CSLA2, a Glucomannan Synthase, is Involved in Maintaining Adherent Mucilage Structure in Arabidopsis Seed

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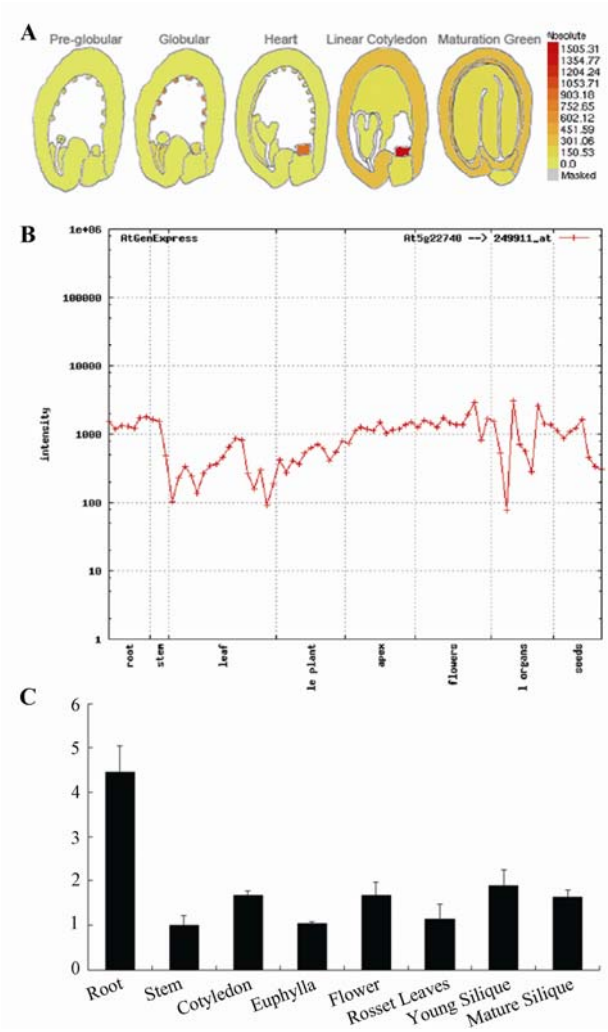
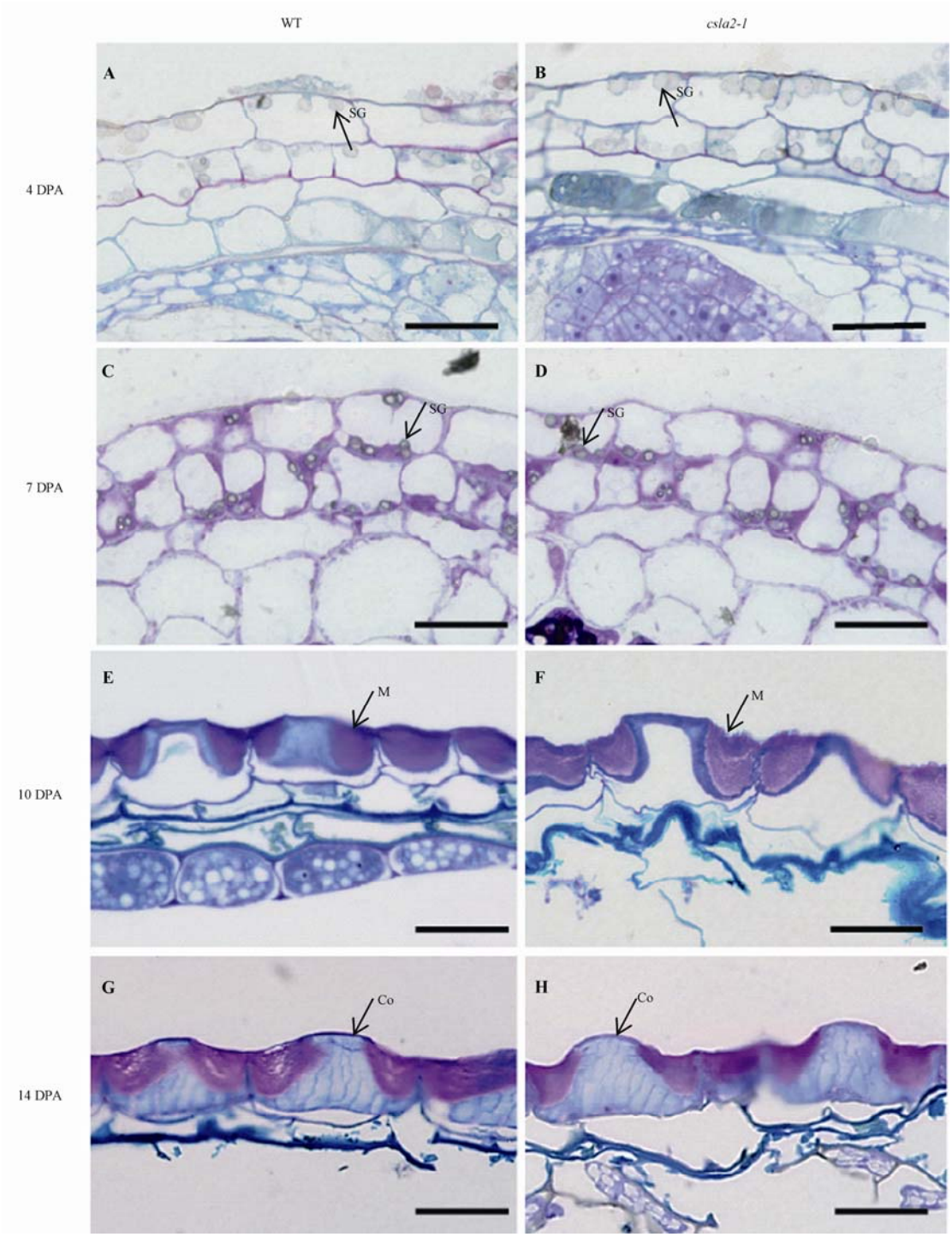
Supplemental Data



