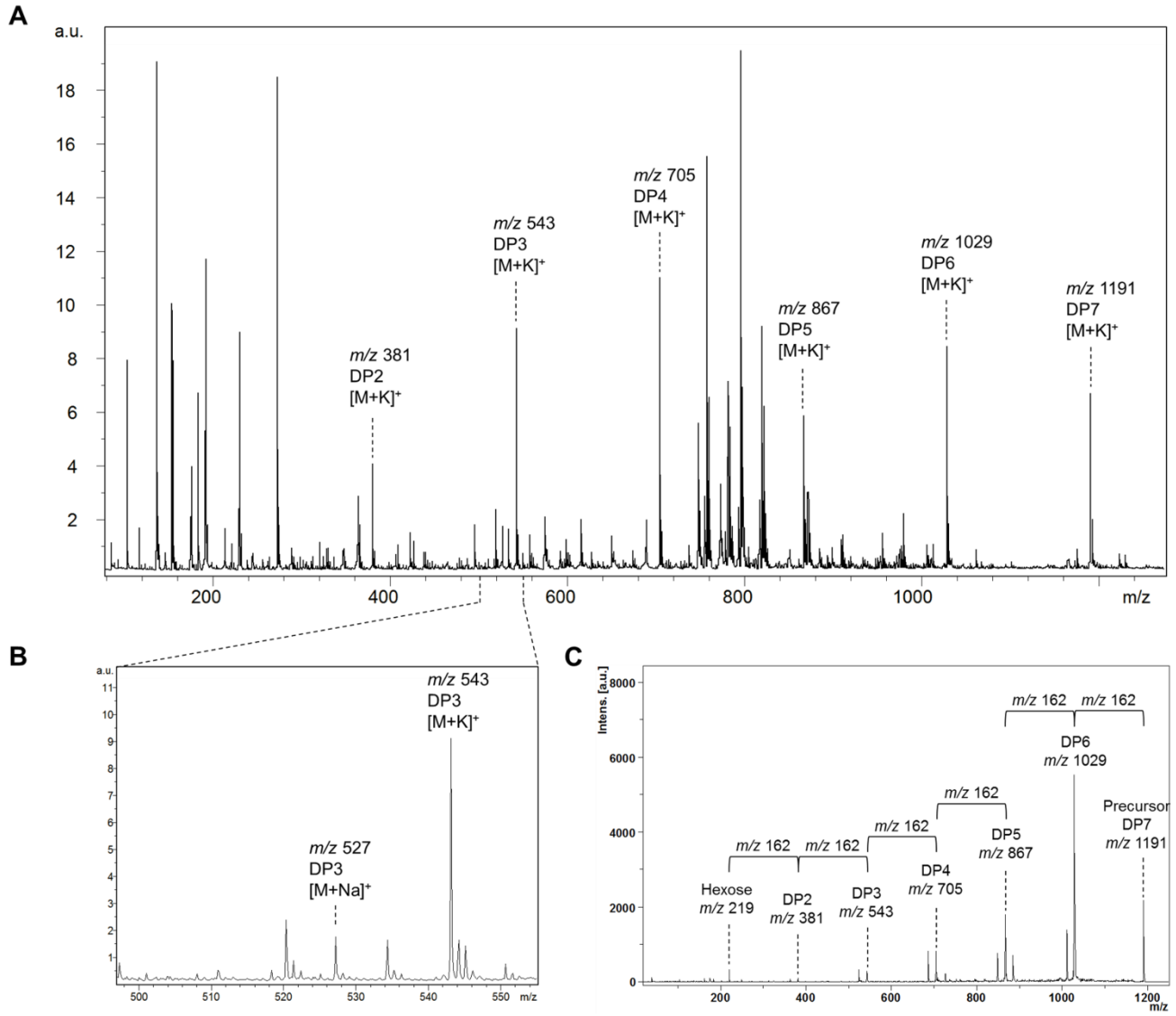
