***AtBXL1* encodes a bifunctional β-D-xylosidase/α-L-arabinofuranosidase required for pectic arabinan modification in *Arabidopsis thaliana* mucilage secretory cells**

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SUPPLEMENTAL MATERIAL

FIGURE LEGENDS

Figure S1. Scanning electron microscopy of *bxl1-1* versus *mum4-1* and wild-type seed coat epidermal cells.
(A) Wild-type Col-2 seed, note cells are hexagonal shape with central raised columella. (B) *mum4-1* seed lacking obvious columella. (C) Wild-type WS seed. (D) *bxl1-1* seed. Scale bars represent 50 μm.

Figure S2. Immunoblot of extracted mucilage and whole seed immunofluorescence of WS versus *bxl1-1* seeds.
(A) Immunoblot of EDTA-extracted mucilage from WS and *bxl1-1* seeds hybridized with unbranched RG I antibody CCRC-M36 and anti-arabinan antibody LM6. Concentrated mucilage from 75 mg of seed was diluted as noted in the figure prior to spotting on the membrane.
(B-E) Confocal images of whole seed immunofluorescence (green) with the seed coat outer cell walls counterstained with propidium iodide (magenta), shown as Z-stack projections. (B-C) Hybridization with CCRC-M36, note thick capsule of stained mucilage. (D-E) Hybridization with LM6. (D) Faint staining close to seed at tops of columellae in WS (arrowhead), with one bit of stained intact cell wall (arrow). (E) *bxl1-1* LM6 staining is more intense, with obvious pieces of intact cell wall (arrows). (F-G) Single confocal slice through controls lacking primary antibodies with contrast enhanced to visualize seed outlines. Scale bar represents 100 μm.

Figure S3. Water absorption of *bxl1-1* versus wild-type mucilage extracts.
Timecourse of water absorption (µg water/mg freeze-dried mucilage) of ammonium oxalate, 0.2 N and 2 N sodium hydroxide extracts of *bxl1-1* versus wild-type mucilage measured every 5 min for 30 min, then every 15 min up to 120 min. Error bars represent SE, n = 3.

Figure S4. Transcription of *AtBXL2* throughout Arabidopsis tissues and during seed and silique development.
Qualitative (35 cycles) RT-PCR using *AtBXL2* primers on RNA isolated from Columbia wild-type leaves, stems, seedlings, roots, inflorescence tips, intact siliques at 4, 7, 10 DPA, plus leaf tissue from wild-type WS and *bxl1-1* plants. The loading control is cytosolic glyceraldehyde-3-phosphate dehydrogenase *(GAPC)*.

Table S1 Quantification of mucilage release of complemented lines of *bxl1-1,* plus *bxl1-2* and *bxl1-3*.

Seeds were shaken in water for 90 min followed by 60 min shaking in 0.01% ruthenium red. The proportion of an individual seed's circumference surrounded by mucilage was then quantified as 100% (seed completely surrounded by mucilage), 75% (3/4 of seed circumference surrounded), 50%, 25% or no mucilage visible. Results are the percent seeds per sample with a particular mucilage category. Vector-only transformed *bxl1-1* seeds (pGR0229) are controls for the PTYg *bxl1-1* complementation lines (to compare to wild type WS see Table I). Col-0 is the wild-type background for *bxl1-2* and *bxl1-3*. Complemented lines were stained in one experiment, while Col-0 wild type and allele samples were stained in separate experiment.

Line	**Percent of seed surrounded by mucilage capsule of specified size**					
	100%	**75%**	**50%**	**25%**	**No mucilage**	**Total # seeds**
PTYg in *bxl1-1* #1	93.0	2.1	2.1	2.8	0	142
PTYg in *bxl1-1* #2	100	0	0	0	0	122
PTYg in *bxl1-1* #3	91.4	6.6	1.3	0.7	0	151
pGR0229 in *bxl1-1* #1	4.4	15.8	36.8	35.1	7.9	114
pGR0229 in *bxl1-1* #2	7.5	12.8	31.6	34.6	13.5	133
Col-0	79.2	3.8	5.7	5.7	5.7	53
bxl1-2	9.5	29.7	40.5	14.9	5.4	74
bxl1-3	6.5	5.2	33.8	50.6	3.9	77

