The present invention relates to transgenic plants with vascular xylem tissue- targeting overexpression of tissue factors involved in vascular xylem cell development.
This application claims the priority benefit of U.S. Provisional Patent Application Serial No. 62/623,279, filed January 29, 2018, which is hereby incorporated by reference in its entirety.

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FIELD OF THE INVENTION

The present invention relates to genetic constructs and transgenic plants with vascular xylem tissue-targeting overexpression of transcription factors (TFs) involved in vascular xylem cell development, as well as their methods of use for enhancing plant growth and yield.

BACKGROUND OF THE INVENTION

Yield is commonly defined as the measurable economic value of agricultural product from a crop. This may be defined in terms of quantity or quality, or a combination of both. Yield is directly dependent on several factors, for example, the number and size of the organs, plant architecture (i.e. number of tillers or branches), seed production, nutrient content, assimilation of metabolic precursors, root development, nutrient uptake, stress tolerance, and early vigor. Optimizing the above-mentioned factors may, therefore, contribute to increasing crop and horticultural yield. Depending on the end use, the modification of certain yield traits may be favored over others. For example, for applications such as forage or wood production, or as a biofuel resource, an increase in the vegetative parts of a plant may be desirable, and for applications such as flour, starch, or oil production, an increase in seed parameters may be particularly desirable. Such plant growth and/or yield-related traits may be improved by enhancing vascular tissue meristematic activity.

In higher plants, vascular tissues are important for transporting water and nutrients throughout the plant and providing physical support for upright growth. Primary constituents of the vascular tissues, xylem and phloem, are derived from the meristematic
vascular procambium and cambium (Esau, Plant Anatomy (1965); Esau, Anatomy of Seed Plants (1977)). Xylem cells are particularly important for developing secondary cell walls that form the largest part of plant lignocellulosic biomass that consists of cellulose, hemicellulose, and lignin. Histochemical studies have indicated that lignification of the secondary cell wall generally occurs after the initial deposition of the cellulosic and hemicellulosic components and that it is initiated in a spatially distinct manner, beginning with the lignification of the middle lamella. Comparative studies of the patterns of secondary cell wall deposition in xylem cells in many different vascular plants have shown that the patterning of secondary cell wall deposition in these cells is a highly conserved process across species (Esau, Plant Anatomy (1965); Meylan and Butterfield, Three-Dimensional Structure of Wood (1972)).

Os09g0l 14500 (OsKIN4A), BC14: 0s02g06l4l00 (OsNSTl), and BC15: 0s09g0494200 (OsCTLl), which mainly consist of gene members coding endomembrane enzyme/protein for the cellulose and hemicellulose deposition and/or lignification. Integrated analysis of the series of mutants and co-expression gene datasets, in particular, revealed that distinct subgroups of CesA genes and proteins involved in cellulose biosynthesis in secondary cell walls are also conserved across plant species. For example, the products of three gene sets, such as the AtCesA4 (IRX5), AtCesA7 (IRX3), and AtCesA8 (IRX1) genes in Arabidopsis, the OsCesA4 (BC7), OsCesA7, and OsCesA9 (BC6) genes in rice, and the ZmCesAlO, ZmCesAl 1, and ZmCesAl2 genes in maize, appear to function non-redundantly to catalyze cellulose biosynthesis in secondary cell walls. Xylan is the most abundant hemicellulose found in the secondary cell walls of plants and is thought to function as the major cellulose cross-linking component in secondary cell walls. Several Golgi-localized glycosyltransferases, including protein members from glycosyltransferases family 43 (i.e. the above-mentioned IRX9 and IRX14), have been involved in the biosynthesis of the xylose sugar backbone in developing xylem cells. Many other orthologous genes co-expressed with the cellulose synthase and xylan synthase genes in vascular xylem tissues are found in both dicot and monocot species, which implies common biological functions (Oikawa et al., “An Integrative Approach to the Identification of Arabidopsis and Rice Genes Involved in Xylan and Secondary Wall Development,” PLoS ONE 5(1):el 548 I (2010)).

Since the expression amount of genes coding endomembrane enzyme/protein for the secondary cell wall cellulose/xylan deposition is specific and enormous in the vascular xylem tissues, their upstream sequence regions, namely promoters and 5'-UTR sequences, would be useful to overexpress heterogenous genes within the xylem tissues (Oikawa et al., “Golgi-Localized Enzyme Complexes for Plant Cell Wall Biosynthesis,” Trends Plant Sci. 18:49-58, (2013); Oikawa et al., “An Integrative Approach to the Identification of Arabidopsis and Rice Genes Involved in Xylan and Secondary Wall Development,” PLoS ONE 5(1):el 548 I (2010)).

As an example of the applications, the transgenic rice plants that expressed a sucrose synthase gene, OsSUS3, driven by the AtCesA8 (the above-mentioned IRX1) promoter maintained a normal growth with slightly increased biomass yields, and also reduced cellulose crystallinity and increased wall thickness, therefore leading to large improvements of both biomass saccharification and lodging (Fan et al., “AtCesA8-driven OsSUS3 Expression Leads to Largely Enhanced Biomass Saccharification and Lodging Resistance by Distinctively Altering Lignocellulose Features in Rice,” Biotechnol. Biofuels 10:221 (2017)). Another recent example of the successful tailoring of biomass properties is tissue-specific overexpression of master TF (Loque and Scheller, “Spatially Modified Gene Expression in Plants,” PCT Publication No. WO
2012/103555; Yang et al., “Engineering Secondary Cell Wall Deposition in Plants,” *Plant Biotechnol. J* 11(3):325-35 (2013)). Tissue-specific overexpression of TF operably linked to a heterologous promoter that induces expression of a gene that is a downstream target of the TF enabled a positive feedback manner that regulates the amplified production of the secondary cell wall production in woody tissue. To accomplish this, a tissue-specific promoter sequence such as an IRX1, IRX3, IRX5, IRX8, IRX9, IRX14, IRX7, and IRX10 promoter was linked in with NAC-MYB TFs such as NST1, NST2, SND1/NST3, SND2, SND3, MYB103, MYB85, MYB46, MYB83, MYB58, or MYB63. This strategy is, however, limited to the accumulation of specific secondary cell wall compounds such as cellulose, xylan, and lignin in xylem and fiber tissues. Overall yield and/or yield-related traits have yet to be improved since the protocol uses the series of TFs only involved in secondary cell wall development, not in the upstream process such as vascular xylem cell differentiation and/or cell development.


Although gene expression of Arabidopsis R2R3-MYB subfamily 4, MYB4, MYB7, and MYB32 are positively regulated by secondary wall MYB TF, MYB46, they are also shown to be involved in fine-tuning the upstream transcriptional regulation of developing vascular xylem cells. Overexpression of two maize homologs from the R2R3-MYB subfamily 4, namely ZmMYB31 and ZmMYB42, in Arabidopsis results in down-regulation of the lignin pathway and a patchy secondary cell wall deposition phenotype in fiber cells, which supports a repressive role for these proteins. In addition, there is molecular evidence showing that MYB4, MYB7, and MYB32 repress not only their own promoters but also the promoter of the secondary cell wall NAC master TF SND1/NST3 that regulates MYB46. Such negative regulations suggest that R2R3-MYB subfamily 4 may fine-tune the expressions and activities of secondary wall NAC-MYB-based transcriptional regulatory network for vascular xylem and fiber cells development.

The secondary cell wall master regulators in the endothecium of anthers include NST2, which is co-expressed with the SHN/WIN genes from ERF/AP2 subfamily B-6 and also with WRKY DNA-Binding Protein 12 (Wang et al., “Mutation of WRKY Transcription Factors Initiates Pith Secondary Wall Formation and Increases Stem Biomass in Dicotyledonous Plants,” *Proc. Natl. Acad. Sci. U. S. A.* 107(51):22338-43 (2010); Yang et al., “PtrWRKY19, a Novel WRKY Transcription Factor, Contributes to the Regulation of Pith Secondary Wall Formation in Populus trichocarpa,” *Sci. Rep.* 6:18643 (2016)) that are believed to be upstream transcriptional regulators. The OsSHNL gene, a homolog of Arabidopsis AtSHN2 in rice, is also tightly co-expressed with TFs and biosynthetic genes associated with the formation of the secondary cell wall in xylem and fiber cells. Although both AtSHN2 and OsSHNL genes are suggested to regulate wax and lipid biosynthesis, they can also (a) enhance cellulose synthase genes expression; and (b) suppress lignin biosynthetic gene expression when they are overexpressed in rice. Additional molecular evidence shows that AtSHN2 can bind the promoters of secondary
cell wall NAC-MYB TFs in rice, indicating an upstream mechanism of transcriptional regulation by SHN gene family in monocots.

[0011] The expression modulation of the TFs for the fundamental studies, however, has been controlled by constitutive promoters, which often show detrimental effects as yield drag. The previously noted vascular xylem tissue-targeting overexpression with the above-mentioned promoters has not been applied to the TFs that can potentially act before the secondary wall NAC-MYB TFs.

[0012] The present invention seeks to cure these deficiencies through the combination of promoters which preferably target vascular xylem tissue and a series of DNA transcription factors (TFs) involved in the transcriptional regulation of developing vascular xylem cells to enhance multiple yield-related traits in plants.

**SUMMARY OF THE INVENTION**

[0013] A first aspect of the present invention is directed to a nucleic acid construct that includes a polynucleotide encoding a transcription factor polypeptide and a heterologous, tissue-specific promoter operably linked to the polynucleotide encoding the transcription factor polypeptide, wherein the promoter specifically directs expression of the transcription factor polypeptide in vascular xylem tissue of a plant.

[0014] A second aspect of the present invention is directed to an expression vector that includes a nucleic acid construct of the present invention.

[0015] A third aspect of the present invention is directed to a recombinant host cell that includes a nucleic acid construct according to the first aspect of the invention or a recombinant expression vector according to the second aspect of the invention. In certain embodiments, the recombinant host cells are bacterial cells or plant cells.

[0016] A fourth aspect of the present invention is directed to a transgenic plant or transgenic plant seed that includes a nucleic acid construct according to the first aspect of the invention or a recombinant host cell according to the third aspect of the invention.

[0017] A fifth aspect of the present invention is directed to a plant having (i.e., including) a transgene that includes a heterologous, tissue-specific promoter operably linked to a polynucleotide encoding a transcription factor involved in vascular xylem cell development, wherein the promoter specifically directs expression of the transcription factor in vascular xylem tissue of the plant.
[0018] A sixth aspect of the present invention is directed to a rootstock, cutting, or seed obtained from a transgenic plant according to the fourth aspect of the invention or a plant according to the fifth aspect of the invention.

[0019] A seventh aspect of the invention is directed to a method of enhancing plant growth or yield by providing a transgenic plant or transgenic plant seed that is transformed with a nucleic acid construct according to the first aspect of the invention (which includes a transgenic plant or transgenic plant seed according to the fourth aspect of the invention). In one embodiment, the transgenic plant is provided, and then grown under conditions effective to permit the nucleic acid construct to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance plant growth or yield. In another embodiment, the transgenic plant seed is provided, planted in a growth medium, and a transgenic plant is then propagated from the transgenic plant seed to permit the nucleic acid construct to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance plant growth or yield.

[0020] An eighth aspect of the invention is directed to a method of enhancing plant growth or yield by providing a rootstock, cutting, or seed according to the sixth aspect of the invention, which rootstock, cutting, or seed is planted in a growth medium, and a transgenic plant is then propagated from the rootstock, cutting, or seed to permit the nucleic acid construct (or transgene) to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance plant growth or yield.

[0021] A ninth aspect of the invention is directed to a method of enhancing plant growth or yield by providing a plant according to the fifth aspect of the invention, and growing the plant under conditions effective to permit the transgene to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance plant growth or yield.

[0022] A tenth aspect of the present invention is directed to a method of planting, cultivating, or harvesting a part or all of a plant according to the first aspect of the invention or fifth aspect of the invention.

[0023] An eleventh aspect of the present invention is direct to a method of making a plant according to the fourth aspect of the invention or a plant according to the fifth aspect of the present invention. The method includes introducing a nucleic acid construct or transgene of the invention into a plant cell and propagating the plant from the plant cell.

[0024] A twelfth aspect of the present invention is directed to a method of enhancing degradability of plant biomass by providing a transgenic plant or transgenic plant seed that is transformed with a nucleic acid construct according to the first aspect of the invention (which
includes a transgenic plant or transgenic plant seed according to the fourth aspect of the invention). In one embodiment, the transgenic plant is provided, and then grown under conditions effective to permit the nucleic acid construct to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance degradability of plant biomass. In another embodiment, the transgenic plant seed is provided, planted in a growth medium, and a transgenic plant is then propagated from the transgenic plant seed to permit the nucleic acid construct to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance degradability of plant biomass.

[0025] An thirteenth aspect of the invention is directed to a method of enhancing degradability of plant biomass by providing a rootstock, cutting, or seed according to the sixth aspect of the invention, which rootstock, cutting, or seed is planted in a growth medium, and a transgenic plant is then propagated from the rootstock, cutting, or seed to permit the nucleic acid construct (or transgene) to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance degradability of plant biomass.

[0026] A fourteenth aspect of the invention is directed to a method of enhancing degradability of plant biomass by providing a plant according to the fifth aspect of the invention, and growing the plant under conditions effective to permit the transgene to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance degradability of plant biomass.

[0027] The minimal cis-genic combination of (i) a promoter sequence that preferably targets vascular xylem tissues, and (ii) a TF polypeptides from an R2R3-MYB subfamily 4 or ERF/AP2 subfamily B-6 is unique. The accompanying Examples surprisingly demonstrate that multiple yield-related traits could be introduced into plant cells by the vascular xylem tissue-targeting overexpression of the TFs that are believed (a) to be upstream regulators of secondary cell wall NAC master TFs (i.e., SND1/NST3, NST1, NST2, VND6, VND7), and (b) to be involved in the vascular xylem cell development. The tissue-targeting manner of the TF overexpression also enables reduced lignin in only the vascular xylem tissue and maintains lignin in other tissue cells that are vital to the structural supports of the plant. This invention generated significantly improved crops with a combination of three beneficial traits: (1) accelerated root growth, (2) increased seeds/grains and vegetative biomass yields, and (3) enhanced degradability of inedible/lignocellulosic biomass. These traits may contribute to enhancing ET.S agricultural production, self-sustainability, the economy, food security, and bioenergy. The invention has wide applicability across plant species, including both monocots and dicots.
BRIEF DESCRIPTION OF THE DRAWINGS

[0028] Fig. 1 illustrates the map and nucleotide sequence for construct No. 001, pAtCTL2- AtMYB32-tRBS (SEQ ID NO:57), which includes the promoter and 5’ UTR from AtCTL2, the open reading frame of AtMYB32, and the 3’ RBS transcription terminator.

[0029] Fig. 2 illustrates the map and nucleotide sequence for construct No. 002, pAtLAC4- AtMYB32-tRBS (SEQ ID NO:58), which includes the promoter from AtLAC4, the open reading frame of AtMYB32, and the 3’ RBS transcription terminator.

[0030] Fig. 3 illustrates the map and nucleotide sequence for construct No. 003, pAtCesA4- AtMYB32-tRBS (SEQ ID NO:59), which includes the promoter from AtCesA4, the open reading frame of AtMYB32, and the 3’ RBS transcription terminator.

[0031] Fig. 4 illustrates the map and nucleotide sequence for construct No. 004, pAtCesA8- AtMYB32-tRBS (SEQ ID NO:60), which includes the promoter and 5’ UTR from AtCesA8, the open reading frame of AtMYB32, and the 3’ RBS transcription terminator.

[0032] Fig. 5 illustrates the map and nucleotide sequence for construct No. 005, pAtFLAI 1- AtMYB32-tRBS (SEQ ID NO:61), which includes the promoter and 5’ UTR from AtFLAI 1, the open reading frame of AtMYB32, and the 3’ RBS transcription terminator.

[0033] Fig. 6 illustrates the map and nucleotide sequence for construct No. 006, pAtCesA7- AtMYB32-tRBS (SEQ ID NO:62), which includes the promoter and 5’ UTR from AtCesA7, the open reading frame of AtMYB32, and the 3’ RBS transcription terminator.

[0034] Fig. 7 illustrates the map and nucleotide sequence for construct No. 007, pAtIRX9- AtMYB32-tRBS (SEQ ID NO:63), which includes the promoter and 5’ UTR from AtIRX9, the open reading frame of AtMYB32, and the 3’ RBS transcription terminator.

[0035] Fig. 8 illustrates the map and nucleotide sequence for construct No. 008, pAtCesA4- AtMYB4-tRBS (SEQ ID NO:64), which includes the promoter from AtCesA4, the open reading frame of AtMYB4, and the 3’ RBS transcription terminator.

[0036] Fig. 9 illustrates the map and nucleotide sequence for construct No. 009, pAtCesA8- AtMYB4-tRBS (SEQ ID NO:65), which includes the promoter and 5’ UTR from AtCesA8, the open reading frame of AtMYB4, and the 3’ RBS transcription terminator.

[0037] Fig. 10 illustrates the map and nucleotide sequence for construct No. 010, pZmCesAl2-ZmMYB31-tRBS (SEQ ID NO:66), which includes the promoter from ZmCesAl2, the open reading frame of ZmMYB3 1, and the 3’ RBS transcription terminator.
Fig. 11 illustrates the map and nucleotide sequence for construct No. 011, pZmCesAl-1-ZmMYB3-l-tRBS (SEQ ID NO:67), which includes the promoter from ZmCesAl, the open reading frame of ZmMYB3, and the 3’ RBS transcription terminator.

Fig. 12 illustrates the map and nucleotide sequence for construct No. 012, pOsCesA4-ZmMYB3-l-tRBS (SEQ ID NO:68), which includes the promoter from OsCesA4, the open reading frame of ZmMYB3, and the 3’ RBS transcription terminator.

Fig. 13 illustrates the map and nucleotide sequence for construct No. 013, pOsCesA7-ZmMYB3-l-tRBS (SEQ ID NO:69), which includes the promoter from OsCesA7, the open reading frame of ZmMYB3, and the 3’ RBS transcription terminator.

Fig. 14 illustrates the map and nucleotide sequence for construct No. 014, pZmCesAl-2-ZmMYB42-tRBS (SEQ ID NO:70), which includes the promoter from ZmCesAl, the open reading frame of ZmMYB42, and the 3’ RBS transcription terminator.

Fig. 15 illustrates the map and nucleotide sequence for construct No. 015, pZmCesAl-1-ZmMYB42-tRBS (SEQ ID NO:71), which includes the promoter from ZmCesAl, the open reading frame of ZmMYB42, and the 3’ RBS transcription terminator.

Fig. 16 illustrates the map and nucleotide sequence for construct No. 016, pOsCesA4-ZmMYB42-tRBS (SEQ ID NO:72), which includes the promoter from OsCesA4, the open reading frame of ZmMYB42, and the 3’ RBS transcription terminator.

Fig. 17 illustrates the map and nucleotide sequence for construct No. 017, pOsCesA7-ZmMYB42-tRBS (SEQ ID NO:73), which includes the promoter from OsCesA7, the open reading frame of ZmMYB42, and the 3’ RBS transcription terminator.

Fig. 18 illustrates the map and nucleotide sequence for construct No. 018, pZmCesAl-2-PvMYB4-tRBS (SEQ ID NO:74), which includes the promoter from ZmCesAl, the open reading frame of PvMYB4, and the 3’ RBS transcription terminator.

Fig. 19 illustrates the map and nucleotide sequence for construct No. 019, pZmCesAl-1-PvMYB4-tRBS (SEQ ID NO:75), which includes the promoter from ZmCesAl, the open reading frame of PvMYB4, and the 3’ RBS transcription terminator.

Fig. 20 illustrates the map and nucleotide sequence for construct No. 020, pOsCesA4-PvMYB4-tRBS (SEQ ID NO:76), which includes the promoter from OsCesA4, the open reading frame of PvMYB4, and the 3’ RBS transcription terminator.

Fig. 21 illustrates the map and nucleotide sequence for construct No. 021, pOsCesA7-PvMYB4-tRBS (SEQ ID NO:77), which includes the promoter from OsCesA7, the open reading frame of PvMYB4, and the 3’ RBS transcription terminator.
[0049] Fig. 22 illustrates the map and nucleotide sequence for construct No. 022, pZmCesAl2-OsSHNl-tRBS (SEQ ID NO:78), which includes the promoter from ZmCesAl2, the open reading frame of OsSHNl, and the 3’ RBS transcription terminator.

[0050] Fig. 23 illustrates the map and nucleotide sequence for construct No. 023, pZmCesAl1-OsSHNl-tRBS (SEQ ID NO:79), which includes the promoter from ZmCesAl1, the open reading frame of OsSHNl, and the 3’ RBS transcription terminator.

[0051] Fig. 24 illustrates the map and nucleotide sequence for construct No. 024, pOsCesA4-OsSHNl-tRBS (SEQ ID NO:80), which includes the promoter from OsCesA4, the open reading frame of OsSHNl, and the 3’ RBS transcription terminator.

