

SUPPLEMENTARY DATA

Supplemental Methods S1. Methods for sugars and starch analysis.

Supplemental Method S2. Formulae for sugar and starch (glucose) analysis.

Supplemental Table T1. Gene names and accession numbers for maize, rice, Arabidopsis, and poplar TPS and TPP.

Supplemental Table T2. Sequence of primers used for qPCR.

Carbohydrate Metabolite Analysis

Frozen tissues (20-100 mg) were weighed and ground for 30-60 s while frozen using a Tissue Lyser II (Qiagen). Sugars (sucrose, hexoses and starch) were then extracted following a method adapted from Lunn et al. 2006. Tissues were resuspended in 500 μL of ice cold $\text{CHCl}_3/\text{CH}_3\text{OH}$ (3:7, v/v). 250 nmols of lactose was added as an internal standard to calculate recovery. Soluble sugars were extracted for 2 h at -10°C with shaking in an orbital mixer (Model 5C25, Cole Parmer) for 5 min every 15 min interval, followed by two extractions with 400 and 200 μL of water. Samples were then vortexed, incubated for 5 min at 4°C , centrifuged for 5 min at 10,000g and 200 μL of the upper aqueous phase was collected and dried using a centrifugal vacuum. The residue was resuspended in 250 μL of water and filtered by centrifugation for 2 h at 2250g using a MultiScreen® Ultracel-10 filter plates (Millipore) with samples covered with mineral oil to prevent evaporation. The filtrate was used directly for sugars analysis as described below. Starch was extracted from the pellet generated while extracting soluble sugars. The remaining upper phase was removed and the pellet was washed with 1 mL ice cold CH_3OH . The supernatant was removed and the pellet was dried using a centrifugal vacuum. The dried material was resuspended with 200 μL 0.5 M NaOH and incubated for 1 h at 60°C with shaking to dissolve the starch. The solution was neutralized with 200 μL of 0.5 M HCl, and the starch digested in 1 U amyloglucosidase (Roche Diagnostics), 600 μL 0.2 M NaOAc (pH 4.5) and 1 μmol lactose as internal standard for 12 to 24 h at 30°C with shaking. The reaction was stopped by boiling samples for 2 min. Samples were then centrifuged to remove debris and the supernatant was transferred to a new microcentrifuge tube. 250 μL of digested starch was filtered as described previously for soluble sugars. Samples were diluted 1:100 in filtered ultrapure water for soluble sugars and 1:10 for hydrolyzed starch.

Carbohydrate Metabolite Analysis

Frozen tissues (20-100 mg) were weighed and ground for 30-60 s while frozen using a Tissue Lyser II (Qiagen). Sugars (sucrose, hexoses and starch) were then extracted following a method adapted from Lunn et al. 2006. Tissues were resuspended in 500 μ L of ice cold $\text{CHCl}_3/\text{CH}_3\text{OH}$ (3:7, v/v). 250 nmols of lactose was added as an internal standard to calculate recovery. Soluble sugars were extracted for 2 h at -10°C with shaking in an orbital mixer (Model 5C25, Cole Parmer) for 5 min every 15 min interval, followed by two extractions with 400 and 200 μ L of water. Samples were then vortexed, incubated for 5 min at 4°C , centrifuged for 5 min at 10,000g and 200 μ L of the upper aqueous phase was collected and dried using a centrifugal vacuum. The residue was resuspended in 250 μ L of water and filtered by centrifugation for 2 h at 2250g using a MultiScreen® Ultracel-10 filter plates (Millipore) with samples covered with mineral oil to prevent evaporation. The filtrate was used directly for sugars analysis as described below. Starch was extracted from the pellet generated while extracting soluble sugars. The remaining upper phase was removed and the pellet was washed with 1 mL ice cold CH_3OH . The supernatant was removed and the pellet was dried using a centrifugal vacuum. The dried material was resuspended with 200 μ L 0.5 M NaOH and incubated for 1 h at 60°C with shaking to dissolve the starch. The solution was neutralized with 200 μ L of 0.5 M HCl, and the starch digested in 1 U amyloglucosidase (Roche Diagnostics), 600 μ L 0.2 M NaOAc (pH 4.5) and 1 μ mol lactose as internal standard for 12 to 24 h at 30°C with shaking. The reaction was stopped by boiling samples for 2 min. Samples were then centrifuged to remove debris and the supernatant was transferred to a new microcentrifuge tube. 250 μ L of digested starch was filtered as described previously for soluble sugars. Samples were diluted 1:100 in filtered ultrapure water for soluble sugars and 1:10 for hydrolyzed starch.