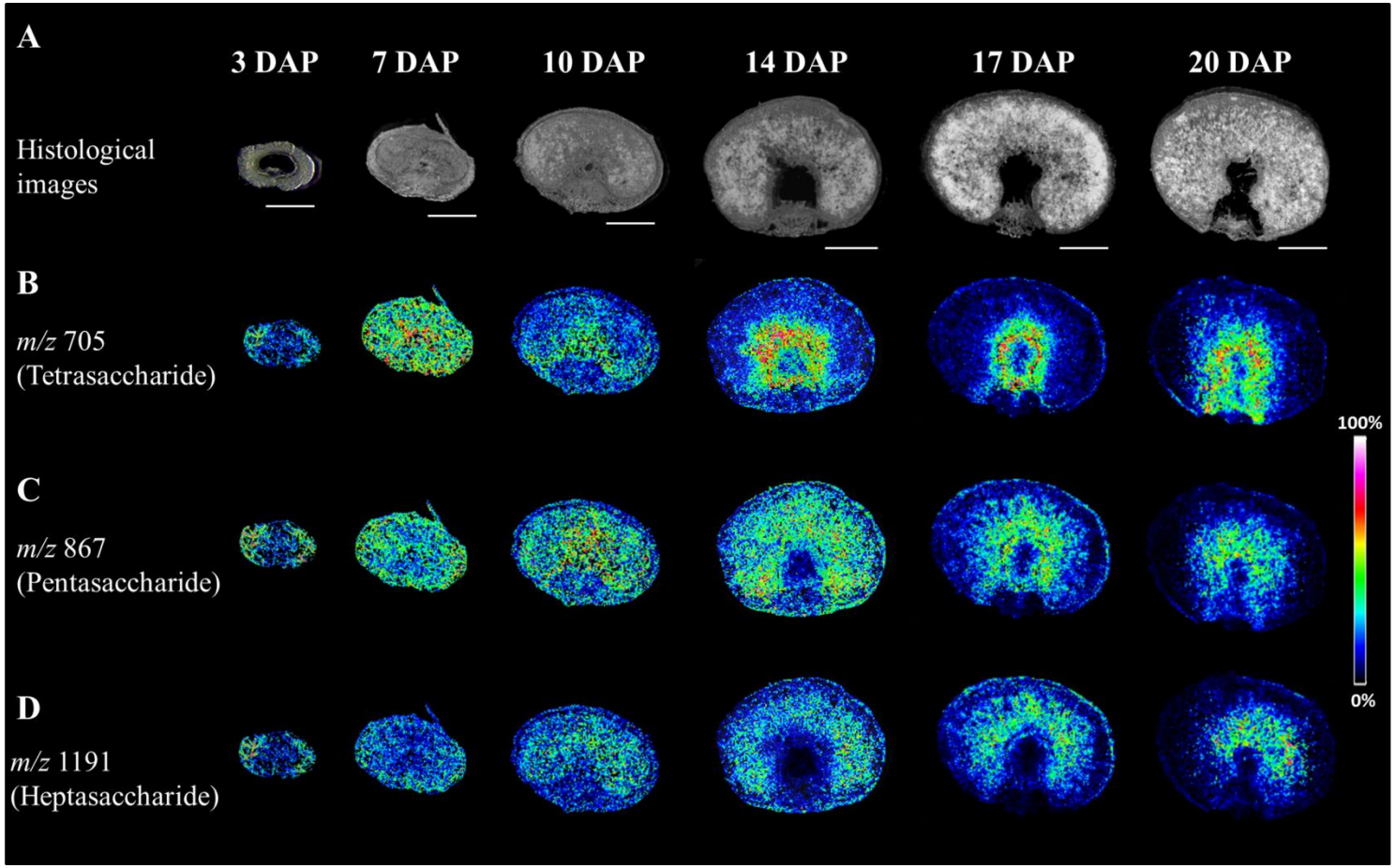


