

A comprehensive overview of grain development in *Brachypodium distachyon* variety Bd21

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Supplementary Tables S1-S2

Supplementary Figures S1-S5

Table S1: Full list of proteins identified from SDS-PAGE displayed in Fig. 3. All analyses were performed with a Q-TOF instrument

DAF-N	Bradi_1.0	MW	NUP	% Cov	Score	Best homologue protein name	E-value	GO Biological process
5-1	Bradi1g25440.1	55160	14	45	1604	Beta-amylase [Triticum aestivum]	0.0	Polysaccharide catabolic process
5-2	Bradi1g09690.1	31817	4	16	255	endotransglucosylase/hydrolase XTH1 [Triticum aestivum]	8 e-119	Cellular glucan metabolic process
	Bradi1g25440.2	50006	4	15	192	Beta-amylase[Triticum aestivum]	0.0	Polysaccharide catabolic process
	Bradi2g17290.1	59201	2	4	118	ATP synthase subunit beta, mitochondrial precursor [Zea mays]	0.0	ATP synthesis; Hydrogen ion transport; Ion Transport; Transport
	Bradi2g37480.1	23630	2	11	62	Dehydroascorbate reductase [Triticum aestivum].	2 e-109	Oxidation reduction
	Bradi2g33320.1	31962	2	14	177	Putative acid phosphatase [Hordeum vulgare subsp. vulgare]	9 e-107	abscisic acid mediated signaling pathway; protein amino acid dephosphorylation
7-1	Bradi1g25440.1	50007	2	5	120	Beta-amylase [Triticum aestivum]	0.0	polysaccharide catabolic process
9-1	Bradi1g25440.1	50007	3	8	165	Beta-amylase [Triticum aestivum]	0.0	polysaccharide catabolic process
9-2	Bradi1g25440.1	50007	6	13	1050	Beta-amylase [Triticum aestivum]	0.0	polysaccharide catabolic process
9-3	Bradi1g25440.1	50007	5	13	342	Beta-amylase [Triticum aestivum]	0.0	polysaccharide catabolic process
	Bradi1g68080.3	48964	2	6	95	Enolase [Zea mays]	0.0	
9-4	Bradi1g09690.1	31817	2	10	153	Endotransglucosylase/hydrolase XTH1 [Triticum aestivum]	8e-119	cellular glucan metabolic process
13-1	Bradi1g09690.1	31817	2	7	60	Endotransglucosylase/hydrolase XTH1 [Triticum aestivum]	8 e-119	Cellular glucan metabolic process
15-1	Bradi2g38060.1	55113	15	34	717	11S globulin [Avena sativa]	0.0	Nutrient reservoir activity
	Bradi1g25440.1	55160	15	41	951	Beta-amylase[Triticum aestivum]	0.0	polysaccharide catabolic process
	Bradi1g05910.1	56331	7	21	390	Globulin 2 [Zea mays]	9 e-99	Nutrient reservoir activity
	Bradi2g20990.1	51250	6	13	243	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Brachypodium distachyon].	0.0	Calvin cycle ; Carbon dioxide fixation ; Photorespiration; Photosynthesis
	Bradi2g13080.1	85082	6	8	283	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Brachypodium distachyon].	0.0	Calvin cycle ; Carbon dioxide fixation ; Photorespiration; Photosynthesis
	Bradi3g27330.1	75538	6	10	264	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Brachypodium distachyon].	0.0	Calvin cycle ; Carbon dioxide fixation ; Photorespiration; Photosynthesis
	Bradi1g13040.1	64973	5	10	268	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Brachypodium distachyon].	1 e-107	Calvin cycle ; Carbon dioxide fixation ; Photorespiration; Photosynthesis
	Bradi4g37350.1	52115	5	16	272	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Brachypodium distachyon].	0.0	Calvin cycle ; Carbon dioxide fixation ; Photorespiration; Photosynthesis

