Callose Synthase GSL7 Is Necessary for Normal Phloem Transport and Inflorescence Growth in Arabidopsis[1]

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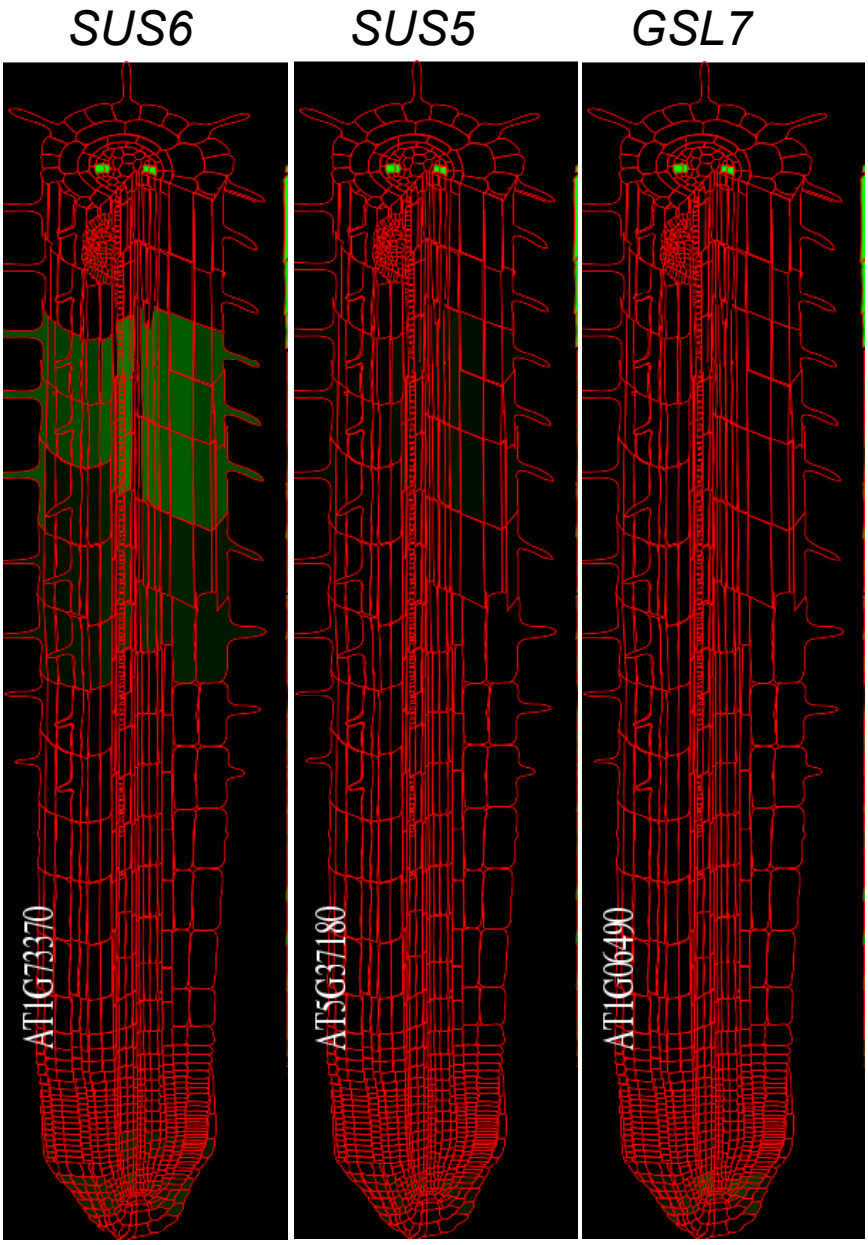
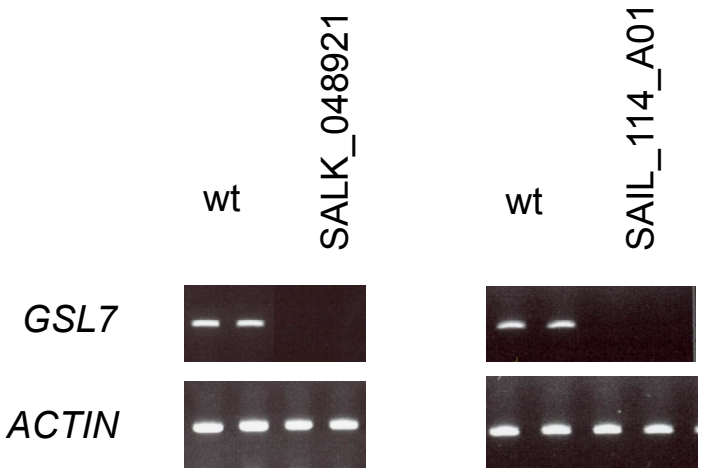


