Supplementary Materials

Semi-overexpressed OsMYB86L2 specifically enhances cellulose biosynthesis to maximize bioethanol productivity by integrating green-like lignocellulose de-polymerization

Hailang Wang[a,b], Sufang Li[a,b], Leiming Wu[a,c], Weihua Zou[a], Mingliang Zhang[a], Youmei Wang[a,d], Zhengyi Lv[a,b], Peng Chen[a], Peng Liu[b], Yujing Yang[b], Liangcai Peng[a,b], Yanting Wang[a,b*]

[a] College of Plant Science & Technology, Huazhong Agricultural University, Wuhan 430070, China.

[b] Key Laboratory of Fermentation Engineering (Ministry of Education), National "111" Center for Cellular Regulation & Molecular Pharmaceutics, Hubei Key Laboratory of Industrial Microbiology, School of Life & Health Sciences, Hubei University of Technology, Wuhan, 430068, China.

[c] The National Engineering Laboratory of Crop Resistance Breeding, School of Life Sciences, Anhui Agricultural University, Hefei, 230036, China.

[d] Houji Laboratory in Shanxi Province, Academy of Agronomy, Taiyuan, 030000, China.

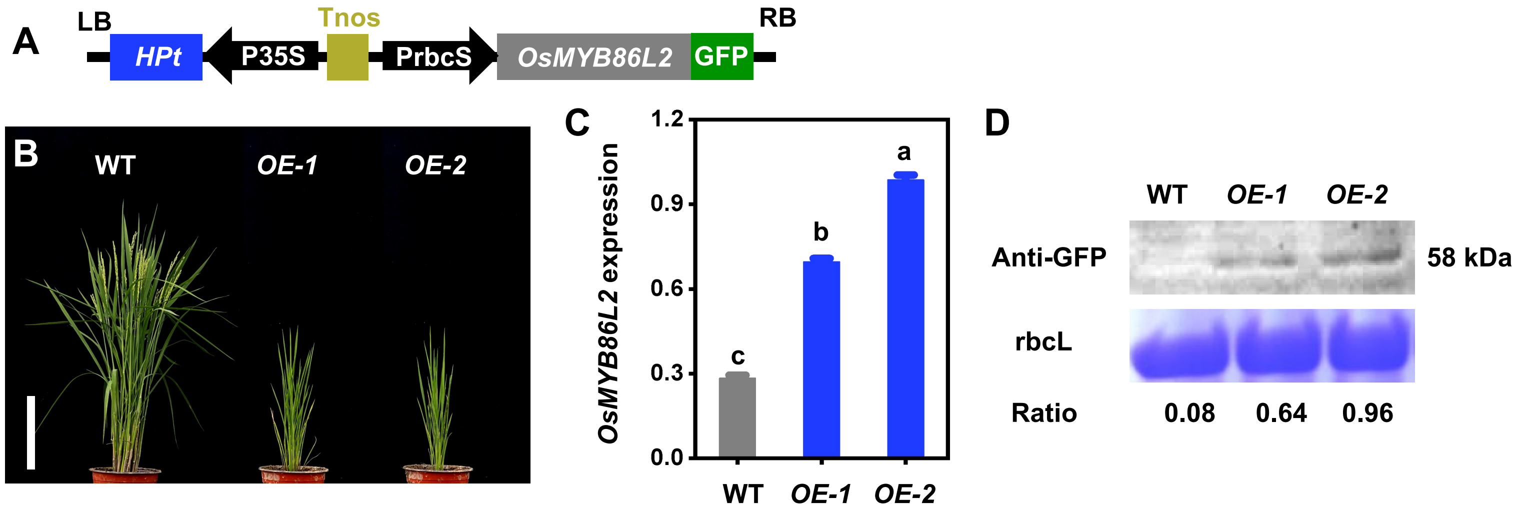


Figure S1. Detection of transgenic rice plants overexpressing *OsMYB86L2* gene. (A) The vector pC1300T-OsMYB86L2-GFP with the OsrbcS promoter; (B) Image of two transgenic rice lines (OE-1, -2) and WT (NPB) at heading stage, scale bar as 20 cm; (C) Real-time PCR assay of *OsMYB86L2* expression; (D) Western blotting analysis of OsMYB86L2 protein levels using anti-GFP, rbcL as rubisco large subunit protein for internal reference from SDS gel running; All primers listed in Table S1; Data as mean $\pm$ SD (n = 3); Lowercases (a, b and c) as multiple significant differences by LSD test at $p < 0.05$, respectively.

