Supplemental Methods

Plant Growth and Seed Harvesting

Seeds for all the lines analyzed in this review were ordered from the Nottingham Arabidopsis Stock Centre (NASC), except from *pmei6-1* seeds, which were obtained from Dr. Helen North (INRA, Versailles). Seeds were germinated under continuous light on ½ Murashige and Skoog (MS) plates, containing 2.15 g/L MS basal salts (Sigma M5519-10L) and 0.7 g/L agar (Cat# 4807.2; Carl Roth, Karlsruhe, Germany). After five to seven days, seedlings were transplanted to individual 7 × 7 × 8 cm pots filled with peat-sand-pumice substrate (SoMi 513 Dachstauden, HAWITA GRUPPE GmbH, Vechta, Germany). Plants were grown in a chamber with constant light (around 170·µE·m^{-2}·s^{-1}), temperature (20 °C) and relative humidity (60%). When starting to bolt, plants were covered with ARACON tubes (Betatech bvba, Gent, Belgium), to prevent cross fertilization of flowers and seed dispersal. The seed to seed generation time under our growth conditions is around 60 days. Mature plants were cut from their base and mature seeds were harvested by shaking each plant into a different large brown paper bag.

Touch-and-Go PCR Genotyping

The PCR genotyping was carried out using the previously described Touch-and-Go method [96], and a mixture of two gene-specific primers and a T-DNA-specific primer. Col-0 WT and negative controls were always included alongside the unknown samples. The gene-specific primers used are listed in Supplemental Table S4. One of the following primers used to amplify each T-DNA insert: SALK LBb1.3 (5'-ATTTTGCCGATTTCGGAAC-3'), SAIL LB3 (5'-TAGCATCTGAATTTCATAACCAATCTCGATACAC-3'), GK o8409 (5'-ATATTGACCATCATA CTCATTGC-3'), SM Spm32 (5'-TACGAATAAGAGCGTCCATTTTAGAGTGA-3').

Ruthenium Red Staining

Staining was carried out using cell culture plates with 24 wells (Cat# 734-2325, VWR International GmbH, Darmstadt, Germany). Wells were first filled with 500 µL water, then around 30 seeds were added to each well. The seeds were hydrated for 5 min, with occasional mixing. The water was then removed and 300 µL of 0.01% (*w*/*v*) ruthenium red as added. After staining for 5 min, the solution was replaced with 300 µL of water. Each well was imaged with a Leica MZ12 stereomicroscope equipped with a Leica DFC 295 camera. Image size and contrast of images were processed in Fiji (available online: http://fiji.sc/Fiji).

Total Mucilage Extraction

Around 5 mg seeds were precisely weighed in 2 mL Safe-Lock Eppendorf tubes. A serial dilution of a nine-sugar mixture (Fuc, Rha, Ara, Gal, Glc, Xyl, Man, GalA, GlcA) added to 2 mL screw-cap tubes. 1 mL of water, containing 30 µg of ribose as an internal standard, was added to all the samples and standards. Total mucilage was extracted by vigorously shaking the seed-containing tubes for 15 min at 30 Hz in a Retsch Mill using two 24 TissueLyser Adapters (Qiagen, Hilden, Germany). The adapters were then rotated 180 degrees and mixed for an additional 15 min at 30 Hz. The seeds

were allowed to settle at the bottom of each tube, and 800 µL of the supernatant was transferred to a screw-cap tube. Samples and standards were dried under pressurize air at 45 °C using a Techne Dri-Block DB 3D heater. Once dry, 300 µL of 2 M trifluoroacetic acid (TFA) was added to each tube. Tubes were capped tightly, vortexed, and heated for 90 min at 121 °C. The heating blocks and the samples were then rapidly cooled on ice. After brief centrifugation, tubes were uncapped and the TFA was evaporated under pressurized air at 45 °C. Dried samples and standards were then re-suspended in 600 µL water. Monosaccharides were quantified by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) using a Dionex system equipped with a CarboPac PA20 column and GP50, ED50, and AS50 modules. Neutral sugars were separated using 2 mM NaOH at a flow rate of 0.4 mL/min over the course of 20 min. Afterwards, 513 mM NaOH was used for 10 min to detect uronic acids. The amount of each monosaccharide was normalized to the internal standard and quantified using a standard calibration curve.

Table S2. An overview of Arabidopsis genes implicated in seed mucilage production. Gene expression during seed coat development was qualitatively assessed using the eFP seed (available online: http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi?dataSource=Seed), and the eFP seed coat (SC) (available online: http://bar.utoronto.ca/efp_seedcoat/cgi-bin/efpWeb.cgi) microarrays. The expression profiles show strong (green), moderate (yellow), or weak (red) specificity to mucilage production.

