	G	Q	G	Y	G	M	T	E	A	G	P	V	L	A	M	C	L	A	F	A	K	E	P	F	E	I	K	S	G	A	C	G	T	V	V	R	N	A	E	M	K	I	V	D	P	E	T	G	A	S	L	P	R	N	Q	A	G	E	I	C	I	R	G	E	Q	I	M	K	G	Y	L	N	D	P	E	A	T	408	
Nt4CL2	A	K	L	G	Q	G	Y	G	M	T	E	A	G	P	V	L	A	M	C	L	A	F	A	K	E	P	F	E	I	K	S	G	A	C	G	T	V	V	R	N	A	E	M	K	I	V	D	P	K	T	G	N	S	L	P	R	N	Q	S	G	E	I	C	I	R	G	E	Q	I	M	K	G	Y	L	N	D	P	E	A	T	406	
Pt4CL	A	I	F	G	Q	G	Y	G	M	T	E	A	G	P	V	L	A	M	N	L	A	F	A	K	N	P	P	F	V	K	S	G	S	C	G	T	V	V	R	N	A	E	I	K	I	L	D	T	E	T	G	E	S	L	P	H	N	Q	A	G	E	I	C	I	R	G	E	P	I	M	K	G	Y	L	N	D	P	E	S	T	406	
Os4CL3	A	V	L	G	Q	G	Y	G	M	T	E	A	G	P	V	L	A	M	C	L	A	F	A																																																											

Fig. 3 (Cont'd)

Majority ANTIDKDGWLHTGDIGYIDDDDEL FIVDR LKEXIKYKGFQVAPAELEALLIXHPEISDAAVVPMKDELAGEVPVAFVRS

	490	500	510	520	530	540	550	560	
At4CL2	ASTIDKDGWLHTGDVGF	IDDDDEL	FIVDR	LKELIKYKGFQVAPAELE	SL	LICHPEINDVAVVAMKE	ED	AGEVPVAFVRS	500
At4CL1	AETIDKDGWLHTGDI	GLIDDDDEL	FIVDR	LKELIKYKGFQVAPAELE	ALLI	CHPDIIDVAVVAMKE	EA	AGEVPVAFVRS	507
Nt4CL1	TRTIDKEGWLHTGDIG	IDE	DEL	FIVDR	LKELIKYKGFQVAPAE	TEALL	LNHPNISDAAVVPMKDE	QAGEVPVAFVRS	491
Ec4CL	ANTIDKEGWLHTGDIGY	IDDDDEL	FIVDR	LKELIKYKGFQVAPAELE	AM	LIAHP	SISDAAVVPMKDE	VAGEVPVAFVRS	488
Pt4CL1	SRTIDKEGWLHTGDIGY	IDDDDEL	FIVDR	LKELIKYKGFQVAPAELE	ALL	IAHP	EISDAAVVGLKDE	DAGEVPVAFVRS	483
Gm4CL1	ERTVDKEGWLHTGDIG	GFIDDDDEL	FIVDR	LKELIKYKGFQVAPAELE	ALL	IAHP	NISDAAVVGMKDE	AAGEVPVAFVRS	488
Ec4CL1	ANTIDKEGWLHTGDIGY	IDDDDEL	FIVDR	LKELIKYKGFQVAPAELE	AM	LIAHP	SISDAAVVPMKDE	VASEVPVAFVRS	488
Ec4CL2	ANTIDKEGWLHTGDIGY	IDDDDEL	FIVDR	LKELIKYKGFQVAPAELE	AM	LIAHP	SISDAAVVPMKDE	VASEVPVAFVRS	488
Nt4CL2	ARTIDKEGWLHTGDIGY	IDDDDEL	FIVDR	LKELIKYKGFQVAPAELE	ALL	LNHP	NISDAAVVPMKDE	QAGEVPVAFVRS	486
Pt4CL	AATIDEEGWLHTGDVGY	IDDDDEL	FIVDR	LKELIKYKGFQVAPAELE	ALL	VIAHP	SIADAAVVPCKE	EAAGEVPVAFVRS	486
Os4CL3	KNTIDEDGWLHTGDIG	YVDDDDDEL	FIVDR	LKELIKYKGFQVAPAELE	ALL	IHP	EIKDAAVVSMKDE	LAGEVPVAFVRS	485
Sb4CL	KNTIDKDGWLHTGDIGY	VDDDDDEL	FIVDR	LKELIKYKGFQVAPAELE	ALL	IHP	EIKDAAVVSMKDE	LAGEVPVAFVRS	500
Zm4CL	KNTIDQDGWLHTGDIGY	VDDDDDEL	FIVDR	LKELIKYKGFQVAPAELE	ALL	IHP	EIKDAAVVSMKDE	LAGEVPVAFVRS	500
Pv4CL	KNTIDKDGWLHTGDIGY	VDDDDDEL	FIVDR	LKELIKYKGFQVAPAELE	ALL	IHP	EIKDAAVVSMKDE	LAGEVPVAFVRS	487
Os4CL4	KNTIDKDGWLHTGDIGY	VDDDDDEL	FIVDR	LKELIKYKGFQVAPAELE	ALL	IHP	EIKDAAVVPMKDE	LAGEVPVAFVRS	495
Lp4CL3	KNTIDKDGWLHTGDIG	LVDDDDDEL	FIVDR	LKELIKYKGFQVAPAELE	ALL	LN	PEVKDAAVVGVKDD	LCGEVPVAFVRS	494
Sm4CL1	RSTVDKDGWLHTGDVAL	IDDDDEL	FIVDR	LKELIKYKGFQVAPAELE	ALL	IS	PSIADAAVVAKKDD	LITGEVPVAFVRS	485
Pp4CL1	ANTIDKDGFLHTGDVAF	IDEDE	EM	FIVDR	LKELIKYKGFQVAPAELE	ALL	ISHKEIQDAAVVSRKDD	VAGEVPVAFVRS	535

Majority EGSEITEDEIKQFVSKQVVFYKRIRXRVFFDTSIPKXPSGKILRKDLRARLAAGVX-----

	570	580	590	600	610	620		
At4CL2	KDSN	ISEDEIKQFVSKQVVFYKR	IRNKVFF	DTSIPK	APSGKILRKDLRARLA	GLMN	556	
At4CL1	KDSEL	SEDDV	KQFVSKQVKS	-----	CVLQ	ENQQSVLH	539	
Nt4CL1	NGS	ATEDEIKQFVSKQVVFYKR	IRKRVFF	VETVPK	SPSGKILRKDLRARLA	AGVFN	547	
Ec4CL	NGS	VTEDEIKQYISKQVVFYKR	IRKRVFF	DAIPK	APSGKILRKDLRAK	LASGVYN	544	
Pt4CL1	EKS	QATEDEIKQYISKQVVFYKR	IRKRVFF	TEAIPK	APSGKILRKDLRAK	LAG-I	536	
Gm4CL1	NGS	EIAEDEIKKYISQVVFYKR	IRGRVFF	DTSIPK	APSGKILRKVL	IARLNEG	LVVAN	546
Ec4CL1	NGS	VTEDEIKQYISKQVVFYKR	IRKRVFF	DAIPK	APSGKILRKDLRAK	LASGVYN	544	
Ec4CL2	NGS	VTEDEIKQYISKQVVFYKR	IRKRVFF	DAIPK	APSGKILRKDLRAK	LASGVYN	544	
Nt4CL2	NGS	TEDEIKQFVSKQVVFYKR	IRKRVFF	DAIPK	SPSGKILRKDLRAK	LAAGLPN	542	
Pt4CL	SE--	ISEDEIKQFVSKQVVFYKR	IRKRVFF	DAIPK	SPSGKILRKDLR	SRLAAK	537	
Os4CL3	EGS	EITEDEIKQFVSKQVVFYKR	IRNKVFF	DTSIPK	NPSGKILRKDLRARLA	AGIPDAVAAAAADAPKSS	554	
Sb4CL	EGS	EITEDEIKQFVSKQVVFYKR	IRNKVFF	DTSIPK	NPSGKILRKDLRARLA	AGVH	555	
Zm4CL	EGS	QVTEDEIKQFVSKQVVFYKR	IRNKVFF	DTSIPK	NPSGKILRKDLRARLA	AGVH	555	
Pv4CL	EGS	QVTEDEIKQFVSKQVVFYKR	IRNKVFF	DTSIPK	NPSGKILRKDLRARLA	AGVH	542	
Os4CL4	EGS	AISENEIKQFVSKQVVFYKR	IRNKVFF	ADSIPK	SPSGKILRKDLRAK	LAAGIPTNDNTQLKS	559	
Lp4CL3	EGS	EINENEIKQFVSKQVVFYKR	IRNKVFF	DTSIPK	NPSGKILRKDLRARLA	AGIPTEVAAPRS	557	
Sm4CL1	ADSH	ISEDDIKQFVSKQVVFYKR	IRKRVFF	DTSIPK	NPSGKILRKELR	SRLSS	537	
Pp4CL1	TSST	ISEDEIKQDYIAKQVVFYKR	IRKRVFF	DTSIPK	SPSGKILRKDLR	KNV	585	

Decoration 'Decoration #1': Shade (with solid black) residues that match the Consensus exactly.

Fig. 4

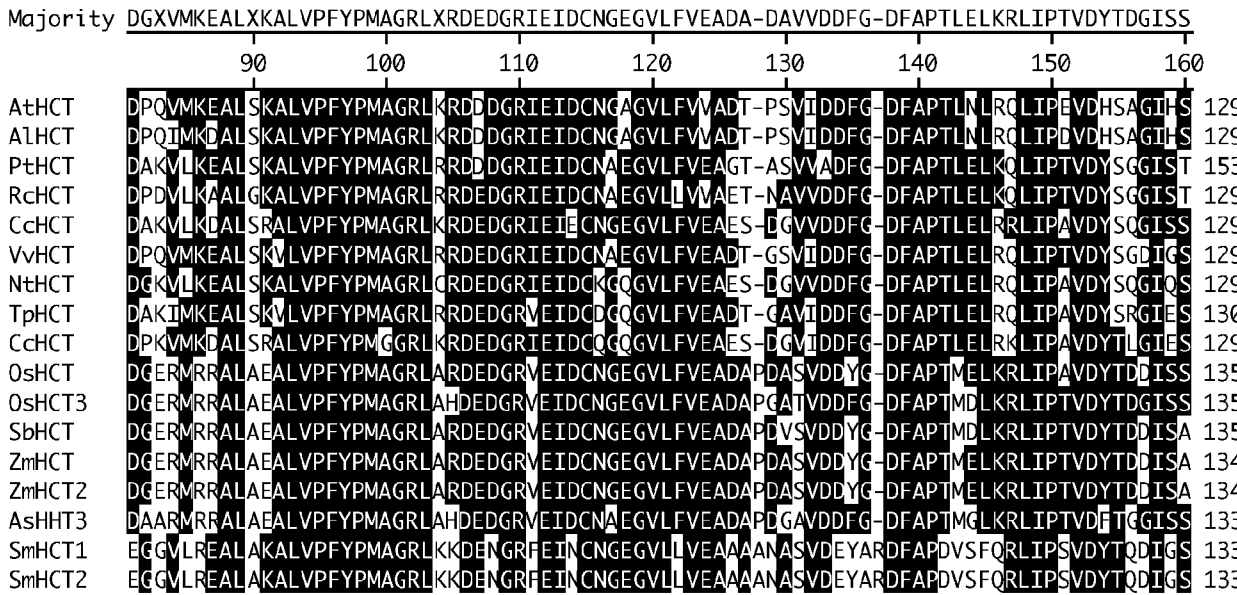
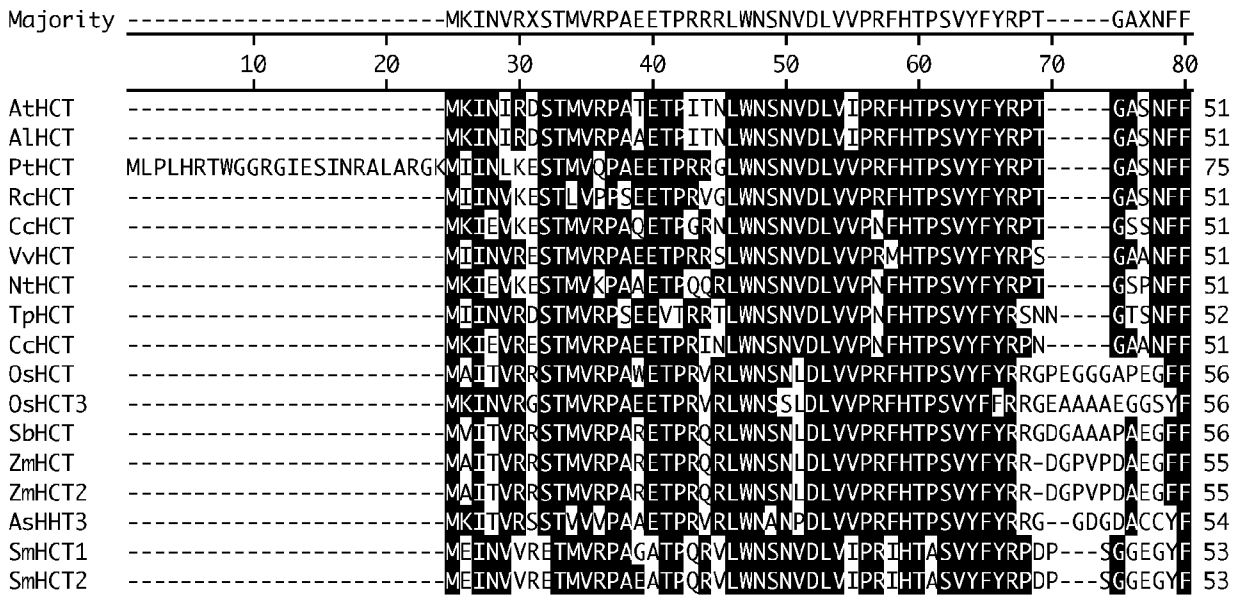


Fig. 4 (Cont'd)

Majority FPLLVLQVTYFKCGGASLVGGMQHHAADGFSGLHFINTWSDMARGLDITIPPFIDRTLLRARDPPQPFPHIEYQPAPAM
 170 180 190 200 210 220 230 240

AtHCT	FPLLVLQVTYFKCGGASLVGGMQHHAADGFSGLHFINTWSDMARGLDITIPPFIDRTLLRARDPPQPFPHIEYQPAPAM	209
AlHCT	FPLLVLQVTYFKCGGASLVGGMQHHAADGFSGLHFINTWSDMARGLDITIPPFIDRTLLRARDPPQPFPHIEYQPAPAM	209
PtHCT	FPLLVLQVTYFKCGGASLVGGMQHHAADGFSGLHFINTWSDMARGLDITIPPFIDRTLLRARDPPQPFPHIEYQPAPAM	233
RcHCT	FPLLVLQVTYFKCGGASLVGGMQHHAADGFSGLHFINTWSDMARGLDITIPPFIDRTLLRARDPPQPFPHIEYQPAPAM	209
CcHCT	YALLVLQVTYFKCGGASLVGGMQHHAADGFSGLHFINTWSDMARGLDITIPPFIDRTLLRARDPPQPFPHIEYQPAPAM	209
VvHCT	YSLLLQVTYFKCGGASLVGGMQHHAADGFSGLHFINTWSDMARGLDITIPPFIDRTLLRARDPPQPFPHIEYQPAPAM	209
NtHCT	YALLVLQVTYFKCGGASLVGGMQHHAADGFSGLHFINTWSDMARGLDITIPPFIDRTLLRARDPPQPFPHIEYQPAPAM	209
TpHCT	YPLLVLQVTYFKCGGASLVGGMQHHAADGFSGLHFINTWSDMARGLDITIPPFIDRTLLRARDPPQPFPHIEYQPAPAM	210
CcHCT	YSLLLQVTYFKCGGASLVGGMQHHAADGFSGLHFINTWSDMARGLDITIPPFIDRTLLRARDPPQPFPHIEYQPAPAM	209
OsHCT	FSLLLQVTYFKCGGASLVGGMQHHAADGFSGLHFINTWSDMARGLDITIPPFIDRTLLRARDPPQPFPHIEYQPAPAM	215
OsHCT3	FPLLVLQVTYFKCGGASLVGGMQHHAADGFSGLHFINTWSDMARGLDITIPPFIDRTLLRARDPPQPFPHIEYQPAPAM	215
SbHCT	FPLLVLQVTYFKCGGASLVGGMQHHAADGFSGLHFINTWSDMARGLDITIPPFIDRTLLRARDPPQPFPHIEYQPAPAM	215
ZmHCT	FSLLLQVTYFKCGGASLVGGMQHHAADGFSGLHFINTWSDMARGLDITIPPFIDRTLLRARDPPQPFPHIEYQPAPAM	214
ZmHCT2	FSLLLQVTYFKCGGASLVGGMQHHAADGFSGLHFINTWSDMARGLDITIPPFIDRTLLRARDPPQPFPHIEYQPAPAM	214
AsHHT3	YPLLVLQVTYFKCGGASLVGGMQHHAADGFSGLHFINTWSDMARGLDITIPPFIDRTLLRARDPPQPFPHIEYQPAPAM	213
SmHCT1	FPLLVLQVTYFKCGGASLVGGMQHHAADGFSGLHFINTWSDMARGLDITIPPFIDRTLLRARDPPQPFPHIEYQPAPAM	213
SmHCT2	FPLLVLQVTYFKCGGASLVGGMQHHAADGFSGLHFINTWSDMARGLDITIPPFIDRTLLRARDPPQPFPHIEYQPAPAM	213

Majority KXSXXXQK-----APPXTAVSIFKLRXQLGRLKAKXKEGENXPYSSYEMLAGHVWRXVCLARGLPDDQ
 250 260 270 280 290 300 310 320

AtHCT	KIPLDPSKS-----GPENTTIVSIFKLRDQLVALKAKSKEDGNTVSYSSYEMLAGHVWRXVCLARGLPDDQ	275
AlHCT	RIPLDPSKS-----GPDNTTIVSIFKLRDQLVALKAKSKEDGNTVSYSSYEMLAGHVWRXVCLARGLPDDQ	275
PtHCT	KTVLETSK-----PESTAVSIFKLRDQLNLLKAKAKEGNNIGYSSYEMLAGHVWRXVCLARGLPDDQ	297
RcHCT	KASAETLK-----PDSTTIVSIFKLRDQLNLLKAKAKEGNTINYSYEMLAGHVWRXVCLARGLPDDQ	273
CcHCT	KVSPQTAK-----SD---SVPETAVSIFKLTREQISALKAKSKEDGNTISYSSYEMLAGHVWRXVCLARGLPDDQ	276
VvHCT	KTPLPNTQ-----N---TIVGIFRITRDQLNLLKAKSKEDGNTISYSSYEMLAGHVWRXVCLARGLPDDQ	271
NtHCT	KVTPENTPI-----SE---AVPETSVSIFKLRDQINTLAKAKSKEDGNTVNYSSYEMLAGHVWRXVCLARGLPDDQ	277
TpHCT	KTTQSTK-----P---GSDGAAVSIFKLTREQISTLAKAKSKEDGNTIHYSSYEMLAGHVWRXVCLARGLPDDQ	276
CcHCT	KTAPTPTP-----TD---DESVPETTVSIFKLRDQVNALKAKSKEDGNTVNYSSYEMLAGHVWRXVCLARGLPDDQ	276
OsHCT	LSS-VPQSV-----TANKTTPPPTAVDIFKLRSDLGRLRSQLPSGEGAPRFSTYAVLAAHVWRXVCLARGLPDDQ	285
OsHCT3	LAPEPPQAL-----TA-KPAPPPTAVDIFKLRSDLGRLRSQLPRGEGAPRYSTYAVLAAHVWRXVCLARGLPDDQ	285
SbHCT	LSS-TPQFL-----AS-KSKPPATAVDIFKLRSDLGRLRSQLAAGEGAPRFSTYAVLAAHVWRXVCLARGLPDDQ	284
ZmHCT	LSS-TTQFL-----AS-KSKPPATAVDIFKLRSDLGRLRSQLPAEGEGAPRFSTYAVLAAHVWRXVCLARGLPDDQ	283
ZmHCT2	LSS-TTQFL-----AS-KSKPPATAVDIFKLRSDLGRLRSQLPAEGEGAPRFSTYAVLAAHVWRXVCLARGLPDDQ	283
AsHHT3	LGSEEPQAL-----AG-KPESPPTAVDIFKLRSDLGRLRSQLPTGEGAPRFSTYAVLAAHVWRXVCLARGLPDDQ	283
SmHCT1	KHAAATNGH--SNGKAKPQAGDDAPPRIAVGLFKFTKEQLQALKSQATDEETNTTYSSYEMLAGHVWRXVCLARGLPDDQ	291
SmHCT2	KQAAATNGHSVNSGKAKPHTGDDAPPRIAVGLFKFTKEQLQALKSQATDEETNTTYSSYEMLAGHVWRXVCLARGLPDDQ	293

Fig. 4 (Cont'd)

Majority ETKLYIATDGRXRLRPPLPPGYFGNVIFTATPLAXAGDLTSKPLWYAAXVIHDALXRMDNDYLRSAIDYLELQPDLSALV
 330 340 350 360 370 380 390 400