Supplemental Figure 1. Detection of Oligosaccharides in a Barley Grain Section.

A) Average mass spectrum of a MALDI MSI experiment from a 14 DAP barley grain cross section showing the detected oligosaccharides.

B) Predominant detection of the sugar potassium adduct ion $[M+K]^+$, preceded by the detection of the sodium adduct ion $[M+Na]^+$.

C) MS/MS spectrum of *m/z* 1191; neutral losses of *m/z* 162 are diagnostic for the hexose cleavage.



Supplemental Figure 2. The Pattern of Sugar Accumulation During Barley Grain Development, as Observed by MALDI MSI Analysis.

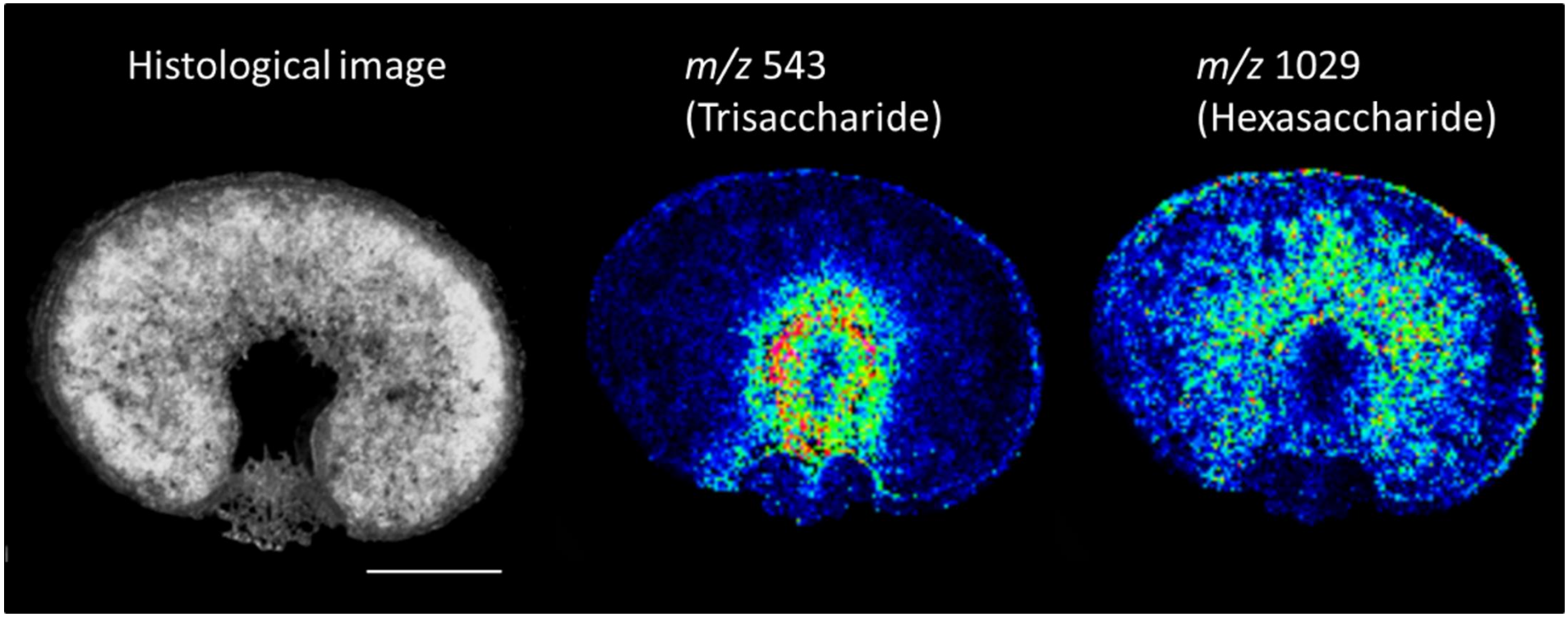
A) Histological images illustrating the various developmental stages.

B) The accumulation of tetrasaccharide (DP 4), around the endosperm cavity at the beginning of the storage phase.

C) The movement of pentasaccharide (DP 5), which had accumulated at the pericarp during the prestorage phase (3 DAP), to the endosperm at beginning of the storage phase (from 10 DAP onwards).

D) The movement of heptasaccharide (DP 7), which had accumulated at the pericarp during the prestorage phase (3 DAP), to the endosperm at beginning of the storage phase (from 10 DAP onwards).

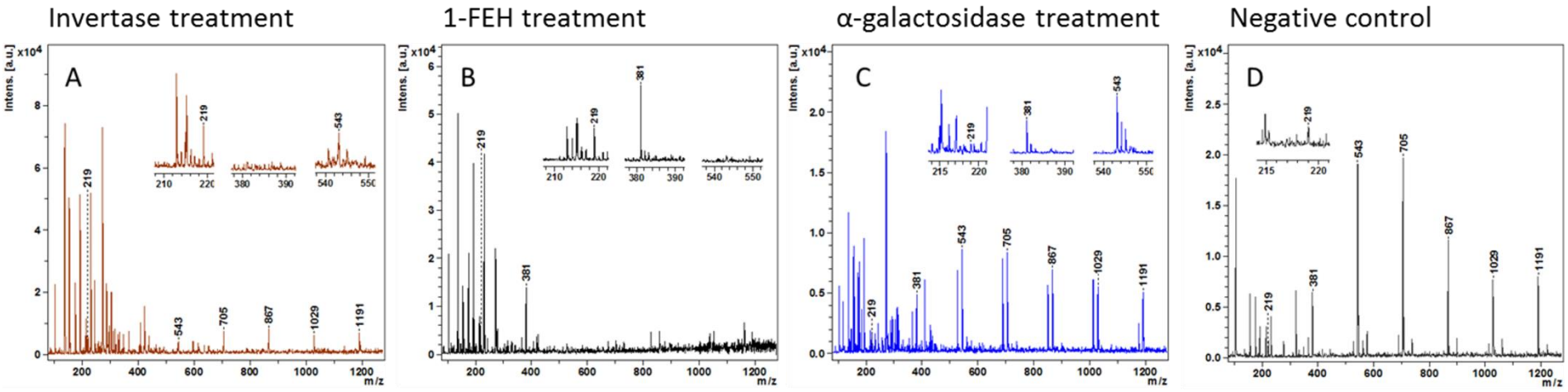
Bars = 1 mm.



Supplemental Figure 3. Enlarged View of Single Ion Intensity Maps for *m/z* 543 and *m/z* 1029.

The trisaccharide (*m/z* 543, DP 3) accumulates in the tissue around the endosperm cavity during the storage phase (17 DAP), while the hexasaccharide (m/z 1029, DP 6) localizes in the endosperm during the storage phase (17 DAP).

Bar = 1 mm.



Supplemental Figure 4. Enzymatic Digestion of Cavity Sap.

A) Digestion with invertase leads to the complete degradation of sucrose (*m/z* 381, $M+K^+$), whereas other sugars were degraded to a lesser extent.

