EFFECTS OF GENETIC MANIPULATION (HCT AND C_3H DOWN-REGULATION) ON MOLECULAR CHARACTERISTICS OF LIGNIN AND ITS BIOCONVERSION TO FERMENTABLE SUGARS

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The molecular characteristics of lignin from hydroxycinnamoyl transferase (HCT) and *p*-coumarate 3-hydroxylase (C_3H) down-regulated 84 k poplar (after four years of growth in field) have not been investigated in detail. In the present study, modified enzymatic mild acid lignin (EMAL) samples were extracted with high yield and purity. NMR characterization of the lignins demonstrated that all the EMALs presented typical and common substructures (β-*O*-4′, β-β′ and β-5′), although the down-regulated poplar lignin had a high content of β-*O*-4′ linkages. Interestingly, the total OCH_3 contents in the HCT-EMAL and C_3H-EMAL were found to be decreased, while the corresponding S/G ratios were increased. In addition, it was observed that the *p*-hydroxybenzoate (PB) content was increased in the lignin from HCT and C_3H down-regulated poplar, respectively. Moreover, HCT and C_3H down-regulation slightly enhanced the enzymatic hydrolysis of 84 k feedstock to 33% (40-60 mesh) as compared to 26% of CK, while the enzymatic hydrolysis of down-regulated poplar (ball-milled) were obviously improved to 78% as compared to 61% of CK poplar. These findings are vital in assessing and evaluating the effects of down-regulated HCT and C_3H genes on the molecular characteristics of lignin and the digestibility of HCT and C_3H down-regulated poplar in the fuel production.

Keywords: transgenic poplar, 2D-HSQC NMR, S/G ratio, *p*-hydroxybenzoate (PB)

INTRODUCTION

Lignocellulosic biomass is the only sustainable and renewable resource that can provide alternatives of crude oil and fossil fuels. Unfortunately, lignocellulosic biomass is a complex composite, comprising primarily cellulose, hemicelluloses and lignin, which is recalcitrant to enzymatic and microbial deconstruction due to the rigid and compact structure of plant cell walls.[1] The recalcitrance has been attributed to several factors (e.g. cellulose accessibility to enzymes, lignin content/structure, lignin-carbohydrate complexes, as well as the presence of structural hemicelluloses).[2]

Currently, most integrated biologically based biorefinery concepts comprise four major steps: feedstock harvest and storage, pretreatment, enzymatic hydrolysis, and sugar fermentation to ethanol or other fuels.[3] Among them, the pretreatment step is crucial to overcome natural recalcitrance of biomass toward biological deconstruction to simple sugars. For many years, several promising pretreatment technologies have been developed, such as steam explosion, hot water, organosolv pretreatment and so on.[4,5] Although some pretreatments have achieved varying degrees of progress in breaking through the recalcitrance of lignocelluloses, the pretreatment process still remains the most expensive step in the current biomass-to-biofuel production. Therefore, addressing biomass recalcitrance via alternative approaches is a crucial issue for the cellulosic biofuel production.[6,7] A promising approach for reducing biomass recalcitrance is the development of genetically engineered plants, consisting in

down-regulation of key enzymes involved in lignin biosynthesis, which can achieve an improved sugar release performance with reduced lignin content and modified lignin structures.[8]

The researchers have made many efforts to tailor the chemical composition, structures and reactivity of lignocelluloses, especially lignin.[8-10] Lignin modification in plants has been extensively investigated to reduce lignin levels or to alter its structure to facilitate pulping, to improve forage digestibility or to overcome recalcitrance for bioenergy feedstocks.[7] More importantly, bioengineering to modify lignin structure or incorporate atypical components has shown a good prospect toward facilitating extraction and chemical transformation of lignin under biorefinery conditions.[8]

Genetic manipulation of biosynthesis pathways has produced lignin feedstocks with unique properties for coproduct development.[8] In addition, lignin content and composition are related to the digestibility of plant cell walls. Hydroxycinnamoyl CoA:shikimate/quinate hydroxycinnamoyl transferase (HCT) and *p*-coumarate 3-hydroxylase (C_3H) gene down-regulation of alfalfa lignin was reported to reduce the methoxyl content, as well as increase the relative level of phenylcoumaran and resinol substructures in the ball-milled lignin.[11] Ralph and coauthors[9,12] have also investigated the effects of C_3H down-regulation on the content and structures of lignin in poplar via NMR characterization of acetylated lignins. The differences in lignin composition indicated that C_3H down-regulation significantly reduced lignin content and obviously increased the content of H units as compared to those of G and S units.[9] Similarly, MWL and CEL have been sequentially isolated from HCT down-regulated hardwood (poplar) to characterize the chemical structures of native lignin. However, the NMR characterization of lignin polymers indicated that only the *p*-hydroxybenzoate (PB) content was increased in the lignin from the HCT poplar.[13]

In the present study, a high-yield lignin extracting method, EMAL, was applied to extract native lignin from CK poplar, HCT and C_3H down-regulated poplars. The lignins obtained were evaluated by means of carbohydrate analysis, gel permeation chromatography (GPC), quantitative ^{13}C, 2D heteronuclear single quantum coherence (2D-HSQC), and ^{31}P-NMR techniques. The effects of HCT and C_3H down-regulation on the molecular characteristics of lignin from transgenic poplars (84 K) were investigated to determine how the genetic modification affects the lignin structure after four years of growth in the field. Moreover, the digestibility of HCT and C_3H (40-60 mesh and ball-milled) down-regulated poplar was also evaluated.