Supplemental Figure S1. Expression profile of *CSLA2*.

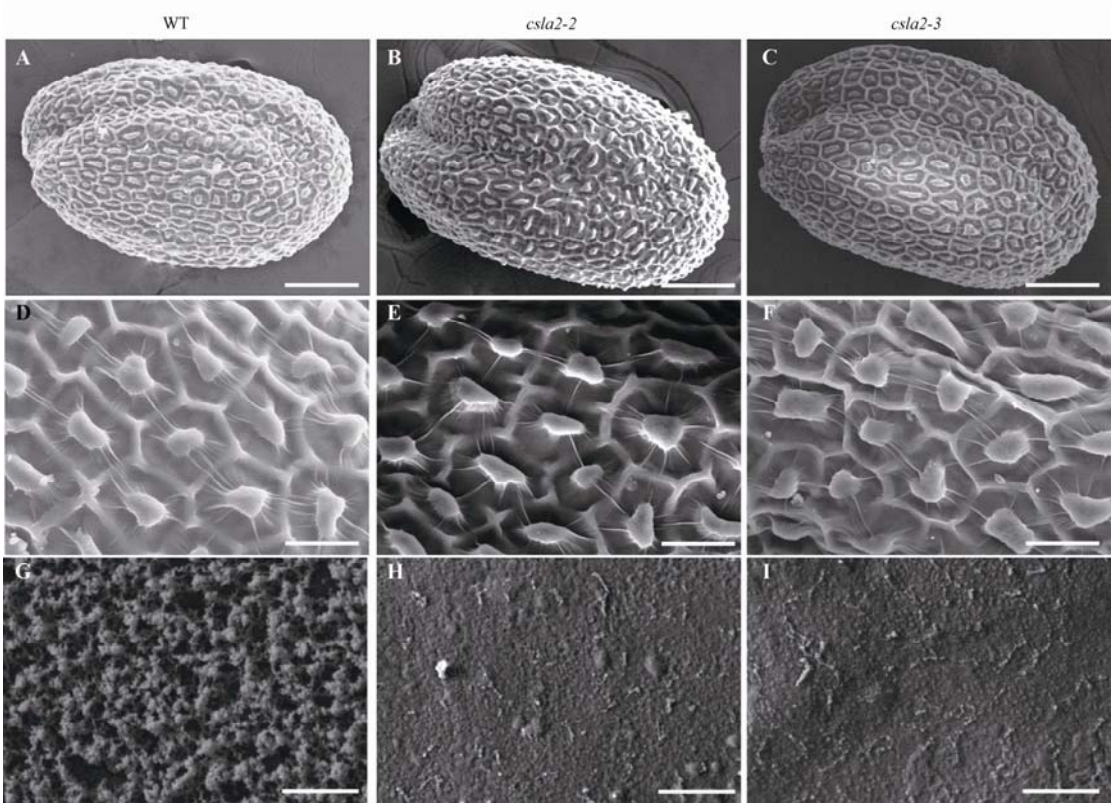
A, *CSLA2* transcript levels in the developing seed coat depicted by the BAR eFP browser (Winter et al., 2007) based on the dataset generated by Le et al. (2010) of gene expression profiling from laser-capture micro-dissected seed coat cells. B, Expression profile of *CSLA2* in different tissues and seed developmental stages. The data were generated by AtGenExpress (Development) and presented using the AtgenExpression Visualization Tool (Schmid et al., 2005, http://jsp.weigelworld.org/expviz/expviz.jsp). Mean signal intensities of *CSLA2* expression were detected in root, apex, and flowers in all stages. *CSLA2* was also expressed in leaf, whole plant, floral organs, and seeds in different intensities at different stages. C, Expression of *CSLA2* in different tissues determined by qRT-PCR.



Supplemental Figure S2. Sections of developing seeds stained with toluidine blue O.