AtHCT	ETKLYIATDGRSRLRPQLPPGYFGNVIFTATPLAVAGDLLSKPTWYAAGQIHDFLVRMDNDYLRSAIDYLELQPDLSALV	355
AlHCT	ETKLYIATDGRSRLRPQLPPGYFGNVIFTATPLAVAGDLLSKPTWYAAGLIHDVLRMDNDYLRSAIDYLELQPDLSALV	355
PtHCT	ETKLYIATDGRSRLRPQLPPGYFGNVIFTATPIAVAGEIQSKPTWYAAGKIHDVLRMDNDYLRSAIDYLELQPDLSALV	377
RcHCT	ETKLYIATDGRSRLNPPLPPGYFGNVIFTATPIAVAGDLESKPTWYAAGKIHDALARMNDYLRSAIDYLELQPDLSALV	353
CcHCT	GTKLYIATDGRARLRPSLPPGYFGNVIFTATPIAVAGDLEKPVWYAASKIHDALARMNDYLRSAIDYLELQPDLSALV	356
VvHCT	DSKLYIATDGRSRLRPALPPGYFGNVIFTTPVAVAGELMSKPLWYAASKIHDALARMNDYLRSAIDYLELQPDLSALV	351
NtHCT	ETKLYIATDGRSRLRPSLPPGYFGNVIFTTPVAVAGDIQSKPTWYAASKIHDALARMNDYLRSAIDYLELQPDLSALV	357
TpHCT	ETKLYIATDGRARLRPPPPGYFGNVIFTTPVAVAGDLSKPTWYAASRIHDALSRMDNDYLRSAIDYLELQPDLSALV	356
CcHCT	DDTKLYIATDGRARLRPSLPRGYFGNVIFTTPVAVAGDLQSKPTWYAASKIHDALARMDDYLRSAIDYLELQPDLSALV	356
OsHCT	PTKLYCATDGRQRRLQPLPEGYFGNVIFTATPLAAGKVTSGLADCAAVIQEALDRMDNDYLRSAIDYLELQPDLSALV	364
OsHCT3	PTKLYCATDGRQRRLQPLPDGYFGNVIFTATPLAAGRVITGSLADCAATIQSALDRMDNDYLRSAIDYLELQPDLSALV	364
SbHCT	PTKLYCATDGRQRRLQPLPDGYFGNVIFTATPLAAGKVTSGLAEGCAAVIQGALDRMDNDYLRSAIDYLELQPDLSALV	363
ZmHCT	PTKLYCATDGRQRRLQPLPDGYFGNVIFTATPLAAGKVTSELAECAAVIQGALDRMDNDYLRSAIDYLELQPDLSALV	362
ZmHCT2	PTKLYCATDGRQRRLQPLPDGYFGNVIFTATPLAAGKVTSELAECAAVIQGALDRMDNDYLRSAIDYLELQPDLSALV	362
AsHHT3	PTKLYCATDGRQRRLTPPLPDGYFGNVIFTATPLAAGKVTGSLADCAATIQDALAKMDDEYLRSAIDYLELQPDLSALV	362
SmHCT1	ETKLYIATDGRARVVPPLPKHYFGNVIFTCTPMALAGDLVSRPLYYAASVIHDALVSRMDNDYLRSAIDYLELQPDLYKLV	371
SmHCT2	ETKLYIATDGRARVVPPLPKHYFGNVIFTCTPMALAGDLVSRPLYYAASVIHDALVSRMDNDYLRSAIDYLELQPDLYKLV	373

Majority RGAHTRFCPNLGLTSWVRLPIHDADFGWRPXFMGPGGIAYEGLXFVLPSPNTDGSLSVAISLQAEHMKLFEKFIYEI--
 410 420 430 440 450 460 470 480

AtHCT	RGAHTYKCPNLGITSWVRLPIYDADFGWRPIFMGPGGIPEYGLSFVLPSPNTDGSLSVAIALQSEHMKLFEKFLFEI	433
AlHCT	RGAHTYKCPNLGITSWVRLPIYDADFGWRPIFMGPGGIPEYGLSFVLPSPNTDGSLSVAIALQSEHMKLFEKFLYEI	433
PtHCT	RGAHTRFCPNLGLTSWVRLPIHDADFGWRPIFMGPGGIAYEGLSFTIPSPNTDGSLSVAISLQAEHMKLFEKFIYDIKE	457
RcHCT	RGAHTRFCPNLGLTSWVRLPIHDADFGWRPIFMGPGGIAYEGLSFTIPSPNTDGSLSVAIALQSEHMKLFEKFIYEL	431
CcHCT	RGAHTRFCPNLGLTSWVRLPIHDADFGWRPIFMGPGGIAYEGLSFTIPSPNTDGSLSVAISLQGEHMKLFSFLYDI	434
VvHCT	RGAHTRFCPNLGLTSWVRLPIYDADFGWRPIFMGPGGIALGLAFALPSPNTDGSLSVAISLQEDHMKLFCYLYEI	429
NtHCT	RGAHTRFCPNLGLTSWVRLPIHDADFGWRPIFMGPGGIAYEGLSFTIPSPNTDGSLSVAISLQAEHMKLFEKFLYDF	435
TpHCT	RGAHTRFCPNLGLTSWVRLPIHDADFGWRPIFMGPGGIAYEGLSFTIPSPNTDGSLSVAIALQSEHMKVFEKFLYDI	434
CcHCT	LVRGAHTRFCPNLGLTSWVRLPIHDADFGWRPIFMGPGGIAYEGLSFVLPSPINDGSLSVAISLQAEHMKLFSKFLYDI	436
OsHCT	RGAHTRFCPNLGLTSWVRLPIHDADFGWRPVFMGPGGIAYEGLAFVLPASANKDGSLSVAISLQAEHMEKFRKLIFFEV	442
OsHCT3	RGAHTRFCPNLGLTSWVRLPIHDADFGWRPVFMGPGGIAYEGLAFVLPASAGDGSLSVAISLQAEHMEKFRKLIFFDF	442
SbHCT	RGAHTRFCPNLGLTSWVRLPIHDADFGWRPVFMGPGGIAYEGLAFVLPASANGDGSLSVAISLQAEHMEKFRKLIICEV	441
ZmHCT	RGAHTRFCPNLGLTSWVRLPIHDADFGWRPVFMGPGGIAYEGLAFVLPASANGDGSLSVAISLQAEHMEKFRKLIIGEA	440
ZmHCT2	RGAHTRFCPNLGLTSWVRLPIHDADFGWRPVFMGPGGIAYEGLAFVLPASANGDGSLSVAISLQAEHMEKFRKLIIGANC	442
AsHHT3	RGAHTRFCPNLGLTSWVRLPIHDADFGWRPVFMGPGGIAYEGLAFVLPASNRDGSLSVAISLQAEHMEKFRKLIFFDF	440
SmHCT1	RGAHTRFCPNLGLTSWVRLPVYDADFGWRPVFMGPAVIAFEGLVYVLPSTGTDGSLSISLGLQPEHMPRFELQIGCI	449
SmHCT2	RGAHTRFCPNLGLTSWVRLPVYDADFGWRPVFMGPAVIAFEGLVYVLPSTGTDGSLSISLGLQPEHMPRFELQIGCI	451

Decoration 'Decoration #1': Shade (with solid black) residues that match the Consensus exactly.

Fig. 5

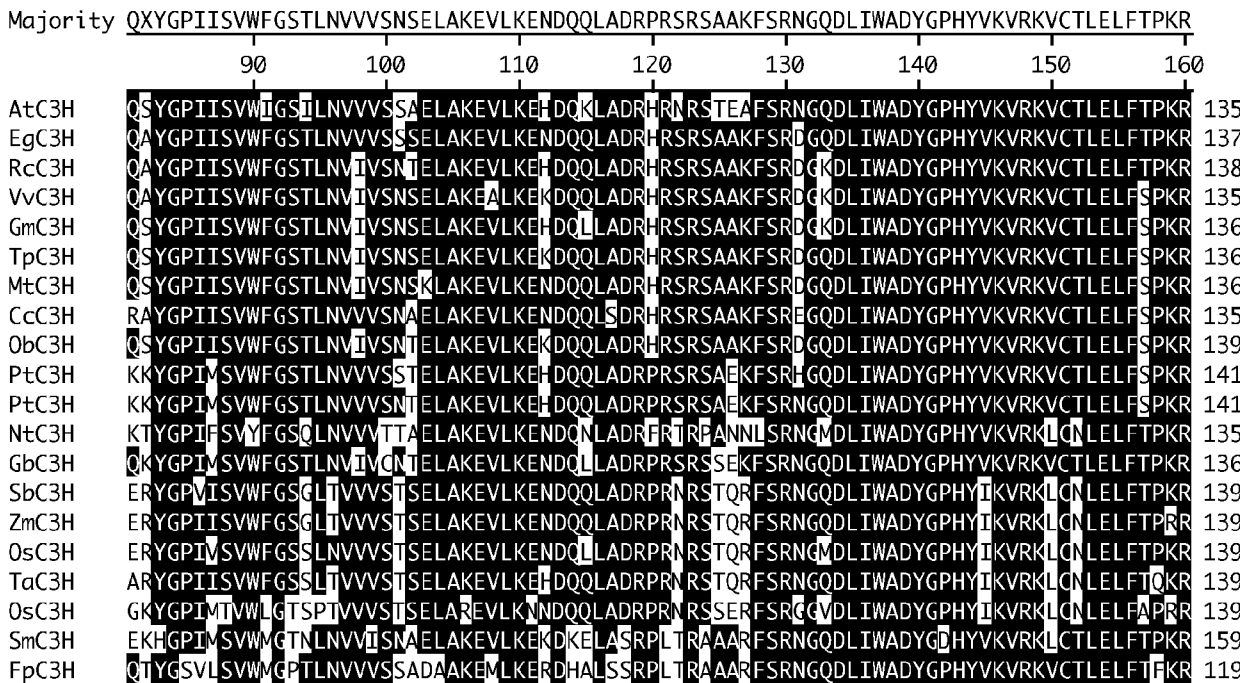
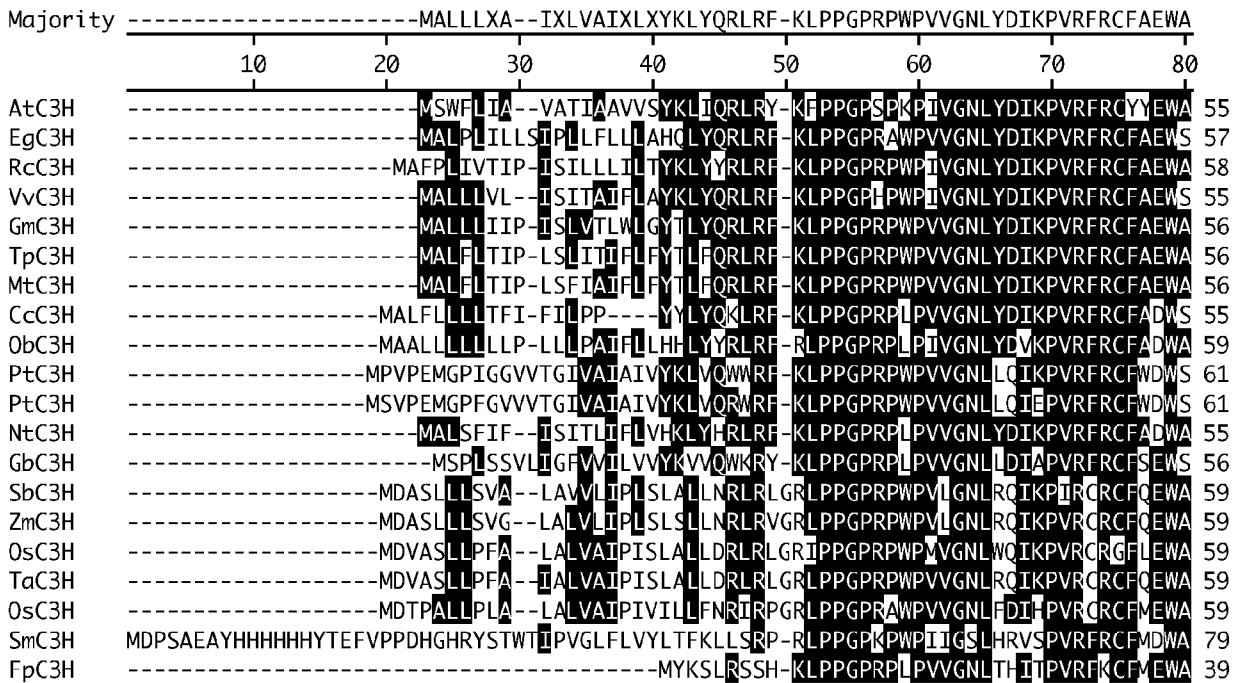


Fig. 5 (Cont'd)

Majority LEALRPIREDEVTAMVESIFXDCTNPENEGKPLLXKYLGAFAFNITRLAFGKRFVNAEGVMDEQGVFEKATVANGLLK

170 180 190 200 210 220 230 240

AtC3H LESLRPIREDEVTAMVESIFRDCNLPENRAKGLQLRKYLGAFAFNITRLAFGKRFVNAEGVVDEQGLFEKATVSNGLKL 215

EgC3H LEALRPIREDEVTAMVESIFKDCCTNPDNSGKTLVKKYLGAFAFNITRLAFGKRFVNAEGVIDEQGLFEKATVSNGLKL 217

RcC3H LDALRPIREDEVTAMVESIFMDCNPNENKSVLVKKYLGAFAFNITRLAFGKRFVNAEGIMDEQGLFEKATVANGLLK 218

VvC3H LEALRPIREDEVTAMVESIFKDCVTPNENLGSILVKKYLGAFAFNITRLAFGKRFVNSEGVIDEQGLFEKATVANGLLK 215

GmC3H LEALRPIREDEVTSMVD SVYNHCTSTENLGKGLLRKHLGVAFNITRLAFGKRFVNSEGVMDEQGVFEKATVENGLKL 216

TpC3H IEALRPIREDEVTAMVESIFNDSTNSENLGKGLMRKYIIVAFNITRLAFGKRFVNSEGVMDEQGVFEKATVANGLLK 216

MtC3H IEALRPIREDEVTAMVESIFNDSTNSENLGKGLMRKYIIVAFNITRLAFGKRFVNSEGVMDEQGVFEKATVANGLLK 216

CcC3H LEALRPIREDEVTAMVESIYKDCITLREGSQSLLVKKYLGVAFNITRLAFGKRFVNSEGVMDEQGLFEKATVANGLLK 215

ObC3H LEALRPIREDEVTAMVESIYHDCITAPDNAGKSLVKKYLGAFAFNITRLAFGKRFVNSEGITDKQGLFEKATVSNGLKL 219

PtC3H LEALRPIREDEVTAMVESIFNDCKQEGIGKPLVKKYLGAFAFNITRLAFGKRFVNSEGKMDPQGLFEKATVATGLKL 221

PtC3H LEALRPIREDEVTAMVESIFNDCKQEGIGKPLVKKYLSGAFNITRLAFGKRFVNSEGKMDPQGVFEKATVATGLKL 221

NtC3H LEALRPIREDEVTAMVENIFKDCITKPDNIGKSLVREYLSGAFNITRLAFGKRFVNSEGKMDPQGVFEKATVSNGLKL 215

GbC3H LESLRPIREDEVTAMVESIFKDCGSGQEGVGVKTVVKKYPSAVAFNITRLAFGKRFVNSEGKMDPQGLFEKATVATGLKL 216

SbC3H LEALRPIREDEVTAMVESVYRAATAPGNEGKPMVVRNHLVAFNITRLAFGKRFVNSAGDIDEQGVFEKATVSNGLKL 219

ZmC3H LEALRPIREDEVTAMVESVHRAATAPGNEGKPMVVRKHLVAFNITRLAFGKRFVNSAGDIDEQGVFEKATVSNGLKL 219

OsC3H LEALRPIREDEVTAMVESVHRAATAPGSEHKPIVVRNHLVAFNITRLAFGKRFVNSAGDIDEQGVFEKATVSNGLKL 219

TaC3H LEALRPIREDEVTAMVESVHRAAAGPNEGKPLVVRNHLVAFNITRLAFGKRFVNSAGDIDEQGVFEKATVSNGLKL 219

OsC3H MEALRPIREDEVTAMVESIYRATITAPGEEGKPMVVRKHLVAFNITRLAFGKRFVNSAGELDEQGLFEKATVSNGLKL 219

SmC3H LESLRPIREDEVTAMVESIFKDCVKNNG--GAAVTVKTVYSAVAFNITRLAFGKRFVNSAGELDEQGLFEKATVSNGLKL 237

FpC3H LESLRPIREDEVTAMVAALFKDCADS----RPLNLLKTVYSAVAFNITRLAFGKRFVNSAGELDEQGLFEKATVSNGLKL 195

Majority GASLMAEHIPWLRWMPLEEEAFKHGARRDRLTRAIMEEHTLARQKSGGAKQHFVDALLTLQDKYDLSSETIIGLLWD

250 260 270 280 290 300 310 320

AtC3H GASLMAEHIPWLRWMPLEEEAFKHGARRDRLTRAIMEEHTLARQKSGGAKQHFVDALLTLQDKYDLSSETIIGLLWD 295

EgC3H GASLMAEHIPWLRWMPLEEEAFKHSARRDRLTRAIMEEHTLARQKSGAKQHFVDALLTLQDKYDLSSETIIGLLWD 296

RcC3H GASLMAEHIPWLRWMPLEEEAFKHGARRDRLTRAIMEEHTLARQKSGGAKQHFVDALLTLQDKYDLSSETIIGLLWD 298

VvC3H GASLMAEHIPWLRWMPLEEEAFKHGARRDRLTRAIMEEHTLARQKSGGAKQHFVDALLTLQDKYDLSSETIIGLLWD 295

GmC3H GASLMAEHIPWLRWMPLEEEAFKHGARRDRLTRAIMEEHTLARQKSGGAKQHFVDALLTLQDKYDLSSETIIGLLWD 296

TpC3H GASLMAEHIPWLRWMPLEEEAFKHGARRDRLTRAIMEEHTLARQKSGGAKQHFVDALLTLQDKYDLSSETIIGLLWD 296

MtC3H GASLMAEHIPWLRWMPLEEEAFKHGARRDRLTRAIMEEHTLARQKSGGAKQHFVDALLTLQDKYDLSSETIIGLLWD 296

CcC3H GASLMAEHIPWLRWMPLEEEAFKHGARRDRLTRAIMEEHTLARQKSGGAKQHFVDALLTLQDKYDLSSETIIGLLWD 295

ObC3H GASLMAEHIPWLRWMPLEEEAFKHGARRDRLTRAIMEEHTLARQKSGGAKQHFVDALLTLQDKYDLSSETIIGLLWD 299

PtC3H GASLMAEHIPWLRWMPLEEEAFKHGARRDRLTRAIMEEHTLARQKSGGAKQHFVDALLTLQDKYDLSSETIIGLLWD 300

PtC3H GASLMAEHIPWLRWMPLEEEAFKHGARRDRLTRAIMEEHTLARQKSGGAKQHFVDALLTLQDKYDLSSETIIGLLWD 300

NtC3H GGLPLAEYVWLRWMPLEEEAFKHSARRDRLTRAIMEEHTLARQKSGGAKQHFVDALLTLQDKYDLSSETIIGLLWD 295

GbC3H GASLMAEHIPWLRWMPLEEEAFKHGARRDRLTRAIMEEHTLARQKSGGAKQHFVDALLTLQDKYDLSSETIIGLLWD 295

SbC3H GASLMAEHIPWLRWMPLEEEAFKHGARRDRLTRAIMEEHTLARQKSGGAKQHFVDALLTLQDKYDLSSETIIGLLWD 298

ZmC3H GASLMAEHIPWLRWMPLEEEAFKHGARRDRLTRAIMEEHTLARQKSGGAKQHFVDALLTLQDKYDLSSETIIGLLWD 298

OsC3H GASLMAEHIPWLRWMPLEEEAFKHGARRDRLTRAIMEEHTLARQKSGGAKQHFVDALLTLQDKYDLSSETIIGLLWD 298

TaC3H GASLMAEHIPWLRWMPLEEEAFKHGARRDRLTRAIMEEHTLARQKSGGAKQHFVDALLTLQDKYDLSSETIIGLLWD 298

OsC3H GASLMAEHIPWLRWMPLEEEAFKHGARRDRLTRAIMEEHTLARQKSGGAKQHFVDALLTLQDKYDLSSETIIGLLWD 298

SmC3H GASLMAEHIPWLRWMPLEEEAFKHGARRDRLTRAIMEEHTLARQKSGGAKQHFVDALLTLQDKYDLSSETIIGLLWD 317

FpC3H GASLMAEHIPWLRWMPLEEEAFKHGARRDRLTRAIMEEHTLARQKSGGAKQHFVDALLTLQDKYDLSSETIIGLLWD 274

Fig. 5 (Cont'd)

Majority	LHHFEWAPPEGVKPEDIDMTENPGLVTXMRTPLQAVATPRLP-SELYKRVPVDM-	
	490 500 510 520 530	
AtC3H	LHHFVWTPPQGTKPEEIDMSENPLVTYMRTPVQAVATPRLP-SDLYKRVPVDM	508
EgC3H	LHHFVWTPPQGTKPEEIDMSENPLVTYMRTPVQAVATPRLP-SELYKRVPVEE	509
RvC3H	LHHFRWTPPEGVKPEEIDMSENPLVTYMRTPVQAVATPRLP-SELYKRVAVDM	511
VvC3H	LHHFNWAPPEGVNPEEIDMSENPLVSYMRTPVQAVATPRLP-ASLYKRMAVDI	508
GmC3H	LHHFGWTPPEGVKPEEIDMGENPGLVTYMRTPVQAVATPRLP-SHLYKRVPAAEI	509
TpC3H	LHHFCWAAPEGVNPEEIDMTENPGMVTYMRTPVQAVATPRLP-SELYKRVPADI	509
MtC3H	LHHFCWAPPEGVNPAEIDMAENPGMVTYMRTPVQAVATPRLP-SELYKRVTADI	509
CcC3H	LHHFNWAPPVGLSPDEIDMGESPLVTYMRTPVQAVATPRLP-SHLYKRVAVDM	508
ObC3H	LHHFNWAPPVGVSSDEIDMGENPGLVTYMRTPVQAVATPRLP-SDLYKRVAVDL	512
PtC3H	LHHFEWAPPEGMKAEDIDLTENPGLVTYMRTPVQAVATPRLP-AHLYKRQPLN	512
PtC3H	LHHFEWAPPEGMQAEDIDLTENPGLVTYMRTPVQAVATPRLP-DHLYKRQPLN	512
NtC3H	LHHFTWAPAPGVNPEEIDLEESPGVTYMRTPVQAVATPRLP-AHLYKRVPVDM	508
GbC3H	LHHFTWAPPEGMKSEIDLEESPGVTYMRTPVQAVATPRLP-APLYKREPNNW	508
SbC3H	LHHFEWVSLPEGTRPEDVNMESPLVTYMRTPVQAVATPRLP-EKEELYKRVPVEE	512
ZmC3H	LHHFEWVSLPEGTRPEDVNMESPLVTYMRTPVQAVATPRLP-EKEELYKRVPVEE	512
OsC3H	LHQFEWVSLPEGTRPEDVNMESNGLVTYMRTPVQAVATPRLP-DNPDKRFPVEE	512
TaC3H	LHHFEWVSLPEGTRPEDVNMESPLVTYMRTPVQAVATPRLP-EKEELYKRVPVEE	512
OsC3H	LHQFTWALPDGTRPEDVNMESPLVTYMRTPVQAVATPRLP-EKEELYKRVPVDM	513
SmC3H	LHQFTWAPPVGVKPEEIDLTERPGVTFMNPVQAVATPRLA-EKLYE	524
FpC3H	LHHFSWAPPVGVTPAAIDMTERPGVTFMNPVQAVATPRLA-AALYKNGSSPS	487

Decoration 'Decoration #1': Shade (with solid black) residues that match the Consensus exactly.