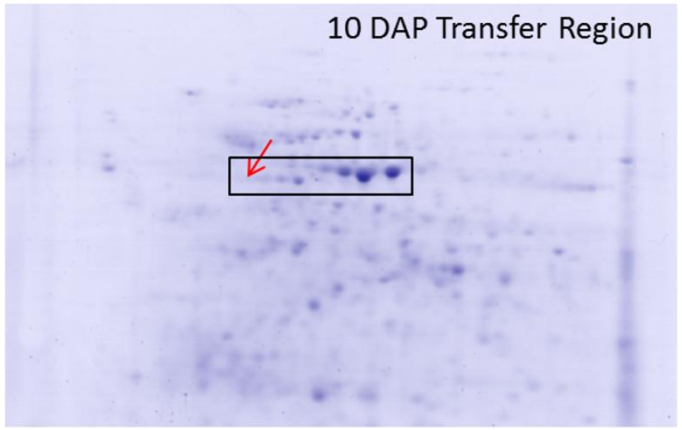
B) Fructan 1-exohydrolase (1-FEH) completely degraded DP 3-7 oligosaccharides, indicating a high concentration of oligofructans in the endosperm cavity. End products of the reaction were sucrose and fructose (m/z 219, $M+K^+$).

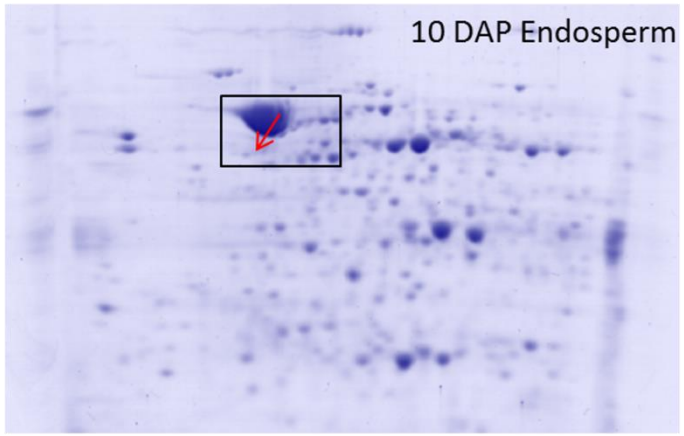
C) Treatment of the cavity sap with α-galactosidase had no effect on the sugar profile with *m/z* 543 and 705 being the most intense signals. In addition to the $[M+K]^+$ molecular ions, intense signals for the $[M+Na]^+$ molecular ions were also detected.

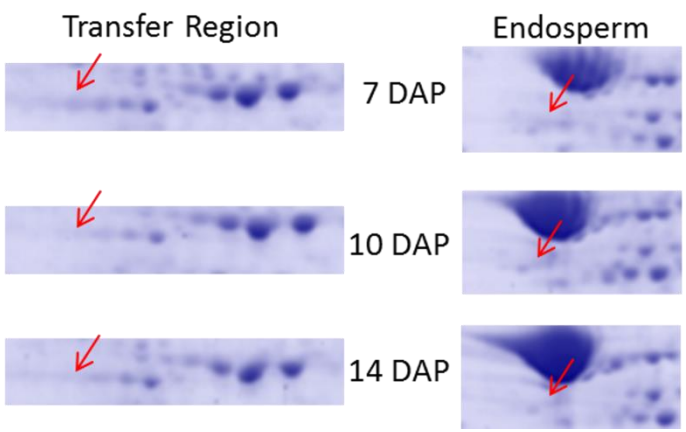
D) As a negative control the cavity sap was treated with only buffer. The DP3 (*m/z* 543) and DP4 (*m/z* 705) sugar molecular ions were most intense, whereas hexose was barely detected.

Digestion of standard compounds to test enzyme specificity. +, substrate degradation was observed; -, no degradation.

Compound	Digestion with INV	Digestion with 1-FEH	Digestion with α-GAL
Sucrose	+	-	-
Trehalose	-	-	-
Maltose	-	-	-
Raffinose	-	-	+
1-Kestose	+	+	-
Maltotriose	-	-	-
Nystose	+	+	-
Stachyose	-	-	+







Supplemental Figure 5. Identification of 6-SFT on Two Dimensional SDS-PAGE (2DE) Gels.

A) Representative 2DE separation of protein extract from transfer region (10 DAP).

B) Representative 2DE separation of protein extract from endosperm (10 DAP).