[0052] Fig. 25 illustrates the map and nucleotide sequence for construct No. 025, pOsCesA7-OsSHNl-tRBS (SEQ ID NO:81), which includes the promoter from OsCesA7, the open reading frame of OsSHNl, and the 3’ RBS transcription terminator.

[0053] Fig. 26 is a map of an exemplary plasmid operable in dicots, which includes construct No. 003 shown in Fig. 3. Similar dicot-functional plasmids containing construct Nos. 001, 002 and 004-009 were also prepared.

[0054] Fig. 27 is a map of an exemplary plasmid operable in monocots, which includes construct 018 shown in Fig. 18. Similar monocot-functional plasmids containing construct Nos. 010-017 and 019-025 were also prepared.

[0055] Fig. 28 is an image of representative control (empty vector) and vector-transformed alfalfa (Medicago sativa L. cv Regen S) using construct No. 003 (center) and construct 008 (right). The height difference between the control and transgenic plants is evident.

[0056] Fig. 29 is an image of representative control (empty vector) and vector-transformed canola (Brassica napus L. cv Westar) using construct No. 004. The height difference between the control and transgenic plants is evident.

[0057] Fig. 30 is an image of representative control (empty vector) and vector-transformed sorghum (Sorghum bicolor P898012) using construct No. 018. The height difference between the control and transgenic plant is evident.

[0058] Fig. 31 is an image of representative control (empty vector) and vector-transformed switchgrass (Panicum virgatum Alamo) using construct No. 018. The height difference between the control and transgenic plants is evident.
DETAILED DESCRIPTION OF THE INVENTION

[0059] The present invention is directed to recombinant nucleic acid constructs and transgenes as well as expression vectors and host cells useful for generating transgenic plants that preferentially express the transgenes in vascular xylem tissue of the plant. Transgenic plant parts are also encompassed by the present invention, as are various methods for making the transgenic plants and plant parts. Also encompassed by the present invention are methods that utilize the transgenic plants or plant parts, including methods for enhancing plant growth, enhancing plant yield, modifying plant lignin content, promoting earlier reproductive maturation, and enhancing degradability of plant biomass. These recombinant materials and their use in practicing the various methods are described below.

[0060] One aspect of the present invention is directed to a nucleic acid construct that includes a polynucleotide encoding a transcription factor ("TF") polypeptide and a heterologous, tissue-specific promoter operably linked to the polynucleotide encoding the TF polypeptide, wherein the promoter specifically directs expression of the TF polypeptide in vascular xylem tissue of a plant.

[0061] According to one embodiment, the nucleic acid construct takes the form of a transgene that includes a heterologous, tissue-specific promoter operably linked to a polynucleotide encoding the TF polypeptide involved in vascular xylem cell development, and a 3’ transcription termination sequence that is operably linked to the polynucleotide encoding the TF, wherein the promoter specifically directs expression of the TF in vascular xylem tissue of the plant.

[0062] Thus, this invention involves the formation and use of synthetic oligonucleotides or nucleotide sequences. A synthetic sequence is one that is initially produced or reproduced in a laboratory setting. The structure of the synthetic sequence is altered or different from that found in the sequence that is directly isolated from its natural setting. A polynucleotide sequence 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 promoter is said to be operably linked to a heterologous coding sequence, it means that the coding sequence 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). "Operably linked" is intended to mean a functional linkage between two or more elements.
In these and other aspects of the invention, the TF polypeptide encoded by the nucleic acid construct, or transgene, is one that modulates expression of at least one gene, and possibly a series of genes (i.e., two or more), involved with cell wall and secondary metabolite biosynthetic pathways. Transcription factors are proteins that are involved in the process of transcribing DNA into RNA. Transcription factors have DNA-binding domains that allow them to bind to specific DNA sequences (e.g., promoter sequences, enhancer sequences, and silencers). In certain embodiment of the present invention, the TF polypeptide is a polypeptide that can act as an upstream transcriptional regulator to secondary wall master TFs as an upstream transcriptional regulator.

Suitable classes of TF polypeptides include, without limitation, an R2R3-MYB subfamily 4 TF polypeptide, an ERF/AP2 subfamily B-6 TF polypeptide, and combinations thereof (i.e., when co-expressed). Both R2R3-MYB subfamily 4 TFs and ERF/AP2 subfamily B-6 TFs are widely conserved among both monocots and dicots, and therefore it is contemplated that any of a variety of TFs from these classes can be utilized.

Non-limiting examples of both the R2R3-MYB subfamily 4 TF polypeptide and an ERF/AP2 subfamily B-6 TF polypeptide are provided in the examples, and include those listed below.

- **ERF/AP2 subfamily B-6 Transcription Factors**: Arabidopsis AtSHN3 (SEQ ID NOS: 1,37); Arabidopsis AtSHN1/WIN1 (SEQ ID NOS: 2,38); Arabidopsis AtSHN2 (SEQ ID NOS: 3,39); rice OsSHN1 (OsEREBl9) (SEQ ID NOS: 7,43); rice OsSHN2 (OsEREBl14) (SEQ ID NOS: 8,44); sorghum ShERE63 (SEQ ID NOS: 13,49); sorghum ShEREBl50 (SEQ ID NOS: 14,50); and maize ZmAERE46 (SEQ ID NOS: 17,53).

- **R2R3-MYB Subfamily 4 Transcription Factors**: Arabidopsis AtMYB32 (SEQ ID NOS: 4,40); Arabidopsis AtMYB4 (SEQ ID NOS: 5,41); Arabidopsis MYB7 (SEQ ID NOS: 6,42); rice OsMYB108-L (SEQ ID NOS: 9,45); rice OsMYB108 (SEQ ID NOS: 10,46); poplar PdMYB221 (SEQ ID NOS: 11,47); poplar PdMYB156 (SEQ ID NOS: 12,48); sorghum SbMYB86 (SEQ ID NOS: 15,51); sorghum SbMYB23 (SEQ ID NOS: 16,52); maize ZmMYB42 (SEQ ID NOS: 18,54); maize ZmMYB31 (SEQ ID NOS: 19,55); and switchgrass PvMYB4 (SEQ ID NOS: 20,56).

As will be appreciated by persons of skill in the art, polynucleotides encoding homologous TFs can be isolated from other monocots and dicots. Such homologous TFs can be substantially similar to one another at the protein level, and polynucleotides encoding those TFs can be substantially identical at the nucleic acid level. "Substantially identical," as used in the
context of two nucleic acids or polypeptides, refers to a sequence that has at least 60% sequence identity with a reference sequence. Alternatively, percent identity can be any integer from 60% to 100% inclusive. In some embodiments, this identity is at least: 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. Embodiments of the present invention provide for nucleic acids encoding polypeptides that are substantially identical to any of the provided TFs sequences. 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. The BLAST algorithm also performs a statistical analysis of the similarity between two sequences. One measure of similarity provided by the BLAST algorithm is the smallest sum probability, 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 $10^{-5} (0.00001)$, and most preferably less than about $10^{-10} (0.0000000001)$.

[0067] The polynucleotides encoding such TFs can also be used to isolate corresponding sequences from other plants. In this manner, methods such as PCR and the like can be used to identify such sequences based on their sequence homology to the sequences set forth herein. Sequences isolated based on their sequence identity to the entire sequences set forth herein or to variants and fragments thereof are encompassed by the present invention. Such sequences include sequences that are orthologs of the disclosed sequences. "Orthologs" is intended to mean genes derived from a common ancestral gene and which are found in different species as a result of speciation. Genes found in different species are considered orthologs when their nucleotide sequences and/or their encoded protein sequences share at least 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or greater sequence identity. Functions of orthologs are often highly conserved among species. Thus, isolated polynucleotides that have transcription activation or enhancer activities, and which hybridize under stringent conditions to the sequences disclosed herein, or to variants or fragments thereof, are encompassed by the present invention.
Variant sequences may also be identified by analysis of existing databases of sequenced genomes. In this manner, corresponding TF or enhancer sequences can be identified and used in the methods of the invention.

As noted above, the nucleic acid construct, or transgene, or the present invention includes tissue-specific promoters that specifically direct expression of the TF polypeptide in vascular xylem tissue of a plant, fiber tissues of a plant, or both.

A promoter is a polynucleotide sequence capable of driving transcription of a coding sequence in a cell. Thus, promoters used in the polynucleotide constructs of the invention include cis-acting transcriptional control elements and regulatory sequences that are involved in regulating or modulating the timing and/or rate of transcription of a gene, in this case the nucleic acid construct, or transgene, that includes a coding sequence for a TF of the type described above. A plant promoter is a promoter capable of initiating transcription in plant cells. Whereas a constitutive promoter is one that is capable of initiating transcription in nearly all tissue types, a tissue-specific promoter initiates transcription in one or a few particular tissue types, and a cell type-specific promoter initiates transcription only in one or a few particular cell types. As used herein, “tissue-specific” does not preclude the promoter from causing initiation of transcription in multiple different types of plant tissues. Rather, the term “tissue-specific” is intended to connote that the promoter causes preferential expression in one or more, but not all, plant tissues. In preferred embodiments, the tissue-specific promoter induces a high level of expression in the one or more plant tissues or, alternatively, where the tissue-specific promoter induces a high level of expression in the one or more plant tissues, the expression level is preferably elevated in vascular xylem and fiber tissues.

Expression levels of a TF can be increased (e.g., by up-regulation or overexpression) relative to the expression level of TF in a wild-type or control plant. With respect to the promoters of the present invention, "specifically directs expression in vascular xylem and fiber tissues" or "vascular xylem tissue-targeting expression" means that the promoter causes expression of a TF of the present invention that is at least 3-fold (e.g., 5-fold, 10-fold, 20-fold, 50-fold, etc.) greater in at least a portion of the vascular xylem tissue of a plant compared to other cell types (e.g., compared to epidermal or mesophyll cells). Vascular xylem tissues of a plant include plant procambium/cambium, xylem, and fiber cell types. In some embodiments, specific expression in plant vascular xylem tissues can be limited to a portion of the vasculature, e.g., above ground (aerial), below ground (roots), cambium cells only, xylem cells only, or both cambium and xylem cells. Further, in certain embodiment of the present invention, the tissue-
specific promoter directs expression of the TF polypeptide in aerial parts of the plant, in roots of
the plant, or in both the aerial parts and the roots of the plant.

[0072] The tissue-specific promoter directs expression of the TF polypeptide involved in
developmental process of vascular xylem tissue cells that occurs before secondary wall
thickening progresses with polysaccharide deposition and lignification.

[0073] The expression level of the TF may be measured, for example, by assaying for the
level of the TF in the plant. Measurement of TF levels can be carried out directly using any of a
variety of protein assays (e.g., by Western Blot) or indirectly by measuring the level of RNA
transcripts (e.g., by northern blot).

[0074] Classes of suitable tissue-specific promoter include gene promoters for secondary
cell wall development, an endomembrane protein gene promoter, or a secondary wall cellulose
synthase (CesA) promoter. Exemplary tissue-specific promoters that induce elevated expression
in vascular xylem and/or fiber tissues include, without limitation, Arabidopsis AtCTL2 promoter
(SEQ ID NO:21); Arabidopsis AtLAC4 promoter (SEQ ID NO:22); Arabidopsis AtCesA4
promoter (SEQ ID NO:23); Arabidopsis AtCesA8 promoter (SEQ ID NO:24); Arabidopsis
AtFLA1 1 promoter (SEQ ID NO:25); Arabidopsis AtCesA7 promoter (SEQ ID NO:26);
Arabidopsis AtIRX9 promoter (SEQ ID NO:27); rice OsFLA9 promoter (SEQ ID NO:28); rice
OsCTL1 promoter (SEQ ID NO:29); rice OsCesA4 promoter (SEQ ID NO:30); rice OcCesA7
promoter (SEQ ID NO:31); rice OsLacIO promoter (SEQ ID NO:32); rice OsGT43J promoter
(SEQ ID NO:33); maize ZmCesAIO promoter (SEQ ID NO:34); maize ZmCesAl2 promoter
(SEQ ID NO:35); maize ZmCesAl 1 promoter (SEQ ID NO:36).

[0075] As will be appreciated by persons of skill in the art, promoters from homologous
genes can be isolated from other monocots and dicots. Such homologous promoters can be
substantially identical at the nucleic acid level as defined above.

[0076] Alternative tissue-specific promoters that induce elevated expression in vascular
xylem and/or fiber tissues can be identified by examining native protein expression levels in the
specified plant tissues over the course of development. See Oikawa et al., “An Integrative
Approach to the Identification of Arabidopsis and Rice Genes Involved in Xylan and Secondary
Wall Development,” PLoS ONE 5(1):el 548 l (2010), which is hereby incorporated by reference
in its entirety.

[0077] As noted above, the nucleic acid construct, or transgenes, of the invention include
5′ and 3′ regulatory sequences operably linked to a TF polynucleotide.

[0078] As noted above, the nucleic acid construct, or transgene, also includes an operable
3′ regulatory region, selected from among those which are capable of providing correct
transcription termination and polyadenylation of mRNA for expression in the host cell of choice, operably linked to a modified trait nucleic acid molecule of the present invention. A number of 3’ regulatory regions are known to be operable in plants, and any suitable 3’ regulatory region can be used in accordance with the present invention.


[0080] Further aspects of the present invention include expression vectors including the nucleic acid constructs, or transgenes, described herein, as well as host cells, transgenic plants (plant cells and plant seeds produced from such transgenic plants), and transgenic plant seeds or plant parts transformed with the nucleic acid constructs described herein.

[0081] The nucleotide sequences used in the present invention may be inserted into any of the many available expression vectors and cell systems using reagents that are well known in the art. Suitable vectors include, but are not limited to, the following viral vectors such as lambda vector system gtI, gt WES.tB, Charon 4, and plasmid vectors such as pG-Cha, p35S- Cha, pBR322, pBR325, pACYC177, pACYC1084, pUC8, pUC9, pUC18, pUC19, pLG339, pR290, pKC37, pKCIOL, SV 40, pBluescript II SK+/- or KS +/- (see “Stratagene Cloning Systems” Catalog (1993) from Stratagene, La Jolla, CA, which is hereby incorporated by reference in its entirety), pQE, pIH821, pGEX, pET series (see Studier et al., “Else of T7 RNA Polymerase to Direct Expression of Cloned Genes,” Gene Expression Technology vol. 185 (1990), which is hereby incorporated by reference in its entirety), and any derivatives thereof. Recombinant molecules can be introduced into cells via transformation, particularly transduction, conjugation, mobilization, or electroporation.

[0082] The DNA sequences are cloned into the vector using standard cloning procedures in the art, as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Second
In preparing a nucleic acid construct for expression, the various nucleic acid sequences may normally be inserted or substituted into a bacterial plasmid. Any convenient plasmid may be employed, which will be characterized by having a bacterial replication system, a marker which allows for selection in a bacterium, and generally one or more unique, conveniently located restriction sites. Numerous plasmids, referred to as transformation vectors, are available for plant transformation. The selection of a vector will depend on the preferred transformation technique and target species for transformation. A variety of vectors are available for stable transformation using *Agrobacterium tumefaciens*, a soilborne bacterium that causes crown gall. Crown gall is characterized by tumors or galls that develop on the lower stem and main roots of the infected plant. These tumors are due to the transfer and incorporation of part of the bacterium plasmid DNA into the plant chromosomal DNA. This transfer DNA (T-DNA) is expressed along with the normal genes of the plant cell. The plasmid DNA, pTi, or Ti-DNA, for “tumor inducing plasmid,” contains the vir genes necessary for movement of the T-DNA into the plant. The T-DNA carries genes that encode proteins involved in the biosynthesis of plant regulatory factors, and bacterial nutrients (opines). The T-DNA is delimited by two 25 bp imperfect direct repeat sequences called the “border sequences.” By removing the oncogene and opine genes, and replacing them with a gene of interest, it is possible to transfer foreign DNA into the plant without the formation of tumors or the multiplication of *Agrobacterium tumefaciens* (Fraley et al., “Expression of Bacterial Genes in Plant Cells,” *Proc. Nat’l Acad. Sci. 80*:4803-4807 (1983), which is hereby incorporated by reference in its entirety).

Further improvement of this technique led to the development of the binary vector system (Bevan, “Binary *Agrobacterium* Vectors for Plant Transformation,” *Nucleic Acids Res. 12*:871 1-8721 (1984), which is hereby incorporated by reference in its entirety). In this system, all the T-DNA sequences (including the borders) are removed from the pTi, and a second vector containing T-DNA is introduced into *Agrobacterium tumefaciens*. This second vector has the advantage of being replicable in *E. coli* as well as *A. tumefaciens*, and contains a multiclonal site that facilitates the cloning of a transgene. An example of a commonly-used vector is pBin19 (Frisch et al., “Complete Sequence of the Binary Vector Bin19,” *Plant Mol. Biol. 27*:405-409 (1995), which is hereby incorporated by reference in its entirety). Any appropriate vectors now known or later described for genetic transformation are suitable for use with the present invention.
U.S. Patent No. 4,237,224 to Cohen and Boyer, which is hereby incorporated by reference in its entirety, describes the production of expression systems in the form of recombinant plasmids using restriction enzyme cleavage and ligation with DNA ligase. These recombinant plasmids are then introduced by means of transformation and replicated in unicellular cultures including prokaryotic organisms and eukaryotic cells grown in tissue culture.

The different components described above can be ligated together to produce the expression systems which contain the nucleic acid constructs used in the present invention, using well known molecular cloning techniques as described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition Cold Spring Harbor, NY: Cold Spring Harbor Press (1989), and Ausubel et al. Current Protocols in Molecular Biology, New York, N.Y. John Wiley & Sons (1989), which are hereby incorporated by reference in their entirety.

Once the nucleic acid construct has been prepared, it is ready to be incorporated into a host cell. Basically, this method is carried out by transforming a host cell with the nucleic acid construct under conditions effective to achieve transcription of the nucleic acid molecule in the host cell. This is achieved with standard cloning procedures known in the art, such as described by Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1989), which is hereby incorporated by reference in its entirety. Suitable host cells are plant cells. Suitable host cells also include bacterial cells. Methods of transformation may result in transient or stable expression of the nucleic acid under control of the promoter. Stable transformation is intended to mean that the nucleotide construct introduced into a plant integrates into the genome of the plant and is capable of being inherited by the progeny thereof. Transient transformation is intended to mean that the nucleotide construct introduced into a plant is not stably integrated into the genome of the plant, but is maintained in the plant cell for a sufficient period of time to allow for the expression of the introduced genes. Preferably, the nucleic acid construct of the present invention is stably inserted into the genome of the recombinant plant cell as a result of the transformation, although transient expression can serve an important purpose, particularly when the plant under investigation is slow-growing.

Plant tissue suitable for transformation includes leaf tissue, root tissue, meristems, zygotic and somatic embryos, callus, protoplasts, tassels, pollen, embryos, anthers, and the like. The means of transformation chosen is that most suited to the tissue to be transformed.

Transient expression in plant tissue can be achieved by particle bombardment (Klein et al., “High-Velocity Microprojectiles for Delivering Nucleic Acids Into Living Cells,” Nature 327:70-73 (1987), which is hereby incorporated by reference in its entirety), also known
as biolistic transformation of the host cell, as disclosed in U.S. Patent Nos. 4,945,050; 5,036,006; and 5,100,792, all to Sanford et al., and in Emerschad et al., “Somatic Embryogenesis and Plant Development from Immature Zygotic Embryos of Seedless Grapes (Vitis vinifera),” *Plant Cell Reports* 14:6-12 (1995), which are hereby incorporated by reference in their entirety.

**[0090]** In particle bombardment, tungsten or gold microparticles (1 to 2 pm in diameter) are coated with the DNA of interest and then bombarded at the tissue using high pressure gas. In this way, it is possible to deliver foreign DNA into the nucleus and obtain a temporal expression of the gene under the current conditions of the tissue. Biologically active particles (e.g., dried bacterial cells containing the vector and heterologous DNA) can also be propelled into plant cells. Other variations of particle bombardment, now known or hereafter developed, can also be used.

**[0091]** An appropriate method of stably introducing the nucleic acid construct into plant cells is to infect a plant cell with *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* previously transformed with the nucleic acid construct of the present invention. As described supra, the Ti (or Rl) plasmid of *Agrobacterium* enables the highly successful transfer of a foreign nucleic acid molecule into plant cells. A variation of *Agrobacterium* transformation uses vacuum infiltration in which whole plants are used (Senior, “ETses of Plant Gene Silencing,” *Biotechnology and Genetic Engineering Reviews* 15:79-1 19 (1998), which is hereby incorporated by reference in its entirety).