Fig. 6

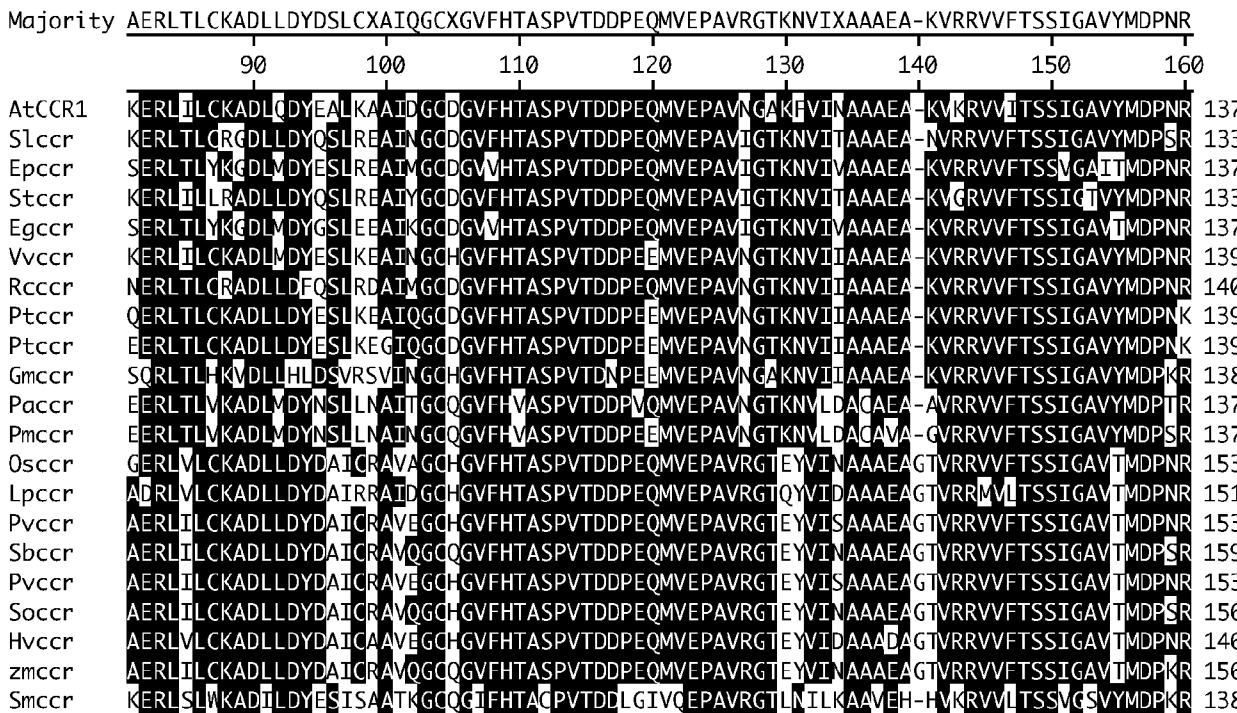
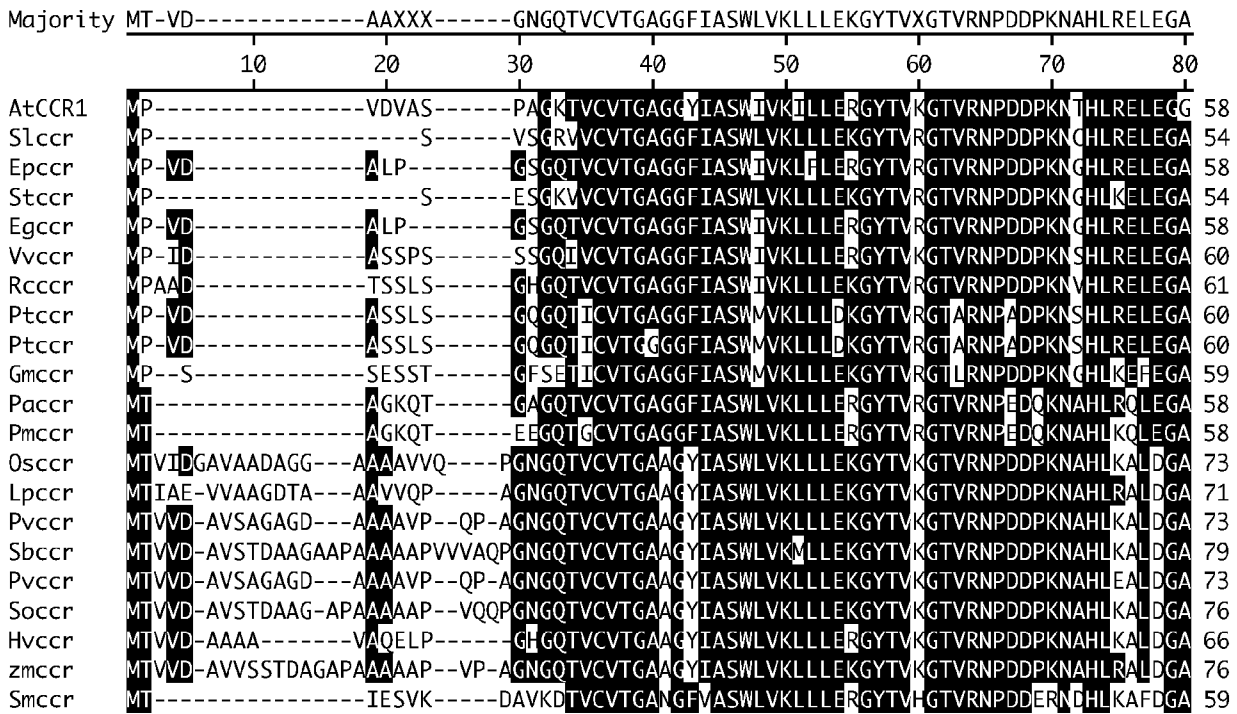


Fig. 6 (Cont'd)

Majority GPDVVVDESCWSDLEFCNKNTKNWYCYGKAVAEQAWEAAEXGVDLVVNPNVVLGPLLQPTVNASIAHILKYLTGSAKT

170 180 190 200 210 220 230 240

AtCCR1 DPEAVVDESCWSDLEFCNKNTKNWYCYGKAVAEQAWEAAEAKERGVDLVVNPNVVLGPLLQPTVNASIAHILKYLTGSAKT 217
Slccr DPEKVVDETCWSDPDEFCKNTKNWYCYGKVAEQAAWDEAREKGVDLVAINPVLVLGPLLQPTVNASIAHILKYLTGSAKT 213
Epccr GPDVVVDESCWSDLEFCNKNTKNWYCYGKAVAEKSAEAKERGVDLVVNPNVVLGPLLQPTVNASIAHILKYLTGSAKT 217
Stccr APDKVVDETCWSDLEFCNKNTKNWYCYGKVAEKTAWDEAREKGVDLVVNPNVVLGPLLQPTVNASIAHILKYLTGSAKT 213
Egccr GPDVVVDESCWSDLEFCNKNTKNWYCYGKAVAEKAAWPEAKERGVDLVVNPNVVLGPLLQPTVNASIAHILKYLTGSAKT 217
Vvccr SPDQVVDESCWSDLEFCNKNTKNWYCYGKAVAEQAWEVAKERGVDLVVNPNVVLGPLLQPTVNASIAHILKYLTGSAKT 219
Rcccr GPDVVVDESCWSDLEFCNKNTKNWYCYGKAVAEQAWEVAKERGLDLVVNPNVVLGPLLQPTVNASIAHILKYLTGSAKT 220
Ptccr GPDVVVDESCWSDLEFCNKNTKNWYCYGKAVAEQAAWDMAKERGVDLVVNPNVVLGPLLQPTVNASIAHILKYLTGSAKT 219
Ptccr GPDVVVDESCWSDLEFCNKNTKNWYCYGKAVAEQAAWDMAKERGVDLVVNPNVVLGPLLQPTVNASIAHILKYLTGSAKT 219
Gmccr SIDLVVDESCWSDLEFCNKNTKNWYCYGKAVAEQAAWDTAKERGVDLVVNPNVVLGPLLQPTVNASIAHILKYLTGSAKT 218
Paccr DYDALVDESCWSDLEFCNKNTKNWYCYGKAVAEKAAWDRAKERGLDLVVNPNVVLGPLLQPTVNASIAHILKYLTGSAKT 217
Pmccr DYDALVDETCWSDLEFCNKNTKNWYCYGKVAEKAAWBRADKGLDLVVNPNVVLGPLLQPTVNASIAHILKYLTGSAKT 217
Oscrr GPDVVVDESCWSDLEFCNKNTKNWYCYGKAVAEQAWEAARRRGLDLVVNPNVVLGPLLQPTVNASIAHILKYLDGSAKT 233
Lpccr GPDVVVDESCWSDLEFCNKNTKNWYCYGKAVAEQAASELARQRGVDLVVNPNVVLGPLLQPTVNASIAHILKYLDGSAKT 231
Pvccr GPDVVVDESCWSDLEFCNKNTKNWYCYGKAVAEQAWEAARRRGLDLVVNPNVVLGPLLQPTVNASIAHILKYLDGSAKT 233
Sbccr GPDVVVDESCWSDLEFCNKNTKNWYCYGKAVAEQAAWDAARQRGVDLVVNPNVVLGPLLQPTVNASIAHILKYLDGSAKT 239
Pvccr GPDVVVDESCWSDLEFCNKNTKNWYCYGKAVAEQAWEAARRRGLDLVVNPNVVLGPLLQPTVNASIAHILKYLDGSAKT 233
Socrr GPDVVVDESCWSDLEFCNKNTKNWYCYGKAVAEQAAWDAARQRGVDLVVNPNVVLGPLLQPTVNASIAHILKYLDGSAKT 236
Hvccr GPDVVVDESCWSDLEFCNKNTKNWYCYGKAVAEQAWEAKARARGLDLVVNPNVVLGPLLQPTVNASIAHILKYLDGSAKT 226
zmccr GPDVVVDESCWSDLEFCNKNTKNWYCYGKAVAEQAWEAARRRGLDLVVNPNVVLGPLLQPTVNASIAHILKYLDGSAKT 236
Smccr PVEEVVSSEEMWSDVQYLKQDTRNGYCLAKTLAESAWEAFANQNHVDMVTNPSVVLGPLLQPTVNASIAHILKYLTGATKV 218

Majority YANXVQAYVHVRDVALAHILVFESPSASGRYLCAESVLHRGDVVEILAKLFPEYVPPTKCSDEVNPRKPKYKFSNQKLRD

250 260 270 280 290 300 310 320

AtCCR1 YANLTVQAYVHVRDVALAHILVFEAPSASGRYLCAESVLRHGEVVEILAKLFPEYVLPPTKCSDEVNPRKPKYKFSNQKLRD 297
Slccr YANSVQAYVHVRDVALAHILVFEAPSASGRYLCAESVLRHGDVVEILAKLFPEYVLPPTKCSDEVNPRKPKYKFSNQKLRD 293
Epccr YANSVQAYVHVRDVALAHILVFEAPSASGRYLCAESVLRHGDVVEILAKLFPEYVLPPTKCSDEVNPRKPKYKFSNQKLRD 297
Stccr YANSVQAYVHVRDVALAHILVFEAPSASGRYLCAESVLRHGDVVEILAKLFPEYVLPPTKCSDEVNPRKPKYKFSNQKLRD 293
Egccr YANSVQAYVHVRDVALAHILVFEAPSASGRYLCAESVLRHGDVVEILAKLFPEYVLPPTKCSDEVNPRKPKYKFSNQKLRD 297
Vvccr YANSVQAYVHVRDVALAHILVFEAPSASGRYLCAESVLRHGDVVEILAKLFPEYVLPPTKCSDEVNPRKPKYKFSNQKLRD 299
Rcccr YANSVQAYVHVRDVALAHILVFEAPSASGRYLCAESVLRHGEVVEILAKLFPEYVLPPTKCSDEVNPRKPKYKFSNQKLRD 300
Ptccr YANSVQAYVHVRDVALAHILVFEAPSASGRYLCAESVLRHGEVVEILAKLFPEYVLPPTKCSDEVNPRKPKYKFSNQKLRD 299
Ptccr YANSVQAYVHVRDVALAHILVFEAPSASGRYLCAESVLRHGEVVEILAKLFPEYVLPPTKCSDEVNPRKPKYKFSNQKLRD 299
Gmccr YANATQAYVHVRDVALAHILVFEAPSASGRYLCAESVLRHGEVVEILAKLFPEYVLPPTKCSDEVNPRKPKYKFSNQKLRD 298
Paccr YANSVQAYVHVRDVALAHILVFEAPSASGRYLCAESVLRHGDVVEILAKLFPEYVLPPTKCSDEVNPRKPKYKFSNQKLRD 297
Pmccr YANSVQAYVHVRDVALAHILVFEAPSASGRYLCAESVLRHGDVVEILAKLFPEYVLPPTKCSDEVNPRKPKYKFSNQKLRD 297
Oscrr FANAVQAYVHVRDVALAHILVFEAPSASGRYLCAESVLRHGEVVEILAKLFPEYVLPPTKCSDEVNPRKPKYKFSNQKLRD 313
Lpccr FANAVQAYVHVRDVALAHILVFEAPSASGRYLCAESVLRHGDVVEILAKLFPEYVLPPTKCSDEVNPRKPKYKFSNQKLRD 311
Pvccr FANAVQAYVHVRDVALAHILVFEAPSASGRYLCAESVLRHGDVVEILAKLFPEYVLPPTKCSDEVNPRKPKYKFSNQKLRD 313
Sbccr FANAVQAYVHVRDVALAHILVFEAPSASGRYLCAESVLRHGDVVEILAKLFPEYVLPPTKCSDEVNPRKPKYKFSNQKLRD 319
Pvccr FANAVQAYVHVRDVALAHILVFEAPSASGRYLCAESVLRHGDVVEILAKLFPEYVLPPTKCSDEVNPRKPKYKFSNQKLRD 313
Socrr FANAVQAYVHVRDVALAHILVFEAPSASGRYLCAESVLRHGDVVEILAKLFPEYVLPPTKCSDEVNPRKPKYKFSNQKLRD 316
Hvccr YANAVQAYVHVRDVALAHILVFEAPSASGRYLCAESVLRHGDVVEILAKLFPEYVLPPTKCSDEVNPRKPKYKFSNQKLRD 306
zmccr FANAVQAYVHVRDVALAHILVFEAPSASGRYLCAESVLRHGDVVEILAKLFPEYVLPPTKCSDEVNPRKPKYKFSNQKLRD 316
Smccr YTNHCQAYVHVRDVALAHILVFEAPSASGRYLCAESVLRHGEVVEILAKLFPEYVLPPTKCSDEVNPRKPKYKFSNQKLRD 297

Fig. 6 (Cont'd)

Majority	LGLEFTPVKQCLYETVKSLEKGHLPVPAQ-----QXGGIAIXA	
	330 340 350 360 370	
AtCCR1	LGLEFTSTKQSLYDITVKSLEKGHLPVPPPPSASQ-----ESVENGIKIGS	344
Slccr	LGMEFTPVKQCLYETVKSLEKGHLPVPTQ-----KDEIIRIQT	332
Epccr	LGLEFTPVKQCLYETVKSLEKGHLPVSP-----PEDSVRIQR	336
Stccr	LGLEFTPVKQCLYETVKSLEKGHLPVPTQ-----NDEPTKIHS	332
Egccr	LGLEFTPVKQCLYETVKSLEKGHLPVSP-----PEDSVRIQG	336
Vvccr	LGLEFTPVKQCLYETVKSLEKGHLPVPPQ-----HDDSLRIQS	338
Rcccr	LGLEFTPVKQCLYETVKSLEKGHLPVPKQ-----AEDSVRIQA	339
Ptccr	LGLEFTPVKQCLYETVKSLEKGHLPVPKQA-----AEEVKTIQ	338
Ptccr	LGLEFTPVKQCLYETVKSLEKGHLPVPKQA-----AEEVKTIQ	338
Gmccr	LGLEFTPVSQCLYEAVKSLQEKGHLPVPAEQ-----QEDSTTVKP	338
Paccr	LGLEFTPVKQCLYETVKSLEKGHIP	322
Pmccr	LGLEFTPVKQCLYETVKSLEKGHIPSK	324
Oscrr	LGLEFRPASQSLYETVKSLEKGHLPVLAEEKTEEE-----AGEVCGGIATRA	361
Lpccr	LGLEFRPVSQSLYETVKSLEKGHLPVLADEQAEADK-----ETLAAELQAGVTIIRA	362
Pvccr	LGLEFRPVSQSLYDITVKSLEKGHLPVLADEQAPEA-----APGAEACGGIATRA	364
Sbccr	LGLEFRPVSQSLYDITVKSLEKGHLPVLADEQAPEA-----APGAEACGGIATRA	374
Pvccr	LGLEFRPVSQSLYDITVKSLEKGHLPVLADEQAPEA-----APGAEACGGIATRA	364
Soccr	LGLEFRPVSQSLYDITVKSLEKGHLPVLADEQAPEA-----APGAEACGGIATRA	372
Hvccr	LGLEFRPVSQSLYDITVKSLEKGHLPVLADEQAPEA-----APGAEACGGIATRA	348
zmccr	LGLEFRPVSQSLYDITVKSLEKGHLPVLADEQAPEA-----APGAEACGGIATRA	371
Smccr	LGLEFRPVSQSLYDITVKSLEKGHLPVLADEQAPEA-----APGAEACGGIATRA	323

Decoration 'Decoration #1': Shade (with solid black) residues that match the Consensus exactly.