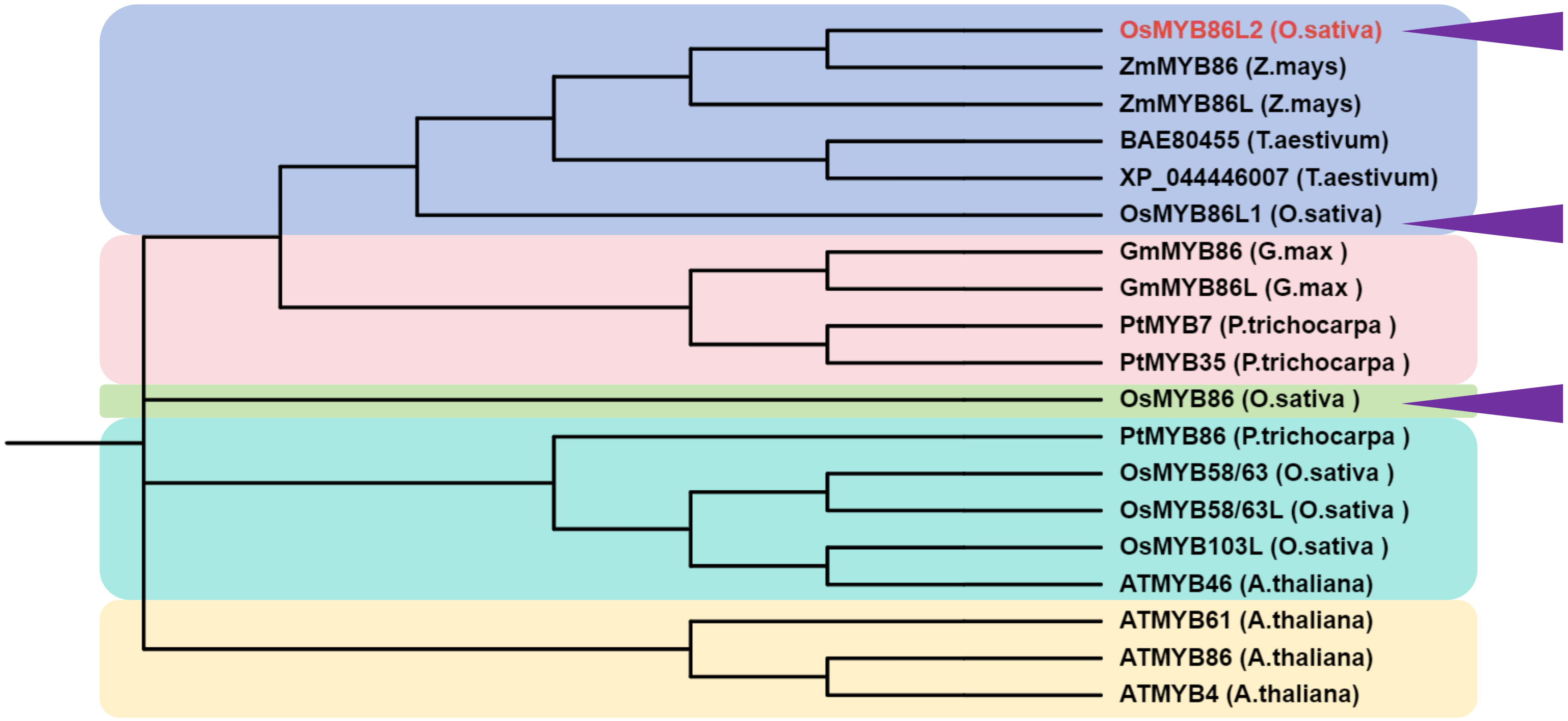


Figure S2. Unrooted neighbour-joining tree of plant MYB86 homologs, bootstrap analysis performed with 1000 iterations.

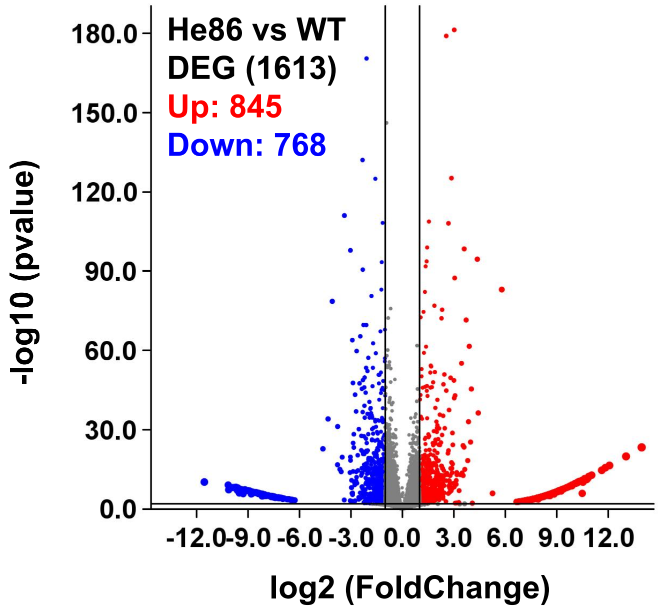


Figure S3. Volcano plot of differentially expressed genes between WT and *He86*.

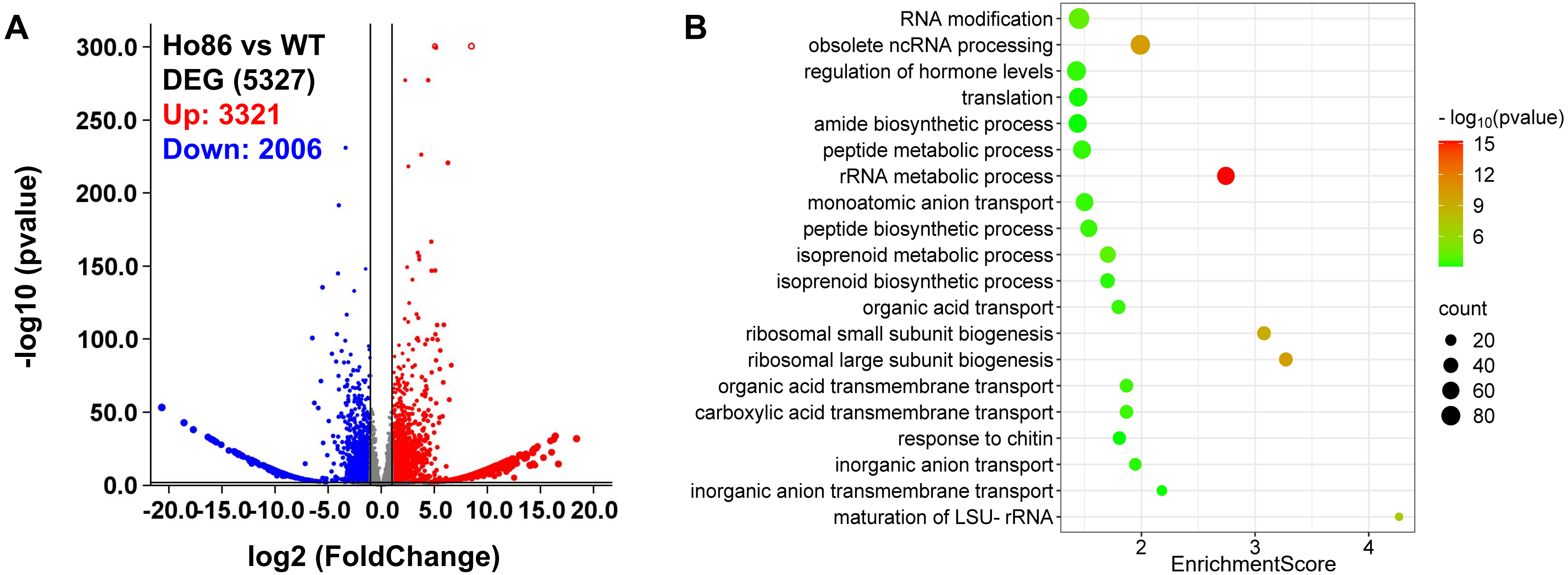


Figure S4. Volcano plot (A) and GO analysis (B) of differentially expressed genes between WT and *Ho86*.