EXPERIMENTAL

Materials

Control 84 K (CK) and down-regulated (HCT and C_3H) transgenic poplar 84 K (*Populus* alba×*P.* glandulosa, 4 years) were kindly supplied by the Chinese Academy of Forestry Sciences.[13] The HCT and C_3H genes coding the enzymes are involved in lignin biosynthesis to construct their RNAi vectors. Poplar was transformed via the leaf-disc method. Transgenic poplar plants with RNAi constructs were obtained and vegetatively propagated by cutting for each line in the greenhouse. The residual C_3H gene activity was 25.0%, while the residual HCT gene activity was 31.0%. The HCT and C_3H down-regulated poplar wood was transplanted from the greenhouse into the field. After four years of growth, the CK and HCT transgenic poplar was harvested. The poplar wood was debarked, chipped and dried in an oven at 50 °C for 24 h, followed by grinding to obtain particles with a size distribution between 20 and 40 mesh. The subsequent treatment with a mixture of toluene/ethanol (2:1, v/v) in a Soxhlet extractor for 6 h removed most of extractives. The composition of CK and HCT poplar (20-40 mesh) was determined by the NREL standard analytical procedure.[14] The Klason lignins in CK, HCT and C_3H poplar wood were determined to be 19.4%, 19.5% and 20.0%, respectively. The ball-milled wood was prepared using a planetary ball mill (FritschGMBH, Idar-Oberstein, Germany) for 5 hours, in which the milling bowl was made of zirconium dioxide. The milling was conducted under N_2 atmosphere with a milling frequency of 500 rpm/min. All the chemical reagents used were of analytical grade or the best available.

Methods

Isolation and purification of native lignins

The EMALs from these poplar woods were prepared as described previously, with minor modifications (Fig. 1).[15] The ball-milled wood (10 g) was subjected to enzymatic hydrolysis for 48 hours (Cellulase, 50 FPU/g, pH 4.8, sodium acetate buffer, 2% substrate concentration). Subsequently, the freeze-dried enzymatic hydrolysis residue (EHR) was extracted with 85% dioxane/water containing 0.01 M hydrochloric acid at 86 °C for 2 h. After filtrating, neutralizating, concentrating and it was poured into 3 volumes of ethanol to remove carbohydrates (hemicelluloses and some of the

lignin-carbohydrate complex). The combined filtrates were then concentrated to ~60 mL and then precipitated in ~600 mL acidified water (two-step isolation procedure).

Physicochemical characterization of lignin

The associated carbohydrates in the lignin samples were determined by hydrolysis with dilute sulfuric acid according to the procedure described previously.[16] The weight-average (M_w) and number-average (M_n) molecular weights of the acetylated lignin samples were determined by GPC with an ultraviolet detector (UV) at 280 nm on a PL-gel 10 µm Mixed-B 7.5 mm ID column, calibrated with PL polystyrene standards.[16] The acetylation of the lignin was conducted as reported previously.[17]