Fig. 13 (Cont'd)

Majority -----TX-VSNVAG----AAQEEGWVCRVFKKKNLVKTLXSS-----SSSSXADXRK

170 180 190 200 210 220 230 240

AtNST1 -----TVHEVVSITIG-E-ASQDEGWVCRVIFKKNLHKTLLNSPV--GGASLISGGGDTPK 204

AtNST2 -----DHDVNVETCD-V--IGSDEGWVCRVFKKKNLCKNMIS-----SSP--ASSVK 192

SND1 -----NGYADVITEDP-MSYN EEGWVCRVFRKKNYOKIDDCPKI-----TLSSLPDTE 199

PtNAC023 -----NSKRKG-----SMRLDDWVLCRVROKNSIPRNTWODQ-----NIFSCASAPT 187

MtNAC1 -----DTNLVSNMIG-D--GQEEGWVCRVIFKKNHLKTLDS-----P--SG---EGRRS 192

GmNAM1 -----DTNLVSNVMG-D--AAQEEGWVCRVIFKKNHLKTLDS-----PLASG---EDRRS 194

PtNAC065 -----DTN-VSNVMEEE-AAQEEGWVCRVIFKKNLNLKTLDK-----PFSSSPIADTRN 196

VvNST -----DTQ-VSNAVA---EAQEEGWVCRVIFKKNHKTVESSS---PMSSSSITAETRT 196

RcNST -----DTN-VSNVMG-E-AAQEEGWVCRVIFKKNHKTLLDS-----PLSSSSFFTEART 195

EgNST -----SSVGPSLVCE---SSFEDGWVCRVFRKKNHSHQRTLESPPK-----TSTGSMQTEM 193

ZmNST AAAAASSDGGQEDGWVCRVQKHHHKESSGRCSRK-RGSKTEHGHGEAKTAAHQRHGGLQYSSNDDTLDHMLGRRSC 230

SbNST TAAAAATVAAAASSDGGQEDAWVVYRVFKKHHHKESSGGGGSKHGGSNNEHGHGGGKAAAAA-----AAAAHQHHG 226

OsNAC7 NLPSYYS-----SSSSSSPMHGVAGDQ--GAQEEGWVCRVFKKKNLVHHGGGAA-----AASHHAAAK 209

SbNST ASAHHAAAGAAADHHPYYTSSSPALPTAIRGAAGDQAAQEEGWVCRVFKKKNLVHHGQSSG-----GGVTAAGSK 226

PtNAC -----NVMEEEAAQE---EGWVCRVIFKKNLNLKTLDPFSSSPI-----SADTRNQML 196

PtNAC -----ETNVSNIPIGE---AIFEEGWVCRVFRKKNYOKTLESPPK-----SSSLDSKAH 196

SbNST ASAHHAAAGAAADHHPYYTSSSPALPTAIRGAAGDQAAQEEGWVCRVFKKKNLVHHGQSSG-----GGVTAAGSK 226

PsNST -----QANTFN-----ELQEDGWVCRVFRKKNHKSQESAELSPKYGSKQFSNNKI 192

AppleT -----ETTIVSSMGE---SMTEEGWVCRVFKKKNYOKALESPPK-----ASFMSDSSNN 196

OsNST NLPSYYS-----SSSSSSPMHGVAGDQ--GAQEEGWVCRVFKKKNLVHHGGGSA-----AASHHAAAK 209

ZmNST AHLLLLPAAEHPPYYTSPQAPSSTTIAIRGAAGDQ-AAQEEGWVCRVFKKKNLVHHGQSSGVKQQAEGDDHAASHT 230

SmNST1 -----INNTAT-----NEEGWVCRVFRKKNLVKTPSSD-----FDGACCYDPEE 185

Majority -----QLAASCNEGSLDQILE--YMGRT-CKEEN-----RP--HS--X-----XDHIXXFMKLPXLES

250 260 270 280 290 300 310 320

AtNST1 T----TSSQIFNEDTLDOFLE--LMGRS-CKEEL-----NLDPFMKLPLNLES 244

AtNST2 T----P-S--FNEETIEQLLE--VMGQS-CKGET-----VLDPFMKLPLNLEC 229

SND1 EEKGPTFHNTQVTLGHVLL--YMDRTGSNICMPESQ-----TTTQH-----QDDVLFVGLPSLET 254

PtNAC023 G----LFPKVNELQOMNINPNTENVTYFYDDCP-----MLPCIFS-----PONFPSTERASSINF 239

MtNAC1 H----HLYDTCDEGALQIILQ--QMGRG-CKEEN--YEANYNNYGRFARPFEST-LNNN-----GGYNNERFMKLPNLES 258

GmNAM1 -----HLFDSQDEGALQIILE--QMGRS-CKEESSYEGNYRN-YGRFTRPYETTGLNNG-----GGYN-DRFMKLPNLES 259

PtNAC065 -----QMLSQDEGTIDQTFH--YMGRT-CKEEN--VADNSA-TARYLRPVDTA-IN-----YVHH-DGFMKLPNLES 256

VvNST -----QLLNSQDEGALQIIFQ--FMGRT-CKEDN-NEANN---SRRFLRSIDTV-INNG-----STLH-DRFMKLPNLES 257

RcNST -----QIFNSQNEGALQIILE--YMGRT-CKEEN--EANN---CVRFLRPIDTT-INSG-----SSYH-DKFMKLPNLES 256

EgNST -----LMNASADSSVLDQILS--YMGRTGESPENYQAP-----INISS-----MQTQLIHGKFLHL 243

ZmNST K----QEHLLPLPPPAARAASRYIRP-IETVLGGHGMKLPPLPSPAAAEALTPHA-----VSAGDATAAGALDGLH 300

SbNST G----LQYSSD DALDQILQYMGRSCKQ-EHELLS-----PPFPGRAASRYLR-----PIETVLGGHGMKLP 285

OsNAC7 L----AAAA--MEGSPSNCSTVT-VSDH-VKAQML-----HSASDD-----ALDHILOQYMGSGCKQ 259

SbNST M----ASAAAPMEGSPSHCSSVTVISDH-TMNKHQ-----AQAMLOHSASDDD-----ALDHILOQYMGGGGKQ 285

PtNAC -----SCDEGTIDQTFHYMGR--TCKEE-NVADNSATARY-----LRPVDTAINVYHHDGFMKLPNLES 263

PtNAC -----QILGSGNEGLDQILM--YMGRT-CKMENETFSNM-----NISNSNSSLRFLSNNCISEGLHERFMHLPNLES 263

SbNST M----ASAAAPMEGSPSHCSSVTVISDH-TMNKHQ-----AQAMLOHSASDDD-----ALDHILOQYMGGGGKQ 285

PsNST LSD--SDCPNDLTDPLHYTHQLNMCKQELMQQYAFPHDQFMQLPQLESKIPTCNNP-----PSSGIIKRFPPDSRSGF 265

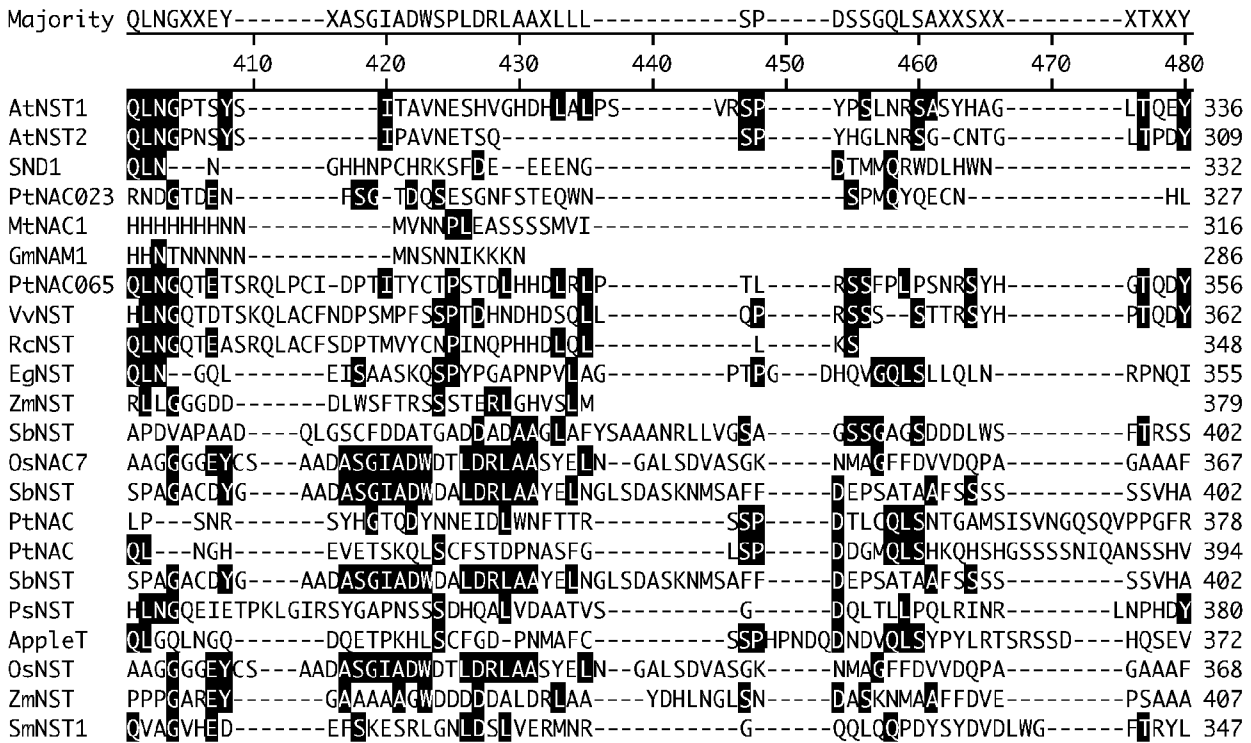
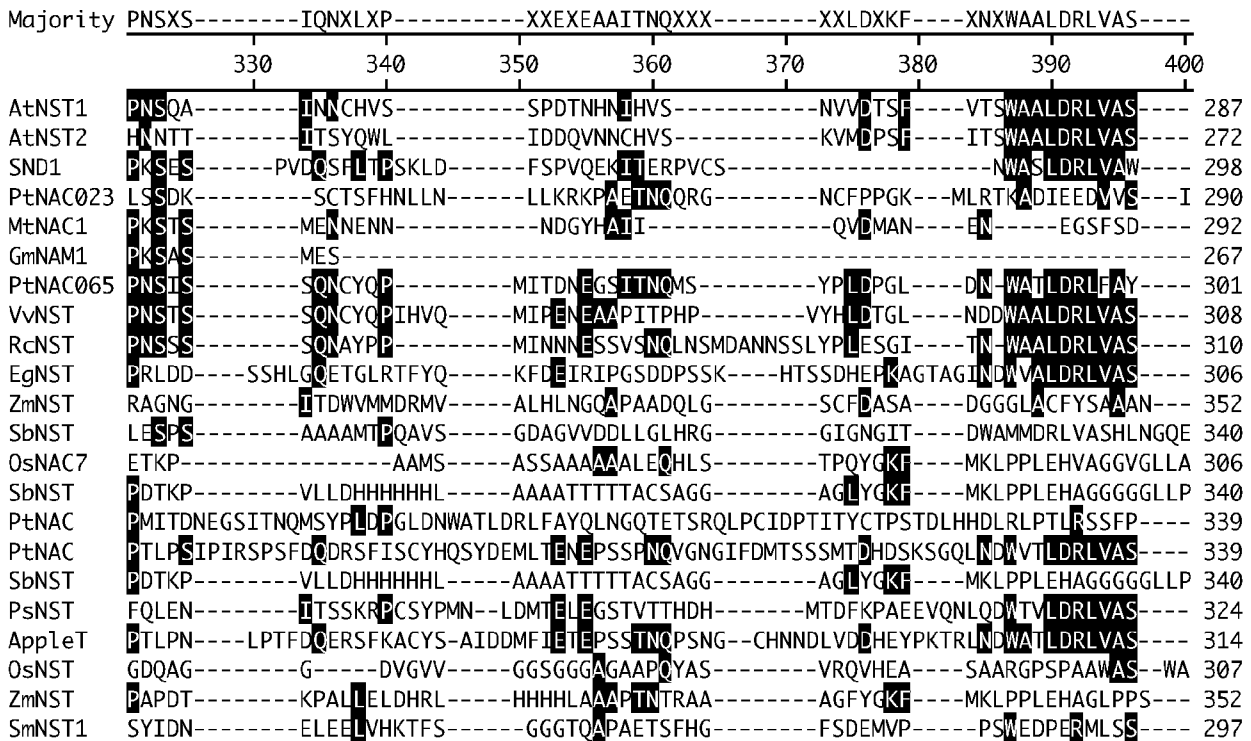
AppleT -----QMHCNRNDGMLDQILM--YMGRTCKLENHDQS-----LTMNN-----ISERFMHLPNLES 245

OsNST L----AAAA--MEGSPSNCSTVT-VSDH-VKAQML-----HSASDD-----ALDHILOQYMGSGLQA 259

ZmNST A----AAAAHMDSSPSQCSSVTVISDH-VHANVNDKQQQAASLLMMHTHSASDDD-----ALDHILOQYMGGGROA 300

SmNST1 N---MDKMYSSNSSFVKQELASAGIYFPFAFDHSLPHLE-----SPKRAQSPMSYN-----PPPEDHEHHHDLIKAS 248

Fig. 13 (Cont'd)



Majority	ASXEGDL-WSLXXS-S--S----L----N---	
	490 500 510	
AtNST1	-TPEMEL-WNTTTSLS-SSPGPFCHVSN GSG	365
AtNST2	YIPEIDL-WNEADFART-T-----CHLLNGSG	334
SND1	NDDNVDLWSSFTESSS---SLDPLHLHSV	358
PtNAC023	TFTGNHLLTD	337
MtNAC1	-S--MW	319
GmNAM1		286
PtNAC065	-NNEIDL-WNFT--TRS-SP-DTLCQLSN	379
VvNST	-NTEIDL-WSFTRSCQLQ-SS-DPLCHISNTPS	390
RcNST		348
EgNST	YSSSSFDWVFGKSSS-VTPSNPLHHLSV	383
ZmNST	LRKKVAS	379
SbNST	AAAAAAT-STERLSHV--SLMSISV	419
OsNAC7	SSGDGDL-WSLARSVSS-SLHADLTTMNV	395
SbNST	AAVDGDL-WSLARSVS--ALHADLT-MNV	428
PtNAC	FHPTEEE	378
PtNAC	YSNNDL-WSLTKSSSPSSSDPLCHLSV	422
SbNST	AAVDGDL-WSLARSVS--ALHADLT-MNV	428
PsNST	ASCEIDL-WNFVK	392
AppleT	YNNNDL-WNFTKSSSPSSSDPLCHLSV	400
OsNST	SSGDGDL-WSLARSVSS-SLHADLTTMNV	396
ZmNST	AAVDGDL-WSLARSVS--ALHADLT-MNV	433
SmNST1	PIQFAGFCYALLSVSLP-SVVSWISERAWK	376

Decoration 'Decoration #1': Shade (with solid black) residues that match the Consensus exactly.

Fig. 14

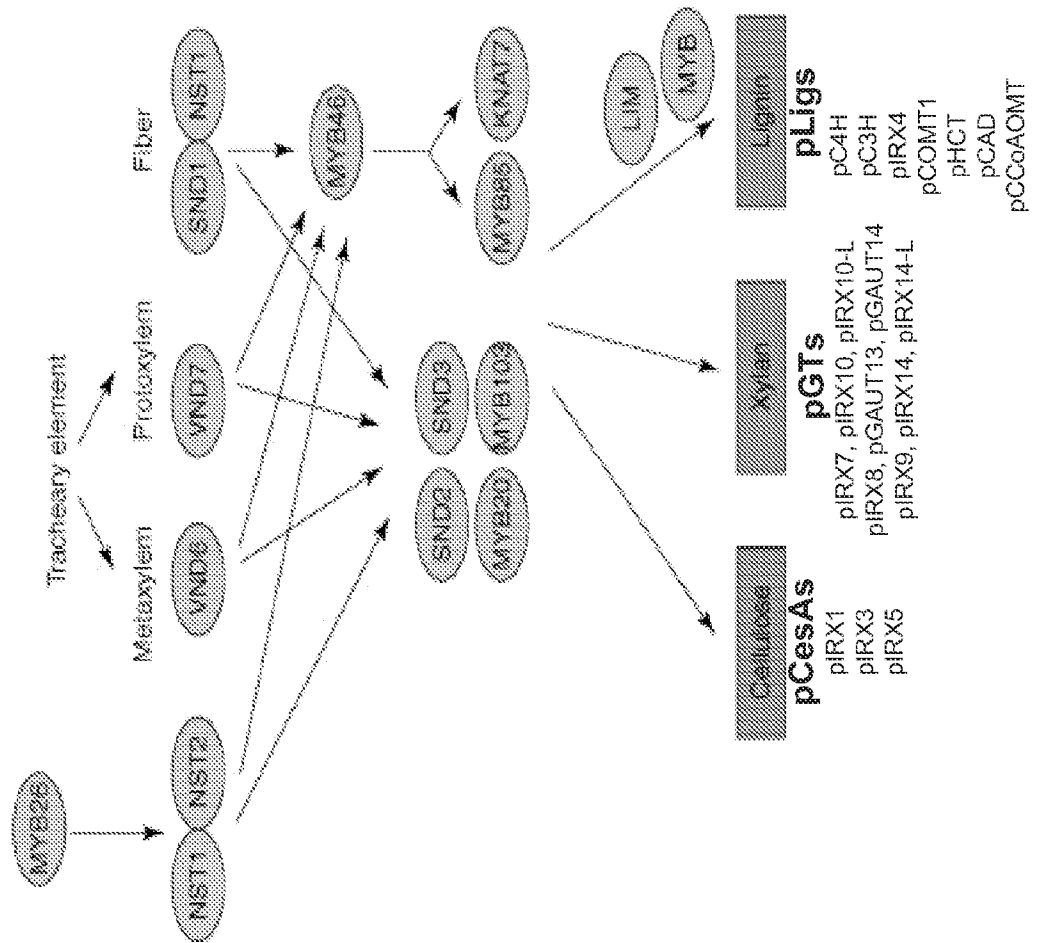


Fig. 15A-B

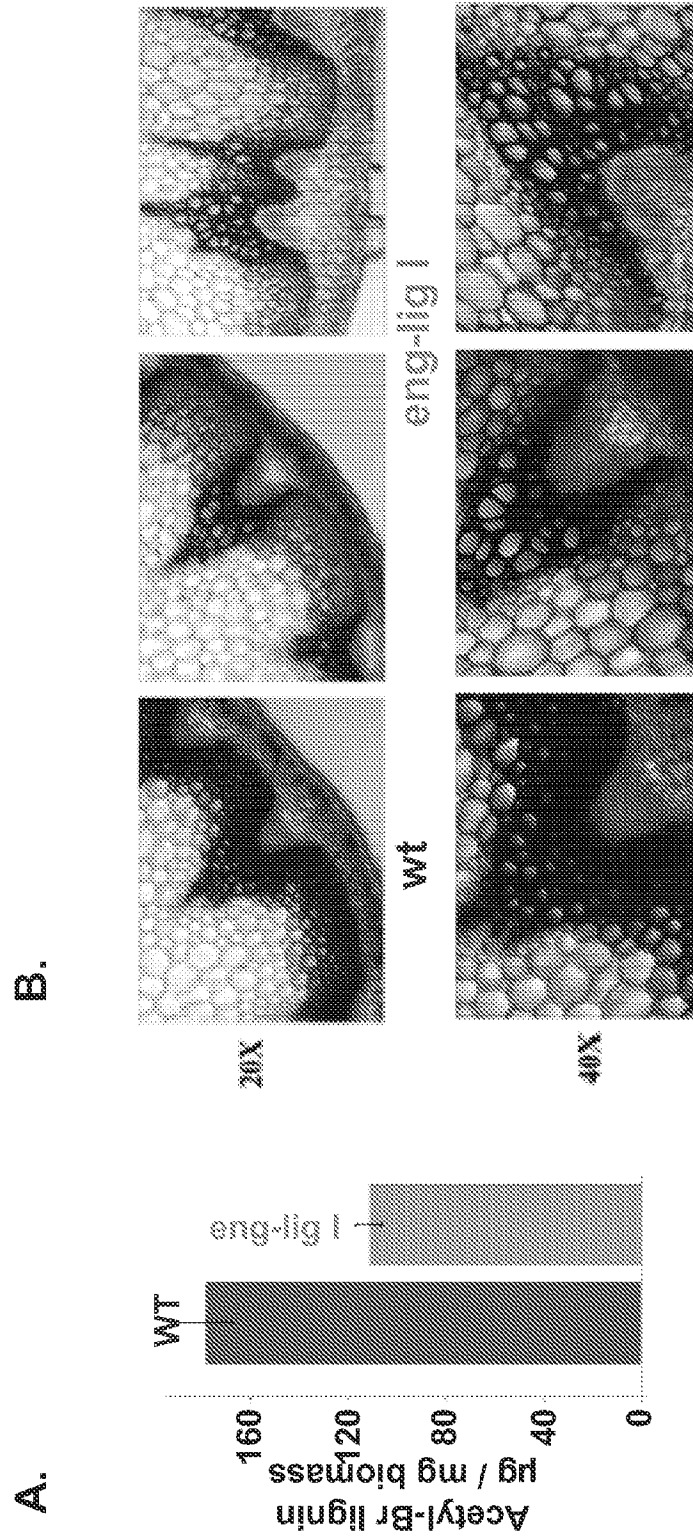
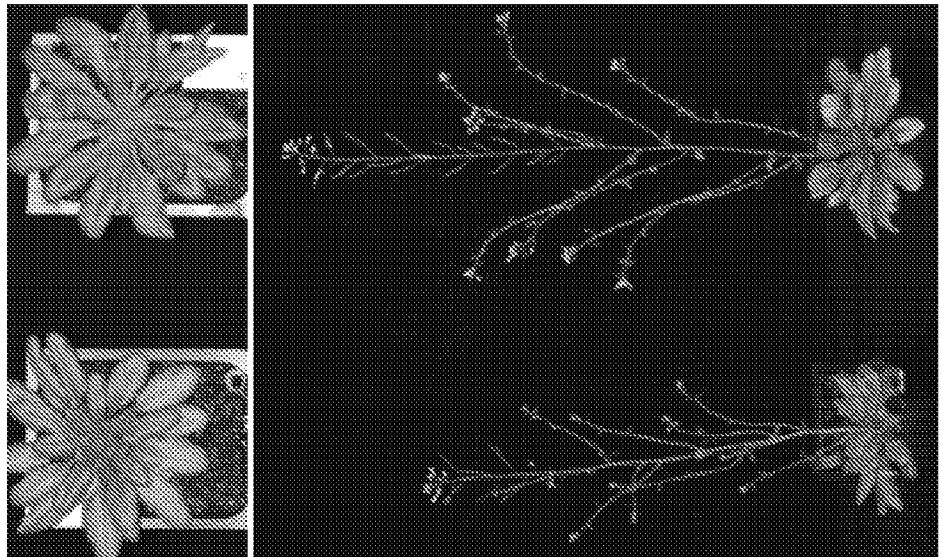


Fig. 16A-B

A.