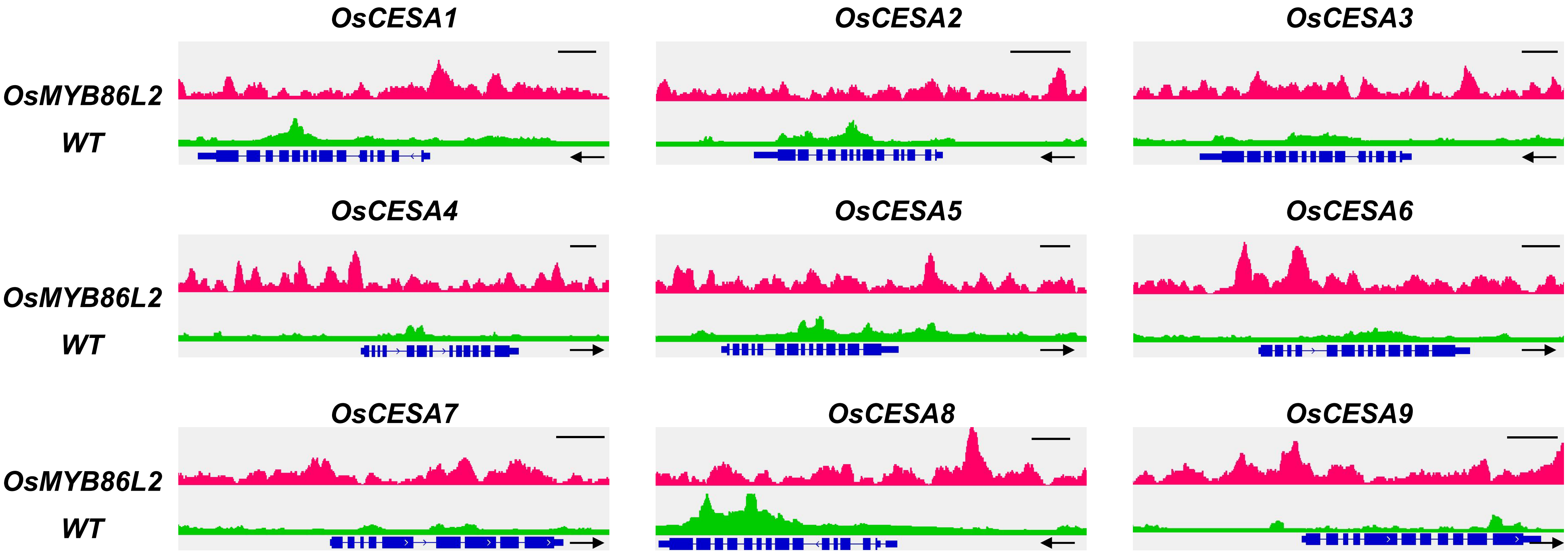
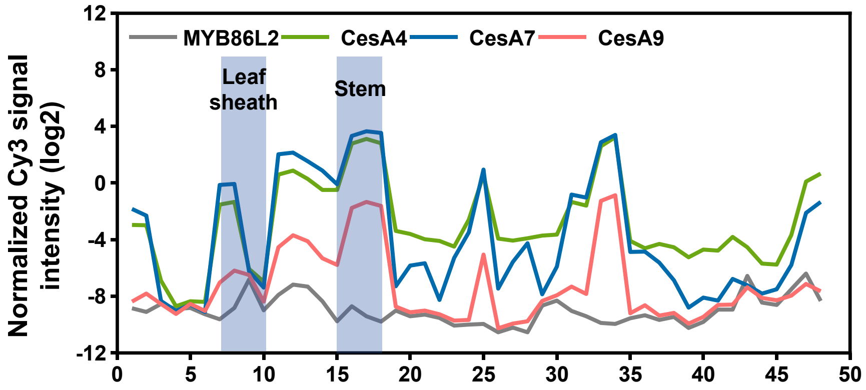


Figure S5. Visualization of MYB86L2 binding peaks for all CesA genes analyzed through DAP-seq.



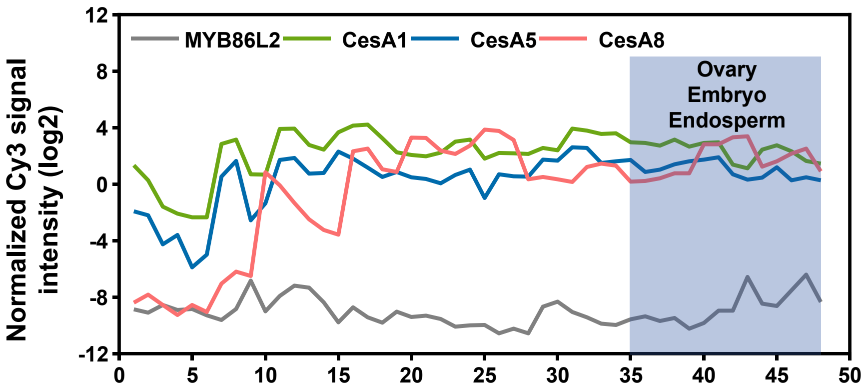


Figure S6. OsMYB86 L2 and OsCesAs gene expression profiling of entire life cycle of cultivar (NPB) using public data (https://ricexpro.dna.affrc.go.jp/), X-axis as different tissues during plant growth and developmental stages.

