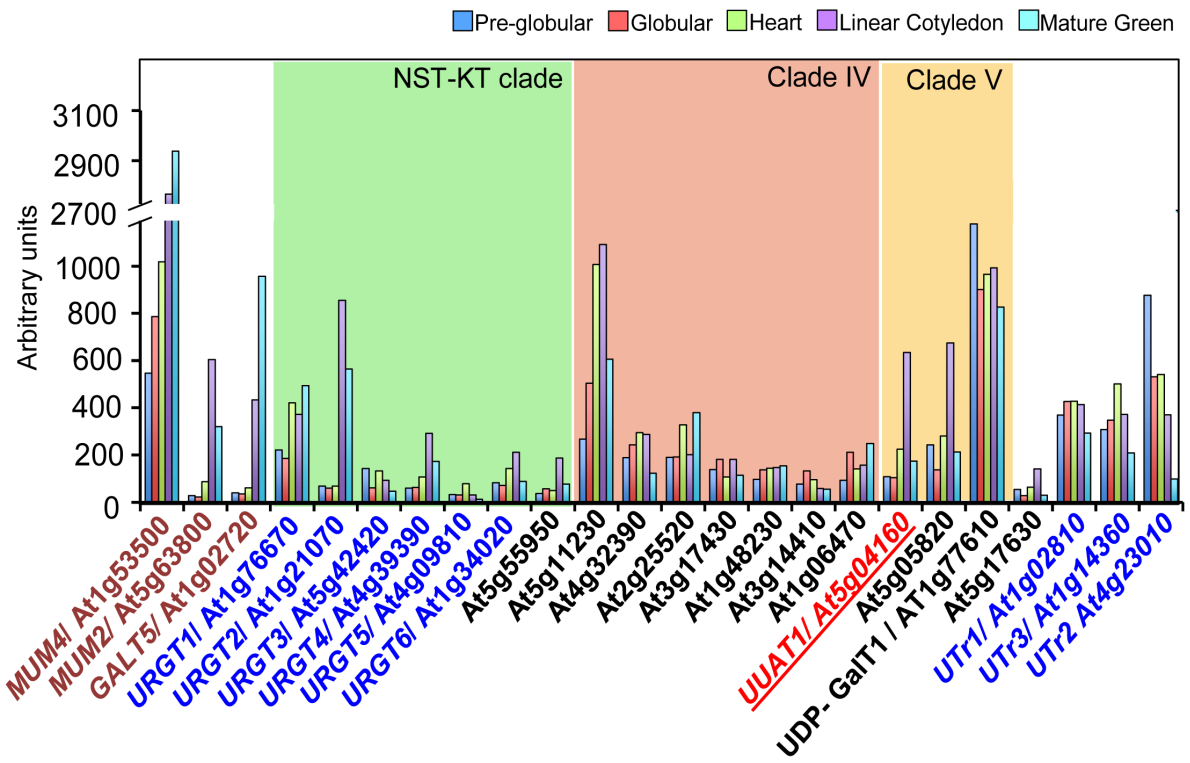
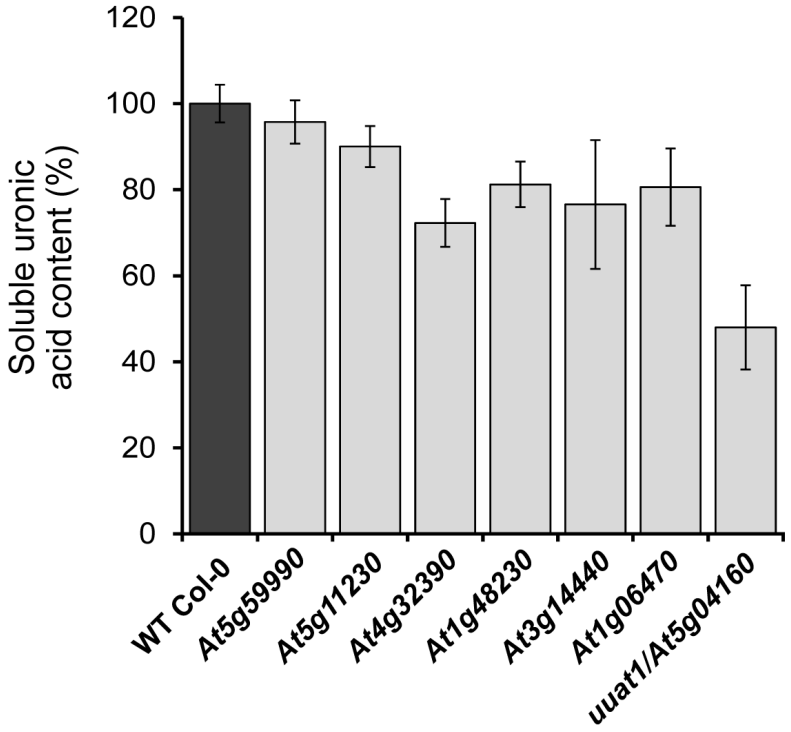