**[0092]** Yet another method of introduction is fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies (Fraley et al., “Liposome-mediated Delivery of Tobacco Mosaic Virus RNA Into Tobacco Protoplasts: A Sensitive Assay for Monitoring Liposome-protoplast Interactions,” *Proc. Natl. Acad. Sci. USA* 79:1859-63 (1982), which is hereby incorporated by reference in its entirety). The nucleic acid molecule may also be introduced into the plant cells by electroporation (Fromm et al., “Expression of Genes Transferred into Monocot and Dicot Plant Cells by Electroporation,” *Proc. Natl. Acad. Sci. USA* 82:5824 (1985), which is hereby incorporated by reference in its entirety). In this technique, plant protoplasts are electroporated in the presence of plasmids containing the expression cassette. Electrical impulses of high field strength reversibly permeabilize biomembranes allowing the introduction of the plasmids. Electroporated plant protoplasts reform the cell wall, divide, and regenerate. Other methods of transformation include polyethylene-mediated plant transformation, micro-injection, physical abrasives, and laser beams (Senior, “Lises of Plant Gene Silencing,” *Biotechnology and Genetic Engineering Reviews* 15:79-1 19 (1998), which is hereby incorporated by reference in its entirety). The precise method
of transformation is not critical to the practice of the present invention. Any method that results in efficient transformation of the host cell of choice is appropriate for practicing the present invention.

[0093] Yet a further method for introduction is by use of known techniques for genome editing or alteration. Such techniques for targeted genomic insertion involve, for example, inducing a double stranded DNA break precisely at one or more targeted genetic loci followed by integration of a chosen transgene or nucleic acid molecule (or construct) during repair. Such techniques or systems include, for example, zinc finger nucleases (“ZFNs”) (Umov et al., “Genome Editing with Engineered Zinc Finger Nucleases,” Nat Rev Genet. 11: 636-646 (2010), which is hereby incorporated by reference in its entirety), transcription activator-like effector nucleases (“TALENs”) (Joung & Sander, “TALENs: A Widely Applicable Technology for Targeted Genome Editing,” Nat Rev Mol Cell Biol. 14: 49-55 (2013), which is hereby incorporated by reference in its entirety), clustered regularly interspaced short palindromic repeat (“CRISPR”)–associated endonucleases (e.g., CRISPR/CRISPR-associated (“Cas”) 9 systems) (Wiedenheft et al., “RNA-Guided Genetic Silencing Systems in Bacteria and Archaea,” Nat 482:331-338 (2012); Zhang et al., “Multiplex Genome Engineering Using CRISPR/Cas Systems,” Science 339(6121): 819-23 (2013); and Gaj et ak, “ZFN, TALEN, and CRISPR/Cas-based Methods for Genome Engineering,” Cell 31(7):397-405 (2013), each of which is hereby incorporated by reference in its entirety).

[0094] In certain embodiments, transformation described herein is carried out by microinjection, Agrobacterium-mediated transformation, direct gene transfer, ballistic particle acceleration, whisker method transformation, vacuum infiltration, biolistic transformation, electroporation, micro-injection, polyethylene-mediated transformation, or laser-beam transformation.


[0096] Means for regeneration vary from species to species of plants, but generally a suspension of transformed protoplasts or a petri plate containing explants is first provided. Callus tissue is formed and shoots may be induced from callus and subsequently rooted.
Alternatively, embryo formation can be induced in the callus tissue. These embryos germinate as natural embryos to form plants. The culture media will generally contain various amino acids and hormones, such as auxin and cytokinins. Efficient regeneration will depend on the medium, on the genotype, and on the history of the culture. If these three variables are controlled, then regeneration is usually reproducible and repeatable.

[0097] Preferably, transformed cells are first identified using a selection marker simultaneously introduced into the host cells along with the nucleic acid construct of the present invention. Suitable selection markers include, without limitation, markers encoding for antibiotic resistance, such as the neomycin phosphotransferase II (“nptII”) gene which confers kanamycin resistance (Fraley et al., “Expression of Bacterial Genes in Plant Cells,” Proc. Natl. Acad. Sci. USA 80:4803-4807 (1983), which is hereby incorporated by reference in its entirety), and the genes which confer resistance to gentamycin, G418, hygromycin, streptomycin, spectinomycin, tetracycline, chloramphenicol, and the like. Cells or tissues are grown on a selection medium containing the appropriate antibiotic, whereby generally only those transformants expressing the antibiotic resistance marker continue to grow. Other types of markers are also suitable for inclusion in the expression cassette of the present invention. For example, a gene encoding for herbicide tolerance, such as tolerance to sulfonylurea is useful, or the dhfr gene, which confers resistance to methotrexate (Bourouis et al., “Vectors Containing a Prokaryotic Dihydrofolate Reductase Gene Transform Drosophila Cells to Methotrexate-resistance,” EMBO J. 2:1099-1 104 (1983), which is hereby incorporated by reference in its entirety). Similarly, “reporter genes,” which encode for enzymes providing for production of an identifiable compound are suitable. The most widely used reporter gene for gene fusion experiments has been uidA, a gene from Escherichia coli that encodes the β-glucuronidase protein, also known as GETS (Jefferson et al., “GUS Fusions: β Glucuronidase as a Sensitive and Versatile Gene Fusion Marker in Higher Plants,” EMBO J. 6:3901-3907 (1987), which is hereby incorporated by reference in its entirety). Similarly, enzymes providing for production of a compound identifiable by luminescence, such as luciferase, are useful. The selection marker employed will depend on the target species; for certain target species, different antibiotics, herbicide, or biosynthesis selection markers are preferred.

[0098] Plant cells and tissues selected by means of an inhibitory agent or other selection marker are then tested for the acquisition of the transgene (Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, New York: Cold Spring Harbor Press (1989), which is hereby incorporated by reference in its entirety).
After a transgene containing a nucleic acid construct is stably incorporated in transgenic plants, the transgene can be transferred to other plants by sexual crossing. Any of a number of standard breeding techniques can be used, depending upon the species to be crossed. Once transgenic plants of this type are produced, the plants themselves can be transplanted to a suitable growth medium and cultivated in accordance with conventional procedure so that the nucleic acid construct is present in the resulting plants. Alternatively, transgenic seeds are recovered from the transgenic plants. These seeds can then be planted in a suitable growth medium and cultivated using conventional procedures to produce transgenic plants.

In these embodiments, suitable growth medium includes soil, soil-less particulate medium, or a liquid growth medium. Conditions for cultivating and harvesting may different depending on the type of growth medium and location, e.g., field, greenhouse, hydroponic environment, etc.

During subsequent growth and cultivation of the transgenic plants of the invention, it is also contemplated that individual plants may be selected based on their exhibiting one or more of the following properties: faster vegetative growth including that which leads to early maturation, increased biomass yields, enhanced root development, increased seed/grain production, improved nutrient contents in biomass, increased release of glucose saccharides, increased release of xylose saccharides, reduced lignin composition, and any combinations thereof.

The present invention may be used for transformation of any plant species, including, but not limited to, monocots and dicots. Examples of plant species of interest include, but are not limited to, the genus Abies, Acacia, Acer, Aegilops, Aesculus, Agave, Ailanthus, Alnus, Amborella, Amelanchier, Arabidopsis, Arbutus, Arctostaphylos, Artemisia, Asimina, Asparagus, Atriplex, Atropa, Aucuba, Avena, Berberis, Betula, Brachypodium, Brassica, Buddleia, Buxus, Calocedrus, Calotropis, Camellia, Camptotheca, Campsis, Cannabis, Capsicum, Capsella, Carpinus, Carya, Castanea, Catalpa, Ceanothus, Cedrus, Celastrus, Celtis, Cephalanthus, Cercidium, Cercis, Chaenomeles, Chamaecyparis, Chilopsis, Chionanthus, Chrysothamnus, Cicer, Cistus, Citrus, Citrullus, Cladrastis, Clematis, Coleogyria, Cornus, Corylus, Cotinus, Cotoneaster, Cowania, Crataegus, Crataegus, Cucumis, Cupressus, Cytisus, Daphne, Daucus, Deutzia, Diospyros, Dioscorea, Elaeagnus, Ephedra, Erythranthe, Escallonia, Eucalyptus, Euonymus, Eutrema, Fagus, Forsythia, Fragaria, Fraxinus, Gaultheria, Gelsemium, Genlisea, Ginkgo, Gleditsia, Glycine, Grevillea, Gymnocladus, Gossypium, Hamamelis, Hebe, Helianthus, Heliamphora, Hibiscus, Heterocallis, Hordeum, Hydrangea, Hyoscyamus, Hypericum, Lactuca, Linum, Lolium, Lycopersicon, Ilex, Ipomea, Juglans, Juniperus, Kalmia,
Further aspects of the invention relates to the planting, cultivating, or harvesting a part or all of a transgenic plant of the present invention.

In addition to transgenic plants, the present invention also relates to transgenic plant parts including plant seeds, rootstock, and cuttings removed from the transgenic plant (including both woody and herbaceous cuttings). In certain embodiments, the plant, plant seed, rootstock, or cutting is (or is from) a monocot, including but not limited to those identified above. In other embodiments, the plant, plant seed, rootstock, or cutting is (or is from) a dicot, including but not limited to those identified above.

The present invention is also directed to one or more methods of enhancing plant growth or plant yield. As used herein, "yield" is defined as the measurement of the amount of a crop that was harvested per unit of land area. Crop yield is the measurement often used for grains or cereals and is typically measured as the amount of plant harvested per unit area for a given time, i.e., metric tons per hectare or kilograms per hectare. Crop yield can also refer to the actual seed or biomass produced or generated by the plant. Thus, an “enhanced yield” refers to an increase in yield relative to a non-transgenic control plant. As used herein, “enhanced plant growth” encompasses a number of aspects including, without limitation, faster vegetative growth including that which leads to early maturation, increased biomass yields, enhanced root development, increased seed/grain production, improved nutrient contents in biomass, and any combinations thereof.

A control plant or plant cell may comprise, for example: (a) a wild-type plant or cell, i.e., of the same genotype as the starting material for the genetic alteration which resulted in the subject plant or cell; (b) a plant or plant cell of the same genotype as the starting material but which has been transformed with a null construct (i.e. with a construct which has no known
effect on the trait of interest, such as a construct comprising a marker gene); (c) a plant or plant cell which is a non-transformed segregant among progeny of a subject plant or plant cell; or (d) the subject plant or plant cell itself, under conditions in which the gene of interest is not expressed. A "subject plant or plant cell" is one in which genetic alteration, such as transformation, has been effected as to a gene of interest, or is a plant or plant cell which is descended from a plant or cell so altered and which comprises the alteration. A "control" or "control plant" or "control plant cell" provides a reference point for measuring changes in phenotype of the subject plant or plant cell.

According to one embodiment, this method is carried out by providing a transgenic plant transformed with a nucleic acid construct of the present invention and growing the plant under conditions effective to permit the nucleic acid construct to express the TF polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance plant growth or yield.

According to a second embodiment, this method is carried out by providing a transgenic plant seed transformed with a nucleic acid construct of the present invention, planting the transgenic plant seed in a growth medium, and propagating a transgenic plant from the transgenic plant seed to permit the nucleic acid construct to express the TF polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance plant growth or yield.

According to a third embodiment, this method is carried out by providing a rootstock, cutting, or seed from a transgenic plant of the present invention, introducing the rootstock, cutting, or seed into a growth medium; and propagating a transgenic plant from the rootstock, cutting, or seed to permit the nucleic acid construct to express the TF polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance plant growth or yield.

According to a fourth embodiment, this method is carried out by providing a plant comprising a transgene that includes a heterologous, tissue-specific promoter operably linked to a polynucleotide encoding a TF involved in vascular xylem cell development, wherein the promoter specifically directs expression of the TF in vascular xylem tissue of the plant, and growing the plant under conditions effective to permit the transgene to express the TF polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance plant growth or yield.

According to a fifth embodiment, this method is carried out by providing a rootstock, cutting, or seed obtained from a plant comprising a transgene that includes a heterologous, tissue-specific promoter operably linked to a polynucleotide encoding a TF involved in vascular xylem cell development, wherein the promoter specifically directs
expression of the TF in vascular xylem tissue of the plant, introducing the rootstock, cutting, or seed into a growth medium, and propagating a plant from the rootstock, cutting, or seed to permit the transgene to express the TF polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance plant growth or yield.

[0112] The present invention is also directed to one or more methods of enhancing degradability of plant biomass. As used herein, enhanced degradability of plant biomass refers to the rate of biomass degradation when otherwise exposed to similar environmental conditions, using comparable amounts of plant biomass, as compared to the biomass of a control plant. Enhanced degradability may refer to any one or more of: (i) increased release of glucose saccharides, (ii) increased release of xylose saccharides, (iii) reduced lignin composition, and any combinations thereof.

[0113] According to one embodiment, this method is carried out by providing a transgenic plant of the present invention and growing the plant under conditions effective to permit the nucleic acid construct to express the TF polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance degradability of plant biomass.

[0114] According to a second embodiment, this method is carried out by providing a transgenic plant seed of the present invention, planting the transgenic plant seed in a growth medium, and propagating a transgenic plant from the transgenic plant seed to permit the nucleic acid construct to express the TF polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance degradability of plant biomass.

[0115] According to a third embodiment, this method is carried out by providing a rootstock, cutting, or seed of the present invention, introducing the rootstock, cutting, or seed into a growth medium, and propagating a transgenic plant from the rootstock, cutting, or seed to permit the nucleic acid construct to express the TF polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance degradability of plant biomass.

[0116] According to a fourth embodiment, this method is carried out by providing a plant of the present invention and growing the plant under conditions effective to permit the transgene to express the TF polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance degradability of plant biomass.

[0117] According to a fifth embodiment, this method is carried out by providing a rootstock, cutting, or seed of the present invention, introducing the rootstock, cutting, or seed into a growth medium, and propagating a plant from the rootstock, cutting, or seed to permit the transgene to express the TF polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance degradability of plant biomass.
EXAMPLES

[0118] The examples below are intended to exemplify the practice of embodiments of the disclosure but are by no means intended to limit the scope thereof.

Example 1 - Gene Combinations of a Promoter and a TF

[0119] A series of simple gene cassettes comprising TFs driven by promoters active in the target tissues were generated (see Tables 1 and 2). The promoters in Table 2 were selected based on their expression profile corresponding to the development of xylem tissue (Oikawa et al., “An Integrative Approach to the Identification of Arabidopsis and Rice Genes Involved in Xylan and Secondary Wall Development,” PLoS ONE 5(1):el 5481 (2010), which is hereby incorporated by reference in its entirety).

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### Table 1: Examples of Transcription Factors for Gene Combination

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2. Relative gene expression value in vascular tissues or xylem-related organ.

[0120] The sequences referenced in Table 1 are set forth below.

**SEQ ID NO:1**

Met Val His Ser Lys Lys Phe Arg Gly Val Arg Gin Arg Gin Trp Gly Ser Trp Val Ser Glu lie Arg His Pro Leu Leu Lys Arg Arg Val Trp Leu Gly Thr Phe Asp Thr Ala Glu Thr Thr Ala Ala Asp Tyr Asp Gin Ala Ala Val Leu Met Asn Gly Gin Ser Ala Lys Thr Asn Phe Pro Val lie Lys Ser Asn Gly Ser Asn Ser Leu Glu lie Asn Ser Ala Leu Arg Ser Pro Lys Ser Leu Ser Glu Leu Leu Asn Ala Lys Leu Arg Lys Asn Cys Lys Asp Gin Thr Pro Tyr Leu Thr Cys Leu Arg Leu Asp Asn Asp Ser Ser His lie Gly Val Trp Gin Trp Gin Lys Arg Ala Gly Ser Lys Thr Ser Pro Asn Trp Val Lys Leu Val Glu Gly Asp Lys Val Asn Ala Arg Pro Gly Gly Asp lie Glu Thr Asn Lys Met Lys Val Arg Asn Gly Asp Val Gin Glu Asp Asp Gin Met Ala Gin Met Gin Met lie Glu Leu Leu Asn Trp Thr Cys Pro Ser Gly Ser Gly Ser lie Ala Gin Val

**SEQ ID NO:2**

Met Val Gin Thr Lys Lys Phe Arg Gly Val Arg Gin Arg Gin His Trp Gly Ser Trp Val Ala Glu lie Arg His Pro Leu Leu Lys Arg Arg lie Trp Leu Gly Thr Phe Glu Thr Ala Glu Glu Ala Ala Arg Ala Tyr Asp Glu Ala Ala Val Leu Met Ser Gly Arg Asn Ala Lys Thr Asn Phe Pro Leu Asn Asn Asn Thr Gly Glu Thr Ser Glu Gly Lys Thr Asp lie Ser Ala Ser Ser Met Ser Ser Thr Ser Ser Ser Ser Ser Leu Ser Ser lie Leu Ser Ala Lys Leu Arg Lys Cys Lys Ser Pro Ser Pro Ser Leu Thr Cys Leu Arg Leu Asp Thr Ala Ser Ser His lie Gly Val Trp Gin Lys Arg Ala Gly Ser Lys Ser Asp Ser Ser Trp Val Met Thr Val
Glu Leu Gly Pro Ala Ser Ser Ser Gin Glu Thr Thr Ser Lys Ala Ser
Gin Asp Ala Ile Leu Ala Pro Thr Thr Glu Val Glu H e Gly Gly Ser
Arg Glu Glu Val Leu Asp Glu Glu Glu Lys Val Ala Leu Gin Met H e
Glu Glu Leu Leu Asn Thr Asn

SEQ ID NO:3
Met Val His Ser Arg Gln Lys Leu Arg His Pro Leu Leu Lys Arg Arg Val Trp
Leu Gly Thr Phe Glu Thr Ala Glu Ala Ala Ala Ala Tyr Asp Gin
Ala Ala Leu Leu Met Asn Gly Gin Gin Ala Lys Thr Asn Phe Pro Val
Val Lys Ser Glu Glu Gly Ser Asp His Val Lys Asp Val Asn Ser Pro
Leu Met Ser Pro Lys Ser Leu Ser Glu Leu Leu Asn Ala Lys Leu Arg
Lys Ser Cys Lys Asp Leu Thr Pro Ser Leu Thr Cys Leu Arg Leu Asp
Thr Asp Ser Ser His Ile Gin Val Trp Gin Lys Arg Ala Gin Ser Lys
Thr Ser Pro Thr Trp Val Met Arg Leu Glu Leu Gly Asn Val Asn Val
Glu Ser Ala Val Asp Leu Gly Leu Thr Met Asn Lys Gin Asn Val
Gl Lys Glu Glu Glu Glu Glu Glu Ala Ile H e Ser Asp Glu Asp Gin
Leu Ala Met Glu Met Ile Glu Glu Leu Leu Asn Trp Ser

SEQ ID NO:4
Met Gly Arg Ser Pro Cys Cys Glu Lys Asp His Thr Asn Lys Gly Ala
Trp Thr Lys Glu Glu Asp Asp Lys Leu H e Ser Tyr H e Lys Ala His
Gly Glu Gly Cys Trp Arg Ser Leu Pro Arg Ser Ala Gly Leu Gin Arg
Cys Gly Lys Ser Cys Arg Leu Arg Trp H e Asn Tyr Leu Arg Pro Asp
Leu Lys Arg Gly Asn Phe Thr Leu Glu Glu Asp Asp Leu H e H e Lys
Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu H e Ala Thr Arg Leu
Pro Gly Arg Thr Asn Glu H e Lys Asn Tyr Trp Asn Thr His Val
Lys Arg Lys Leu Leu Arg Lys Gly H e Asp Pro Ala Thr His Arg Pro
H e Asn Glu Thr Lys Thr Ser Gin Asp Ser Ser Asp Ser Ser Lys Thr
Glu Asp Pro Leu Val Lys H e Leu Ser Phe Gly Pro Gin Leu Glu Lys
H e Ala Asn Phe Gly Asp Glu Arg H e Gin Lys Arg Val Glu Tyr Ser
Val Val Glu Arg Cys Lys Leu Leu Asp Leu Asn Leu Glu Leu Arg H e Ser
Pro Pro Trp Gin Asp Lys Leu His Asp Glu Arg Asn Leu Arg Phe Gly
Arg Val Lys Tyr Arg Cys Ser Ala Cys Arg Phe Gly Phe Gly Asn Gin
Lys Glu Cys Ser Cys Asn Asn Val Lys Cys Gin Thr Glu Asp Ser Ser
Ser Ser Ser Tyr Ser Ser Thr Asp H e Ser Ser Ser H e Gly Tyr Asp
Phe Leu Gly Leu Asn Asn Thr Arg Val Leu Asp Phe Ser Thr Leu Glu
Met Lys