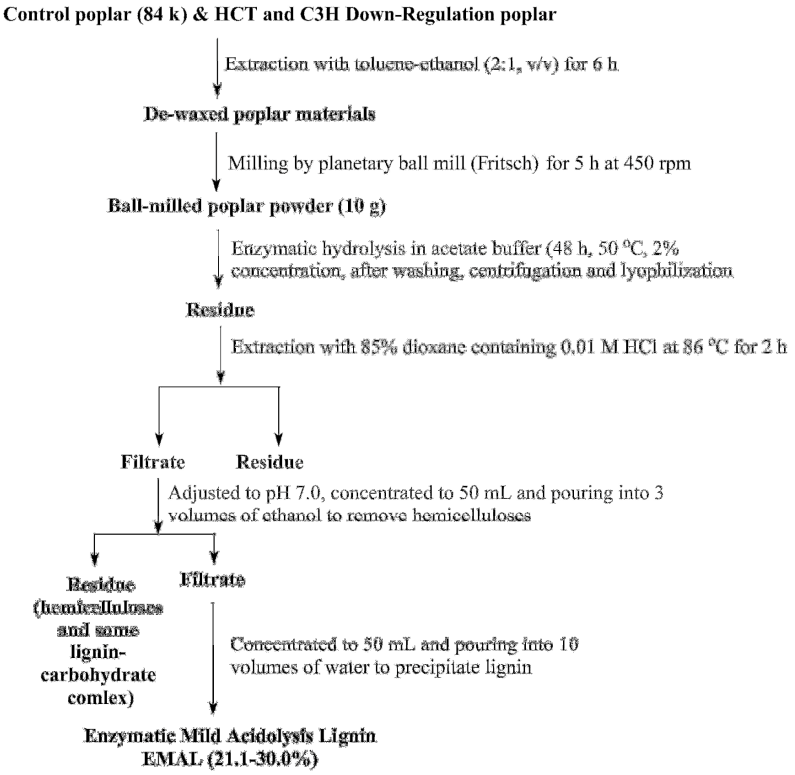

Figure 1: Overall scheme of the isolation process of lignins

NMR spectra were recorded on a Bruker AVIII 400 MHz spectrometer. For the quantitative ^{13}C NMR experiments (Program: C13IG), 140 mg of lignin was dissolved in 0.5 mL of DMSO-d_6, 20 µL of chromium (III) acetylacetonate (0.01 M) was added to the lignin solution to provide complete relaxation of all nuclei.[18] For the 2D HSQC NMR, 60 mg of lignin was dissolved in 0.5 mL of DMSO-d_6. 2D HSQC NMR spectra were recorded in HSQC experiments according to a previous report.[16] Quantitative ^{31}P NMR spectrum was recorded according to the literature.[19,20]

Enzymatic hydrolysis

The experiments were carried out at 2% of substrate (w/v) in 25 mL of 50 mM sodium acetate buffer (pH 4.8) using a double layer shaking incubator (ZWYR-2102C) (Shanghai, China) at 150 rpm for 72 h. The temperature was controlled at 50 °C. Cellulase (15 FPU/g substrate), which was supplied by Shanghai Youtell Biochemical Co., Ltd., was used for all the hydrolysis experiments.

RESULTS AND DISCUSSION

Traditionally, HCT and C_3H down-regulation results in the decrease of the content of Klason lignin.[9,11-12] However, in this study, it was observed that the content of Klason lignin in the CK poplar wood was slightly increased after HCT and C_3H down-regulation. The HCT and C_3H down-regulated poplar used in this study was four-year old poplar grown in the field. After four years of growth, the HCT and C_3H down-regulated poplar probably restored growth, if there was an active cell wall feedback signalling responsible for dwarfing existing in lignin deficient mutants.[21] Similarly, the content of other components, such as cellulose and hemicelluloses, remained unchanged.

Although the content of the main components of poplar wood after HCT and C_3H down-regulation remained unchanged, the question whether HCT and C_3H down-regulation affects the chemical composition and structural features of lignin and the subsequent enzymatic hydrolysis still needs to be confirmed.

Generally, the most used native lignin samples for structural characterization are MWL and CEL.[22,23] However, the low yield of MWL and CEL in a previous study impeded their representtativeness in lignin characterization.[13] The aim of the present study was to delineate the consequences of C_3H and HCT down-regulation on lignin structure. In addition, to obtain a more representative lignin sample for delineating the molecular characteristics of native lignin in the control and gene down-regulated poplars (C_3H and HCT), modified EMAL fractions were extracted from these poplars in the current study. The yields of the lignin from CK, HCT and C_3H are listed in Table 1. It was found that the yields of EMAL from CK, HCT and C_3H poplar wood were 21.1, 30.0 and 28.2% (based on respective content of Klason lignin), respectively. Therefore, the increased yields of the EMALs from down-regulated poplars were probably related to the enhanced digestibility of the transgenic feedstocks, which was further confirmed by the improved fermentable sugar yields in the down-regulated poplar wood. For native lignin, the lignin-carbohydrate complex (LCC) was accompanied by the isolation of the lignin. To determine what kind of carbohydrates attached to the lignin molecules, the carbohydrate analysis of the lignin fractions was performed and the results are given in Table 1. As can be seen, CK-EMAL, HCT-EMAL and C_3H-EMAL contained small amounts of associated carbohydrates, amounting to 2.50%, 4.22% and 3.77%, respectively. Especially, it was found that xylose and glucose were the main sugars in all the EMAL samples. Undoubtedly, a lower amount of carbohydrates in lignin facilitates a deeper understanding of the structure of lignin.