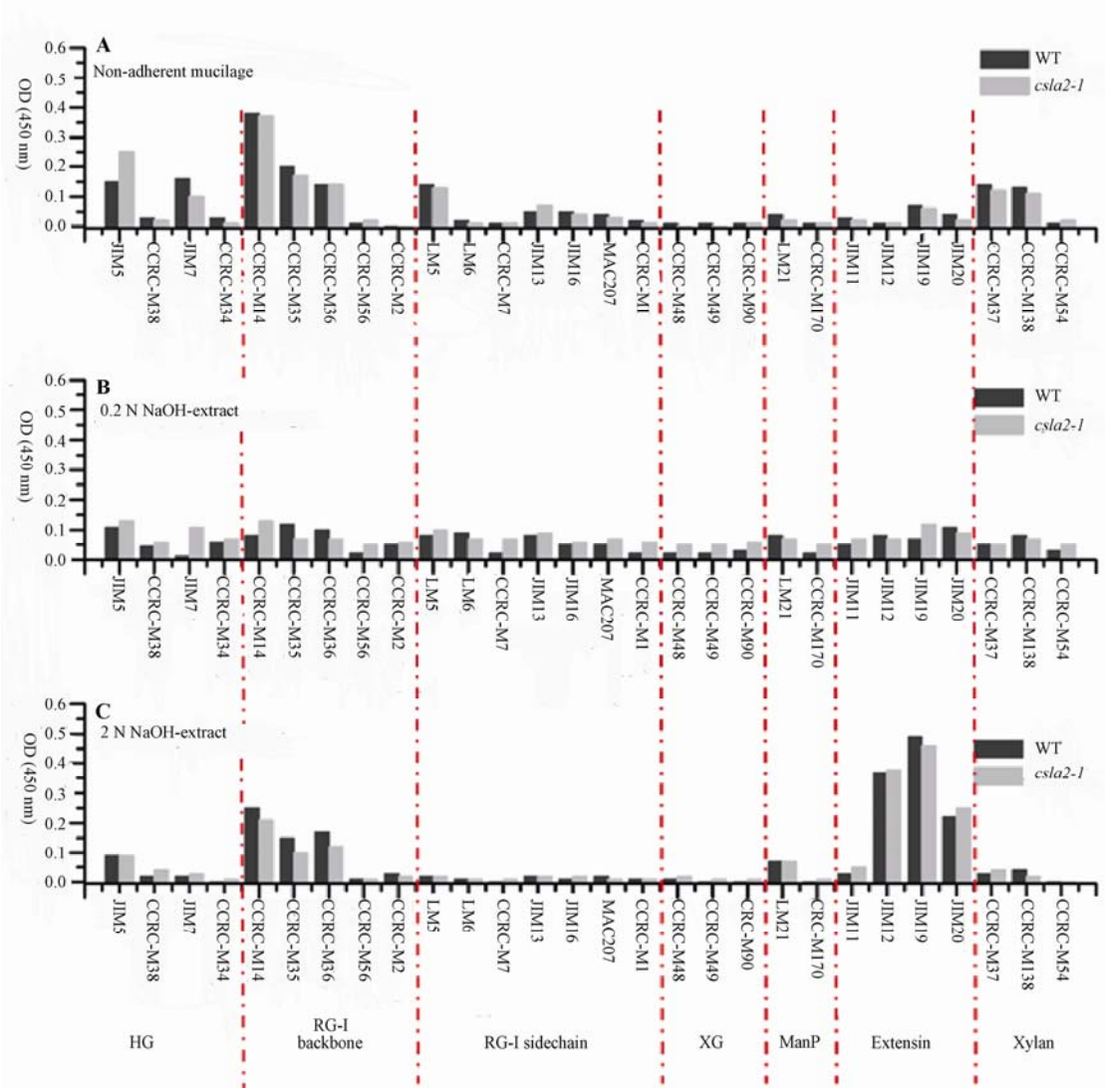
A and B show seeds with heart stage embryos at 4 DPA. C and D show seeds with linear cotyledon stage embryos at 7 DPA. E and F show seeds with bent cotyledon stage embryos at 10 DPA. G and H show seeds with mature embryos at 14 DPA. A, C, E, and

G show wild-type seeds. B, D, F, and H show *csla2-1* seeds. Co, Columella; M, mucilage; SG, starch granule. Bars = 20 μm.



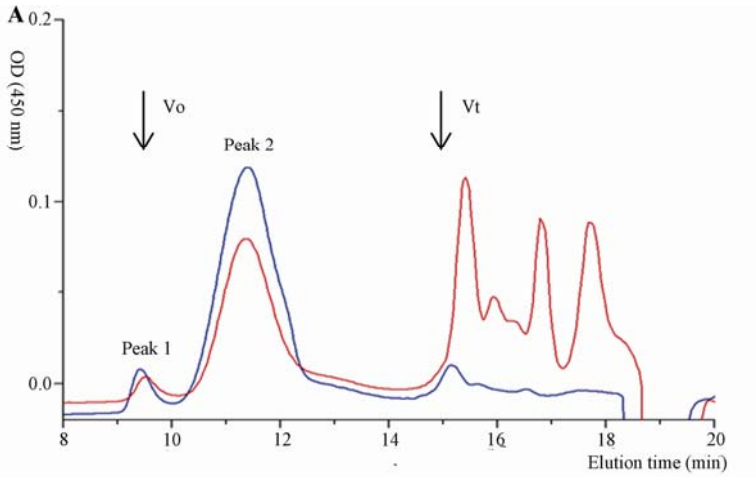
Supplemental Figure S3. Analysis of dry and hydrated seeds by scanning electron microscopy.

