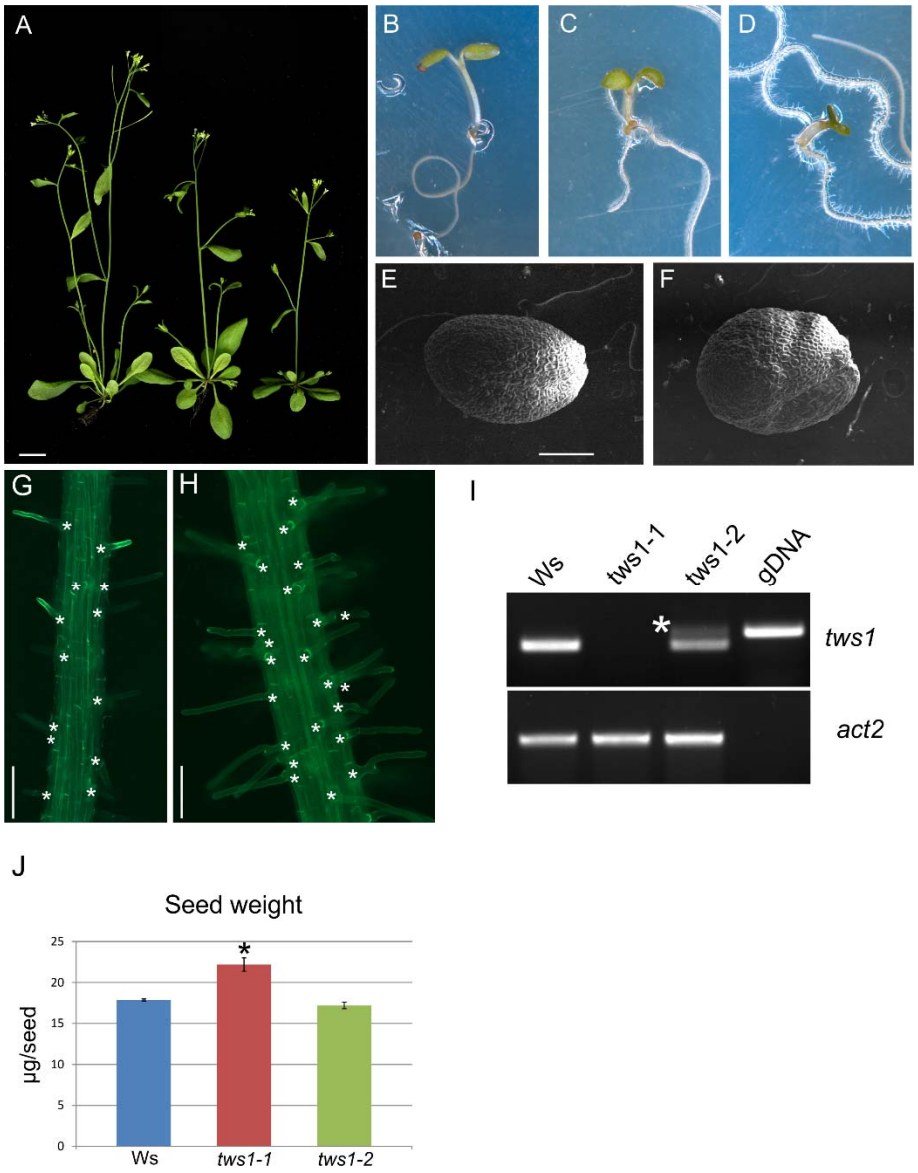
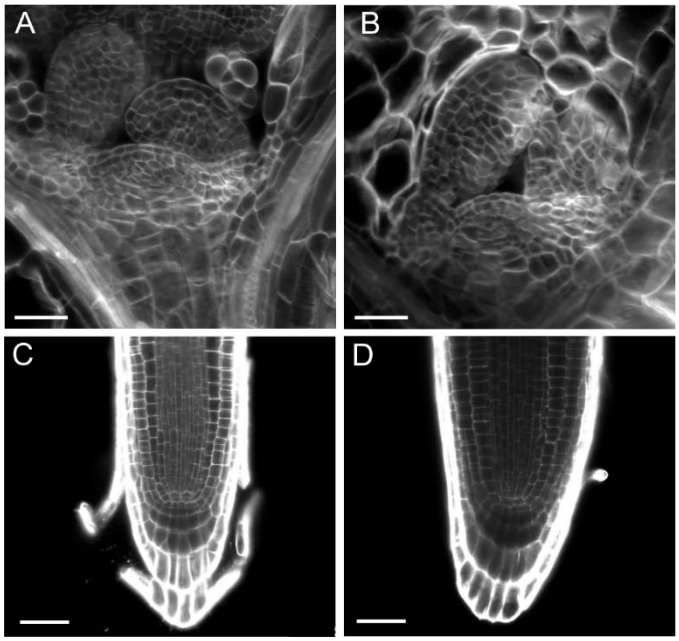
Supplemental Figures

Supplemental Figure 1. Phenotypic and molecular characterization of *tws1-1* and *tws1-2* mutant alleles **(A**) Wild-type **(left),** *tws1-2* **(center)** and *tws1-1* **(right)** 3-week-old bolting plants. Scale bar = 2cm. **(B)** to **(D)** A wild-type **(B),** a *tws1-2* **(C)** and a *tws1-1* **(D)** 7-day-old seedling grown *in vitro*. **(E)** and **(F)** SEM images of a wild-type **(E)** and a *tws1-1* **(F)** mature dry seed. Scale bars = 100µm. **(G)** and **(H)** autofluorescence images of wild-type **(G)** and *tws1-1* **(H)** roots. Stars indicate root hairs base. bars = 40µm. **(I)** RT-PCR run with mRNA extracted from seeds. *TWS1* amplification: 30 cycles, *ACT2* amplification: 25 cycles. **(J)** Seed weight measurements. * indicates values significantly different from wild-type (n = 6, Student's t test, $P < 0.05$)



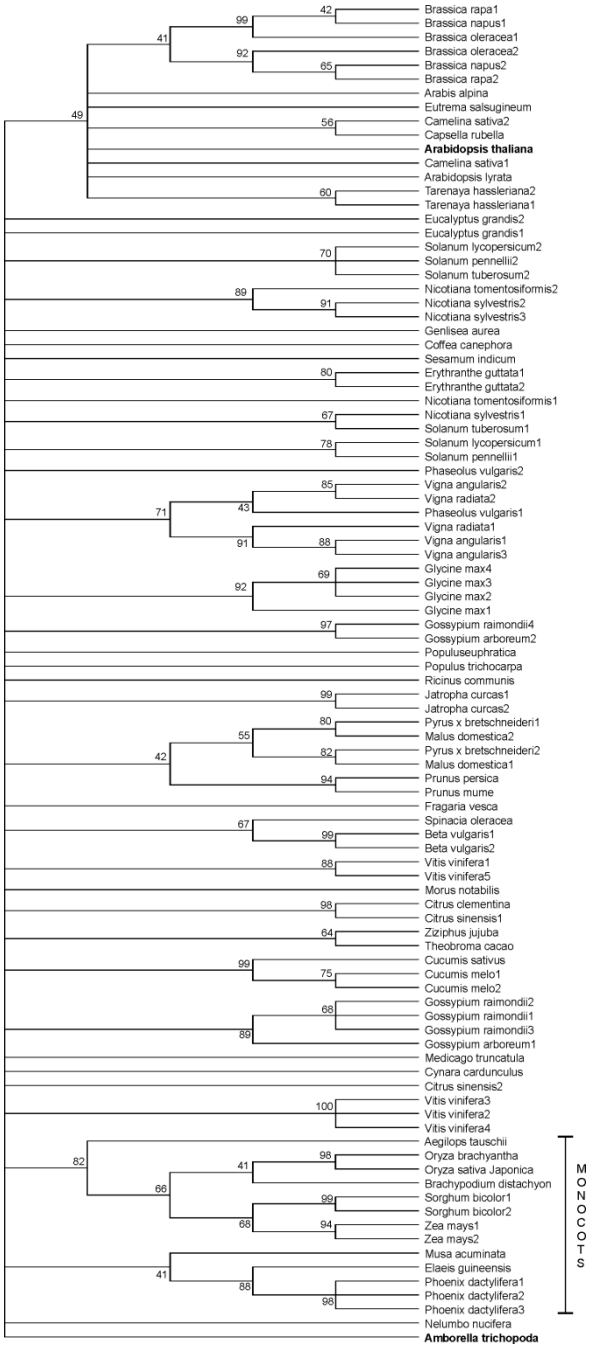
Supplemental Figure 2. *tws1-1 s*hoot and root apical meristem morphology

(A) Wild-type and **(B)** *tws1-1* confocal microscopy images of propidium iodide stained shoot apical meristems. **(C)** Wild-type and **(D)** confocal microscopy images of propidium iodide stained root apical meristems. Scale bars = 40µm.



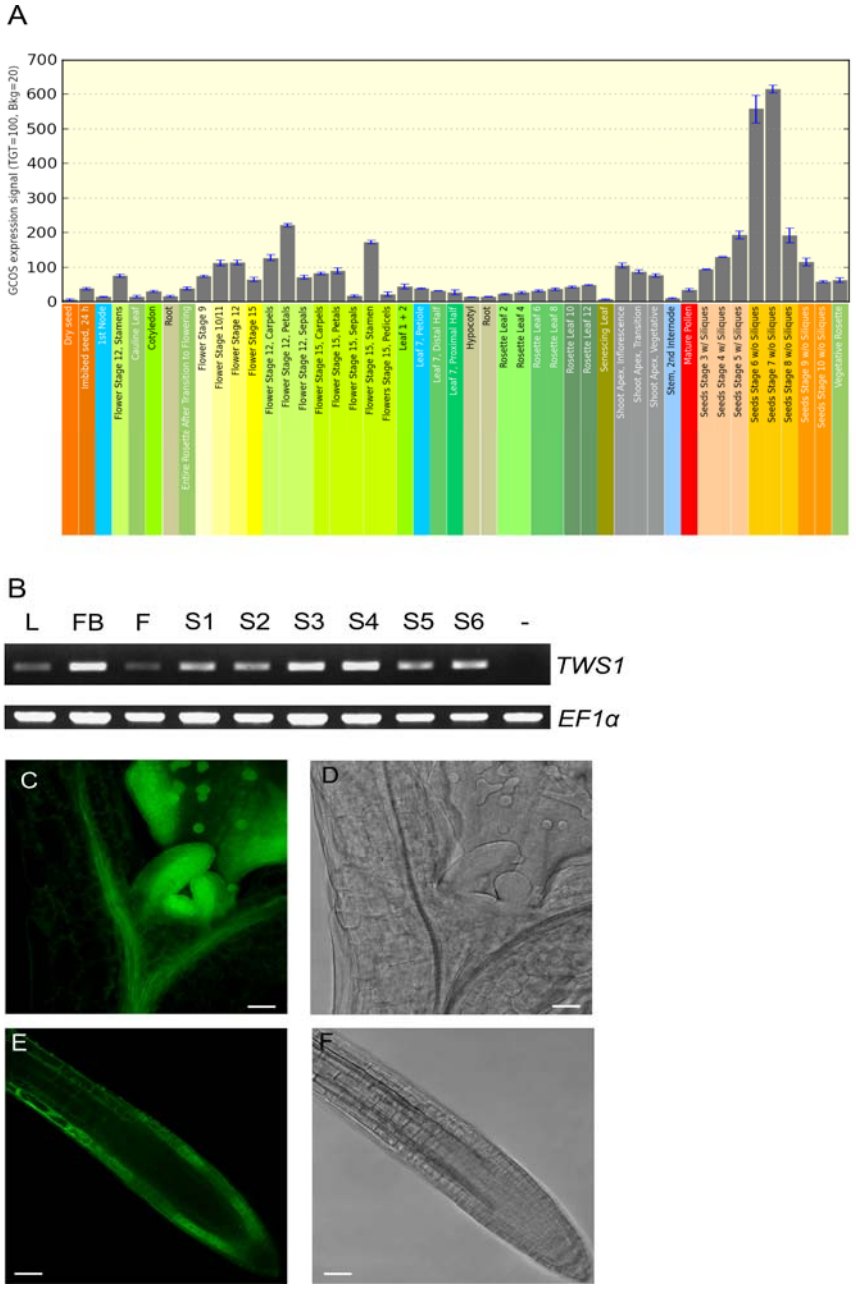
Supplemental Figure 3. TWS1 homologs in plants and their evolutionary relationships

The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. The analysis involved 96 amino acid sequences. All positions containing gaps and missing data were eliminated. There were a total of 40 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al., 2016).



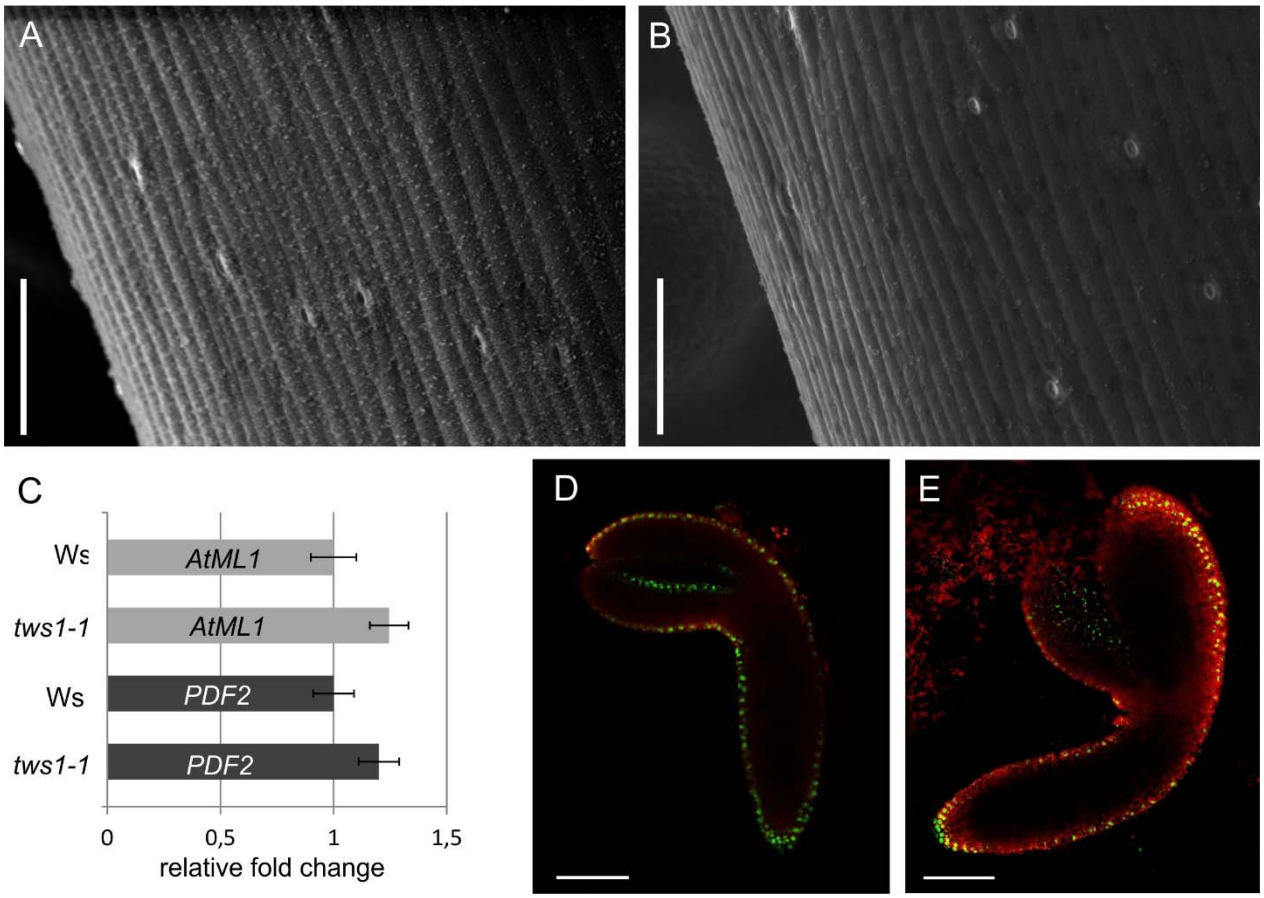
Supplemental Figure 4. *TWS1* expression

(A) Expression profiling of *At5g01075* during *Arabidopsis* development (http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi).**(B)** RT-PCR analysis performed on RNA extracts from different tissues fully expanded vegetative leaves (L), flower buds (FB), open flowers (F), and siliques containing embryos at different stages of development; early globular (S1), globular (S2), heart (S3) torpedo (S4) walking stick (S5) and mature (S6). **(C)** to **(F)** confocal microscopy images of transgenic plants expressing the *uidA-GFP* chimeric gene under the control of *TWS1* promoter. **(C)** GFP signal in the shoot apical meristem region of 7-day-old seedlings and **(D)** the corresponding bright field image. **(E)** GFP signal in the root tip of 7-day-old seedlings and **(F)** the corresponding bright field image. Scale bars = 40µm.



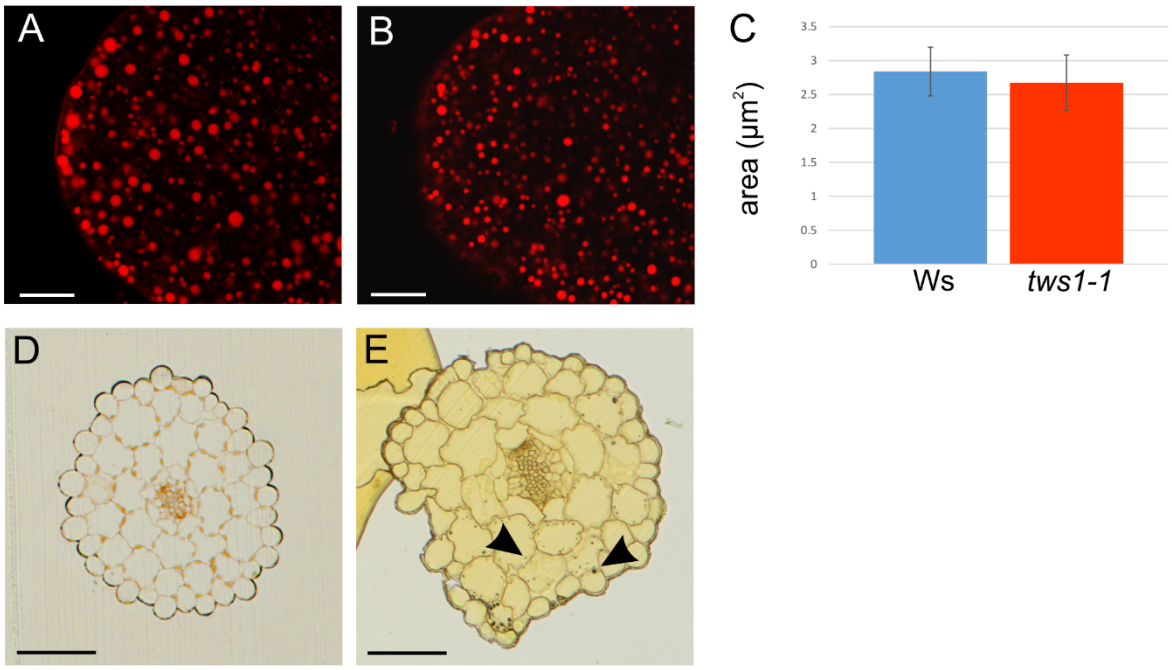
Supplemental Figure 5. Stem cuticle deposition defects and L1 markers analysis in *tws1-1*

SEM images of a wild-type **(A)** and a *tws1-1* **(B)** inflorescence stems. Scale bars = 100µm.
(C) *TWS1* mRNA accumulation in siliques of wild-type (WS) and *tws1-1* plants determined by real-time quantitative RT-PCR. Graph values are means of three biological replicates ± SE. The experimental values do not show statistically significant difference (Student's t test) between wild-type and mutant.
(D) and **(E)** confocal images showing *proATML1::NLS:3xGFP* signal in wild-type (D) and *tws1-1* (E) walking stick embryos. Green: GFP; red: autofluorescence. Scale bars = 100µm.



Supplemental Figure 6. Oleosomes and starch granules visualization in *tws1-1*

(A) and **(B)** Confocal images of Red Nile stained wild-type **(A)** and *tws1-1* **(B)** embryo oleosomes. Scale bars = 10µm. **(C)** Oleosomes area of the largest cross section in Ws and *tws1-1* (n=60, The p-value is 0.387998. The result is not significant at $p < .05$). Measurements were taken on cotyledons of embryos at the torpedo stage.
(D) and **(E)** Lugols' stained longitudinal sections of wild-type **(C)** and *tws1-1* **(D)** hypocotyls. Due to their bending defect, *tws1-1* longitudinal sections are not perfectly oriented. Scale bars = 50µm.



Supplemental tables

Supplemental Table 1. Fatty acids composition of *tws1*-1 and *tws1*-2 mutant seeds. Table values are means of three biological replicates ± SE. Percentage variations are calculated referring to wild-type Ws measurements.

	Ws (µg/mg dry mass)	*tws1-1* (µg/mg dry mass)	*tws1-1* var	*tws1-2* (µg/mg dry mass)	*tws1-2* var
C16:0	23.1 ± 0.4	18.5 ± 0.2	-19.6%	20.5 ± 0.8	-11.2%
C18:0	10.3 ± 0.2	8.5 ± 0.2	-17.5%	9.3 ± 0.5	- 9.7%
C18:1	37.0 ± 1.3	32.1 ± 0.9	-13.5%	33.2 ± 2	-10.3%
C18:2	93.8 ± 1.1	80.9 ± 1.1	-13.8%	84.1 ± 3.5	-10.34%
C18:3	67.6 ± 1	54.8 ± 0.8	-18.9%	61.1 ± 2.7	-9.6%
C20:0	8.3 ± 0.1	7.1 ± 0.1	-14.5%	7.7 ± 0.3	-7.2%
C20:1	76.7 ± 1.3	59.7 ± 0.7	-22.2%	68.4 ± 3.2	-10.8%
C22:1	9.2 ± 0.1	7.8 ± 0.1	-15.2%	7.3 ± 1.5	-20.7%

Supplemental Table 2. Primers used in the experimental procedures.

Purpose	Name	Sequence
Cloning		
Promoter region	TWS1prom_attbfw	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTGAATGAAAGTTGTGTTTTGAC-3'
	TWS1prom_attbrv	5'-

		GGGGACCACTTTGTACAAGAAAGCTGGGTCTTCTTCTCTAGAGATTGAG-3'
Coding sequence	TWS1atg _attbfw	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTATGAAGACGTCAAGCTTTGTC-3'
	TWS1no_stop_attbrv	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTTCCACCGTCTTGGGATTGCT-3'
	TWS1stop_attbrv	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTTTATCCACCGTCTTGGGATT-3'
In situ probe	TWS1insituF	5'-CTCTTCCTCAATCTCTAGAGAAGA-3'
	TWS1insituR	5'-TTCACGGGGCAAATAAATCAGA-3'
PCR		
RT-PCR	TWS1_RT_fw	5'-GACGTCAAGCTTTGTCTTCCT-3'
	TWS1_RT_rv	5'-CGTCTTGGGATTGCTGTGG-3'
	Act2_fw	5'-GTTAGCAACTGGGATGATATGG-3'
	Act2_rv	5'-CAGCACCAATCGTGATGACTTGCCC-3'
qPCR	qPDF2_fw	5'-GATCAGTGCCTTGAAGGAAA-3'
	qPDF2_rv	5'-ATCCTATCAATCTCTTCGCG-3'
	qATML1_fw	5'-TGGGATATACAGGCAGAAGA-3'
	qATML1_rv	5'-CTCTCATGTTGTGCCTTCAT-3'
	qTub4Fw	5'-GGTCAATACGTCCGGGATTC-3'
	qTub4Rv	5'-TCTGACCGAACGGACCAGAT-3'