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Gene Profiling of Plant Cell Wall Biosynthesis for Genetic Enhancing Biomass Enzymatic Saccharification in Cereal Crops

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Abstract

Cereal crops, including rice, wheat, corn, and sweet sorghum, produce major food grains for humans and generate huge amount biomass for biofuels production. Due to lignocellulose recalcitrance, however, the most biomass residues of cereal crops are not well used for biofuel application, and genetic modification of plant cell walls has thus proposed as a promising solution for enhancing biomass enzymatic saccharification. In the present review, we described plant cell wall compositions and wall polymer features in four cereal crops, and presented their major wall factors that significantly affect biomass enzymatic digestibility under various pretreatments including cellulose crystallinity, arabinose substitution degree of hemicellulose and three monomers proportions of lignin. Furthermore, using globe genomic sequence database and gene expression information, we compared all candidate genes that are involved in cell wall biosynthesis in the four cereal crops, and discussed the potential cell wall modifications for increasing both biomass yields and lignocellulose enzymatic saccharification in the cereal crops by using desire genes and selecting appropriate genetic manipulation approaches.

Keywords: Cereal crops; Plant cell walls; Gene profiling; Genetic engineering; Biomass saccharification; Chemical pretreatment; Biofuels

Introduction

Rice, wheat, corn, and sweet sorghum are four major cereal crops around the world. Over the past years, great efforts have been made to improve grain yields by using genetic breeding and biotechnology. For instance, the annual grain yield in 2014 year reached 6.76, 4.96 and 5.87 tons per hectare for rice, wheat, and corn in China (National data: http://data.stats.gov.cn). Sweet sorghum has been considered as an ideal bioenergy crop for its high fermentable sugar content in stalk and degradable lignocellulose in bagasse with high tolerance to environmental stress [1,2]. Those four cereal crops can provide large amount of lignocellulose residues, accounting for major biomass resources harvested in the farming fields all over the world. However, biomass residues are currently far from good applications, and alternatively some residues are burned in the field, leading to biomass waste and air pollution [3].

As fossil fuels are constantly used as major energy source, bioenergy has been considered as a renewable energy derived from biomass resources for heat, electricity, fuel and chemical products [4,5]. Due to food security, however, bioenergy crops could not be allowed to grow in large arable lands, and lignocellulose-based residues of food crops are alternative source of biomass. Because lignocellulose recalcitrance leads to a costly biomass process coupled with secondary environmental contamination, genetic modification of plant cell walls is thus proposed as a promising solution for enhancing biomass enzymatic digestibility [5,6].

Since plants contain different cell types with complicated cell wall structures and diverse biological functions, genetic modification of plant cell walls may cause defects in plant growth and development with consequent effects on important agronomic traits in crops such as plant lodging resistance, biomass yield and environmental stress tolerance [7-9]. Furthermore, as lignocellulose recalcitrance is mainly determined by plant cell wall composition and wall polymer features [10,11], it becomes essential to find out the key factors of plant cell walls that could not only enhance biomass saccharification, but also have slight impacts on plant growth and development. In this review, we describe recent research advances on lignocellulose recalcitrance and biomass enzymatic digestibility in different plant species, and then discuss the optimal genetic manipulation approaches by genome-wide analyses of the genes involved in cell wall biosynthesis in four major cereal crops.

Biomass Production in Four Cereal Crops

Among four major cereal crops, rice and wheat are the typical C3 plants cultivated in the most regions of Asia, whereas maize and sweet sorghum are the highly photosynthetic-efficient C4 crops that can be widely grown with high biomass yields around the world (Figure 1). Despite that the total grain yields of rice, wheat and maize reached to 206.5074, 126.2084 and 215.6463 million tons in 2004 (National data: http://data.stats.gov.cn), China government should import grains as foods from the overseas each year, leading to the national policy launched that all arable lands must be utilized for food crop cultivations. On the other hands, those three crops provide 75% biomass of total agricultural residues in China [12], but almost half biomass could not be well used for any economical purpose [13]. For instance, besides the crop residues used for livestock feed, heat and fertilizers, 25.7% biomass was unused and 16.3% crop residues were burn in the fields in 2008 (Table 1) [13,14]. Although significant amount of crop residues need to remain in the fields for soil conservation and sustainable grain production, there are approximately 30%-50% of total lignocellulose

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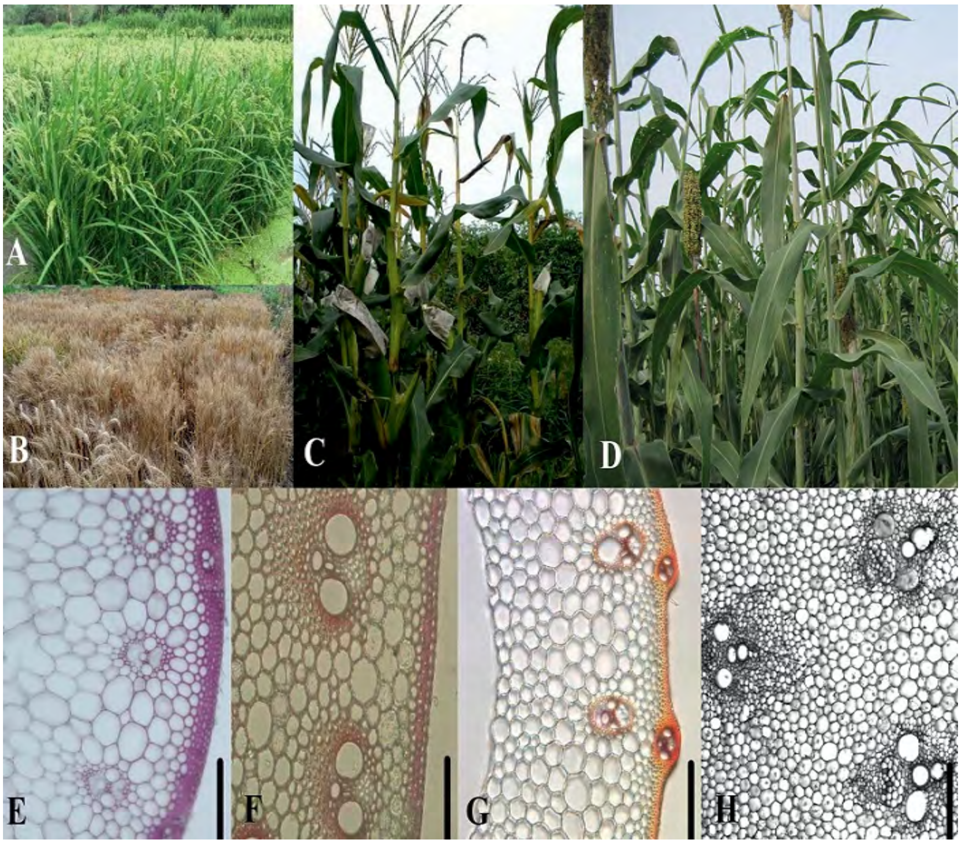

Figure 1: Plant growth and stem tissues in rice (A, E), wheat (B, F), maize (C, G) and sweet sorghum (D, H). (E, F, G) as phloroglucin staining and (H) as bright field image provided by Leiming Wu, Ying Li, Mingliang Zhang, Weichen Hu and Huanhuan Hao; bars indicate 200 µm.