AGI Code	Gene Name	Functional Class	eFP	eFP SC
At1g02720	*GATL5*	GT		
At1g09330	*ECH*	Secretion		
At1g09540	*MYB61*	TF	no probe	
At1g18580	*GAUT11*	GT		
At1g21070	*URGT2*	Sugar Transporter		
At1g52880	*NARS2*	TF		
At1g53500	*RHM2/MUM4*	Sugar Synthase		
At1g56650	*MYB75*	TF		
At1g62990	*KNAT7*	TF		
At1g63650	*EGL3*	TF		
At1g76880	*DF1*	TF		
At1g79840	*GL2*	TF		
At2g18840	*YIP4a*	Secretion		
At2g32700	*MUM1/LUH*	TF		
At2g35620	*FEI2*	Receptor		
At2g37260	*TTG2*	TF		
At2g47670	*PMEI6*	Protein Modification		
At3g10380	*SEC8*	Secretion		
At3g13540	*MYB5*	TF		
At3g15510	*NARS1*	TF		
At3g46550	*SOS5/FLA4*	AGP	no probe	
At3g50990	*PER36*	Peroxidase		
At4g09820	*TT8*	TF		
At4g28370	*FLY1*	Protein Modification		
At4g30260	*YIP4b*	Secretion		
At4g36920	*AP2*	TF		
At5g03540	*Exo70A1*	Secretion	no probe	
At5g09870	*CESA5*	GT		
At5g22740	*CSLA2*	GT		
At5g23940	*DCR2*	Cuticle		
At5g24520	*TTG1*	TF		
At5g35550	*TT2*	TF		
At5g42630	*ATS*	TF		
At5g49360	*BXL1*	GH		
At5g63800	*MUM2/BGAL6*	GH		
At5g63840	*RSW3*	Secretion		
At5g67360	*SBT1.7/ARA12*	Protein Modification		

Table S3. Monosaccharide composition of mucilage in transcription factor mutants. The relative composition of each monosaccharide is shown as mol %, while the total mucilage content was calculated as nmol sugar/mg seed. Values represent the mean ± SD of three biological replicates for each mutant and six for the WT. Total mucilage was extracted by vigorously shaking 5 mg seeds in 1 mL water using a Retsch Mill for 30 min at 30 Hz.

	WT	***myb5-2***	***knat7-1***	***myb61-7***	***df1-1***	***ttg2-3***
Rha	40.22 ± 1.04	44.82 ± 1.26	41.48 ± 0.47	41.23 ± 2.24	43.20 ± 0.42	47.24 ± 2.88
Ara	1.55 ± 0.16	1.75 ± 0.22	2.36 ± 0.16	1.79 ± 0.30	1.93 ± 0.08	3.43 ± 1.42
Gal	2.61 ± 0.31	2.44 ± 0.31	3.48 ± 0.15	2.51 ± 0.26	2.51 ± 0.13	3.54 ± 0.73
Glc	0.95 ± 0.07	2.13 ± 1.26	1.14 ± 0.12	1.11 ± 0.20	0.98 ± 0.04	4.98 ± 1.43
Xyl	4.32 ± 0.35	3.58 ± 0.32	2.46 ± 0.05	3.10 ± 0.14	4.34 ± 0.21	2.88 ± 0.51
Man	0.83 ± 0.05	1.11 ± 0.06	0.71 ± 0.01	0.66 ± 0.03	0.80 ± 0.01	1.11 ± 0.12
GalA	49.52 ± 0.83	44.18 ± 1.33	48.37 ± 0.73	49.59 ± 1.52	46.24 ± 0.07	36.83 ± 5.96
Total	132.38 ± 10.80	54.69 ± 5.93	138.85 ± 4.92	123.56 ± 9.96	79.70 ± 6.02	65.78 ± 7.55

Table S4. Gene-specific primers used to genotype insertional mutants.

Mutant	**Polymorphism**	**Forward Primer (5' to 3')**	**Reverse Primer (5' to 3')**
gatl5-1	SALK_106615C	ACACTCCCCTCTCTCTCTCAC	TCCATTTCTCAATTCGTTTCG
myb61-7	SALK_106556C	GAAATTTAAATTTGGCTCTGTTTG	AAAGGCCATCTGATTTATCCG
gaut11-3	SAIL_567_H05	CAACCAATTGCCCAAATATTG	GAAAACCCGAAAGGAGAAAAG
knat7-1	SALK_002098C	GAGATTAGTGTTTGCGCTTGG	TATGCGTAAGGGCATATCAGG
df1-1	SALK_106258C	AGACGCTAGCGTTAAAGGTCC	CAAGAATTTGCGTTGAAGCTC
gl2-8	SALK_130213C	ACCACCGATCAGATCAGACAC	GGAGTTTTCGAGGTGGAGATC
fei2-2	SALK_044226	TTGCCATCTATGGGAACTTTG	ATTACAACCATTGTTGCAGGC
ttg2-3	SALK_148838	TAAAACCAAACGACACCGTTC	TCCAAGTTTGTTGACGATTCC
pmei6-1	SM_3.19557	ATGAACCTCCCAAATACCCTC	GTTCCATGGCAATCACGATAC
myb5-2	SALK_105723C	GAACACAATTGCTCCTCAAGG	GACGACAGCTCTTTCCACATC
sos5-2	SALK_125874	GAAACTGGGAATAACCTTCGG	AGCTTCTCGAGACCAAACCTC
per36-1	SAIL_194_G03	AGATTAAAGAGAAGCTGCCGG	CAAGGCAGACTTGATCTCGTC
fly1-2	SALK_067290	CGCAAGTTCAGATGCTAATGC	AAAAAGGAACCGACAAACCTG
cesa5-4	SALK_207154C	CGTCTTCATCAAGTTCTTCGG	AAATAGACGACACCACCTTCTTC
csla2-3	SALK_149092C	TAGATGGTCTTGTGGACCTGC	CAAAAGAACCCTTGGAGCTTC
dcr-2	SALK_128228C	ATCCACGTGGCATTTTATGAG	ACAATTCCAAACCAAACACAC
mum2-14	SALK_060221C	TATACCGAACCGACAATGAGC	CGCGTCTTCAAAGACATAAGC
sbt1.7-2	GK-544E06.01	ATCCGACCATTCAATACTCCC	TCAAACGTAGCTCCATGGATC