B.

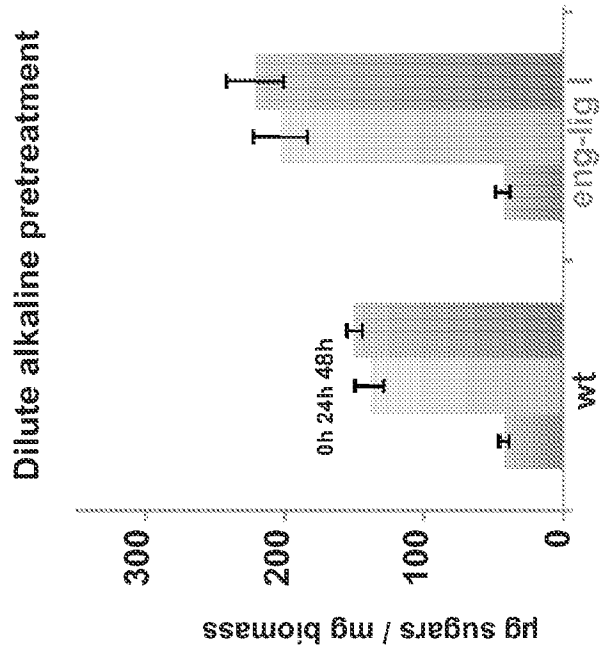


Fig. 16C-D

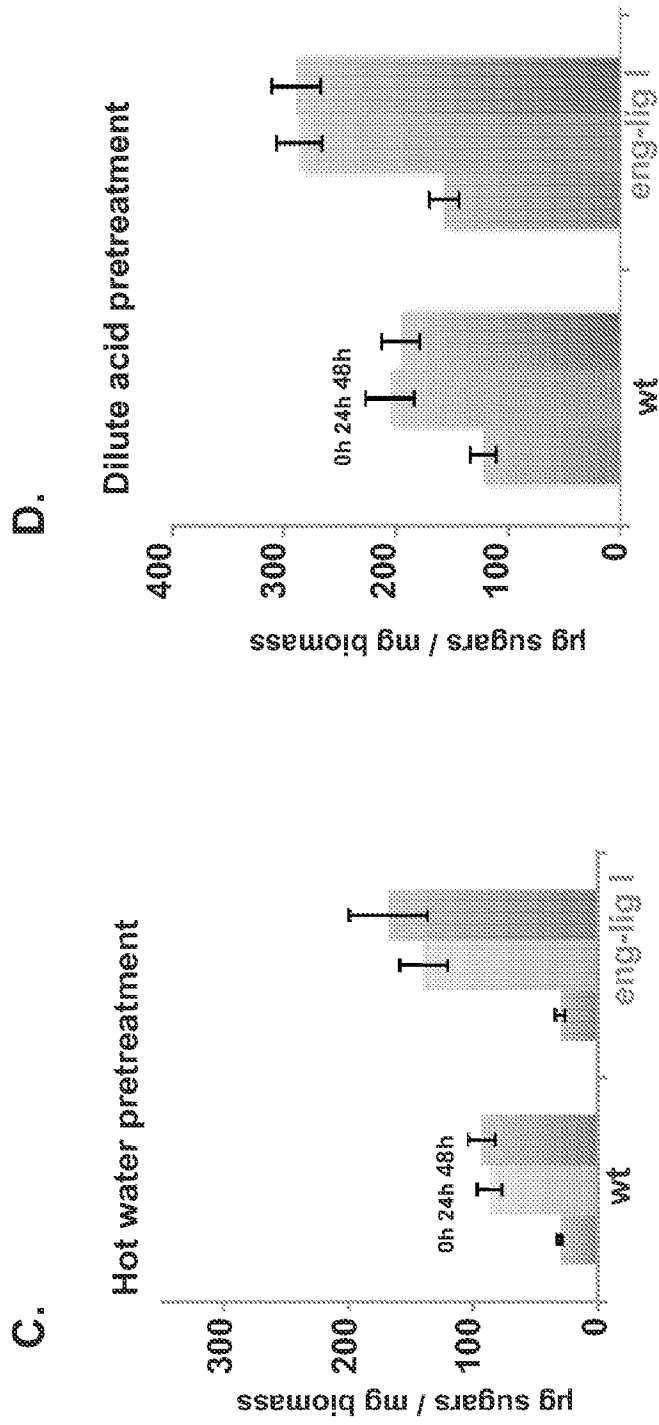


Fig. 17A

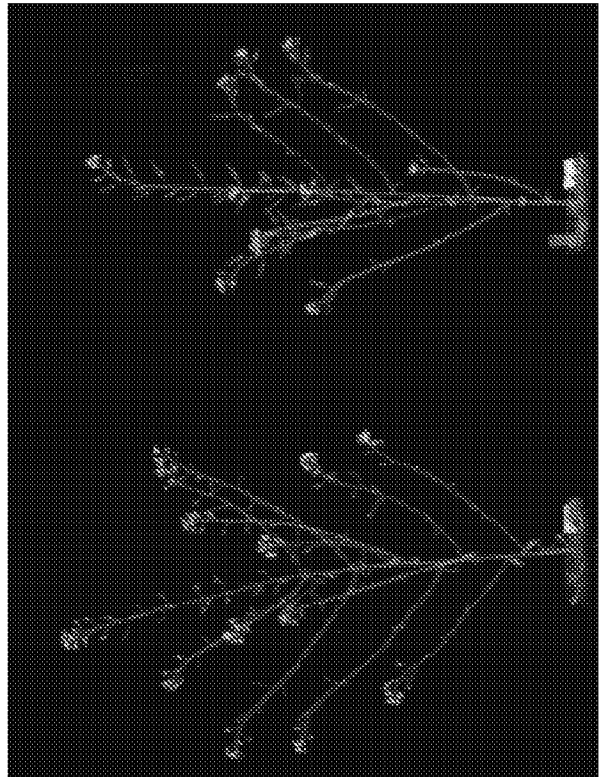
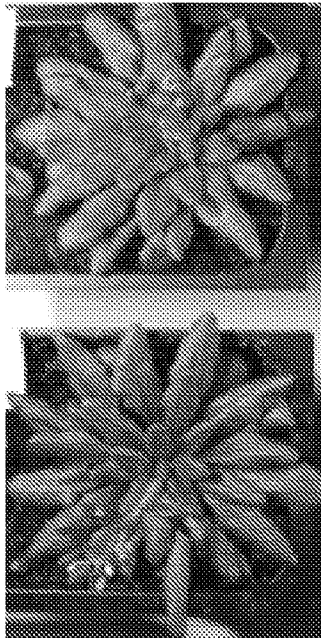


Fig. 17B-C

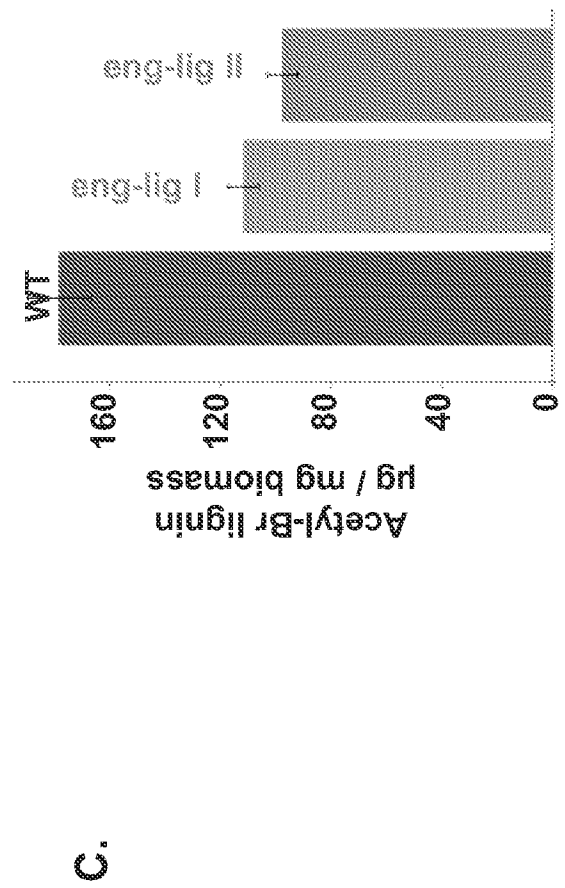
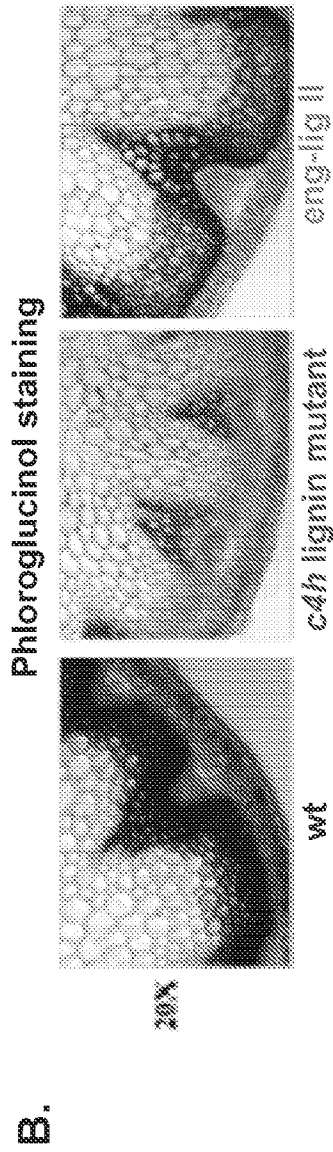


Fig. 18A-D

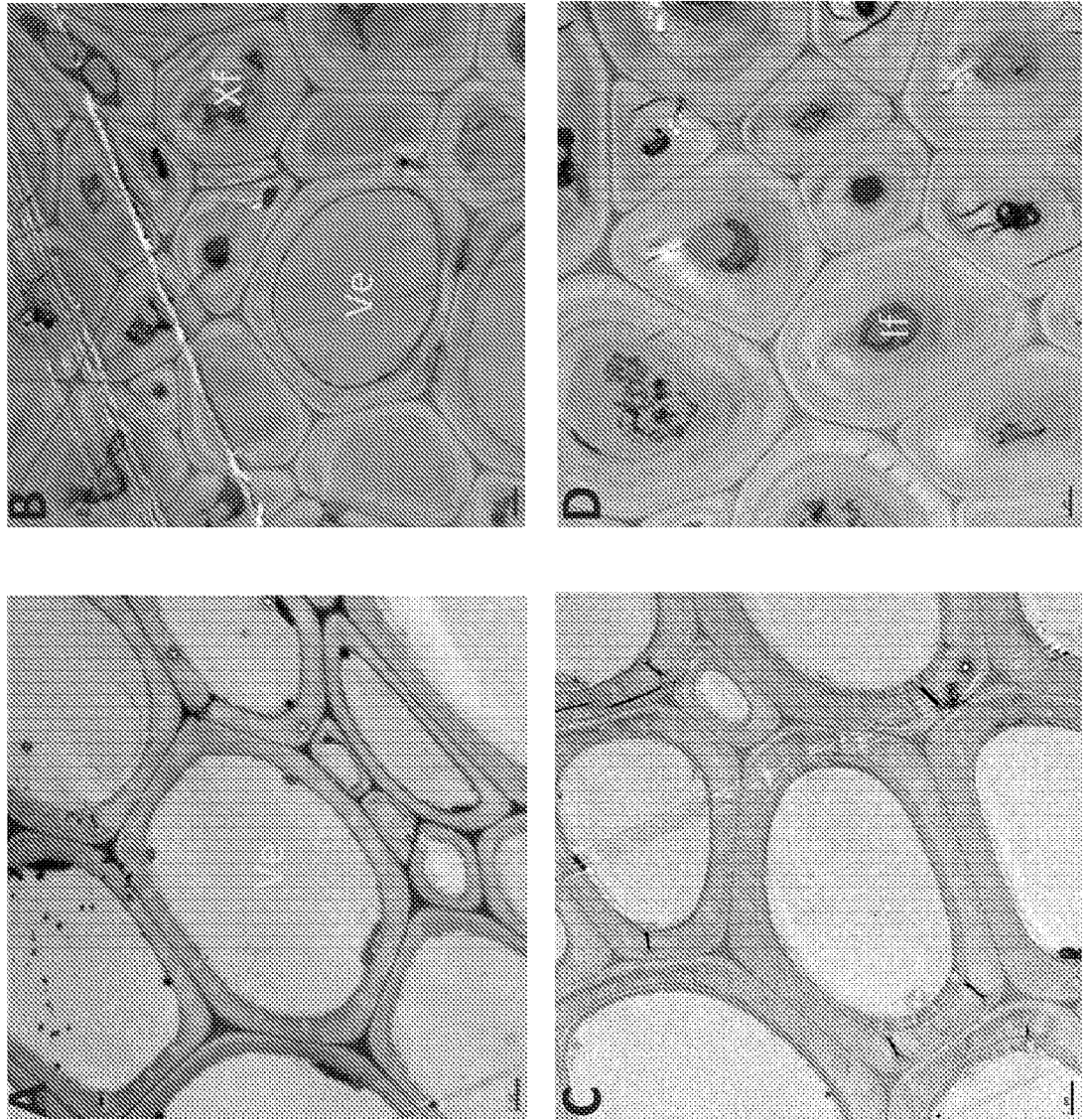


Fig. 19A-B

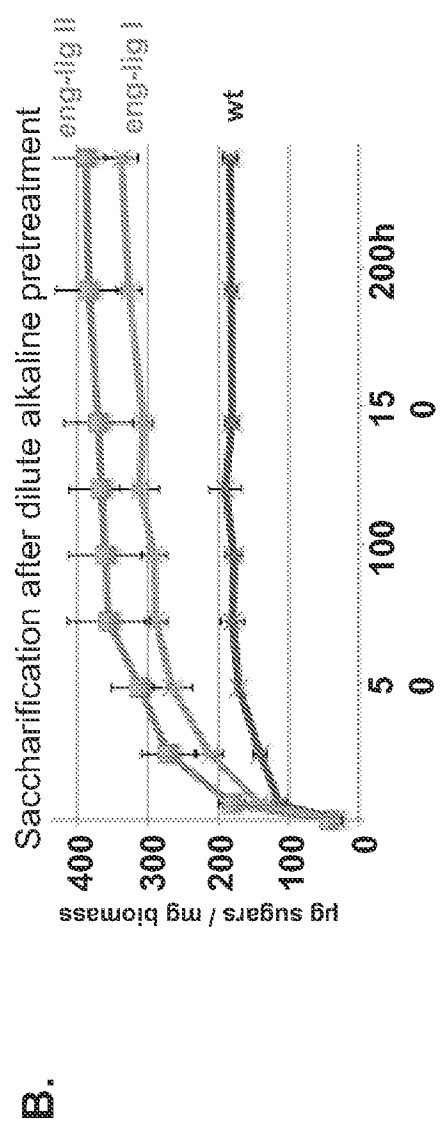
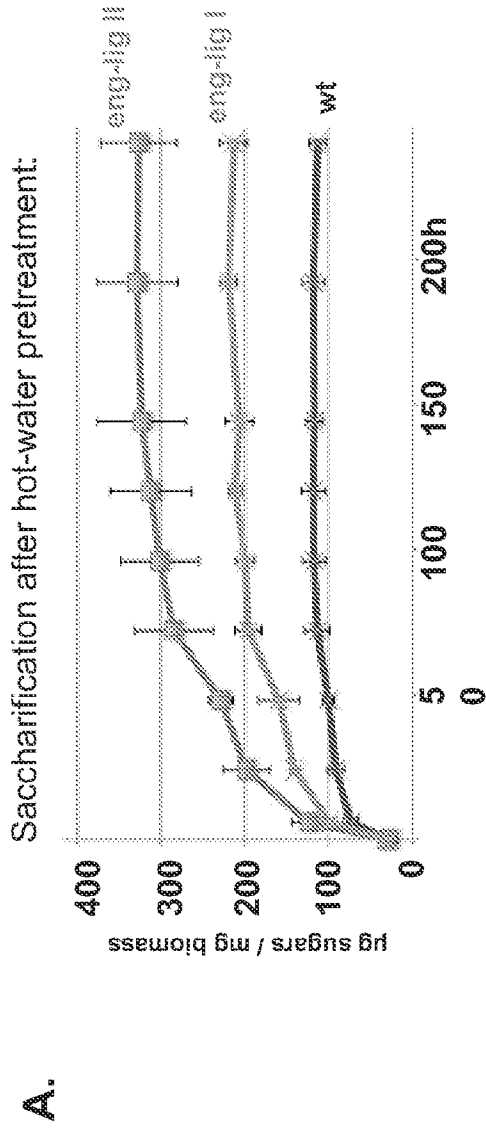