Species	Biomass yield (Tons/ha/year)[a]	Utilization of total biomass (% total)[b]
Rice	15-30	Residues in fields (16.3%)
Wheat	15-30	Fertilizers (12.5%)
		Feed (25.9%)
Maize	15-45	Fuels (15.8%)
		Industrial materials (2.0%)
Sweet	10-30	Other uses (1.8%)
Sorghum		Non-use (25.7%)

Data modified from [a]Xie et al. [13]; [b]Hu et al. [14]

Table 1: Biomass output and current utilization in China.

residues could be applied to biofuel production in the future [15,16].

Plant Cell Wall Composition and Features

As predominate components of crop biomass, plant cell walls are composed of cellulose, hemicelluloses and lignin with minor pectin and wall proteins. Cellulose is the β-1, 4-glucan polymer that accounts for 30%-45% in biomass. In four cereal crops, cellulose levels are variable from 14% to 38%, but wheat and maize have high cellulose contents in average (Table 2) [17-20]. As the high molecular weight polymer, cellulose microfibrils contain crystalline and amorphous regions, and cellulose crystallinity could be characterized by measuring the crystalline index (CrI) of biomass materials. Despite of large variations of cellulose CrI values in four cereal crops, wheat and sweet sorghum have relatively higher avenge CrI values than those of rice and maize (Table 2) [17-20].

Hemicelluloses are branched polymers of heterogeneous polysaccharides that are composed of mainly xylose (Xyl) and arabinose (Ara) in grasses [5,6,16]. Recent reports have indicated that there are great variations of either total hemicellulose levels or monosaccharide compositions in large population of biomass samples of four cereal crops (Table 2) [17-20]. Lignin is the phenolic polymer consisting of three monomers: p-hydroxyphenyl (H), guaiacyl (G), and syringyl (S). By comparison, four cereal crops have relatively smaller amounts of lignin (11%-24%) than that of hemicelluloses, but they also show much variable three monolignol compositions in the biomass residues (Table 2) [17-20].

Species	Cellulose		Hemicellulose				Lignin		
	Level (% dry matter)	CrI (%)	Level (% dry matter)	Xyl (%)[c]	Ara (%)[c]	Level (% dry matter)	G (%)[c]	S (%)[c]	H (%)[c]
Rice [17]	21.99[a]	36.53	13.91	79.88	11.94	14.52	36.66	24.85	38.49
	(14.05~31.62)[b]	(27.46~43.38)	(8.00~23.37)	(69.62~84.84)	(9.23~14.52)	(10.63~18.50)	(23.72~48.27)	(19.73~34.04)	(32.00~45.17)
Wheat [18]	31.82	51.73	31.88	88.92	8.77	21.95	54.1	34.8	11.1
	(27.53~35.89)	(46.80~57.60)	(29.66~34.15)	(87.43~90.18)	(7.91~9.72)	(19.28~24.48)	(47.42~58.14)	(31.85~38.04)	(9.05~18.61)
Maize [19]	30.01	43.96	26.89	86.49	9.28	17.4	33.64	27.08	39.29
	(19.94~38.35)	(39.70~49.27)	(20.89~32.04)	(83.70~89.20)	(7.30~10.80)	(12.83~21.16)	(25.20~40.60)	(21.60~30.80)	(31.50~45.90)
Sweet sorghum [20]	25.59	48.75	23.44	73.7	9.59	15.12	40.64	25.18	34.17
	(18.03~34.06)	(42.81~53.15)	(12.97~31.33)	(69.48~77.42)	(9.08-10.19)	(12.07~19.09)	(37.50~44.84)	(19.58~31.48)	(25.47~42.01)

[a]Mean value; [b]Minimun and maximum values; [c]As percentage of total. Data modified from [d]Li et al. [17], [e]Wu et al. [18], [f]Jia et al. [19], [g]Li et al. [20].

Table 2: Cell wall polymer contents and features of rice, wheat, maize and sweet sorghum.

Biomass Digestibility in Four Cereal Crops

Lignocellulose-based biofuel conversion principally involves in three major steps: physical and chemical pretreatments to deconstruct biomass, subsequent enzymatic hydrolysis to release soluble sugars, and yeast fermentation to produce bioethanol [21,22]. As biomass pretreatment is considered as an initial and crucial step to enhance lignocellulose enzymatic hydrolysis, various pretreatments have been applied using physical, chemical and biological approaches. For instance, hot water, steam explosion, and hydrothermal microwave have been used as physical pretreatments in different biomass materials [23-25]. Alkalis (NaOH, CaO, $NH_3{\cdot}H_2O$), acids (H_2SO_4, H_3PO_4) and ionic liquids are broadly applied as effective chemical pretreatments. In addition, white rot fungi and termites are incubated with biomass materials as biological pretreatments [26,27]. The biomass digestibility (saccharification) could be detected by measuring either hexoses yield (% cellulose) released from hydrolysis by a crude cellulase mixture of lignocellulose after pretreatment or total sugars yield (% dry matter) released from both enzymatic hydrolysis and pretreatment [28,29]. Due to their diverse cell wall compositions and features, the four cereal crops exhibit great variation of hexoses yields released from enzymatic hydrolysis after chemical pretreatments (Table 3) [23-25]. As a comparison, several biomass samples of rice and sweet sorghum could be completely degraded from 4% NaOH pretreatments, whereas wheat and maize samples show relatively low biomass saccharification.