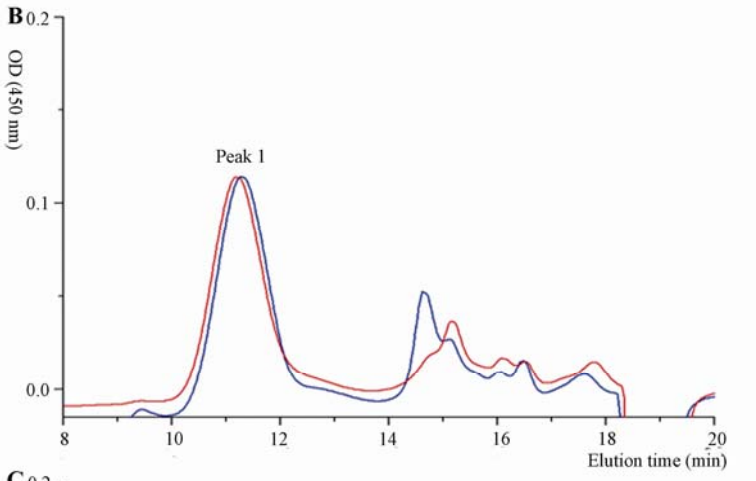
A to F, The surface morphology of dry mature Arabidopsis wild-type (A and D), *csla2-2* (B and E) and *csla2-3* (C and F) seeds viewed with scanning electron microscopy. G to I, Cryo-scanning electron microscopy of adherent mucilage extruded from dry mature Arabidopsis wild-type (G), *csla2-2* (H) and *csla2-3* (I) seeds hydrated in water. Bars = 100 μm (A, B, and C), 25 μm (D, E, and F), and 5 μm (G, H, and I).

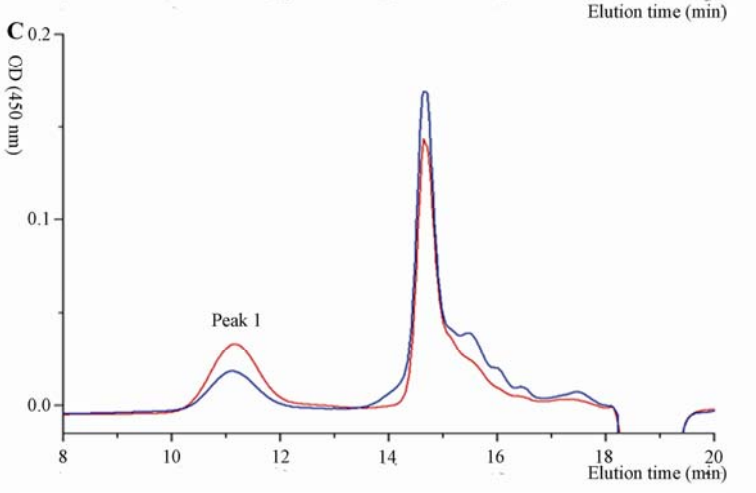


Supplemental Figure S4. ELISA assays of the mucilage extracted with H_2O, 0.2 N NaOH, and 2 N NaOH from wild-type and cs*la2-1* seeds.

A, Non-adherent mucilage. B, 0.2 N NaOH extracted mucilage. C, 2 N NaOH extracted mucilage. Values indicate the means of three replicates. Background signal is lower than 0.05 for OD (450 nm). HG, homogalacturonan; RG-I, rhamnogalacturonan I; ManP, mannan polysaccharide.







