

Table S1. Carbohydrates content in (A) mature barley grain (embryo removed) and (B) mature embryo as percentage on a weight per weight basis (w/w) and per grain basis (milligram, mg). In case of MLG and starch measurements for mature grain ‘outer tissues’ and endosperm were analysed together. The embryo, containing low amounts of storage carbohydrates, was removed from mature grain and used for analysis of soluble sugars. The term “other” relates to grain constituents other than carbohydrates and proteins, including lipids, cellulose, lignin and other secondary metabolites (e.g. tocopherol, phytosterols), minerals and vitamins. * indicates a significant difference from the WT by t-test with P-values of < 0.05. † indicates values for glucose and fructose, which were very low in mature tissues and are expressed as µg/grain. For soluble sugars two biological replicates with two tech. replicates each were analysed.

A

	WT	WT(tc)	15-3	16-5	18-6	25-5
1000 ‘outer tissue’ and endosperm weight (g)	31.230±2.821	40.155±3.679	21.213±3.317	14.430±2.879	13.410±4.152	15.863±3.901
MLG (%w/w)	3.854±0.306	5.645±0.454*	8.904±1.222*	9.576±0.244*	8.682±0.389*	9.485±0.242*
Starch (%w/w)	66.588±1.603	59.769±2.475*	49.133±0.983*	36.493±0.606*	23.640±0.193*	37.664±1.154*
Total fructan (%w/w)	1.555±0.218	1.505±0.335	1.541±0.049	2.372±0.101*	3.872±0.050*	2.714±0.572*
Glucose (%w/w)	0.067±0.017	0.054±0.027	0.038±0.001	0.061±0.010	0.089±0.012	0.076±0.001
Fructose (%w/w)	0.081±0.022	0.061±0.013	0.043±0.010	0.046±0.009	0.087±0.026	0.056±0.013
Sucrose (%w/w)	0.936±0.212	0.963±0.184	1.785±0.241*	3.197±0.043*	4.977±0.109*	3.013±0.365*
Raffinose (%w/w)	0.340±0.024	0.332±0.007	0.320±0.028	1.351±0.221*	1.323±0.320*	0.826±0.092*
Arabinoxylan (%w/w)	4.421±0.365	4.336±0.325	6.622±1.250*	9.136±0.373*	13.156±0.501*	8.171±0.038*
Protein (%w/w)	20.977±1.717	17.600±2.131	17.643±4.553	21.301±1.763	22.997±0.867	21.940±0.544
Other (%w/w)	1.181	9.735	13.972	16.469	21.179	16.056
MLG (mg)	1.147±0.025	2.116±0.018*	1.973±0.180*	1.148±0.102	0.852±0.001*	1.201±0.128*
Starch (mg)	20.796±0.50	24.000±0.994*	10.422±0.209*	5.266±0.087*	3.170±0.026*	5.974±0.183*
Total fructan (mg)	0.486±0.068	0.604±0.135	0.327±0.011	0.342±0.015	0.519±0.007	0.431±0.091
Glucose (µg)[†]	21.0±5.3	21.7±10.6	8.1±0.3	8.8±1.4	11.9±1.6	12.1±0.2
Fructose (µg)[†]	25.4±7.0	24.4±5.2	9.2±2.2	6.6±1.4	11.6±3.5	8.8±2.1
Sucrose (mg)	0.292±0.066	0.387±0.074	0.379±0.051	0.461±0.06	0.667±0.015	0.478±0.058
Raffinose (mg)	0.137±0.010	0.133±0.003	0.068±0.006	0.195±0.032	0.177±0.043	0.131±0.015
Arabinoxylan (mg)	1.381±0.114	1.741±0.131*	1.405±0.265	1.318±0.054	1.764±0.067*	1.296±0.006
Protein (mg)	6.551±0.536	7.067±0.856	3.743±0.966*	3.074±0.254*	3.084±0.116*	3.480±0.086*

B

	WT	WT(tc)	15-3	16-5	18-6	25-5
1000 embryo weight (g)	1.173±0.660	1.293±0.541	0.943±0.200	1.258±0.361	1.758±0.578	0.978±0.275
Total fructan (%w/w)	5.159±0.591	5.861±0.365	4.963±0.185	3.958±0.232*	4.963±0.638	4.493±0.455
Glucose (%w/w)	0.128±0.032	0.120±0.001	0.127±0.026	0.052±0.010	0.088±0.019	0.105±0.026
Fructose (%w/w)	0.101±0.034	0.050±0.007	0.038±0.010	0.027±0.008	0.028±0.06	0.029±0.008
Sucrose (%w/w)	9.217±1.121	9.918±0.065	9.073±0.406	7.266±0.773*	9.343±1.302	8.312±0.722
Raffinose (%w/w)	12.716±0.903	12.026±0.152	9.998±1.719*	12.957±0.865	11.681±0.001	11.757±0.142
Other (%w/w)	72.680	72.025	75.801	75.740	73.897	75.304
Total fructan (mg)	0.061±0.007	0.076±0.005	0.047±0.002	0.005±0.003	0.087±0.011	0.044±0.004
Glucose (µg)[†]	1.5±0.4	1.6±0.0	1.2±0.2	0.7±0.1	1.6±0.3	1.0±0.3
Fructose (µg)[†]	1.2±0.4	0.7±0.1	0.4±0.1	0.3±0.1	0.5±0.1	0.3±0.1
Sucrose (mg)	0.108±0.013	0.128±0.001	0.086±0.004	0.091±0.001	0.164±0.023	0.081±0.007
Raffinose (mg)	0.149±0.011	0.155±0.002	0.094±0.011	0.163±0.011	0.205±0.007	0.115±0.001

Table S2. List of primers used for real-time PCR. # indicates primers that flanked between *HvCslF6* gene and NOS terminator. * ** indicates β -fructofuranosidase (invertase) genes (INV* indicates *HORVU6Hr1G011260* gene, INV** indicates *HORVU2Hr1G109120* gene) which are involved in cleaving O-C (fructose) bond.