	Bradi4g19460.1	51499	4	11	232	Ribulose biphosphate carboxylase large chain [stellaria media]	0.0	Nutrient reservoir activity
	Bradi2g17290.1	59201	3	7	149	Globulin 3 [Triticum aestivum].	0.0	metabolic process
	Bradi4g28220.1	54630	3	9	82	UDP-glucose pyrophosphorylase [Bambusa oldhamii].	8 e-119	ATP synthesis; Hydrogen ion transport; Ion Transport; Transport
	Bradi1g09690.1	31817	3	15	141	Adenosylhomocysteinase [Zea mays]	0.0	Nutrient reservoir activity
	Bradi3g22550.2	48319	2	5	52	ATP synthase subunit beta, mitochondrial precursor [Zea mays].	3 e-174	Cellular glucan metabolic process
	Bradi1g02940.3	50072	1	4	112	Seed storage globulin. [Avena sativa]		Glycolysis
						Endotransglucosylase/hydrolase XTH1 [Triticum aestivum]		Protein folding
						Enolase [Oryza sativa (japonica cultivar-group)].		
						Calreticulin2 [Zea mays].		
15-2	Bradi2g38060.1	55113	18	49	1760	11S globulin [Avena sativa]	0.0	Nutrient reservoir activity
	Bradi2g40840.1	55679	10	23	978	11S globulin [Avena sativa]	0.0	Nutrient reservoir activity
	Bradi4g28220.1	54630	8	20	348	Seed storage globulin [Avena sativa]	0.0	Nutrient reservoir activity
	Bradi4g29130.1	37213	6	16	273	GluB-5 short variant [Oryza sativa Japonica Group]	7 e-91	Nutrient reservoir activity
	Bradi2g13080.1	85082	6	6	245	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Brachypodium distachyon].	1 e-107	Calvin cycle ; Carbon dioxide fixation ; Photorespiration; Photosynthesis
	Bradi1g13040.1	64973	4	11	208	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Brachypodium distachyon].	4 e-173	Nutrient reservoir activity
	Bradi3g27270.1	46097	3	10	160	Globulin 3 [Triticum aestivum].	0.0	Calvin cycle ; Carbon dioxide fixation ; Photorespiration; Photosynthesis
	Bradi2g20990.1	51250	3	8	167	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Brachypodium distachyon].	8 e-119	Calvin cycle ; Carbon dioxide fixation ; Photorespiration; Photosynthesis
	Bradi1g25440.2	50007	3	9	135	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Brachypodium distachyon].	9 e-99	Calvin cycle ; Carbon dioxide fixation ; Photorespiration; Photosynthesis
	Bradi1g09690.1	31817	3	14	148	Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit [Brachypodium distachyon].	0.0	polysaccharide catabolic process
	Bradi1g05910.1	56332	2	7	95	Beta-amylase [Triticum aestivum]		Cellular glucan metabolic process
	Bradi1g60320.3	92990	2	2		Endotransglucosylase/hydrolase XTH1 [Triticum aestivum]		Nutrient reservoir activity
						Globulin-like protein [Oryza sativa Japonica Group].		biosynthetic process; sucrose metabolic process
						Sucrose synthase [Bambusa oldhamii]		
15-3	Bradi2g38060.1	55113	9	29	674	11S globulin [Avena sativa]	0.0	Nutrient reservoir activity
	Bradi1g13040.1	64973	9	22	515	Globulin 3 [Triticum aestivum]	1 e-107	Nutrient reservoir activity
	Bradi2g02140.1	39 841	7	31	352	Xylanase inhibitor 725OS [Triticum aestivum]	3 e-44	Xylan degradation
	Bradi3g05220.1	42206	7	29	486		0.0	Glycolysis

