

Electronic Supplementary Information (ESI)

Insights of biomass recalcitrance in *Populus trichocarpa* natural variants for biomass conversion

Chang Geun Yoo,^{a,b} Yongil Yang,^a Yunqiao Pu,*^a Xianzhi Meng,^c Wellington Muchero,^a Kelsey L. Yee,^a Olivia A. Thompson,^a Miguel Rodriguez Jr,^a Garima Bali,^c Nancy L. Engle,^a Erika Lindquist,^d Vasanth Singan,^d Jeremy Schmutz,^{d,e} Stephen P. DiFazio,^f Timothy J. Tschaplinski,^a Gerald A. Tuskan,^a Jin-Gui Chen,^a Brian Davison,^a and Arthur J. Ragauskas*^{a,b,g}

^a BioEnergy Science Center and Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA.

^b UT-ORNL Joint Institute for Biological Science, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA.

^c Renewable Bioproducts Institute, School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332, USA.

^d Department of Energy, Joint Genome Institute, Walnut Creek, CA 94598, USA.

^e HudsonAlpha Institute for Biotechnology, Huntsville, AL 35806, USA.

^f Department of Biology, West Virginia University, Morgantown, WV 26506, USA.

^g Department of Chemical and Biomolecular Engineering & Center for Renewable Carbon, Department of Forestry, Wildlife, and Fisheries, University of Tennessee, Knoxville, TN 37996, USA.

*Email: puy1@ornl.gov; aragausk@utk.edu

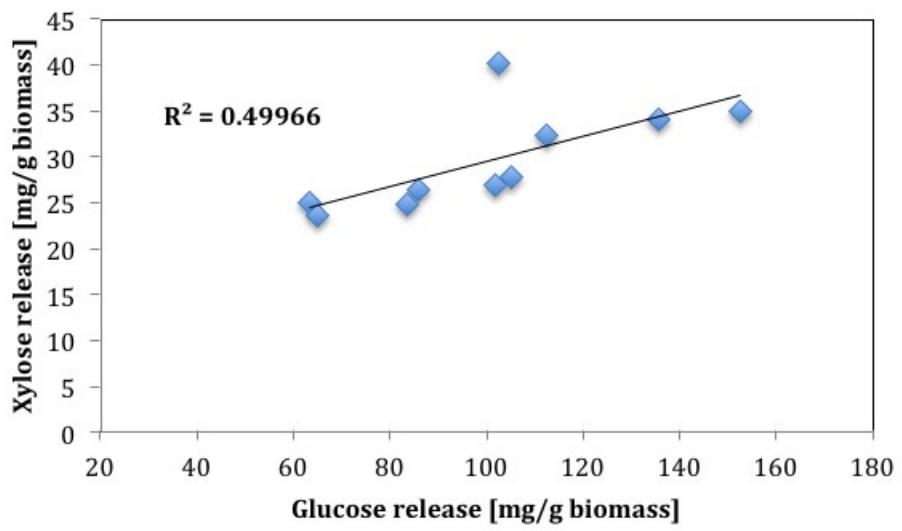


Figure S1. Correlation between glucose release and xylose release of *P. trichocarpa* natural variants ($R^2=0.50$, Pearson coefficient=0.705, p -value=0.023)

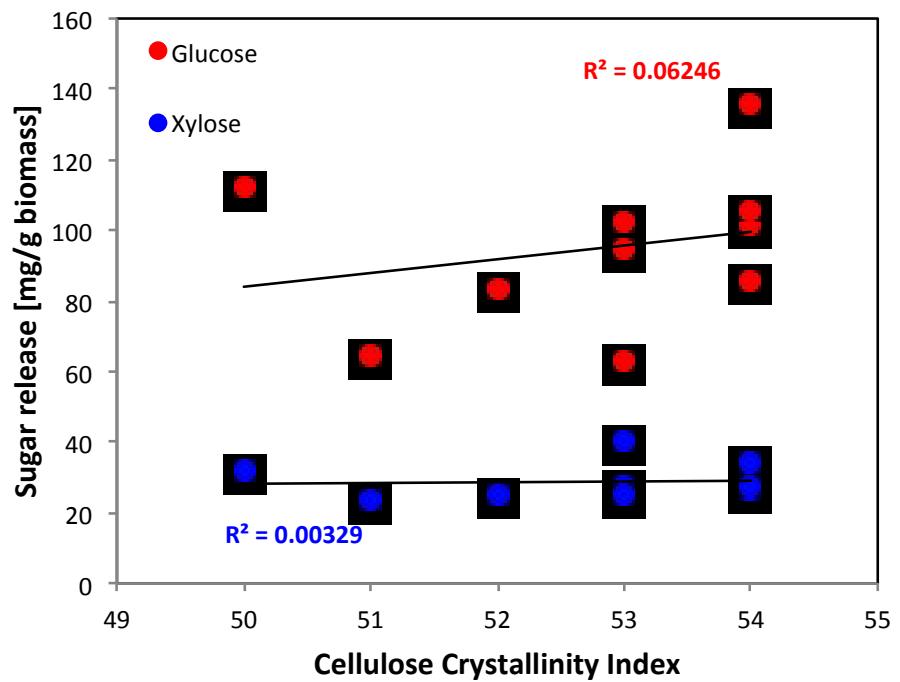


Figure S2. Correlation between sugar release and cellulose crystallinity of *P. trichocarpa* (CrI vs glucose release: $R^2=0.06$, Pearson coefficient=0.078, p -value=0.831; CrI vs xylose release: $R^2=0.003$, Pearson coefficient=-0.001, p -value=0.998)

