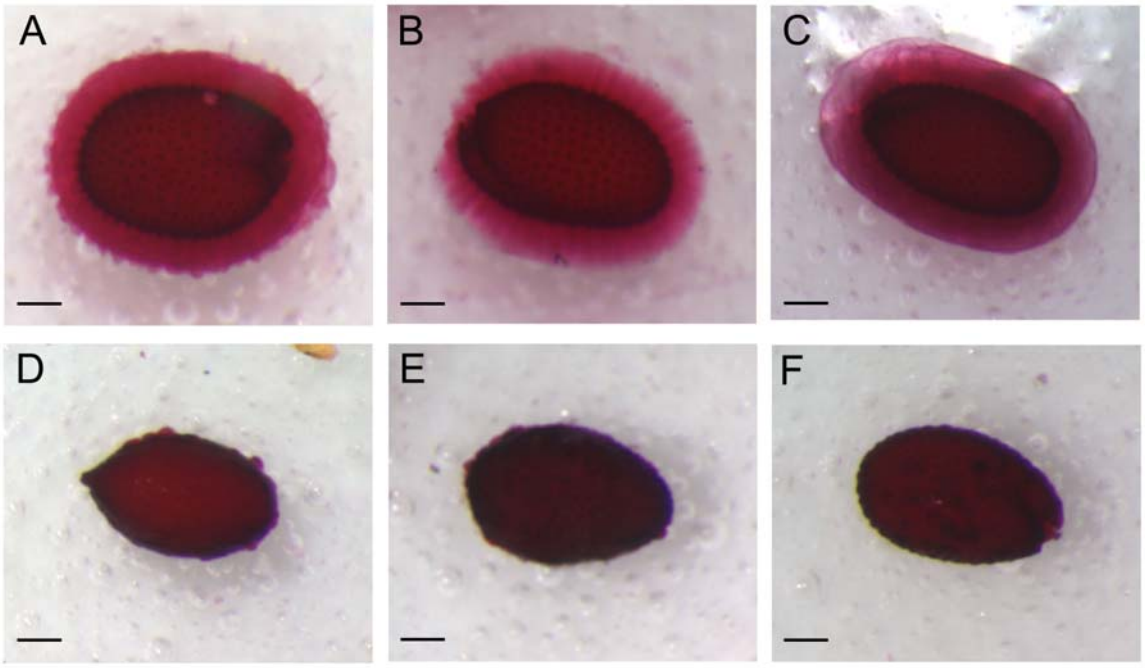
Supplemental Data. Dean et al. (2007). The Arabidopsis *MUM2* gene encodes a β-galactosidase required for the production of seed coat mucilage with correct hydration properties.

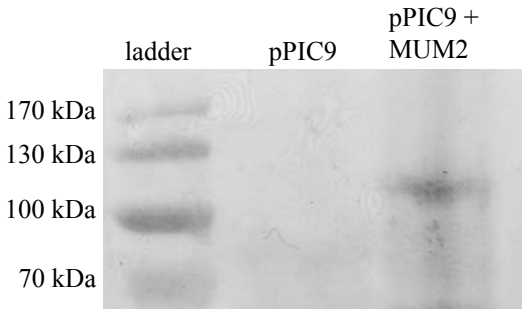


Supplemental Figure 1. Extraction of mucilage from *mum2* seeds using chelating agents.