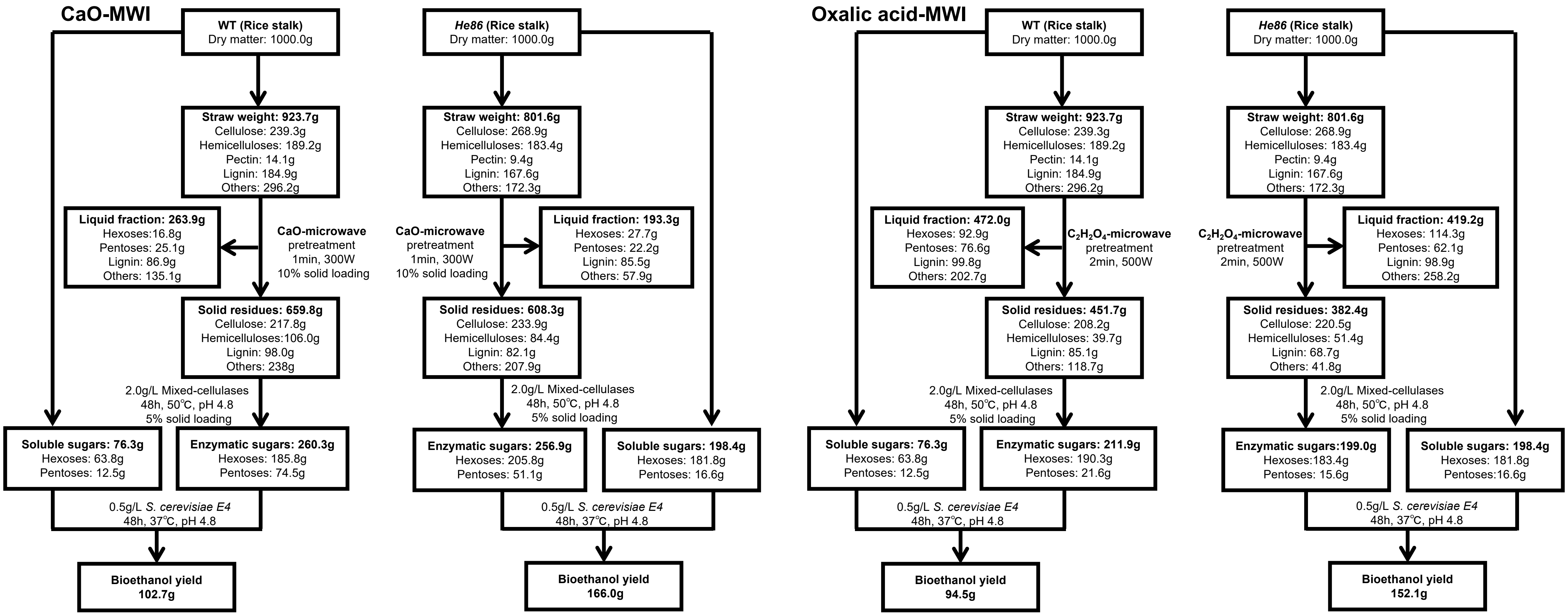


Figure S7. Mass balance analysis for lignocellulose conversion into ethanol between He86 mutant and WT under two optimal green-like pretreatments.

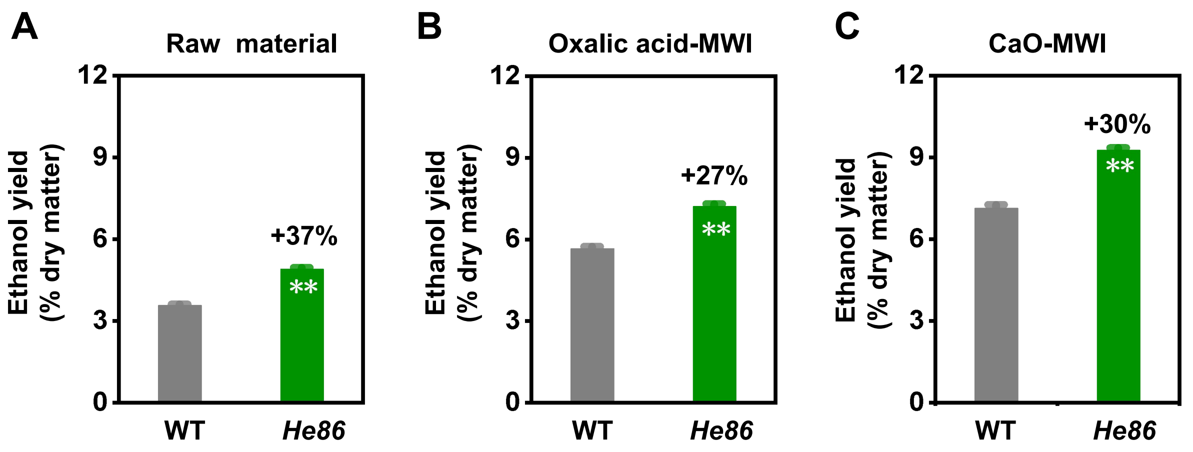


Figure S8. Bioethanol yields (% dry matter) using commercial yeast strain (Angel) hexoses released from enzymatic hydrolysis of two optimal-pretreated lignocellulose in *He86* mutant. Data as mean ± SD (n = 3); Increased or decreased percentage (%) obtained by subtraction between mutant and WT divided by WT; ** as significant difference between mutant and WT by two-tailed Student's *t*-test at $p < 0.01$ level.

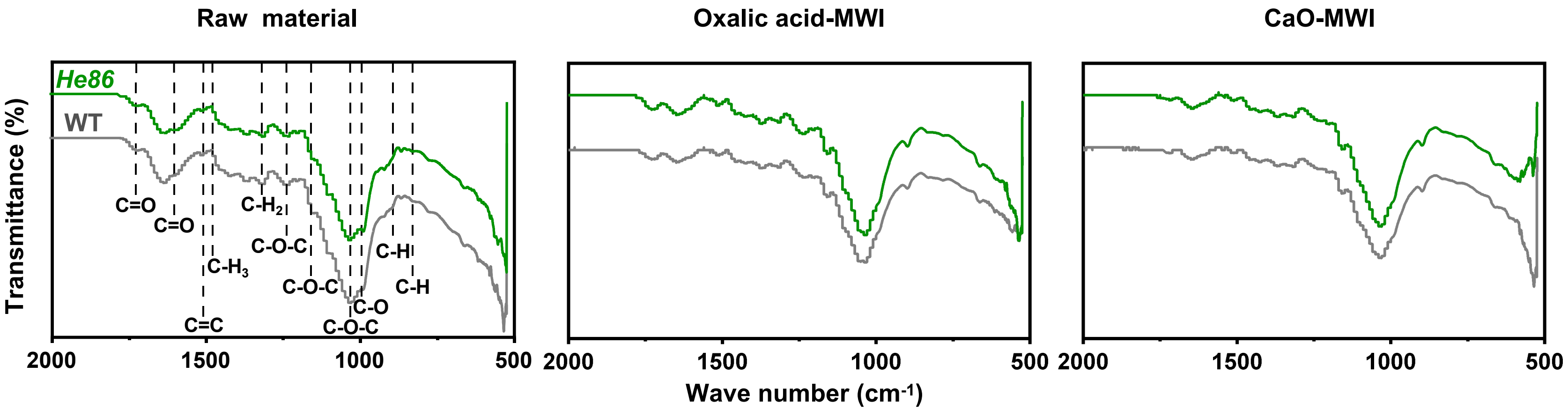


Figure S9. Attenuated Total Reflection-Fourier Transform Infrared (ATR-FTIR) spectroscopic profiling with raw material and two optimal-pretreated lignocelluloses in *He86* mutant. Characteristic peaks corresponding for chemical bonds assigned in **Table S3**.