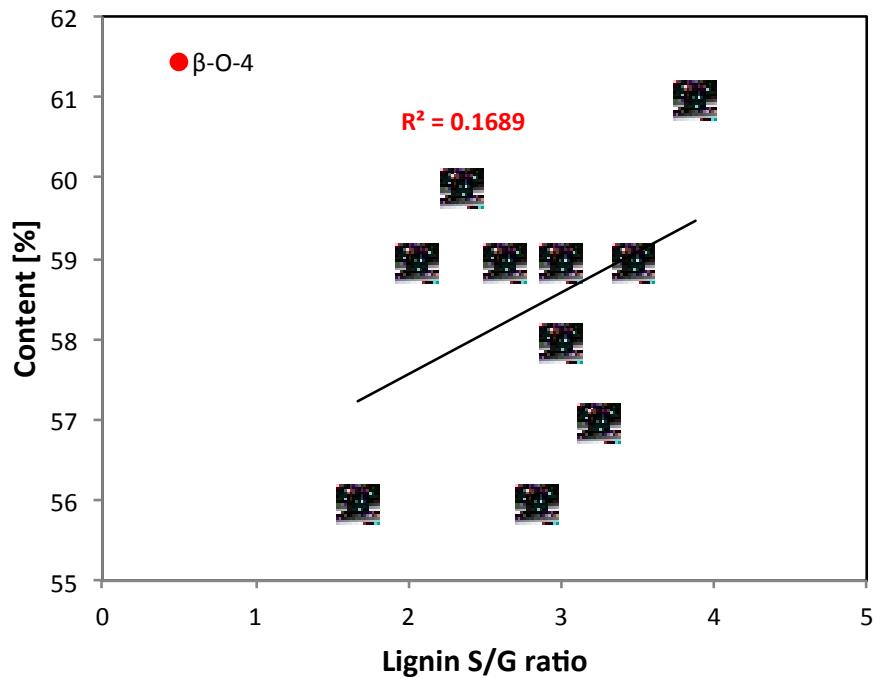


Figure S3. Correlation between lignin S/G ratio and $\beta\text{-O-4}$ linkage content ($R^2=0.17$, Pearson coefficient=0.411, p -value=0.238)

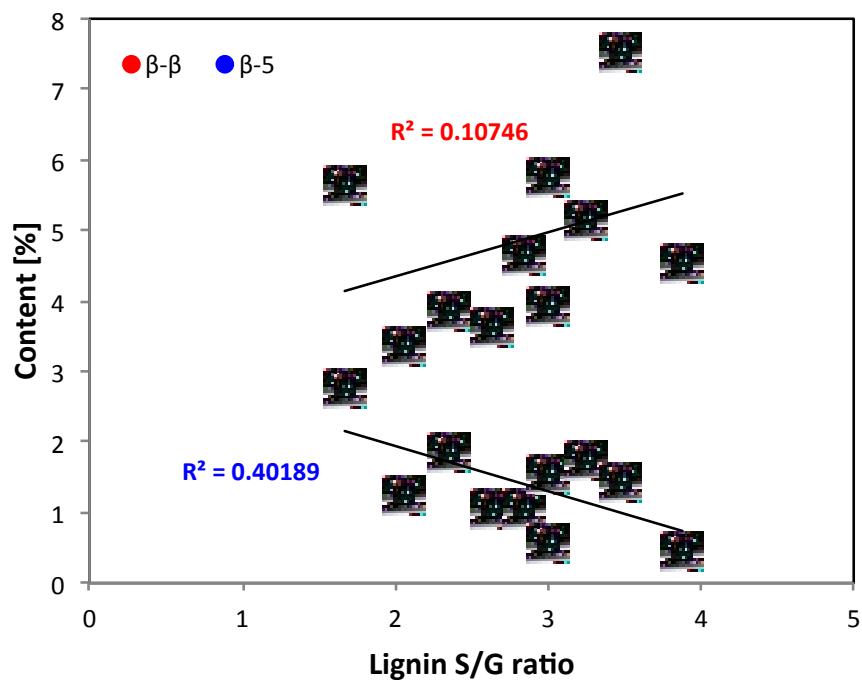


Figure S4. Correlation between lignin S/G ratio and $\beta\text{-}5/\beta\text{-}\beta$ linkage content (S/G ratio vs $\beta\text{-}5$: $R^2=0.40$, Pearson coefficient=0.634, p -value=0.049; S/G ratio vs $\beta\text{-}\beta$: $R^2=0.11$, Pearson coefficient=0.328, p -value=0.355)