SEQ ID NO:5
Met Gly Arg Ser Pro Cys Cys Glu Lys Ala His Thr Asn Lys Gly Ala
Trp Thr Lys Glu Glu Asp Glu Arg Leu Val Ala Tyr H e Lys Ala His
Gly Glu Gly Cys Trp Arg Ser Leu Pro Lys Ala Ala Gly Leu Leu Arg
Cys Gly Lys Ser Cys Arg Leu Arg Trp H e Asn Tyr Leu Arg Pro Asp
Leu Lys Arg Gly Asn Phe Thr Glu Glu Glu Asp Glu Leu H e H e Lys
Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu H e Ala Gly Arg Leu
Pro Gly Arg Thr Glu H e Lys Asn Tyr Trp Asn Thr His H e
Arg Arg Lys Leu H e Asn Arg Gly H e Asp Pro Thr Ser His Arg Pro
H e Gin Glu Ser Ser Ala Ser Gin Asp Ser Lys Pro Thr Gin Leu Glu
Pro Val Thr Ser Asn Thr H e Asn H e Ser Phe Thr Ser Ala Pro Lys
Val Glu Thr Phe His Glu Ser H e Ser Phe Pro Gly Lys Ser Glu Lys
lie Ser Met Leu Thr Phe Lys Glu Glu Lys Asp Glu Cys Pro Val Gin Glu Lys Phe Pro Asp Leu Asn Leu Glu Leu Arg lie Ser Leu Pro Asp Asp Val Asp Arg Leu Gin Gly His Gly Lys Ser Thr Thr Pro Arg Cys Phe Lys Cys Ser Arg Gin Met lie Asn Gly Met Glu Cys Arg Cys Gly Arg Met Arg Cys Asp Val Val Gly Gly Ser Ser Lys Gly Ser Asp Met Ser Asn Gly Phe Asp Phe Leu Gly Leu Ala Lys Lys Glu Thr Thr Ser Leu Leu Gly Phe Arg Ser Leu Glu Met Lys

SEQ ID NO:6
Met Gly Arg Ser Pro Cys Cys Glu Lys Glu His Met Asn Lys Gly Ala
Trp Thr Lys Glu Glu Asp Glu Arg Leu Val Ser Tyr lie Lys Ser His Gly Glu Gly Cys Trp Arg Ser Leu Pro Arg Ala Ala Gly Leu Leu Arg Cys Gly Lys Ser Cys Arg Leu Arg Trp lie Asn Tyr Leu Arg Pro Asp Leu Lys Arg Gly Asn Phe Thr His Asp Glu Asp Glu Leu lie lie Lys Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu lie Ala Ala Arg Leu Pro Gly Arg Thr Asp Glu lie Lys Asn Tyr Trp Asn Thr His lie Lys Arg Lys Leu Ser Lys Gly lie Asp Pro Ala Thr His Arg Gly lie Asn Glu Ala Lys lie Ser Asp Leu Lys Lys Thr Lys Asp Gin lie Val Lys Asp Val Ser Phe Val Thr Lys Phe Glu Glu Thr Asp Lys Ser Gly Asp Gin Lys Gin Asn Lys Tyr lie Arg Asn Gly Leu Val Cys Lys Glu Glu Arg Val Val Glu Glu Lys lie Gly Pro Asp Leu Asn Leu Glu Leu Arg lie Ser Pro Pro Pro Trp Gin Asn Gin Arg Glu lie Ser Thr Cys Thr Ala Ser Arg Phe Tyr Met Glu Asn Asp Met Glu Cys Ser Ser Glu Thr Val Lys Cys Gin Thr Glu Asn Ser Ser Ser Ser Ser Ser Tyr Ser Ser lie Asp lie Ser Ser Ser Asn Val Gly Tyr Asp Phe Leu Gly Leu Lys Thr Arg lie Leu Asp Phe Arg Ser Leu Glu Met Lys

SEQ ID NO:7
Met Gly Gin Ser Lys Lys Lys Phe Arg Gly Val Arg Gin Arg His Trp Gly Ser Trp Val Ser Glu lie Arg His Pro Leu Leu Lys Arg Arg Val Trp Leu Gly Thr Phe Glu Thr Ala Glu Glu Ala Ala Arg Ala Tyr Asp Glu Ala Ala lie Leu Met Ser Gly Arg Asn Ala Lys Thr Asn Phe Pro Val Ala Arg Asn Ala Thr Gly Glu Leu Thr Pro Ala Ala Ala Val Ala Gly Arg Asp Gly Val Gly Gly Gly Gly Ser Gly Ser Ser Ser Ser Met Thr Ala Asn Gly Gly Gly Gly Asn Ser Leu Ser Gin lie Leu Ser Ala Lys Leu Arg Lys Cys Cys Lys Thr Pro Ser Pro Ser Leu Thr Cys Leu Arg Leu Asp Pro Glu Lys Ser His lie Gly Val Trp Gin Lys Arg Ala Gly Ala Arg Ala Asp Ser Ser Trp Val Met Thr Val Glu Leu Asn Lys Asp Thr Ala Val Ser Ser Ala Ala Thr Val Ala Ala Ala Thr Val Val Ser Ser Ser Asp Gin Pro Thr Pro Ser Pro Ser Thr Val Thr Thr Ser Thr Ser Thr Gly Ser Pro Ser Pro Pro Pro Pro Pro Pro Ala Met Asp Asp Glu Glu Arg lie Ala Leu Gin Met lie Glu Leu Leu Arg Gly Ser Gly Pro Gly Ser Pro Ser His Gly Leu Leu His Gly Gly Glu Gly Ser Leu Val lie

SEQ ID NO:8
Met Gly Gin Ser Lys Lys Lys Phe Arg Gly Val Arg Gin Arg His Trp Gly Ser Trp Val Ser Glu lie Arg His Pro Leu Leu Lys Arg Arg Val Trp Leu Gly Thr Phe Glu Thr Ala Glu Glu Ala Ala Arg Ala Tyr Asp Glu Ala Ala lie Leu Met Ser Gly Arg Asn Ala Lys Thr Asn Phe Pro Val Ala Arg Asn Ala Thr Gly Glu Leu Thr Pro Ala Ala Ala Val Ala
Gly Arg Asp Gly Arg Val Gly Gly Gly Ser Gly Ser Ser Ser Ser Ser Ser Ser Met
Thr Ala Asn Gly Gly Gly Asp Ser Leu Ser Gin lie Leu Ser Ala Lys
Leu Arg Lys Cys Cys Lys Thr Pro Ser Pro Ser Leu Thr Cys Leu Arg
Leu Asp Pro Glu Lys Ser His lie Gly Val Trp Gin Lys Arg Ala Gly
Ala Arg Ala Asp Ser Ser Trp Val Met Thr Val Glu Leu Asn Lys Asp
Thr Ala Val Ser Ser Ala Ala Thr Val Ala Ala Ala Thr Val Ala Val Ser
Ser Ser Asp Gin Pro Thr Pro Ser Asp Ser Thr Val Thr Thr Thr Ser
Thr Ser Thr Thr Gly Ser Pro Ser Pro Pro Pro Pro Pro Pro Pro Pro Ala Met Asp Asp
Glu Glu Arg lie Ala Leu Gin Met lie Glu Glu Leu Gly Arg Ser
Gly Pro Gly Ser Pro Ser His Gly Leu Leu His Gly Glu Gly Glu Ser
Leu Val lie

SEQ ID NO: 9
Met Gly Arg Ser Pro Cys Cys Glu Lys Glu His Thr Asn Lys Gly Ala
Trp Thr Lys Glu Glu Asp Glu Arg Leu Val Ala Tyr lie Arg Ala His
Gly Glu Gly Cys Trp Arg Ser Leu Pro Lys Ala Ala Gly Leu Leu Arg
Cys Gly Lys Ser Cys Arg Leu Arg Trp lie Asn Tyr Leu Arg Pro Asp
Leu Lys Arg Gly Asn Phe Thr Ala Asp Glu Asp Leu H e H e Lys
Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu lie Ala Ala Arg Leu
Pro Gly Arg Thr Asp Asn Glu lie Lys Asn Tyr Trp Asn Thr His H e
Arg Arg Lys Leu Leu Gly Arg Gly lie Asp Pro Val Thr His Arg Pro
Val Asn Ala Ala Ala Ala Thr lie Ser Phe His Pro Gin Pro Pro Pro
Thr Thr Lys Glu Glu Gin Leu lie Leu Ser Lys Pro Pro Lys Cys Pro
Asp Leu Asn Leu Asp Leu Cys lie Ser Pro Pro Ser Cys Gin Glu Glu
Asp Asp Asp Tyr Glu Ala Lys Pro Ala Met Ile Val Arg Ala Pro Glu
Leu Gin Arg Arg Arg Gly Glu Leu Cys Phe Gly Cys Ser Leu Gly Leu
Gin Lys Glu Cys Lys Ser Gly Gly Gly Ala Gly Ala Gly Ala Gly
Asn Asn Phe Leu Leu Gly Leu Arg Ala Gly Met Leu Asp Phe Arg Ser Leu
Pro Met Lys

SEQ ID NO: 10
Met Gly Arg Ser Pro Cys Cys Glu Lys Ala His Thr Asn Lys Gly Ala
Trp Thr Lys Glu Glu Asp Asp Arg Leu Ile Ala Tyr lie Lys Ala His
Gly Glu Gly Cys Trp Arg Ser Leu Pro Lys Ala Ala Gly Leu Leu Arg
Cys Gly Lys Ser Cys Arg Leu Arg Trp H e Asn Tyr Leu Arg Pro Asp
Leu Lys Arg Gly Asn Phe Thr Glu Glu Glu Asp Glu Leu H e H e Lys
Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu H e Ala Gly Arg Leu
Pro Gly Arg Thr Asp Asn Glu lie Lys Asn Tyr Trp Asn Thr His H e
Arg Arg Lys Leu Leu Ser Arg Gly lie Asp Pro Val Thr His Arg Pro
lie Asn Asp Ser Ala Ser Asn lie Thr H e Ser Phe Glu Ala Ala Ala
Ala Ala Ala Ala Asp Asp Lys Ala Ala Val Phe Arg Arg Glu Arg Asp
Pro His Gin Pro Lys Ala Val Thr Val Ala Gin Glu Gin Gin Ala Ala
Ala Asp Trp Gly His Gly Lys Pro Leu Lys Cys Pro Asp Leu Asn Leu
Asp Leu Cys lie Ser Leu Pro Ser Gin Glu Glu Pro Met Met Met Lys
Pro Val Lys Arg Glu Thr Val Gly Val Cys Phe Ser Cys Ser Leu Gly Leu
Pro Lys Ser Thr Asp Cys Lys Cys Ser Ser Phe Leu Gly Leu Arg Thr
Ala Met Leu Asp Phe Arg Ser Leu Glu Met Lys

SEQ ID NO: 11
Met Gly Arg Ser Pro Cys Cys Glu Lys Ala His Thr Asn Lys Gly Ala
Trp Thr Lys Glu Glu Asp Asp Arg Leu H e Ala Tyr H e Arg Thr His
Gly Glu Gly Cys Trp Arg Ser Leu Pro Lys Ala Ala Gly Leu Leu Arg
Cys Gly Lys Ser Cys Arg Leu Arg Trp He Asn Tyr Leu Arg Pro Asp
Leu Lys Arg Gly Asn Phe Thr Glu Glu Glu Asp Glu Leu lie lie Lys
Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu lie Ala Gly Arg Leu
Pro Gly Arg Thr Asp Asn Glu lie Lys Asn Tyr Trp Asn Thr His lie
Arg Arg Lys Leu Asn Arg Gly lie Asp Pro Ala Thr His Arg Pro
Leu Asn Glu Pro Ala Gin Glu Ala Ser Thr Thr lie Ser Phe Ser Thr
Thr Thr Ser Val Lys Glu Glu Ser Leu Ser Ser Val Lys Glu Glu Ser
Asn Lys Glu lie lie Ser Ala Ala Ala Phe lie Cys Lys Glu Glu
Lys Thr Pro Val Gin Glu Arg Cys Pro Asp Leu Asn Leu Glu Leu Arg
lie Ser Leu Pro Cys Gin Asn Gin Pro Asp Arg His Gin Ala Phe Lys
Thr Gly Gly Ser Thr Ser Leu Cys Phe Ala Cys Ser Leu Gly Leu Gin
Asn Ser Lys Asp Cys Ser Cys Ser Val He Val Gly Thr lie Gly Ser
Ser Ser Ser Ala Gly Ser Lys Thr Gly Tyr Asp Phe Leu Gly Met Lys
Ser Gly Val Leu Asp Tyr Arg Gly Leu Glu Met Lys

SEQ ID NO: 12
Met Gly Arg Ser Pro Cys Cys Glu Lys Ala His Thr Asn Lys Gly Ala
Trp Thr Lys Glu Glu Asp Asp Arg Leu Val Ala Tyr He Arg Ala His
Gly Glu Gly Cys Trp Arg Ser Leu Pro Lys Ala Ala Gly Leu Leu Arg
Cys Gly Lys Ser Cys Arg Leu Arg Trp He Asn Tyr Leu Arg Pro Asp
Leu Lys Arg Gly Asn Phe Thr Glu Glu Glu Asp Glu Leu lie lie Lys
Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu He Ala Gly Arg Leu
Pro Gly Arg Thr Asp Asn Gly lie lie Asp Pro Ala Thr His Arg Pro
Leu Asn Glu Pro Ala Val Gin Glu Ala Thr Thr Thr He Ser Phe Thr
Thr Thr Thr Ser Val Leu Glu Glu Ser Leu Gly Ser He lie lie lie lie
Lys Glu Glu Asn Lys Glu Lys lie lie Ser Ala Thr Ala Phe Val Cys
Lys Gly Lys Thr Gin Val Gin Glu Arg Cys Pro Asp Leu Asn Leu
Gluc Leu Gly lie Ser Leu Pro Ser Gin Asn Gin Pro Asp His His Gin
Pro Phe Lys Thr Gly Gly Ser Arg Ser Leu Cys Phe Ala Cys Ser Leu
Gly Leu Gin Asn Ser Lys Asp Cys Ser Cys Ser Val He Val Ser Thr
Val Gly Ser Ser Gly Ser Thr Ser Thr Lys Thr Gly Tyr Asp Phe Leu
Gly Met Lys Ser Gly Val Leu Asp Tyr Arg Ser Leu Glu Met Lys

SEQ ID NO: 13
Met Pro Thr Pro Thr Pro Thr Pro Thr Pro Thr Pro Thr Cys Gly Asp Gly Ser
Leu Ala Arg Phe Ala Leu Leu Arg Gly Glu Lys Arg Val Ala Asn
Gly Ala Arg Gly Arg Gly Arg Gly Ile Gly Gly Glu Arg Ala Lys He He He
Arg Arg Arg His Ala Glu Lys Thr His Gly Arg Arg Glu Arg Gly Gly
His His Arg Ser His Arg Leu Ala Tyr Pro Leu Trp Val Leu Asp He He
Arg Ser Pro Asn Gly lie Met Leu Gly He Phe Arg Gly Ala Ala Leu
Trp Leu Trp Thr Leu Ala Trp His Met

SEQ ID NO: 14
Met Val Gin Ser Lys Lys Lys Phe Arg Gly Val Arg Gin Arg His Trp
Gly Ser Trp Val Ser Glu lie Arg His Pro Leu Leu Lys Arg Arg Val
Trp Leu Gly Thr Phe Glu Thr Ala Glu Glu Ala Ala Arg Ala Tyr Asp
Glu Ala Ala Val Leu Met Ser Gly Arg Asn Ala Lys Thr Asn Phe Pro
Val Pro Arg Thr Ala Thr Gly Glu Leu Ala Pro Val Pro Ala Ala Arg
Asp Ala Arg Gly Gly Glu Gly Ser Ser Ser Ala Ala Ala Ala Ala Pro Gly
Gly Gly Thr Ser Asn Leu Ser Gin lie Leu Ser Ala Lys Leu Arg Lys
Cys Cys Lys Thr Pro Ser Pro Ser Leu Thr Cys Leu Arg Leu Asp Pro
Glu Lys Ser His lie Gly Val Trp Gin Lys Arg Ala Gly Ala Arg Ala
Asp Ser Ser Trp Val Met Thr Val Gin Lys Asn Lys Asp Val Pro Pro
Pro Ala Ser Ser Ser Gly Glu Glu Pro Val Pro Ser Asp Gly Gly Ala
Ala Ala Thr Pro Thr Pro Thr Ser Thr Ser Thr Ser Thr Val Thr Thr
Thr Gly Ser Pro Pro Pro Ala Met Met Met Asp Asp Glu Glu Arg H e
Ala Leu Gin Met Ile Glu Glu Leu Leu Gly Ser Ser His Ser His Gly
Met Phe Gin Gly Ala Ala Gly Ser H e Val H e

SEQ ID NO: 15
Met Gly Arg Ser Pro Cys Cys Glu Lys Ala His Thr Asn Lys Gly Ala
Trp Thr Lys Glu Glu Asp Asp Arg Leu Val Ala Tyr H e Arg Ala His
Gly Glu Gly Cys Trp Arg Ser Leu Pro Lys Ala Ala Gly Leu Met Arg
Cys Gly Lys Ser Cys Arg Leu Arg Trp H e Asn Tyr Leu Arg Pro Asp
Leu Lys Arg Gly Asn Phe Thr Ala Asp Glu Asp Leu H e H e Lys
Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu H e Ala Ala Arg Leu
Pro Gly Arg Thr Asp Asn Glu H e Lys Asn Tyr Trp Asn Thr His H e
Arg Arg Gly Leu Gly Arg Gly H e Asp Pro Val Thr His Arg Pro
lie Ala Asp Ala Gly Ala Gly Thr Val Thr Thr H e Ser Phe Gin Pro
Asn Lys Pro Asn Ala Ala Val Ala Ala Gin Ala Pro Gin His Gin Pro
lie Ala Val Ala Val Ala Val Lys Val Pro Arg Cys Pro Asp
Leu Asn Leu Asp Leu Cys H e Ser Pro Pro Cys Gin Gin Lys Glu Asp
Glu Glu Leu Asp Leu Lys Pro Ala Val Val Val Lys Arg Glu Val Leu
Gln Ala Gly His Gly Ser Leu Cys Phe Gly Cys Ser Leu Gly H e
Gin Lys Gly Ala Pro Gly Cys Ser Cys Ser Ser Ser Asn Ser His His
Arg Phe Leu Gly Leu Arg Ser Gly Met Leu Asp Phe Arg Gly Leu Glu
Met Lys

SEQ ID NO: 16
Met Gly Arg Ser Pro Cys Cys Glu Lys Ala His Thr Asn Lys Gly Ala
Trp Thr Lys Glu Glu Asp Asp Arg Leu Val Ala Tyr H e Lys Ala His
Gly Glu Gly Cys Trp Arg Ser Leu Pro Lys Ala Ala Gly Leu Leu Arg
Cys Gly Lys Ser Cys Arg Leu Arg Trp H e Asn Tyr Leu Arg Pro Asp
Leu Lys Arg Gly Asn Phe Thr Glu Glu Asp Glu Leu H e H e Lys
Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu H e Ala Gly Arg Leu
Pro Gly Arg Thr Asp Asn Glu H e Lys Asn Tyr Trp Asn Thr His H e
Arg Arg Gly Leu Leu Ser Arg Gly H e Asp Pro Val Thr His Arg Pro
lie Asn Glu His Thr Ser Asn H e Thr H e Ser Phe Glu Ala Ala Ala
Ala Ala Arg Asp Arg Glu Glu Asn Lys Gly Ala Val Phe Arg Leu Glu
Glu His Gin Asn Lys Ala Thr Ala Ala Ala Ala Ala Ala H e Gly Arg Asp
His His Gin Asn His Pro Ala Gly Asp Trp Gly Gin Gly Lys Pro
Leu Lys Cys Pro Asp Leu Asn Leu Asp Leu Cys H e Ser Pro Pro Ala
Ala Pro Cys Gin Glu Glu Lys Ala Met Val Thr Met Lys Pro Val Lys
Arg Glu Ala Gly Leu Cys Phe Ser Cys Ser Leu Gly Leu Pro Lys Ser
Ala Asp Cys Lys Cys Ser Asn Phe Leu Gly Leu Arg Thr Ala Met Leu
Asp Phe Arg Ser Leu Glu Met Lys

SEQ ID NO: 17
Met Thr Glu Asn Leu His Ser Arg Lys Met Val Gin Pro Lys Lys Phe
Arg Gly Val Arg Gin Arg His Trp Gly Ser Trp Val Ser Glu H e Arg
His Pro Leu Leu Lys Arg Arg Val Trp Leu Gly Thr Phe Glu Thr Ala
Glu Glu Ala Ala Arg Ala Tyr Asp Glu Ala Ala Val Leu Met Ser Gly
Arg Asn Ala Lys Thr Asn Phe Pro lie Gin Arg Ser Ser Thr Gly Glu
Pro Thr Pro Ala Ala Gly Arg Asp Ala Arg Ser Asn Phe Ser Ser Gly
Ser Ser Thr Thr Asn Leu Ser Gin lie Leu Ser Ala Lys Leu Arg Lys
Cys Cys Lys Ala Pro Ser Pro Ser Leu Thr Cys Leu Arg Leu Asp Pro
Glu Lys Ser His lie Gly Val Trp Gin Lys Arg Ala Gly Ala Arg Ala
Asp Ser Asn Trp Val Met Thr Val Glu Leu Asn Lys Asp Ala Ala Ser
Thr Asp Ala Ala Ser Gin Ser Thr Ser Ala Thr Thr Ala Pro Pro Ala
Thr Pro Met Asp Glu Glu Glu Arg lie Ala Leu Gin Met lie Glu Glu
Leu Leu Ser Ser Ser Ser Pro Ala Ser Pro Ser Asn Gly Asp Asp Gin
Gly Arg Phe lie lie