Main Factors of Plant Cell Walls on Biomass Saccharification

It has been reported that biomass saccharification could be affected by plant cell wall compositions and wall polymer features in different plant species [28-30]. In almost all plant biomass materials examined including four cereal crops, cellulose CrI has been examined as the key factor that negatively impacts biomass enzymatic digestions (Table 4) [17-20], probably due to the cellulose CrI accounting for both cellulose crystallinity and wall polymer interlink styles. As hemicelluloses play a central role in wall-network construction, recent reports have indicated that hemicelluloses levels show diverse impacts on biomass saccharification among four cereal crops (Table 4) [17-20]. But, the Xyl/Ara ratios of hemicelluloses exhibit negative effects in all four cereal crops examined, suggesting that the arabinose substitution degree of xylan, a major hemicellulose in grasses, should positively affect biomass enzymatic digestibility. Notably, it has suggested that the substituted Ara may interact with the β-1, 4-glucan chains in amorphous regions of cellulose microfibrils via hydrogen bonding, thereby significantly reducing cellulose crystallinity in rice mutants [29,30].

As lignin is tightly associated with hemicelluloses to maintain plant mechanical strength and biomass recalcitrance [31], it exerts two primary impacts on biomass enzymatic digestions by preventing cellulose microfibrils swelling to reduce surface area access for cellulose enzymes and blocking cellulase action on the cellulose surface [32]. However, recent reports have indicated that lignin could play a dual role in biomass enzymatic hydrolysis distinctive in different plants species, probably due to three monolignol compositions and its interaction styles with other wall polymers [30]. In a conclusion, the lignin level and monolignol ratios of the four cereal crops also exhibit diverse impacts on biomass enzymatic saccharification after chemical pretreatments (Table 4) [17-20].

Gene Profiling for Plant Cell Wall Biosynthesis

During plant growth and development, plant cell walls are systematically formed as primary and secondary walls [33-37]. As the most characteristic component of all plant cell walls, cellulose is synthesized by a large cellulose synthase complex, and the cellulose synthase (CesA) superfamily has been identified in plants. Based on the protein sequence alignment analysis, wheat and maize are respectively identified with 30 and 20 CesA genes, whereas rice and sorghum have 11 CesAs (Figure 2). To date, however, only 8, 18, and 8 CesA genes have been detectable with obvious expression levels in wheat, maize, and sorghum, respectively (Table 5). By comparison, OsCesA family has been well characterized in rice [38,39]. For instance, OsCesA4, OsCesA7, and OsCesA9 are identified as essential isoforms for cellulose biosynthesis of secondary cell walls, whereas other OsCesA genes are involved in primary cell wall formation. Meanwhile, this study also compared the differential expression levels of CesA family in primary and secondary cell walls in the four cereal crops (Figure 3).

Cellulose synthase-like (Csl) genes and glycosyltransferases (GT) genes are increasingly considered as main genes closely related to hemicellulose biosynthesis. In rice, CslD, CslE, CslF, CslH, GT43, GT47 and GT61 are reportedly to associate with hemicellulose biosynthesis [38-41]. For example, rice mutants of OsXAX1 (GT61) [42] and OsIRX10 (GT47) [43] exhibit reduced xylose content and Xyl/Ara value, indicating their involvements in arabinose side-chain substitution and xylan backbone elongation. Using OsCsl and OsGT as references for protein sequence alignment analyses combined with the gene expression data, this study identified the candidate genes associated with hemicellulose biosynthesis in wheat, maize and sorghum (Table 6). Among the candidate genes, TaXAT1 and TaXAT2 genes have been well characterized in wheat by selecting the RNAi transgenic plants that show increased Xyl/Ara value [44].

With respect to lignin biosynthesis, several distinct enzymes have been identified to participate in three monolignols formations including

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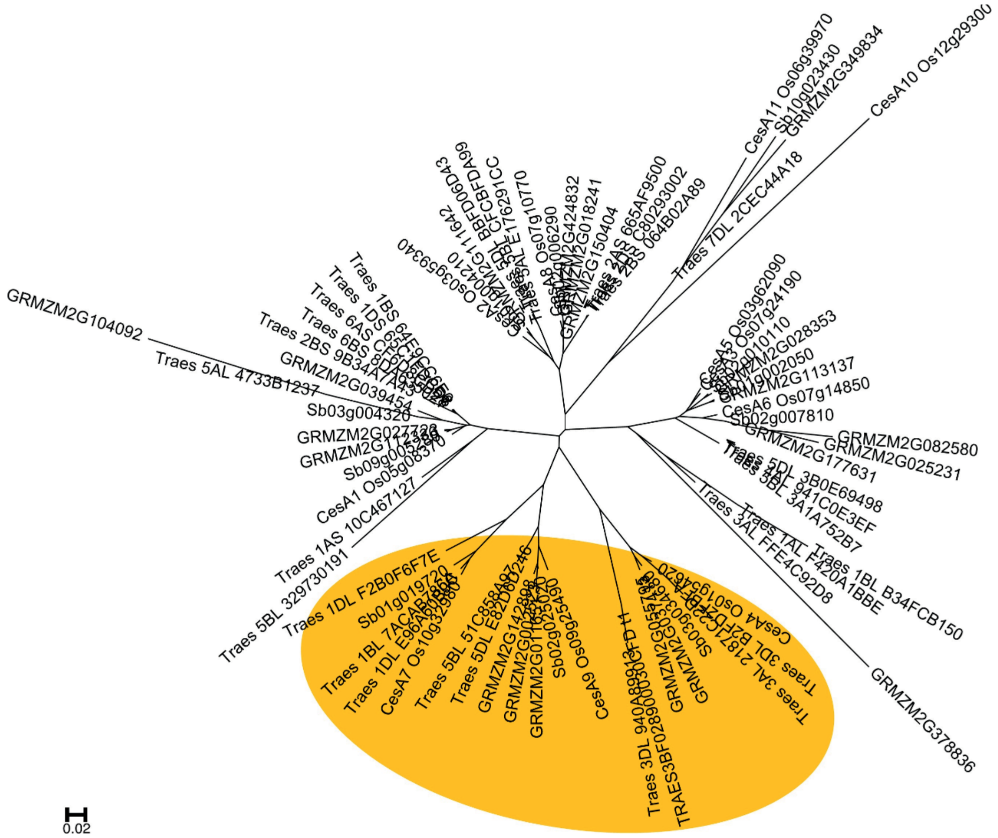

Figure 2: Phylogenetic tree of CesA genes in four cereal crops. Genes associated with secondary cell wall biosynthesis highlighted as orange.

Species	Hexoses (% cellulose)*			
	H_2SO_4		NaOH	
	1%	4%	1%	4%
Rice[d]	64.77[a]	63.03	73.47	93.23
	(58.4~74.3)[b]	(55.4~68.3)	(59.6~93.4)	(79.7~100)
Wheat[d]	43.07	38.52	42.78	51.08
	(34.3~50.3)	(28.0~47.3)	(31.4~54.5)	(37.7~67.0)
Maize[e]	36.33	42.79	49.9	80.19
	(30.3~50.4)	(31.7~51.1)	(35.8~63.0)	(69.7~93.2)
Sweet sorghum[f]	46.95	38.38	76.87	93.26
	(35.6~61.7)	(30.4~46.5)	(56.4~93.8)	(72.9~100.0)

*Hexoses released from cellulases hydrolysis after H_2SO_4 or NaOH pretreatment; [a]Mean value; [b]Minimum and maximum values; Data modified from [d]Wu et al. [18], [e]Jia et al. [19], [f]Li et al. [20].