^{13}C NMR analysis

The quantitative ^{13}C NMR spectra were recorded, and signal intensity could be correlated to the amount of these specific carbon atoms present in lignin (Fig. 2). Detailed signal assignments of lignin were achieved by a recent publication and the identified signals are marked in Fig. 2.[24] For example, the obvious signals were assigned and listed as follows, such as signal 1 (165.6 ppm, C-7 in PB units), signal 2 (162.1 ppm, C-4 in PB units), signal 3 (152.3 ppm, etherified S3,5 units), signal 4 (149.2 ppm, etherified G3), signal 5 (147.6 ppm, G4 in β-β units, non-etherified S3,5 units, non-ether G3 units), signal 6 (145.5 ppm, non-etherified G4), signal 7 (138.2 ppm, etherified S4), signal 8 (134.9 ppm, etherified S1 or G1), signal 9 (131.6 ppm, PB2,6), signal 10 (120.5 ppm, G6), signal 11 (115.4 ppm, G5), signal 12 (111.2 ppm, G2), signal 13 (104.4 ppm, S2,6), signal 14 (86.1 ppm, C-α in β-5 units), signal 15 (85.2 ppm, C-β in β-O-4′ units), signal 16 (83.8 ppm, C-β in acetylated β-O-4′ units), signal 17 (72.4 ppm, C-α in β-O-4′ units), signal 18 (63.0 ppm, C-5 in xylans), signal 19 (59.8 ppm, C-γ in β-O-4′ units), and signal 20 (56.0 ppm, OCH_3). Quantification of the lignin fractions can provide more precise structural details of the lignin from CK and down-regulated poplar wood (HCT and C_3H).[25] For β-O-4′ linkage (integration region from 58.0-62.0 ppm), it was found that the β-O-4′ linkage was 59.3/100Ar, 61.5/100Ar and 62.9/100Ar in the CK-EMAL, HCT-EMAL and C_3H-EMAL, respectively.

Table 1
Composition analysis of the feedstock

	CK	HCT	C3H
Rhamnose	0.79	1.19	0.81
Arabinose	0.44	0.42	0.45
Galactose	0.42	0.45	0.89
Glucose	47.50	47.39	45.96
Xylose	18.48	18.14	18.15
Mannose	2.47	3.06	2.91
Klason lignin	19.40	19.50	20.00
Total	89.49	90.15	89.16