SEQ ID NO: 18
Met Gly Arg Ser Pro Cys Cys Glu Lys Ala His Thr Asn Arg Gly Ala
Trp Thr Lys Glu Glu Asp Glu Arg Leu Val Ala Tyr Val Arg Ala His
Gly Glu Gly Cys Trp Arg Ser Leu Pro Arg Ala Ala Gly Leu Leu Arg
Cys Gly Lys Ser Cys Arg Leu Arg Trp lie Asn Tyr Leu Arg Pro Asp
Leu Lys Arg Gly Asn Phe Thr Ala Asp Glu Asp Leu lie Val Lys
Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu lie Ala Ala Arg Leu
Pro Gly Arg Thr Asp Asn Glu lie lie Asn Tyr Trp Asn Thr His lie
Arg Arg Lys Leu Leu Gly Ser Gly lie Asp Pro Val Thr His Arg Arg
Val Ala Gly Ala Ala Thr lie Ser Phe Gin Pro Ser Pro Asn
Thr Ala Val Ala Ala Ala Glu Thr Ala Ala Gin Ala Pro lie Lys
Ala Glu Glu Thr Ala Ala Val Lys Ala Pro Arg Cys Pro Asp Leu Asn
Leu Asp Leu Cys lie Ser Pro Pro Cys Gin His Glu Asp Asp Gly Glu
Glu Glu Glu Glu Leu Asp Leu lie Lys Pro Ala Val Val Lys Arg
Glu Ala Leu Gin Ala Gly His Gly His Gly His Gly Leu Cys Leu Gly
Cys Gly Leu Gly Gly Gin Lys Gly Ala Ala Gly Cys Ser Cys Ser Asn
Gly His His Phe Leu Gly Leu Arg Thr Ser Val Leu Asp Phe Arg Gly
Leu

SEQ ID NO: 19
Met Gly Arg Ser Pro Cys Cys Glu Lys Ala His Thr Asn Lys Gly Ala
Trp Thr Lys Glu Glu Asp Glu Arg Leu Val Ala His lie Arg Ala His
Gly Glu Gly Cys Trp Arg Ser Leu Pro Lys Ala Ala Gly Leu Leu Arg
Cys Gly Lys Ser Cys Arg Leu Arg Trp lie Asn Tyr Leu Arg Pro Asp
Leu Lys Arg Gly Asn Phe Thr Glu Glu Glu Asp Glu Leu lie Val Lys
Leu His Ser Val Leu Gly Asn Lys Trp Ser Leu lie Ala Gly Arg Leu
Pro Gly Arg Thr Asp Asn Glu lie Lys Asn Tyr Trp Asn Thr His lie
Arg Arg Lys Leu Leu Ser Arg Gly lie Asp Pro Val Thr His Arg Pro
Val Thr Glu His His Ala Ser Asn lie Thr lie Ser Phe Glu Thr Glu
Val Ala Ala Ala Ala Arg Asp Asp Lys Lys Gly Ala Val Phe Arg Leu
Glu Glu Glu Glu Arg Asn Lys Ala Thr Met Val Val Gly Arg Asp
Arg Gin Ser Gin Ser Gin Ser His Ser His Pro Ala Gly Glu Trp Gly
Gin Gly Lys Arg Pro Leu Lys Cys Pro Asp Leu Asn Leu Asp Leu Cys
lie Ser Pro Pro Cys Gin Glu Glu Glu Glu Met Glu Glu Ala Ala Met
Arg Val Arg Pro Ala Val Lys Arg Ala Gly Leu Cys Phe Gly Cys
Ser Leu Gly Leu Pro Arg Thr Ala Asp Cys Lys Cys Ser Ser Ser
Phe Leu Gly Leu Arg Thr Ala Met Leu Asp Phe Arg Ser Leu Glu Met
Lys
SEQ ID NO:20
Met Gly Arg Ser Pro Cys Cys Glu Lys Ala His Thr Asn Lys Gly Ala
Trp Thr Lys Glu Glu Asp Asp Arg Leu Val Ala Tyr lie Arg Ala His
Gly Gly Gly Trp Arg Ser Leu Pro Lys Ala Ala Gly Leu Leu Arg
Cys Gly Lys Ser Cys Arg Leu Arg Trp lie Asn Tyr Leu Arg Pro Asp
Leu Lys Arg Gly Asn Phe Thr Ala Asp Glu Asp Asp Leu lie Val Lys
Leu His Ser Leu Leu Gly Asn Lys Trp Ser Leu lie Ala Ala Arg Leu
Pro Gly Arg Thr Asp Glu lie Lys Asn Tyr Trp Asn Thr His lie
Lys Arg Lys Leu Ser Arg Gly lie Asp Pro Val Thr His Arg Pro
lie Ala Asp Ala Ala Arg Asn Val Thr lie Ser Phe Gin Pro Asp Ala
Pro Ser Gin Gin Gin Leu Ser Asp Asp Ala Glu Ala Pro Pro Pro Pro
Pro Pro Gin Gin Gin Gin Leu Lys Pro Pro Pro Pro Arg Cys Pro Asp
Leu Asn Leu Pro lea Ser Pro Pro Cys His Lys Glu Glu Glu
Asp Gin Glu Leu Val Lys Pro Ala Ala Val Lys Arg Glu Met Leu Gin
Ala Gly His Gly Thr Leu Gly Leu Cys Phe Gly Cys Ser Leu Gly Leu
Gin Lys Gly Ala Ala Gly Cys Thr Cys Ser Ser Asn Ser His Phe Leu
Gly Leu Arg Val Gly Met Leu Leu Asp Phe Arg Gly Leu Glu Met Lys

SEQ ID NO:37
atg gta cat teg aag aag ttc ega ggt gtc ege cag cgt cag tgg ggt
tct tgg gtt tct gag att cgt cat cct etc tgg aag aga aga gtt tgg
etag gga aca ttc gac aeg geg gaa aca geg get aga gcc tac gac caa
gcc geg gtt eta atg aac ggc cag age geg age act aac ttc ccc gtc
tct ccc aaa tta teg gaa etg tgg gag att tct cgg tgg age tga agg
tgt ctg aag cag aca cct tgg gag aag cgg tgg aag gaa aag cgg
tgg aag gaa cag gga cag gga act cag cag atg atg cag cgg aag tgg
tac tgt cca ggt cag gga tgg atg aag aag cag gga gga gag cgg

SEQ ID NO:38
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tct aag cag ina gta tgg gtc get gag att gta cat cct etc tgg aag agg atg tgg
etag ggg aeg ttc gac ace gca gag gag gca cga gca tcc gac gac
ccc gtc ctt taa atg age ggc ege aac gcc aag aca cgg cgg aag cgg cgg
ac aac aac cag gcg gaa gga act tee gag ggc aa cgc act atc
get teg tee aca atg tea tee tea aca tea tec tec tec tec tec tec tec
tcc ccc cgg ccc cgg aac cgg cgg cgg cgg cgg cgg cgg cgg cgg cgg
ctc cg cgg cgc cta tgg cgg cgg cgg cgg cgg cgg cgg cgg cgg cgg

SEQ ID NO:39
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tct tgg gtc tct gag att ege cat cct etc tgg aag aga aga gtt tgg
ett gga act ttc gaa aeg gca gaa geg get gca aga gca tac gac caa
geg get ett eta atg aac ggc caa aac get aag ace aat ttc cct gtc
gta aaa tea gag aat ggc tee gat ccc gtt aaa gat gtt aac tct ccc
ttg atg tea cca aag tea tta tct gat ett ttg aac get aag eta agg
aag age tgc aaa gag eta aeg cct tct ttk aeg tgg etc cgt ett gat
act gac agt tee cac att gga gtt ttk cag aaa eeg gee ggg teg aaa
aca agt ccc act ttk gtc atg ege etc gaa ett ggg aac gta gtc aac
gaa agt geg ttk gac tta gtt ttk act aeg atg aac aaa caa aac gtt
gag aaa gaa gaa gaa gaa gag att att att agt gat gag gat gag tca
tta get atg gag agt ate gat ggc gat ctt ctc aat ttk agt tga

SEQ ID NO:40
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tgc gga aaa age ttk cgt etc ega ttk att aac tat etc ega cct gat
etc aag agg gtt aac ttk ace etc gaa gaa gat gat etc ate ate aaa
eta cat age ett etc gtt aac aag ttk cct ett att geg aeg aga tta
cca gga aga aca gat aac gag att aag aat tac ttk aag aca caa cat gtt
aag agg aag eta tta aga aaa ggg att gat ccc geg act cat ega cct
ate aac gag ace aaa act tct cca gat teg tct gat ttk aag aca aca
gag gac cct ett gtc aag att etc ttk cct gtt cag ctc gag aaaa
ata gca aat ttk ggg gac gag aga att caa aag aga gtt gag tac tea
gtt gta gaa gaa gaa ggg ctg gac gtt aat ett gag ett agg ate agt
cca cca tgg cca gac aag etc cat gat gag agg aec etg aag ttk ggg
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aag gag tgt age tgt aat aat gtt aca aac gaa gac aag agt aag
age age agt tat ttk tea aca aec att aeg aat aag aag att ggt tat gac
ttc ttk ggt etta aac aac act agg gtt ttk gat ttk age act ttk gaa
atg aaa tga

SEQ ID NO:41
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tgg ggc aag age ttk cgt etc ege tgg aag etc tat etc ega cct gac
ett aag cgt gga aac ttk ace gag gaa gaa gac gaa etc ate ate aag
etc cat age ett ett ggg aac aaa ttk teg ett att gee ggg aga tta
ccg gga aga aca gat aac gac gat aag aat cac tgg aac aeg cat ata
ega aga aag ett ata aac aga ggg att gat ccc aeg aag agt cat aga cca
ate cca gaa tea tea get ttk cca gat ttk ccc cct aca caa eta gaa
cca gtt aeg aat cac att aet cta aac ggg ggg gggt cag ccc ggg ggg
atg gtc gaa ggg aag tgg cat aag agt ata age tgg cgg gga aac tea gag aag
ate tea atg gga aca gat aec act ttk caa gaa gaa gaa gaa gat ggc ggc gtt caa
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gat gtt gat cgt ett caa ggg cat gga aag tea aca aeg cca cgt ttk
ttc aag tgg age ttk ggg atg ata aac ggc atg gag ttk aga ttk ggg
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ggt gaa ggt tgg tgg ega tet ett cct aga gee get ggt etc ett ege
tgc ggt aaa age tgc cgt ett egg tgg att aac tat etc ega cct gat
etc aaa aga aac ttt ctt aga cat gat gaa gat gaa ett ate ate aag
ett cat age etc eta ggc aac aag tgg tet ttg att geg geg aga tta
cct gga aga aca gat aac gag ate aag aac tac tgg aac aca cat ata
aag agg aag tgg age aaa ggg att gat cca gee act cat aga ggg
ate aac gag gca aaa att tet gat ttg aag aaa aca aag gag cac caa att
gta aaa gat gtt tet ttt gtt aca aag ttt gag gaa aca gag aag tet
ggc gac cag aag caa aat aag tat att ega aat ggg tta gtt tgc aaa
ga aag gag gta gtt gtt gaa gaa aaa ata ggc cca gat ttt aat ett
gag ett aag get ttt gtt age aca aag gaa ata tet act
tgc act geg tee cgt ttt tac atg gaa aac gag atg gag tgt aat aag
gaa act tgt aag tgt cca aca gag aat agt age age att age tat tet
tet att gat att aaggt aag tgt tat gac ttc tgt ggt tgt
aag aca aga att ttg gat ttt ega age ttg gaa atgaaa taa

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gaa cgt age aat ggc ggt age agg aec aag aca acc tgg tea cag att etc aag gtt etc age ege aag tgg tgc tgg aag cgc cca act etc gca ctg ccc
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gtg gag etc aac aag gag gta gaa cca act gaa cct gca get cag ccc
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SEQ ID NO:44
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tgg ctt ggt acc ttt gag aeg geg gag gag geg geg eeg geg tac gac
gag gee gee ctt ctg atg age ggc ege aec aeg acc aac tcc cca
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etc gtc ate tga
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ggc gag ggc tgc tgg ege teg etc ccc aag gee gee ggc etc etc ege
tgc ggc aag age tgc ege etc ege tgg ate aac tac etc ege ccc gac
etc aag ege ggc aac ttc acc gee gac gag gac gac etc ate ate aag
etc cac age etc etc ggc aac aag tgg tet ctg ate geg geg agg ctg
ccg ggg aag aeg gac aac gag ate aag aac tac tgt aac aeg cac ate
ege eeg aag aag aag tgg aag ggg ate gac ccc gtc aeg cac ege ccc
gtc aac gee gee gee acc ate tee ttc cat ccc cag ccc ccc cca
aeg aeg aag gag gag cag etc ata etc age aag ccc ccc aag tgt ccc
gac etc aac ctg gac etc tgc age age ccc cgg teg tgc cag gaa gaa
gac gat gac tat gag geg aag ccc geg atg ate gtg agg geg cgg gag
ctg cag gat gac tat gag tat gag aag ccc cgg cgg cgg
cag aag gag tgc aag tgt age ggc ggc ggc gee ggc gee ggc gee ggc
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SEQ ID NO:46

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ggc gaa ggt tgc tgg ega teg ctg ccc aag gee gee ggc etc etc ege
tgt ggc aag age tgc ege etc eeg tgg ate aac tac etc egg cct gac
etc aag ege ggc aee etc ctc acc gag gac gag gat ctg ate ate aag
ett cac age ett tta ggc aac aai tgg tet ctg ata gee ggg agg ttt
cca gga agra eeg gee ccc cgg gaa aac tac tgt aac aag cac ate
agg agg aag ctg ctg age ctg ggc ate gac ccc gttg aca cac egg ccc
ate aac gac age geg tee aac ate acc ata tea ttc gag geg gee geg
geg geg egg aag gac gac aag gee gee gtg ttg eeg ega gag gac cat
cct cat cag ccg aag geg gttg aca gttg gca cag gag cag cag gca gee
gee gat tgtg ggc cat cgg aac cca etc aag gct ccc gac etc aat ctg
gac etc tgc ate age etc cct tee cca gaa gag ccc atg atg atg aag
ccg gttg aag agg gag acc ggc gtc tgc tgc ttc age tgc age ctg ggg etc
ccc aag age caa gac tgt aag tgt age age ttc ctt gga etc agg aca
gee atg etc gac etc ctc aga age ttc gga atg aaa tga

SEQ ID NO:47

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tgc ggc aag age tga aga ett cgt tgg ate aac tat tta aga cct gac
ett aag cgt ggc aat ttt act gaa gaa gaa gat gat gac etc att ate aag
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aga agg aag etc ttg aat aga ggc ata gat cct geg act cat agg cca
etc aat gaa cca gee cca gaa get tea aca aca ata tet ttc age act
act acc tea ttg aag gaa gaa gat teg tgg agt tet gtt aab gag gaa aag
aat aag gag aag aat att age gca get get ttt ata tgc aab gaa gag
aaa acc cca gtt cca gaa agg tgt cca gac ttg aat ett gaa ett aga
att age ett cct tgc cca aac cag cct gat cgt cag cca ctc aab
act gga gga aht aca aht ett ttt get tgt age ttg ggg eta caa
SEQ ID NO:48

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tgg ace aag gag gaa gac gat ege ett gtt get tac att aca get cac

ggt gaa ggt tgg ege tea ett cct aca gaa gee get ggc ett ett aca

tgg ggc aag agt tgc aga ett cgt tgg aca gat act act gga age

ett aca gtt ggc aat ttc ace gaa gca gaa gat gac etc att act aa

etc cat age etc ett gga aac aa aag gtc tea etc atg get gga gaa act ccc
cca ggg aca gat act aag gat gta gataa aag atg tgg aac aca act ata

SEQ ID NO:49

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cat ccc etc ett aac agg agg gtg gtc tgg cgg ace tcc gag aca get
gag gag gca gac aca act cag tet ccc tet act cag etc aca aag etc

ege aac gaa gca gac gaa ac gga gtc cgc cca aac agg act aca age

cca aca cca get gac gaa agg gag aca age aag gaa gac ggc age aac
tee tet ace gaa ctc gag act act ctc atg ggt act gc act age aca aag

tgc tgc aag ggc gcc gtg ccc cca tet ccc tcg atg ege ett aca gcc
gag gaa tet ca ca ctc act ctt ggt gtt tgg gac aag cgg gaa gg cgt get
gac tee aac ggc ggt ggc act gcg atg act tgg ggt gcg gcc ac aag etc

SEQ ID NO:50

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tgg ctc gtc gc ac aag gca gag aga cgg gag ac gcc aac gca aca

gac gca gct ggc cgg ggc gtc ctc ggt cag cca gct ctc aag gca gac

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etc aag ege ggc aac ttc acc gcc gac gag gac gac etc ate aee aag
c tg cac age etc etc ggc aac aag tgg teg etc ate gcc gcg egg etc
c cg ggg egg aeg gac aac gat aee aac tac tgg aac aeg cac ate
eeg egg aag age ett ggc aac aag gac etc gcc gtc aeg ccc gac
eate gcc gac gcc ggc gcc ggc acc gtc acc acc aee aeg etc cg ccc
aac aag gcc ccc gcc gtc gca ggc gcg cca caa cat ccg ccc
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caa aag ggc gcc ccc ggg tgc age tgc age age aac age cac cac
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gac atg aag tga

SEQ ID NO:52
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etc aag tgc ccc gcc etc aac ctg gac etc tgc ate age ccc ccg ccg gcg
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gac ttc aga age etc gag atg aag tga

SEQ ID NO:53
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cat ccc etc ett aag agg aag gtc tgc ctg gtt acc ttt gag aeg get
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cct acc cca get gcg gga agg gac gcc ege age aac ttc age age ggc
tee tet acc acc aac ctg tee cag acc etc agt gcg aag etc ege aee
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gag aag tcc cac att ggt gtt tgg cag aag cgt gca gga gee cgt get
gac tcc aac tgg gtc atg aca gtt gac etc aac aaa gat gca gca tcc
act gat get gca tea cag tcc aca tea gca aca act get cca cca gee
aeg ccg atg gat gag cag gau agg ate gca ctg caa atg ate gaa gag
ttg ctg age age age cca get tea ccc tea aac gga gat gac caa
ggt ege atte atte tga

SEQ ID NO:54
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tcg cac age etc etc ggg aac aag tgg teg etc atte gee geg egg etc
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SEQ ID NO:55
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tgc ggc aag age tgc ege etc ege tgg aac tac etc ege ccc gag
etc aag ege ggc aac ttc aeg gag gaa gag gac gag etc atte gtc aag
cgg cac age etc etc ggg aac aag tgg ctc atg ege gag gag ctc
ccc ggc agg aeg gac aac gag ate aag aac tac tgg aac aeg cac atc
egg aag aag ctg ctg age aag ggg ate gag ccc gtg aeg cac ege cgg
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ggc gag ggg tgc tgg ege tcc etc ccc aag gee geg ggc ctg ctg ege
Table 2: Examples of Vascular Xylem Tissue Targeting Promoters for Gene Combination

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<th>Referenced expression database¹</th>
<th>Expression²</th>
<th>Gene name</th>
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<th>Sequence Feature and Location</th>
<th>Protein family (Pfam)</th>
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<th>Gene ID</th>
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Table 2: Examples of Vascular Xylem Tissue Targeting Promoters for Gene Combination

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<th>Referenced expression database</th>
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<th>Sequence Feature and Location</th>
<th>Protein family (Pfam)</th>
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2 Relative gene expression value in vascular tissues or xylem-related organ.

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cttaccttact actattattt cccagatttt gttttatattt gtttaaaaat gtttaatttaa
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cccaagggaa atcagccttc gaattggaat atgaaaagaa gagtgtgagc caatacaaag cagatttgag gacatggctt
tgcaactgcg gataatgctg cacttctcag caaatatttt ttttcccttt
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Twenty-five independent plasmids suitable for Agrobacterium-mediated plant transformation were generated (see Table 3). Nine (9) combinations (construct 001 to construct 009 shown in Table 3) and one vector control were integrated into a dicot binary vector for alfalfa, canola, and other dicot transformation, and sixteen (16) combinations (construct 010 to construct 025 shown in Table 3) and one vector were generated into a monocot binary vector for sorghum, switchgrass, and other monocot transformation.