Table 3: Biomass saccharification under chemical pre-treatments in four cereal crops.

Wall polymers and wall features	Hexoses (% cellulose)							
	Rice [16]		Wheat [17]		Maize [18]		Sweet sorghum [19]	
	1% NaOH	1% H_2SO_4	1% NaOH	1% H_2SO_4	1% NaOH	1% H_2SO_4	1% NaOH	1% H_2SO_4
Cellulose	-	-	-	-	-		-	
CrI	-	-	-	-	-	-	-	-
Hemicellulose	+	+	-	-	/[a]	/	/	
Xylose	+	+						
Arabinose	+	+						
Xly/Ara	-	-	-	-	-[b]	-[b]	-[b]	-[b]
Lignin	+	+	-	-	-		-	
G	+	+					-	-

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S	+	+					/	/
H	+	+					/	/
S/G	/	/	/	/	+[b]	+[b]	/	/
H/G	+[g]	+[g]	+[g]	+[g]	/	/	/	/
H/S	/	/	/	/			/	/

[a]No significant effect; [b]Xyl/Ara and S/G are that of non-KOH extractable hemicellulose and lignin respectively; [g]H/G are that of KOH extractable lignin.

Table 4: Impacts of wall polymer features on biomass digestibility after chemical retreatments in four cereal crops.

Wall polymer	Gene family	*Oryza sativa* Japonica	*Triticum aestivum*	*Zea mays*	*Sorghum bicolor*
Cellulose	CesA	LOC_Os07g24190	Traes_2BS_064B02A89	GRMZM2G113137_T01	Sb02g010110
		LOC_Os05g08370	Traes_1DS_65C1FDCD8	GRMZM2G111642_T01	Sb09g005280
		LOC_Os07g14850	TRAES3BF028900030CFD_t1	GRMZM2G028353_T01	Sb02g006290
		LOC_Os07g10770	Traes_6AS_CF6D8CD28	GRMZM2G112336_T01	Sb02g025020
		LOC_Os03g62090	Traes_1AL_F420A1BBE	GRMZM2G039454_T01	Sb03g034680
		LOC_Os01g54620	Traes_5BL_51C858A97	GRMZM2G018241_T01	Sb03g004320
		LOC_Os10g32980	Traes_1BS_64E9CC6E0	GRMZM2G150404_T01	Sb02g007810
		LOC_Os09g25490	Traes_5DL_3B0E69498	GRMZM2G424832_T01	Sb10g023430
		LOC_Os03g59340		GRMZM2G027723_T01	
		LOC_Os03g59330		GRMZM2G025231_T01	
		LOC_Os06g39970		GRMZM2G177631_T01	
				GRMZM2G037413_T01	
				GRMZM2G055795_T01	
				GRMZM2G002523_T01	
				GRMZM2G011651_T01	
				GRMZM2G142898_T01	
				GRMZM2G378836_T01	
				GRMZM2G349834_T01	

Table 5: List of cellulose synthase (CesA) genes with highest expressions in four cereal crops.

Wall polymer	Gene family		*Oryza sativa* Japonica	*Triticum aestivum*	*Zea mays*	*Sorghum bicolor*
Hemicellulose	CslD	Expressed gene number	5	0	5	5
		Genes with highest expression	LOC_Os06g02180		GRMZM5G870176_T01	Sb10g000980
			LOC_Os06g22980		GRMZM2G015886_T01	Sb08g017750
			LOC_Os08g25710		GRMZM2G044269_T01	Sb07g011890
	CsLE	Expressed gene number	2	5	3	4
		Genes with highest expression	LOC_Os09g30120	Traes_5AL_9C9E55228	GRMZM2G012044_T01	Sb02g027610
			LOC_Os02g49332	Traes_5BL_B89047CEB	GRMZM2G014558_T01	Sb04g029420
				Traes_6BL_38857B770	GRMZM2G122431_T01	Sb02g027570
	CslF	Expressed gene number	6	1	7	8
		Genes with highest expression	LOC_Os08g06380	Traes_2BS_F0846FCC2	GRMZM2G110145_T01	Sb07g004110
			LOC_Os07g36630		GRMZM2G122277_T01	Sb02g036020
			LOC_Os07g36750		GRMZM2G113432_T01	Sb02g035980
	CslH	Expressed gene number	3	2	1	2
		Genes with highest expression	LOC_Os10g20090	Traes_2BL_5D736B4B7	GRMZM2G074546_T01	Sb06g016770
			LOC_Os04g35020	Traes_2AL_E6C429BE0		Sb06g016750
			LOC_Os04g35030			
	GT43	Expressed gene number	10	5	12	10
		Genes with highest expression	LOC_Os04g55670	Traes_2BL_BF242FF5C	GRMZM2G113655_T01	Sb10g027970
			LOC_Os06g47340	Traes_1AS_1FD6B3E2D	GRMZM2G150302_T01	Sb03g030990
			LOC_Os01g48440	Traes_4AS_FD9FAE3D8	GRMZM2G150264_T01	Sb09g028220
	GT47	Expressed gene number	36	16	49	36
		Genes with highest expression	LOC_Os04g32670	Traes_3AL_69C3C3A87	GRMZM2G056702_T01	Sb09g027440
			LOC_Os01g70200	Traes_2AL_29B1F83B2	GRMZM2G076688_T01	Sb03g044530
			LOC_Os02g32110	Traes_5AL_E799C43E9	GRMZM2G018962_T01	Sb03g044520
	GT61	Expressed gene number	25	13	33	29
		Genes with highest expression	LOC_Os01g72610	Traes_6AS_5DD54182D	GRMZM2G096946_T01	Sb03g008020
			LOC_Os06g27560	Traes_7AL_8895EDF48	GRMZM2G176576_T01	Sb03g008010
			LOC_Os03g37010	Traes_1AL_9EC1E6F0C	GRMZM2G419267_T01	Sb10g029380

Table 6: List of highly expressed genes involved in hemicellulose synthesis in four cereal crops.

phenylalanine ammonia lyase (PAL), cinnamate 4-hydroxylase (C4H), 4-coumarate-CoA ligase (4CL), shikimate hydroxycinnamoyl transferase (HCT), coumarate 3-hydroxylase (C3H), caffeoyl-CoA 3-O-methyltransferase (CCoAOMT), ferulate 5-hydroxylase (F5H), and caffeic acid/5-hydroxyferulic acid O-methyltransferase (COMT) [45]. Using PAL, C4H, 4CL, HCT, C3H, CCoAOMT, F5H, and COMT gene families in rice as a reference, the genes related to these families are identified in wheat, maize, and sweet sorghum based on protein sequence homology (Table 7). In particular, functions of Os4CL [46], SbCOMT [47], and ZmCOMT [48] have been analyzed using RNAi transgenic plants or genetic mutants.