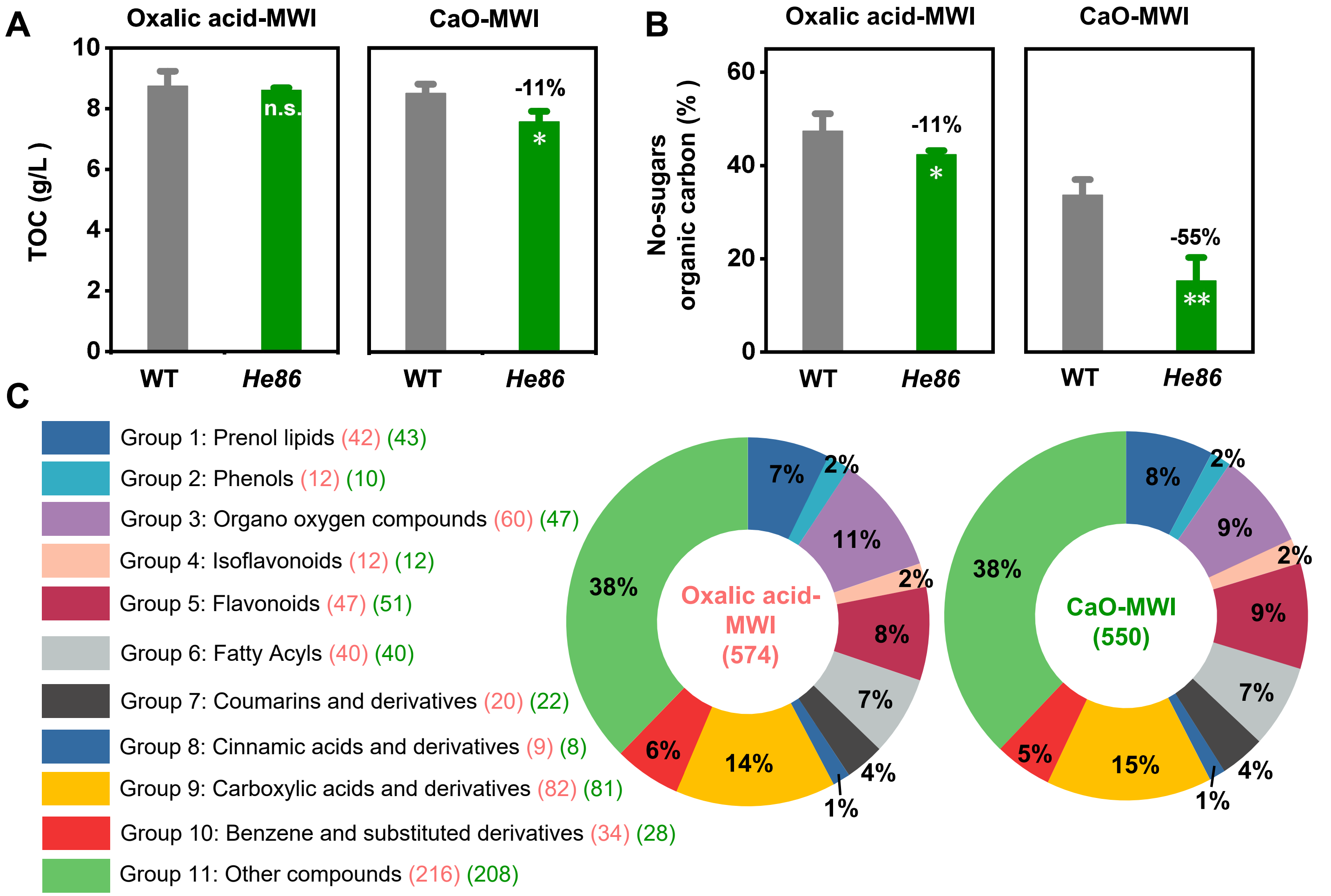


Figure S10. Detection of chemical compounds generated from enzymatic hydrolyses of two optimal pretreated lignocelluloses in *He86* mutant and WT. (A) Total organic carbon (TOC) content; (B) No-sugar organic carbon (organic carbon excluding glucose and xylose); (C) LC-MS/MS identification of total compounds in mutant only; Data as mean ± SD (n = 3); Increased or decreased percentage (%) obtained by subtraction between mutant and WT divided by WT; * and ** as significant difference between mutant and WT by two-tailed Student's *t*-test at $p < 0.05$ and 0.01, n.s., no significant, respectively.

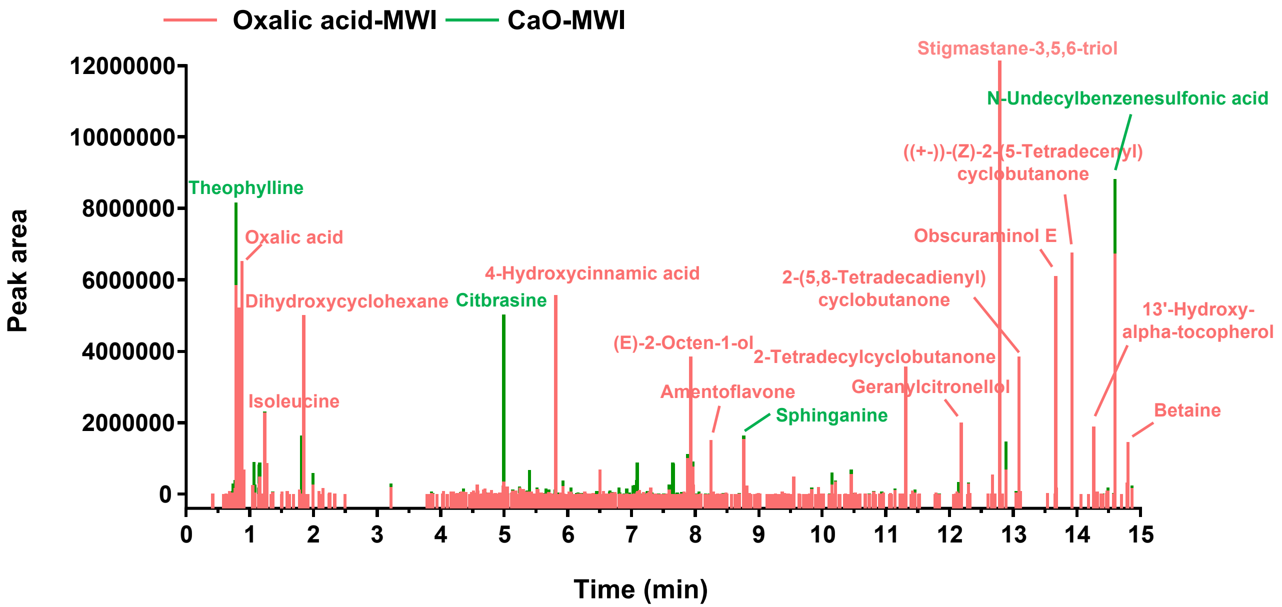


Figure S11. LC-MS/MS analysis of characteristic compounds released from enzymatic hydrolyses of two pretreated-lignocelluloses in *He86* mutant.