Table 3: List of Constructs

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</tbody>
</table>
A similar approach was used for preparation of each of the constructs and plasmids. Briefly, approximately 1.0 kb of genome sequences upstream of the respective AtCTL2 (AT3G16920), AtLAC4 (AT2G38080), AtCesA4 (AT5G44030), AtCesA8 (AT4G18780), AtFLA1 (AT5G03170), AtCesA7 (AT5G17420), and AtIRX9 (AT2G37090) start codons were amplified by PCR from Arabidopsis thaliana genomic DNA; and the nucleotide sequences from start codon to stop codon of transcription factor AtMYB32 (At4g34990) and AtMYB4 (At4g38620) coding region were amplified by PCR from Arabidopsis thaliana cDNA derived from reverse transcription reaction using stem tissue RNA. The two nucleotide sequences (promoter and coding regions) were then combined with RBS terminator region into a commonly used binary vector plasmid (including Kanamycin selection marker) through Gibson cloning (Gibson et al., “Enzymatic Assembly of DNA Molecules up to Several Hundred Kilobases,” Nature Methods 6(5):343-345 (2009), which is hereby incorporated by reference in its entirety). The result was assembly of constructs 001-009: pAtCTL2-AtMYB32-tRBS (Fig. 1), pAtLAC4-AtMYB32-tRBS (Fig. 2), pAtCesA4-AtMYB32-tRBS (Fig. 3), pAtCesA8-AtMYB32-tRBS (Fig. 4), pAtFLA1-AtMYB32-tRBS (Fig. 5), pAtCesA7-AtMYB32-tRBS (Fig. 6), pAtIRX9-AtMYB3 2-tRBS (Fig. 7), pAtCesA4-AtMYB4-tRBS (Fig. 8), and pAtCesA8-AtMYB4-tRBS (Fig. 9), respectively. A representative plasmid map for construct 003 is shown in Fig. 26. Similar dicot-functional plasmids containing constructs 001, 002 and 004-009 were also prepared.

Using this same approach, approximately 1.0 kb of genome sequences upstream of the respective ZmCesA12 (GRMZM2G142898) ZmCesA1 (GRMZM2G037413), OsCesA4 (LOC_Os05g4620), and OcCesA7 (LOC_Os20g32980) start codons were amplified by PCR from corn and rice genomic DNA; and the nucleotide sequences from start codon to stop codon of transcription factors ZmMYB31 (GRMZM2G050305), ZmMYB42 (GRMZM2G19239), PvMYB4 (Pavir.J16675), and OsSHN1 (LOC_Os06g40l50) were amplified by PCR from corn, rice, and switchgrass cDNA derived from reverse transcription reaction using stem tissue RNA. The two nucleotide sequences (promoter and coding regions) were then combined with RBS terminator region into a commonly used binary vector plasmid (including Kanamycin selection marker) through Gibson cloning (Gibson et al., “Enzymatic Assembly of DNA Molecules up to Several Hundred Kilobases,” Nature Methods 6(5):343-345 (2009), which is hereby incorporated by reference in its entirety). The result was assembly of constructs 010-025: pZmCesA12-ZmMYB3 1-tRBS (Fig. 10), pZmCesA1-1-ZmMYB31-tRBS (Fig. 11), pOsCesA4-ZmMYB31-tRBS (Fig. 12), pOsCesA7-ZmMYB31-tRBS (Fig. 13), pZmCesA12-ZmMYB42-tRBS (Fig. 14), pZmCesA1-1-ZmMYB42-tRBS (Fig. 15), pOsCesA4-ZmMYB42-tRBS (Fig. 16),
pOsCesA7-ZmMYB42-tRBS (Fig. 17), pZmCesA12-PvMYB4-tRBS (Fig. 18), pZmCesAl1-PvMYB4-tRBS (Fig. 19), pOsCesA4-PvMYB4-tRBS (Fig. 20), pOsCesA7-PvMYB4-tRBS (Fig. 21), pZmCesAl2-OsSHN1-tRBS (Fig. 22), pZmCesAl1-OsSHN1-tRBS (Fig. 23), pOsCesA4-OsSHN1-tRBS (Fig. 24), and pOsCesA7-OsSHN1-tRBS (Fig. 25). A representative plasmid map for construct 018 is shown in Fig. 27. Similar monocot-functional plasmids containing constructs 010-017 and 019-025 were also prepared.

Empty vectors corresponding to those shown in Figs. 26 and 27, but lacking the constructs 001-025, were used as controls in the following molecular biology analysis and phenotypic analysis described in the following Examples.

Example 2 - Introduction of Construct Nos. 003 and 008 into Alfalfa (Medicago sativa L. cv Regen S)

The vectors containing construct Nos. 003 and 008, along with the control vector, were used to generate transgenic alfalfa (Medicago sativa L. cv Regen S) using tissue culture and the Agrobacterium-mediated transformation method. After selection of primary transgenic plants using kanamycin, multiple events were obtained from the regeneration medium, cloned and propagated vegetatively, and transferred to soil after roots were developed. Introduction of the transgene cassette in the genome of the regenerated plants was confirmed by PCR using genomic DNA extracted from leaves.

The experimentally confirmed and propagated plants were grown in individual pots inside growth chambers with 18/6-hour light/dark cycles. Table 4 shows biomass yield and stem internode length at approximately 250 days after propagation along with similar data for the control alfalfa plant with the construct. Engineered alfalfa shows approximately 15-18% longer internodes and 58-63% increased yields compared to the control plant. Representative images of control and inventive vector-transformed alfalfa plants are shown in Fig. 28.

<table>
<thead>
<tr>
<th>Construct</th>
<th>Events (n)</th>
<th>Stem length</th>
<th>Fresh weight</th>
<th>Dry weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8 (17)</td>
<td>57.56 ± 5.42</td>
<td>11.42 ± 2.99</td>
<td>2.20 ± 0.51</td>
</tr>
<tr>
<td>No. 008</td>
<td>8 (21)</td>
<td>66.47 ± 7.77***</td>
<td>17.74 ± 5.80***</td>
<td>3.49 ± 1.10***</td>
</tr>
<tr>
<td>No. 003</td>
<td>7 (16)</td>
<td>68.37 ± 6.28***</td>
<td>17.47 ± 3.95***</td>
<td>3.60 ± 0.73***</td>
</tr>
</tbody>
</table>

*** p<0.001

RNA was extracted from the leaves and stems in the engineered alfalfa lines for quantitative RT-PCR. Results show that the expression level of the target TF was similar to that
of the native UbiQ gene. Moreover, transcript levels of the target TF were approximately 50 times higher in stem-enriched tissues compared to leaves, which highlights the tissue-preferential expression pattern enabled by the promoter used to drive expression of the TF. During the course of propagation, engineered alfalfa No. 003 also showed better development not only in stems but also significantly in roots. Table 5 shows regeneration efficiency and root length of alfalfa control and engineered No. 003 at 13 days after initiation of the regeneration step using sectioned internodes.

Table 5: Regeneration efficiency of new roots from sectioned stems

<table>
<thead>
<tr>
<th>Construct</th>
<th>Prepared stem fragments (n)</th>
<th>Number of rooted stems</th>
<th>Rooting efficiency (%)</th>
<th>Regenerated root length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>59</td>
<td>10</td>
<td>16.95</td>
<td>1.91 ± 2.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Max: 19.0)</td>
</tr>
<tr>
<td>No. 003</td>
<td>62</td>
<td>24</td>
<td>38.71</td>
<td>8.25** ± 5.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(Max: 29.0)</td>
</tr>
</tbody>
</table>

** pO.01

[0129] The construct No. 003 and No. 008 alfalfa lines also showed a reduction of insoluble lignin and ash content (Table 6) and changes in lignin monomeric composition (Table 7), which indicate the inventive constructs also enhance biomass degradability through reduced lignin composition. Moreover, 12% and 16% less insoluble lignin content and 20% and 38% less ash content were observed in the No. 003 and No. 008 alfalfa lines, respectively, compared to control lines.

Table 6: Ash and insoluble lignin content in cell wall biomass

<table>
<thead>
<tr>
<th>Construct</th>
<th>Events (n)</th>
<th>Insoluble lignin (%)</th>
<th>Ash (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>8 (8)</td>
<td>12.96 ± 1.94</td>
<td>0.26 ± 0.09</td>
</tr>
<tr>
<td>No. 008</td>
<td>11 (11)</td>
<td>10.93* ± 0.86</td>
<td>0.16 ± 0.04</td>
</tr>
<tr>
<td>No. 003</td>
<td>10 (10)</td>
<td>11.45* ± 0.57</td>
<td>0.21* ± 0.04</td>
</tr>
</tbody>
</table>

* p<0.05

Table 7: Lignin monomeric composition in cell wall biomass

<table>
<thead>
<tr>
<th>Construct (events)</th>
<th>H unit (%)</th>
<th>G unit (%)</th>
<th>S unit (%)</th>
<th>S/G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (3)</td>
<td>1.77 ± 0.74</td>
<td>63.58 ± 1.24</td>
<td>34.65 ± 0.50</td>
<td>0.55 ± 0.02</td>
</tr>
<tr>
<td>No. 008 (3)</td>
<td>1.48 ± 0.34</td>
<td>48.39 ± 1.47</td>
<td>50.13 ± 1.76</td>
<td>1.04** ± 0.07</td>
</tr>
<tr>
<td>No. 003 (3)</td>
<td>1.48 ± 0.39</td>
<td>51.08 ± 3.28</td>
<td>46.99 ± 3.08</td>
<td>0.93* ± 0.11</td>
</tr>
</tbody>
</table>

** pO.01, * p<0.05
Table 8 summarizes the composition of saccharide released from cell wall fraction in the control lines and construct No. 003 and No. 008 lines. After a mild thermochemical treatment, glucose, xylose, and arabinose saccharides from constructs No. 003 and No. 008 biomass cell wall fraction were released two to three times more efficiently than the control lines.

<table>
<thead>
<tr>
<th>Construct (events)</th>
<th>Glucose (%)</th>
<th>Xylose (%)</th>
<th>Arabinose (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (11)</td>
<td>4.71 ± 2.46</td>
<td>8.01 ± 3.32</td>
<td>1.56 ± 0.18</td>
</tr>
<tr>
<td>No. 008 (11)</td>
<td>14.14** ± 3.79</td>
<td>15.78** ± 3.57</td>
<td>1.82** ± 0.18</td>
</tr>
<tr>
<td>No. 003 (11)</td>
<td>14.11** ± 4.05</td>
<td>15.84** ± 3.48</td>
<td>1.98*** ± 0.23</td>
</tr>
</tbody>
</table>

*** p<0.001, ** p<0.01

Example 3 - Introduction of Construct No. 004 into Canola (Brassica napus L. cv Westar)

The vector containing construct No. 004 and the control vector were used to generate transgenic canola (Brassica napus L. cv Westar) using tissue culture and the Agrobacterium-mediated transformation method. After selection of primary transgenic plants using kanamycin, multiple events were obtained on the regeneration agar plates and transferred to soil after vegetative tissue and roots were developed and cloned by vegetative propagation.

Table 9 summarizes the measured heights of five (5) transformed control canola lines compared to the four (4) No. 004 lines. The height of the plants transformed with the inventive method is approximately two times taller than the height of the control plants at 130 days after regeneration. These results indicate the inventive constructs enhance stem internode development and may increase biomass yield. Representative images of control and inventive vector-transformed canola plants are shown in Fig. 29.

<table>
<thead>
<tr>
<th>Construct</th>
<th>Events (n)</th>
<th>Days after regeneration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>100_</td>
</tr>
<tr>
<td>Control</td>
<td>5 (5)</td>
<td>18.58 ± 1.78</td>
</tr>
<tr>
<td>No. 004</td>
<td>4 (4)</td>
<td>18.90 ± 0.70</td>
</tr>
</tbody>
</table>

*** p<0.001, ** p<0.01, *p<0.05

The measured number of branches and flowers for five (5) control lines compared to the four (4) lines engineered with inventive constructs are shown in Table 10. Among the
examined lines at day 150 after regeneration, two control lines and three No. 004 lines possessed grain pods, although grain in mature pods was observed in only No. 004 lines as shown in Table 11. All of the results indicate the inventive constructs enhance vegetative growth, root redevelopment as well as reproductive tissue development, and may increase overall yields.

### Table 10: Numbers of Branch and Flower at 130 and 140 days after regeneration (DAR)

<table>
<thead>
<tr>
<th>Construct</th>
<th>Events (n)</th>
<th>Number of Branch 130 DAR</th>
<th>Number of Branch 140 DAR</th>
<th>Number of Flower 130 DAR</th>
<th>Number of Flower 140 DAR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5 (5)</td>
<td>1.00 ± 0.00</td>
<td>1.40 ± 0.48</td>
<td>0.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>No. 004</td>
<td>4 (4)</td>
<td>4.00** ± 1.00</td>
<td>4.00* ± 1.00</td>
<td>12.00** ± 4.50</td>
<td>41.50*** ± 8.75</td>
</tr>
</tbody>
</table>

** p<0.01 , *** p<0.001 , * p<0.05

### Table 11: Number of seed pods and seeds at 150 days after regeneration

<table>
<thead>
<tr>
<th>Construct</th>
<th>Number of plant with grain pods</th>
<th>Number of grain pods per event</th>
<th>Diameter of pods (cm)</th>
<th>Number of grain per pod</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2 (2)</td>
<td>5.50 ± 1.50</td>
<td>1.60 ± 0.10</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>No. 004</td>
<td>3 (3)</td>
<td>25.67** ± 15.6</td>
<td>3.17** ± 1.38</td>
<td>6.67** ± 4.44</td>
</tr>
</tbody>
</table>

** p<0.01

### Example 4 - Introduction of Construct No. 018 into Sorghum (Sorghum bicolor P898012)

[0134] The vector containing construct No. 018 and the control vector were used to generate transgenic sorghum (Sorghum bicolor P898012) using tissue culture and the Agrobacterium-mediated transformation method. After selection of primary transgenic plants using glufosinate, multiple lines were obtained on the regeneration agar plates and transferred to soil after vegetative issue and roots were developed and cloned by vegetative propagation. Introduction of the transgene cassette was confirmed by PCR using genomic DNA extracted from the regenerated plant’s leaves as the template.

[0135] The experimentally confirmed and propagated plants were grown in individual pots inside the greenhouse with 18/6-hour light/dark cycles. Table 12 shows biomass yield, number of branches and plant height at approximately 250 days after propagation, along with similar data for sorghum control lines. The engineered sorghum lines showed approximately 80% more dry weight than the control lines, probably due to enhanced branching specific to the engineered lines. The obtained grain number and weight data, summarized in Table 13, indicate improved grain production. Representative images of control and inventive vector-transformed sorghum plants are shown in Fig. 30.
Example 5 - Introduction of Construct No. 018 into Switchgrass (*Panicum virgatum Alamo*)

[0136] The vector containing construct No. 018 and the empty vector control were used to generate transgenic switchgrass (*Panicum virgatum Alamo*) using tissue culture and the Agrobacterium-mediated transformation method. After selection of primary transgenic plants using hygromycin, multiple events were obtained on the regeneration agar plates and transferred to soil after vegetative tissue and roots were developed and cloned by vegetative propagation. Introduction of the transgene cassette was confirmed by PCR using genomic DNA extracted from the regenerated plant’s leaves as the template.

[0137] The experimentally confirmed and propagated plants were grown in individual pots with 18/6 hours light/dark cycles at a green house facility. RNA was extracted from the engineered switchgrass leaves and stems for quantitative RT-PCR. The analysis confirmed the target TF genes are mainly expressed in the stem rather than the leaves, suggesting that the tissue-preferred expression was enabled by the used cellulose synthase gene promoters.

[0138] During the course of transgenic plant generation, the engineered switchgrass with the gene cassette tended to show better differentiation and vegetative development. Table 14 compares plant height and number of tillers at 40 days after ratooning. In comparison to the control lines, approximately double the growth and 2-3x times more tillers were observed in the engineered lines. Representative images of control and inventive vector-transformed switchgrass plants are shown in Fig. 31.
Table 14: Plant height and number of tillers (40 days after ratooning)

<table>
<thead>
<tr>
<th>Construct</th>
<th>Event (n)</th>
<th>Plant height (cm)</th>
<th>Number of tillers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3 (3)</td>
<td>83.00 ± 9.42</td>
<td>6.00 ± 2.83</td>
</tr>
<tr>
<td>No. 010</td>
<td>5 (5)</td>
<td>159.00** ± 18.14</td>
<td>22.80** ± 9.66</td>
</tr>
<tr>
<td>No. 012</td>
<td>8 (8)</td>
<td>178.00*** ± 10.09</td>
<td>32.88*** ± 8.33</td>
</tr>
<tr>
<td>No. 013</td>
<td>5 (5)</td>
<td>161.60*** ± 4.63</td>
<td>25.20** ± 10.72</td>
</tr>
<tr>
<td>No. 020</td>
<td>7 (7)</td>
<td>139.71** ± 23.14</td>
<td>24.00** ± 7.80</td>
</tr>
<tr>
<td>No. 022</td>
<td>8 (8)</td>
<td>164.13** ± 15.81</td>
<td>16.38** ± 4.47</td>
</tr>
</tbody>
</table>

*** p<0.001, ** p<0.01

The switchgrass engineered by MYB TFs showed not only faster growth but also approximately 10-20% less insoluble lignin content (see Table 15: Constructs No. 010, No.012, No. 013, and No. 020). Plants engineered by ERF TFs, however, maintained a similar amount of insoluble lignin (see Table 15: Construct No. 022), suggesting that the R2R3-MYB subfamily 4 and ERF/AP2 subfamily B-6 contribute to faster growth through distinguished mechanisms.

Table 15: Insoluble lignin content in upper and lower stem biomass

<table>
<thead>
<tr>
<th>Construct</th>
<th>Event (n)</th>
<th>Upper stem biomass</th>
<th>Lower stem biomass</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>6</td>
<td>17.46 ± 0.59</td>
<td>21.13 ± 0.46</td>
</tr>
<tr>
<td>Control</td>
<td>3 (11)</td>
<td>17.06 ± 0.41</td>
<td>20.00 ± 0.90</td>
</tr>
<tr>
<td>No. 010</td>
<td>5 (5)</td>
<td>15.92** ± 0.32</td>
<td>18.62** ± 0.54</td>
</tr>
<tr>
<td>No. 012</td>
<td>5 (5)</td>
<td>15.95** ± 0.92</td>
<td>18.33** ± 0.90</td>
</tr>
<tr>
<td>No. 013</td>
<td>4 (4)</td>
<td>15.15** ± 0.25</td>
<td>17.44** ± 0.34</td>
</tr>
<tr>
<td>No. 020</td>
<td>4 (4)</td>
<td>15.51** ± 0.41</td>
<td>16.96** ± 0.16</td>
</tr>
<tr>
<td>No. 022</td>
<td>7 (7)</td>
<td>17.59 ± 1.47</td>
<td>21.18 ± 0.85</td>
</tr>
</tbody>
</table>

** p<0.01

Faster development in the reproductive phase was also observed in the engineered lines. Mature seeds produced after the completion of reproductive development were harvested and quantitatively analyzed (Table 16). Among engineered lines, construct No. 012, No. 013, and No. 018 switchgrass produced large quantities of seeds, approximately 6-7x more seeds than the control lines.
Table 16: Number of mature seeds produced

<table>
<thead>
<tr>
<th>Construct</th>
<th>Event (n)</th>
<th>Number of seeds produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11 (11)</td>
<td>165.4 ± 39.9</td>
</tr>
<tr>
<td>No. 012</td>
<td>12 (12)</td>
<td>915.3** ± 153.7</td>
</tr>
<tr>
<td>No. 013</td>
<td>5 (5)</td>
<td>1012.0** ± 153.2</td>
</tr>
<tr>
<td>No. 018</td>
<td>7 (7)</td>
<td>1109.0* ± 451.4</td>
</tr>
</tbody>
</table>

** p<0.01, * p<0.05

[0141] Germinating efficiency from the obtained T1 seeds was examined by using a 96 well format system. Two containers that included pre-soaked sponges with 96 well halls were prepared for seed planting and used for the germination process in dark conditions at 25 °C. Time-course observation of the germinated seedlings confirmed that the seeds from construct No. 012 and No. 018 switchgrass enhance not only the germination rate but also seedling development (seedling length) in comparison to the control lines (Table 17).