	Bradi3g14040.1	36623	5	29	545	Phosphoglycerate kinase, cytosolic.	4 e-179	Glycolysis
	Bradi2g50500.1	50459	3	8	173	Glyceraldehyde-3-phosphate dehydrogenase [Hordeum vulgare] Aspartate aminotransferase [Oryza sativa]	0.0	2-oxoglutarate metabolic process Aspartate metabolic process Biosynthetic process Glutamate metabolic process
15-4	Bradi1g09690.1	31817	11	45	1692	Endotransglucosylase/hydrolase XTH1 [Triticum aestivum]	8 e-119	Cellular glucan metabolic process
	Bradi2g38060.1	55113	7	20	493	11S globulin [Avena sativa]	0.0	Nutrient reservoir activity
	Bradi2g09600.1	37736	2	6	83	Peroxidase 1 [Triticum aestivum].	3 e-138	Hydrogen peroxide catabolic process ; oxidation reduction
	Bradi2g39940.1	21332	2	10	53	Prolamine [Brachypodium distachyon].	1e-15	Nutrient reservoir activity
15-5	Bradi2g38060.1	55113	12	32	1486	11S globulin [Avena sativa]	0.0	Nutrient reservoir activity
	Bradi1g09690.1	31817	5	24	452	Endotransglucosylase/hydrolase XTH1 [Triticum aestivum]	8 e-119	Cellular glucan metabolic process
	Bradi2g40840.1	55679	5	12	288	12s globulin [Avena sativa].	0.0	Nutrient reservoir activity
	Bradi4g29130.1	37213	4	12	282	GluB-5 short variant [Oryza sativa Japonica Group].	7 e-91	Nutrient reservoir activity
	Bradi4g28220.1	54630	2	6	137	Seed storage globulin [Avena sativa]	0.0	Nutrient reservoir activity
15-6	Bradi2g38060.1	55113	1	4	180	11S globulin [Avena sativa]	0.0	Nutrient reservoir activity
19-1	Bradi1g13040	64973	11	23	3396	Globulin 3 [Triticum aestivum]	1e-107	Nutrient reservoir activity
	Bradi2g38060.1	55113	7	26	759	11S globulin [Avena sativa]	0.0	Nutrient reservoir activity
	Bradi1g05910.1	56332	3	8	174	Globulin-2 [Zea mays]	5e-97	Nutrient reservoir activity
	Bradi4g29130.1	37213	3	12	280	GluB-5 short variant [Oryza sativa Japonica Group].	7e-91	Nutrient reservoir activity
19-2	Bradi2g38060.1	55113	11	33	1056	11S globulin [Avena sativa]	0.0	Nutrient reservoir activity
	Bradi1g05910.1	56332	8	25	864	Globulin-2 [Zea mays] Globulin 3 [Triticum aestivum]	5e-97	Nutrient reservoir activity
	Bradi1g13040.1	64973	7	17	17	Seed storage globulin [Avena sativa]	1e-107	Nutrient reservoir activity
	Bradi4g28220.1	54630	5	22	364	Xylanase inhibitor 725OS [Triticum aestivum]	2e-175	Nutrient reservoir activity
	Bradi2g02140.1	40214	4	17	355	GluB-5 short variant [Oryza sativa Japonica Group].	3e-44	Xylan degradation
	Bradi4g29130.1	37213	3	12	379		7e-91	Nutrient reservoir activity
19-3	Bradi2g38060.1	55113	11	32	3305	11S globulin [Avena sativa]	0.0	Nutrient reservoir activity
	Bradi4g29130.1	37213	3	12	1041	GluB-5 short variant [Oryza sativa Japonica Group].	7e-91	Nutrient reservoir activity
	Bradi1g13040	64973	2	6	208	Globulin 3 [Triticum aestivum]	1e-107	Nutrient reservoir activity
	Bradi4g28220.1	54630	2	5	155		2e-175	Nutrient reservoir activity

						Seed storage globulin [Avena sativa]		
19-4	Bradi2g38060.1	55113	9	25	4735	11S globulin [Avena sativa]	0.0	Nutrient reservoir activity
	Bradi4g29130.1	37213	3	10	1279	GluB-5 short variant [Oryza sativa Japonica Group].	7e-91	Nutrient reservoir activity
	Bradi1g50200.1	18870	3	25	171	Avenin-like b [Triticum aestivum].	1e-17	Nutrient reservoir activity
	Bradi4g28220.1	54630	3	7	92	Seed storage globulin [Avena sativa]	2e-175	Nutrient reservoir activity
19-5	Bradi2g38060.1	55113	8	24	2644	11S globulin [Avena sativa]	0.0	Nutrient reservoir activity
	Bradi2g39940.1	21322	3	16	371	Prolamine [Brachypodium distachyon]	1e-15	Nutrient reservoir activity
	Bradi4g29130.1	37213	2	3	264	GluB-5 short variant [Oryza sativa Japonica Group].	1e-107	Nutrient reservoir activity
	Bradi1g13040.1	64973	3	8	654	Globulin 3 [Triticum aestivum]	0.0	Nutrient reservoir activity
	Bradi4g28220.1	54630	2	5		Seed storage globulin [Avena sativa]	2e-175	Nutrient reservoir activity
23-1	Bradi2g38060.1	55113	10	29	680	11S globulin [Avena sativa]	0.0	Nutrient reservoir activity
	Bradi2g02140.1	40214	8	29	2530	Xylanase inhibitor 725OS [Triticum aestivum]	3e-44	Xylan degradation
	Bradi1g13040	64973	9	20	1726	Globulin 3 [Triticum aestivum]	1e-107	Nutrient reservoir activity
	Bradi4g29130.1	37213	2	8	169	GluB-5 short variant [Oryza sativa Japonica Group].	7e-91	Nutrient reservoir activity
23-2	Bradi2g38060.1	55113	11	27	4009	11S globulin [Avena sativa]	0.0	Nutrient reservoir activity
	Bradi2g40840.1	55679	4	10	1349	11S globulin [Avena sativa]	0.0	Nutrient reservoir activity
	Bradi2g02140.1	40214	3	13	194	Xylanase inhibitor 725OS [Triticum aestivum]	3e-44	Xylan degradation
	Bradi4g29130.1	37212	3	10	956	GluB-5 short variant [Oryza sativa Japonica Group]	7e-91	Nutrient reservoir activity
	Bradi1g13040.1	64973	2	6	156	Globulin 3 [Triticum aestivum]	1e-107	Nutrient reservoir activity
23-3	Bradi2g38060.1	55113	13	34	1968	11S globulin [Avena sativa]	0.0	Nutrient reservoir activity
	Bradi2g40840.1	55679	8	26	954	11S globulin [Avena sativa]	0.0	Nutrient reservoir activity
	Bradi2g02140.1	40214	3	16	248	Xylanase inhibitor 725OS [Triticum aestivum]	3e-44	Xylan degradation
	Bradi4g29130.1	37213	10	10	670	GluB-5 short variant [Oryza sativa Japonica Group]	7e-91	Nutrient reservoir activity
	Bradi1g13040.1	64973	3	8	142	Globulin 3 [Triticum aestivum]	1e-107	Nutrient reservoir activity
	Bradi4g28220.1	54630	3	9	172	Seed storage globulin [Avena sativa]	2e-175	Nutrient reservoir activity
	Bradi1g29880.1	27692	3	18	202	Endochitinase 1 Hordeum vulgare].	2e-132	Carbohydrate metabolism
	Bradi1g09690.1	31817	2	11	270	Endotransglucosylase/hydrolase XTH1 [Triticum aestivum]	8e-119	Chitin degradation; Plant defense; Polysaccharide degradation
								Cellular glucan metabolic process