Table S1. Primers used in this study

Primer name	Primer Sequence (5'-3')	Primer use
Primer-1	TAGTAGTAAACACAGTGATTCCCAGT	Mutants identification
Primer-2	CACGACCCAAAAAACCAAAAT	Mutants identification
Primer-3	CTGGAGATTATTGCTCGGGTAGATCGTC	Mutants identification
OsMYB86L2-F	GCTCTAGAATGGGACGGCTGTCGTC	Overexpression vector construction
OsMYB86L2-R	GGGGTACCGAAGTATTCCAAGTTGAAGTCGA	Overexpression vector construction
Hyg-F	CGCTTCTGCGGGCGATTTGTGT	Transgenic rice screening
Hyg-R	TGAAAAAGCCTGAACTCACCGC	Transgenic rice screening
OsMYB86L2-RT-F	AGCGAGGTGCTCAACTGGG	qRT-PCR
OsMYB86L2-RT-R	GCTCTGCTCATGGCATGGTAG	qRT-PCR
OsCesA1-RT-F	CTGGAGAGGCTGGCTTACATTA	qRT-PCR
OsCesA1-RT-R	GCCACCAATGACCCAGAACT	qRT-PCR
OsCesA5-RT-F	TTCTGGAAATGAGATGGAGTGG	qRT-PCR
OsCesA5-RT-R	CAATAGGAGCAAAGTGGTAGGG	qRT-PCR
OsCesA8-RT-F	TTTTGCCTGCTATCTGTTTGC	qRT-PCR
OsCesA8-RT-R	AGGAGACCCTGGAAGACGG	qRT-PCR
OsCesA4-RT-F	CATTCCCACGCTCTCCAAC	qRT-PCR
OsCesA4-RT-R	TGTCCAGCCCAGCAATCAT	qRT-PCR
OsCesA7-RT-F	TCACCTCCATTCCCCTCC	qRT-PCR
OsCesA7-RT-R	CACCAGTCCTCGATGCTCAC	qRT-PCR
OsCesA9-RT-F	CTACGGCTACAAGAACGGCA	qRT-PCR
OsCesA9-RT-R	CTCCACCACTCCTCGATGCT	qRT-PCR
OsUBQ-F	CCAGGACAAGATGATCTGCC	qRT-PCR
OsUBQ-R	AAGAAGCTGAAGCATCCAGC	qRT-PCR

Table S2. Major agronomic traits of *He86* and *Ho86* mutants and WT

Traits	WT	*He86*	*Ho86*
Plant height (cm)	72.76±2.98	60.11±3.87**	47.85±3.48**
Number of tillers per plant	28.61±6.25	20.97±4.81**	9.67±2.94**
Number of panicles per plant	13.43±2.60	10.80±2.66**	4.50±1.85**
Grains per panicle	46.17±4.97	64.30±7.61**	19.07±6.99**
Seed-setting rate (%)	78.33±5.61	87.53±3.15**	36.36±5.93**
1000-grain weight (g)	26.18±0.45	22.62±0.59**	19.60±0.19**
Seed length (mm)	7.39±0.02	7.00±0.04**	6.33±0.01**
Seed width (mm)	3.26±0.01	3.12±0.01**	3.02±0.01**
Grain yield per plant (g)	16.21±3.40	15.78±4.19	1.71±1.14**

Data as mean ± SD (n = 30); ** As significant difference between the mutant and WT by two-tailed Student's *t*-test at $p < 0.01$.

Table S3. Candidate genes for downstream regulation were analyzed by combination of RNA-seq and DAP-seq

Locus_ID	Description	Name	Function	Expression	
				He86	Ho86
Os02g0237100	Thermospermine synthase, Regulation of leaf development, plant height and grain size	OsACL5	Tolerance and resistance	/	up
Os01g0354200	Similar to Sterol C-14 reductase.	OsFK1	Development	/	up
Os10g0356000	Similar to ribulose-1, 5-bisphosphate carboxylase/oxygenase large subunit.	OsrbcL1	Photosynthesis	down	down
Os07g0248600	Similar to Protein kinase domain containing protein, expressed.	OsRLCK225	Phosphorylation	down	down
Os05g0427400	Similar to Phenylalanine ammonia-lyase.	OsPAL7	Cell wall synthesis	/	down
Os09g0356000	Similar to OsD305.	OsRLCK266	Tolerance and resistance	/	up
Os04g0515900	Similar to NAM / CUC2-like protein.	ONAC8	Transcription factors	down	down
Os02g0504000	Similar to Cytochrome P450 CYP81L6.	OsCYP81L6	Tolerance and resistance	/	down
Os02g0467600	Similar to Cinnamate 4-hydroxylase CYP73.	OsC4H1	Cell wall synthesis	/	up
Os03g0214100	Replication protein A 70kDa subunit b	OsRPA70b	Tolerance and resistance	/	up
Os11g0497350	Promotion of cell division, Regulation of grain and organ development, Abiotic stress tolerance	OsMAPKKK67	Tolerance and resistance	/	up
Os01g0126200	Multicopper oxidase	OsLPR2	Development	/	down
Os02g0125500	K Homology domain containing protein.	OsKH8	Tolerance and resistance	/	up
Os05g0530400	Heat stress transcription factor, Plant growth and balancing reactive oxygen species (ROS) during biotic and abiotic stress	OsSPL7	Tolerance and resistance	/	down
Os10g0577500	Concanavalin A-like lectin/glucanase, subgroup domain containing protein.	OsXTH25	Cell wall synthesis	up	up
Os11g0116300	Class IV chalcone isomerase (CHI) protein family member, Chalcone isomerase like protein, Biosynthesis of extractable flavones and tricin-lignin	OsCHIL1	Tolerance and resistance	/	down
Os01g0184100	Class II small heat shock protein, Positive regulation in both Xoo resistance and heat/salt tolerance	OsHSP18	Tolerance and resistance	down	down
Os06g0604300	Chloroplast-localized phospholipase D, Herbivore defense	OsPLDa5	Tolerance and resistance	down	down
Os08g0191000	Auxin efflux carrier domain containing protein.	OsPILS2	Tolerance and resistance	/	up
Os03g0281900	ATP-binding cassette (ABC) transporter, Hypodermal suberization of roots, Salt stress tolerance	OsABCG5	Tolerance and resistance	up	up

Table S4. Characteristic chemical bonds of FTIR spectra as referenced from previous studies