Table 17: Time course of T1 seedling length (cm)

<table>
<thead>
<tr>
<th>Construct</th>
<th>0</th>
<th>8</th>
<th>10</th>
<th>13</th>
<th>16</th>
<th>20</th>
<th>23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.00</td>
<td>0.19</td>
<td>0.49</td>
<td>1.35</td>
<td>1.42</td>
<td>1.75</td>
<td>3.31</td>
</tr>
<tr>
<td>Container 1</td>
<td>± 0.00</td>
<td>± 0.48</td>
<td>± 0.84</td>
<td>± 1.14</td>
<td>± 1.17</td>
<td>± 1.20</td>
<td>± 1.78</td>
</tr>
<tr>
<td>Control</td>
<td>0.00</td>
<td>0.00</td>
<td>0.29</td>
<td>1.13</td>
<td>1.45</td>
<td>2.06</td>
<td>3.76</td>
</tr>
<tr>
<td>Container 1</td>
<td>± 0.00</td>
<td>± 0.00</td>
<td>± 0.51</td>
<td>± 1.32</td>
<td>± 1.37</td>
<td>± 1.50</td>
<td>± 2.49</td>
</tr>
<tr>
<td>No. 012</td>
<td>0.00</td>
<td>2.22**</td>
<td>3.57**</td>
<td>4.40**</td>
<td>4.61**</td>
<td>4.92**</td>
<td>5.48**</td>
</tr>
<tr>
<td>Container 2</td>
<td>± 0.00</td>
<td>± 1.92</td>
<td>± 2.36</td>
<td>± 2.61</td>
<td>± 2.70</td>
<td>± 2.56</td>
<td>± 2.43</td>
</tr>
<tr>
<td>No. 012</td>
<td>0.00</td>
<td>2.22**</td>
<td>3.25**</td>
<td>4.03**</td>
<td>4.19**</td>
<td>4.78**</td>
<td>5.74**</td>
</tr>
<tr>
<td>Container 2</td>
<td>± 0.00</td>
<td>± 1.57</td>
<td>± 1.85</td>
<td>± 1.34</td>
<td>± 1.22</td>
<td>± 0.99</td>
<td>± 0.96</td>
</tr>
<tr>
<td>No. 018</td>
<td>0.00</td>
<td>1.77**</td>
<td>2.20**</td>
<td>3.10**</td>
<td>3.80**</td>
<td>4.77**</td>
<td>6.35**</td>
</tr>
<tr>
<td>Container 1</td>
<td>± 0.00</td>
<td>± 1.72</td>
<td>± 2.32</td>
<td>± 2.35</td>
<td>± 2.52</td>
<td>± 2.48</td>
<td>± 2.94</td>
</tr>
<tr>
<td>No. 018</td>
<td>0.00</td>
<td>2.07**</td>
<td>2.61**</td>
<td>3.37**</td>
<td>4.16**</td>
<td>5.06**</td>
<td>6.50**</td>
</tr>
<tr>
<td>Container 2</td>
<td>± 0.00</td>
<td>± 1.97</td>
<td>± 2.59</td>
<td>± 2.63</td>
<td>± 2.82</td>
<td>± 2.67</td>
<td>± 2.54</td>
</tr>
</tbody>
</table>

** p<0.01

[0142] Growth and morphology of the engineered lines were also examined in a field environment. Switchgrass plantlets were grown under greenhouse conditions and transplanted to field plots with a total of 1,000 square feet. A total of 30 plantlets was distributed to each plot, and a total of 120 plantlets were planted per construct. Table 18 shows biomass data for constructs No. 012 and No. 018 switchgrass that yielded approximately 35% more than wild-type and control lines.
Table 18: Biomass yield (total dry weight) from the field test

<table>
<thead>
<tr>
<th>Construct</th>
<th>n per plot</th>
<th>Number of plots</th>
<th>Biomass yield (kg) from one plot</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>30</td>
<td>4</td>
<td>8.82 ± 1.04</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>4</td>
<td>8.44 ± 1.09</td>
</tr>
<tr>
<td>No. 012</td>
<td>30</td>
<td>4</td>
<td>12.14** ± 1.29</td>
</tr>
<tr>
<td>No. 018</td>
<td>30</td>
<td>4</td>
<td>12.96** ± 1.21</td>
</tr>
</tbody>
</table>

** p<0.01

Construct No. 012 and No. 018 plants grown in field conditions also showed cell wall characteristics similar to those observed in laboratory-grown plants. As shown in Table 19, the constructs had 10% less insoluble lignin content and higher S/Gunit composition ratios in comparison to wild-type switchgrass, suggesting the engineered switchgrass could be useful as potential forage with better digestibility and/or as less recalcitrant feedstock for a cost-effective biorefinery process.

Table 19: Lignin characteristics from the field test.

<table>
<thead>
<tr>
<th>Construct</th>
<th>Insoluble lignin (%)</th>
<th>G unit (%)</th>
<th>S (%)</th>
<th>S/G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT</td>
<td>22.74 ± 1.94</td>
<td>75.01 ± 0.05</td>
<td>24.99 ± 0.47</td>
<td>0.33 ± 0.0008</td>
</tr>
<tr>
<td>No. 012</td>
<td>20.48** ± 1.58</td>
<td>69.47** ± 0.70</td>
<td>30.53** ± 0.53</td>
<td>0.44** ± 0.01</td>
</tr>
<tr>
<td>No. 018</td>
<td>20.90** ± 0.78</td>
<td>65.49** ± 0.53</td>
<td>34.51** ± 0.54</td>
<td>0.53** ± 0.01</td>
</tr>
</tbody>
</table>

** p<0.01

Although preferred embodiments have been depicted and described in detail herein, it will be apparent to those skilled in the relevant art that various modifications, additions, substitutions, and the like can be made without departing from the spirit of the invention and these are therefore considered to be within the scope of the invention as defined in the claims which follow.
WHAT IS CLAIMED IS:

1. A nucleic acid construct comprising:
   a polynucleotide encoding a transcription factor polypeptide; and
   a heterologous, tissue-specific promoter operably linked to the
polynucleotide encoding the transcription factor polypeptide, wherein the promoter specifically
directs expression of the transcription factor polypeptide in vascular xylem tissue of a plant.

2. The nucleic acid construct according to claim 1, wherein the vascular
   xylem tissue comprises one or more of (i) procambium/cambium cell, (ii) xylem cell, and (iii) a
   fiber cell type of a plant.

3. The nucleic acid construct according to claim 1, wherein the tissue-
specific promoter directs expression of the transcription factor polypeptide during a time when
   initial deposition of cellulosic and hemicellulosic components in vascular xylem tissue occurs.

4. The nucleic acid construct according to any one of claims 1 to 3, wherein
   the tissue-specific promoter directs expression of the transcription factor polypeptide in aerial
   parts of the plant.

5. The nucleic acid construct according to any one of claims 1 to 3, wherein
   the tissue-specific promoter directs expression of the transcription factor polypeptide in roots of
   the plant.

6. The nucleic acid construct according to any one of claims 1 to 3, wherein
   the tissue-specific promoter is a gene promoter for secondary cell wall development, an
   endomembrane protein gene promoter, or a secondary wall cellulose synthase (CesA) promoter.

7. The nucleic acid construct according to any one of claims 1 to 6, wherein
   the transcription factor polypeptide modulates expression of a series of genes involved with cell
   wall and secondary metabolite biosynthetic pathways.

8. The nucleic acid construct according to any one of claims 1 to 6, wherein
   the transcription factor polypeptide is a polypeptide that can act before secondary wall master
   transcription factors as an upstream transcriptional regulator.
9. The nucleic acid construct according to any one of claims 1 to 6, wherein the transcription factor polypeptide is an R2R3-MYB subfamily 4 polypeptide.

10. The nucleic acid construct according to any one of claims 1 to 6, wherein the transcription factor polypeptide is an ERF/AP2 subfamily B-6 polypeptide.

11. The nucleic acid construct according to any one of claims 1 to 10, wherein the nucleic acid construct further comprises a 3’ transcription termination polynucleotide.

12. The nucleic acid construct according to any one of claims 1 to 11, wherein the nucleic acid construct comprises DNA.

13. A recombinant expression vector comprising the nucleic acid construct according to any one of claims 1 to 12.

14. The recombinant expression vector according to claim 13, wherein the vector is operable in dicots.

15. The recombinant expression vector according to claim 13, wherein the vector is operable in monocots.

16. The recombinant expression vector according to claim 13, wherein the vector is a plasmid.

17. The recombinant expression vector according to claim 13, wherein the vector is Agrobacterium comprising the plasmid.

18. A recombinant host cell comprising the nucleic acid construct of any of any one of claims 1 to 11 or the recombinant expression vector according to any one of claims 13 to 17.

19. The recombinant host cell according to claim 18, wherein the host cell is a plant cell.

20. The recombinant host cell according to claim 18, wherein the host cell is a bacterium.
21. A transgenic plant comprising a recombinant host cell according to claim 19.

22. A transgenic plant comprising the nucleic acid construct according to any one of claims 1 to 12.

23. The transgenic plant according to claim 21 or 22, wherein the transgenic plant is a monocot.

24. The transgenic plant according to claim 21 or 22, wherein the transgenic plant is a dicot.

25. A transgenic plant seed comprising a recombinant host cell according to claim 19.

26. A transgenic plant seed comprising the nucleic acid construct according to any one of claims 1 to 12.

27. The transgenic plant seed according to claim 25 or 26, wherein the transgenic plant is a monocot.

28. The transgenic plant seed according to claim 25 or 26, wherein the transgenic plant is a dicot.

29. A rootstock, cutting, or seed obtained from the transgenic plant according to any one of claims 21 to 24.

30. A method of enhancing plant growth or yield comprising:
   providing a transgenic plant transformed with the nucleic acid construct according to any one of claims 1 to 12; and
   growing the plant under conditions effective to permit the nucleic acid construct to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance plant growth or yield.

31. A method of enhancing plant growth or yield comprising:
   providing a transgenic plant seed transformed with the nucleic acid construct according to any one of claims 1 to 12;
planting the transgenic plant seed in a growth medium; and
propagating a transgenic plant from the transgenic plant seed to permit the nucleic
acid construct to express the transcription factor polypeptide in vascular xylem tissue of the
transgenic plant, and thereby enhance plant growth or yield.

32. A method of enhancing plant growth or yield comprising:
providing a rootstock, cutting, or seed according to claim 29;
introducing the rootstock, cutting, or seed into a growth medium; and
propagating a transgenic plant from the rootstock, cutting, or seed to permit the
nucleic acid construct to express the transcription factor polypeptide in vascular xylem tissue of the
transgenic plant, and thereby enhance plant growth or yield.

33. The method of any one of claims 30 to 32, wherein the enhanced plant
growth or yield is in comparison to a non-transgenic plant of the same variety.

34. The method of any one of claims 30 to 32, wherein the enhanced plant
growth or yield is selected from:
   (i) faster vegetative growth,
   (ii) increased biomass yields,
   (iii) enhanced root development,
   (iv) increased seed/grain production,
   (v) improved nutrient contents in biomass, and
   any combinations thereof.

35. The method of any one of claims 30 to 32, wherein the growth medium
comprises soil, soil-less particulate medium, or a liquid growth medium.

36. A plant comprising a transgene that includes a heterologous, tissue-
specific promoter operably linked to a polynucleotide encoding a transcription factor involved in
vascular xylem cell development, wherein the promoter specifically directs expression of the
transcription factor in vascular xylem tissue of the plant.

37. The plant according to claim 36, wherein the transgene is stably integrated
into the genome of the plant.
38. The plant according to claim 36, wherein the transgene is present on a plasmid.

39. The plant according to claim 36, wherein the plant is a monocot.

40. The plant according to claim 36, wherein the plant is a dicot.

41. The plant according to any one of claims 36 to 40, wherein the vascular xylem tissue comprises one or more of (i) procambium/cambium cell, (ii) xylem cell, and (iii) a fiber cell type of the plant.

42. The plant according to any one of claims 36 to 40, wherein the tissue-specific promoter directs expression of the transcription factor polypeptide during a time when initial deposition of cellulosic and hemicellulosic components in vascular xylem tissue occurs.

43. The plant according to any one of claims 36 to 42, wherein the tissue-specific promoter directs expression of the transcription factor polypeptide in aerial parts of the plant.

44. The plant according to any one of claims 36 to 42, wherein the tissue-specific promoter directs expression of the transcription factor polypeptide in roots of the plant.

45. The plant according to any one of claims 36 to 40, wherein the tissue-specific promoter is a gene promoter for secondary cell wall development, an endomembrane protein gene promoter, or secondary wall cellulose synthase (CesA) promoter.

46. The plant according to any one of claims 36 to 45, wherein the transcription factor polypeptide modulates expression of a series of genes involved with cell wall and secondary metabolite biosynthetic pathways.

47. The plant according to any one of claims 36 to 45, wherein the transcription factor polypeptide is a polypeptide that can act before secondary wall master transcription factors as an upstream transcriptional regulator.

48. The plant according to any one of claims 36 to 45, wherein the transcription factor polypeptide is an R2R3-MYB subfamily 4 polypeptide.
49. The plant according to any one of claims 36 to 45, wherein the transcription factor polypeptide is an ERF/AP2 subfamily B-6 polypeptide.

50. The plant according to any one of claims 36 to 45, wherein the transgene further comprises a 3’ transcription termination polynucleotide.

51. A rootstock, cutting, or seed obtained from the plant according to any one of claims 36 to 50.

52. A method of enhancing plant growth or yield comprising:
providing a plant according to any one of claims 36 to 50; and
-growing the plant under conditions effective to permit the transgene to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance plant growth or yield.

53. A method of enhancing plant growth or yield comprising:
-providing a rootstock, cutting, or seed according to claim 51;
-introducing the rootstock, cutting, or seed into a growth medium; and
-propagating a plant from the rootstock, cutting, or seed to permit the transgene to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance plant growth or yield.

54. The method of claim 52 or 53, wherein the enhanced plant growth or yield is in comparison to a non-transgenic plant of the same variety.

55. The method of claim 52 or 53, wherein the enhanced plant growth or yield is selected from:

(i) faster vegetative growth,
(ii) increased biomass yields,
(iii) enhanced root development,
(iv) increased seed/grain production,
(v) improved nutrient contents in biomass, and
any combinations thereof.

56. The method of claim 52 or 53, wherein the growth medium comprises soil, soil-less particulate medium, or a liquid growth medium.
57. A method of planting, cultivating, or harvesting a part or all of the plant of any one of claims 21 to 24 and claims 36 to 50.

58. A method of making the plant of any one of claims 36 to 50, the method comprising introducing the transgene into a plant cell and propagating the plant from the plant cell.

59. The method of claim 58, wherein said introducing comprises infective transformation, electroporation, direct uptake, microinjection, biolistic transformation.

60. The method of claim 58 further comprising selecting plants that exhibit one or more of the following properties:

   (i) faster vegetative growth,
   (ii) increased biomass yields,
   (iii) enhanced root development,
   (iv) increased seed/grain production,
   (v) improved nutrient contents in biomass, and any combinations thereof.

61. The method of claim 58 further comprising selecting plants that exhibit one or more of the following properties:

   (i) increased release of glucose saccharides,
   (ii) increased release of xylose saccharides,
   (iii) reduced lignin composition, and any combinations thereof.

62. A method of enhancing degradability of plant biomass comprising:

   providing a transgenic plant transformed with the nucleic acid construct according to any one of claims 1 to 12; and

   growing the plant under conditions effective to permit the nucleic acid construct to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance degradability of plant biomass.
63. A method of enhancing degradability of plant biomass comprising:
providing a transgenic plant seed transformed with the nucleic acid construct
according to any one of claims 1 to 12;
planting the transgenic plant seed in a growth medium; and
propagating a transgenic plant from the transgenic plant seed to permit the nucleic
acid construct to express the transcription factor polypeptide in vascular xylem tissue of the
transgenic plant, and thereby enhance degradability of plant biomass.

64. A method of enhancing degradability of plant biomass comprising:
providing a rootstock, cutting, or seed according to claim 29;
introducing the rootstock, cutting, or seed into a growth medium; and
propagating a transgenic plant from the rootstock, cutting, or seed to permit the
nucleic acid construct to express the transcription factor polypeptide in vascular xylem tissue of the
transgenic plant, and thereby enhance degradability of plant biomass.

65. The method of any one of claims 62 to 64, wherein the enhanced
degradability of plant biomass is in comparison to a non-transgenic plant of the same variety.

66. The method of any one of claims 62 to 64, wherein the enhanced
degradability of plant biomass is selected from:
(i) increased release of glucose saccharides,
(ii) increased release of xylose saccharides,
(iii) reduced lignin composition, and
any combinations thereof.

67. The method of any one of claims 62 to 64, wherein the growth medium
comprises soil, soil-less particulate medium, or a liquid growth medium.

68. A method of enhancing degradability of plant biomass comprising:
providing a plant according to any one of claims 36 to 50; and
growing the plant under conditions effective to permit the transgene to express the
transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby
enhance degradability of plant biomass.

69. A method of enhancing degradability of plant biomass comprising:
providing a rootstock, cutting, or seed according to claim 51;
introducing the rootstock, cutting, or seed into a growth medium; and propagating a plant from the rootstock, cutting, or seed to permit the transgene to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance degradability of plant biomass.

70. The method of claim 68 or 69, wherein the enhanced degradability of plant biomass is in comparison to a non-transgenic plant of the same variety.

71. The method of claim 68 or 69, wherein the enhanced degradability of plant biomass is selected from:
   (i) increased release of glucose saccharides,
   (ii) increased release of xylose saccharides,
   (iii) reduced lignin composition, and any combinations thereof.

72. The method of claim 68 or 69, wherein the growth medium comprises soil, soil-less particulate medium, or a liquid growth medium.
[Claim 1] [Amended] A nucleic acid construct comprising a heterologous, plant tissue-specific promoter operably linked to a polynucleotide encoding a transcription factor polypeptide selected from the group of a R2R3-MYB subfamily 4 or an ERF/AP2 subfamily B-6, wherein the promoter specifically directs an increased expression of the transcription factor polypeptide in vascular xylem tissue within a plant transfected with the nucleic acid construct as compared to a wildtype plant.

[Claim 2] The nucleic acid construct of claim 1, wherein the vascular xylem tissue comprises one or more of (i) procambium/cambium cell, (ii) xylem cell, and (iii) a fiber cell type of a plant.

[Claim 3] [Amended] The nucleic acid construct of claim 1, wherein the tissue-specific promoter directs an increased expression of the transcription factor polypeptide comprising an amino acid sequence of one of SEQ ID NOS: 4, 5, 7, 19, and 20 during a time when an initial deposition of cellulosic and hemicellulosic components in vascular xylem tissue occurs.

[Claim 4] [Amended] The nucleic acid construct of claim 1, wherein the tissue-specific promoter directs an increased expression of the transcription factor polypeptide comprising an amino acid sequence of one of SEQ ID NOS: 4, 5, 7, 19, and 20 in aerial parts of the plant, as compared to a wildtype plant of the same variety.

[Claim 5] [Amended] The nucleic acid construct of claim 1, wherein the tissue-specific promoter directs expression of the transcription factor polypeptide comprising an amino acid sequence of one of SEQ ID NOS: 4, 5, 7, 19, and 20 in roots of the plant, as compared to a wildtype plant of the same variety.

[Claim 6] [Amended] The nucleic acid construct of claim 1, wherein the tissue-specific promoter is a gene promoter for secondary cell wall development, an endomembrane polypeptide gene promoter comprising a nucleotide sequence of one of SEQ ID NOS: 21-36.

[Claim 7] [Amended] The nucleic acid construct of claim 1, wherein the transcription factor polypeptide comprises an amino acid sequence of one of SEQ ID NOS: 4, 5, 7, 19, and 20 and modulates expression of a series of genes involved with cell wall and secondary metabolite biosynthetic pathways.
[Claim 8]  The nucleic acid construct of claim 1, wherein the transcription factor polypeptide is a polypeptide comprising an amino acid sequence of one of SEQ ID NOS: 4, 5, 7, 19, and 20 that can act before secondary wall master transcription factors as an upstream transcriptional regulator.

[Claim 9]  The nucleic acid construct of claim 1, wherein the transcription factor polypeptide from the R2R3-MYB subfamily 4 is Arabidopsis AtMYB32 (SEQ ID NOS: 4,40), Arabidopsis AtMYB4 (SEQ ID NOS: 5,41), Arabidopsis MYB7 (SEQ ID NOS: 6,42), rice OsMYB108 (SEQ ID NOS: 9,45), rice OsMYB108 (SEQ ID NOS: 10,46), poplar PdMYB22 (SEQ ID NOS: 11,47), poplar PdMYB21 (SEQ ID NOS: 12,48), sorghum SbMYB86 (SEQ ID NOS: 15,51), sorghum SbMYB23 (SEQ ID NOS: 16,52), maize ZmMYB42 (SEQ ID NOS: 18,54), maize ZmMYB31 (SEQ ID NOS: 19,55), switchgrass PvMYB4 (SEQ ID NOS: 20,56), or a combination thereof.

[Claim 10]  The nucleic acid construct according to any one of claims 1-6, wherein the transcription factor from the ERF/AP2 subfamily B-6 is Arabidopsis AtSHN3 (SEQ ID NOS: 1,37), Arabidopsis AtSHN1/WIN1 (SEQ ID NOS: 2,38), Arabidopsis AtSHN2 (SEQ ID NOS: 3,39), rice OsSHN1 (OsEREBI9) (SEQ ID NOS: 7,43), rice OsSHN2 (OsEREBIU) (SEQ ID NOS: 8,44), sorghum SbERE63 (SEQ ID NOS: 13,49), sorghum SbERE50 (SEQ ID NOS: 14,50), and maize ZmERE46 (SEQ ID NOS: 17,53), or a combination thereof.

[Claim 11]  The nucleic acid construct of claims 1, wherein the nucleic acid construct further comprises a 3' transcription termination polynucleotide.

[Claim 12]  The nucleic acid construct of claims 1, wherein the nucleic acid construct comprises DNA.

[Claim 13]  A recombinant expression vector comprising a heterologous, plant tissue-specific promoter operably linked to a polynucleotide encoding a transcription factor polypeptide selected from the group of a R2R3-MYB subfamily 4 or an ERF/AP2 subfamily B-6, wherein the promoter specifically directs an increased expression of the transcription factor polypeptide in vascular xylem tissue within a plant transfected with the recombinant expression vector as compared to a wildtype plant.