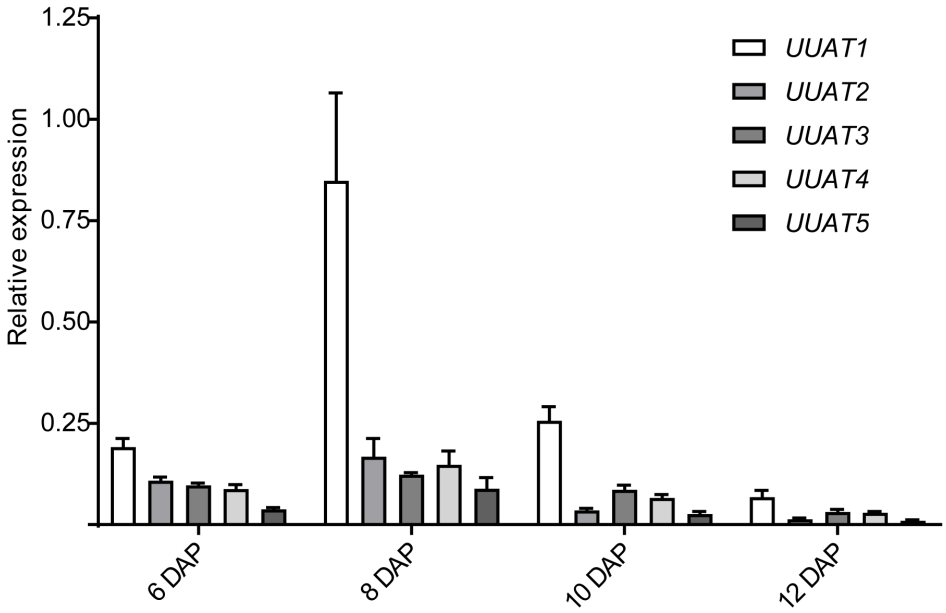
Supplemental Figure 1. NST Genes Expressed During Seed Development

Genes were identified and expression levels of each were obtained from the eFP Browser (Le et al., 2010; Winter et al., 2007) and plotted along with the expression of genes coding enzymes catalyzing mucilage biosynthesis (brown fonts). The background color represents the NST clade as described by Rautengarten et al., (2014). NSTs with known functions were disregarded from the screening (blue fonts). *UUAT1* (At5g04160, red font) from clade V exhibited a high level of expression at stages where the biosynthesis of mucilage takes place.