23-4	Bradi2g38060.1	55113	12	33	1790	11S globulin [Avena sativa]	0.0	Nutrient reservoir activity
	Bradi4g28220.1	54630	6	15	962	Seed storage globulin [Avena sativa]	2e-175	Nutrient reservoir activity
	Bradi4g29130.1	37213	4	14	261	GluB-5 short variant [Oryza sativa Japonica Group].	7e-91	Nutrient reservoir activity
	Bradi2g02140.1	40214	3	12	233	Xylanase inhibitor 725OS [Triticum aestivum]	3e-44	Xylan degradation
23-5	Bradi2g38060.1	55113	9	22	797	11S globulin [Avena sativa]	0.0	Nutrient reservoir activity
	Bradi1g13040.1	64973	6	17	205	Globulin 3 [Triticum aestivum]	1e-107	Nutrient reservoir activity
	Bradi4g28220.1	54630	4	11	286	Seed storage globulin [Avena sativa]	2e-175	Nutrient reservoir activity
	Bradi2g02140.1	40214	2	9	120	Xylanase inhibitor 725OS [Triticum aestivum]	3e-44	Xylan degradation
23-6	Bradi2g38060.1	55113	7	18	3608	11S globulin [Avena sativa]	0.0	Nutrient reservoir activity
	Bradi4g29130.1	37213	3	10	1704	GluB-5 short variant [Oryza sativa Japonica Group].	7e-91	Nutrient reservoir activity
	Bradi4g28220.1	54630	4	13	903	Seed storage globulin [Avena sativa]	2e-175	Nutrient reservoir activity
	Bradi2g39940.1	21322	1	5	45	Prolamine [Brachypodium distachyon]	1e-15	Nutrient reservoir activity

Table S2: Fatty acid composition and content of *B. distachyon* grain.

Top Fatty acid composition and content of *B. distachyon* grain. Lipids from manually dehulled grains were derivatised using 2% H₂SO₄ in methanol, and the fatty acid methyl esters were analysed by gas chromatography – mass spectrography, as described under "Materials and Methods". Data are mol% ± S.E. (n=4).

Bottom Fatty acid composition of total lipids from various cereals.

References: 1: E. Ucciani TEC&DOC, Lavoisier , ISBN 2-7430-0009-0

2 : Dubois et al (2008) OCL vol 15 N°1

3 : Banas, A.,et al. (2007).J Exp Bot 58, 2463-2470.