Potential Modification of Plant Cell Walls

Genetic modification of plant cell wall has been considered to enhance biomass digestibility by selecting transgenic plants and genetic mutants. Because plant cell walls have complicated structures and diverse biological functions, it remains a challenge to maintain plant normal growth and mechanism strength with high biomass yields in the transgenic plants and mutants. To address this issue, it becomes essential to selection appropriate genes and genetic engineering approaches and a model was thus proposed based on current research progress (Figure 4).

Wall polymer	**Gene family**		***Oryza sativa* Japonica**	***Triticum aestivum***	***Zea mays***	***Sorghum bicolor***
Lignin	PAL	Expressed gene number	7	15	12	8
		Genes with highest expression	LOC_Os02g41630	Traes_6DL_1AEA7B869	GRMZM2G074604_T01	Sb06g022750
			LOC_Os02g41650	Traes_2BL_13D5272D7	GRMZM2G029048_T01	Sb04g026510
			LOC_Os04g43760	Traes_2AL_9EC3226F7	GRMZM2G160541_T01	Sb06g022740
	C4H	Expressed gene number	3	4	4	3
		Genes with highest expression	LOC_Os05g25640	Traes_3AS_44927A043	GRMZM2G147245_T01	Sb02g010910
			LOC_Os02g26810	Traes_7DL_33BB5BE33	GRMZM2G139874_T01	Sb03g038160
			LOC_Os01g60450	Traes_3AL_395DABDE6	GRMZM2G010468_T01	Sb04g017460
	4CL	Expressed gene number	3	3	5	4
		Genes with highest expression	LOC_Os06g44620	Traes_6DS_B3BD3031B	GRMZM2G075333_T01	Sb10g026130
			LOC_Os08g34790	TRAES3BF234900010CFD_t1	GRMZM2G054013_T01	Sb04g005210
			LOC_Os02g08100	Traes_7BL_5ABCB74E1	GRMZM2G055320_T01	Sb07g022040
	HCT	Expressed gene number	7	9	17	19
		Genes with highest expression	LOC_Os02g39850	Traes_6AL_D8A91F983	GRMZM2G158083_T01	Sb04g025760
			LOC_Os09g25460	Traes_5DL_EA74A6BF3	GRMZM2G156816_T01	Sb06g021640
			LOC_Os11g07960	Traes_5BL_C43CADB04	GRMZM2G035584_T01	Sb02g022440
	C3H	Expressed gene number	1	3	3	3
		Genes with highest expression	LOC_Os05g41440	Traes_1AL_A0B81FF76	GRMZM2G140817_T01	Sb09g024210
				Traes_4BL_C2714B866	GRMZM2G138074_T01	Sb03g037380
				Traes_4DL_4C9D6ADB4	GRMZM2G138248_T01	Sb07g002610
	CCoAOMT	Expressed gene number	5	3	7	8
		Genes with highest expression	LOC_Os08g38900	Traes_7AS_460CD64C3	GRMZM2G127948_T01	Sb10g004540
			LOC_Os08g38910	Traes_7AL_96299B626	GRMZM2G099363_T01	Sb07g028520
			LOC_Os06g06980	Traes_7AS_6BB29FB77	GRMZM2G077486_T01	Sb07g028530
	F5H	Expressed gene number	3	1	1	2
		Genes with highest expression	LOC_Os10g36848	Traes_1BL_8617E565C	GRMZM2G100158_T01	Sb02g002630
			LOC_Os03g02180			Sb05g007210
			LOC_Os06g24180			
	COMT	Expressed gene number	7	11	10	15
		Genes with highest expression	LOC_Os08g06100	Traes_7DL_930094B08	GRMZM2G082998_T01	Sb07g003860
			LOC_Os04g01470	Traes_5DS_554AEC0FE	GRMZM2G060886_T02	Sb08g021580
			LOC_Os02g57760	Traes_1BL_22EDF8650	GRMZM2G170400_T01	Sb08g021610

Table 7: List of highly expressed genes involved in lignin synthesis in four cereal crops.

From large rice mutagenesis pools, Li et al. have selected two desire rice mutants (Osfc17 and Osfc30) that show enhanced biomass production and lignocellulose saccahrification with improved lodging resistances by reducing cellulose CrI value and increasing hemicellulosic arabinose level [31]. Both mutants exhibit elevated transcription levels of the genes responsible for the reduction of cellulose CrI (GH9B1, B3 and B16) and the elongation of arabinose side chain (OsXAT2 and OsXAT3). Obviously, those homologous genes should be considered for genetically enhancing both biomass enzymatic digestibility and lodging resistance in the four cereal crops. More recently, our preliminary data have suggested that it might enhance biomass yield and lignocellulose enzymatic digestion by either selecting transgenic plants that overexpress CesA3, 6 genes of primary cell walls or generating genetic mutants with site-specific mutation at conserve regions of CesA7, 9 proteins for secondary cell wall biosynthesis in the four cereal crops (Figure 4).

As OsIRX14, OsIRX9, and OsIRX9L are involved in xylan biosynthesis [49], those homologous genes should be considered genes for potential genetic modifications of hemicelluloses in four cereal crops. Because RNAi silencing of TaXAT1, T2 leads to increased Xyl/Ara value of hemicelluloses with little effect on biomass yields in the transgenic wheat plants [44], overexpressing those homologous genes may increase Ara substitution degree of xylan for enhancing biomass enzymatic saccharification in the four cereal crops (Figure 4). In addition, since several Csl family genes are reportedly involved in hemicellulose biosynthesis [50], they should be also considered for genetic modification of plant cell walls.

Furthermore, genetic mutants and transgenic plants associated with lignin biosynthesis have been reported in the cereal crops. For instances, site-mutations of COMT genes lead to reduced lignin levels for improved biomass saccharification in sorghum [47], and RNAi silencing of the 4CL1 gene could decrease lignin level for enhanced biomass digestibility in switchgrass [51]. However, most mutants and transgenic plants exhibit a defect in plant growth and stress tolerance with a significantly reduced biomass yields [8,31,52,53]. Hence, genetic alteration of three monolignol proportions, rather than reduction of total lignin levels, should be an alternative approach for cell wall modification. As the biomass samples with high-level of G-monomer show an effective wall polymer extraction from physical and chemical pretreatments [20], increasing G-monomer may lead to enhanced biomass digestibility in the four cereal crops (Figure 4).