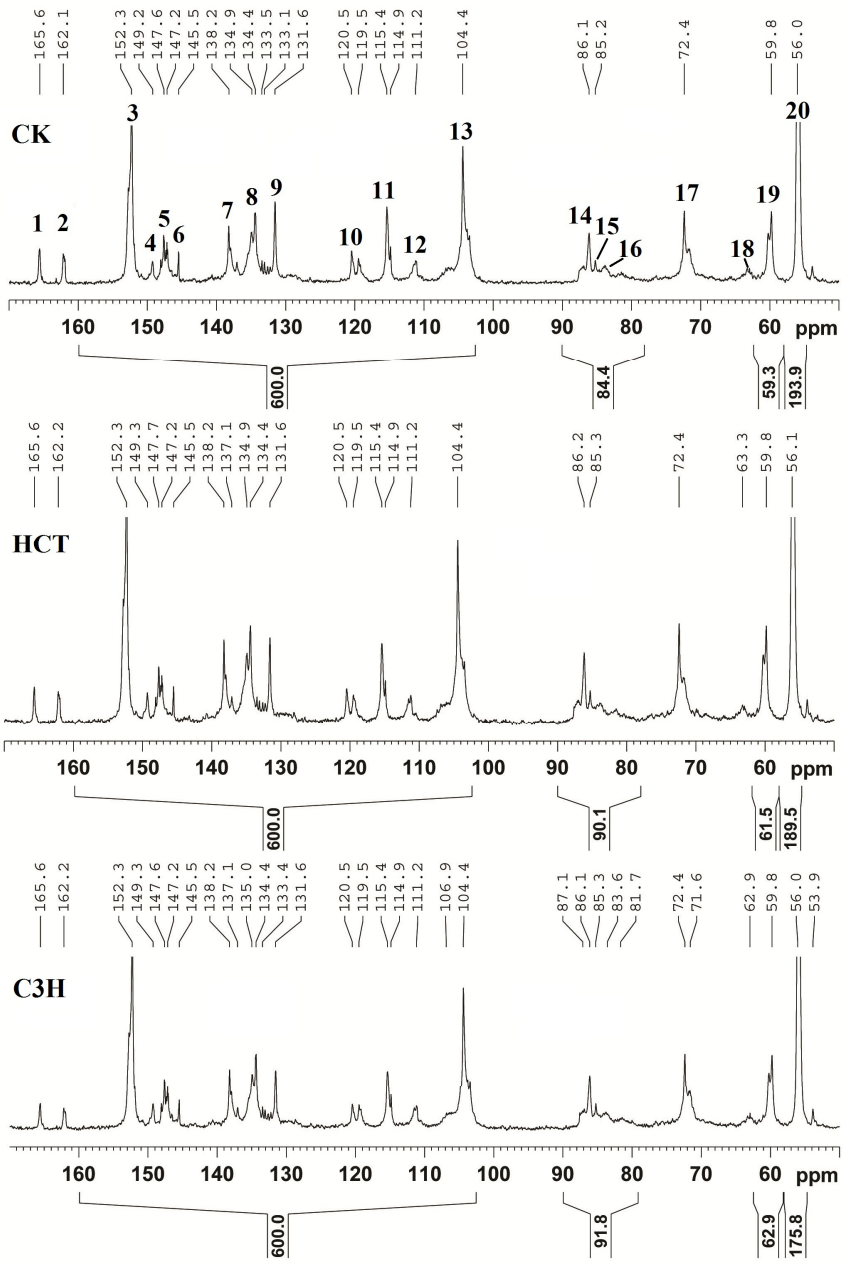

Figure 2: Quantitative ^{13}C NMR spectra of lignins

This suggested that C_3H-EMAL and HCT-EMAL contained a relatively higher amount of β-O-4′ linkage, as compared to CK-EMAL, as also revealed by 2D HSQC spectra. Interestingly, the total OCH_3 contents in the HCT-EMAL and C_3H-EMAL were decreased, while the corresponding S/G ratios were increased, implying that the demethoxylation of G-units was faster than that of S-units during down-regulation of these genes. In short, the quantitative ^{13}C-NMR results were in accordance with the information obtained from 2D HSQC spectra.

2D-HSQC spectral analysis

To investigate the chemical composition and detailed structural features of the lignin, the

EMALs were analyzed by the 2D HSQC NMR technique. The side-chain and aromatic region of the HSQC spectra of the MWL and CEL are plotted in Fig. 3 and the main linkages detected in the lignin are depicted in Fig. 4. Signal assignments of various lignin components and inter-unit linkages have been reported in several previous publications.[24,26-28] The side-chain region of 2D HSQC spectra revealed detailed information regarding the types and abundance of linkages present in the lignins (Fig. 3). The spectra showed prominent signals corresponding to β-*O*-4′ ether units (substructures A and A′). The C_α-H_α correlations in β-*O*-4′ linkages were located at δ_C/δ_H 71.8/4.84 ppm, while the C_β-H_β correlations were observed at δ_C/δ_H 84.3/4.21 and 85.7/4.12 for the β-*O*-4′ linkages linked to G and S units, respectively. The C_γ-H_γ correlations in β-*O*-4′ substructures were exhibited at δ_C/δ_H 59.5/3.66 and 3.41. In addition, the C_γ-H_γ correlations in γ-acylated lignin units (A′) were also observed at δ_C/δ_H 63.0/4.30. These signals indicated that lignin in CK, HCT and C_3H down-regulated poplar wood was partially acylated at the γ-carbon of side chains in β-*O*-4′ aryl ether linkages.[28] Moreover, a signal for the C_β-H_β correlations of γ-acylated β-*O*-4′ substructures linked to a G-unit ($A'_{\beta(G)}$) was clearly noted at δ_C/δ_H 80.6/4.54.[29] In a recent publication, whether *p*-hydroxybenzoates acylated solely S units in transgenic poplar has not been verified.[9] However, the assignment here adds some clues to the partial acylation on the G units. Besides the abundant β-*O*-4′ linkages, the other linkages observed were β-β′ (resinol, B), β-5′ (phenylcoumaran, C), and β-1′ (spirodienone, D, not shown in the current contour level) linkages. For example, resinol substructures (B) appeared in the spectra as indicated by their C_α-H_α, C_β-H_β, and the double C_γ-H_γ correlations at δ_C/δ_H 84.7/4.66, 53.8/3.08, 71.0/3.81 and 4.18 ppm, respectively. Phenylcoumaran (C) was observed in a small amount. The signals for their C_α-H_α correlation were discovered at δ_C/δ_H 86.8/5.44, whereas the C_γ-H_γ correlations were overlapped with other signals around δ_C/δ_H 62.5/3.82. In addition to these linkages, a signal located at δ_C/δ_H 61.4/4.09 was assigned to C_γ-H_γ correlation of *p*-hydroxycinnamyl alcohol end groups (F) in the HSQC spectra.

In the aromatic region of the 2D HSQC spectra of the lignin samples (Fig. 2), syringyl (S) and guaiacyl (G) lignin units were readily observed. The S-lignin units showed a prominent signal for the $C_{2,6}$-$H_{2,6}$ correlations at δ_C/δ_H 103.8/6.68, the $C_{2,6}$-$H_{2,6}$ correlations in C_α-oxidized S units (S′) were observed at δ_C/δ_H 106.2/7.26. The G units showed different correlations for C_2-H_2, C_5-H_5 and C_6-H_6 at δ_C/δ_H 110.9/6.96, 114.9/6.77 and 118.9/6.79, respectively. Obviously, a prominent signal located at δ_C/δ_H 130.4/7.62 was assigned to the $C_{2,6}$-$H_{2,6}$ correlations of the *p*-hydroxybenzoate substructures (PB).