A

Grain Weight (mg)	C12:0	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1 (mol%)	C18:2	C18:3	C20:0	C20:1	C22:0	C24:0	C26:0	Total fatty acids		
															µg/grain	µg/mgDW	nmol/grain
4.93 ± 0.23	2.13 ±0.7	2.78 ±0.12	0.18 ±0.01	15.92 ±0.12	0.32 ±0.01	0.65 ±0.02	35.71 ±0.49	36.97 ±0.32	3.86 ±0.04	0.16 ±0.01	0.61 ±0.01	0.26 ±0.01	0.24 ±0.01	0.21 ±0.01	80.89 ±3.84	16.42 ±0.13	280.53 ±13.29

B

	C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C22:0	% oil/grain	Ref.
<i>Avena fatua</i>		0.6	23.4	3.3	40.9	29.5	0.9	0.8			1.4	1
<i>Avena sativa</i>			18.0	1.7	39.8	37.7	1.3					3
<i>Oryza sativa</i>		0.4		1.9 - 2.6	31.9 - 37.9	31.9 - 43.6	1.0 - 3.0	0 - 0.6			16.0	1
<i>Triticum sativum</i>		10.5	16.5		27.5	41.9	2.4				10.7	1
<i>Zea mais</i>				2.3 - 2.7	28.4 - 36.9	45.9 - 55.5	0 - 0.9	0 - 0.5	0 - 0.3		17 - 37.8	1
<i>Triticum aestivum (germ)</i>			17.5	1,0	19.1	55.2	6.1	0.2	1.6			2

(1) E. Ucciani, TEC&DOC, Lavoisier, Paris, ISBN 2-7430-0009-0

(2) Dubois et al., OCL, vol 15 N°A, 2008

(3) Banas et al., J. Exp. Bot., 58, pp. 2463-2470, 2007

Supplementary Figures

Figure S1: Multiplanar reconstructions of the embryo

A- Sections at 2 (a), 11 (b), 13 (c) and 16 (d) DAF respectively. e-f: 3D reconstruction of embryo at 11 and 14 DAF respectively. g-h : highlights epidermis differentiation (green), coleorhiza (orange) and root (blue).

B- Three dimensional multiplanar reconstruction (3D MPR) of a 30 DAF embryo within the seed coat. a frontal, b transversal, c sagittal.

Figure S2: The *Brachypodium* embryo at 28 DAF. Longitudinal section.

c: coleorhiza, rc: root cap, r: radicle, am: apical meristem, co: coleoptile, c: cotyledon, fl: leaf primordium , s: scutellum

Figure S3: Bright field micrograph of cross-sections from a *B. distachyon* grain at 22 DAF

Ac : aleurone cell, ne : nucellus epidermis, sc : seed coat, se : storage endosperm, va : vascular tissue

Figure S4: Synchrotron UV microspectroscopy (wavelength excitation 275 nm)

A- *B. distachyon* grain cross-section at 17 DAF, visible image (a), fluorescence (290-360nm) image (b), merged image obtained by superposition of the visible and fluorescence images (c). Scale bar: 5 microns

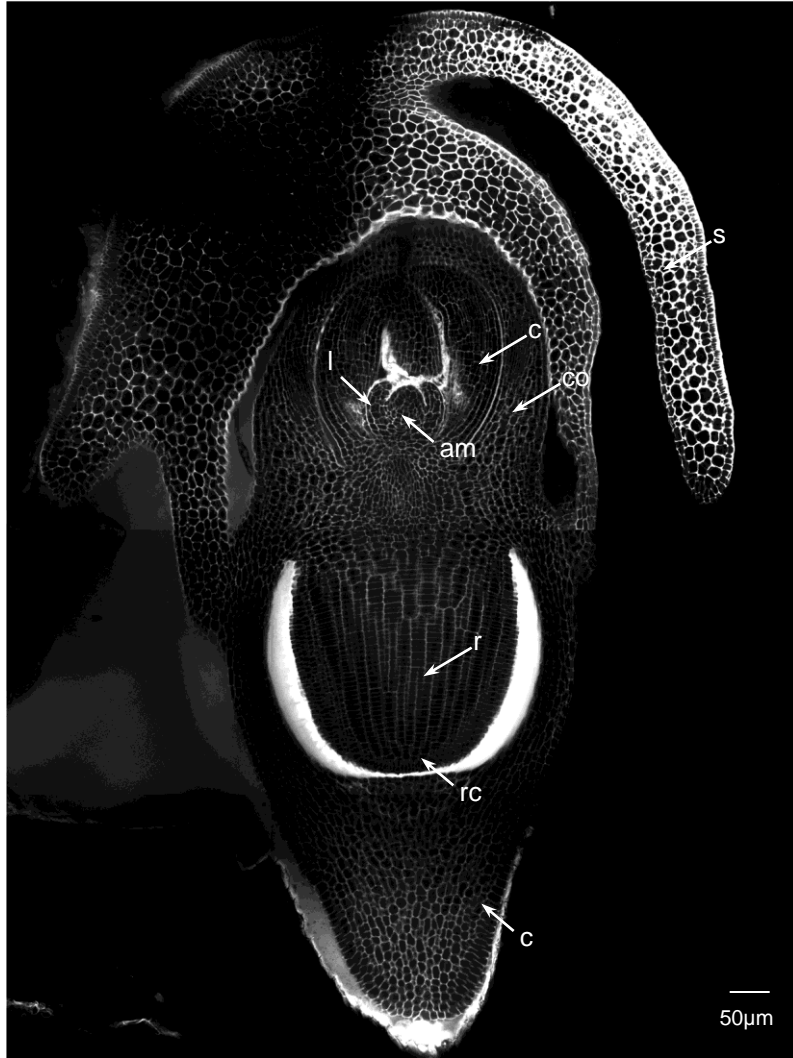
B- Mature grain cross-sections, visible image (a), fluorescence (290-360nm) image (b), merged image(c). Scale bar: 4 microns

