Supporting Information

Table S1. Orthogonal experiment results of soluble saccharides from leaves of *Populus tomentosa* Carr. ($\overline{X}$±SD）（mg/100g）

No.	Combination	α-D-galactose	α-D-glucose	α-D-fructose	myo-inositol	D-mannitol	D-sorbitol	trehalose	sucrose
1	$A_1B_1C_1$	22.923±2.115	37.473±2.312	ND	612.353±3.334	5.845±0.142	2.544±0.131	2.211±0.047	55.821±2.314
2	$A_1B_1C_2$	21.015±1.214	50.535±2.656	ND	620.755±5.365	16.556±1.171	ND	2.164±0.123	53.314±5.778
3	$A_1B_1C_3$	20.047±0.387	34.231±1.173	19.332±0.103	610.262±7.792	5.137±0.426	2.631±0.259	2.445±0.234	54.835±3.559
4	$A_1B_2C_1$	21.942±1.381	35.333±3.419	ND	615.195±4.512	5.415±0.255	2.431±0.044	2.119±0.033	57.335±6.333
5	$A_1B_2C_2$	23.156±0.633	52.565±2.067	ND	604.253±5.343	16.426±2.096	ND	2.134±0.147	53.489±5.334
6	$A_1B_2C_3$	21.556±0.562	35.185±2.574	18.399±1.741	625.867±2.035	5.306±1.289	2.372±0.125	2.262±0.113	54.811±4.315
7	$A_1B_3C_1$	22.925±1.724	33.534±4.163	ND	619.256±3.737	5.832±0.215	2.579±0.268	2.432±0.039	51.836±1.647
8	$A_1B_3C_2$	23.054±0.752	51.734±1.351	ND	611.134±9.596	15.845±1.122	ND	2.342±0.357	53.821±3.411
9	$A_1B_3C_3$	22.342±0.102	37.322±0.431	19.541±1.418	616.508±3.328	5.756±0.264	2.539±0.011	2.305±0.012	56.863±6.434
10	$A_2B_1C_1$	2.276±0.243	37.498±3.218	ND	621.368±7.284	5.671±0.243	2.221±0.243	2.461±0.230	43.487±4.343
11	$A_2B_1C_2$	2.473±0.375	52.465±1.159	ND	615.443±2.272	16.527±1.135	ND	2.263±0.032	42.532±2.234
12	$A_2B_1C_3$	2.369±0.083	34.243±1.443	18.365±0.645	611.365±2.513	5.245±0.523	2.436±0.155	2.124±0.317	45.444±3.114
13	$A_2B_2C_1$	2.177±0.166	37.521±0.341	ND	608.106±2.016	5.834±0.144	2.359±0.191	2.460±0.155	46.686±3.231
14	$A_2B_2C_2$	2.242±0.217	51.425±2.256	ND	609.231±3.553	16.435±2.365	ND	2.531±0.071	43.287±8.734
15	$A_2B_2C_3$	2.133±0.051	35.519±1.615	19.422±2.403	616.143±4.211	5.563±0.178	2.531±0.081	2.259±0.022	46.487±8.334
16	$A_2B_3C_1$	2.191±0.113	37.636±0.332	ND	620.805±6.798	5.537±0.254	2.372±0.134	2.334±0.341	44.111±5.715
17	$A_2B_3C_2$	2.276±0.142	53.479±3.051	ND	607.349±5.984	16.568±2.185	ND	2.503±0.019	42.536±7.984
18	$A_2B_3C_3$	2.372±0.232	37.533±0.047	20.395±1.279	625.585±4.667	5.346±0.045	2.351±0.231	2.328±0.325	52.441±3.561
19	$A_3B_1C_1$	23.028±0.113	36.385±0.673	ND	614.055±2.094	5.765±0.353	2.552±0.165	2.267±0.211	54.233±7.451
20	$A_3B_1C_2$	22.055±1.258	51.551±2.482	ND	623.893±5.395	16.533±0.241	ND	2.361±0.042	52.659±3.334
21	$A_3B_1C_3$	23.093±0.122	37.488±3.177	19.427±0.553	602.125±6.452	5.659±0.167	2.434±0.046	2.512±0.156	56.133±7.192
22	$A_3B_2C_1$	21.348±0.252	34.775±2.211	ND	615.743±8.823	5.833±0.537	2.405±0.390	2.468±0.228	54.864±5.233
23	$A_3B_2C_2$	21.325±2.198	52.554±3.283	ND	613.455±5.473	16.428±2.195	ND	2.112±0.037	57.323±2.324
24	$A_3B_2C_3$	23.057±0.553	34.494±1.121	21.491±2.303	611.755±2.905	5.870±0.445	2.732±0.359	2.260±0.014	53.319±4.333
25	$A_3B_3C_1$	22.176±1.325	37.161±0.422	ND	622.873±5.433	5.832±0.549	2.449±0.214	2.261±0.033	56.693±5.239
26	$A_3B_3C_2$	21.928±0.442	52.526±1.275	ND	619.145±3.131	16.543±1.167	ND	2.263±0.011	55.485±3.624
27	$A_3B_3C_3$	23.035±1.271	35.386±0.501	18.498±1.212	607.435±4.379	5.814±0.243	2.336±0.031	2.359±0.152	57.283±1.535