Sugar peaks were identified in comparison with known sugars and following formulas:

Soluble sugars:

(1)

$$\text{Initial sugar quantity } [\mu\text{mol g}^{-1} \text{ of FW}] = \frac{FSC \times FV \times L}{LCF}$$

Where:

Final sugar concentration:

FSC = Sugar peak area [nC] / Slope of standard curve for this sugar [nC = f(sugar concentration)]

Final volume of resuspension:

$$FV = 2.5 \times 10^{-4} \text{ L}$$

Lost correction factor:

LCF = Standard lactose peak area for a final concentration of 100 μM / Actual lactose peak area

**A final concentration of 100 μM lactose would have been expected if adding 25 μL internal standard and not diluting samples before running. Since Dionex optimal sensor concentrations from 1 to 100 μM , and that kernel samples contains high quantity of lactose, we used a 1/100 dilution to increase initial lactose to sugar ratio by 100.*

Lactose-Sugar Ratio:

$$L / S \text{ Ratio} = 10$$

(2) Glucose from starch digestion:

$$\text{Initial glucose quantity } [\mu\text{mol g}^{-1} \text{ of FW}] = \frac{FGC \times FV \times L}{LCF}$$

Where:

Final glucose concentration:

FGC = Glucose peak area [nC] / Slope of standard curve for glucose [nC = f(glucose concentration)]

Final volume of resuspension:

$$FV = 10^{-3} \text{ L}$$

Lactose-Glucose Ratio:

$$L / G \text{ Ratio} = 10$$

d data analysed using the

$$\frac{LCF \times L / S \text{ Ratio}}{FW}$$

concentration)]

*ose peak area **
of 1 mM of lactose as an
sitivity applies to sugar
sugars, we had to dilute them to

$$\frac{LCF \times L / G \text{ Ratio}}{FW}$$

se concentration)]

Supplemental table S1: TPS and TPP genes names and accession numbers in maize, rice, Arabidopsis and poplar

Specie	Gene name	Accecion number in PLAZA	Accecion number in other databases	Database		
Maize	ZmTPSI.1.1_tps1	ZM08G19270	GRMZM2G068943_T01	www.maizesequence.org		
	ZmTPSI.1.2	ZM06G28170	GRMZM2G001304_T01			
	ZmTPSII.2.1	ZM07G13460	GRMZM2G019183_T02			
	ZmTPSII.2.2	ZM01G08410	GRMZM2G099860_T01			
	ZmTPSII.3.1	ZM03G31900	GRMZM2G304274_T01			
	ZmTPSII.3.2	ZM03G31920	GRMZM2G123277_T01			
	ZmTPSII.3.3	ZM08G19270	GRMZM2G118462_T01			
	ZmTPSII.4.1	ZM02G30010	GRMZM2G527891_T01			
	ZmTPSII.4.2	ZM01G39130	GRMZM2G008226_T01			
	ZmTPSII.4.3	ZM04G13350	GRMZM2G366659_T01			
	ZmTPSII.5.1	ZM04G11490	GRMZM2G007736_T02			
	ZmTPSII.5.2	ZM01G37720	GRMZM2G079928_T01			
	ZmTPSII.5.3	ZM04G26220	GRMZM2G312521_T01			
	ZmTPSII.5.4	ZM05G42890	GRMZM2G122231_T01			
	ZmTPPA.1	ZM09G18330	GRMZM2G178546_T01			
	ZmTPPA.3	ZM05G35700	GRMZM2G112830_T01			
	ZmTPPB.1.1	ZM01G39020	GRMZM2G347280_T01			
	ZmTPPB.1.2	ZM02G29850	GRMZM2G140078_T01			
	ZmTPPB.1.3	ZM07G12490	GRMZM2G174396_T01			
	ZmTPPB.1.4	ZM05G40760	GRMZM2G055150_T01			
	ZmTPPB.1.5	ZM04G27640	GRMZM2G151044_T01			
	ZmTPPB.1.6	ZM09G00990	GRMZM2G080354_T01			
	ZmTPPB.2.1_ramora3	ZM07G27620	GRMZM2G014729_T01			
	ZmTPPB.2.2	ZM02G39290	GRMZM2G117564_T01			
	ZmTPPB.2.3	ZM07G27610	GRMZM5G840145_T01			
	Rice	OsTPS1	OS05G44210		HM050424	Genebank
		OsTPS2	OS01G54560		HM050425	
		OsTPS3	OS01G53000		HM050426	
		OsTPS4	OS03G12360		HM050427	
		OsTPS5	OS02G54820		HM050428	
		OsTPS6	OS05G44100		HM050434	
		OsTPS7	OS08G31980		HM050429	
		OsTPS8	OS08G34580		HM050430	
OsTPS9		OS09G25890	HM050431			
OsTPS10		OS09G23350	HM050432			
OsTPS11		OS09G20990	HM050433			
OsTPP1		OS02G44230	AB120515			