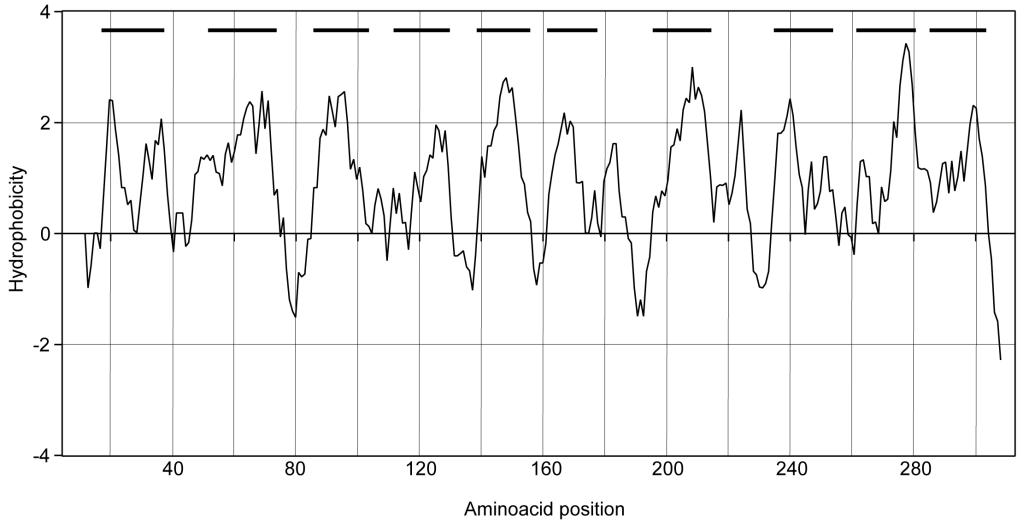
Supplemental Figure 2. Uronic Acid Content in the Soluble Mucilage Fraction from Mutants in NST Genes.

Quantification was carried out on one T-DNA line per gene indicated. For At2g25520, At3g17430 and At5g05820, we were not able to obtain homozygous mutants. Dry seeds were imbibed for 10 min in water before uronic acid quantification using the *m*-hydroxybiphenyl method (Saez-Aguayo et al., 2013).



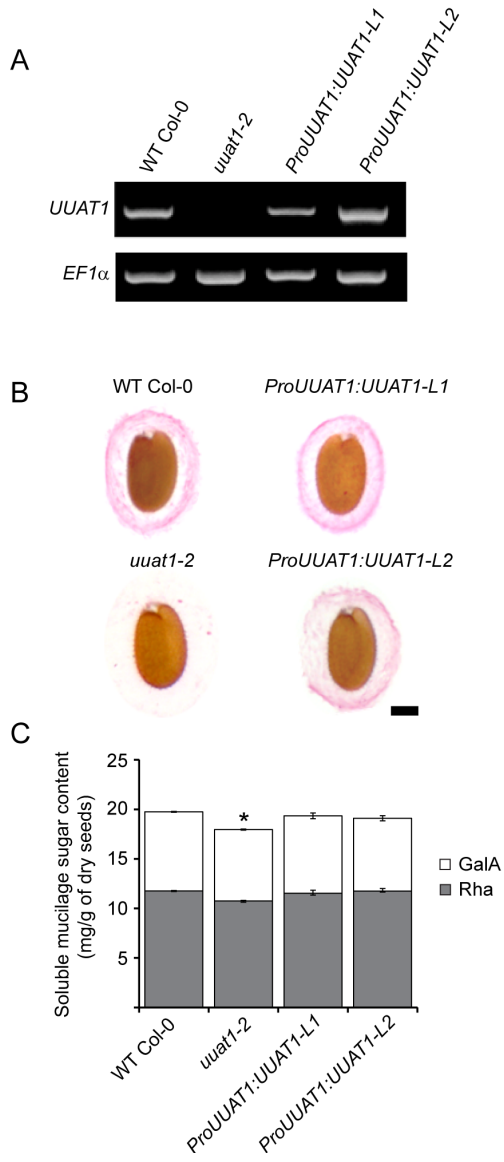
Supplemental Figure 3. UUATs Expression in Developing Seeds.

Quantitative RT-PCR analysis of *UUAT1* and the closest paralogues during seed development at various days after pollination (DAP). Steady state mRNA levels are represented relative to expression to the seed reference housekeeping gene *At4g12590*, described in Dekkers et al., 2012. Error bars represent SE of 3 independent biological replicates measured in triplicate.