ND: not detected

Mass Balance of extraction from plant tissues by developed method

Plant materials of roots, stems and leaves of poplar were used to determine whether the polymeric carbohydrates extracted by the new method are the main compounds and the mass balance error (%) between extracted amounts and true amounts. While detection of soluble saccharides by GC-MS, quantitation was also conducted by phenol-sulfuric acid method[3]. The dectected amount of saccharides by the latter method was defined as total input amount while the total output amount was determined by GC-MS. As can be seen from table S2, the mass balance errors (%) were about 5%. This result indicates that the main sugar compounds in polar materials are monosaccharides and disaccharides rather than polysaccharoses.

Table S2. Mass balance data of soluble sugar extracted by Me$_2$SO from different tissues of poplar.

Items (mg/100g)	Leaves	Stems	Roots
Total input amountof sugar	817.15	587.16	1006.02
α-D-galactose	23.05	15.16	29.33
α-D-glucose	52.54	45.12	104.32
α-D-fructose	21.49	14.22	32.11
myo-inositol	611.76	433.23	701.99
D-mannitol	5.87	6.92	7.05
D-sorbitol	2.73	2.73	3.15
Trehalose	2.26	ND	1.05
Sucrose	53.32	47.66	89.29
Total output amountof sugar	773.02	565.04	968.29
Balance error/%	5.41	4.75	4.23

ND: not detected

Reference

1. Persson, S.; Caffall, K.H.; Freshour, G.; Hilley, M.T.; Bauer, S.; Poindexter, P.; Hahn, M.G.; Mohnen, D.; Somerville, C. The Arabidopsis irregular xylem8 mutant is deficient in glucuronoxylan and homogalacturonan, which are essential for secondary cell wall integrity. *Plant Cell.* **2007**, 19(1), 237-55.

2. F. Lu and Ralph, J.; Non-degradative dissolution and acetylation of ball-milled plant cell walls: high resolution solution-state NMR. *The Plant Journal.* **2003**, 35, 535-544.

3. Masuko, T.; Minami, A.; Iwasaki, N.; Majima, T.; Nishimura, S. Y.C. Lee. Carbohydrate analysis by a phenol–sulfuric acid method in microplate format. *Analytical Biochemistry.* **2005**, 339(1), 69-72.

Optimization of derivation conditions in the new method

The conditions of new derivatization method should be optimized in two aspects: 1. Time of derivatization. 2. Amounts of reagents needed for quantitative derivatization of saccharides. In the optimal assays, D-glucose (aldose), D-fructose (ketose) and mannitol (alditol) were selected for optimization of the derivatization method. The analytical procedures, results and discussion are described below.

Analytical Procedures

1. Optimization of reagent amounts needed for quantitative derivatization of saccharides

Two hundred μl (2.5μg/μl) of saccharides in Me$_2$SO solution with 30μl of 1-methylimidazole was added to seven tubes, followed by adding 25, 50, 100, 150, 300, 600 and 1200μl of acetic anhydride, respectively. Six replications of each level were prepared. After stirring for 30 min, 1500μl of ddH$_2$O was added to each tube to remove the excess acetic anhydride. Then 2ml of CH$_2$Cl$_2$ were added to extract the acetylated derivatives. These tubes were centrifuged for 1 min to partition the organic phase. Finally, 1μl of the lower methylene chloride layers was injected into the GC-MS system.

2.   Optimization of derivativation time

Two hundred μl (2.5μg/μl) of saccharides in Me$_2$SO solution with 30μl of 1-methylimidazole and 150μl acetic anhydride were added to seven tubes respectively. 1 min, 2.5 min, 5 min, 10 min, 20 min, 40 min and 80 min were selected for the acetylation reaction. Six replications of each level were prepared. After the reaction, 1500μl of ddH$_2$O was added to remove the excess acetic anhydride. Then 2ml of CH$_2$Cl$_2$ were added to extract the acetylated derivatives. These tubes were centrifuged for 1 min to partition the organic phase. Finally, 1μl of the lower methylene chloride layers was injected into the GC-MS system.