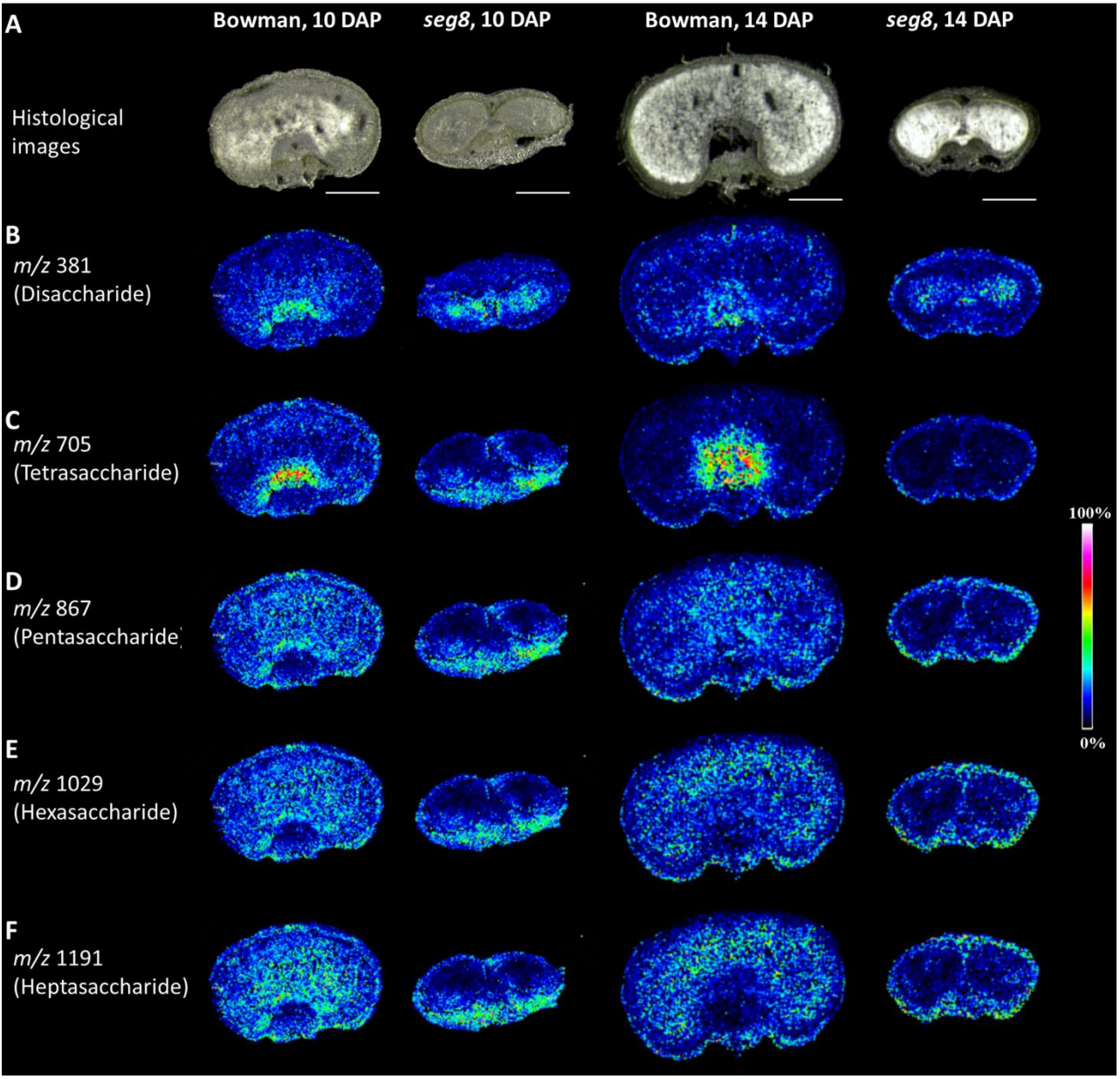
C) Enlarged sections from 2DE gels of transfer region and endosperm from 7-10 DAP; shown in rectangles in A and B.

D) Results table of mass spectrometric (MS) analysis for protein identification. The indicated spots (red arrows) were identified as sucrose:fructan 6-fructosyltransferase (6-SFT; Accession number MLOC_67531.6 of the barley genome sequences).

6-SFT has not been identified in samples from 14 DAP. The methods used for MS identification are described in Methods.

D

	Protein match	Peptide Sequences (ions score)	Prot. Score	Sequ. Cov.
7 DAP Endosperm	67531.6	K.HPANPVIWSPPGVGTK.D K.TKDFLNYELIPGILHR.V (3) K.DFLNYELIPGILHR.V R.TGEWECIDFYPVGR.R (Propionamid C) K.FYASTSFYDPAK.K (34) K.GWASIQSVPR.T (22) R.VYLFNNATGASVTAER.L	88	12 %
Transfer Region	67531.6	K.TKDFLNYELIPGILHR.V K.DFLNYELIPGILHR.V R.TGEWECIDFYPVGR.R (Propionamid C) K.FYASTSFYDPAK.K K.GWASIQSVPR.T (37) R.TNLLLWPVEEIETLR.L R.TTMTSR.V (Oxidation M) R.VYLFNNATGASVTAER.L	77	13 %
10 DAP Endosperm	67531.6	R.TGCMRWSACATVLTASAMAVVVVGAT LLAG.M (Oxidation M, Propionamid C) R.NLVQWR.T K.HPANPVIWSPPGVGTK.D K.TKDFLNYELIPGILHR.V (7) R.TGEWECIDFYPVGR.R (Propionamid C) K.GWASIQSVPR.T R.VLVDHSIVQGFAMGGR.T (Oxidation M)	45	16 %
Transfer Region	67531.6	R.NLVQWR.T K.TKDFLNYELIPGILHR.V (3) K.DFLNYELIPGILHR.V R.TGEWECIDFYPVGR.R (Propionamid C) K.FYASTSFYDPAK.K K.GWASIQSVPR.T (6)	41	8 %



Supplemental Figure 6. The Pattern of Sugar Accumulation During Grain Development, as Observed by MALDI MSI Analysis of *H. vulgare* L. var. Bowman and *seg8*.