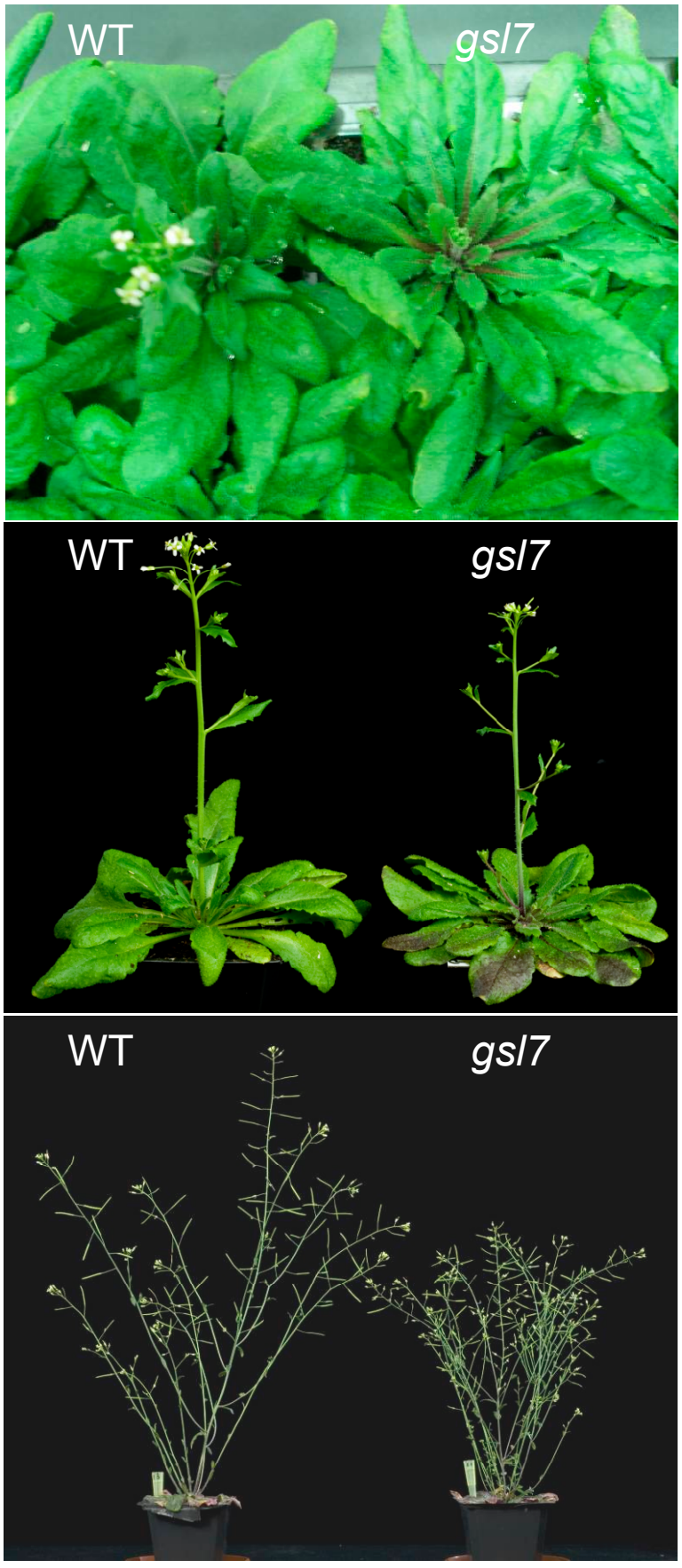
Supplemental Figure S1. Location in roots of transcripts for *SUS5*, *SUS6*, and *GSL7*.

Data are from the Deconvolved Root Expression Map Visualizer tool in the Arex database (http://www.arexdb.org/). Methods and cautions on interpretation are presented in Birnbaum et al. (2003), Brady et al. (2007), and Cartwright et al. (2009).