Fig. 20A-B

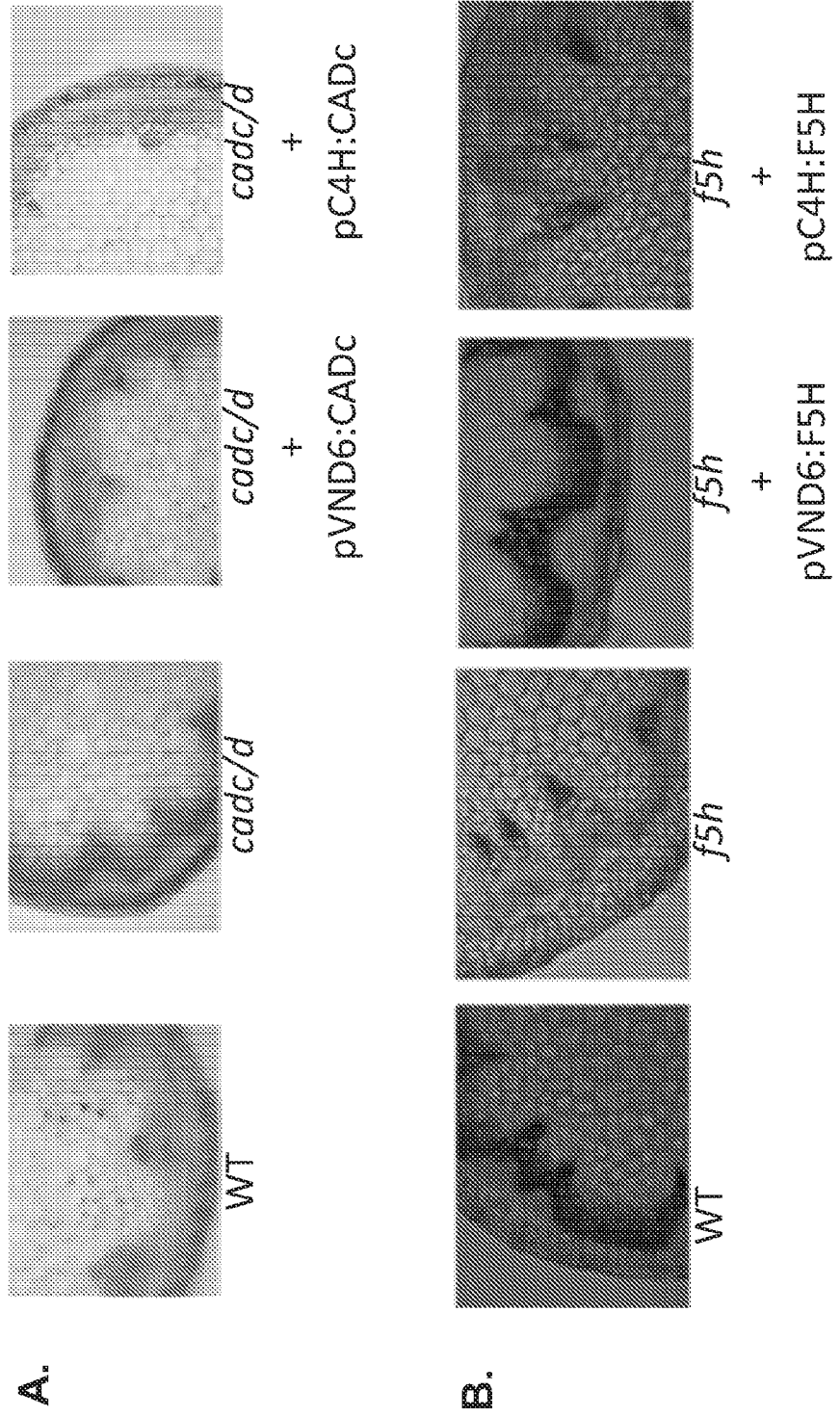


Fig. 21A-C

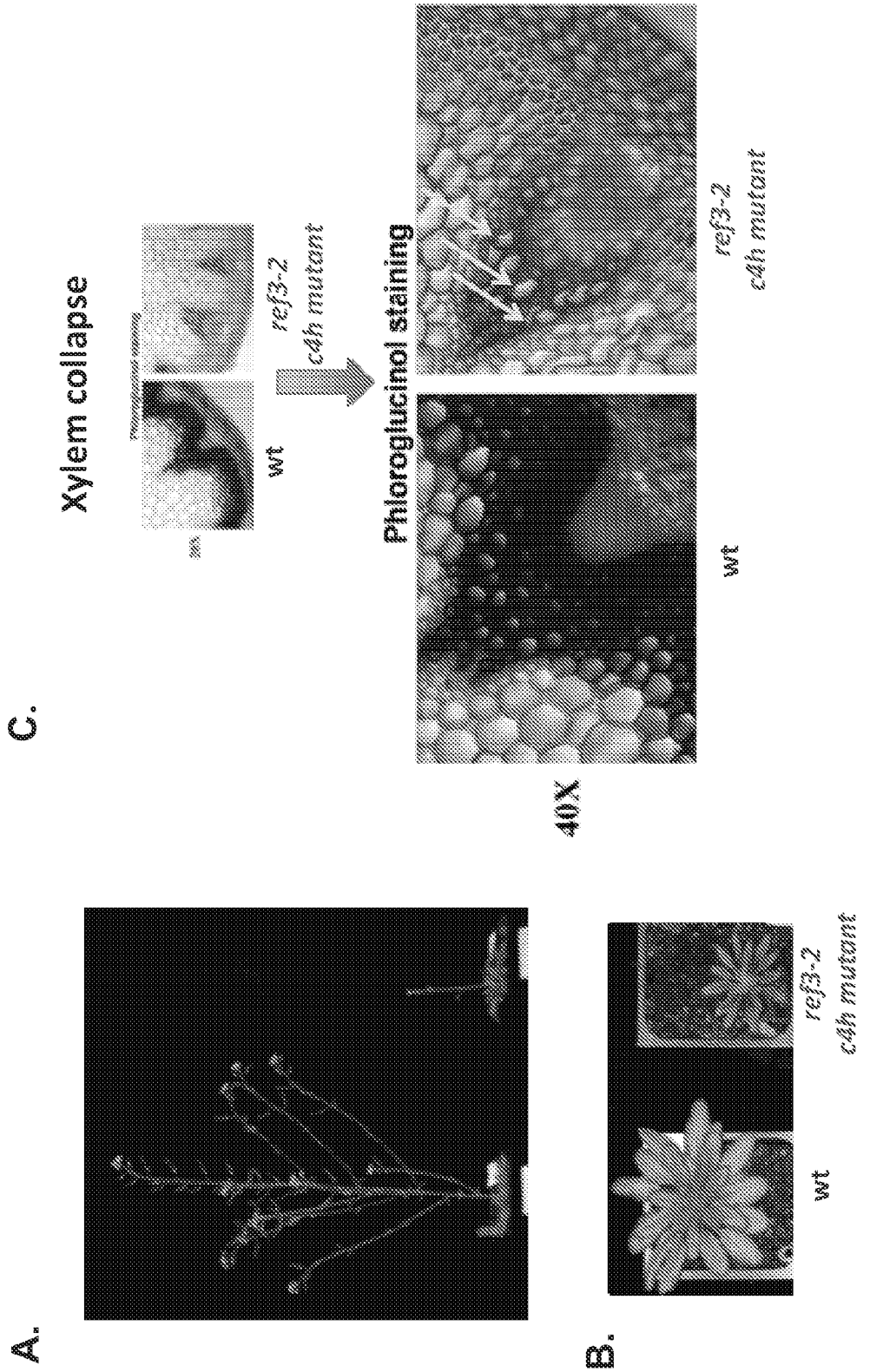


Fig. 22

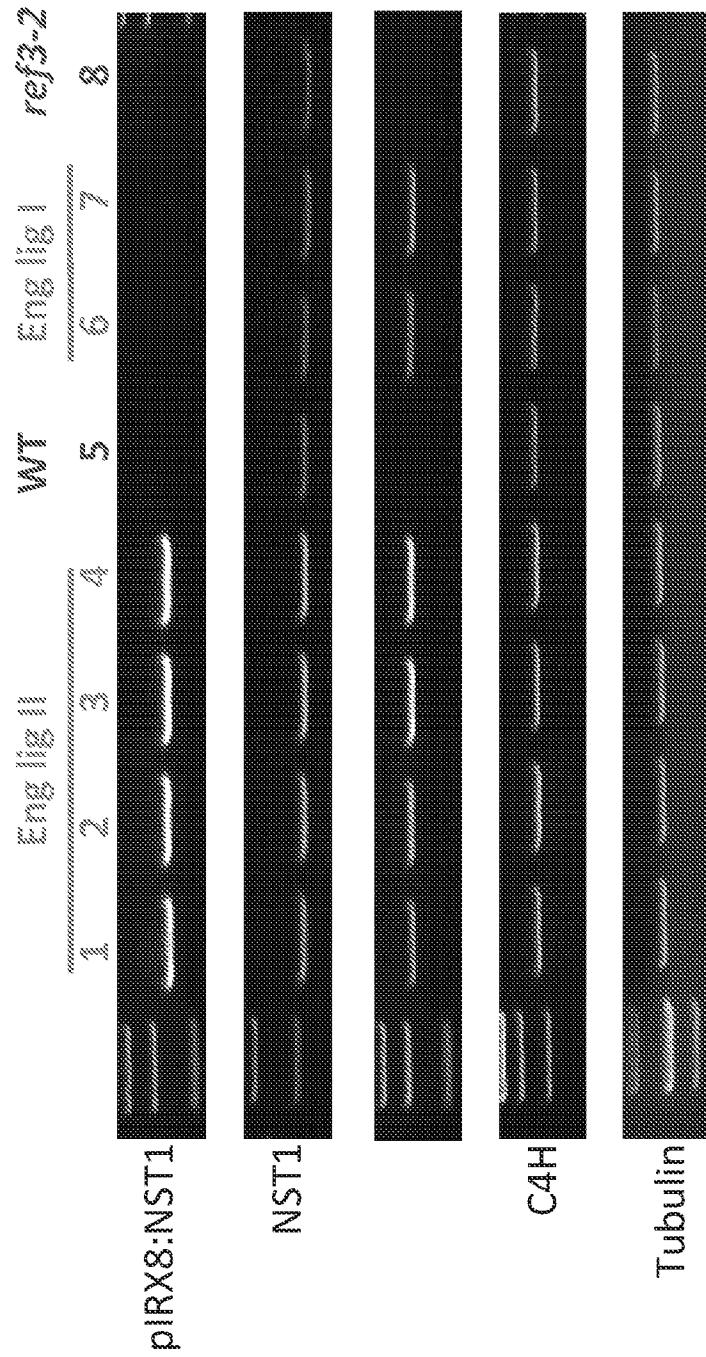


Fig. 23A

WT fiber cell number	cell diameter um	cell wall a um	cell wall b um	cell wall ratio
1	12.72	1.56	1.43	0.24
2	15.94	1.86	2.02	0.24
3	14.93	1.54	1.56	0.21
4	10.92	1.51	1.15	0.24
5	14.44	2.23	2.56	0.33
6	13.84	2.48	2.66	0.37
7	13.87	2.29	2.06	0.31
8	15.73	2.21	2.22	0.28
9	12.40	1.84	2.02	0.31
10	14.48	2.10	1.97	0.28
11	6.67	1.59	1.51	0.46
12	14.32	2.31	2.18	0.31
13	17.95	2.02	2.26	0.24
14	17.99	2.78	2.08	0.27
15	13.45	1.82	1.73	0.26
16	5.46	0.87	1.00	0.34
17	13.81	2.04	2.46	0.33
18	13.69	1.58	1.88	0.25
19	14.66	2.23	2.26	0.31
20	13.44	2.54	2.55	0.38
Average	13.53	1.97	1.98	0.30
SD	3.05	0.45	0.46	0.06

Fig. 23B

<i>ref3-2</i> fiber cell number	cell diameter um	cell wall a um	cell wall b um	cell wall ratio
1	18.618	1.455	1.195	0.14
2	20.152	0.798	0.885	0.08
3	8.446	0.609	0.749	0.16
4	18.273	0.837	1.095	0.11
5	14.586	0.965	1.439	0.16
6	20.771	1.154	1.329	0.12
7	17.48	0.971	0.908	0.11
8	17.302	0.924	1.029	0.11
9	15.033	1.108	0.793	0.13
10	15.666	0.858	0.713	0.10
11	20.286	0.943	0.772	0.08
12	14.743	1.455	9.59	0.75
13	14.779	0.951	1.265	0.15
14	13.775	0.876	0.985	0.14
15	11.573	0.991	1.106	0.18
16	8.891	1.042	0.742	0.20
17	12.123	0.842	0.841	0.14
18	10.792	0.841	0.898	0.16
19	15.869	1.221	2.016	0.20
20	17.271	0.916	1.012	0.11
Average	15.32	0.99	1.47	0.17
SD	3.61	0.21	1.94	0.14

Fig. 23C

eng lig I fiber cell number	cell diameter um	cell wall a um	cell wall b um	cell wall ratio
1	16.782	2.304	1.837	0.25
2	8.778	1.143	1.168	0.26
3	18.989	1.472	1.724	0.17
4	14.428	1.564	1.177	0.19
5	10.773	1.099	1.068	0.20
6	15.385	1.402	1.742	0.20
7	13.22	1.341	1.306	0.20
8	12.012	1.657	1.543	0.27
9	11.785	2.077	1.955	0.34
10	17.73	2.188	2.005	0.24
11	9.623	1.837	1.814	0.38
12	8.368	1.399	1.39	0.33
13	7.623	1.879	1.823	0.49
14	8.335	1.719	1.624	0.40
15	11.459	1.463	1.622	0.27
16	9.711	1.836	1.864	0.38
17	12.242	2.219	2.193	0.36
18	6.267	1.937	1.624	0.57
19	12.517	1.577	1.507	0.25
20	10.306	1.368	1.344	0.26
Average	11.82	1.67	1.62	0.30
SD	3.46	0.35	0.30	0.10

Fig. 23D

eng lig II fiber cell number	cell diameter um	cell wall a um	cell wall b um	cell wall ratio
1	10	3.677	3.63	0.73
2	9.868	3.241	3.749	0.71
3	8.086	2.93	3.513	0.80
4	5.835	2.185	2.085	0.73
5	5.884	2.328	2.609	0.84
6	8.13	3.603	3.424	0.86
7	14.167	5.39	5.024	0.74
8	10.703	3.084	2.971	0.57
9	8.346	2.502	3.069	0.67
10	7.707	3.315	2.117	0.70
11	8.23	3.097	3.226	0.77
12	13.635	4.412	4.459	0.65
13	7.484	2.998	2.923	0.79
14	12.488	5.834	5.863	0.94
15	9.364	2.43	2.727	0.55
16	15.601	4.86	6.058	0.70
17	10.592	3.519	3.48	0.66
18	13.644	3.571	4.525	0.59
19	9.567	3.112	3.737	0.72
20	9.833	3.622	3.673	0.74
Average	9.96	3.49	3.64	0.72
SD	2.73	0.98	1.08	0.10

Fig. 23E

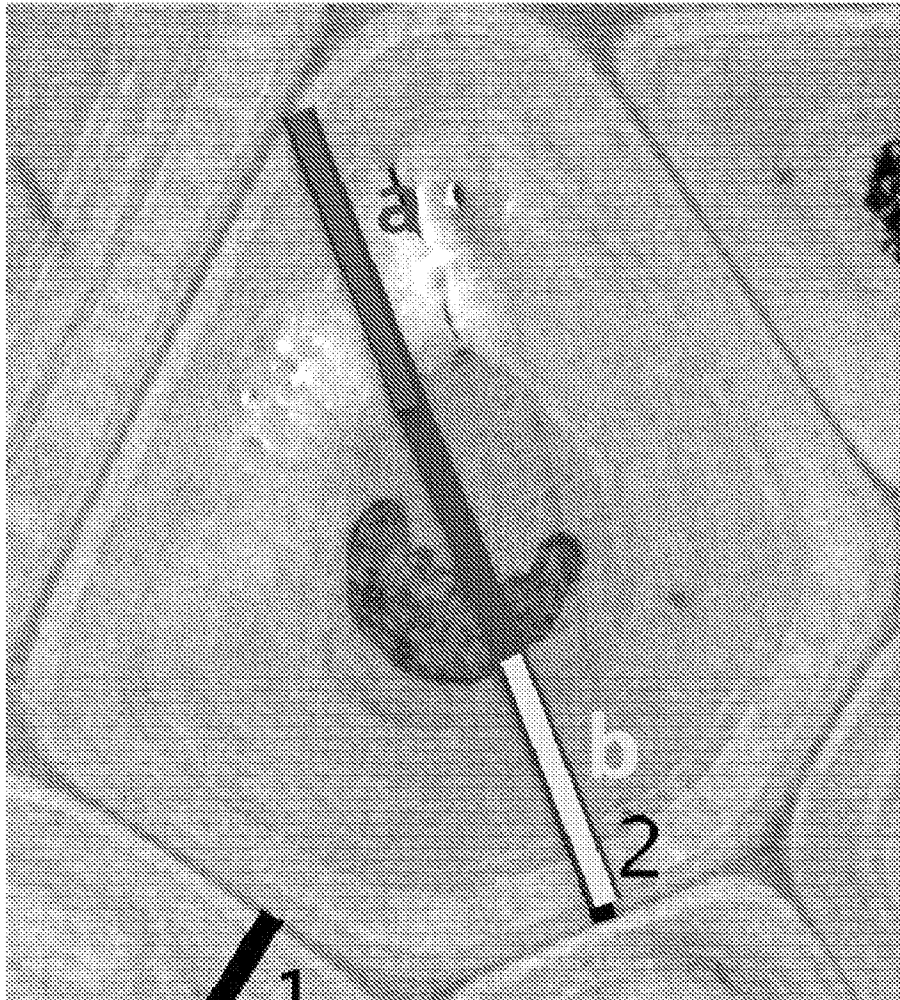
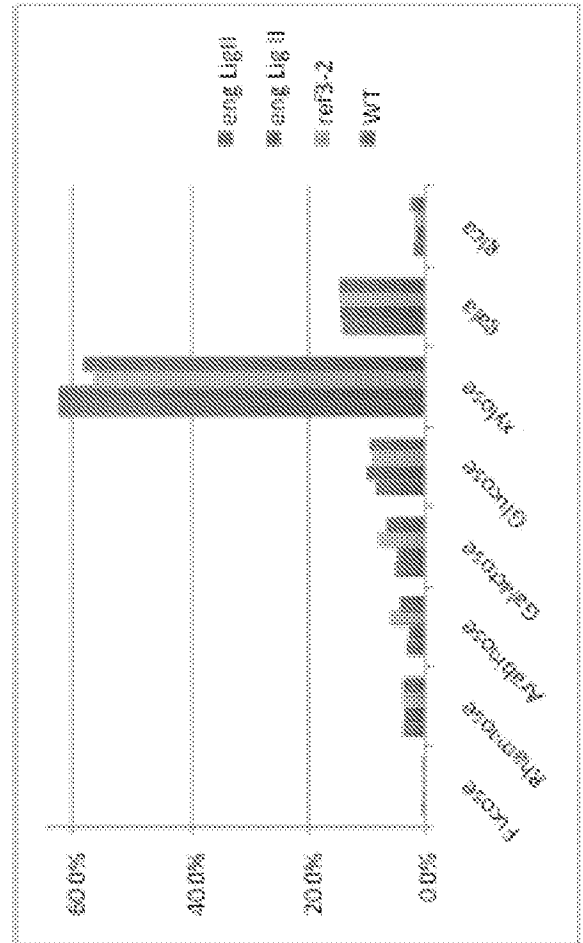


Fig. 24A-B

	eng Lig1	eng Lig 4	ref3-2	WT	eng Lig1	eng Lig 4	SD	SD	SD	SD	SD	SD
aver=EC												
Fucose	0.0014	0.0012	0.0014	0.0011	0.0007	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002	0.0002
Phannose	0.0080	0.0075	0.0083	0.0074	0.0016	0.0010	0.0005	0.0005	0.0005	0.0005	0.0003	0.0003
Arabinose	0.0068	0.0050	0.0131	0.0081	0.0022	0.0008	0.0019	0.0019	0.0019	0.0019	0.0007	0.0007
Galactose	0.0112	0.0104	0.0177	0.0129	0.0031	0.0004	0.0018	0.0018	0.0018	0.0018	0.0007	0.0007
Glucose	0.0187	0.0212	0.0200	0.0186	0.0018	0.0019	0.0019	0.0019	0.0019	0.0019	0.0009	0.0009
xylose	0.1377	0.1360	0.1269	0.1159	0.0072	0.0031	0.0031	0.0031	0.0031	0.0031	0.0041	0.0041
glu	0.0215	0.0318	0.0332	0.0296	0.0039	0.0033	0.0015	0.0015	0.0015	0.0015	0.0028	0.0028
gluc	0.0042	0.0035	0.0040	0.0049	0.0012	0.0002	0.0002	0.0002	0.0002	0.0002	0.0005	0.0005
Total amt.	0.2192	0.2174	0.2248	0.1989	0.0062	0.0039	0.0050	0.0050	0.0050	0.0050	0.0043	0.0043

A.



B.

Fig. 24C

C.

mg/mg	eng LigI	eng LigII	ref3-2	WT
Rhamnose	0.007	0.009	0.011	0.011
Arabinose	0.006	0.009	0.014	0.009
Galactose	0.011	0.014	0.022	0.017
Glucose	0.305	0.330	0.318	0.341
xylose	0.196	0.216	0.173	0.166

Fig. 26

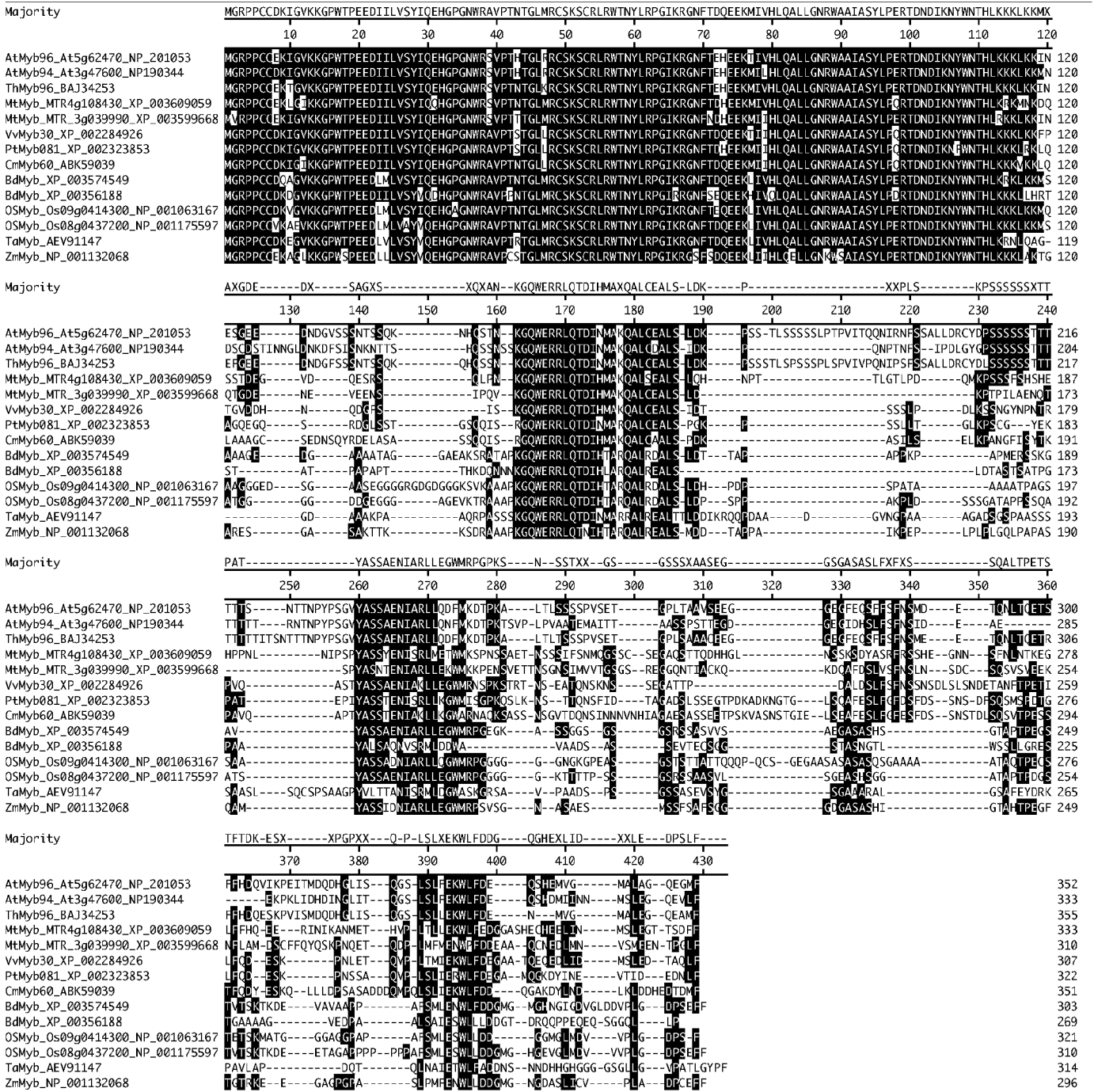
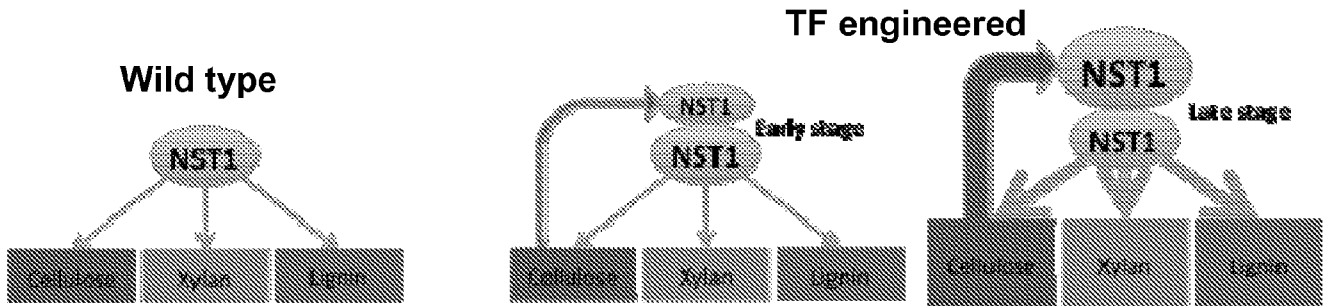