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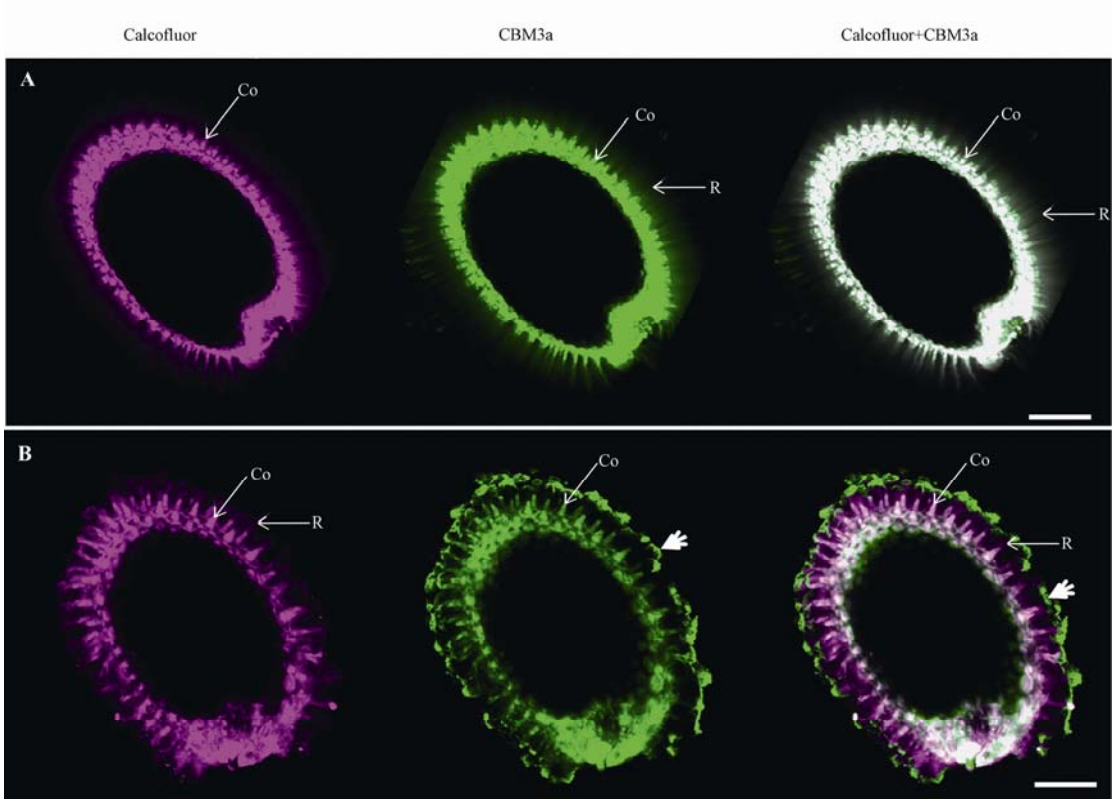
	Non-adherent mucilage				0.2 N NaOH-extracted		2 N NaOH-extracted	
	WT		*csla2-1*		WT	csla2-1	WT	csla2-1
	Peak 1	Peak2	Peak1	Peak2	Peak1	Peak1	Peak1	Peak1
Mn (kD)	16,470	344.9	11,680	214.4	299.2	283.2	561.9	573.2
Mp (kD)	15,590	305.8	15,960	301.6	294.7	253.8	548.3	553.3
Mw (kD)	16,820	376.9	12,540	317.8	314.6	294.5	587.0	581.1
Mw/Mn	1.021	1.093	1.074	1.482	1.052	1.039	1.045	1.014

Supplemental Figure S5. Analysis of the mucilage extracted with H_2O, 0.2 N NaOH, and 2 N NaOH from wild-type and *csla2-1* seeds by size-exclusion