Reported wave number (cm^{-1})	Observed wave number (cm^{-1})	Functional group	Assignment	References
829	831	C-H breathing	H-lignin	1
898	895	C-H vibration	Cellulose	2
990	996	C-O vibration	Cellulose	3
1051	1033	C-O-C ring skeletal vibration	Lignin/ Hemicelluloses	4
1163	1160	C-O-C asymmetric stretching	Cellulose	5
1247	1240	C-O-C stretching of aryl-alkyl ether	Lignin	6
1373	1320	C-H_2 scissoring	Cellulose	7
1460	1480	C-H_3 asymmetric bending	Lignin	8
1510	1510	C=C stretching of the aromatic ring	Lignin	9
1603	1605	C=C stretching	Lignin	10
1735	1730	-C=O stretching from CO-OR	Hemicelluloses/ lignin	11

Table S5. The maximum adsorption capacity of Congo red (Langmuir model)

Sample	Pretreatment	$1/q_{max}$	q_{max}[a]	$1/b*q_{max}$	b[b]	R^2
WT	Raw	0.0033	303.0303	0.3847	0.0086	0.9998
	CaO-MWI	0.0020	502.5126	0.2841	0.0070	0.9994
	Oxalic acid-MWI	0.0026	384.6154	0.3506	0.0074	0.9982
He86	Raw	0.0027	370.3704	0.4812	0.0056	0.9972
	CaO-MWI	0.0015	666.6667	0.3319	0.0045	0.9952
	Oxalic acid-MWI	0.0021	478.4689	0.4425	0.0047	0.9938

a q_{max} is the maximum adsorption capacity (mg/g)
b b is the equilibrium adsorption constant (L/mg)

Table S6. Chemical compounds generated from enzymatic hydrolyses of two optimal-pretreated lignocelluloses in *He86* mutant by LC-MS/MS

Classification	Metabolite	Formula	Retention time(min)	m/z	Mode	Fragmentation Score	Peak area			
							Oxalic acid-P[a]	CaO-P[a]	Oxalic acid-E[b]	CaO-E[b]
Carboxylic acids	13,14,15,16-Tetranor-8(17)-labden-12-oic acid	$C_{16}H_{26}O_2$	10.26	295.19	neg	56	355.34	0.00	11039.95	2550.13
	Maleic acid	$C_4H_4O_4$	1.23	115.00	neg	77.6	266050.59	16686.15	3312.84	8760.76
	2,2'-(3-Methylcyclohexane-1,1-diyl)diacetic acid	$C_{11}H_{18}O_4$	7.29	213.11	neg	69	2595.54	4901.52	59195.72	5869.78
	Elenaic acid	$C_{11}H_{14}O_6$	6.03	223.06	neg	68.8	142984.74	16677.64	205039.03	46361.18
	Oxalic acid	$C_2H_2O_4$	0.88	88.99	neg	56.3	84369924.08	6529726.67	1796749.75	1308061.00
	Citrate	$C_6H_8O_7$	1.15	191.02	neg	85.3	4145339.12	148578.03	2792.48	66145.02
	Cichoric acid	$C_{22}H_{18}O_{12}$	5.77	473.07	neg	87.7	239434.59	172.30	2529.01	0.00
	Kansuinine B	$C_{38}H_{42}O_{14}$	1.15	767.26	neg	56.7	2012.23	243788.87	0.00	819831.36
Benzoic acids and derivatives	Norbergenin	$C_{13}H_{14}O_9$	4.21	359.06	neg	75.3	74713.98	0.00	0.00	0.00
	Vanillic acid	$C_8H_8O_4$	4.31	167.04	neg	68.1	37018.73	44351.88	391514.14	53913.44
	5-Methoxysalicylic acid	$C_8H_8O_4$	5.13	167.04	neg	64.9	125919.65	94128.97	36771.83	134770.21
	4-{hydroxy[(3,4,5,6-tetrahydroxyoxan-2-yl)methoxy]methylidene}cyclohexa-2,5-dien-1-one	$C_{13}H_{16}O_8$	4.36	299.08	neg	64.2	21372.51	448.64	0.00	0.00
	Methyl gallate	$C_8H_8O_5$	4.90	183.03	neg	63.9	21638.11	14169.84	49005.88	16758.87
	Obscurolide A1	$C_{15}H_{17}NO_5$	7.06	328.06	neg	63.4	2105.67	0.00	7178.60	5018.32
	Propyl gallate	$C_{10}H_{12}O_5$	6.61	211.06	neg	62.8	12376.09	6310.37	6838.06	6093.35
	Ethyl 4-methoxysalicylate	$C_{10}H_{12}O_3$	5.57	225.08	neg	54.8	15552.63	5042.27	79844.09	5039.67
	Dioctyl Phthalate	$C_{24}H_{38}O_4$	12.16	391.28	pos	97.7	37020.09	23063.92	71812.73	41627.13
	3,4-Dimethoxybenzoic acid	$C_9H_{10}O_4$	1.16	165.05	pos	55.5	94732.40	59922.72	0.00	57701.64
	Benzamide	C_7H_7NO	4.83	122.06	pos	54.9	11989.34	6892.28	10852.35	7531.01
	Agastenol	$C_{27}H_{26}O_8$	7.56	479.17	pos	53.2	49819.48	5584.78	0.00	0.00
Furan	Moracin P	$C_{19}H_{18}O_5$	6.00	371.11	neg	78.4	11688.24	266.64	39572.32	4494.10
	Moracin L	$C_{19}H_{16}O_5$	6.80	369.10	neg	78.2	3400.71	0.00	2342.68	179459.83
	1-[2-(2H-1,3-benzodioxol-5-yl)-1-benzofuran-5-yl]propane-1,3-diol	$C_{18}H_{16}O_5$	7.96	357.10	neg	76.2	9658.57	0.00	206859.14	94728.87
	Moracin G	$C_{19}H_{16}O_4$	7.72	353.10	neg	70.4	28931.77	2419.16	0.00	0.00
	Moracin K	$C_{19}H_{16}O_5$	7.37	369.10	neg	62.9	17875.02	750.28	2868.60	11662.02
	Demethoxyegonol	$C_{18}H_{16}O_4$	8.79	341.10	neg	57.2	0.00	0.00	18466.02	13533.39
	Massonianoside B	$C_{25}H_{32}O_{10}$	6.00	491.19	neg	50.6	0.00	0.00	278073.20	0.00
	Mulberrofuran M	$C_9H_{10}ClNO_2$	3.88	200.05	pos	89.1	5360.49	14220.02	619201.73	31042.93
	Glycosmisic acid	$C_{20}H_{20}O_7$	7.72	355.12	pos	62.2	50073.50	2205.32	362.15	3311.96
	Massarilactone B	$C_{11}H_{14}O_5$	5.53	207.07	neg	77.4	29990.10	4889.26	2934.93	4494.77
	8alpha-(2-Methylacryloyloxy)hirsutinolide	$C_{19}H_{24}O_7$	7.77	329.14	pos	51.8	23941.52	0.00	0.00	0.00
	Isosalvipuberulin	$C_{20}H_{14}O_5$	7.75	367.12	pos	58.1	9240.91	0.00	0.00	0.00
	trans-Grandmarin	$C_{15}H_{16}O_6$	7.46	275.09	pos	66	9512.18	212.52	0.00	908.44
	3'-Hydroxyxanthyletin	$C_{14}H_{12}O_4$	7.24	245.08	pos	66.8	20773.33	1820.42	0.00	2901.84
	Loliolide	$C_{11}H_{16}O_3$	7.10	241.11	neg	51	56718.46	82813.56	1145.15	56381.43

