

1126

1127

1128

1129

1130 **Tables**

1131 **Supplemental Table S1.** CESA expression in seed coat cells during seed development
1132 (Le et al., 2010).

	Pre-globular	Globular (~3 DPA)	Heart	Linear embryo (~6 DPA)	Mature green (~10 DPA)
<i>CESA1</i>	268 (20)	260 (20)	201 (52)	275 (35)	294 (48)
<i>CESA2</i>	543 (85)	611 (91)	1024 (38)	435 (86)	192 (109.48)
<i>CESA3</i>	811 (101)	677 (12)	1094 (58)	1765 (185)*	1115 (192)
<i>CESA4</i>	8 (7.31)	2 (0.47)	8.21 (7.22)	5 (4.49)	34 (19.84)
<i>CESA5</i>	101 (20)	74 (16)	51 (1)	362 (123)	239 (52)
<i>CESA6</i>	168 (86)	80 (33)	140 (58)	123 (24)	121 (12)
<i>CESA7</i>	5 (1.77)	13 (0.97)	1 (0.31)	17 (8.1)	22 (9.53)
<i>CESA8</i>	1 (0.41)	21 (10)	33 (16.69)	80 (27.23)	62 (5.67)
<i>CESA9</i>	12 (1.52)	15 (1.55)	10 (7.65)	26 (6.83)	45 (0.06)
<i>CESA10</i>	62 (7.15)	59 (21.19)	303 (53.98)	887 (376.26)	420 (2.89)
<i>MUM4</i>	547.9 (131)	787 (196.81)	1018.76(33.19)	7764 (503)	7937 (610)

1133 Values in parenthesis indicate standard deviation. Values in bold indicate the highest
1134 expression level of each *CESA* throughout seed development. Asterisks indicate highest
1135 expression level of all *CESAs* in seeds. <http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi?dataSource=Seed>

1137

1138

1139

1140

1141

1142

1143

1144
 1145
 1146
 1147
 1148 **Supplemental Table S2.** *CESA* expression in the seed coat during development (Dean et
 1149 al., 2011).

Seed stage	3 DPA	7 DPA	11 DPA
Gene			
<i>CESA1</i>	380 (105.88)	620.78 (256.58)	467.66 (175.3)
<i>CESA2</i>	5321.52 (7049.24)	1440.8 (846.67)	2620.3 (1453.59)
<i>CESA3</i>	9692.38 (6893.56)	13475.48 (9182.12)*	9165.4 (4859.99)
<i>CESA4</i>	151.76 (114.61)	127.24 (49.46)	122.34 (54.39)
<i>CESA5</i>	149.13 (92.44)	159.94 (45.2)	165.48 (37.39)
<i>CESA6</i>	10656.71 (4871.29)	7786.39 (4196.9)	12414.76 (7440.4)
<i>CESA7</i>	622.9 (1148.5)	142.11 (30.75)	163.67 (46.87)
<i>CESA8</i>	203.64 (76.79)	293.03 (65.99)	216.49 (52.6)
<i>CESA9</i>	155.9 (29.18)	152.34 (41.39)	184.3 (93.18)
<i>CESA10</i>	1021.04 (719.53)	2207.19 (3105.38)	416.8 (203.13)
<i>MUM4</i>	380 (105.88)	620.78 (256.58)	209.31 (89.37)

1150 Values in parenthesis indicate standard deviation. Values in bold indicate the highest
 1151 expression level of each *CESA* throughout seed development. Asterisks indicate highest
 1152 expression level of all *CESAs* in seeds. http://bar.utoronto.ca/efp_seedcoat/cgi-bin/efpWeb.cgi
 1153

Supplemental Table S3. CESA expression in whole seeds during development (Winter et al., 2007).