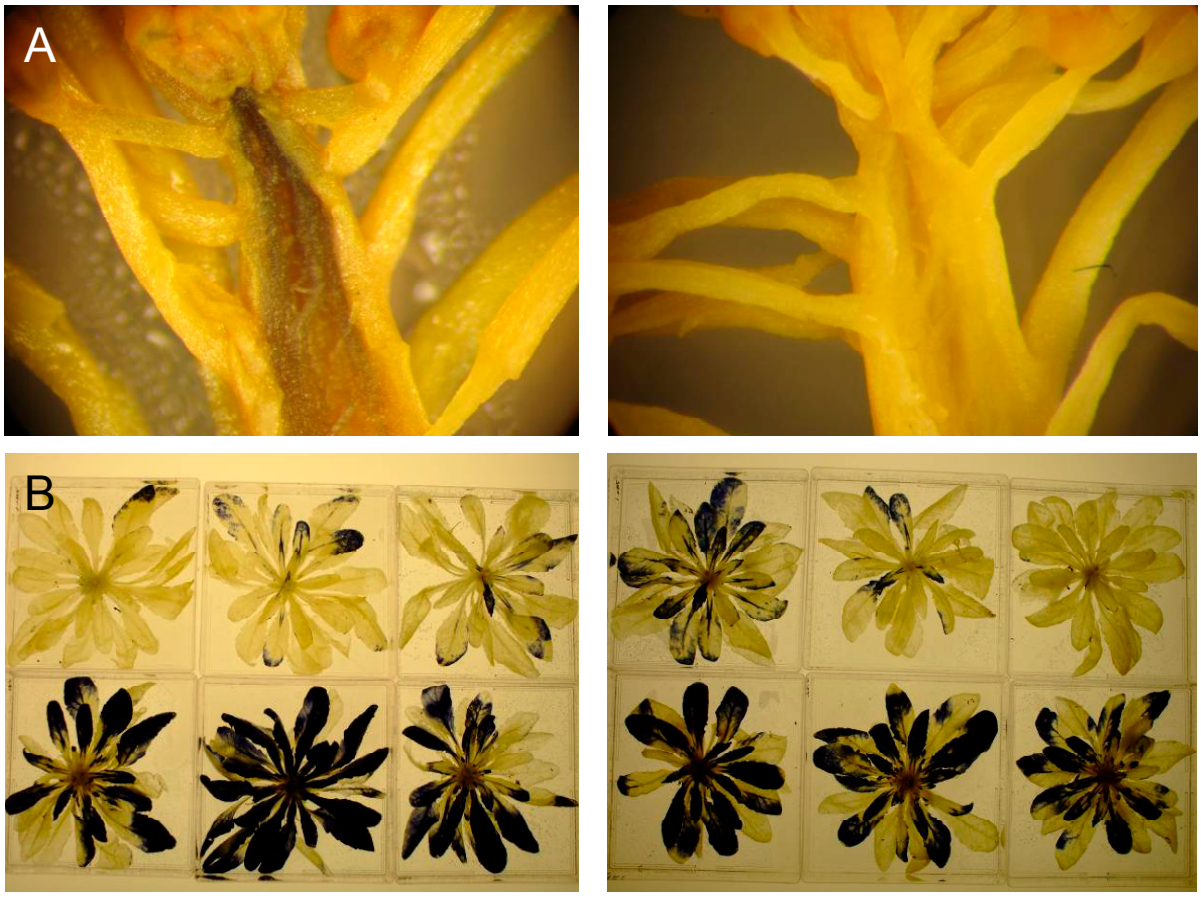
Supplemental Figure S2. Absence of *GSL7* transcript in two lines carrying T-DNA insertions in the *GSL7* gene.

Two T-DNA insertion lines were obtained from public collections. Wild-type (wt) lines were selected from the segregating F2 population from which mutants were selected. Total RNA was isolated from the basal 4 cm of flowering stems of 6 to 7-week-old plants. Two independent samples, each of two stem sections, were prepared for each line. First-strand cDNA was synthesised from 5µg of total RNA (Bieniawska et al., 2007). Primers for an *ACTIN* gene were used to verify the quality of the cDNA and to ensure equal loadings of cDNA templates. RT-PCR was performed using primers given in Supplemental Table S5. Amplification cycles of 24 and 30 were performed for *ACTIN* and *GSL7* respectively. Ten µL of each product were separated by electrophoresis on a 1% w/v agarose gel and stained with ethidium bromide.