Supplemental Figure 4. UUAT1 Hydropathy Plot.

A Kite-Doolitle hydropathy plot and predicted transmembrane domains (black bars) are depicted in the graph and were predicted using the HHMMTOP program (Tusnády and Simon, 2001).

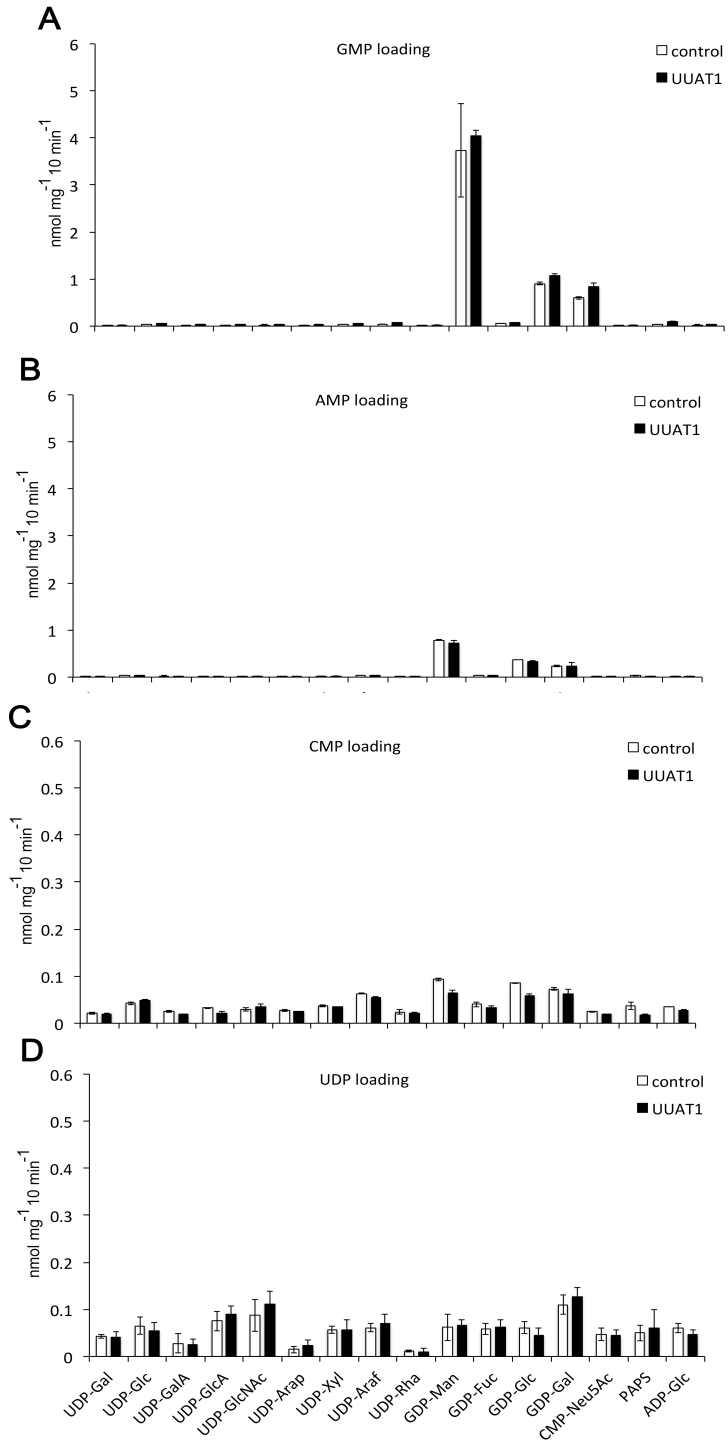


Supplemental Figure 5. Rescue of the *uuat1-2* Mutant Phenotype Using the *UUAT1 ProUUAT1:UUAT1-GFP* Construct.