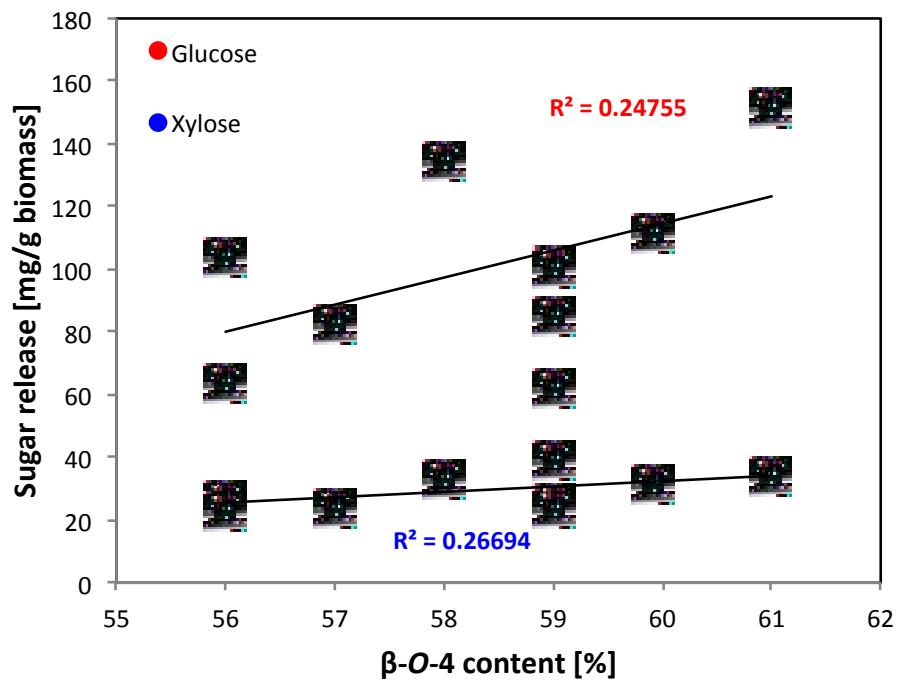


Figure S5. Correlation between β -O-4 linkage content and sugar release
(β -O-4 vs glucose release: $R^2=0.25$, Pearson coefficient=0.498, p -value=0.143; β -O-4 vs xylose release: $R^2=0.27$, Pearson coefficient=0.517, p -value=0.126)

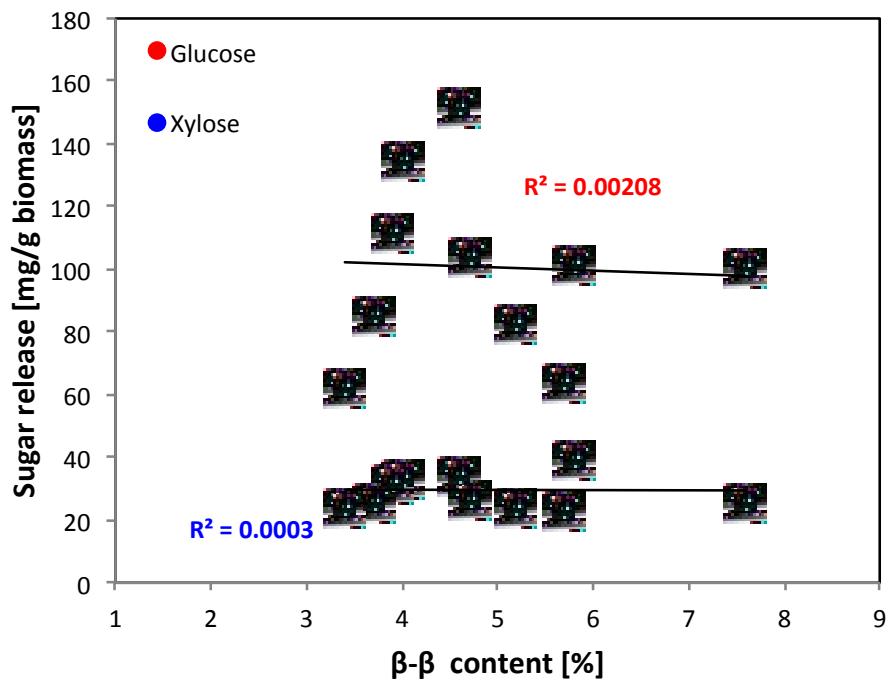


Figure S6. Correlation between β - β linkage content and sugar release
(β - β vs glucose release: $R^2=0.002$, Pearson coefficient=-0.045, p -value=0.901; β - β vs xylose release: $R^2=0.0003$, Pearson coefficient=-0.017, p -value=0.963)