A) Histological images illustrating the various developmental stages.

B) The distribution of the disaccharide (DP 2) at the beginning of the storage stage. In Bowman a higher concentration in the region of the nucellar projection and cavity was observed, while in *seg8* the disaccharide was most concentrated in the endosperm.

C) Tetrasaccharide (DP 4) accumulated around the endosperm cavity at the beginning of the storage phase in Bowman, whereas no accumulation was observed in this region in *seg8*.

D) Distribution of the pentasaccharide (DP 5), which slightly accumulates in the endosperm in Bowman, but mainly concentrates in the pericarp in *seg8*.

E) Distribution of the hexasaccharide (DP 6), which slightly accumulates in the endosperm in Bowman, but concentrates in the pericarp in *seg8*.

F) Distribution of the heptasaccharide (DP 7), which accumulates in the endosperm in Bowman, but concentrates in the pericarp in *seg8*.

Bars = 1 mm.

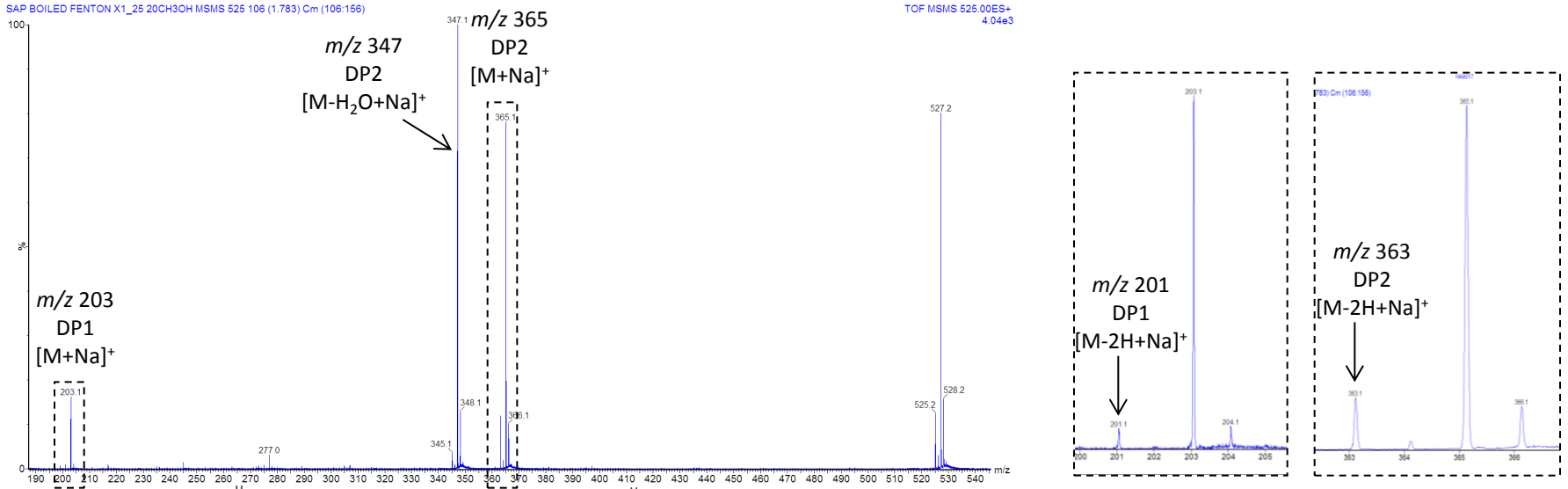
Supplemental Data. Peukert et al. (2014). Plant Cell 10.1105/tpc.114.130211

Supplemental Figure 7. ESI-MS/MS Fragmentation of a DP3 Molecular Ion (*m/z* 525.2 [M-2H+Na]$^+$).