Quantification of the lignin fractions can provide more valuable information. Based on a previous report, the different signal intensities in the side-chain and aromatic regions of HSQC spectra can be expressed in a comparable mode.[24] As shown in Table 2, HCT-EMAL and C_3H-EMAL had higher contents of β-O-4′ linkages as compared to CK-EMAL. However, a different result has also been reported in a previous paper,[3] in which the β-*O*-4′ linkages decreased in relative abundance in the C_3H- and HCT-deficit grass lignin samples. One of the reasons was probably related to the HCT-regulated poplar used in this study, which was four-year-old poplar grown in the field. In general, genetical modification during lignin biosynthesis leads to dwarfing or developmental abnormalities of the transgenic plants.[30] However, after four years of growth, the HCT-regulated poplar probably restores growth if there is an active cell wall feedback signaling responsible for dwarfing existing in lignin deficient mutants.[30]

With regard to the S/G ratio of the lignin, the S/G ratio of CK-EMAL was calculated to be 3.10, while the S/G ratio of HCT-EMAL and C_3H-EMAL slightly increased to 3.25 and 3.33, respectively. Similarly, the content of PB was also higher in HCT-EMAL and C_3H-EMAL than that in CK-EMAL in the present study. This fact suggested that HCT and C_3H down-regulation enhanced the PB content in the lignin. However, as a pendant group, the PB content in the lignin was increased in the HCT and C_3H down-regulated poplar wood, as compared to that in CK wood. In plants, the *p*-hydroxybenzoates are similar to H units, thus the compatibility is unlikely to be determined. The related transferase in poplar has not been identified.[9] The identified transferase will help understand how the transgene affects the pendant group, such as *p*-hydroxybenzoates in hardwood and *p*-coumarate in gramineous plants.

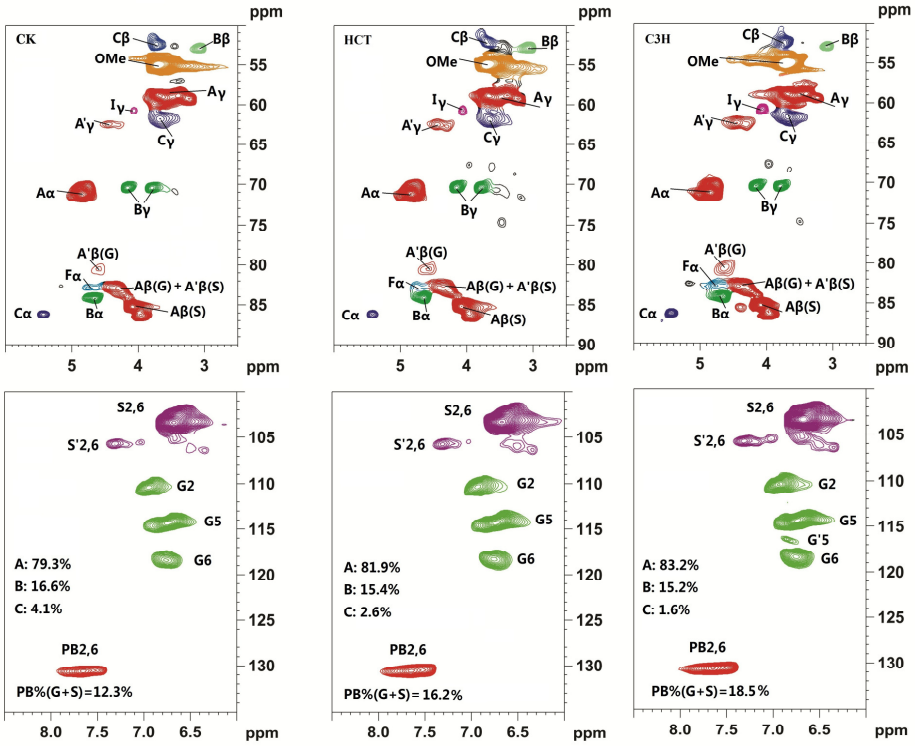

Figure 3: 2D-HSQC spectra of lignins

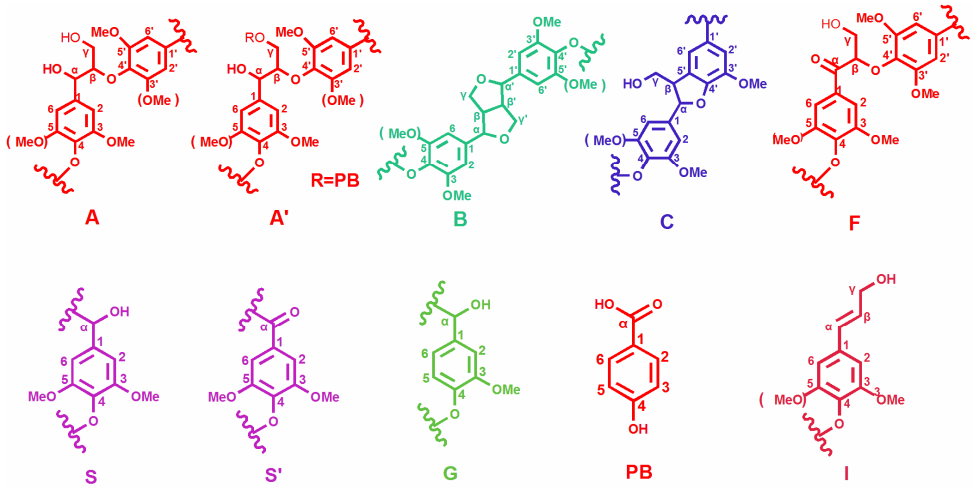

Figure 4: Main classical substructures, involving different side-chain linkages and aromatic units identified by 2D NMR of lignins