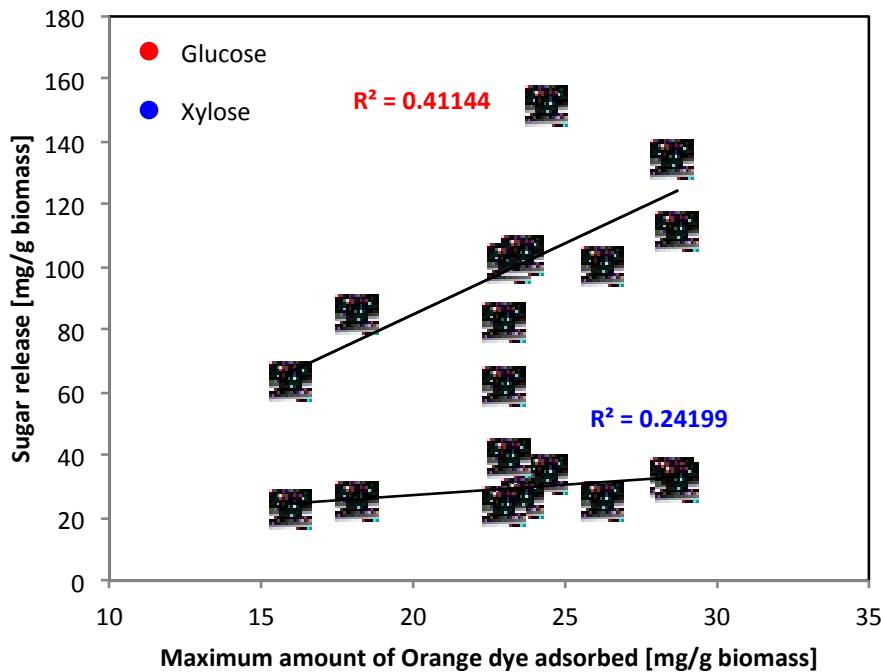


Figure S7. Correlation between maximum amount of orange dye adsorption and sugar release (Orange dye vs glucose release: $R^2=0.41$, Pearson coefficient=0.641, p -value=0.046; Orange dye vs xylose release: $R^2=0.24$, Pearson coefficient=0.493, p -value=0.148)

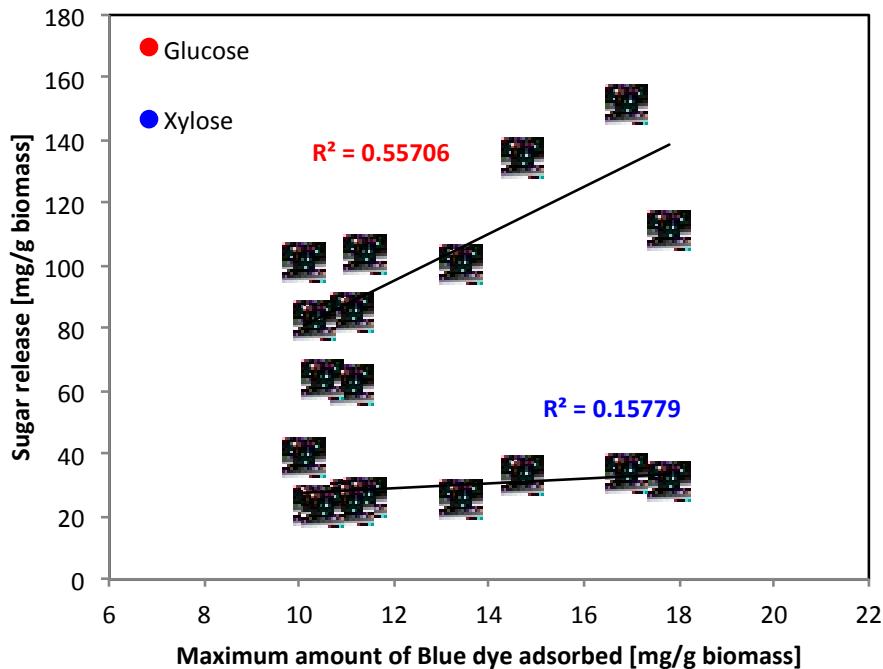


Figure S8. Correlation between maximum amount of blue dye adsorption and sugar release (Blue dye vs glucose release: $R^2=0.56$, Pearson coefficient=0.747, p -value=0.013; Blue dye vs xylose release: $R^2=0.16$, Pearson coefficient=0.397, p -value=0.256)