[Claim 14]  The recombinant expression vector of claim 13, wherein
the vector is operable in dicots comprising alfalfa and canola, and the
promoter and 5' UTR comprise a polynucleotide sequence of one of
SEQ ID NOS: 21-36; and the transcription factor polypeptide encoded
by a nucleotide sequence of one of SEQ ID NOS: 40, 41, 43 55, and 56

[Claim 15] [Amended] The recombinant expression vector of claim 13, wherein
the vector is operable in monocots such as sorghum and switchgrass,
and the promoter and 5' UTR comprise a nucleotide sequence of one of
SEQ ID NOS: 21-36; and the transcription factor polypeptide encoded
by a nucleotide sequence of one of SEQ ID NOS: 40, 41, 43 55, and 56.

[Claim 16] The recombinant expression vector of claim 13, wherein the vector is a
plasmid.

[Claim 17] The recombinant expression vector of claim 13, wherein the vector is
Agrobacterium comprising the plasmid.

[Claim 18] [Amended] A recombinant host cell consisting of a nucleic acid
construct or a recombinant expression vector that comprise a heterologous,
plant tissue-specific promoter operably linked to a polynucleotide encoding a transcription factor polypeptide selected from the
group of a R2R3-MYB subfamily 4 or an ERF/AP2 subfamily B-6,
wherein the promoter specifically directs an increased expression of the
transcription factor polypeptide in vascular xylem tissue within a plant
transfected with the nucleic acid construct or the recombinant expression vector as compared to a wildtype plant.

[Claim 19] The recombinant host cell of claim 18, wherein the host cell is a plant
cell.

[Claim 20] The recombinant host cell of claim 18, wherein the host cell is a
bacterium.

[Claim 21] [Amended] A transgenic plant from a recombinant host plant cell
consisting of a nucleic acid construct, or a recombinant expression
vector, that include a heterologous, plant tissue-specific promoter
operably linked to a polynucleotide encoding a transcription factor polypeptide selected from the group of a R2R3-MYB subfamily 4 or an
ERF/AP2 subfamily B-6, wherein the promoter specifically directs an increased expression of the transcription factor polypeptide in vascular
xylem tissue within a plant transfected with the nucleic acid construct or the recombinant expression vector as compared to a wildtype plant.

[Claim 22] [Amended] A transgenic plant consisting of a nucleic acid construct
that includes a heterologous, plant tissue-specific promoter operably
linked to a polynucleotide encoding a transcription factor polypeptide
selected from the group of a R2R3-MYB subfamily 4 or an ERF/AP2
subfamily B-6, wherein the promoter specifically directs an increased
expression of the transcription factor polypeptide in vascular xylem
tissue within the transgenic as compared to a wildtype plant.

[Claim 23] The transgenic plant as in claim 21 or 22, wherein the transgenic plant
is a monocot such as a sorghum and a switchgrass.

[Claim 24] The transgenic plant as in claim 21 or 22, wherein the transgenic plant
is a dicot such as an alfalfa and a canola.

[Claim 25] [Amended] A transgenic plant seed consisting of a nucleic acid
construct or a recombinant expression vector that includes a hetero-
logous, plant tissue-specific promoter operably linked to a polynu-
cleotide encoding a transcription factor polypeptide selected from the
group of a R2R3-MYB subfamily 4 or an ERF/AP2 subfamily B-6,
wherein the promoter specifically directs an increased expression of the
transcription factor polypeptide in vascular xylem tissue within a plant
transfected with the nucleic acid construct or the recombinant expression vector as compared to a wildtype plant.

[Claim 26] [Amended] A transgenic plant seed consisting of a nucleic acid
construct that includes a heterologous, plant tissue-specific promoter operably linked to a polynucleotide encoding a transcription factor polypeptide selected from the group of a R2R3-MYB subfamily 4 or an ERF/AP2 subfamily B-6, wherein the promoter specifically directs an increased expression of the transcription factor polypeptide in vascular xylem tissue within the transgenic plant as compared to a wildtype plant.

[Claim 27] The transgenic plant seed as in claim 25 or 26, wherein the transgenic plant is a monocot such as sorghum and switchgrass.

[Claim 28] The transgenic plant seed as in claim 25 or 26, wherein the transgenic plant is a dicot such as alfalfa and canola.

[Claim 29] [Amended] A rootstock, cutting, or seed obtained from a transgenic plant with a recombinant plant cell comprising a nucleic acid construct or a recombinant expression vector that include a heterologous, plant tissue-specific promoter operably linked to a polynucleotide encoding a transcription factor polypeptide selected from the group of a R2R3-MYB subfamily 4 or an ERF/AP2 subfamily B-6, wherein the promoter specifically directs an increased expression of the transcription factor polypeptide in vascular xylem tissue within the transgenic plant as compared to a wildtype plant.
[Claim 30] [Amended] A method of enhancing plant growth or yield comprising:
a) providing a transgenic plant transformed with a nucleic acid construct that includes a heterologous, plant tissue-specific promoter operably linked to a polynucleotide encoding a transcription factor polypeptide selected from the group of a R2R3-MYB subfamily 4 or an ERF/AP2 subfamily B-6, wherein the promoter specifically directs an increased expression of the transcription factor polypeptide in vascular xylem tissue in the transgenic plant as compared to a wildtype plant; and
b) growing the plant under conditions effective to permit the nucleic acid construct to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance plant growth or yield.

[Claim 31] [Amended] A method of enhancing plant growth or yield comprising:
a) providing a transgenic plant seed transformed with a nucleic acid construct that comprise a heterologous, plant tissue-specific promoter operably linked to a polynucleotide encoding a transcription factor polypeptide selected from the group of a R2R3-MYB subfamily 4 or an ERF/AP2 subfamily B-6, wherein the promoter specifically directs an increased expression of the transcription factor polypeptide in vascular xylem tissue within the transgenic plant as compared to a wildtype plant;
b) planting the transgenic plant seed in a growth medium; and
c) propagating a transgenic plant from the transgenic plant seed to permit the nucleic acid construct to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance plant growth or yield.

[Claim 32] [Amended] A method of enhancing plant growth or yield comprising:
a) providing a rootstock, cutting, or seed obtained from a transgenic plant consisting of a recombinant plant cell comprising a nucleic acid construct or a recombinant expression vector that includes a heterologous, plant tissue-specific promoter operably linked to a polynucleotide encoding a transcription factor polypeptide selected from the group of a R2R3-MYB subfamily 4 or an ERF/AP2 subfamily B-6, wherein the promoter specifically directs an increased expression of the transcription factor polypeptide in vascular xylem tissue within the transgenic plant as compared to a wildtype plant;
b) introducing the rootstock, cutting, or seed into a growth medium;
and

c) propagating a transgenic plant from the rootstock, cutting, or seed to permit the nucleic acid construct to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance plant growth or yield.

[Claim 33] The method as in one of claims 30-32, wherein the enhanced plant growth or yield is in comparison to a wildtype plant of the same variety.

[Claim 34] The method as in one of claims 30-32, wherein the enhanced plant growth or yield is selected from:

(i) faster vegetative growth,
(ii) increased biomass yields,
(iii) enhanced root development,
(iv) increased seed/grain production,
(v) improved nutrient contents in biomass, or any combinations thereof.

[Claim 35] The method as in one of claims 30-32, wherein the growth medium comprises soil, soil-less particulate medium, or a liquid growth medium.

[Claim 36] [Amended] A plant comprising a transgene that includes a heterologous, plant tissue-specific promoter operably linked to a polynucleotide encoding a transcription factor polypeptide selected from the group of a R2R3-MYB subfamily 4 or an ERF/AP2 subfamily B-6, wherein the promoter specifically directs an increased expression of the transcription factor polypeptide in vascular xylem tissue of the plant as compared to a wildtype plant.

[Claim 37] The plant of claim 36, wherein the transgene is stably integrated into the genome of the plant.

[Claim 38] The plant of claim 36, wherein the transgene is present on a plasmid.

[Claim 39] The plant of claim 36, wherein the plant is a monocot such as sorghum and switchgrass.

[Claim 40] The plant of claim 36, wherein the plant is a dicot such as alfalfa and canola.

[Claim 41] The plant of claim 36, wherein the vascular xylem tissue comprises one or more of (i) procambium/cambium cell, (ii) xylem cell, and (iii) a fiber cell type of the plant.

[Claim 42] [Amended] The plant of claim 36, wherein the tissue-specific promoter directs an increased expression of the transcription factor polypeptide
comprising an amino acid sequence of one of SEQ ID NOS: 4, 5, 7, 19, and 20 during a time when an initial deposition of cellulosic and hemicellulosic components in vascular xylem tissue occurs.

[Claim 43] [Amended] The plant of claim 36, wherein the tissue-specific promoter directs an increased expression of the transcription factor polypeptide comprising an amino acid sequence of one of SEQ ID NOS: 4, 5, 7, 19, and 20 in aerial parts of the plant as compared to a wildtype plant from the same variety.

[Claim 44] [Amended] The plant of claim 36, wherein the tissue-specific promoter directs an increased expression of the transcription factor polypeptide comprising an amino acid sequence of one of SEQ ID NOS: 4, 5, 7, 19, and 20 in roots of the plant as compared to a wildtype plant from the same variety.

[Claim 45] [Amended] The plant of claim 36, wherein the tissue-specific promoter is an endomembrane polypeptide gene promoter for secondary cell wall development that comprises a nucleotide sequence of one of SEQ ID NOS: 21-36.

[Claim 46] [Amended] The plant of claim 36, wherein the transcription factor polypeptide comprise an amino acid sequence of one of SEQ ID NOS: 4, 5, 7, 19, and 20 and modulates expression of a series of genes involved with cell wall and secondary metabolite biosynthetic pathways.

[Claim 47] [Amended] The plant of claim 36, wherein the transcription factor polypeptide is a polypeptide comprising an amino acid sequence of one of SEQ ID NOS: 4, 5, 7, 19, and 20 that can act before secondary wall master transcription factors as an upstream transcriptional regulator.

[Claim 48] [Amended] The plant of claim 36, wherein the transcription factor polypeptide from the R2R3-MYB subfamily 4 is Arabidopsis AtMYB32 (SEQ ID NOS: 4,40), Arabidopsis AtMYB4 (SEQ ID NOS: 5,41), Arabidopsis MYB7 (SEQ ID NOS: 6,42), rice OsMYB108-L (SEQ ID NOS: 9,45), rice OsMYB108 (SEQ ID NOS: 10,46), poplar PdMYB221 (SEQ ID NOS: 11,47), poplar PdMYB156 (SEQ ID NOS: 12,48), sorghum SbMYB86 (SEQ ID NOS: 15,51), sorghum SbMYB23 (SEQ ID NOS: 16,52), maize ZmMYB42 (SEQ ID NOS: 18,54), maize ZmMYB31 (SEQ ID NOS: 19,55), switchgrass PvMYB4 (SEQ ID NOS: 20,56) or a combination thereof.

[Claim 49] [Amended] The plant of claim 36, wherein the transcription factor polypeptide from the ERF/AP2 subfamily B-6 is Arabidopsis AtSHN3
(SEQ ID NOS: 1,37), Arabidopsis AtSHNl/WINl (SEQ ID NOS: 2,38), Arabidopsis AtSHN2 (SEQ ID NOS: 3,39), rice OsSHNl (OsEREBl9) (SEQ ID NOS: 7,43), rice OsSHN2 (OsEREBlU) (SEQ ID NOS: 8,44), sorghum SbEREBl50 (SEQ ID NOS: 13,49), sorghum SbEREBl50 (SEQ ID NOS: 14,50), and maize ZmEREBl46 (SEQ ID NOS: 17,53), or a combination thereof.

[Claim 50] The plant of claim 36, wherein the transgene further comprises a 3’ transcription termination polynucleotide.

[Claim 51] A rootstock, cutting, or seed obtained from the plant comprising a transgene that includes a heterologous, plant tissue-specific promoter operably linked to a polynucleotide encoding a transcription factor polypeptide selected from the group of a R2R3-MYB subfamily 4 or an ERF/AP2 subfamily B-6, wherein the promoter specifically directs an increased expression of the transcription factor polypeptide in vascular xylem tissue of the plant as compared to a wildtype plant.

[Claim 52] A method of enhancing plant growth or yield comprising:

a) providing a plant comprising a transgene that includes a heterologous, plant tissue-specific promoter operably linked to a polynucleotide encoding a transcription factor polypeptide selected from the group of a R2R3-MYB subfamily 4 or an ERF/AP2 subfamily B-6, wherein the promoter specifically directs an increased expression of the transcription factor polypeptide in vascular xylem tissue of the plant as compared to a wildtype plant; and

b) growing the plant under conditions effective to permit the transgene to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance plant growth or yield.

[Claim 53] A method of enhancing plant growth or yield comprising:

a) providing a rootstock, cutting, or seed obtained from a plant comprising a transgene that includes a heterologous, plant tissue-specific promoter operably linked to a polynucleotide encoding a transcription factor polypeptide selected from the group of a R2R3-MYB subfamily 4 or an ERF/AP2 subfamily B-6, wherein the promoter specifically directs an increased expression of the transcription factor polypeptide in vascular xylem tissue of the plant as compared to a wildtype plant;

b) introducing the rootstock, cutting, or seed into a growth medium; and
c) propagating a plant from the rootstock, cutting, or seed to permit the transgene to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance plant growth or yield.

[Claim 54] The method as in claim 52 or 53, wherein the enhanced plant growth or yield is in comparison to a wildtype plant of the same variety.

[Claim 55] The method as in claim 52 or 53, wherein the enhanced plant growth or yield is selected from:
(i) faster vegetative growth,
(ii) increased biomass yields,
(iii) enhanced root development,
(iv) increased seed/grain production,
(v) improved nutrient contents in biomass, and any combinations thereof.

[Claim 56] The method as in claim 52 or 53, wherein the growth medium comprises soil, soil-less particulate medium, or a liquid growth medium.

[Claim 57] [Amended] A method of planting, cultivating, or harvesting a part or all of a plant comprising a nucleic acid construct, a recombinant expression vector or a transgene that includes a heterologous, plant tissue-specific promoter operably linked to a polynucleotide encoding a transcription factor polypeptide selected from the group of a R2R3-MYB subfamily 4 or an ERF/AP2 subfamily B-6, wherein the promoter specifically directs an increased expression of the transcription factor polypeptide in vascular xylem tissue in the plant as compared to a wildtype plant.

[Claim 58] [Amended] A method of making a plant comprising a nucleic acid construct, a recombinant expression vector or a transgene that includes a heterologous, plant tissue-specific promoter operably linked to a polynucleotide encoding a transcription factor polypeptide selected from the group of a R2R3-MYB subfamily 4 or an ERF/AP2 subfamily B-6, wherein the promoter specifically directs an increased expression of the transcription factor polypeptide in vascular xylem tissue in the plant as compared to a wildtype plant, the method comprising introducing the transgene into a plant cell and propagating the plant from the plant cell.

[Claim 59] The method of claim 58, wherein said introducing comprises infective transformation, electroporation, direct uptake, microinjection, biolistic...
transformation.

[Claim 60] The method of claim 58, further comprising selecting plants that exhibit at least 5% increase in one or more of the following properties compared to a wild type of the same variety:
(i) faster vegetative growth,
(ii) increased biomass yields,
(iii) enhanced root development,
(iv) increased seed/grain production,
(v) improved nutrient contents in biomass, or any combinations thereof.

[Claim 61] The method of claim 58, further comprising selecting plants that exhibit at least a 5% change in one or more of the following properties, as compared to a wild type of the same variety:
(i) an increased release of glucose saccharides,
(ii) an increased release of xylose saccharides,
(iii) a reduced lignin content,
(iv) an altered lignin composition, or any combinations thereof.

[Claim 62] [Amended] A method of enhancing degradability of plant biomass comprising:

a) providing a transgenic plant transformed with a nucleic acid construct comprising a heterologous, plant tissue-specific promoter operably linked to a polynucleotide encoding a transcription factor polypeptide selected from the group of a R2R3-MYB subfamily 4 or an ERF/AP2 subfamily B-6, wherein the promoter specifically directs an increased expression of the transcription factor polypeptide in vascular xylem tissue in the transgenic plant as compared to a wildtype plant; and

b) growing the plant under conditions effective to permit the nucleic acid construct to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance degradability of plant biomass.

[Claim 63] [Amended] A method of enhancing degradability of plant biomass comprising:

a) providing a transgenic plant seed transformed with a nucleic acid construct comprising a heterologous, plant tissue-specific promoter operably linked to a polynucleotide encoding a transcription factor polypeptide selected from the group of a R2R3-MYB subfamily 4 or an
ERF/AP2 subfamily B-6, wherein the promoter specifically directs an increased expression of the transcription factor polypeptide in vascular xylem tissue in the transgenic plant as compared to a wildtype plant; b) planting the transgenic plant seed in a growth medium; and c) propagating a transgenic plant from the transgenic plant seed to permit the nucleic acid construct to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance degradability of plant biomass.

[Claim 64] [Amended] A method of enhancing degradability of plant biomass comprising:

a) providing a rootstock, cutting, or seed obtained from a transgenic plant transformed with a nucleic acid construct comprising a heterologous, plant tissue-specific promoter operably linked to a polynucleotide encoding a transcription factor polypeptide selected from the group of a R2R3-MYB subfamily 4 or an ERF/AP2 subfamily B-6, wherein the promoter specifically directs an increased expression of the transcription factor polypeptide in vascular xylem tissue within the transgenic plant as compared to a wildtype plant;

b) introducing the rootstock, cutting, or seed into a growth medium; and

c) propagating a transgenic plant from the rootstock, cutting, or seed to permit the nucleic acid construct to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance degradability of plant biomass.

[Claim 65] The method as in any one of claims 62-64, wherein the enhanced degradability of plant biomass is in comparison to a wildtype plant of the same variety.

[Claim 66] The method as in any one of claims 62-64, wherein the enhanced degradability of plant biomass is selected from:

(i) an increased release of glucose saccharides,
(ii) an increased release of xylose saccharides,
(iii) a reduced lignin content,
(iv) an altered lignin composition, or any combinations thereof.

[Claim 67] The method as in any one of claims 62-64, wherein the growth medium comprises soil, soil-less particulate medium, or a liquid growth medium.

[Claim 68] [Amended] A method of enhancing degradability of plant biomass
comprising:
a) providing a plant comprising a transgene that includes a heterologous, plant tissue-specific promoter operably linked to a polynucleotide encoding a transcription factor polypeptide selected from the group of a R2R3-MYB subfamily 4 or an ERF/AP2 subfamily B-6, wherein the promoter specifically directs an increased expression of the transcription factor polypeptide in vascular xylem tissue of the plant as compared to a wildtype plant; and
b) growing the plant under conditions effective to permit the transgene to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance degradability of plant biomass.

[Claim 69] [Amended] A method of enhancing degradability of plant biomass comprising:
a) providing a rootstock, cutting, or seed obtained from a plant comprising a transgene that includes a heterologous, plant tissue-specific promoter operably linked to a polynucleotide encoding a transcription factor polypeptide selected from the group of a R2R3-MYB subfamily 4 or an ERF/AP2 subfamily B-6, wherein the promoter specifically directs an increased expression of the transcription factor polypeptide in vascular xylem tissue of the plant as compared to a wildtype plant;
b) introducing the rootstock, cutting, or seed into a growth medium; and
c) propagating a plant from the rootstock, cutting, or seed to permit the transgene to express the transcription factor polypeptide in vascular xylem tissue of the transgenic plant, and thereby enhance degradability of plant biomass.

[Claim 70] The method as in claim 68 or 69, wherein the enhanced degradability of plant biomass is in comparison to a wildtype plant of the same variety.

[Claim 71] The method as in claim 68 or 69, wherein the enhanced degradability of plant biomass is selected from:
(i) an increased release of glucose saccharides,
(ii) an increased release of xylose saccharides,
(iii) a reduced lignin content,
(iv) an altered lignin composition, or
any combinations thereof.

[Claim 72] The method as in claim 68 or 69, wherein the growth medium
comprises soil, soil-less particulate medium, or a liquid growth medium.
FIG. 1
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**002 pAtLC4-A Myb32-tRBS**

2778 bp
FIG. 3
FIG. 4
FIG. 5
Figure 8
FIG. 9
FIG. 15
FIG. 22
FIG. 23
**FIG. 24**
**FIG. 25**
FIG. 26
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

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<td>C12B 15/8216, C12N 15/8237</td>
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According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
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| X         | US 2017/0298375 A1 (BOARD OF TRUSTEES OF MICHIGAN STATE UNIVERSITY) 19 October 2017 (19.10.2017); para [0004], [0005], [0013], [0032], [0060], [0080], [0087], [0121], [0150], [0173], claim 19, claim 23 | 1-6, 36-42, 45 |

**Date of the actual completion of the international search**

20 March 2019

**Date of mailing of the international search report**

15 April 2019

Name and mailing address of the ISA/US

Mail Step PCT, Attn: ISA/US, Commissioner for Patents

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Authorized officer: Lee W. Young

PCT Helpdesk: 571-272-4300

PCT OSP: 571-273-7774

Form PCT/ISA/210 (second sheet) (January 2015)
INTERNATIONAL SEARCH REPORT

Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 7-35, 43, 44, 46-72 because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims, it is covered by claims Nos.:

Remark on Protest  ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (January 2015)