Embryo stage		Globular	Heart	Torpedo	Bent cotyledon			Mature green
Seed stage	Seed stage 3	Seed stage 4	Seed stage 5	Seed stage 6	Seed stage 7	Seed stage 8	Seed stage 9	Seed stage 10
Silique	+	+	+	-	-	-	-	-
Gene								
<i>CESA1</i>	348 (10.79)	359 (26.4)	385 (57.16)	377 (11.58)	410 (19.78)	321 (15.7)	295 (13.53)	256 (11.47)
<i>CESA2</i>	327 (7.43)	573 (18.27)	507 (3.58)	561 (3.15)	605 (14.84)	379 (23.14)	294 (5.31)	246 (16.25)
<i>CESA3</i>	912 (29.81)	1222 (65.07)	1197 (26.17)*	987 (29.18)	930 (16.47)	798 (11.64)	630 (25.65)	624 (24.53)
<i>CESA4</i>	98 (19.69)	1141 (22.18)	664 (30.6)	23 (3.35)	27 (2.51)	12 (7.22)	21 (7.61)	30 (2.17)
<i>CESA5</i>	195 (5.18)	385 (3.87)	352 (3.54)	269 (19.43)	292 (9.41)	132 (9.55)	120 (4.57)	99 (4.59)
<i>CESA6</i>	460 (32.77)	429 (9.91)	439 (6.43)	483 (11.76)	505 (33.26)	416 (3.34)	326 (18.24)	316 (23.76)
<i>CESA7</i>	41 (4.87)	489 (53.28)	224 (7.06)	19 (0.53)	24 (0.73)	21 (5.85)	31 (3.63)	38 (2.03)
<i>CESA8</i>	81 (5.62)	669 (46.14)	328 (7.12)	4 (0.8)	2 (1.46)	2 (0.53)	1 (0.37)	2 (0.45)
<i>CESA9</i>	22 (4.3)	40 (5.08)	56 (1.64)	135 (11.66)	125 (4.15)	85 (7.01)	65 (2.54)	61 (7.11)
<i>CESA10</i>	94 (1.87)	1327 (92.07)	1058 (48.6)	547 (11.27)	490 (6.18)	151 (8.87)	119 (3.75)	98 (2.92)
<i>MUM4</i>	192 (10.81)	1097 (21.88)	1075 (21.88)	410 (18.43)	326 (5.57)	65 (7.01)	66 (3.04)	81 (16.55)

1155 Values in parenthesis indicate standard deviation. Values in bold indicate the highest expression level of each *CESA* throughout seed
1156 development. Asterisks indicate highest expression level of all *CESAs* in seeds. The “+” and “-“ indicate presence or absence,
1157 respectively, of siliques tissue used for RNA extraction. <http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi>

1158
 1159 **Supplemental Table S4.** List of the top 30 genes coexpressed with CESA5 in seeds
 1160 (http://bbc.botany.utoronto.ca/ntools/cgi-bin/ntools_expression_angler.cgi). The r-value
 1161 represents the Pearson's correlation coefficient of the expression pattern of CESA5 with
 1162 all other genes (Toufighi et al., 2005).

Locus	Gene	r-value
AT5G09870	<i>CESA5</i>	1
AT2G04780	<i>FLA7</i>	0.742
AT5G05170	<i>CESA3</i>	0.733
AT1G75680	<i>GH9B7</i>	0.713
AT3G11700	<i>FLA18</i>	0.709
AT3G25780	<i>AOC3</i>	0.696
AT2G25220	N/A	0.691
AT1G28600	N/A	0.681
AT5G49720	<i>GH9A1/KOR1</i>	0.676
AT1G33800	N/A	0.674
AT3G28340	<i>GATL10</i>	0.671
AT1G23200	N/A	0.669
AT4G30440	<i>GAE1</i>	0.668
AT1G28590	N/A	0.659
AT1G08280	N/A	0.656
AT5G45770	<i>RLP55</i>	0.656
AT5G50750	<i>RGP4</i>	0.652
AT5G15180	N/A	0.649
AT4G32410	<i>CESA1</i>	0.648
AT2G24550	N/A	0.642
AT5G04530	<i>KCS19</i>	0.641
AT3G54810	<i>BME3</i>	0.639
AT3G11600	N/A	0.638
AT2G23570	<i>MES19</i>	0.637
AT4G35060	<i>HIPP25</i>	0.637
AT2G24240	N/A	0.636
AT2G25450	N/A	0.636
AT2G25540	<i>CESA10</i>	0.633
AT5G06530	<i>ABCG22</i>	0.631
AT5G03170	<i>FLA11</i>	0.63

1163 *Genes in bold denote cellulose synthases

1164
 1165
 1166
 1167
 1168
 1169

1170
 1171
 1172
 1173
 1174 **Supplemental Table S5.** Pearson correlations and Manders coefficients for GFP-
 1175 CESA10 (M1) colocalization with RFP-VHA-1a (M2) in seed coat epidermal cells.