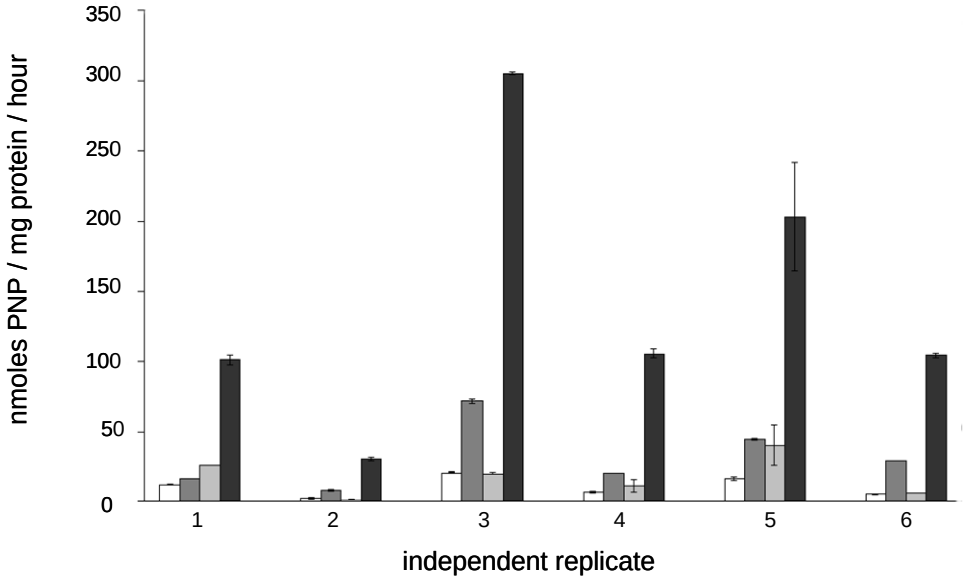
Mature wild type (A, B, C) and *mum2* (D, E, F) seed treated with EGTA (A), CDTA (B) and ammonium oxalate (C) prior to staining with Ruthenium Red. In wild type seed, mucilage has been released and seeds have a similar appearance to those stained directly with Ruthenium Red (compare with Figure 1A). In *mum2* seed, a small amount of mucilage is released from seeds treated with EGTA and CDTA (compare with Figure 1P). No mucilage appears to be released from seeds treated with ammonium oxalate.

Scale bars: 100 μm

A

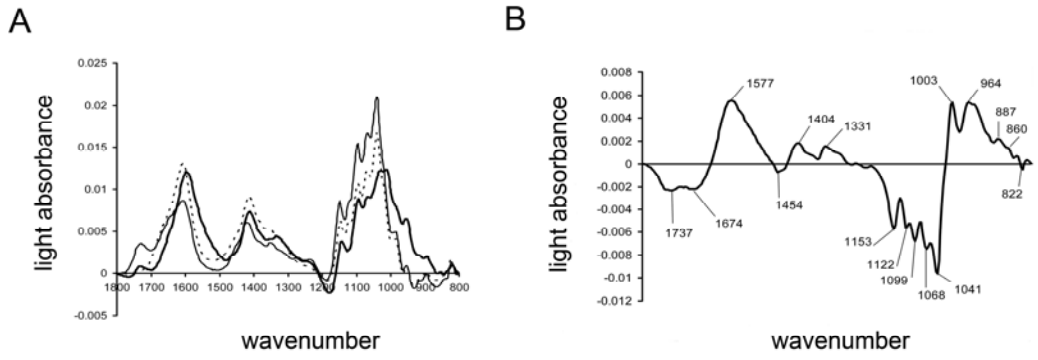


B



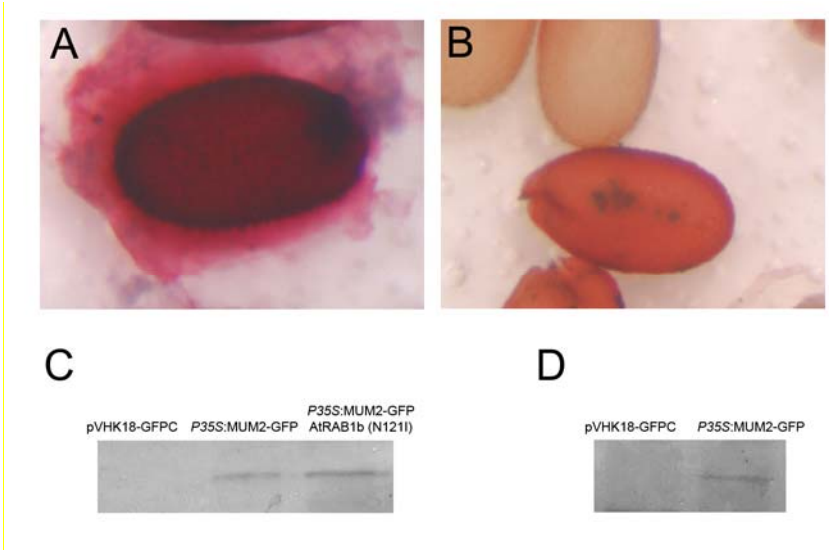
Supplemental Figure 2. Heterologous MUM2 expression in *Pichia pastoris*.