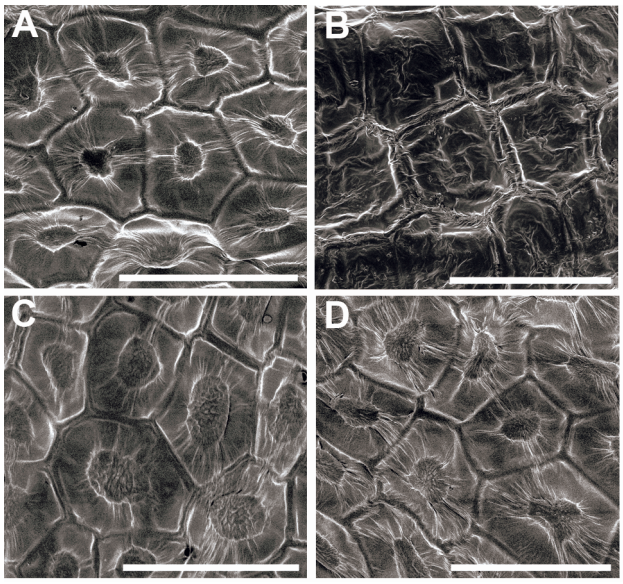
Table S2 Monosaccharide quantitation of *bxl1-1* versus wild type seedlings.

Samples were alcohol insoluble residue prepared from seedlings 12 days after germination that were hydrolyzed with trifluoroacetic acid and derivatized to alditol acetates. Results are average µg sugar plus standard error per 100 mg fresh tissue calculated from three independent samples

Sugar	**WS**	***bxl1-1***
Rha	9.3 ± 0.4	8.4 ± 0.8
Fuc	2.6 ± 0.1	2.3 ± 0.1
Ara	18.9 ± 1.1	21.6 ± 1.6
Xyl	18.0 ± 0.6	18.4 ± 1.6
Man	3.3 ± 0.2	2.7 ± 0.3
Gal	14.6 ± 1.1	12.9 ± 1.1
Glc	5.7 ± 0.3	4.9 ± 0.4
Total	72.4 ± 3.8	71.2 ± 4.0

Table S3 Primers used for RT-PCR, real-time PCR and genotyping of double mutants.

Gene	Primer Pair	Sequence
AtBXL1	At5g49360 p3/p4	ACTAGCACTCCGGAAGAAGC
		CAATCCTTTCTTCACTGCACCT
	At5g49360 p8/p11	ACGCCATTCCATTATCAAG
		GGTCCAGGCGCTAAGTTCGG
	At5g49360 p15/p16	TGGTGGACCAATCGATGTAA
		ATTACCGGATGCTCTCATGG
GAPC	GAPC p1/p2	TCAGACTCGAGAAAGCTGCTAC
		GATCAAGTCGACCACACGG
	RT GAPC p5/p6	GACAGATTTGGAATTGTTGAGG
		GGCCCATCAACAGTCTTCTG
AtBXL2	BXL2 p1/p2	AGGAAACTCCCGGTGAAGAT
		ATACGGAACGGTACGTCGAA
MYB61	MYB61 p1/p2	TTTGCAGAGATGTGGGAAGA
		GCCATTGTCGAAGAAATTTGA
	DSPM11	GGTGCAGCAAAACCCACACTTTTACTTC
MUM4	At1g53500 p1/p8	TTGCAGATTTCAAGGATGGA
		CATGGTTTCCTACAGCAGCA