Supplemental Figure S3. Growth of wild-type and *gsl7* mutant plants.

All plants were grown with 12 h light, 12 h dark. In each picture wild-type plants are on the left; *gsl7* plants are on the right. Top: onset of flowering. The *gsl7* plant has more purple pigmentation that the wild-type plant. Middle: plants at six weeks old. The flowering stem of the wild-type plant in 13 cm high. Bottom: plants at 8 weeks old.



Supplemental Figure S4. Loss of starch from the inflorescence and altered starch turnover in the leaves of the *gsl7* mutant.

Seven-week-old plants were harvested immediately before the end of the night. Samples were decolorized in ethanol and stained for starch with Lugol's iodine solution.

(A) The inside of the apex of the flowering stem of a wild-type (left) and a mutant (right) plant. The presence of starch in wild-type plants and its near absence in mutant plants at this time point was observed consistently on different batches of plants.

(B) Rosettes after removal of the flowering stem. Plants were washed in water for 24 h after iodine staining. The upper row is wild-type plants. In the lower row the left three plants are *gsl7-1* mutants; the right three plants are *gsl7-2* mutants.

Supplemental Table S1.

Top 20 Genes Co-expressed with *SUS5* (At5g37180), from Atted-II.

Data are from the *Arabidopsis thaliana* trans-factor and cis-element prediction database, http://atted.jp/.

Rank	MR	CORR	Locus	Gene description
1	3.5	0.52	At1g73370	SUS6 (SUCROSE SYNTHASE 6)
2	5.0	0.44	At1g06490	ATGSL07 (glucan synthase-like 7)
3	8.5	0.45	At3g58350	meprin and TRAF homology domain-containing protein / MATH domain-containing protein
4	19.3	0.43	At5g38770	AtGDU7 (Arabidopsis thaliana GLUTAMINE DUMPER 7)
5	19.8	0.43	At4g33810	glycosyl hydrolase family 10 protein
6	23.6	0.40	At3g18670	ankyrin repeat family protein
7	25.8	0.44	At4g24430	lyase
8	29.7	0.47	267343_at	At2g44255; At2g44260
9	33.7	0.43	At4g35970	APX5 (ASCORBATE PEROXIDASE 5)
10	36.9	0.42	At2g17940	unknown protein
11	38.1	0.38	At5g11990	proline-rich family protein
12	38.2	0.41	At2g27140	heat shock family protein
13	39.0	0.44	At1g02950	ATGSTF4 (GLUTATHIONE S-TRANSFERASE F4)
14	43.6	0.44	At4g15093	catalytic LigB subunit of aromatic ring-opening dioxygenase family
15	44.2	0.42	At3g08490	unknown protein
16	47.1	0.39	At5g56720	malate dehydrogenase, cytosolic, putative
17	52.5	0.36	At1g75720	unknown protein
18	56.5	0.43	At3g56230	speckle-type POZ protein-related
19	58.5	0.42	At5g03260	LAC11 (laccase 11)
20	60.5	0.39	At3g49070	unknown protein

MR: mutual rank, based on Pearson's correlation coefficient (see http://atted.jp/help/coex_cal.shtml). CORR: Pearson's correlation coefficirent.

When genes co-expressed with SUS6 are searched, ATGSL07 (glucan synthase-like 7) ranks 2 on the list, with a CORR value of 0.51, MR value 3.7.

Supplemental Table S2.

Top 40 Genes Co-expressed with *SUS5* (At5g37180), from CSB.DB.

Data are from AthCoR@CSB.DB - The *A.thaliana* Co-Response Database (http://csbdb.mpimp-golm.mpg.de/csbdb/dbcor/ath/ath_tsgq.html), searching "developmental series".