OsTPP2	OS10G40550	AB277360
OsTPP3	OS07G43160	NM_001066861
OsTPP4	OS02G51680	NM_001054678
OsTPP5	OS04G46760	TPP5_ORYSJ
OsTPP6	OS08G31630	TPP6_ORYSJ
OsTPP7	OS09G20390	BAD25928
OsTPP8	OS06G11840	BAD37685
OsTPP9	OS03G26910	AAT78804
OsTPP10	OS07G30160	NP_001059655
OsTPP11	OS02G44235	<i>none</i>
OsTPP12	OS10G40555	Osl_34594
OsTPP13	OS12G09060	LOC_Os12g09060

Arabidops

AtTPS1	AT1G78580
AtTPS2	AT1G16980
AtTPS3	AT1G17000
AtTPS4	AT4G27550
AtTPS5	AT4G17770
AtTPS6	AT1G68020
AtTPS7	AT1G06410
AtTPS8	AT1G70290
AtTPS9	AT1G23870
AtTPS10	AT1G60140
AtTPS11	AT2G18700

AtTPPA	AT5G51460
AtTPPB	AT1G78090
AtTPPC	AT1G22210
AtTPPD	AT1G35910
AtTPPE	AT2G22190
AtTPPF	AT4G12430
AtTPPG	AT4G22590
AtTPPH	AT4G39770
AtTPPI	AT5G10100
AtTPPJ	AT5G65140

Poplar

PtTPS1	PT11G09730
PtTPS2	PT01G38460
PtTPS3	PT03G08380
PtTPS4	PT08G13400
PtTPS5	PT01G02000
PtTPS6	PT10G10370
PtTPS7	PT11G06720
PtTPS8	PT04G05810
PtTPS9	PT12G07420
PtTPS10	PT15G07580
PtTPS11	PT06G17480

PtTPS12	PT18G09730
PtTPS14	PT04G06150
PtTPPA.1.1	PT12G13940
PtTPPA.1.2	PT15G13860
PtTPPA.1.3	PT01G00080
PtTPPA.1.4	PT03G10240
PtTPPB.1.1	PT05G15960
PtTPPB.1.2	PT02G09320
PtTPPB.1.3	PT05G07700
PtTPPB.1.4	PT07G05480

Supplemental table S2: Maize TPS, TPP, SnRK1 targets and reference genes RT-qPCR primers sequence, product size and efficiency