Results and Discussion

Figure S1 shows that the peak areas of three saccharide derivatives reached the maximum in 30 min when 100μl of acetic anhydride was used. The reaction could not be completed when 50μl of acetic anhydride was used. After the dosage of acetic anhydride was more than 100μl,

the peak areas remained substantially unchanged. In order to make sure that the acetylation reaction can be completed, 150μl of acetic anhydride was selected in the assay.
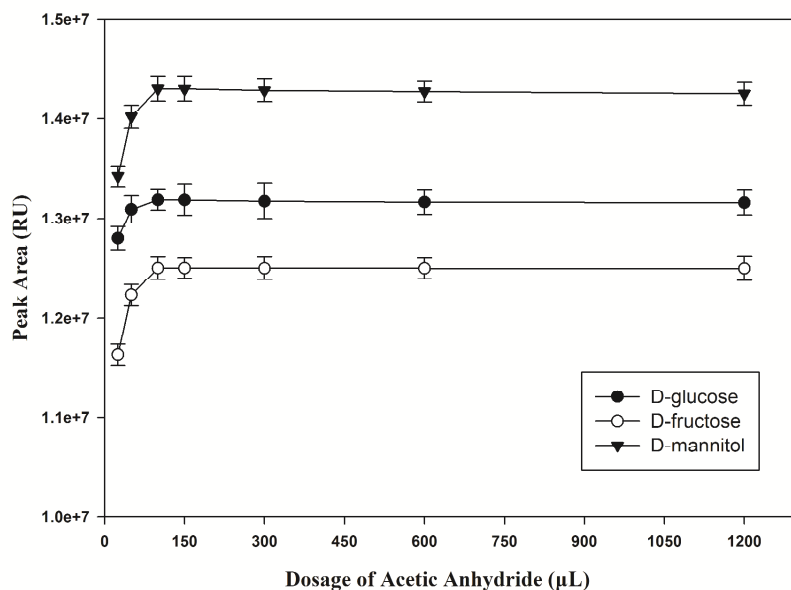

Figure S1. Optimization of reagent dosage needed for quantitative derivatization of saccharides
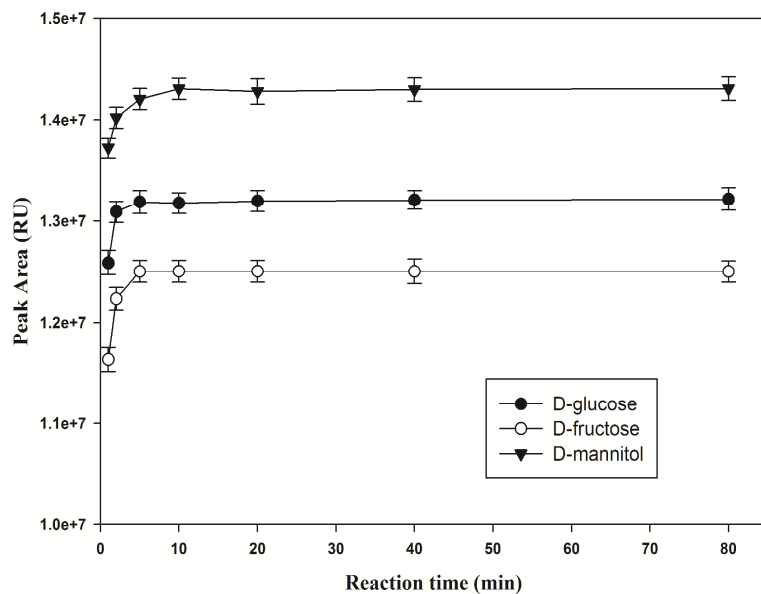

Figure S2. Optimization of time for completion of derivatization reaction

To optimize derivatization time, as can be seen in Figure S2, the maximum peak areas of 3 saccharide derivatives could be reached in 5 min. After 5 min, the peak areas were unchanged.

To complete the reaction and save time, 10 min was selected for the acetylation of saccharides in plant materials.

Stability test of sugar derivatives by the new method

Two hundred μl (50ng/μl) of 23 authentic standard saccharides in Me$_2$SO solution was derivatized according to the description in the section of derivatization of authentic standard soluble saccharides in manuscript. After the reaction, the lower methylene chloride layer was collected and sealed to be stored at -20°C. Ten different time periods were designed to perform the stability test of derivative products. As can be seen from table S3, RSDs (%) of 23 saccharides ranged from 0.15% to 3.21%, which indicates that the derivatives are relatively stable.

Table S3. Stability test of sugar derivatives

RSD(%), n=10 (0.5h, 1h, 3h，6h，12h, 24h, 48h, 96h, 192h, 384h)					
meso-erythritol	1.34	D-ribitol	0.12	myo-inositol	0.15
2-deoxy-β-d-ribose	1.99	L-fucitol	2.54	D-mannitol	0.13
2-deoxy-d-ribitol	2.31	L-arabinitol	2.13	D-sorbitol	0.54
α-D-xylose	2.23	D-xylitol	1.12	D-galactitol	0.54
α-L-rhamnose	0.55	α-D-galactose	3.12	N-acetyl-D-glucosamine	1.35
α-L-fucose	1.48	α-D-glucose	2.14	trehalose	2.33
β-L-arabinose	3.21	α-D-fructose	2.77	sucrose	2.53
L-rhamnitol	1.49	α-D-mannose	1.98		