Rank	Spearman	Locus	Gene description
1	0.8033	At1g73370	SuSy / arab6 / SuSy1 [SUS6]
2	0.6673	At5g56540	arabinogalactan-protein (AGP14)|
3	0.6475	At1g76200	expressed protein
4	0.6465	At3g27200	plastocyanin-like domain-containing protein|
5	0.6384	At1g14240	atapy3-apyrase
6	0.6372	At1g52910	expressed protein
7	0.6306	At1g62990	homeodomain transcription factor (KNAT7)
8	0.626	At1g06490	glucan synthase, putative, AtGsl07
9	0.6247	At1g08340	rac GTPase activating protein, putative
10	0.6111	At3g13590	DC1 domain-containing protein
11	0.6103	At5g04470	expressed protein
12	0.5979	At3g14570	hypothetical protein, glucan synthase, putative, AtGsl04
13	0.5917	At5g04890	small heat shock-like protein (RTM2)
14	0.5903	At3g44730	kinesin motor protein-related
15	0.5889	At4g38470	protein kinase like protein protein kinase 6 (EC 2.7.1.-) – soybean, PIR2:S29851
16	0.5881	At3g21550	expressed protein
17	0.5874	At1g50890	expressed protein
18	0.5836	At3g22400	putative lipoxygenase similar to lipoxygenase GB:AAB67865 from [Solanum tuberosum]
19	0.5827	At3g03070	NADH-ubiquinone oxidoreductase-related
20	0.5817	253227_at	no agi hits found
21	0.5817	At1g26820	ribonuclease 3 (RNS3)
22	0.579	At2g35120	d glycine cleavage system H protein, mitochondrial, putative
23	0.5779	At5g65660	hydroxyproline-rich glycoprotein family protein
24	0.5761	At3g17420	serine/threonine protein kinase, putative
25	0.5757	At3g54860	vacuolar protein sorting protein 33a
26	0.5736	At3g52370	beta-lg-H3 domain-containing protein / fasciclin domain -containing protein
27	0.5718	At1g26450	beta-1,3-glucanase-related
28	0.5667	At1g80950	phospholipid/glycerol acyltransferase family protein
29	0.5664	At4g00230	subtilisin-like serine protease XSP1
30	0.566	At5g23170	serine/threonine protein kinase-like protein
31	0.5659	At2g02510	expressed protein
32	0.5648	At1g51540	kelch repeat-containing protein
33	0.5617	At1g03920	putative protein kinase
34	0.56	At5g53590	auxin-responsive family protein
35	0.5578	At5g55550	RNA recognition motif (RRM)-containing protein
36	0.5545	At4g18425	expressed protein| contains Pfam profile PF05078:
37	0.5539	At5g15490	b UDP-glucose 6-dehydrogenase, putative (UGD3)

38	0.553	At2g33310	auxin regulated protein (IAA13)
39	0.551	At5g65390	arabinogalactan-protein (AGP7)
40	0.5507	At3g21240	putative 4-coumarate:CoA ligase 2

When genes co-expressed with *SUS6* are searched, ATGSL07 (glucan synthase-like 7) ranks 2 on the list, with a Spearman correlation coefficient of 0.7548. ATGSL04 (glucan synthase-like 4) ranks 14 on the list, Spearman correlation coefficient of 0.6482.

Supplemental Table S3

Weights of rosettes, leaves and stem bases of wild-type and mutant plants.

	Wild-type		*gsl7* mutant	
	Fresh weight (g)	Dry weight[c] (g)	Fresh weight (g)	Dry weight[c] (g)
Rosette[a]	1.44 ± 0.31[d]	0.163 ± 0.032	1.22 ± 0.19[e]	0.140 ± 0.028
Root system[a]	0.37 ± 0.07	0.030 ± 0.004	0.32 ± 0.07	0.027 ± 0.004
Basal 4 cm of flowering stem[b]	0.137 ±0.012	n.d.[g]	0.099 ± 0.006[f]	n.d.

[a]Rosettes and root systems were harvested from plants grown with 12 h light, 12 h dark in a 1:1 mixture of sand and Terragreen medium (Oil-Dri, http://www.oildri.com) supplemented with a slow-release fertilizer. Plants were 35 days old at the time of harvest: flowering stems were approximately one cm high.

[b]Flowering stems were harvested from plants grown with 12 h light, 12 h dark in compost. Plants were 43 days old at the time of harvest

[c]Dry weights were determined after drying for 7 days at 50°C.

[d]Values are means ± SD of measurements on 12 plants for rosettes and root systems, and for 15 plants for flowering stems

[e]Mutant value statistically significantly different from wild-type value, P = 0.04, Student's t-test)

[f]Mutant value statistically significantly different from wild-type value, P = 3 x 10^{-11}, Student's t-test)

[g]n.d.: not determined

Supplemental Table S4.

Starvation marker genes used in this study.