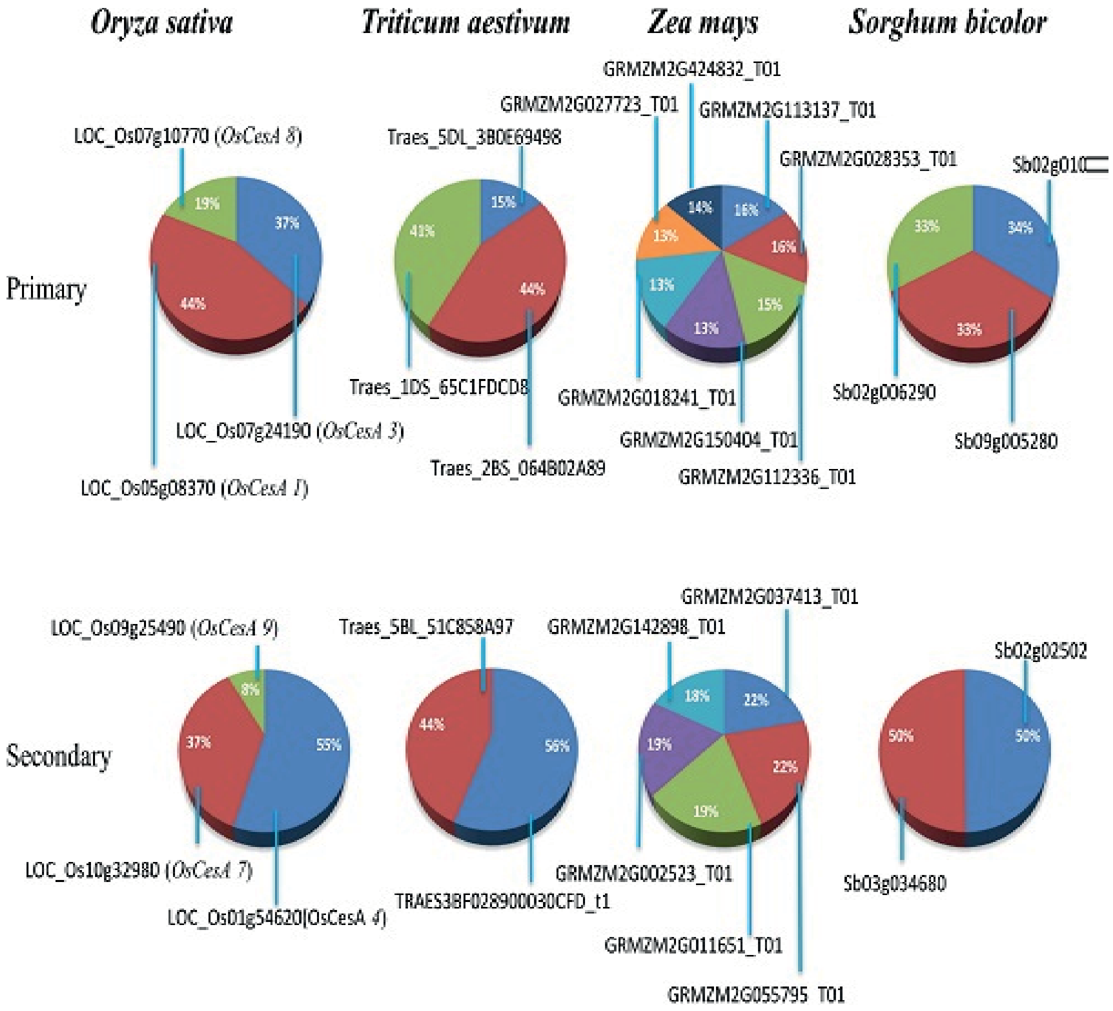

Figure 3: Comparison of CesA expressions in primary and secondary cell walls in four cereal crops.

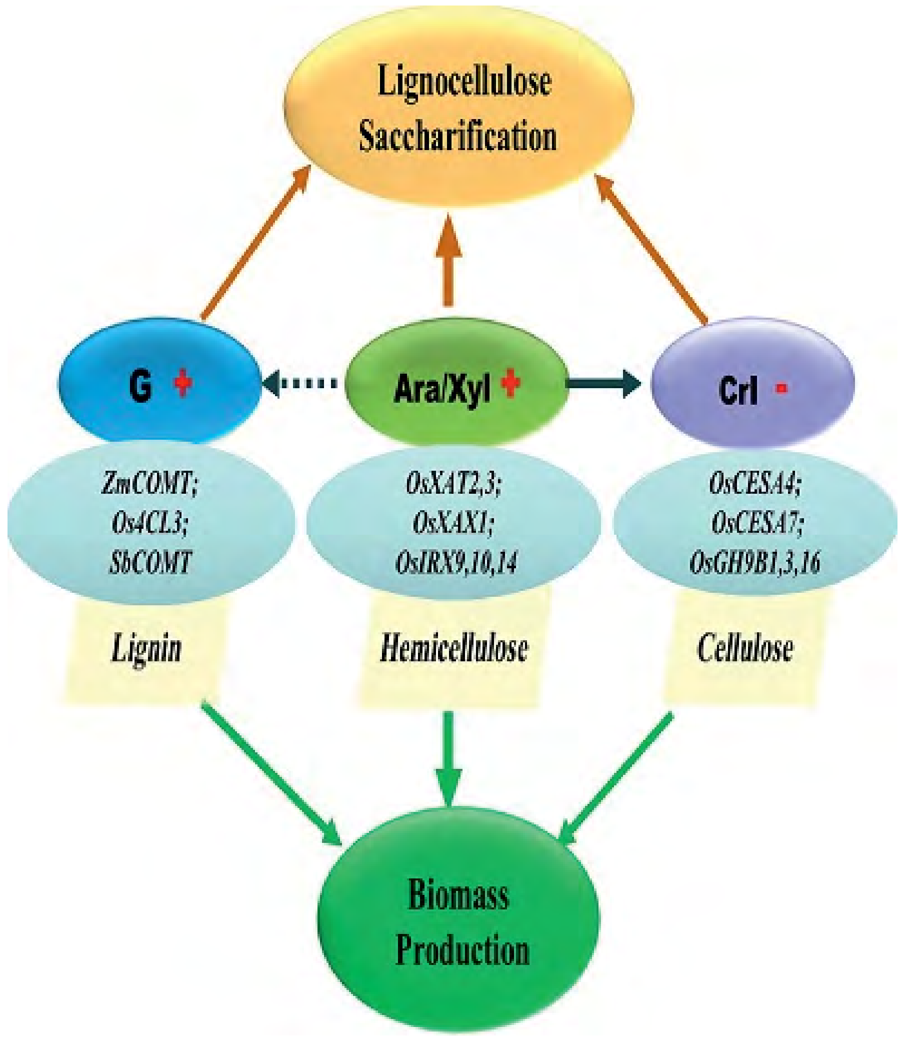

Figure 4: A model of potential cell wall modifications for genetically enhancing both biomass production and lignocellulose enzymatic digestibility in four cereal crops using the candidate genes. +/- for increasing and decreasing cell wall polymer features.