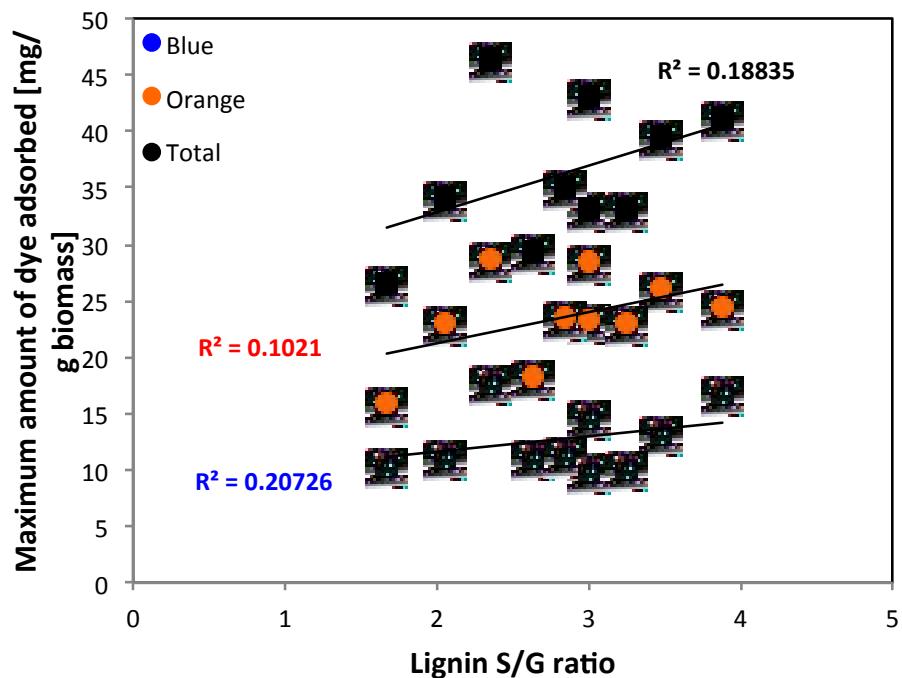


Figure S9. Correlation between cellulose accessibility and lignin S/G ratio

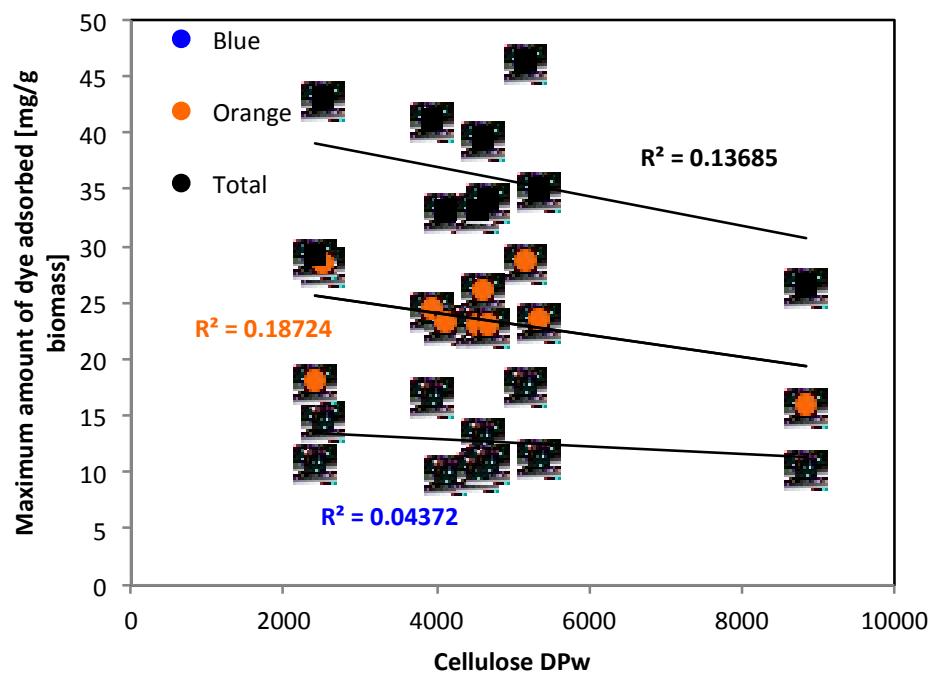


Figure S10. Correlation between cellulose accessibility and cellulose DP_w

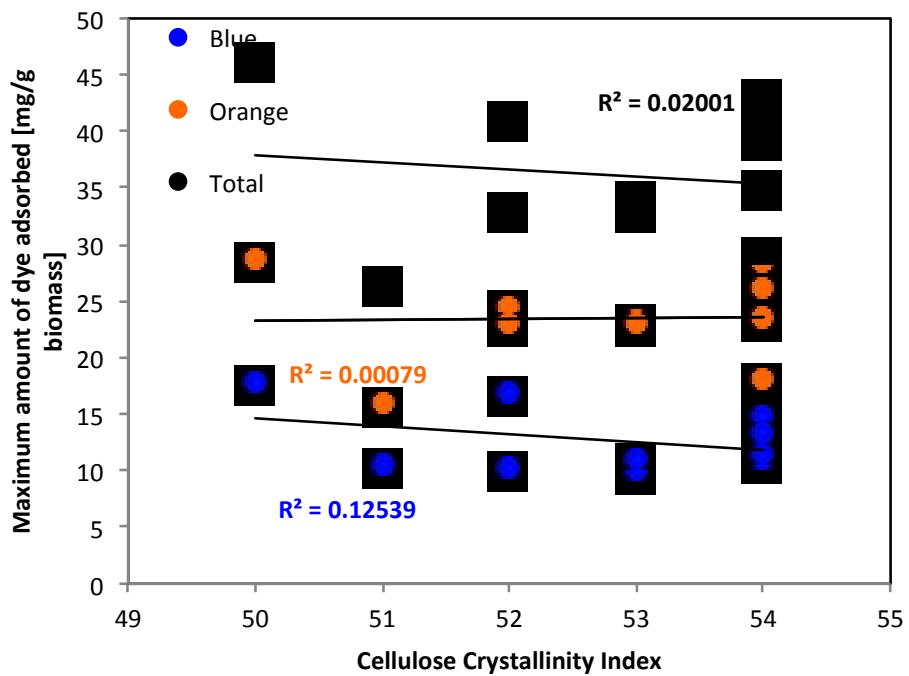


Figure S11. Correlation between cellulose accessibility and cellulose crystallinity index