Usadel et al. (2008) identified a set of specifically carbohydrate-responsive genes based on transcriptomic data from experiments in which plants were subjected to low carbon availability including sucrose starvation (Osuna et al., 2007), growth in low CO_2 levels (Bläsing et al., 2005) and extensions of the normal night period (Usadel et al., 2008). The analysis also included transcript profiles obtained from the *pgm* mutant that faces early carbon starvation during the night due to its inability to produce starch (Bläsing *et al.*, 2005). Consequently, the classification as carbohydrate-responsive is not based on gene-function but on a common transcriptional response to changes in carbohydrate availability. We previously quantified transcript levels of carbohydrate-responsive genes to monitor the onset of carbon-starvation following the exhaustion of starch reserves in Arabidopsis rosettes (Graf et al., 2010).

Transcripts of all five genes studied here show no or very little change in abundance during a normal diurnal cycle but are rapidly induced or repressed under conditions of low-carbon availability such as extended night periods (see cited references).

Gene	Effect of sugars on expression	Annotation
At1g10070	Repressed	Chloroplastic branched-chain amino acid aminotransferase (ATBCAT-2)
At1g76410	Repressed	RING/FYVE/PHD-type zinc finger protein (ATL8),
At1g08630	Repressed	Threonine aldolase (THA1)
At3g59940	Repressed	Kelch repeat-containing F-box family protein
At3g13470	Induced	Chaperonin

Supplemental Table S5.

Primers used in this study.

Experiment	Primer sequences
In situ hybridization: partial cDNA for *GSL7*	5'- ATGGCGAGTACTAGTAGTGGTGGAAGA-3' 5'-GGTCAACACGTTTCTAAAGACATCCTCAGTA-3'
In situ hybridization: T3 (sense) and T7 (antisense) promoter sequences for 150 bp probe	5'-AATTAACCCTCACTAAAGGGCTAGTAGTGGTGGAA-3'; 5'-TAATACGACTCACTATAGGGAAGAAGGAACAAGCT-3'
In situ hybridization: T3 (sense) and T7 (antisense) promoter sequences for 350 bp probe	5'-AATTAACCCTCACTAAAGGGATGGCGAGTACTAGT-3'; 5'-TAATACGACTCACTATAGGGGTAATCTCTTCCTCC-3'
Identification of homozygous T-DNA insertion lines: *gsl7* (At1g06490) SALK_048921	5'-GCTTTACCAGATAGCGACAGTCTTGTATGA-3'; 5'-GGTCAACACGTTTCTAAAGACATCCTCAGTA-3'
Identification of homozygous T-DNA insertion lines: *gsl7* (At1g06490) SAIL_114_A01	5'-CAGAGTGGTGATATTGAAAGCGACAAGAA-3'; 5'-CCTCCATGCAAAATCGTCCTTCCAAAGTAA-3'
Identification of homozygous T-DNA insertion lines: T-DNA primer for SALK lines	5'-GCGTGGACCGCTTGCTGCAACT-3'
Identification of homozygous T-DNA insertion lines: T-DNA primer for SAIL line	5'-TAGCATCTGAATTTCATAACCAATCTCGATACAC-3'
ACTIN2 (At3g18780) control for RT-PCR	5'-CTTCCTCAATCTCATCTTCT-3'; 5'-TTAACATTGCAAAGAGTTTCAAGGT-3'
Real-time quantitative PCR analysis: *UBIQUITIN10*	F5'- GGCTTGTATAATCCCTGATGAATAAG-3'; R5'-AAAGAGATAACAGGAACGGAAACATAGT-3'
Real-time quantitative PCR analysis: At1g76410	F5'-AGACGAGCTTAGGGTGTTGC-3'; R5'-CGCCACATTTATGACACCTG-3'
Real-time quantitative PCR analysis: At3g59940	F5'-TGGAACGATGATGGTGAAGA-3'; R5'-AACCAGAGGGAGTGTTGACG-3'
Real-time quantitative PCR analysis: At1g10070	F5'-ACCGGGGATGAATCTGTCT-3'; R5'-TCTGTGACCCATCCCTTGTT-3'
Real-time quantitative PCR analysis: At3g13470	F5'-CCCGACAAAGGTTGTGAGAT-3'; R5'-TACCAGCAGGAACTGGCTCT-3'
Real-time quantitative PCR analysis: At1g08630	F5'-CTCCTTATCCGGGGAAACTC-3'; R5'-GGTTCCTGCATCGTTAGCAT-3'