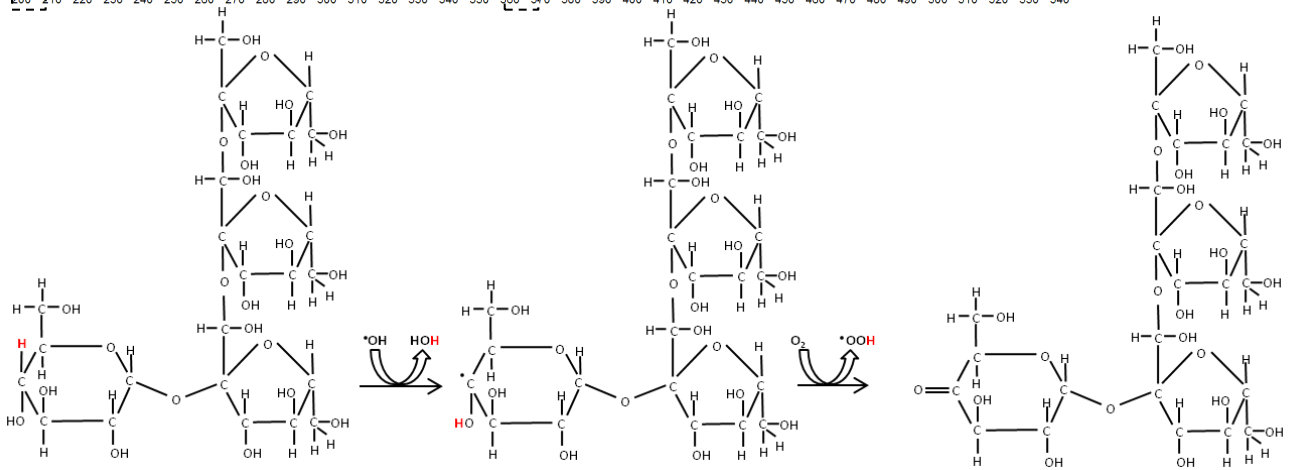
A) Fragmentation of the oxidized DP 3 precursor *m/z* 525.2 [M-2H+Na]$^+$ generated molecular ions with an *m/z* of 365.1 ([DP2+Na]$^+$), 203.1 ([DP1+Na]$^+$) and 347.1 ([DP1-H_2O+Na]$^+$, indicated by an arrow) confirming the *m/z* 525.2 molecular ion to be an oligosaccharide. Additionally, corresponding oxidized fragmentation products were detected (enlargements of dotted boxes are represented on the right with *m/z* 201.1 and *m/z* 363.1, indicated by arrows). Please also refer to Figure 6D for a summarizing table of detected molecular ions for oligosaccharides and corresponding oxidation products.

B) Scheme for the spontaneous 2H (red) elimination as proposed by Von Sontag and Schuchmann (2001).

A



B



Von Sonntag C and Schuchmann H.-P.: Carbohydrates. In: Radiation Chemistry: Present Status and Future Trends, Elsevier Science 2001, C.D. Jonah and BSM Rao (Editors).

Supplemental Table 1. Quantities of Individual Sugars Present in Extracts of the Whole Barley Grain and the Endosperm Cavity.

Quantification was carried out by LC and electrochemical detection from grains of three independent sowings. Per sample extracts of 20 grains were pooled. Raffinose was not detected.

	Glucose		Fructose		Sucrose		1-Kestose		Maltose		6-Kestose		Nystose		Bifurcose	
	µmol/g		µmol/g		µmol/g		µmol/g		µmol/g		µmol/g		µmol/g		µmol/g	
Samples	**FW**	**σ**	**FW**	**σ**	**FW**	**σ**	**FW**	**σ**	**FW**	**σ**	**FW**	**σ**	**FW**	**σ**	**FW**	**σ**
7 DAP total grain	22.21	6.06	14.45	4.24	56.11	4.45	12.29	1.89	34.33	3.16	5.07	0.32	2.66	0.23	18.84	3.01
7 DAP transfer region	16.57	1.32	13.32	0.97	68.16	7.57	12.33	1.50	0.00	0.00	7.44	0.68	3.04	0.24	23.36	2.20
10 DAP total grain	6.00	0.94	5.41	0.68	32.94	2.17	6.82	1.19	25.34	2.60	2.55	0.15	2.35	0.27	5.89	0.89
10 DAP cavity extract	4.55	0.84	3.25	0.37	34.10	5.95	24.67	7.86	0.00	0.00	1.85	0.42	10.21	2.31	4.82	0.58
14 DAP total grain	2.39	0.15	3.72	0.39	23.17	2.71	7.13	0.82	22.77	7.49	0.78	0.33	2.27	0.12	1.47	0.68
14 DAP cavity extract	4.02	0.85	3.09	0.61	48.68	9.52	53.42	11.71	0.00	0.00	0.52	0.06	16.53	2.50	2.62	0.64
17 DAP total grain	2.29	0.50	2.59	0.46	23.09	2.35	9.56	2.11	43.65	5.92	0.27	0.04	2.63	0.61	0.88	0.30
17 DAP cavity extract	6.07	1.30	3.24	0.25	55.88	4.97	64.17	9.39	0.00	0.00	0.53	0.08	18.43	4.62	6.29	1.89
20 DAP total grain	1.95	0.55	1.66	0.38	20.45	7.83	8.21	4.11	54.86	14.29	0.17	0.07	2.46	0.83	1.28	0.80
20 DAP cavity extract	9.31	2.72	4.79	2.75	57.55	5.68	69.11	1.41	0.00	0.00	0.94	0.38	20.53	0.67	10.42	5.61

Supplemental Table 2. Expression Profiles of Genes Involved in Fructan Metabolism in Isolated Barley Grain Tissues.

NP1 – nucellar projection with dividing cells; NP2 – nucellar projection with differentiated / degrading cells; ETC – endosperm transfer cells; nd – not detectable.