Conclusion

Based on biomass enzymatic saccharification under various physical and chemical pretreatments, this study reveals three major factors applicable for mild genetic modification of plant cell walls in cereal crops (rice, wheat, corn, and sweet sorghum), including cellulose crystallinity, arabinose substitution degree of hemicellulose and G-monomer proportion of lignin. According to global gene expression profiling and genomic sequences analysis, several related candidate genes are considered to select the transgenic cereal plants that could enhance both biomass production and lignocellulose enzymatic digestibility. Hence, this study has proposed a hypothetic model for potential cell wall modification under appropriate genetic manipulation in cereal crops.

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References

1. Zegada-Lizarazu W, Monti A (2012) Are we ready to cultivate sweet sorghum as a bioenergy feedstock? A review on field management practices. Biomass Bioenergy 40: 1-12.
2. Zegada-Lizarazu W, Zatta A, Monti A (2012) Water uptake efficiency and aboveand belowground biomass development of sweet sorghum and maize under different water regimes. Plant Soil 351: 47-60.
3. Chen L, Xing L, Han L (2009) Renewable energy from agro-residues in China: solid biofuels and biomass briquetting technology. Renewable and Sustainable Energy Reviews 13: 2689-2695.
4. Himmel ME, Bayer EA (2009) Lignocellulose conversion to biofuels: Current challenges, global perspectives. Curr Opinion in Biotechnology 20: 316-317.
5. Yuan JS, Tiller KH, Al-Ahmad H, Stewart NR, Stewart Jr CN (2008) Plants to power: Bioenergy to fuel the future. Trends in Plant Science 13: 421-429.
6. Chen P, Peng LC (2013) The diversity of lignocellulosic biomass resources and their evaluation for use as biofuels and chemicals. In: Sun JZ, Ding SY, Peterson JD (eds.). Biological conversion of biomass for fuels and chemicals: explorations from natural biomass utilization systems. Royal Society of Chemistry, pp: 83-109.