(A) Expression of the *UUAT1* coding sequence (CDS) in the *uuat1-2* mutant background. Determination of *UUAT1* mRNA steady state in two *uuat1-2* rescue lines L1 and L2 (*ProUUAT1:UUAT1-GFP-L1 and L2)*. RT-PCR analysis was used to determine the presence of the *UUAT1* CDS in transformant lines. *EF1α* amplification was used as a control.

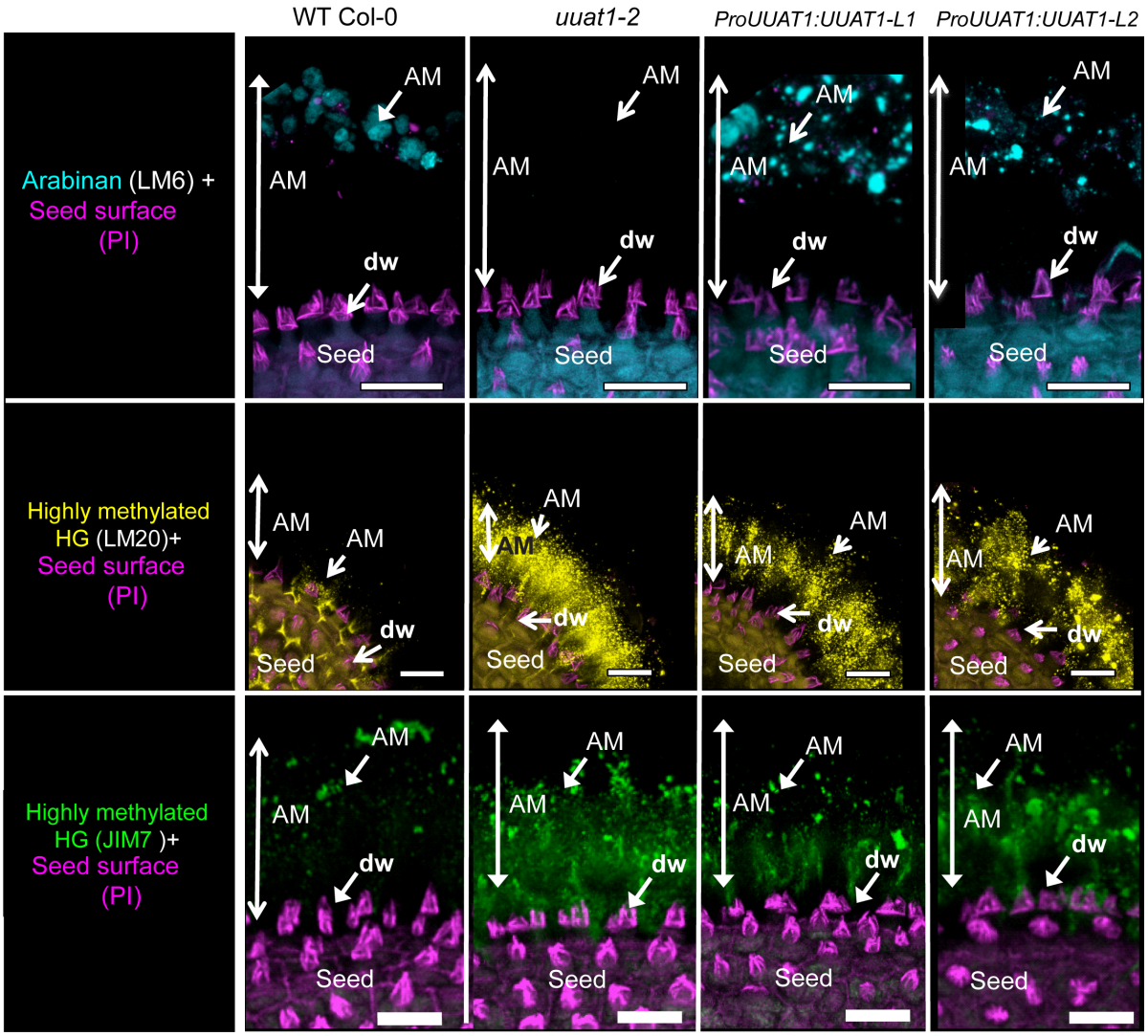
(B) The Ruthenium red adherent mucilage phenotype is restored in T2 seeds of independent *uuat1-2* transformant lines expressing *UUAT1* CDS fused to GFP under the control of the *UUAT1* promoter (Pro*UUAT1:UUAT1- GFP*). Seeds were imbibed for 1h in 0.5 M EDTA and stained with Ruthenium red (0.03%). The Pro*UUAT1*:*UUAT1-L1* and *-L2* lines recover the WT Col-0 adherent mucilage staining when compared to the *uuat1-2* mutant, which shows an unstained adherent mucilage phenotype. Bar = 100 μm