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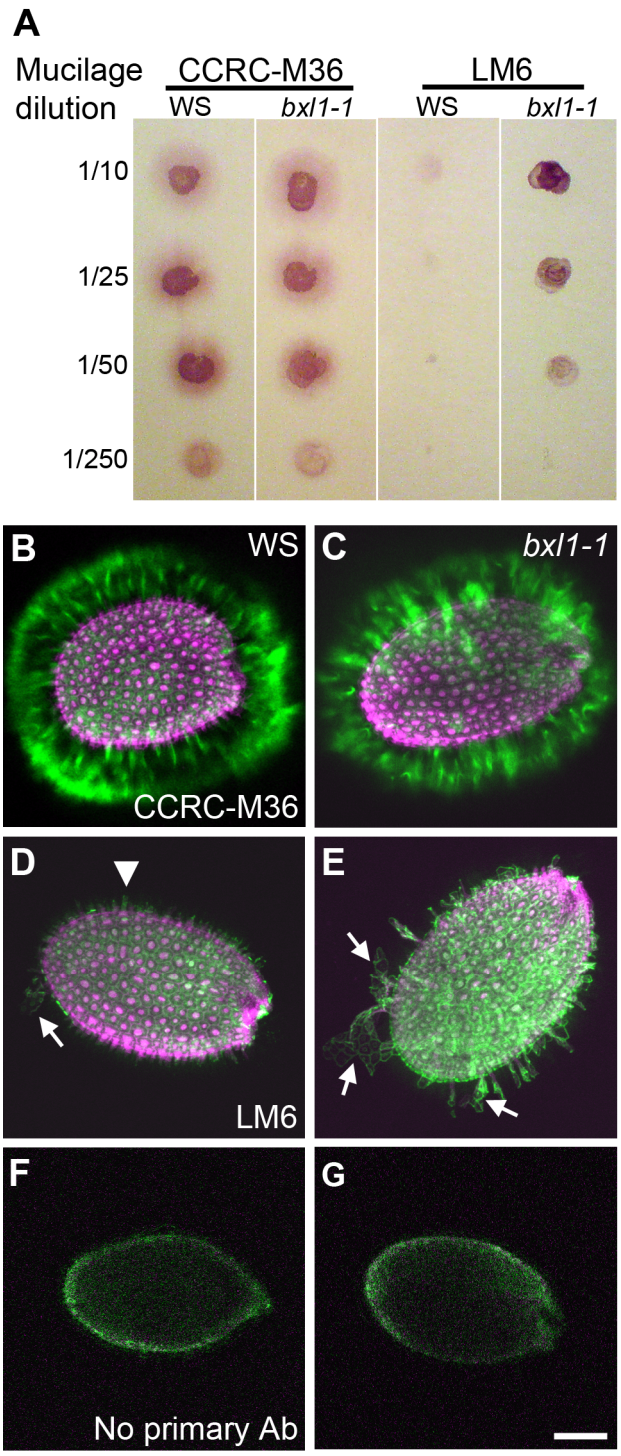

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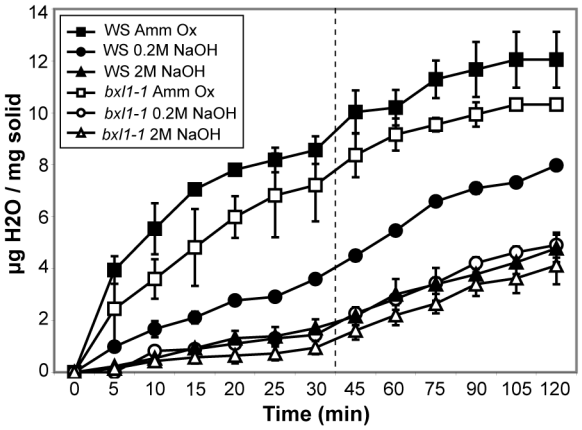

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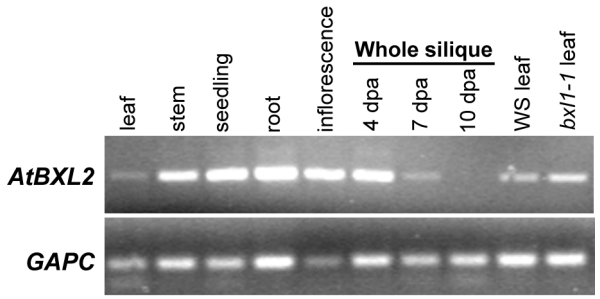

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