Fig. 27

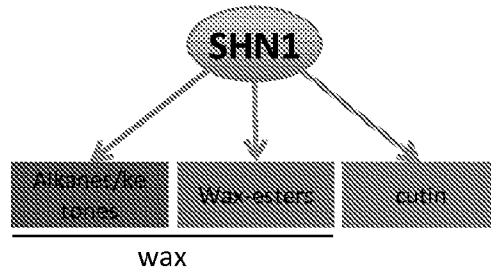
Representaion of the cell wall positive feed back loop



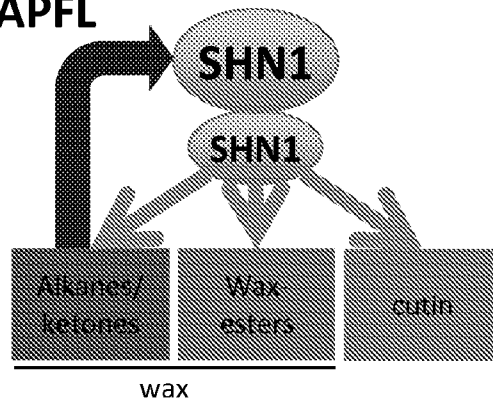
Cell wall densification strategy is based on the creation of an artificial positive feedback loop to enhance the expression of fiber specific transcription factor. It is created by the expression of a new copy of a fiber specific transcription factor (eg. NST1) under the control of a downstream induced promoter from xylan or cellulose biosynthesis. Furthermore, this approach is designed to be compatible with the xylan and lignin engineering strategies.

Fig. 28

A native system



B native system + wax-APFL

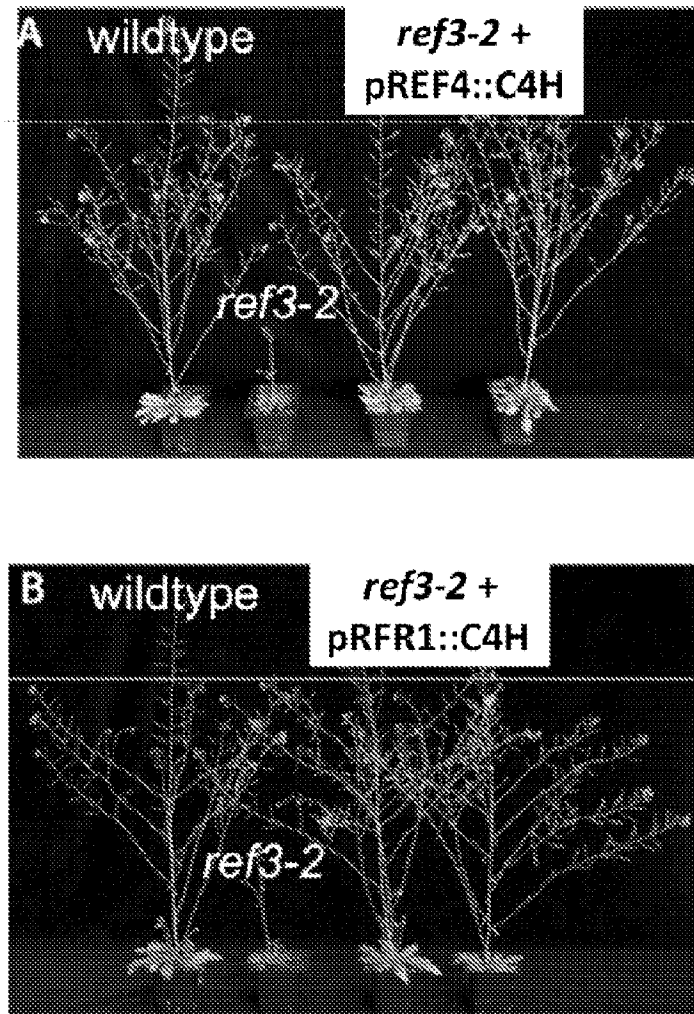


Illustrates an artificial positive feed back loop for wax deposition.

(A) Represent the native regulation of wax and cutin biosynthesis in wildtype plants which is under the control of the master transcription factor SHN1.

(B) Represent the wax-APFL which is used to enhance the biosynthesis of wax and cutin components in wildtype plants. The wax-APFL has been created by using an induce-SHN1 promoter to express a new copy of SHN1 transcription factor which allows to enhance the amount of SHN1 transcription factor when the native SHN1 is expressed and increase the biosynthesis of waxes and cutin components.

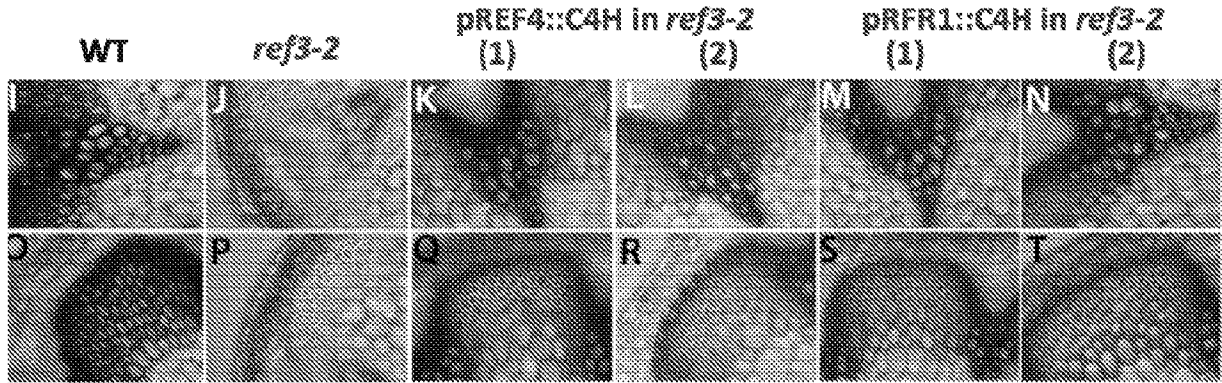
Fig. 29



Plant growth phenotype of the engineered cell wall plant lines

Growth comparison of wildtype, *ref3-2* (*c4h* mutant) and the engineered plant lines: *ref3-2* mutant complemented with either pREF4::C4H (A) or pRFR1::C4H (B) dna construct.

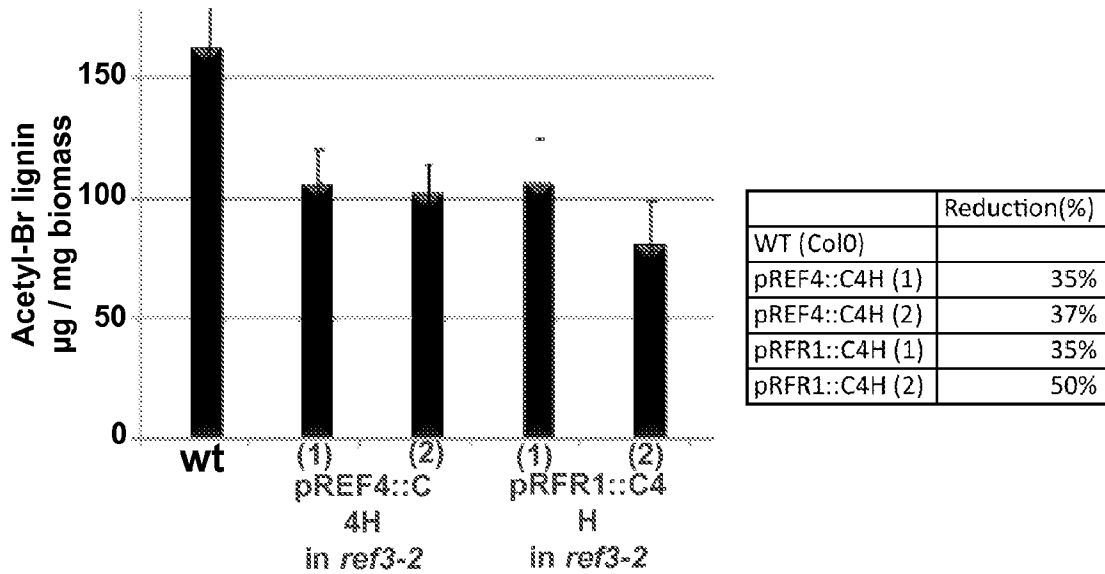
Fig. 30 **Lignin distribution**



Lignin distribution analysis of the engineered cell wall plant lines

Bright light images of stem cross sections stained with phloroglucinol of same age wildtype (wt), *ref3-2* mutant (*c4h* mutant) and the engineered plant lines: *ref3-2* mutant complemented with either pREF4::C4H or pRFR1::C4H dna construct. Middle panels (I-N): staining of xylem bottom panels (O-T): staining of interfascicular fibers.

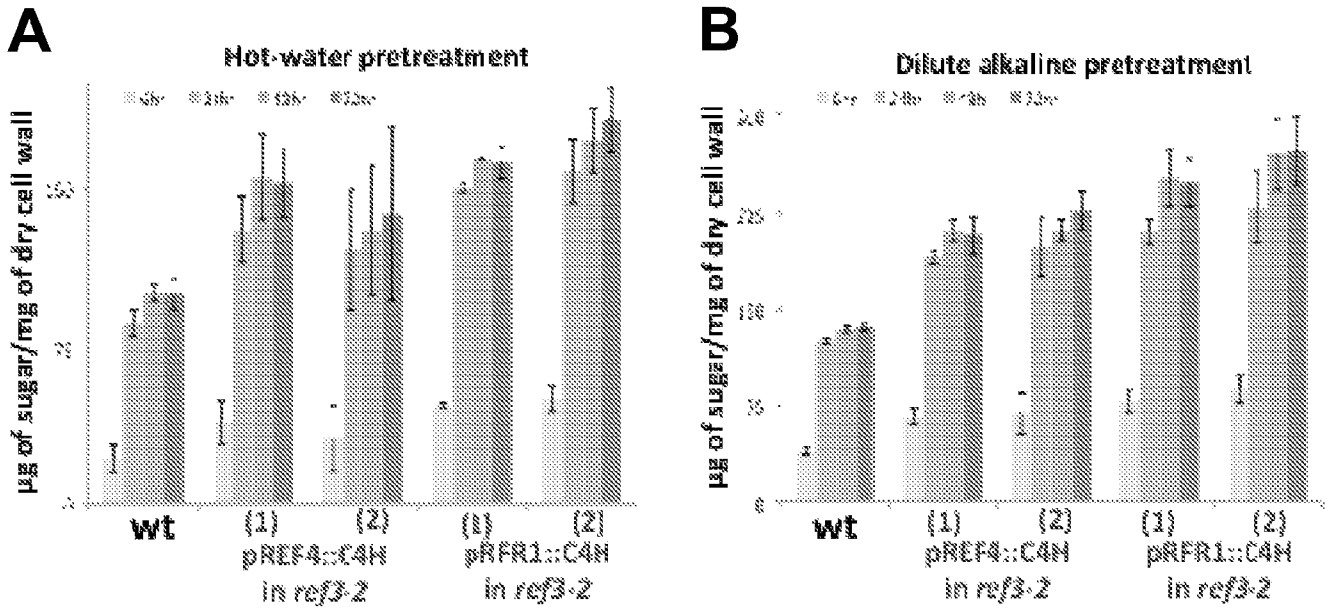
Lignin content



Lignin content analysis of the engineered cell wall plant lines

Lignin quantification using acetyl bromide method of senescence stems from wildtype (WT) and the engineered plant lines: *ref3-2* mutant complemented with either pREF4::C4H or pRFR1::C4H dna construct.

Fig. 31 **Saccharification**



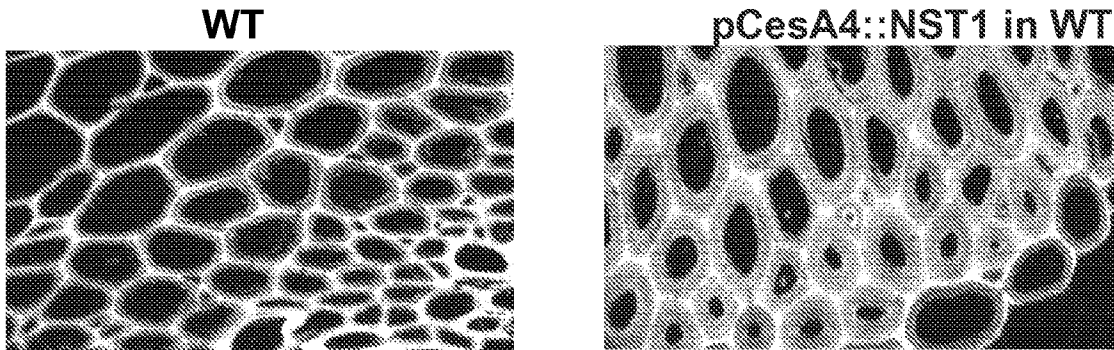
C

	Hot Water	Dilute Alkaline
WT (Col0)	Improvement after 72h (%)	
pREF4::C4H (1)	53%	51%
pREF4::C4H (2)	38%	65%
pRFR1::C4H (1)	62%	81%
pRFR1::C4H (2)	82%	98%

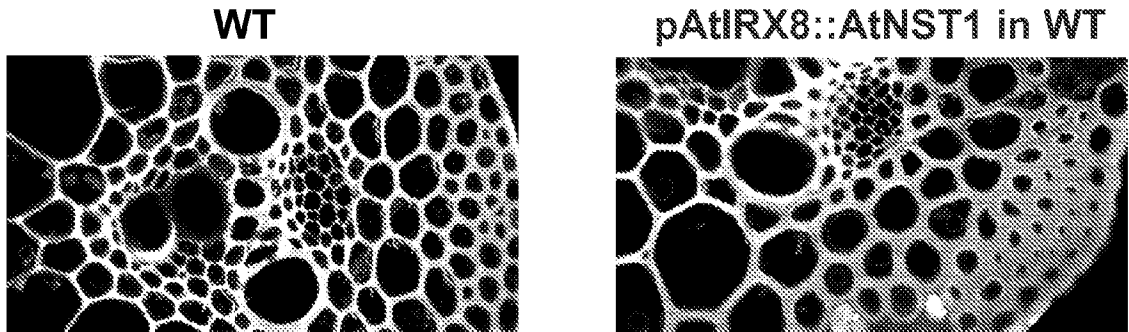
Saccharification efficiency of the lignin engineer lines

Sugar released from dry stems hot-water (A) or alkali (B) pretreated with followed by an incubation with a cellulase cocktail for 0 to 72h. Stem are from Wildtype (wt) plants and several complemented *ref3-2* lines with pREF4::C4H or pRFR1::C4H DNA construct.

(C) Summary of saccharification improvement after hot-water and alkali pretreated of dry stems from the lignin engineered lines (*ref3-2* mutant complemented with either pREF4::C4H or pRFR1::C4H)

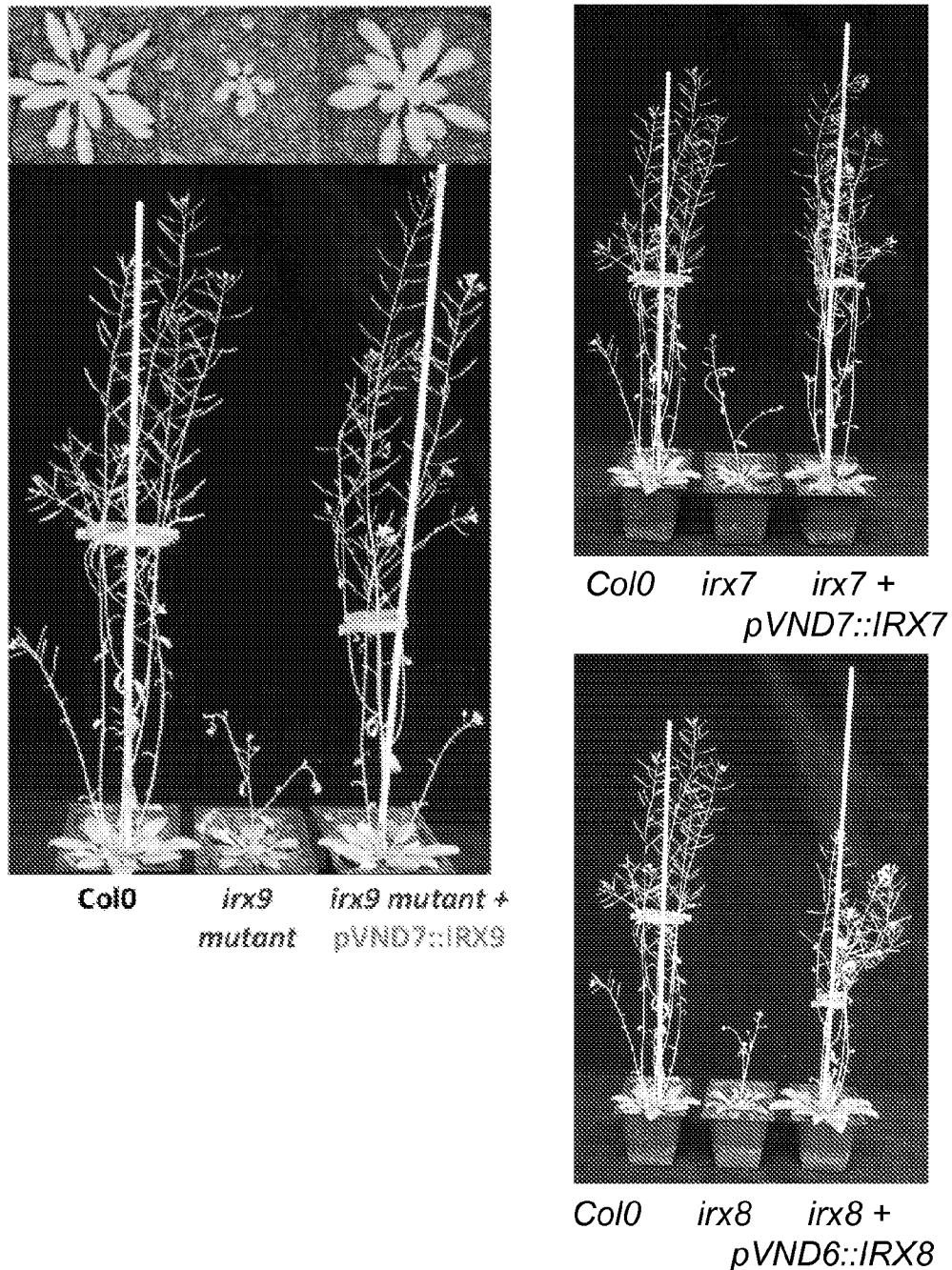
Fig. 32A. Density loop *pCesA4::NST1* in wild type Arabidopsis (dicot)

Cell wall densification strategy in Arabidopsis wild type plants (dicotyledon)
UV images of stem cross sections from wildtype and wildtype containing the *pCesA4::NST1* DNA construct. The creation of a positive feedback loop with the secondary cell wall cellulose promoter (*pCesA4*) and the secondary cell wall transcription factor (*NST1*) enhances secondary cell wall deposition in fiber cells.