(C) Soluble mucilage content of the transgenic lines *ProUUAT1:UUAT1-L1 and –L2*. The Rha and GalA content was quantified using HPAEC-PAD. The result shows the average of 3 independent experiments,* t-test $p<0.05$.



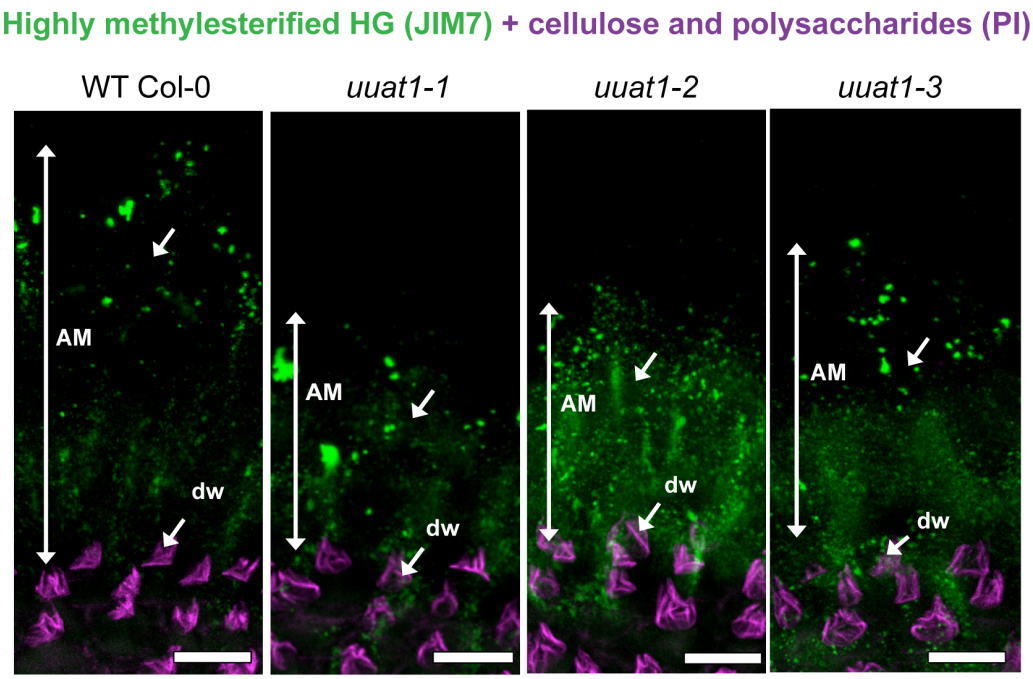
Supplemental Figure 6. Exchange of Nucleotide Sugars with GMP, AMP, CMP or UDP by UUAT1.

Transport assays were performed as described in Figure 2, except that proteoliposomes were loaded with
(A) GMP
(B) AMP
(C) CMP
(D) UDP