Phenols	Coniferaldehyde	C10H10O3	6.6984	177.06	neg	92.6	110767.14	38077.34	28756.54	55285.26
	Syringaldehyde	C9H10O4	6.0473	181.05	neg	94.3	46673.26	75403.33	17005.35	190361.10
	6-Gingerol	C17H26O4	9.5546	293.18	neg	89	227073.85	490586.27	779592.94	323856.98
	Vanillin	C8H8O3	5.92145	151.04	neg	88.1	99373.56	227056.48	55339.21	381372.08
	1,2,3-Trihydroxybenzene	C6H6O3	3.218583333	125.02	neg	86.1	1405691.16	193384.41	159415.27	288544.64
	(E)-Antibiotic BE 23372M	C17H12O6	6.322866667	357.06	neg	79.4	87974.89	15319.26	11475.94	8197.16
	6-(2,4-dihydroxyphenyl)-2-(2,6-dihydroxyphenyl)-5-hydroxy-4-methylcyclohex-3-ene-1-carboxylic acid	C20H20O7	8.084316667	371.11	neg	75	519070.43	19973.69	66.81	0.00
	2-Methoxy-4-vinylphenol	C9H10O2	7.075283333	149.06	neg	72.5	894.22	1415.73	2067.05	13813.21
	4-Nitrophenol	C6H5NO3	6.687016667	138.02	neg	63.1	46604.20	44774.20	228127.72	34348.77
	[(2R)-1-[(2S)-6-oxo-2,3-dihydropyran-2-yl]propan-2-yl] 3-(3,4-dihydroxyphenyl)propanoate	C17H20O6	12.71583333	338.16	pos	63.8	0.00	14271.27	2835.57	0.00
	Sinapaldehyde	C11H12O4	6.667583333	209.08	pos	61.7	89523.10	22669.66	58483.32	89603.51
	3-(3,4-dihydroxy-5-methoxyphenyl)oxirane-2-carboxylic acid	C10H10O6	6.0349	207.03	neg	58.5	11392.67	6609.99	24747.80	17922.37
	Isoeugenol acetate	C12H14O3	5.736883333	189.09	pos	60.6	0.00	0.00	13699.71	0.00
Coumarins and derivatives	Hydramicromelin D	C15H14O7	5.18	287.06	neg	63.9	485.14	654.73	32416.94	1841.26
	trans-Grandmarin	C15H16O6	7.46	275.09	pos	66	9512.18	212.52	0.00	908.44
	3'-Hydroxyxanthyletin	C14H12O4	7.24	245.08	pos	66.8	20773.33	1820.42	0.00	2901.84
	Isofraxidin	C11H10O5	6.8	221.05	neg	73.8	3635.94	2654.89	5597.44	12524.68
	6,8-Dimethyl-4-hydroxycoumarin	C11H10O3	5.72	189.06	neg	51	7680.27	138.14	98.47	241.27
	7,8-Dihydroxy-4-methylcoumarin	C10H8O4	5.18	175.04	pos	54	8634.32	0.00	0.00	0.00
	4-Methyl-6,7-dihydroxycoumarin	C10H8O4	7.08	193.05	pos	54.5	9920.82	7394.97	10617.38	40542.87
	Esculetin	C9H6O4	5.66	177.02	neg	79.8	12240.87	11198.72	1092729.94	19007.94
	7-Hydroxy-R-phenprocoumon	C18H16O4	8.06	341.10	neg	67	16208.81	407.85	30728.95	70233.46
	7-hydroxy-6-(3-oxobutyl)-2H-chromen-2-one	C13H12O4	6.89	233.08	pos	77.3	18761.37	732.68	0.00	0.00
	7-hydroxy-6-(3-oxobut-1-en-1-yl)-2H-chromen-2-one	C13H10O4	7.24	229.05	neg	72.8	48747.02	5496.99	350.87	236.22
	Isoscopoletin	C10H8O4	4.79	193.05	pos	54.7	54485.42	7307.45	8137.56	10350.51
	Bergapten	C12H8O4	7.24	261.04	neg	75.3	902.08	0.00	506.01	70349.25
	Aflatoxin P1	C16H10O6	8	343.05	neg	61.1	20802.12	576.23	283.90	1211.63
	Bergaptol	C11H6O4	5.03	247.03	neg	69.1	23922.00	0.00	0.00	0.00
	4-Hydroxy-8-methoxy-2H-furo[2,3-h]-1-benzopyran-2-one	C12H8O5	6.14	231.03	neg	56.7	35336.68	0.00	0.00	0.00
	Marmesin rhamnoside	C20H24O8	6.03	373.13	neg	84.7	42187.61	2651.97	0.00	656.10
	Isoimperatorin	C16H14O4	8.45	271.10	pos	74.4	450899.60	385.19	6486.05	0.00
	Aflatoxin B2a	C17H14O7	7.96	331.08	pos	83.9	7660833.40	760713.16	513848.81	900304.17
	Xanthotoxol glucoside	C17H16O9	7.28	329.07	pos	75.5	2336.70	0.00	25309.56	21182.54
	4-Methylcoumarin	C10H8O2	7.62	193.09	pos	60.8	10247.25	641.39	3089.42	2675.76
	Hydramicromelin B	C15H14O7	5.98	351.07	neg	68.6	16607.59	0.00	0.00	0.00
	Ethyl coumarin-3-carboxylate	C12H10O4	5.02	217.05	neg	61.1	30441.41	3796.45	1010.09	2256.26
	Coumarin	C9H6O2	4.77	164.07	pos	71.8	39158.05	15401.38	8927.19	37663.60
	7-Methoxycoumarin	C10H8O3	6.39	209.08	pos	59.8	78279.21	13892.26	64269.55	30256.07

a P is pretreated liquid
b E is enzymatic liquid

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