C- Fluorescence emission spectrum associated with the pixel +A in the fluorescence image of mature grain cross-sections and spectra of isolated components obtained as Gaussian functions represented with their emission maxima (a) tryrosine, (b) tryptophan (c) esterified ferulic acid.

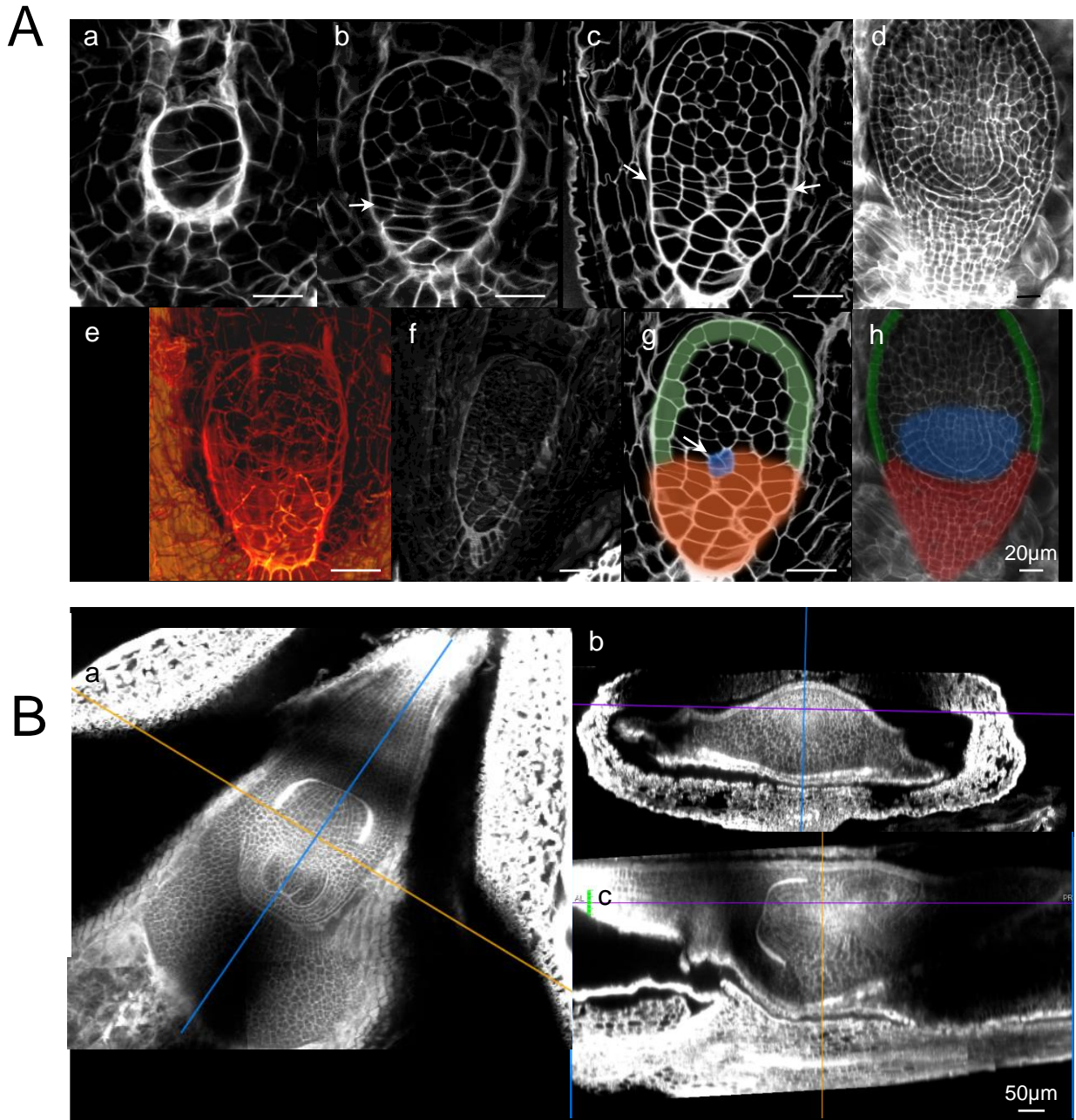
Figure S5: DIC (left panel) and immunofluorescence (right panel) micrographs of grain cross-sections from diverse cereals. Sections were labelled with anti-(1-3)(1-4)- β -glucan.