Gene	Gene accession number	Primers sequence	Product size (bp)	Efficiency
ZmTPSI.1.1_tps1	GRMZM2G068943	ACAGAGCTACACCCGTAGCTAGTCA	107	1.81
		TCCTTTATCCTTTCCATTTGCTA		
ZmTPSII.2.1	GRMZM2G019183	AGCTACGGTCAGTCCCTCAACC	116	1.84
		GAAGATATCCATGTCATCAACACCA		
ZmTPSII.3.2	GRMZM2G123277	GCATCGGCGATGATAGGTCC	210	1.99
		AATCAGATTCCAGTTCAGCTCCAGT		
ZmTPSII.3.3	GRMZM2G118462	TTTGAAAATATTGCTGATATCATTGG	233	2
		GATTGTTTCGTCACCAATATCAAGTG		
ZmTPSII.4.1	GRMZM2G527891	CTCCAAGCGCTGAACTTATCTCTAC	216	1.92
		GCTTCCATTTCAGAATAAATACCTGAGA		
ZmTPSII.4.2	GRMZM2G008226	ATTTCTTGATTACGATGGCACACTT	185	1.97
		CCGCTAGACCAAGCTTCTCACAC		
ZmTPSII.4.3	GRMZM2G366659	TGTGAAGTGTGGCCATTATATCGTA	98	1.93
		CGTTGTTGTTGGCCAGTGCT		
ZmTPSII.5.3	GRMZM2G312521	ATGTTTGGACCACCTTTGTATATGG	100	1.71
		CGGAGTGAATGAATCAACTTCTCTT		
ZmTPSII.5.4	GRMZM2G122231	CCATGGGATACCTCCGGG	113	2
		CTCTCCTTGTGCTCGATGTAGGAG		
ZmTPPA.1	GRMZM2G178546	GGCGGAAGATGACTATAAAAAGGTT	156	2.05
		AGCGATTCAAGTAAAACTCCACAG		
ZmTPPA.3	GRMZM2G112830	GTCACCTGTATCACCAGTCC	157	1.95
		ATTGACAAGGACCTCCTCGATTTTA		
ZmTPPB.1.3	GRMZM2G174396	GCCAAGGCCTCCTCTTCTTCT	158	2.11
		CAGAACCTGTTGTTCTCCACCTTG		
ZmβGal	ZM03G38190	GGATTGCCAGGGTTACAGGA	100	2.07
		CTAACCACCTTCTCCATGCAAGTCT		
ZmAKINβ	ZM09G22070	GTTTGCTGTTACAGAGAGCCAAGG	95	2.12
		TTTCATTCTGGGATGGGATG		
ZmARG10 *	ZM10G26580	CAACCACGAGACTTTGCTTCTAAAC	100	2.03
		CTAGGCAGGAAAAGTAAAAAGGAT		
ZmMDH	ZM04G14160	ATGGTTGCTGTTTGTCTTAATTG	90	2.11
		GTGTAAATAAGGCCTGGTTCAGAAA		
ZmbZIP11	ZM04G40980	GTGTACTGTGTCACCTCCACTCCAAC	92	1.93
		ATTGTATGGTGCACCTTCTTTGTTT		
ZmDPS *	ZM06G24060	CGGCTCAGGACTCCCATTT	87	2.05
		GCCGAGGCTTGAGATTGATAG		
ZmEF1α-1	GRMZM2G153541 (at1g07920)	AATCTCTGGTTTTGAAGGTGACAAC	230	1.9
		CAAAAGTAACAACCATACCAGGCTTA		
ZmEF1α-2	GRMZM2G343543 (at5g60390)_1	AAGACTTATCCGAACATCTGGTGAG	163	2.05
		AGATTTAAGCGCAAGAGAAATTTGA		
ZmEF1α-3	GRMZM2G112158 (at1g07940)	ACAGATGAAGTTGCTAAATCCAAGC	170	2.05
		GTACTIONTTCAGGAAGCAGGTCAT		
ZmPP2AA2-2	GRMZM2G122135 (at3g25800)_3	TACCTGTAATTTGTTGGCCTTTTA	151	1.97
		TACGTGTTGTGCTCCTGCTCAATTAT		
ZmCACS	GRMZM2G331032 (at5g46630)	CTGGGATTAATGACAAGATTGGAC	201	2.14
		ACCCTCTGTGATTCGATACTTCATC		
ZmCDC27	GRMZM2G392710 (at2g20000)	GATGGAAGATCCTTTGAGCAAGATA	164	2
		TTTCTGTAGACTTCCAGTGCTTCCT		