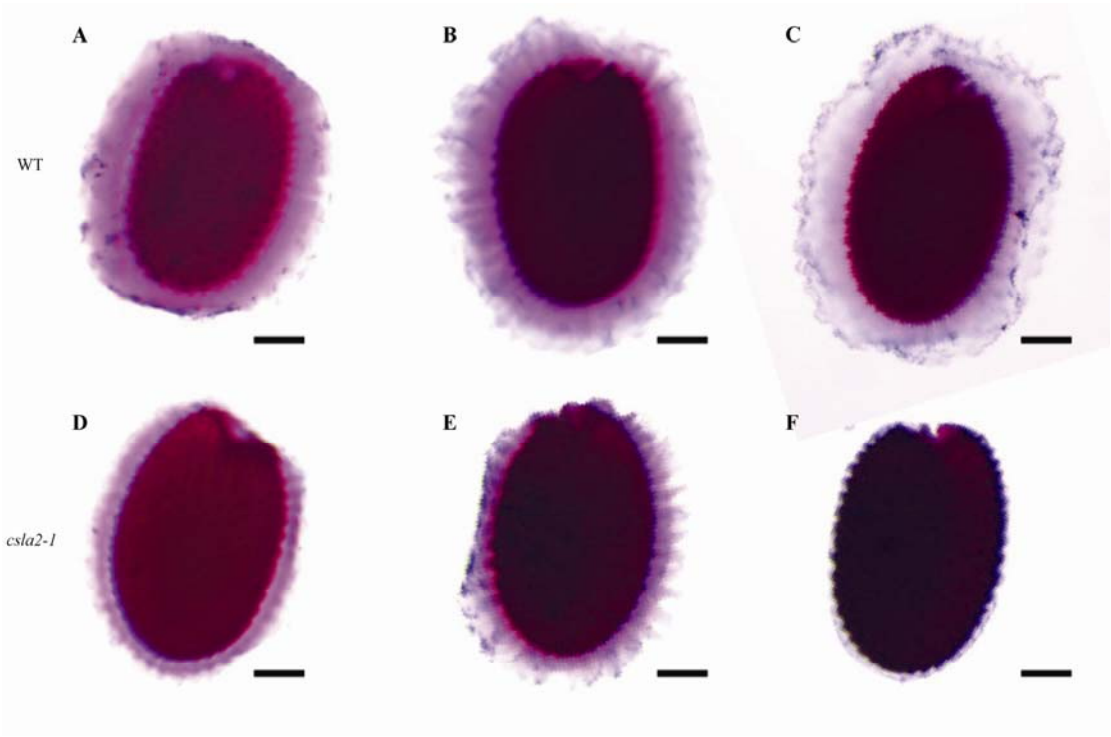
chromatography combined with refractive index detection.

A, Elution profile of non-adherent mucilage. B, Elution profile of 0.2 N NaOH extracted mucilage. C, Elution profile of 2 N NaOH extracted mucilage. D, A summary of average molecular weight of mucilage. Refractive index signals: blue line, wild type; red line, *csla2-1*. Vo, column void volume; Vt, column total volume.



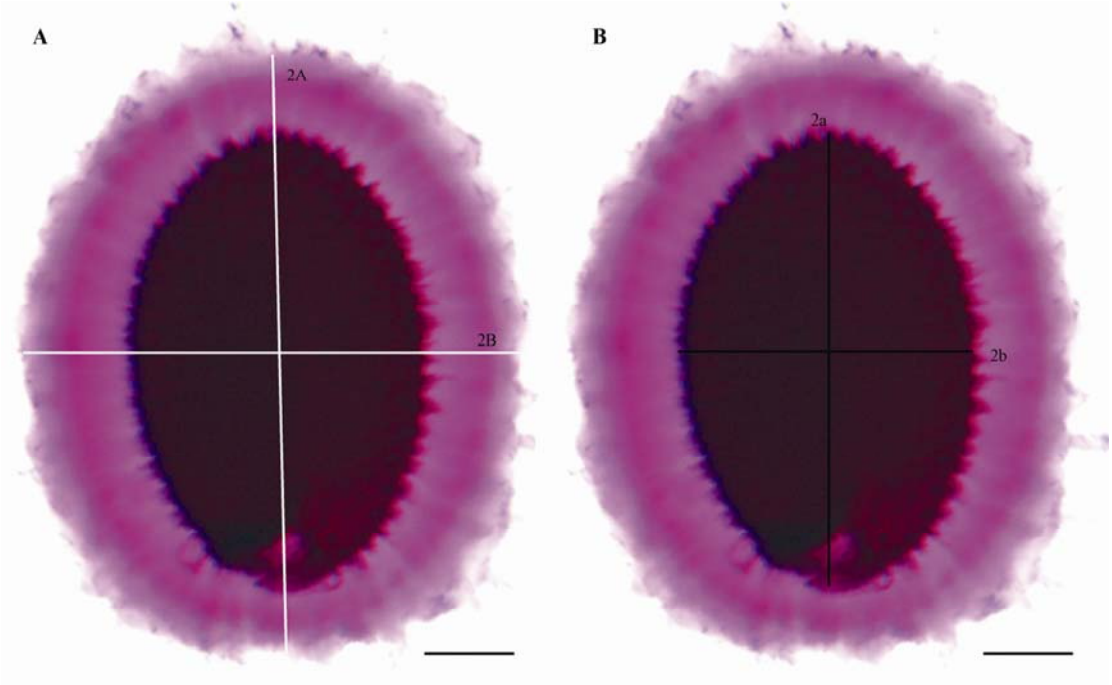
Supplemental Figure S6. 3D structure of Arabidopsis wild-type and *csla2-1* adherent mucilage stained with Calcofluor and CBM3a.