Fig. 32B. Density loop *pAtIRX8::AtNST1* in wild type Brachypodium (monocot) using Arabidopsis promoter (*pAtIRX8*) and transcription factor (*AtNST1*)

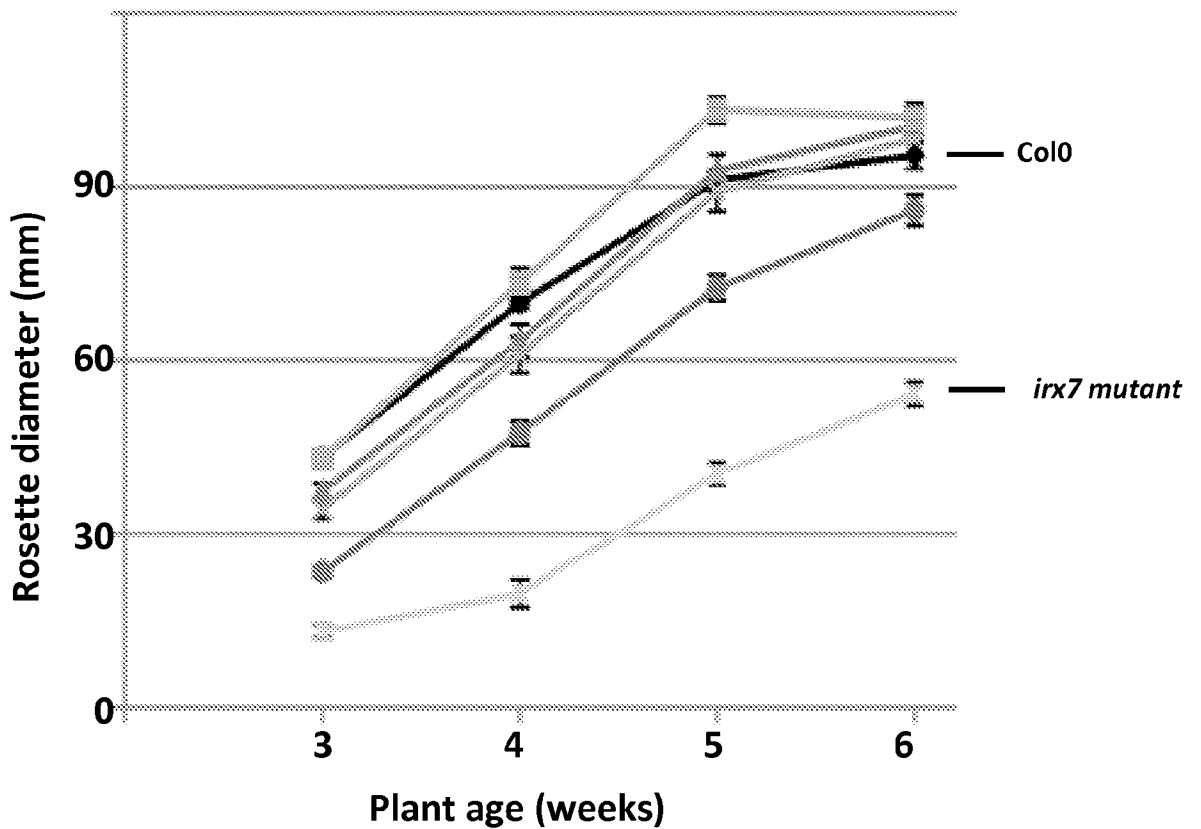
Cell wall densification strategy in Brachypodium wild type plants (monocotyledon)
UV images of stem cross sections from wildtype and wildtype containing the *pAtIRX8::AtNST1* DNA construct. The creation of a positive feedback loop with the secondary cell wall cellulose promoter (*pAtIRX8*) and the secondary cell wall transcription factor (*AtNST1*), both from Arabidopsis, enhances secondary cell wall deposition in fiber cells in Brachypodium.

Fig. 33



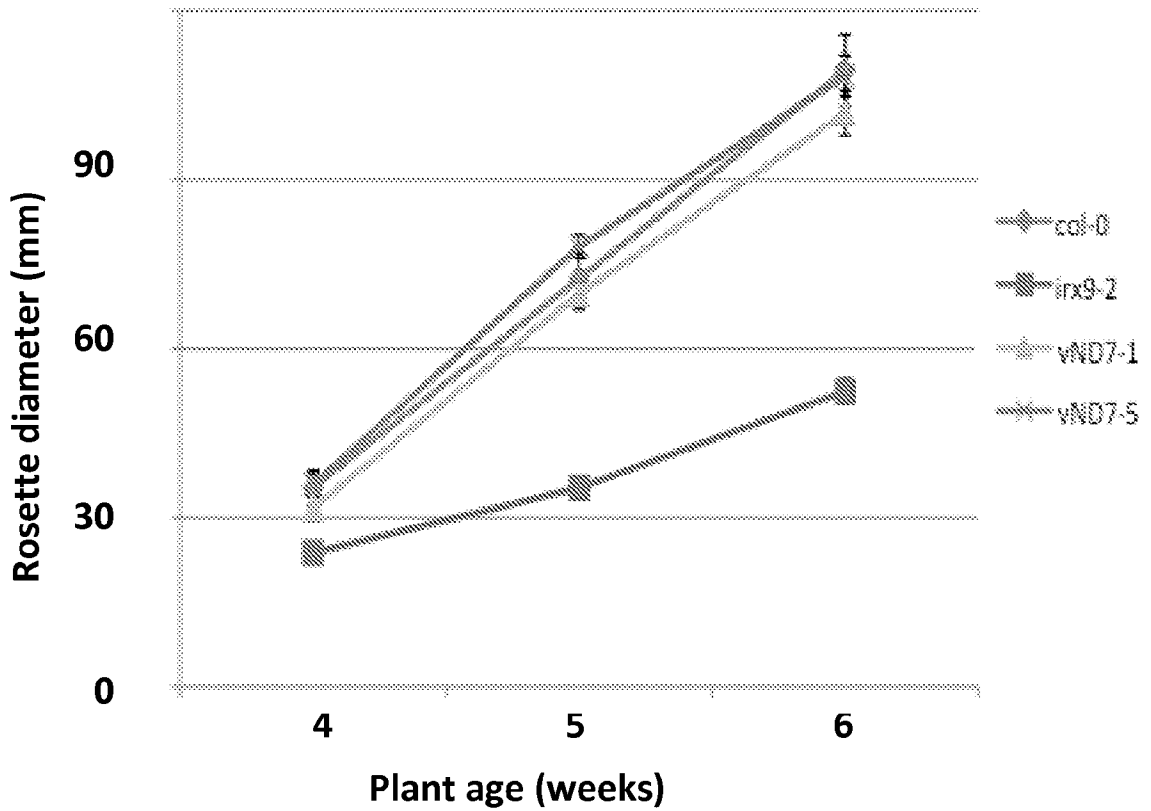
Examples of xylan engineering. Mutants in the *IRX7*, *IRX8* or *IRX9* genes exhibit strong growth reduction. Transformation of the mutants with constructs where the wild type version of the mutated gene is driven by *pVND6* or *pVND7* promoter restores the growth. Similar results were obtained with *pVND6::IRX9* and *pVND7::IRX7*.

Fig. 34



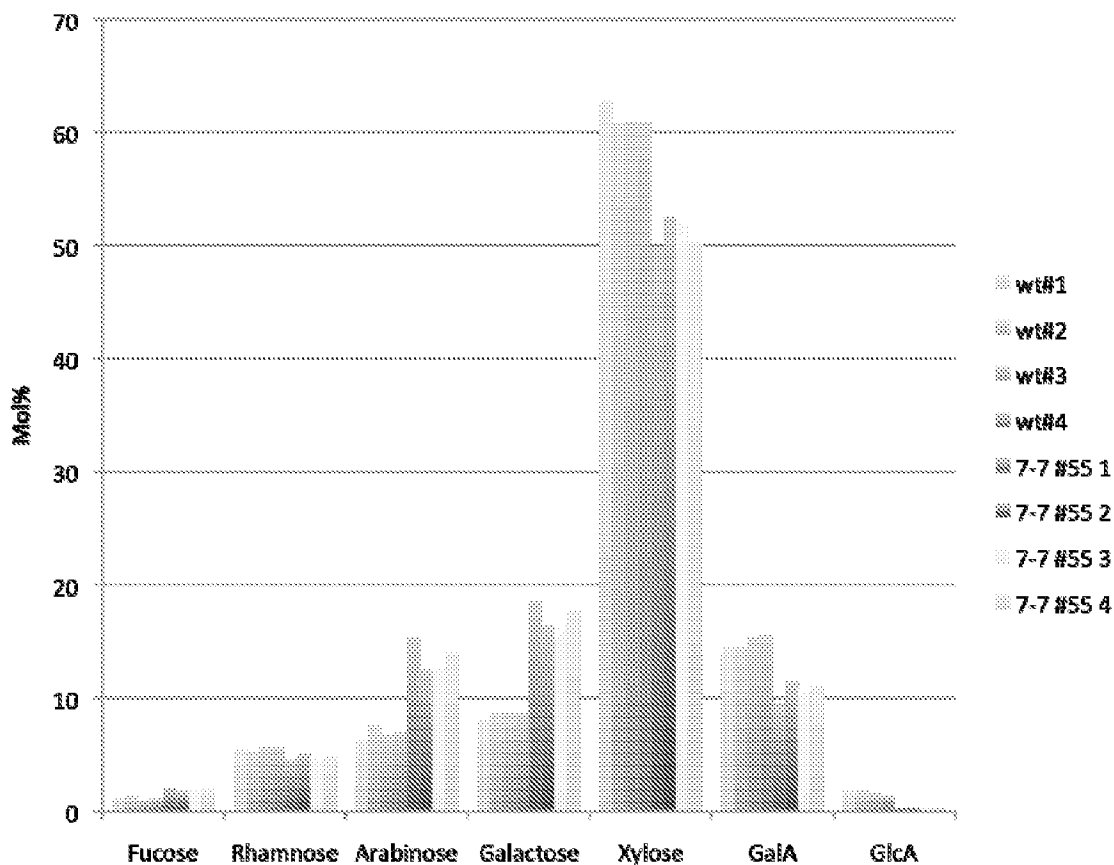
Growth of offspring of four individual transformants made by transforming *irx7* mutant with the *pVND7::IRX7* construct was quantified by measuring rosette diameter. Two of the plant lines grow identically to wild type (Col0), while one plant line grows slightly better and one plant line is only partially restored.

Fig. 35



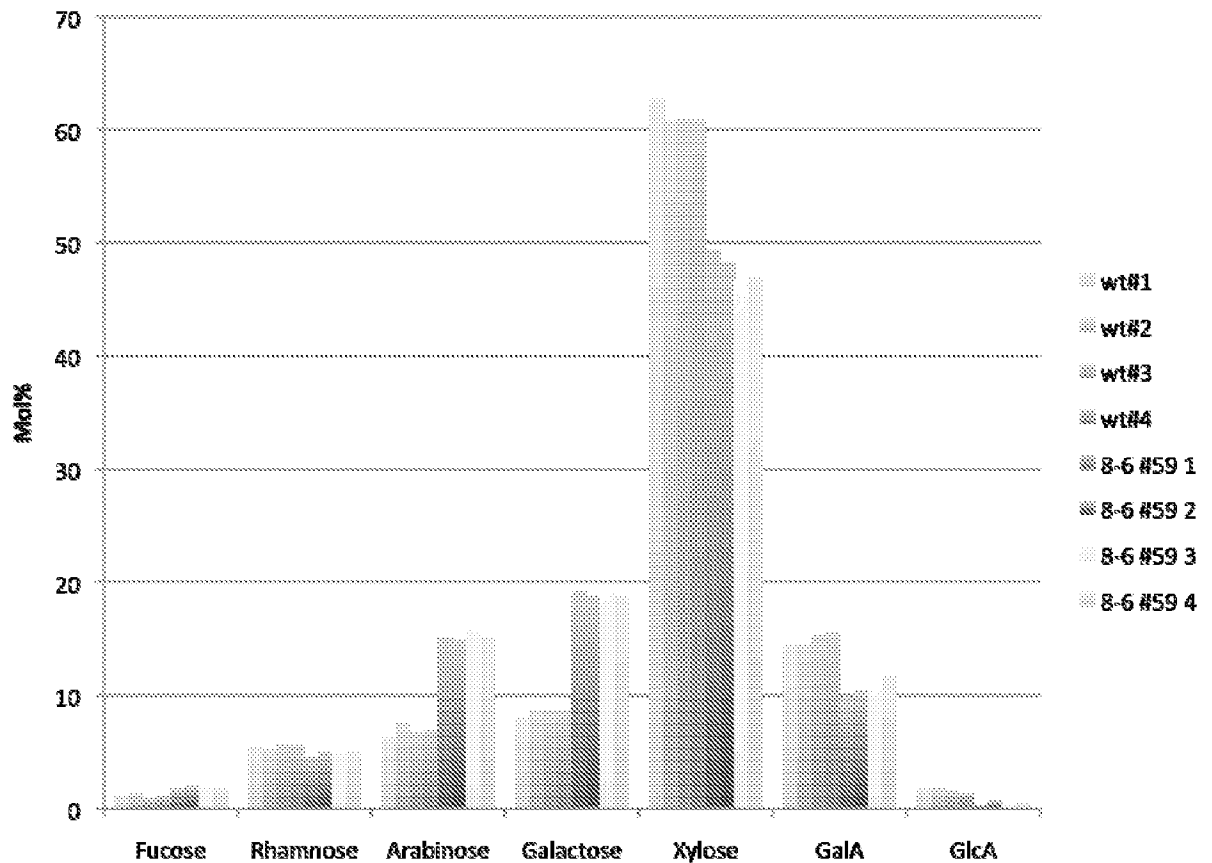
Growth of offspring of two individual transformants made by transforming *irx9* mutant with the *pVND7::IRX9* construct was quantified by measuring rosette diameter. The transformed plant lines grow identically to wild type (Col0). Similar results were obtained with plants transformed with *pVND6::IRX9*.

Fig. 36



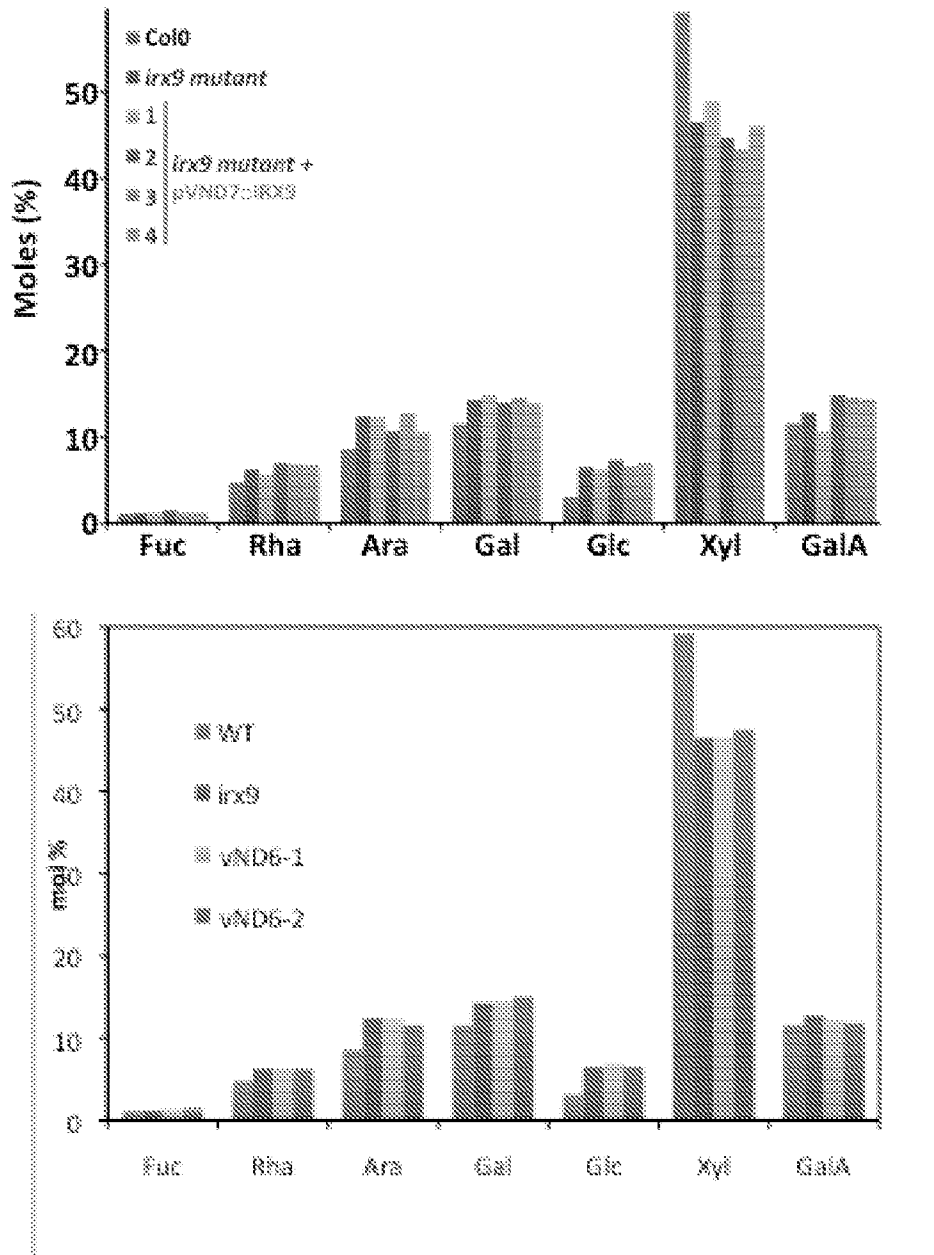
Non-cellulosic monosaccharide composition of cell walls prepared from four individual transformants made by transforming *irx7* mutant with the *pVND7::IRX7* construct. All the transformants still exhibit the low xylan content of the original *irx7* mutant in spite of the restored growth.

Fig. 37



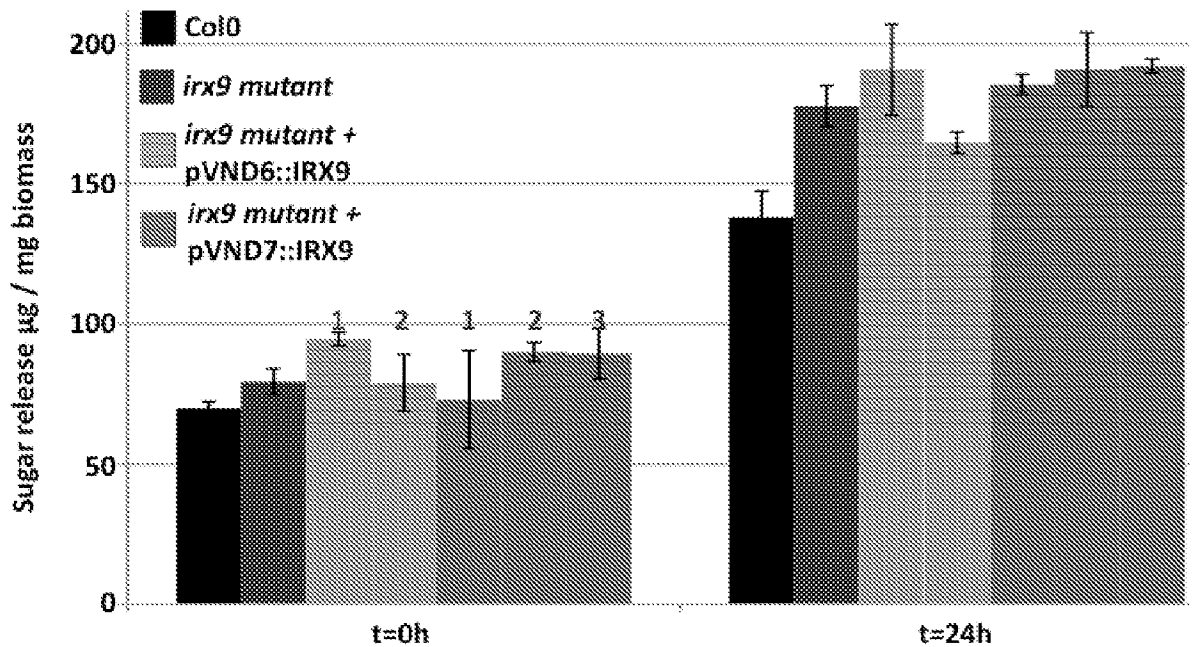
Non-cellulosic monosaccharide composition of cell walls prepared from offspring of four individual transformants made by transforming *irx8* mutant with the *pVND6::IRX8* construct. All the transformants still exhibit the low xylan content of the original *irx8* mutant in spite of the restored growth.

Fig. 38



Non-cellulosic monosaccharide composition of stem cell walls prepared from offspring of four individual transformants made by transforming *irx9* mutant with the *pVND7::IRX9* construct and two individual transformants with the *pVND6::IRX9* construct. All the transformants still exhibit the low xylan content of the original *irx9* mutant in spite of the restored growth.

Fig. 39



Saccharification analysis of cell walls prepared from offspring of two individual transformants made by transforming *irx9* mutant with the *pVND6::IRX9* construct and three individual transformants made by transforming *irx9* mutant with the *pVND7::IRX9* construct. All the transformants exhibit improved saccharification similar to the original *irx9* mutant in spite of the restored growth.

Fig. 40