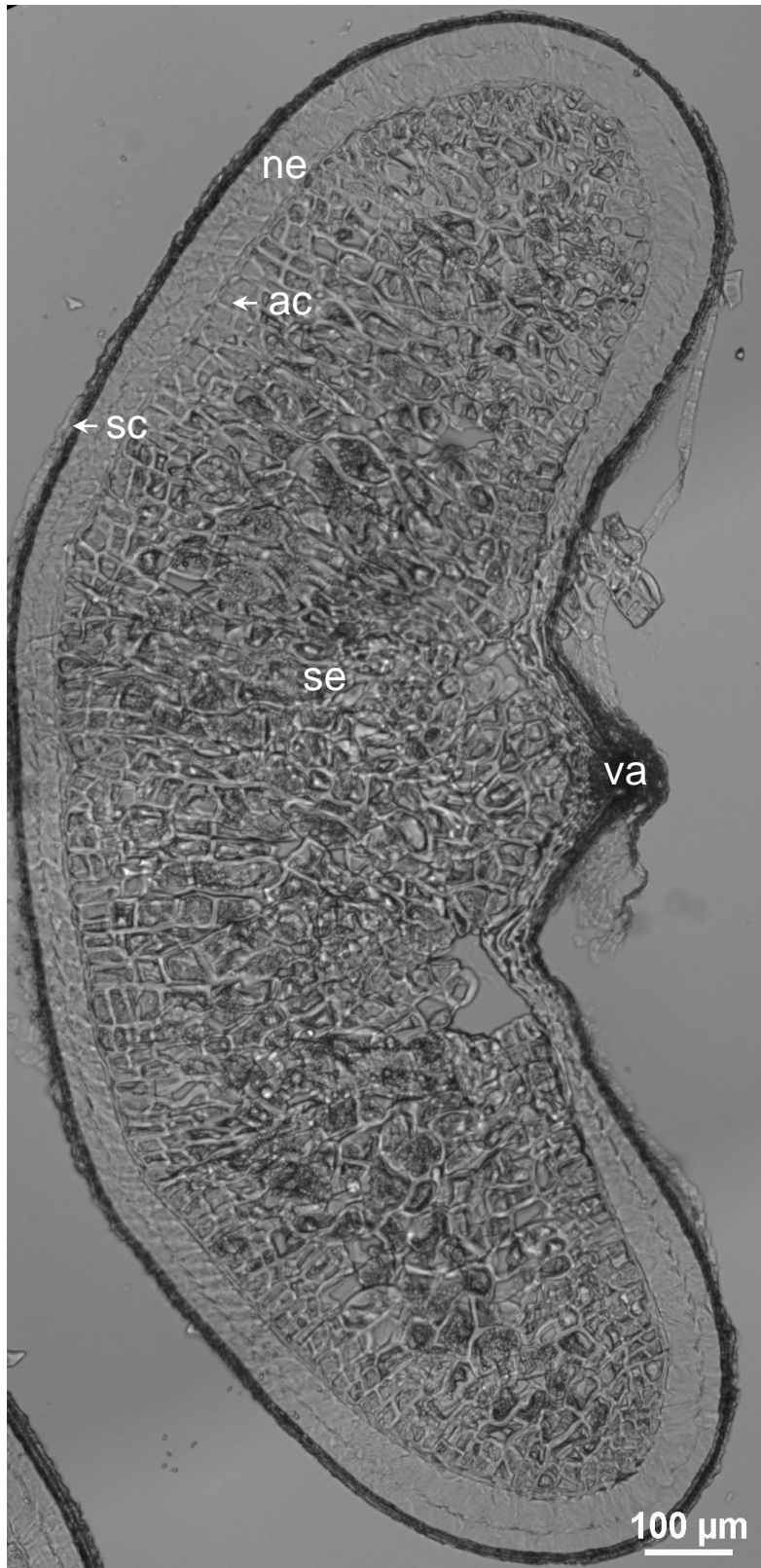
al aleurone layer, ne nucellus epidermis