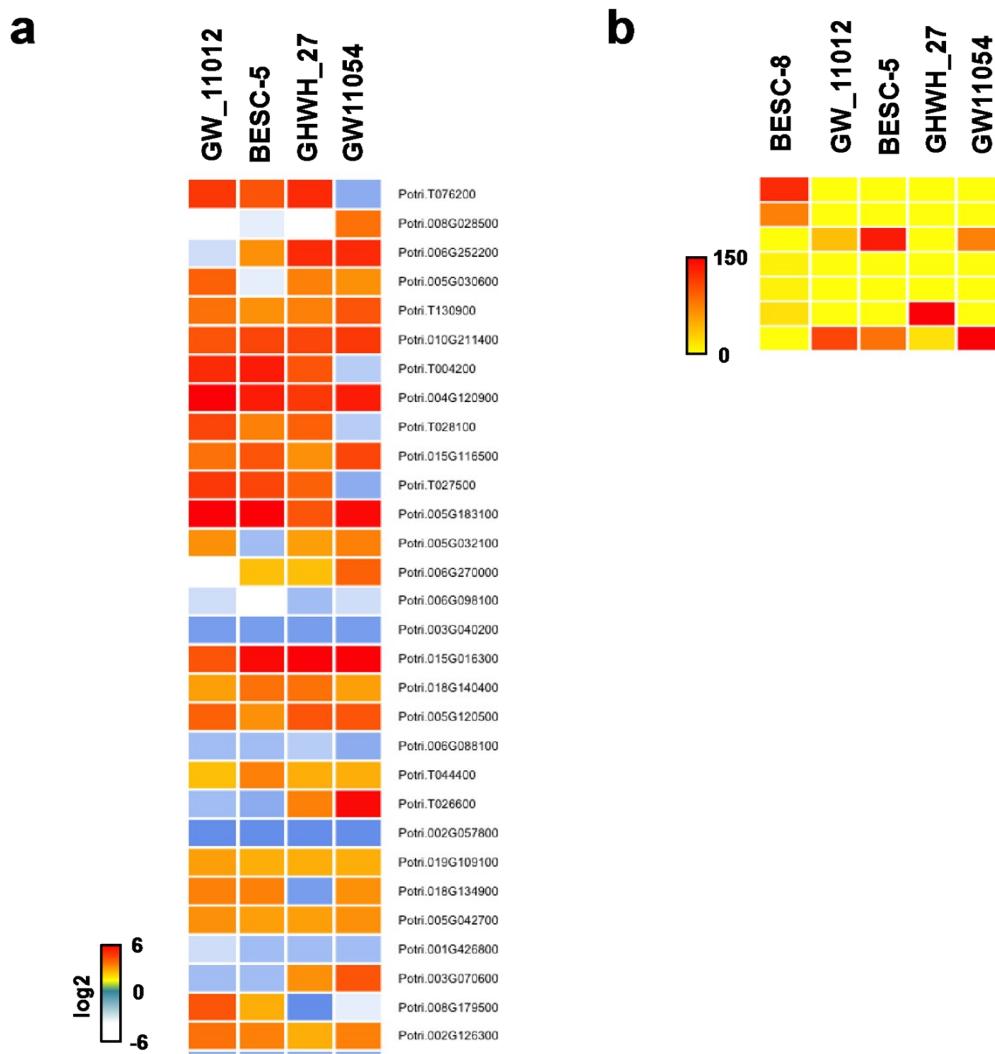


Figure S12. Differentially expressed genes between *P. trichocarpa* natural variant genotypes

Table S1. The list of correlation efficient between physicochemical property analysis and differentially expressed genes over natural variant.