Gene full name	Gene	HORVU/Accession number	Sequence for forward primer (5'→3')	Sequence for reverse primer (5'→3')
Granule bound starch synthase 1a	GBSS 1a	FN179380.1	AGGACGTGCTTCTGG AACTG	CGAGAACCCTGAAGA TGGAA
Starch synthase 2	SS2	AH011942.2	GCCTCAACACGTACT GGAAC	ATCGCGTGATCCTTG GTTACT
Starch branching enzyme 1	SBE1	FN179382.1	AACTTTGTCTTCCGG TCACCT	TTTACACAGCTCGAT AATACTGCT
Starch branching enzyme 2b	SBE2b	AF064561	CGGAAACACGAGGA AGATAAG	TGGAGCATAGACAAC ACAGGT
Starch branching enzyme 2a	SBE2a	AK373114.1	ACCAAGTAGCCGTTT TGGAAC	TGTGTATCAGTGCCA TCGAAA
Isoamylase 1	Iso1	AF490375.1	AGATGAAACAAAAGG CGAGAT	AGGGCGTGATACAA GGATGAC
Limit dextrinase	LD	AF252635.1	AAGTGAAAAGAACGA AGATAA	CCATCTCGGGGCTCT CACCTC
Cellulose synthase-like F6	<i>CslF6</i> (wild type)	KP260639.1	TGGGCATTACCTTC GTCAT	TGTCCGGGCAAAC CATCAA
Cellulose synthase-like F6	<i>CslF6</i> (transgene)	n/a#	TGGGCATTACCTTC GTCAT	TGATAATCATCGCAA GACCG
Cellulose synthase-like H1	<i>CslH1</i>	FJ459581.1	TGCTGTGGCTGGATG GTGTT	GCTTTATTATTGAGA GAGATTGGGAGA
Cellulose synthase-like F9	<i>CslF9</i>	EU267184.1	CTGCCACCGCGTCC GTGTA	AGGTTTTGCAGCATT ACTTGA
(1,3;1,4)-beta-glucanase E1	<i>Endo E1</i>	AK252046.1	AACGAGAACCAGAAG GACAAC	TACGGACATACGGG CACTA
Cellulose synthase 2	<i>CesA2</i>	AY483152.1	CAGCCAGCCAGCAAT TCTTTAT	AACCGCATTCTTGCC TTACAGA
Sucrose synthase 1	<i>SuSy1</i>	X65871.1	GTACGTGAGCAACCT GGAGAG	AATGTGCCGAGACAT CAAATC
Sucrose-phosphate synthase	<i>SPS</i>	AK366557	AACCACAGCGCTACC AGACT	TGATCCGCATTTGTA TTGGA
Sucrose-phosphatase	<i>SPP</i>	AF493562.1	AAGTACCGATCATGG GTGGA	CTCAACCCCGGGATA TTCTT
Sucrose transporter 1	<i>SUT1</i>	AM055812.1	TCGCATAGGCGTCGT AACTA	GGTTTGCTACACGCA AGTCA
Sucrose transporter 2	<i>SUT2</i>	AJ272308.1	ACAAGGAAGGAAGG CAGTT	CCCACGGAGGAAAG TAACAA
Cell wall invertase 1	<i>CWINV1</i>	AJ534447.1	CTCCGAGGGTATGG ACGTAG	TTTTTCACTCATCCG GCAAT
Vacuolar invertase	<i>VIN</i>	JQ411255.1	CGGTGAGGCTCTATG TGTGA	CTAAGACGTCGAGG CCATCT
Sucrose:sucrose-1-fructosyltransferase	<i>1-SST</i>	AK366020.1	GGGCTACGGTACGA CTGG	GGGTCTTCTCGTCAA GCTC
Fructan:fructan 1-fructosyltransferase	<i>1-FFT</i>	JQ411253.1	GACGACCGGCGAGA CTATTACG	GACGCGTCGTACCT GCCATA
Sucrose:fructan 6-fructosyltransferase	<i>6-SFT</i>	JQ411254.1	GACCCGGAGCTCGA CTTG	CCGGGTCTTCTCATC CAGAG
β -fructofuranosidase	<i>INV*</i>	HORVU6Hv1G011260	TTATTGTCACAGAAG AGCAATGTG	GATAACCATACATAG TTGAGCAATTT
β -fructofuranosidase	<i>INV**</i>	HORVU2Hv1G109120/ AK367512.1	CCAATTGTGGCTCAG GTGTA	GGGCATGCGTGCTAT ACTTT
Lipid transfer protein 2	<i>Ltp2</i>	X69793.1	TGTGCCAGTACGTCA AGGAC	GCTAGCCAGGAAGC AAGCTA