Table 2
Yield and carbohydrate contents of EMALs

Lignin	Yield[a]	Sum (%)	Arabinose	Galactose	Glucose	Xylose	Mannose	Glucuronic acid
CK-EMAL	21.1%	2.50	0.12	0.11	1.06	0.99	0.22	0.08
HCT-EMAL	30.0%	4.22	0.18	0.21	2.08	1.40	0.25	0.10
C3H-EMAL	28.2%	3.77	0.23	0.20	1.14	1.81	0.31	N.D

[a] Based on the respective Klason lignin content

Table 3
Quantification of lignin by quantitative 2D-HSQC NMR (% of total side chains involved)

Samples	β-*O*-4′	β-β′	β-5′	PB (%)	S/G
CK-EMAL	79.3	16.6	4.1	12.3	3.10
HCT-EMAL	81.9	15.4	2.6	16.2	3.25
C3H-EMAL	83.2	15.2	1.6	18.5	3.33

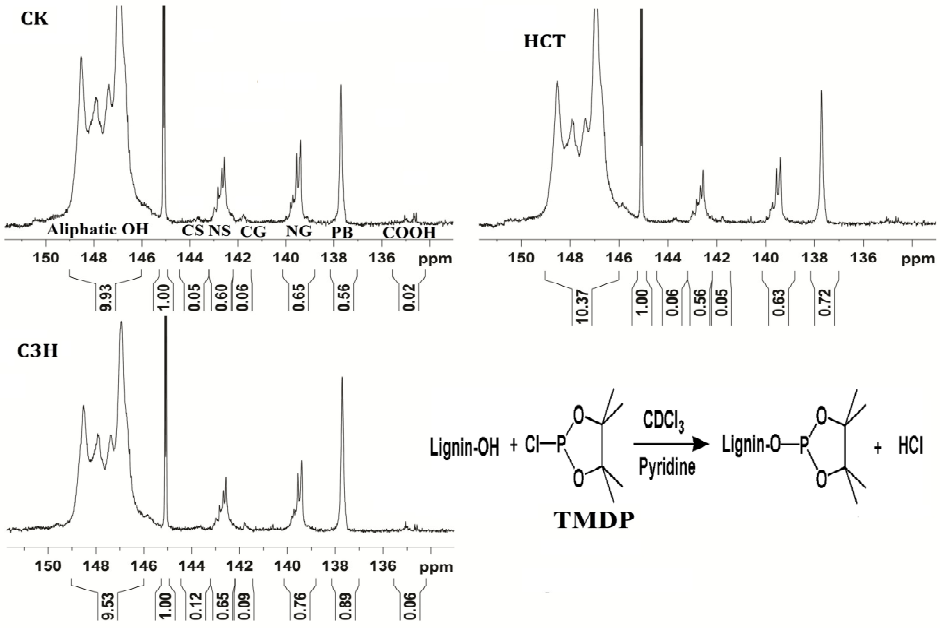

Figure 5: Quantitative ^{31}P NMR spectra of lignins

^{31}P-NMR spectra

^{31}P-NMR methodology is an effective analytical method for quantizing the different hydroxyl groups in lignin (Fig. 5).[19,20] As shown in Table 3, all the lignin samples showed similar aliphatic OH (5.16-5.61 mmol/g). In addition, there were no obvious differences in G and S-type phenolic hydroxyl groups among these samples. It was observed that HCT-EMAL and C_3H-EMAL contained more H-type phenolic hydroxyl groups (*p*-hydroxybenzoate units, 0.39-0.48 mmol/g) than those of CK-EMAL (0.30 mmol/g), suggesting that HCT and C_3H poplar wood contained more *p*-hydroxybenzoate substructures, as also revealed by the 2D HSQC spectra.

Molecular weight analysis

As shown in Table 4, the weight-average (M_w) molecular weight of CK-EMAL, HCT-EMAL and C_3H-EMAL was 14160 g/mol, 12260 g/mol and 15480 g/mol, respectively. The EMAL from C_3H poplar presented a slightly higher M_w and M_n. Moreover, all lignins had narrow molecular weight distributions (low polydispersity, 2.09-2.15). The GPC analysis showed that the HCT and C_3H transgenic lignin had comparable molecular weights, as compared to the wild-type control (Fig. 6), indicating that HCT and C_3H down-regulation under the conditions given appeared to have no significant impact on the molecular weights and polydispersity of lignin.