A. Western blot of cell pellet extracts from yeast transformed with pPIC9 empty vector or the MUM2 expression cassette after 26 hours induction with methanol. Recombinant MUM2 was detected using Anti-His antibody and is the expected size of approximately 110 kDa (80 kDa MUM2 protein plus the 30 kDa α-Factor secretion signal).
B. β-Galactosidase assays using crude protein extracts from *Pichia pastoris* transformed with either the pPIC9 parent vector or the MUM2 expression cassette. Results are shown for six independent experiments. White bars, pPIC9 parent (boiled); dark grey bars, pPIC9 parent (unboiled); light grey bars, MUM2 expression cassette (boiled); black bars, MUM2 expression cassette (unboiled). Protein extracts from yeast expressing the MUM2 cassette have consistently greater activity toward PNP-β-D-Gal than do yeast expressing the pPIC9 parent. Error bars represent the range of two technical replicates performed for each assay.



Supplemental Figure 3. Fourier Transform Infrared Spectroscopy (FTIR) analysis of wild type and *mum2* mucilage extracted with water or Na_2CO_3

A. Average spectra of wild type mucilage extracted with water, wild type mucilage extracted with Na_2CO_3 and *mum2* mucilage extracted with Na_2CO_3. Spectra for wild type mucilage extracted with water (dotted line) and Na_2CO_3 (thin line) are typical of RG-I (peaks at 1126, 1068, 1041, 987 and 821 cm-1). Samples extracted with Na_2CO_3 are also acetylated (1735 and 1238 cm^{-1}) but acetylation is lost on extraction with water. Peaks at 1612 and 1423 cm-1 are carboxylate ion stretches from the galacturonic acid. The *mum2* spectrum (thick line) also shows peaks characteristic of RG-I, acetylation and galacturonic acid as well as peaks at 1095, 1014, 956, 898 and 860 cm-1 that can be assigned to pectin. B. Digital subtraction of an average Na_2CO_3-extracted wild type spectrum from an average Na_2CO_3-extracted *mum2* spectrum. Wavenumbers, indicating absorbance by different polysaccharide components, are labeled. Negative peaks at 1153, 1122, 1068, 1041 and 822 cm^{-1} indicate a higher proportion of RG-I in wild type mucilage. Positive peaks at 1577 and 1404 cm^{-1} indicate that *mum2* mucilage may contain more carboxylic acid groups and other pectic polysaccharide components (peaks at 964, 887, 860 cm^{-1}).



Supplemental Figure 4. Complementation of *mum2* with P_{35S}:MUM2-GFP.
A. The *mum2* plants transformed with P_{35S}:MUM2-GFP show complementation of the *mum2* phenotype as indicated by a restoration of mucilage extrusion. B. The *mum2* plants transformed with empty pVHK18-GFPC retain a *mum2* phenotype. C. Western blot of total protein from tobacco transiently expressing pVHK18-GFPC, P_{35S}:MUM2-GFP alone or P_{35S}:MUM2-GFP plus AtRab1b(N121I). Full length MUM2-GFP fusion protein of 110 kDa (80 kDa MUM2 plus 30 kDa GFP) can be detected in plants transformed with P_{35S}:MUM2-GFP and P_{35S}:MUM2-GFP plus AtRab1b(N121I) but not in plants expressing pVHK18-GFPC. D. Western blot of total protein from Arabidopsis seedlings expressing pVHK18-GFPC or P_{35S}:MUM2-GFP. Full length MUM2-GFP fusion protein of 110 kDa (80 kDa MUM2 plus 30 kDa GFP) can be detected in plants transformed with P_{35S}:MUM2-GFP but not in plants expressing pVHK18-GFPC.

Supplemental Table 1

Monosaccharide composition of mucilage determined by HPAEC expressed as mole percentage of each monosaccharide

Sugar	wild type mucilage	*mum2* mucilage	wild type whole seed	*mum2* whole seed
Fuc	0.12	0.37	0.73	0.69
Ara	1.20	3.98	19.41	18.93
Rha	35.61	24.83	18.72	18.86
Gal	1.67	5.87	13.43	14.75
Glc	5.84	12.48	11.39	12.55
Xyl	3.06	3.19	8.53	7.95
Man	1.06	1.11	2.42	2.91
GalA	51.45	48.17	25.37	23.36