Replicate	Pearson Correlation	Manders coefficients	
1	0.503	M1	0.319
		M2	0.342
2	0.391	M1	0.101
		M2	0.608
3	0.5	M1	0.324
		M2	0.597
4	0.396	M1	0.283
		M2	0.699
5	0.404	M1	0.139
		M2	0.281
Average	0.4388	M1	0.2332
		M2	0.5054

1176

1177
 1178
 1179
 1180
 1181
 1182
 1183
 1184
 1185
 1186
 1187
 1188
 1189
 1190
 1191

1192

1193

1194

1195

1196

Supplemental Table S6. Genes, accession numbers, and mutant lines used in this study.

Gene	Accession	Mutant line	T-DNA line/TAIR accession	Reference
<i>CESA1</i>	AT4G32410	<i>any1-1</i>		Fujita et al., 2013
<i>CESA3</i>	AT5G05170	<i>ixr1-1</i>	CS6201	Scheible et al., 2001
		<i>ixr1-2</i>	CS6202	Scheible et al., 2001
<i>CESA5</i>	AT5G09870	<i>cesa5-1</i>	SALK_118491	Mendu et al., 2011
<i>CESA6</i>	AT5G64740	<i>prc1-1</i>	CS297	Fagard et al., 2000
<i>CESA10</i>	AT2G25540	<i>cesa10-1</i>	SALK_150533	
		<i>cesa10-2</i>	SALK_052533	
<i>BXL1</i>	AT5G49360	<i>bxl1-1</i>	CS16299	Arsovski et al., 2009
<i>FLY1</i>	AT4G28370	<i>fly1-1</i>	CS67936	Voiniciuc et al., 2013

1197

1198

1199

1200

1201

1202

1203

1204

1205

1206

1207

1208

1209

1210

1211

1212

1213

1214

1215

1216

1217

1218

1219

1220

1221

1222

1223

1224

1225

1226

1227

1228

1229

1230 **Supplemental Table S7:** Monosaccharide composition and whole seed acid insoluble
1231 cellulose amounts.

	Col	<i>cesa5-1</i>	<i>ixr1-1</i>	<i>ixr1-2</i>
Soluble mucilage				
Fucose	0.037 +/- 0.01	0.043 +/- 0.01	0.033 +/- 0.01	0.05 +/- 0.01
Arabinose	0.27 +/- 0.023	0.49 +/- 0.04*	0.38 +/- 0.02*	0.45 +/- 0.03*
Rhamnose	36.40 +/- 0.58	45.48 +/- 0.38*	35.31 +/- 0.25	30.53 +/- 0.69*
Galactose	2.02 +/- 0.152	1.57 +/- 0.12	1.70 +/- 0.06	1.62 +/- 0.174
Glucose	0.98 +/- 0.061	1.58 +/- 0.385	2.80 +/- 0.98	2.63 +/- 1.16
Xylose	2.77 +/- 0.15	3.46 +/- 0.05*	2.97 +/- 0.08	2.34 +/- 0.08
Galacturonic acid	44.08 +/- 7.42	50.33 +/- 1.15	36.92 +/- 0.54	31.19 +/- 0.92
Whole seed AIR				
Fucose	7.68 +/- 0.23	4.71 +/- 0.21*	8.26 +/- 0.34	7.41 +/- 0.11
Arabinose	236.91 +/- 4.25	230.91 +/- 3.80	257.34 +/- 7.34	245.76 +/- 4.03
Rhamnose	228.63 +/- 3.91	214.66 +/- 2.31*	216.24 +/- 3.95	226.85 +/- 5.04
Galactose	154.14 +/- 3.37	134.94 +/- 5.04*	153.03 +/- 5.58	165.45 +/- 3.97*
Glucose	197.65 +/- 23.29	102.47 +/- 26.74*	225.44 +/- 29.39	182.66 +/- 20.23
Xylose	78.54 +/- 5.60	64.78 +/- 3.43*	89.29 +/- 1.66	81.88 +/- 1.77
Galacturonic acid	323.13 +/- 10.13	300.81 +/- 10.11	286.34 +/- 11.81	315.87 +/- 10.49
Acid insoluble cellulose				
Cellulose	110.06 +/- 12.15	76.04 +/- 5.57*	81.76 +/- 5.17*	88.7 +/- 6.54