Enzymatic digestibility

The efficiency of the CK and transgenic poplar in enzymatic hydrolysis was demonstrated by enzymatic digestibility and the results are shown in Fig. 7. In some cases, lignification was highly affected by the C_3H and HCT down-regulation, thus the molecular characteristics of lignin were significantly changed in these transgenic lines.[11-12] The altered content, composition, and the structural features of lignin will affect the subsequent

enzymatic hydrolysis of the biomass. However, few studies focus on the enzymatic hydrolysis of C_3H and HCT transgenic poplar wood. In this study, although the content and structural features of lignin were not obviously changed after C_3H and HCT down-regulation, the cellulose digestibility still needs to be investigated.

Table 4
Quantification of lignin fractions by quantitative ^{31}P-NMR method

	Hydroxyl content (mmol/g of lignin)						
Lignin samples	Aliphatic OH	Syringyl OH		Guaiacyl OH		*p*-Hydroxybenzoate (PB)	Carboxylic OH
		C[a]	NC[b]	C	NC		
CK-EMAL	5.38	0.03	0.32	0.03	0.35	0.30	0.01
HCT-EMAL	5.61	0.03	0.30	0.03	0.34	0.39	0.01
C3H-EMAL	5.16	0.06	0.35	0.05	0.41	0.48	0.03

C[a] condensed; NC[b] non-condensed

Table 5
Weight-average molecular weight (M_w), number-average (M_n) molecular weight and polydispersity (M_w/M_n) of lignin fractions

	CK-EMAL	HCT-EMAL	C3H-EMAL
M_w	14160	12260	15480
M_n	6580	5700	7390
M_w/M_n	2.15	2.15	2.09

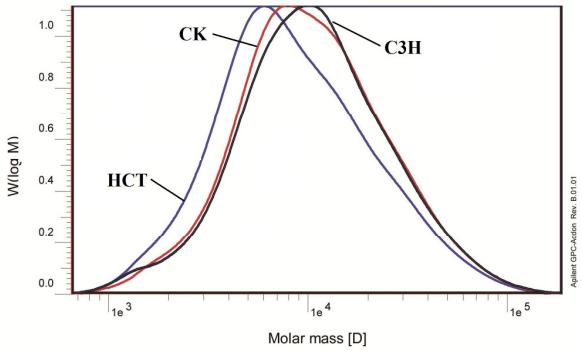

Figure 6: GPC curves of lignins

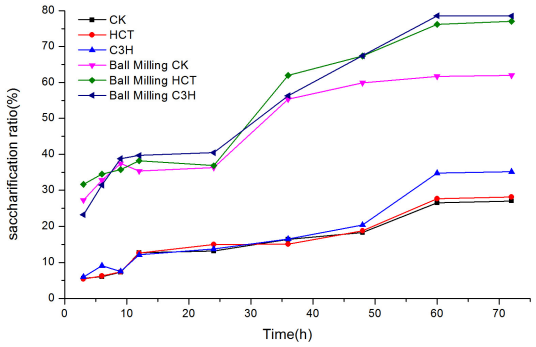

Figure 7: Glucose yields of enzymatic hydrolysis of the control and transgenic feedstock (40-60 mesh and ball-milled 84k poplar)

As shown in Fig. 7, the cellulose digestibility of the CK was 26.7%, whereas the cellulose digestibility of the HCT transgenic poplar slightly increased to 27.4%. By contrast, the cellulose digestibility of the C_3H line increased to 35.0%. The phenomenon suggested that the four years of growth of the transgenic poplars in the field probably reduced the original effects of C_3H and HCT down-regulation. In addition, ball-milling was also applied for evaluating the cellulose digestibility of these poplars. For CK poplar, the cellulose digestibility was 62.0%, while the cellulose digestibility of HCT and C_3H increased to 78.1% and 80.2%, respectively. The data implied that the ball-milling process also helped visualize the effects of C_3H and HCT down-regulation on cellulose digestibility.

CONCLUSION

The down-regulation of C_3H and HCT genes in poplar wood produced plants with slight structural differences in their lignins. The *p*-hydroxybenzoate content was increased in the lignin from HCT and

C_3H down-regulated poplar wood. With regard to the structural changes induced by HCT and C_3H down-regulation, the total OCH_3 contents in the HCT-EMAL and C_3H-EMAL were decreased, while the S/G ratios were increased, suggesting that the demethoxylation of G-units was faster than that of S-units during the down-regulation of these genes. Moreover, HCT-EMAL and C_3H-EMAL had increased contents of β-O-4′ linkage, as compared to that of CK-EMAL, while they had decreased contents of β-β′ and β-5′ linkages. Furthermore, it has been demonstrated that the cellulose digestibility of HCT and C_3H down-regulated wood increased significantly, as compared to that of the control wood. In short, understanding the lignin structures of transgenic feedstock is beneficial for selecting optimal lignin characteristics required for various applications of lignocellulosic materials.

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