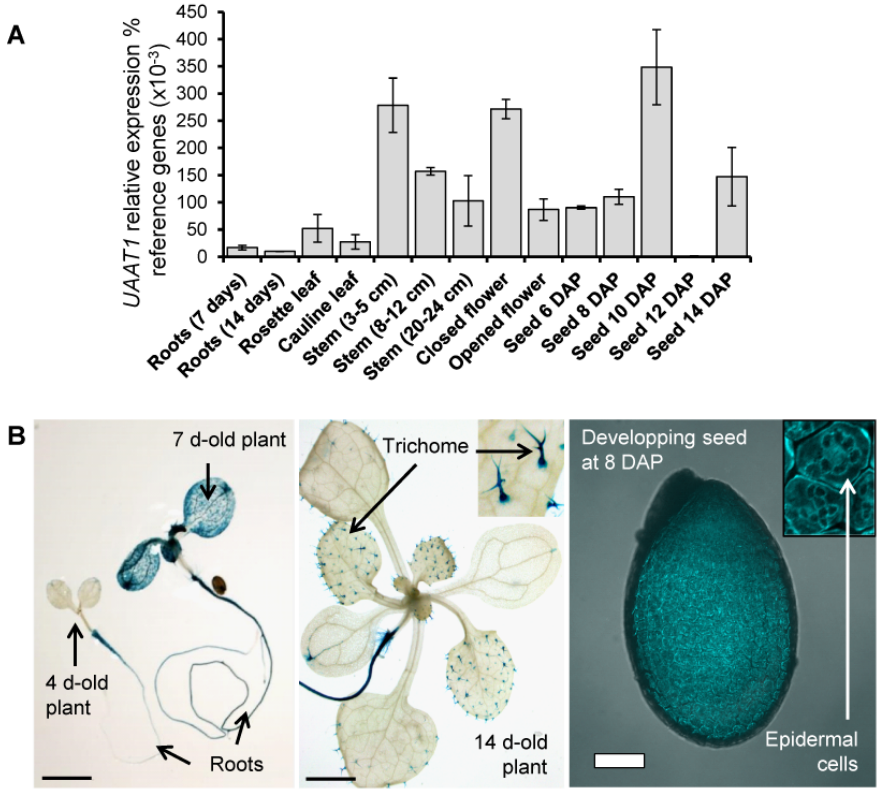
Supplemental Figure 7. Analyses of the Mucilage Phenotypes of uuat1-2 and the Rescued Lines Using Immunolocalization.

WT Col-0, *uuat1-2* and two *uuat1-2* lines transformed with the construct *ProUUAT1*:*UUAT1-GFP* (*ProUUAT1:UUAT1-L1* and *ProUUAT1:UUAT1-L2*) were used to perform labeling of arabinan epitopes (top row) and highly methylated HG using the antibodies LM20 (middle row) and JIM7 (bottom row). Propidium iodide was used to visualize seed surface polysaccharides (purple). AM: adherent mucilage; dw: distal wall. Bars = 50 µm.



Supplemental Figure 8. Changes in Methylesterification Degree in *uuat1* Allelic Mutants.

Labeling of highly methylesterified HG in the adherent mucilage from seeds of WT Col-0 and *uuat1-1*, *uuat1-2* and *uuat1-3* allelic lines. Confocal microscopy optical section reconstruction of AM released from dry imbibed seeds. The JIM7 antibody (green) was used to label highly methylesterified HG domains and propidium iodide was used to stain the seed coat surface (purple). Bar = 100 µm; AM, adherent mucilage; dw: distal wall.



Supplemental Figure 9. *UUAT1* is Highly Expressed in Roots, Trichomes, Stems and the Seed Coat.

(A) A qPCR analysis of *UUAT1* transcript steady state levels in Arabidopsis organs and during silique development with days after pollination (DAP) indicated. For roots, stems, cauline and rosette leaves and flowers, the level of *UUAT1* expression was calculated relative to the *Ef1α* and *UBC9*. For developing seeds and siliques, the expression was calculated relative *to Ef1α* and a seed specific reference gene (At4g12590).

(B) GUS activity patterns in transgenic Arabidopsis plants expressing *ProUUAT1-GUS*. GUS activity was detected in the roots of young plants (4, 7 and 14 d-old), in differentiated trichomes (inset) and in epidermal cells of developing seeds (8 DAP). Bars: left panels = 1 mm, right panel = 50 µm.

Supplemental Table 1. Percentage of Protein Identity among UUATs Family Members.