A, Wild-type seeds exposed to Calcofluor and CBM3a were so bright in three dimensional reconstructions that the rays originating from columella were not obvious. B, In *csla2-1* seeds, Calcofluor labels columella and rays were weaker, but CBM3a labels columella and external adherent mucilage mushroom-cap. Co, Columella; R, ray. Double arrows indicate CBM3a binding of external domain of adherent mucilage like mushroom-cap in *csla2-1*. Bars = 150 μm.



Supplemental Figure S7. Ruthenium red staining of the adherent mucilage after digestion with enzymes.

A and D, Seeds as control. B and E, Seeds following endo-β-Mannanase treatment. C and F, Seeds following endo-β-glucanase treatment. Bars = 100 μm.



Supplemental Figure S8. Illustration of how the measurements of mucilage and seed volumes of Arabidopsis seed were conducted.

A, Illustration of how the measurements of the volumes of seed including mucilage (seed + mucilage) were conducted. B, Illustration of how the measurements of volumes of seed without mucilage were conducted, using the same seed as in A. 2A and 2a indicate length; 2B and 2b indicate width. The volume of a seed including mucilage and without mucilage was estimated using the formula for a spheroid (volume = 4/3 × 1/8 × length × width × depth). For this calculation, the measured width was also taken as depth. Therefore, the volume of adherent mucilage was determined by subtracting the volume of the seed from the total volume (seed + mucilage). Bars = 100 μm.

Supplemental Table S1. Primers set used in this study

Primer name	Sequence
Primers for identification of T-DNA insertion mutants	
SALK_006803-LP	TCAAGCATTGCGAGTATGTTG
SALK_006803-RP	ACACTACCAAGTGTCGGCAAG
SALK_083877-LP	AATAGCGGCGATAAATGAAGC
SALK_083877-RP	AAAGTCATAGCATCCGCACAC
SALK_149092-LP	TAGATGGTCTTGTGGACCTGC
SALK_149092-RP	CAAAAGAACCCTTGGAGCTTC
LBb1.3	ATTTTGCCGATTTCGGAAC
Primers for gene cloning	
CSLA2F	ATGGACGGTGTATCACCAAAG
CSLA2R	CTAACTCGGGACATAAGTCCC
Primers for RT-PCR and qRT-PCR	
CSLA2RTF	AGTTATGTCAAGCATTGCGAGT
CSLA2RTR	CTGAAGGTCACCGAGGTAGAG
GAPC-F	TCAGACTCGAGAAAGCTGCTAC
GAPC-R	GATCAAGTCGACCACACGG
CSLA2qRTF	TGAGGCAGGAAGGGCTAACG
CSLA2qRTR	AGCATCCGCACACGAACAAG
ACTIN2-F	CCAGAAGGATGCATATGTTGGTGA
ACTIN2-R	GAGGAGCCTCGGTAAGAAGA
Primers for *In situ* hybridization	
CSLA2ProbeF	TCTTCAAATTGCCTGATC
CSLA2ProbeR	TCCTACTTGTTGTTACCG

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