1232 Values are the mean +/- SE of four samples, and are expressed as nmol sugar/mg seed.
 1233 Cellulose amounts are expressed as mg cellulose/g seed. Results were similar for three
 1234 independent biological replicates. Asterisks indicate significantly different from wild type
 1235 based on a Student's t-test, p < 0.05.

1236

1237

1238

1239

1240

1241

1242

1243

1244 **Supplemental Table S8.** Sequences of primers used in this study.

1245

1246

Gene	Purpose	Primer name	Sequence (5'-3')
<i>CESA3</i>	<i>ixr1-1</i> identification	<i>ixr1-1</i> MseI F	CAATCATGGGGACCACTCTTA
		<i>ixr1-1</i> MseI R	GACTCGGCTAGTGAAGGGATCA
<i>CESA10</i>	GFP-CESA10 construction	CESA10-attB1	AAAAAGCAGGCTaATGGTTGCAGGATC TTACCGGAGAT
		CESA10-attB2	AGAAAGCTGGGTaTCACTCACCCATGA AACTGTTGCTCA
		HindIII_CESA10p F	TGTAAGCTTGAAATCATAAAGCAATCA AACCCCTAA
		XbaI_CESA10p R	ATATCTAGAGCCGGCTTGCTGCAA
<i>CESA10</i>	<i>cesa10-2</i> and RT-PCR	CESA10-1R	AAAGGAGCTTCCCCTGAGAGG
		CESA10-2F	CCAATTACATCCATCCCGCTCC
		CESA10-4F	CCTGAAGATGGTTGGACAATGG
		CESA10-3R	TGGCTTCCTTAATGCCCTGC
	<i>cesa10-2</i> genotyping	052533-LP	AACCCCTTCAAGTTGATCTCG
		052533-RP	TACCCAGTGGATAAAGTTGCG GCTGATCCTTAAAGGCCGG
<i>CESA5</i>	Genotyping	CESA5-1 LP	CCCGGATTGACCATCACAAG
		CESA5-1 RP	CCCCAGCTTGGGATGAATACTGGTGGT CGGCTCATCG
	N-Terminal cloning	NCESA5 HindIII F	CCGCTCGAGCGGCGGATTATTTGCT CGACTTATCG
		NCESA5 XhoI R	GAACTAAGTGGACAAACAGCTCAAATC TGTGGAGATGAG
	Site Directed Mutagenesis	CESA5C39	GGAAACCAATCTGTCTCAGGCCAAA ACTCGTTACAAGCGC
		CESA5C84	GGAGCTGTCGTATTCCAGTC AACCCCTCAAGACCCGTTA
	Phage sequencing	T7 F	GGCGGAATTCATGAACTGGTGGTCG GCTC
		T7 R	GCGCGGATCCGGATTATTTGCTCG AC
	Yeast two-hybrid	CESA5 pGADT7-F	GCCGGAAATTCACTGGAATCCGAAGGAG AAACC
		CESA5 pGADT7-R	GATCCACTACCTTGAGCCTCTT
		CESA3 pGBKT7-F	GTGGTGGTCG AC
		CESA3 pGBKT7-R	GTGGTGGTCG AC

1247

1248

1249

1250

1251
1252