		1-SST		1-FFT		6-SFT		1-FEH	
DAP	**Grain part**	**Rel. expression**	**σ**	**Rel. expression**	**σ**	**Rel. expression**	**σ**	**Rel. expression**	**σ**
3	NP	0.056	0.014	0.010	0.003	0.019	0.007	0.002	0.001
5	NP	0.126	0.011	0.010	0.003	0.145	0.058	nd	
7	NP_1	1.621	0.075	0.125	0.023	0.226	0.067	0.010	0.004
7	NP_2	1.025	0.210	0.046	0.018	0.464	0.174	0.010	0.003
10	NP_1	2.333	0.274	0.186	0.043	0.150	0.036	0.169	0.045
10	NP_2	0.101	0.013	0.017	0.003	0.075	0.014	0.042	0.020
12	NP	nd	nd	nd	nd	0.006	0.004	0.045	0.018
14	NP	0.022	0.006	0.015	0.004	0.025	0.009	0.075	0.035
5	ETC	0.049	0.007	nd		0.220	0.127	0.012	0.005
7	ETC	0.014	0.003	nd		0.275	0.097	0.024	0.014
10	ETC	0.009	0.001	nd		0.099	0.025	0.013	0.006
12	ETC	nd		nd		nd	nd	nd	
14	ETC	nd		nd		nd	nd	0.023	0.009
5	Endosperm wings	0.850	0.045			4.702	1.444	0.006	0.004
5	Endosperm centre	0.441	0.037	0.001	0.001	4.979	1.584	0.002	0.001
7	Endosperm wings	0.137	0.042	0.004	0.002	3.000	0.692	0.005	0.003
7	Endosperm centre	0.194	0.021	0.003	0.001	3.915	0.628	0.008	0.008
10	Endosperm wings	0.011	0.005	nd		0.025	0.006	nd	
10	Endosperm centre	0.047	0.062	0.003	0.000	0.044	0.048	nd	
14	Endosperm wings	0.005	0.001	nd		0.021	0.013	nd	
14	Endosperm centre	0.015	0.007	nd		0.008	0.004	nd	

Supplemental Table 3. Expression Profiles of Genes Involved in Fructan Metabolism in Isolated Barley Grain Tissues of 'Bowman' and *seg8*.

NP – nucellar projection ; ETC – endosperm transfer cells; nd – not detectable

qRT-PCR Analysis was performed as described in Methods. Expression levels for each gene are marked by color code with red indicating high and white indicating low values.

Relative expression		1-SST				1-FFT				6-SFT				1-FEH			
DAP	grain part	*Bow*	σ	*seg8*	σ	*Bow*	σ	*seg8*	σ	*Bow*	σ	*seg8*	σ	*Bow*	σ	*seg8*	σ
5	NP	0.066	0.018	0.161	0.015	0.089	0.005	0.067	0.013	0.184	0.032	0.258	0.000	0.019	0.003	0.022	0.008
7	NP	0.136	0.042	0.210	0.029	0.067	0.014	0.059	0.014	0.801	0.059	0.475	0.000	0.068	0.008	nd	
10	NP	0.123	0.017	0.336	0.122	0.130	0.034	nd		0.165	0.023	0.112	0.017	0.084	0.014	nd	
5	ETC	0.544	0.048	0.093	0.024	0.003	0.002	0.010	0.001	0.068	0.029	0.083	0.000	0.053	0.000	0.057	0.000
7	ETC	0.019	0.011	0.146	0.013	nd		nd		0.301	0.085	0.160	0.000	0.049	0.000	0.027	0.000
5	Endosperm centre	2.761	0.221	1.187	0.115	0.003	0.003	0.017	0.006	1.812	0.103	0.130	0.000	0.026	0.007	0.049	0.011
5	Endosperm wings	4.142	0.559	0.408	0.037	0.002	0.001	0.001	0.000	2.860	0.127	0.108	0.000	nd		0.019	0.000

Supplemental Table 4. PCR Primer Sequences used in qRT-PCR Analyses.

Sequences information was adopted from Huynh et al. (2012).

Gene name	Accession	Primer combinations
1-SST	AK366020 AK357135 AJ567377 JQ411252	F-ACTTGGTAGTCGTCGGTTAGGC R-ACCGAGCAATCAATCCACAA
1-FFT	AK354338 JQ411253	F-TTCGAACGCACTTCTGCCA R-TTCGCCACATCGGTAGCAT
6-SFT	AK253058 JQ411254	F-AAAGGCTTGTGGTGCACGAGA R-ATGCCATCGTCCTCATTGGAG
1-FEH	AK252358	F-GCAGAGGAGCAATTCGATTTTC R-CCAGGCAATGCTCATGAATG
6-FEH/CWINV2	AJ534444	F-GCCTCGTGAAGGTTTCCAAA R-ACAACGTTTACAGCAGCCGTG

Huynh B-L, Mather D, Schreiber A, Toubia J, Baumann U, Shoaei Z, Stein N, Ariyadasa R, Stangoulis JR, Edwards J et al: Clusters of genes encoding fructan biosynthesizing enzymes in wheat and barley. Plant Mol Biol 2012, 80(3):299-314