	UUAT1/At5g04160	UUAT2/At3g1090	UUAT3/At5g05820	UUAT4/At3g11320	UUAT5/At1g12500
UUAT1/At5g04160		81	69	70	49
UUAT2/At3g1090	81		59	60	49
UUAT3/At5g05820	69	59		95	52
UUAT4/At3g11320	70	60	95		53
UUAT5/At1g12500	49	49	52	53	

BLAST-based percentage of identical amino acids located at the same position in the consensus sequence among the UUATs family members.

Supplemental Table 2. Sugar Composition of Soluble Mucilage from WT Col-0 and *uuat1* Allelic Lines.

Sugar (mg/g dry seeds)	WT Col-0	*uuat1-1*	*uuat1-2*	*uuat1-3*
GalA	6.37 (0.07)	5.61 (0.1)*	5.81 (0.07)*	6.05 (0.27)*
Rha	9.81 (0.43)	9.59 (0.08)*	7.15 (0.31)*	8.52 (0.25)

Sugar content in soluble mucilage was determined using HPAEC-PAD. Values are means (± SE) of 3 biological replicates with 2 technical replicates for each. * shows significant differences from WT using the ANOVA test ($p < 0.05$).

Supplemental Table 3. Sequences of Primers Used in this Study

Primers for genotyping (5' to 3'):	
uuat1-1For	TCTCTCTCATTCTCTCAACG
uuat1-1 Rev	AAATCGGGAATTTGAATCCG
uuat1-2 For	GTGTCGTCATCGCCAGTGGG
uuat1-2 Rev	TAGATCAAGATCATACATAC
uuat1-3 For	ACTTAATTTAATTAACAAGC
uuat1-3 Rev	TAGATCAAGATCATACATAC
Primers for expresion analysis (5' to 3'):	
UUAT1 Forward	CCCTCCAGGTTCTTGGAAATGCTA
UUAT1 Reverse	ATGGAGTACCCGCCAATTCCCATA
UUAT2 Forward	TACCCTGCAGGTCCTTGGAAAT
UUAT2 Reverse	CCGCCAATTCCCATCACCGTTA
UUAT3 Forward	TGCTCCTTCTGCCTGCTACTCTTA
UUAT3 Reverse	CTCCTTTGGCGTTTCCTAGCAC
UUAT4 Forward	TCAGTCCCTCAGGTAAACAAACAC
UUAT4 Reverse	GGTATTGCTTTCACGCATCCTTTG
UUAT5 Forward	CGGTAGAGCTCTCAAATCCGT
UUAT5 Reverse	AAGATACAAGCCGCCATTGGAG
EF1α Foward	ATGCCCCAGGACATCGTGATTTCAT
EF1α Reverse	TTGGCGGCACCCTTAGCTGGATCA
UBC9 Foward	GCTCTCACAATTTCCAAGGTGCTGC
UBC9 Reverse	AGGGCTCTTCCTTAAGGACAGTATTTGTG
At4g12590 Foward	TGGCATTGACTTGAGCACTGTCG
At4g12590 Reverse	TCGAGGTAGTGCCCATTCGTGCT
Primers for RT-PCR (5' to 3'):	
UUAT1 RT Foward	CCCAGATCAGAAAACATAACAACAATGTCGTCGT
UUAT1 RT Reverse	CTCACTTGCTAATGTCTGAGAGTTCATCTACTAAA
EF1αA4 Foward	ATGCCCCAGGACATCGTGATTTCAT
EF1αA4 Reverse	TTGGCGGCACCCTTAGCTGGATCA
Primers for cloning (5' to 3'):	
UUAT1 CDS Foward	CACCATGTCGTCGTCTGCGAAGAAAC
UUAT1 CDS Reverse	TCTAAATCTGCGTTTTGTCTCTC
UUAT1 Pro Foward	TGTCAGATTGTCAAGGACACT
UUAT1 Pro Reverse	TCTAAATCTGCGTTTTGTCTCTCC