Are provided as supplementary data laser scanning microscopy files of 7 stages of embryo development. The files contain full Z stacks through the grain for additional 3D reconstructions or additional observations

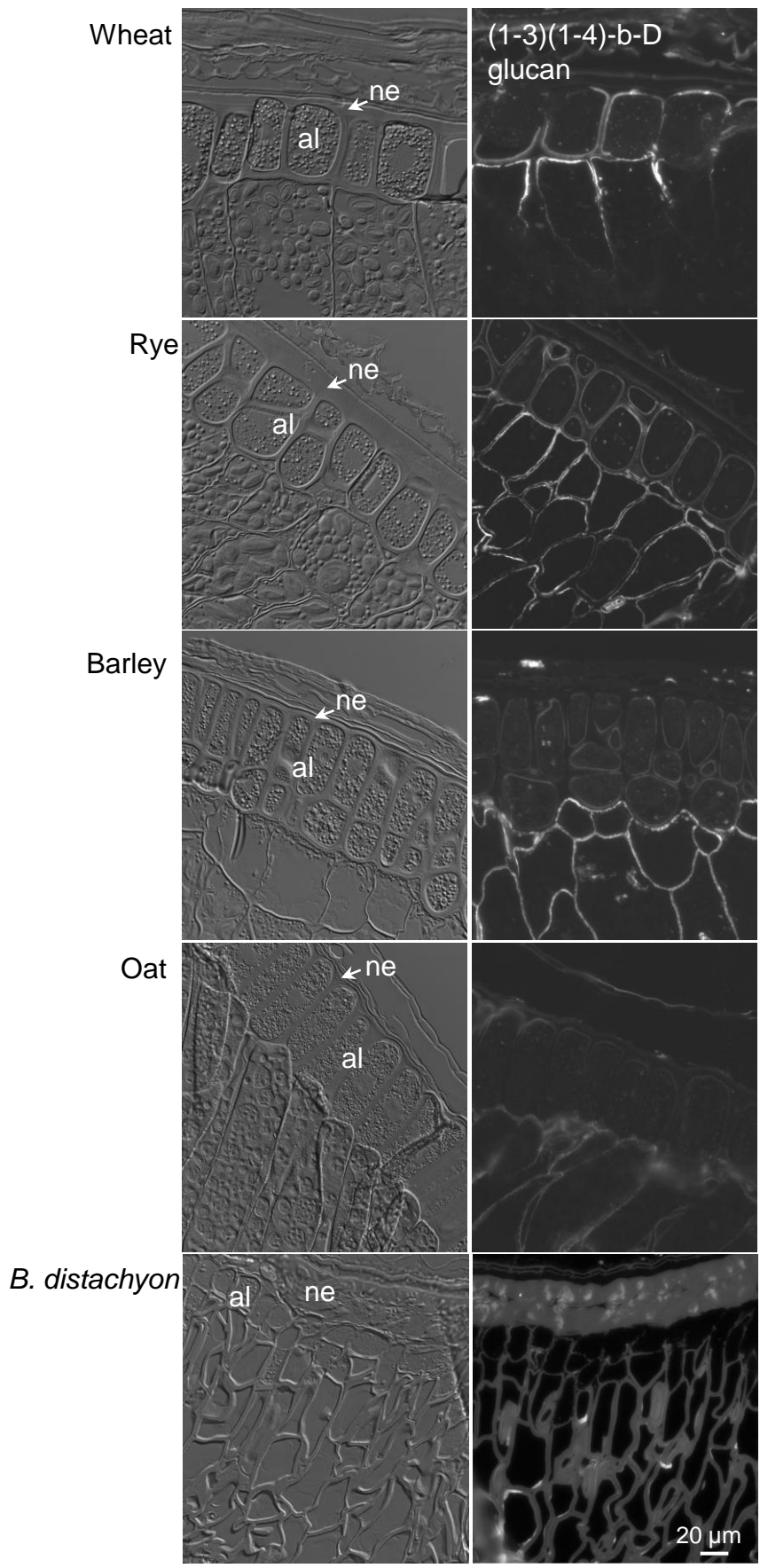


Supplementary figure S1:





Supplementary figure S3:



Supplementary figure S4:

