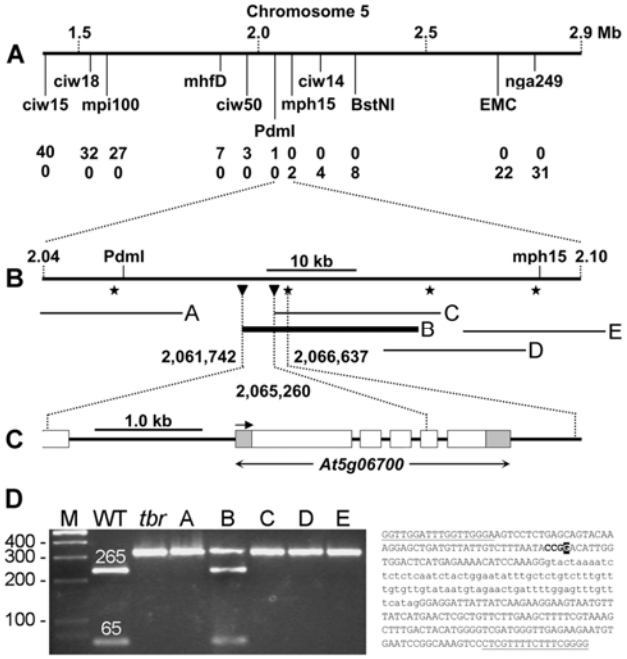
SUPPLEMENTAL DATA

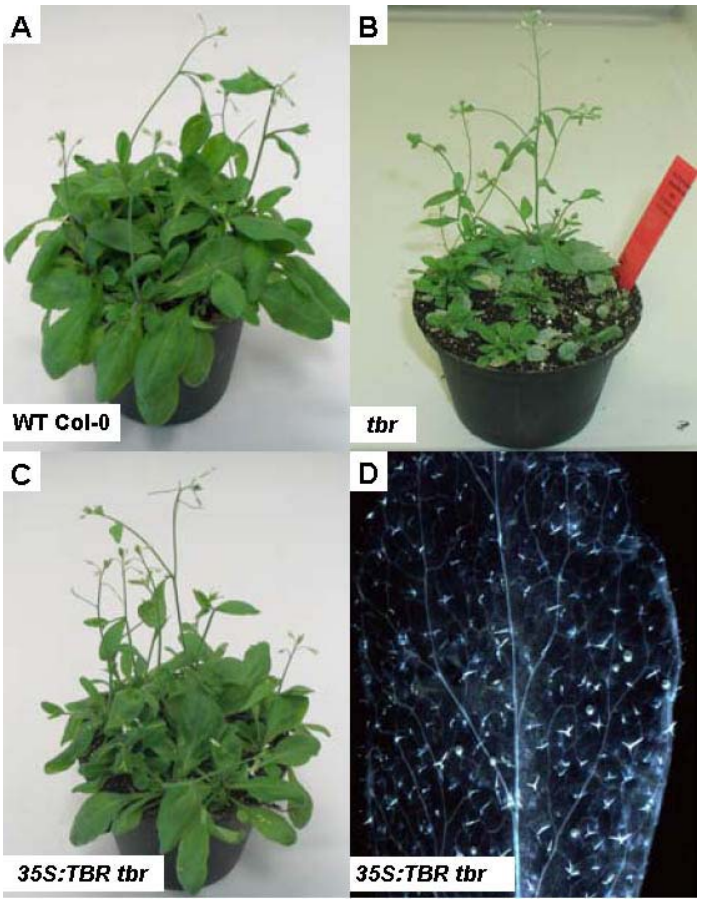
Supplemental Figure S1. Growth Phenotype of *tbr* mutants

Supplemental Figure S2. Identification of *TBR* by recombinational mapping and cosmid complementation.



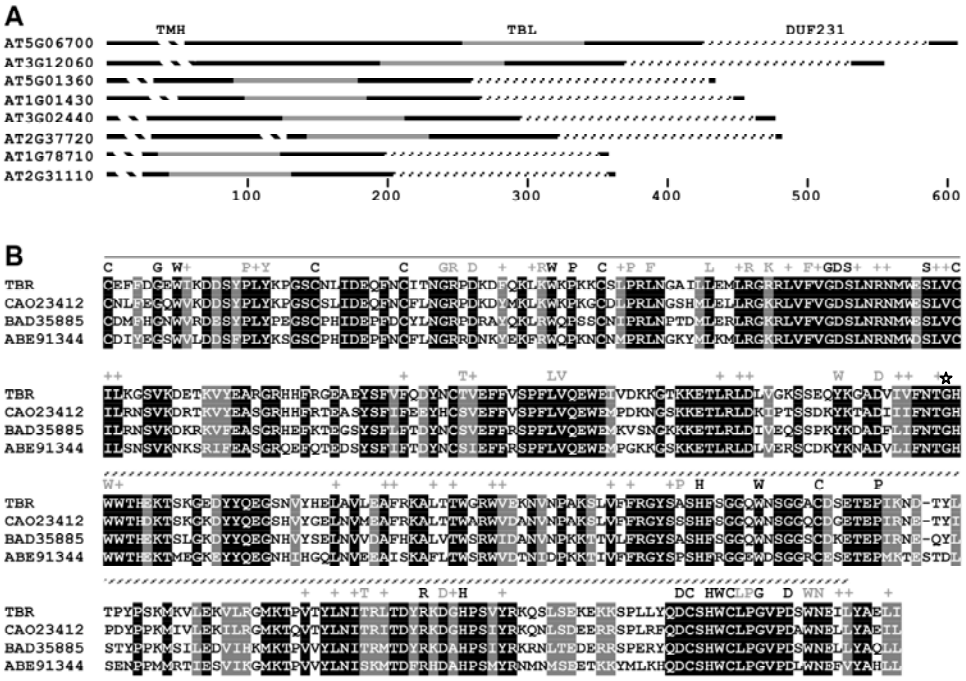
(A) Representation of Arabidopsis chromosome 5 in the region between 1.4 and 2.9 megabases (Mb), showing the positions of PCR-markers used for mapping. The number of recombination events (meiotic breakpoints) found for each marker in a total of 2496 examined chromosomes is given. Recombination events upstream or downstream of *TBR* are shown in the upper or lower row, respectively. **(B)** View of the mapping interval determined by flanking dCAPS marker mpi70 (CER482882) and SSLP marker mpi80 (CER482935). The positions of hybridization probes for cosmid library-screening are shown as asterisks. Complementing and non-complementing cosmid clones are depicted as bold and thin vertical lines, respectively, and labelled with A-E. Sequenced cosmid ends are indicated with triangles. **(C)** View of the ~5kb region responsible for complementation of the *tbr* phenotype. The structure of the one annotated gene (*At5g06700*) in that region is given. Exons are depicted as white boxes and untranslated regions of *At5g06700* i.e. *TBR* as grey boxes. The arrow indicates the direction of transcription. **(D)** The *tbr* point mutation is detectable by PCR using a co-dominant CAPS marker that exploits a *HpaII* restriction site present in the wild-type (giving 265 and 65 bp fragments) but absent in the *tbr* gene sequence. Primary *tbr* transformants that received genomic wild-type cosmid clone B invariably show a heterozygous PCR genotype. CAPS marker primer sequences are underlined and exon sequence is shown in capital letters. The *HpaII* site is shown in bold and the *tbr* polymorphic nucleotide with black background.

Supplemental Figure S3. Complementation of *tbr* mutant by 35S::TBR.



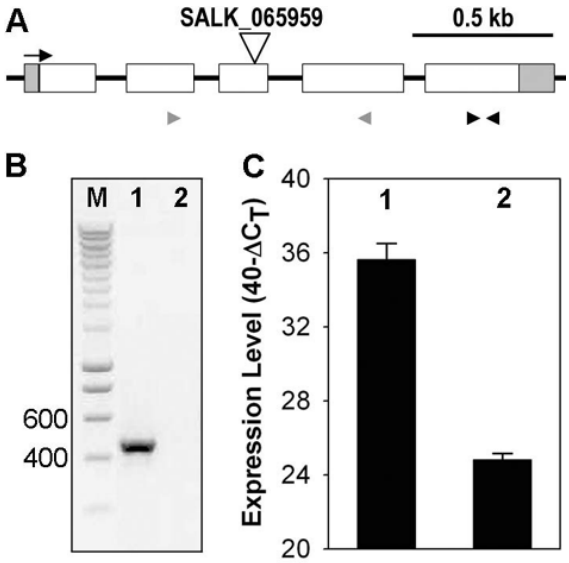
(A) Uniform growth aspect of wild-type Col-0 plants and **(B)** variable growth aspect of *tbr* mutants. **(C, D)** Wild type-like growth and trichome birefringence of *tbr* mutants transformed with a 35S::*TBR* construct.

Supplemental Figure S4. Structure and sequence alignment of DUF231 domain proteins



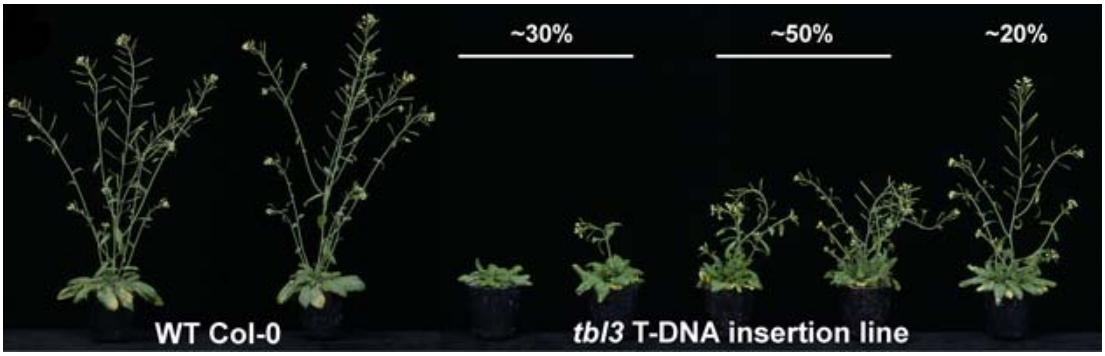
(A) Lengths and domain structure of TBR and a selection of seven additional Arabidopsis TBL proteins. Grey and fine crosshatched areas represent TBL and C-terminal DUF231 domains, respectively. Coarsely crosshatched areas depict putative transmembrane regions (TMH). Numbers below give the lengths in amino acids. **(B)** Partial protein sequence alignment of TBR and its closest grapevine (CAO23412), rice (BAD35885) and barrel medic (ABE91344) homologs. The alignment was created with ClustalW and Boxshade 3.21. Grey and hatched lines above the alignment represent TBL and DUF231 domains, respectively. Amino acids that are identical or similar in all four sequences are shaded. Amino acids that are 100% conserved in all 46 Arabidopsis TBL sequences are shown in black font above the alignment. Positions with similar amino acids in all 46 Arabidopsis sequences are marked with grey font symbols: an amino acid letter is given if this amino acid is present in more than 80% of the sequences, otherwise + is shown. The glycine residue changed in the *tbr* mutant is marked with a star.

Supplemental Figure S5. Molecular Characterization of *tbl3* T-DNA Insertion Mutants.



(A) *TBL3* (*At5g01360*) gene structure. Exons are depicted as white boxes and untranslated transcript regions as grey boxes. The arrow indicates the direction of transcription. The annotated T-DNA insertion site in SALK_065959 is shown as white triangle and primer pairs used for PCR amplifications are shown as grey and black triangles. **(B)** PCR amplification from (lane 1) wild-type and (lane 2) *tbl3* mutant cDNA with primers (grey triangles in (A) spanning the annotated insertion site. 35 PCR cycles were performed. The expected 461 bp product is amplified only from wild-type cDNA. A molecular weight marker (M) is shown to the left. **(C)** Quantitative RT-PCR amplification from (1) wild-type or (2) *tbl3* mutant cDNA with primers amplifying a 61 bp product located downstream of the T-DNA insertion site in the last exon. The expression level is given on a logarithmic scale expressed as 40- C_T, where C_T is the difference in qRT-PCR threshold cycle number between *At5g01360* and the reference gene (*UBQ10*; *At4g05320*); 40 therefore equals the expression level of *UBQ10*; the number 40 was chosen because the PCR run stops after 40 cycles. The fold difference in expression is 2^{CT} when product doubles in each cycle, e.g.: an ordinate value of 36 represents a ~1000-fold higher initial abundance than a value of 26. The results are the mean ± deviation of two biological replicates (pooled material from different growth experiments) with two technical replications for each.

Supplemental Figure S6. Growth Phenotype of *tbl3* mutants

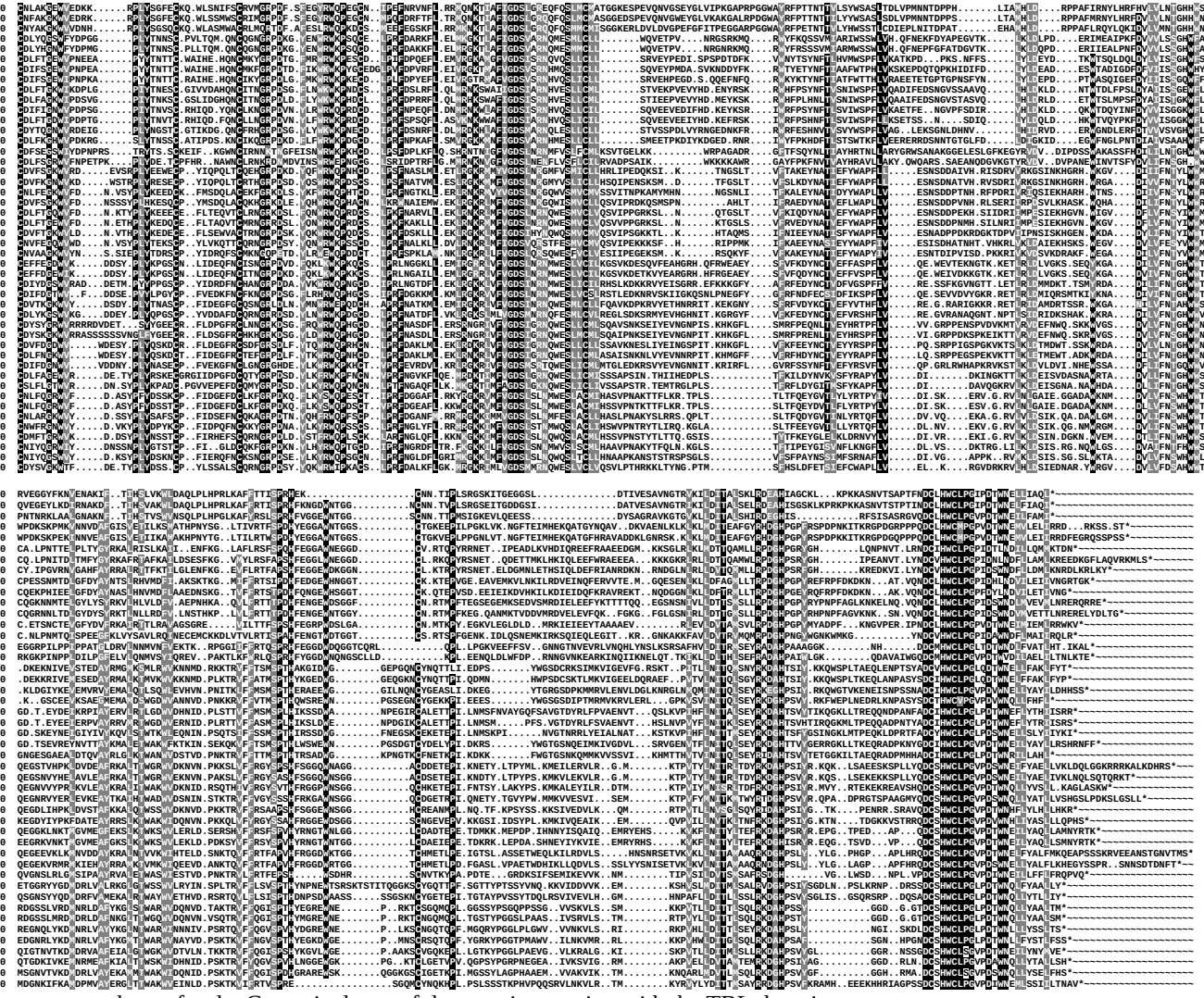


Supplemental Table S1. Specifications and primer sequences of mapping markers and the *tbr* CAPS marker.

Marker Name & Type	Chr. 5 Position (Mb)	BAC Clone	Polymorphism	Primer Sequences (5' → 3')	Product Length (bp) Col / *Ler* / *tbr*	$[Mg^{2+}]$ (mM) T_a (°C)
ciw15 SSLP	1.409	MUK11	INDEL55	TCCAAAGCTAAATCGCTAT CTCCGTCTATTCAAGATGC	177 / 122	2.0 / 55
ciw18 SSLP	1.531	K2A11	INDEL6	AACACAACATGGTTTCAGT GCCGTTTGTCTCTTCAC	135 / 129	2.0 / 53
mpi100	1.577	K18I23	CER454290 INDEL19	AGAACTAAGAGTAGACACGCACT CTGATCCCAAGCCTGTATAT	175 / 156	2.5 / 55
nga158 SSLP	1.698	MJJ3	INDEL4	ACCTGAACCATCCTCCGTC TCATTTTGGCCGACTTAGC	108 / 104	2.5 / 55
MHFD SSLP	1.899	MHF15	INDEL	AAAAACCCAAACTTTCTATTTATAC ACTTCGCTTCAAGTAAAGAGG	124 / ~114	2.5 / 55
mpi50 SSLP	1.968	MHF15	CER455774 INDEL52	CAACATCAATGGTCTTAGTC GATGCACAGAAAGGTCATG	429 / 377	2.5 / 55
mpi70 dCAPS	2.049	F15M7	CER482882 SNP G/T (PdmI)	ATTTTTGTTTGGCGATTGAG CAACTCTTTTCTGCAGAAAAGTTT	128, 20 / 148	2.5 / 52
tbr CAPS	2.065	MPH15	*tbr* SNP G/A (HpaII)	GGTTGGATTTGGTTGGGA GCCCCGAAAGAAAACGAG	265, 65 / 330	2.0 / 50
mpi80 SSLP	2.095	MPH15	CER482935 INDEL12	ACGCCTCATATGCACATTAAC AGCAACTAGTCGAATCGCTTAG	205 / 193	2.5 / 50
ciw14 SSLP	2.174	MOJ9	INDEL	CATGATCCATCGTCTTAGT AATATCGCTTGTTTTTGC	179 / ~160	2.2 / 55
mpi60 CAPS	2.271	T28J14	CER476482 SNP G/A (BstNI)	GTGAACTGTGCAGGCATAAG CACAAGATTGCATCCTTTGAC	291, 163 / 454	2.0 / 50
EMC SSLP	2.666	F8L15	INDEL4	AACAGATCGGAAAATCGTCG AATGACGACGAGACGCTCTT	131 / 135	2.5 / 50
nga249 SSLP	2.770	MAH20	INDEL10	GGATCCCTAACTGTAAAATCCC TACCGTCAATTTCATCGCC	125 / 115	2.5 / 50

Mb: megabase; bp: base pair; Col: wild-type Columbia-0; L*er*: wild-type Landsberg *erecta*; Ta: annealing temperature used for PCR amplification. Mismatched nucleotides in dCAPS marker primers are underlined.

Supplemental Table S2. Partial Alignment of the 46 Arabidopsis DUF231 proteins.



Alignments are shown for the C-terminal part of the proteins starting with the TBL domain.

Supplemental Table S3. Genes co-expressed with *TBR* or *TBL3*

Genes co-expressed with *TBR*, ranked by r-value (>0.7) [a]		
Rank	**Arabidopsis Gene ID**	**Description**
1	At1g05850	chitinase-like protein 1 (CTL1)
2	At5g09870	CESA5
3	At3g49220	pectinesterase family protein
4	At3g23820	NAD-dependent epimerase/dehydratase
5	At5g52060	BAG domain-containing protein
6	At3g02250	expressed protein
7	At5g64740	CESA6 (PRC)
8	At3g23050	auxin-responsive protein (IAA7)
9	At1g12500	phosphate translocator-related
10	At5g60920	COBRA phytochelatin synthetase
11	At5g05170	CESA3 (IXR1)
12	At1g72180	LRR transmembrane protein kinase
13	At1g29670	GDSL-motif lipase/hydrolase family protein
14	At2g35860	fasciclin domain-containing protein
15	At4g27430	CIP7
16	At2g39010	putative plasma membrane aquaporin
Genes co-expressed with *TBL3*, ranked by r-value (>0.75) [a]		
1	At5g40020	pathogenesis-related thaumatin family protein
2	At5g54690	GAUT12, IRX8
3	At3g16920	chitinase-like glycoside hydrolase family 19 protein
4	At3g62020	germin-like protein (GLP10)
5	At5g17420	CESA7 (IRX3)
6	At5g60720	expressed protein, contains DUF547
7	At1g27380	RIC2
8	At5g44030	CESA4 (IRX5)
9	At4g18780	CESA8 (IRX1)
10	At5g15630	IRX6, COBL4 phytochelatin synthetase
11	At2g38080	IRX12, LAC4
12	At5g01190	similar to laccase
13	At4g08160	glycosyl hydrolase family 10 protein
14	At5g03170	FLA11
15	At2g37090	IRX9, GT43 glycosyltransferase-like
16	At5g60020	LAC17
17	At3g18660	glycogenin glucosyltransferase (glycogenin)-related
18	At3g50220	expressed protein, contains DUF579
19	At1g58370	similar to (1,4)-beta-xylan endohydrolase (GH10)
20	At1g79620	LRR transmembrane protein kinase, putative
21	At2g41610	expressed protein
22	At4g27435	expressed protein
23	At5g45970	Rac-like GTP-binding protein (ARAC2)

(a) Results and r-values were obtained from GeneCAT (Mutwil *et al.*, 2008).

Protocol S1: Positional cloning of the *tbr* locus

To obtain a high-resolution map position for the *TBR* locus, 96 *tbr* mutant plants were selected from the F2 progeny of a cross of the *tbr* mutant and the *Landsberg erecta* ecotype, and a mixed DNA sample from these *tbr* mutant F2 plants was prepared and scored with a series of co-dominant PCR-based markers (Lukowitz *et al.,* 2000). This bulked segregant analysis placed the *tbr* mutation on the top of chromosome 5 between simple sequence length polymorphism (SSLP) markers ciw13 (@ 1.00 Mb) and nga249 (@ 2.77 Mb) (Fig. S2). More than 1200 individual DNA samples from homozygous *tbr* mutant plants from the segregating F2 mapping population were subsequently scored for recombination events between markers ciw15 and nga249, resulting in the identification of 40 lines with a recombination event between ciw15 and *tbr* and 31 lines with a recombination event between *tbr* and nga249 (Fig. S2). By analyzing ten additional SSLP, cleaved amplified polymorphic sequence (CAPS) and derived CAPS (dCAPS) markers (Table S1), the interval containing the *TBR* locus was narrowed to a 46 kb genomic region between markers mpi70 and mpi80 (Fig. S2). A set of five cosmid clones (named A-E in Fig. S2) covering the 46 kb interval was identified from a genomic Col-0 cosmid library constructed in the binary vector pBIC20 (Meyer *et al.,* 1994) using colony hybridization with four sequence-specific DNA probes (the positions are marked with asterisks in Fig. S2), and multiplex PCR. The five cosmid clones were individually introduced into *tbr* mutants via *Agrobacterium tumefaciens*–mediated transformation. Kanamycin-resistant progeny of *tbr* mutants transformed with cosmid clone B were complemented for the *tbr* trichome birefringence and growth phenotypes, as judged by the occurrence of bright birefringence and the wild-type growth aspect in 3/4 of the T2 plants. By contrast, the progeny of *tbr* mutants transformed with cosmid clones A, C, D or E still had the typical *tbr*-like dark trichomes under polarized light and retained their mutant growth aspect in the T2 generation, implying that these cosmid clones do not contain the wild-type *TBR* gene and hence cannot complement the *tbr* mutation.

Genetic mapping of the tbr locus

Linkage of the *TBR* locus to the top of chromosome 5, between simple sequence length polymorphism (SSLP) markers ciw13 and nga249, was established by bulked segregant analysis according to Lukowitz *et al.* (2000). Over 1200 *tbr* mutant-like plants were subsequently identified from the *tbr* x Landsberg *erecta* F2 mapping population, and for each plant, a DNA sample was prepared from inflorescence or leaf tissue using a quick alkaline-lysis protocol

(Lukowitz *et al.,* 2000). Co-dominant PCR markers used for fine mapping were identified (ciw14, ciw15, ciw18, mhfD, EMC, nga249) from the genetic maps provided by the Arabidopsis Information Resource (http://www.arabidopsis.org) or designed (mpi50, mpi60, mpi70, mpi80, mpi100) using the CEREON Genomics database of Col-0/Landsberg polymorphisms and dCAPS finder 2.0 (Neff *et al.,* 2002). Detailed information for the new markers is available in Supplemental Table S1. PCR conditions for SSLP markers were 50 mM KCl, 10 mM Tris-HCl, pH 9.0, at 25°C, 0.1% Triton X-100, 200 µM each of dATP, dGTP, dTTP, and dCTP, 10 pmol of each primer, 2.0 to 2.5 mM $MgCl_2$, 1 unit of *Taq* polymerase (Promega, Madison, WI), and 10 to 50 ng of genomic DNA, to a final volume of 22 µL. The PCR program was as follows: 1 min at 94°C; 40 cycles of 20 s at 94°C, 20 s at 50 to 55°C, and 30 s at 72°C; and 2 min at 72°C. Four percent agarose gels (3:1 HR agarose; Amresco, Solon, OH) were used to resolve SSLP markers for mapping.

Cosmid isolation and tbr mutant complementation

Using colony hybridization, according to Roche's DIG application manual (http://www.roche-applied-science.com), with four sequence-specific, digoxigenin-11-dUTP–labeled 301- to 505-bp DNA probes (the positions are marked with asterisks in Fig. S2), multiple clones corresponding to the 46kb *TBR*-containing mapping interval were isolated from a genomic Col-0 cosmid library constructed in the binary vector pBIC20 (Meyer *et al.,* 1994). A minimal set of five overlapping clones, covering the interval, was identified by multiplex PCR using the same primer pairs (sequences available on request) that amplified the hybridization probes. Mutant *tbr* plants were transformed by Agrobacterium tumefaciens (GV3101) carrying the various cosmid clones according to Clough and Bent (1998), and T1 transformants were selected on half-strength Murashige & Skoog agar plates containing kanamycin (50 µg/mL). Trichome birefringence of adult T1 transformants was analyzed as described above. Complementation was also achieved by PCR-amplifying a 2.45 kb genomic fragment comprising the entire TBR coding region with primers 5'–CAATGGATCCCAACCCTAAAACCACTCGTC-3' and 5'–CAATGTCGACAACCTCTCTTTGGAGCTAAAT-3', then inserting the fragment into the *BamHI* / *SalI* restriction sites of pBinAR (Bevan, 1984) followed by Agrobacterium tumefaciens (GV3101) mediated transformation of *tbr* plants, and kanamycin-selection of transformants. Furthermore, complementation of *tbr* mutants could also be largely achieved with a genomic PCR-fragment harboring the *TBL1* (*At3g12060*) gene driven by a 1.64 kb TBR promoter sequence (results not shown). The *TBL1* fragment was amplified with primers 5' –

GGAGATAGAACCATGGCGTTGGACTCCGTTA-3' and 5'–CAAGAAAGCTGGGTCTTAACTCCTATGATCTTTTAGG-3', inserted into pDONR207 (Invitrogen) before recombination with a pMDC32-derived GATEWAY™ destination vector (Curtis and Grossniklaus, 2003), named pVolki, in which the 2x 35S promoter was replaced by 1.64kb of *TBR* promoter sequences. The construct was transferred into *tbr* mutant plants by Agrobacterium mediated transformation (Clough and Bent, 1998), and transgenic plants were selected on hygromycin and analyzed for complementation by assessing *tbr* trichome birefringence.

DNA sequencing

Genomic DNA was prepared using a cetyl-trimethylammonium bromide-detergent extraction method (Lukowitz *et al.*, 2000) from Col-0 wild-type and *tbr* mutant plants. A 2.83-kb fragment encompassing the *TBR* (*At5g06700*) gene sequence, 172 bp 3'-UTR, the 151 bp 5'-UTR and 332 bp promoter region was amplified independently by PCR three times from each genotype using the primers 5'-ATTTCCGGATAATTTAGTTAGA-3' and 5'-ATATTGTATTCGTCGTGACA-3' and a mixture of *Taq* and proofreading *Pfu* polymerases (Promega). Cycle sequencing of both strands of the PCR products was performed by MWG Biotech (Ebersberg, Germany) with a set of eight additional primers (sequences available on request).

Protocol S2: Biochemical analyses of *tbr* and *tbl3* cell walls

Wild-type Col-0 and *tbr* mutant plants were grown on soil for five weeks. Trichomes were harvested from mature rosette leaves as described by Zhang and Oppenheimer (2004, crystalline cellulose measurement), or by shaving them from deep frozen leaves using a razor blade (HPLC analysis). Stems of wild-type Col-0 and *tbl3* mutant plants were harvested 10, 15 and 20 days after bolting. Crude cell wall extracts were prepared as described (Reiter *et al.*, 1993) with minor adaptations. Stem material from wild-type and *tbl3* mutants harvested between the first and second node. Plant material was harvested by snap freezing in liquid nitrogen, and then incubated twice during 60 min at 70°C in 70% ethanol. Pellets were suspended with chloroform:methanol (v/v; 1:1), washed in acetone for 2 min and vacuum-dried. Following suspension in 0.25 M sodium acetate-buffer (pH 4.0), samples were heated (20 min at 80°C) and then chilled on ice. After adjusting the pH to 5.0 with 1M sodium hydroxide, residual starch was removed by incubating the samples overnight at 37°C in 0.01% sodium azide containing amylase (50 µg / ml;

SIGMA, Germany) and pullanase (SIGMA, Germany). Samples were then boiled for 10 min, washed until no reducing sugars could be detected with anthrone reagent, and vacuum-dried.

The dry CW pellet was weighed into a 2 mL screw capped reaction tube suspended with 50 µg of *myo*-Inositol (internal standard) and hydrolized in 250 µL 2 M TFA at 121°C for 1 h. After allowing to cool down, the samples were diluted with 300 µL of 2-propanol and dried under a stream of nitrogen. The procedure was repeated three times. Adding 200 µL ddH2O, the samples were vortexed vigorously, sonicated for 10 min and centrifuged to remove residual TFA-hydrolyzed material. The supernatant was carefully separated from the pellet. 50 µL of the supernatant was reduced in 250 µL of reduction reagent (sodium borohydrate in 1 M ammonium hydroxide) at RT for 1 h. Reduction was terminated by adding 20 µL glacial acetic acid. The reduced samples were suspended with 250 µL of glacial acetic acid:methyl alcohol (v/v; 1:9) and dried under a nitrogen stream. The procedure was repeated three times followed by four evaporations with 250 µL of methyl alcohol. For acetylation, the reduced samples were suspended in 50 µL acetic anhydride and 50 µL pyridine and were incubated at 121°C for 20 min. Adding twice 200 µL of toluene the samples were evaporated, were suspended in 500 µL ddH2O and 500 µL methylene chloride and vortexed. The organic phase (containing per-O-acetylated alditols) was transferred to a new 2 mL reaction tube. The solution was evaporated shortly, suspended with 100 to 300 µL acetone and analyzed using a Agilent 6890 Series GC system equipped with a 5975B inert XL MSD and an SP-2380 fused silica capillary column (30 m 3 0.25 mm i.d. x 20 mm film thickness; Supelco).

50 µL of the TFA hydrolysis supernatant was used to determine uronic acid by a carbazole assay in which sulfamate and m-hydroxydiphenyl were used to completely eliminate interference by neutral sugars (Filisetti-Cozzi and Carpita, 1991). The absorbance was analyzed at 540 nm (Anthros Reader HT II, Eugendorf, Austria).

For the determination of crystalline cellulose, the insoluble TFA pellets (or crude CW material) were suspended with 1.5 mL Updegraff reagent (acetic acid:nitric acid:water; 8:1:2; v/v) and further incubated in a boiling water bath for 30 min. Crystalline cellulose was determined as described (Updegraff,1969; Scott and Melvin, 1953). Absorbance was determined at 620 nm (Anthos Reader HT II, Eugendorf, Austria).

Monosaccharides extracted from trichome cell walls by TFA (2M) hydrolysis were determined by high performance anionen exchange chromatography (HPAEC) as described in Neumetzler

(2010). Trichomes were harvested by leaf hair depilation (LHD) following the protocol described in Ebert *et al*. (2010). HPAEC of monosaccharides was performed on a DX 500 system (Dionex, Sunnyvale, CA, USA) equipped with a GP 50 gradient pump, a CarboPac PA20 Guard column (3 mm ID x 30 mm L) Dionex, Sunnyvale, CA, USA) and a subsequent CarboPac PA20 column (3 mm ID x 150 mm L) both from Dionex (Sunnyvale, CA, USA). Detection was carried out using a pulsed amperometric detector (PAD, Dionex, Sunnyvale, CA, USA). In brief, after equilibration neutral sugars were separated by an isocratic flow (0.15 ml/min) using 5 mM NaOH (solvent A) for 30 min. After raising the proportion of solvent B (800 mM NaOH) from 0% to 35% between 40 min and 50 min, it was further increased up to 100% from 50 min to 60 min before an isocratic flow of 100% solvent B from 60 min to 70 min were kept to separate uronic acids. The column was re-equilibrated by ramping solvent A from 0% to 100% between 70 min and 80 min. Afterwards a constant isocratic flow of 100% solvent A was kept until 100 min and before new samples were loaded. Assignments of all peaks were carried out according to retention times of pure standards.

Protocol S3: Pectin methylesterase (PME) extraction and activity assay.

Etiolated seedlings were snap-frozen in liquid nitrogen and homogenized to fine powder using a ball mill (1 min, 30 Hz, Retsch, Germany). PMEs were extracted by incubating the plant powder for 1h at 4°C and at constant shaking (600 rpm, Thermomixer comfort, Eppendorf, Germany) in 50mM Na_2HPO_4 extraction buffer (pH7) supplemented with 12mM citric acid, 1M NaCl, 0.01% (v:v) Tween20, 0.2% (w:v) polyvinylpyrrolidon (PVPP) and 5µl EDTA free protease inhibitor cocktail per 1ml extraction buffer.

The PME activity was assayed by the measurement of the released methanol. Therefore 10µl of the samples protein extract were added to 75µl Na-phosphate buffer (50mM, pH 7.5), 5µl Alcohol Oxidase (5U/ml, *Pichia pastoris*, Sigma, A2404), 10µl pectin (20 mg/ml solved in 50 mM Na-phosphate buffer, pH 7.5) containing either 34%, 65% or 89% of methylesters (Sigma: P-9311, P-9436, P-9561) and incubated for 20 min at 28°C (600 rpm, Thermomixer comfort, Eppendorf, Germany). The reaction was stopped by adding 100µl revelation buffer (2M ammonium acetate, 0.28% (v:v) acetic acid, 0.2% (v:v) 2,4-pentandione) and incubation for 10 min at 67°C. Absorbance was read at 420 nm and methanol release was calculated as in parallel a standard curve was performed using 0-20 mg/L methanol as sample. All measurements were performed in technical duplicates and indicated numbers of biological replicates.

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