geneID	11012vs8	27vs8	8vs8	5 vs8	11054vs8	lignin content	S/G ratio	celluloseDPw	glucoserelease	xyloserelease	ligninMw	Hemi	description	
Potri_008G179500	3.81975293	-1.332104		1 1.84359984	-5.691667	-0.911323751	0.85581463	-0.674709388	0.952825203	0.572087537	0.54137444	0.609828487	multidrug resistance-associated protein 3	
Potri_T028100	4.33235249	3.58601314		1 2.87452429	-4.1472149	-0.766462965	0.61796706	-0.452174717	0.854398316	0.28022806	0.802255359	0.744175934	NB-ARC domain-containing disease resistance protein	
Potri_T027500	4.52750026	3.62077982		1 4.39221715	-2.9935358	-0.727351302	0.69230093	-0.391721398	0.816870684	0.157105432	0.810267066	0.696825896	NB-ARC domain-containing disease resistance protein	
Potri_T004200	4.98894575	3.86690171		1 5.21454249	-4.3588461	-0.720085421	0.72477041	-0.37508968	0.813054971	0.178186203	0.823054585	0.719100278	NB-ARC domain-containing disease resistance protein	
Potri_T076200	6.54896256	5.01230443		1 3.80369045	-2.8470997	-0.66083187	0.53135559	-0.332799942	0.758156442	0.107269627	0.839155931	0.712584008	Zinc-binding dehydrogenase family protein	
Potri_0196109100	2.18179376	1.65543109		1 1.58330057	1.64916644	-0.469442202	-0.0843557	-0.556489882	0.398253363	-0.388506516	-0.145771316	-0.394934516		
Potri_0196000200	6.9398581	-3.2817515		1 -3.2817515	2.90357465	-0.491085196	-0.1351334	-0.810679683	0.344041264	0.371190666	-0.697867321	-0.549661252		
Potri_018G134900	2.92292587	-2.0278906		1 2.75074803	2.35771369	-0.280975196	0.39781009	-0.4250571	0.17741663	0.068813487	-0.507645112	-0.498337891	Protein kinase superfamily protein	
Potri_0016110400	-3.6706253	4.08316387		1 -3.6706253	-5.7477199	-0.005124478	-0.0585862	0.220713343	0.12733409	0.272850065	0.68372038	0.77105134	sugar transporter 1	
Potri_0046120900	6.25767115	4.68146046		1 5.36246632	5.5293851	-0.141446235	-0.1387976	-0.213398899	0.08134999	-0.702414787	-0.178889066	-0.52218854	Domain of unknown function (DUF3598)	
Potri_T113600	-3.2300527	-3.2300527		1 -3.2300527	-3.2300527	-0.031805917	0.22617267	-0.013787495	0.059102421	0.83885322	0.039968038	0.427337218	P-loop containing nucleoside triphosphate hydrolases superfamily protein	
Potri_0106165400	-2.8450347	-2.8450347		1 -2.8450347	-2.8450347	-0.031805917	0.22617267	-0.013787495	0.059102421	0.83885322	0.039968038	0.427337218	EXS (ERD1/XPR1/SYG1) family protein	
Potri_005G030600	3.63711338	2.73270779		1 -5.7401287	2.46318183	-0.128721495	-0.6829662	-0.406059754	0.045988182	0.19851534	-0.43781429	-0.315288475	Leucine-rich receptor-like protein kinase family protein	
Potri_005G183100	5.94793944	4.02036985		1 5.95382289	5.5731498	-0.100365885	-0.0157232	-0.155389823	0.041494432	-0.70843889	-0.193364021	-0.535610771		
Potri_002G126300	3.05730255	1.76455318		1 2.86906579	2.94903383	-0.130889708	0.00053713	-0.256134871	0.041394834	-0.589148703	-0.3790703	-0.657502469	alpha-amylase-like	
Potri_006G098100	-5.1791433	-3.3565611		1 -6.9759709	-5.3616988	-0.000391913	-0.0617737	-0.004777885	0.034691228	0.769021699	0.087224019	0.451470499	plasma membrane intrinsic protein 1;4	
Potri_005G042700	2.5077552	2.06467857		1 2.25070522	2.37699846	-0.098473086	-0.1812805	-0.188509669	0.033608509	-0.721532245	-0.2222135775	-0.563694496	DCC (Development and Cell Death) domain protein	
Potri_013G033100	-5.72647487	-3.5664764		1 -6.5203475	-7.7264785	0.0279542	0.01727431	0.063600707	0.020839789	0.760503696	0.15863678	0.516432227	Esterase/lipase/thioesterase family protein	
Potri_006G088100	-3.3532627	-4.0814108		1 -3.6789301	-3.0491148	-0.017484954	0.18001595	-0.067701697	0.830896031	-0.134914446	0.26356908	Pathogenesis-related thiamatin superfamily protein		
Potri_003G040200	-1.9542864	-1.5711539		1 -1.7590178	-1.6978483	0.057070184	0.16737847	0.085660369	-0.020410432	0.783389195	0.068873533	0.443831998	Metallo-hydrolase/oxidoreductase superfamily protein	
Potri_002G0507800	-1.4315601	-1.5710929		1 -1.0884879	-1.880844	0.065783897	0.22570138	0.105158474	-0.027317468	0.770630941	0.072680488	0.440902498	vacuoleless1 (VCL1)	