7. Abramson M, Shoseyov O, Shani Z (2010) Plant cell wall reconstruction toward improved lignocellulosic production and processability. Plant Science 178: 61-72.
8. Casler MD, Buxton DR, Vogel KP (2002) Genetic modification of lignin concentration affects fitness of perennial herbaceous plants. Theoretical and Applied Genetics 104: 127-131.
9. Li X, Weng JK, Chapple C (2008) Improvement of biomass through lignin modification. The Plant Journal 54: 569-581.
10. Himmel ME, Ding SY, Johnson DK, Adney WS, Nimlos MR, et al. (2007) Biomass recalcitrance: engineering plants and enzymes for biofuels production. Science 315: 804-807.
11. Lynd LR, Laser MS, Brandsby D, Dale BE, Davison B, et al. (2008) How biotech can transform biofuels. Nature Biotechnology 26: 169-172.
12. Li X, Weng JK, Chapple C (2008) Improvement of biomass through lignin modification. The Plant Journal 54: 569-581.
13. Xie GS, Peng LC (2011) Genetic engineering of energy crops: a strategy for biofuel production in China. Journal of Integrative Plant Biology 53: 143-150.
14. Hu RQ, Qin SP, Fan JC, Zhang QF, Zhao YQ, et al. (2010) Study on China Biomass Energy Technology Development Roadmap. The Renewable Energy Development Center of Energy Research Institute (ERI), China National Development and Reform Commission (NDRC).
15. Antizar-Ladislao B, Turrion-Gomez JL (2008) Second-generation biofuels and local bioenergy systems. Biofuels Bioproducts & Biorefining 2: 455-469.
16. Liu H, Sale KL, Holmes BM, Simmons BA, Singh S (2010) Understanding the interactions of cellulose with ionic liquids: a molecular dynamics study. The Journal of Physical Chemistry B 114: 4293-4301.
17. Li FC, Zhang ML, Guo K, Hu Z, Zhang R, et al. (2015) High-level hemicellulosic arabinose predominately affects lignocellulose crystallinity for genetically enhancing both plant lodging resistance and biomass enzymatic digestibility in rice mutants. Plant Biotechnology Journal 13: 514-525.
18. Wu ZL, Zhang ML, Wang LQ, Tu YY, Zhang J, et al. (2013) Biomass digestibility is predominantly affected by three factors of wall polymer features distinctive in wheat accessions and rice mutants. Biotechnology for Biofuels 6: 183.
19. Jia J, Yu B, Wu LM, Wang HW, Wu ZL, et al. (2014) Biomass enzymatic saccharification is determined by the non-KOH-extractable wall polymer features that predominately affect cellulose crystallinity in corn. PLoS One 9: e108449.
20. Li M, Feng SQ, Wu LM, Li Y, Fan CF, et al. (2014) Sugar-rich sweet sorghum is distinctively affected by wall polymer features for biomass digestibility and ethanol fermentation in bagasse. Bioresource Technology 167: 14-23.
21. Pauly M, Keegstra K (2008) Cell-wall carbohydrates and their modification as a resource for biofuels. The Plant Journal 54: 559-568.
22. Ragauskas AJ, Williams CK, Davison BH, Britovsek G, Cairney J, et al. (2006) The path forward for biofuels and biomaterials. Science 311: 484-489.
23. Aguedo M, Ruizb HA, Richel A (2015) Non-alkaline solubilization of arabinoxylans from destarched wheat bran using hydrothermal microwave processing and comparison with the hydrolysis by an endoxylanase. Chemical Engineering and Processing 96: 72-82.
24. Kapoor M, Raj T, Vijayaraj M, Chopra A, Gupta RP, et al. (2015) Structural features of dilute acid, steam exploded, and alkali pretreated mustard stalk and their impact on enzymatic hydrolysis. Carbohydrate Polymers 124: 265-273.
25. Yu Q, Zhuang XS, Lv SL, He MC, Zhang Y, et al. (2013) Liquid hot water pretreatment of sugarcane bagasse and its comparison with chemical pretreatment methods for the sugar recovery and structural changes. Bioresource Technology 129: 592-598.
26. Ghorbani F, Karimi M, Biria D, Kariminia HR, Jeihanipour A (2015) Enhancement of fungal delignification of rice straw by Trichoderma viride sp. to improve its saccharification. Biochemical Engineering Journal 101: 77-84.
27. Wang W, Yuan TQ, Cui BK, Dai YC (2013) Investigating lignin and hemicellulose in white rot fungus-pretreated wood that affect enzymatic hydrolysis. Bioresource Technology 134: 381-385.
28. Xu N, Zhang W, Ren SF, Liu F, Zhao CQ, et al. (2012) Hemicelluloses negatively affect lignocellulose crystallinity for high biomass digestibility under NaOH and H_2SO_4 pretreatments in Miscanthus. Biotechnology for Biofuels 5: 58.
29. Li FC, Ren SF, Zhang W, Xu Z, Xie GS, et al. (2013) Arabinose substitution degree in xylan positively affects lignocellulose enzymatic digestibility after various NaOH/H_2SO_4 pretreatments in Miscanthus. Bioresource Technology 130: 629-637.
30. Wang YT, Fan CF, Hu HZ, Ying L, Dan S, et al. (2016) Genetic modification of plant cell walls to enhance biomass yield and biofuel production in bioenergy crops. Biotechnology Advances 34: 997-1017.
31. Li FC, Zhang ML, Guo K, Hu Z, Zhang R, et al. (2015) High-level hemicellulosic arabinose predominately affects lignocellulose crystallinity for genetically enhancing both plant lodging resistance and biomass enzymatic digestibility in rice mutants. Plant Biotechnology Journal 13: 514-525.
32. Keating J, Panganiban C, Mansfield S (2006) Tolerance and adaptation of ethanologenic yeasts to lignocellulosic inhibitory compounds. Biotechnology and Bioengineering 93: 1196-1206.
33. Cosgrove D (2005) Growth of the plant cell wall. Nature Reviews Molecular Cell Biology 6: 850-861.
34. Dumville JC, Fry SC (2000) Uronic acid-containing oligosaccharins: Their biosynthesis, degradation and signalling roles in non-diseased plant tissues. Plant Physiology and Biochemistry 38: 125-140.
35. Hall Q, Cannon MC (2002) The cell wall hydroxyproline-rich glycoprotein RSH is essential for normal embryo development in Arabidopsis. The Plant Cell 14: 1161-1172.
36. Motose H, Sugiyama M, Fukuda H (2004) A proteoglycan mediates inductive interaction during plant vascular development. Nature 429: 873-878.
37. Wang YT, Xu ZD, Peng LC (2014) Model of plant cell wall ditches structure for enhancing biomass saccharification and plant resistances. Scientia Sinica Vitae 2014 44: 766-774.
38. Wang LQ, Guo K, Li Y, Tu YY, Hu HZ, et al. (2010) Expression profiling and integrative analysis of the CESA/CSL superfamily in rice. BMC Plant Biology 10: 282.
39. Guo K, Zou WH, Feng Y, Zhang M, Zhang J, et al. (2014) An integrated genomic and metabolomic frame work for cell wall biology in rice. BMC Genomics 15: 596.
40. Pauly M, Gille S, Liu LF, Mansoori N, Souza AD, et al. (2013) Hemicellulose biosynthesis. Planta 238: 627-642.
41. Scheller HV and Ulvskov P (2010) Hemicelluloses. Annual Review of Plant Biology 61: 263-289.
42. Chiniquy D, Sharma V, Schultink A, Baidoo EE, Rautengarten C, et al. (2012) XAX1 from glycosyltransferase family 61 mediates xylosyltransfer to rice xylan. Proceedings of the National Academy of Sciences of the USA 109: 17117-17122.
43. Chen XW, Vega-Sánchez M, Verhertbruggen Y, Chiniquy D, Canlas PE, et al. (2013) Inactivation of OsIRX10 leads to decreased xylan content in rice culm cell walls and improved biomass saccharification. Molecular Plant 6: 570-573.
44. Anders N, Wilkinson MD, Lovegrove A, Freeman J, Tryfona T, et al. (2012) Glycosyl transferases in family 61 mediate arabinofuranosyl transfer onto xylan in grasses. Proceedings of the National Academy of Sciences of the USA 109: 989-993.
45. Vanholme R, Demedts B, Morreel K, Ralph J, Boerjan W (2010) Lignin Biosynthesis and Structure. Plant Physiology 153: 895-905.
46. Gui J, Shen J, Li L (2011) Functional characterization of evolutionarily divergent 4-coumarate: coenzyme a ligases in rice. Plant Physiology 157: 574-586.
47. Sattler SE, Palmer NA, Saballos A, Greene AM, Xin ZG, et al. (2012) Identification and characterization of four missense mutations in Brown midrib 12 (Bmr12), the caffeic O-methyltranferase (COMT) of sorghum. Bioenergy Research 5: 855-865.
48. Guillaumie S, Goffner D, Barbier O, Martinant J, Pichon M, et al. (2008) Expression of cell wall related genes in basal and ear internodes of silking brown-midrib-3, caffeic acid O-methyltransferase (COMT) down-regulated, and normal maize plants. BMC Plant Biology 8: 71.
49. Chiniquy D, Varanasi P, Oh T, Harholt J, Katnelson J, et al. (2013) Three novel rice genes closely related to the Arabidopsis irx9, irx9l, and irx14 genes and their roles in xylan biosynthesis. Frontiers in Plant Science 4: 83.

50. Yin L, Verhertbruggenb Y, Oikawab A, Manisserib C, Knierim B, et al. (2011) The cooperative activities of csld2, csld3, and csld5 are required for normal arabidopsis development. Molecular Plant 4: 1024-1037.
51. Xu B, Escamillatreviño LL, Sathitsuksanoh N, Shen Z, Shen H, et al. (2011) Silencing of 4-coumarate:coenzyme a ligase in switchgrass leads to reduced lignin content and improved fermentable sugar yields for biofuel production. New Phytologist 192: 611-625.
52. Pedersen JF, Vogel KP, Funnell DL (2005) Impact of reduced lignin on plant fitness. Crop Science 45: 812-819.
53. Vega-Sánchez ME, Ronald PC (2010) Genetic and biotechnological approaches for biofuel crop improvement. Current Opinion in Biotechnology 21: 218-224.