Potri_015G115500	-4.8802268	-3.397298		1 -3.397298	-3.397298	0.22495999	0.13532616	-0.274924816	-0.180244714	0.650689924	0.082905449	0.421811631		
Potri_015G115500	3.55078969	3.80193842		1 2.53914438	3.96274838	0.141050247	-0.6047491	-0.014165621	-0.329518352	-0.071504973	0.1366869268	-0.771052611	-0.771156665	
Potri_005G032100	2.5692233	2.20010334		1 -3.52827406	2.72990337	-0.007216905	-0.766494942	-0.317114883	-0.083793786	0.1170287276	-0.526397268	-0.415319033	peptidoglycan-binding LysM domain-containing protein	
Potri_018G140400	1.97372042	3.24724233		1 3.02719138	2.08209585	0.186054992	-0.43046464	0.400795468	-0.107335309	-0.801804213	0.504095817	0.118677824	UDP-Glycosyltransferase superfamily protein	
Potri_011G139400	4.5953431	1.10415823	no_detection	1 6.55673881	5.61751557	0.066280752	0.44709891	0.009504909	-0.122424907	-0.116205041	-0.365167219	-0.413244953		
Potri_016G011300	-8.7709759	-6.4622083		1 -6.4622083	-6.4622083	0.210838285	0.14242706	0.254409011	-0.163080982	0.666475043	0.079976573	0.423447642	Mo25 family protein	
Potri_T044400	1.49551303	1.5946225		1 2.90435474	1.86769276	0.20418731	0.34172013	0.3675554842	-0.166111171	-0.687777168	0.211013864	-0.109582142		
Potri_001G426800	-4.8802268	-3.397298		1 -3.397298	-3.397298	0.22495999	0.13532616	-0.274924816	-0.180244714	0.650689924	0.082905449	0.421811631		
Potri_005G120500	3.55078969	3.80193842		1 2.53914438	3.96274838	0.141050247	-0.6047491	-0.014165621	-0.327753541	-0.071611171	-0.584184961	Glycosyl hydrolase superfamily protein		
Potri_010G211400	3.78108519	4.38468666		1 4.1014219	4.55329346	0.225678514	-0.3704118	0.18356334	-0.250255671	-0.921462901	-0.094207937	-0.482455771	sulfate transporter 4.1	
Potri_T130900	3.19295559	2.87591547		1 2.37636811	3.84034765	0.180421063	-0.5851295	-0.045774333	-0.272670052	-0.752662975	-0.49175594	-0.774526172	Tetratricopeptide repeat (TPR)-like superfamily protein	
Potri_015G116500	3.0073495	2.54089415		1 3.93845341	4.12540016	0.247891767	-0.1529626	0.158226657	-0.305310205	-0.84198645	-0.317323126	-0.655856642	Protein of unknown function (DUF594)	
Potri_015G016300	3.94858441	5.93569236		1 5.80039967	6.73208928	0.469855145	-0.4314128	0.429409476	-0.482472842	-0.991445961	-0.121332077	-0.500536001	OBP3-responsive gene 1	
Potri_008G028500	-6.3948821	-6.2704362		1 -5.9621775	3.02177675	0.608060531	-0.4823248	0.311235419	-0.686037405	0.131121441	-0.739134858	-0.517454024	Phosphorylase superfamily protein	
Potri_003G070600	-3.6250868	2.31419501		1 -3.5294423	3.76779813	0.795613457	-0.8723115	0.592206335	-0.810640103	-0.229322834	-0.384620206	-0.317125513	Chaperone DnaJ-domain superfamily protein	
Potri_006G252200	-5.3358116	5.11364703		1 2.60068421	4.95144963	0.94244724	-0.5010894	0.977094937	-0.878940417	-0.598915997	0.056596366	-0.068631015	plant natriuretic peptide A	
Potri_T026600	-3.7646697	2.99665736		1 -2.9271235	5.8312303	0.857266971	-0.8908425	0.637040187	-0.881910489	-0.35838261	-0.448929102	-0.428673763	LRR and NB-ARC domains-containing disease resistance protein	
Potri_006G270000	-7.4038908	1.41114792		1 1.52417933	3.54704961	0.953609402	-0.3642964	0.969350926	-0.90072064	-0.431172004	-0.041208476	-0.081990829	Disease resistance protein (TIR-NBS-LRR class) family	
lignincontent	17.83	20.63		20.03	20.17	21.91	1	-0.6011638	0.90687104	-0.986250862	-0.557134148	-0.266108747	-0.338388836	
S/G ratio	3	2.38		3.01	3.47	2.05	-0.601163843	1	-0.312190106	0.664198076	0.42017513	0.546662628	0.587484213	
celluloseDPw	2505	4721		4104	4624	4682	0.906876104	-0.3121901	1	-0.827303834	-0.540679229	0.152367846	0.037676617	
glucose release	135.5	95		102.3	101.9	63.2	-0.986250862	0.66419808	-0.827303834	1	0.55590743	0.420264562	0.482370448	
xylose release	34.1	27		40.2	27	25	-0.557134148	0.42017513	-0.540679229	0.55590743	1	0.07816012	0.450623395	
ligninMw	10900	11600		11100	11500	10200	-0.266108747	0.54666263	0.152367846	0.420264562	0.07816012	1	0.914448692	
hemice	373	405		407	396	328	-0.338388836	0.58744821	0.037676617	0.482370448	0.450623395	0.914448692	1	