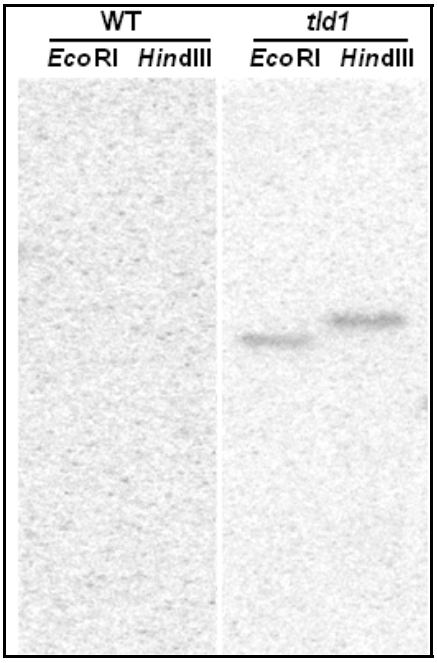
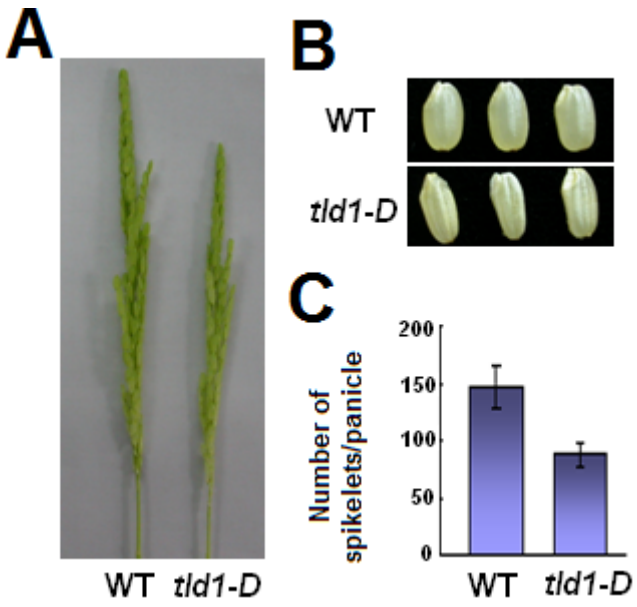
Supplemental data

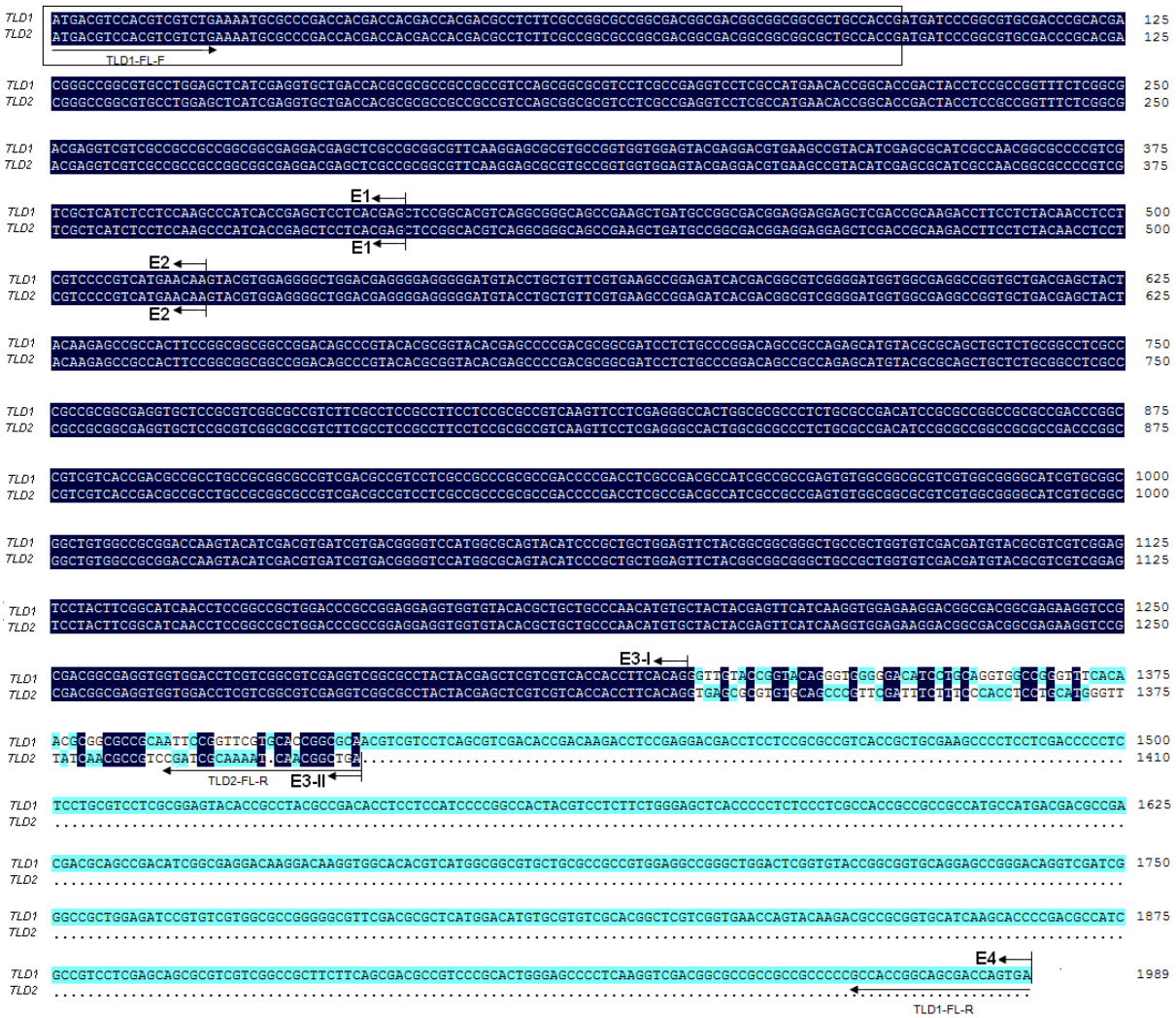
1. Supplemental Figures



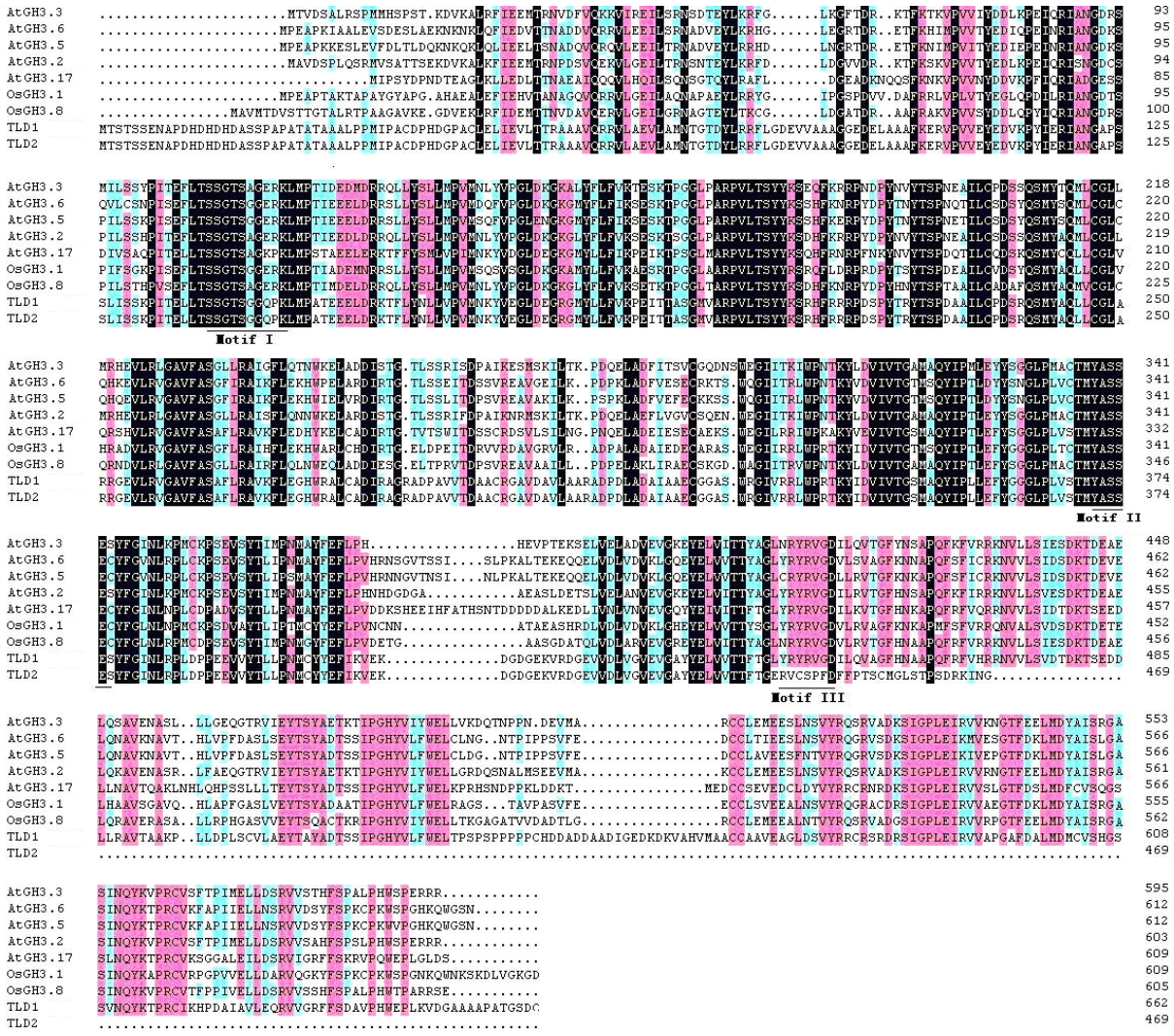
Supplemental Figure S1 Determination of the number of T-DNA insertions in *tld1-D* by Southern blotting. Genomic DNA isolated from wild-type (WT) or homozygous *tld1* plants was digested with *Eco*RI or *Hin*dIII. The blot was probed with α-^{32}P-dCTP-hygromycin coding sequence. A single band was detected in the *tld1* mutant, while no band was detected in WT.



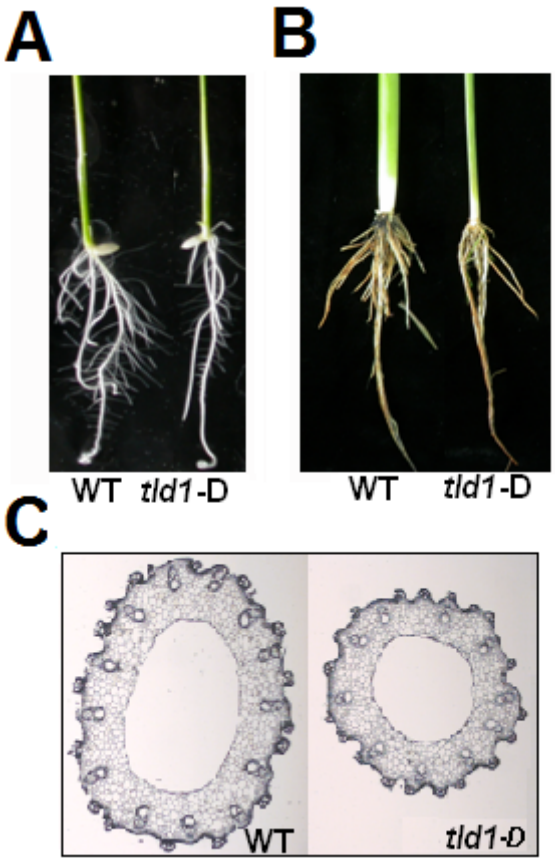
Supplemental Figure S2 Productivity of WT vs. *tld1-D*. (A) Representative panicles from WT and *tld1-D* plants. (B) Mature seeds from WT and *tld1-D* plants. (C) The number of spikelets per panicle (n=10).



Supplemental Figure S3 The coding sequences of the *TLD1* and *TLD2* splice variants. The four exons in *TLD1* are indicated by arrows along the upper edge, while the three exons in *TLD2* are indicated by arrows along the lower edge. The primers used to clone the open reading frames of *TLD1* and *TLD2* are underlined. Similarities are shown in black. The 99 extra base pairs described in the text are boxed.

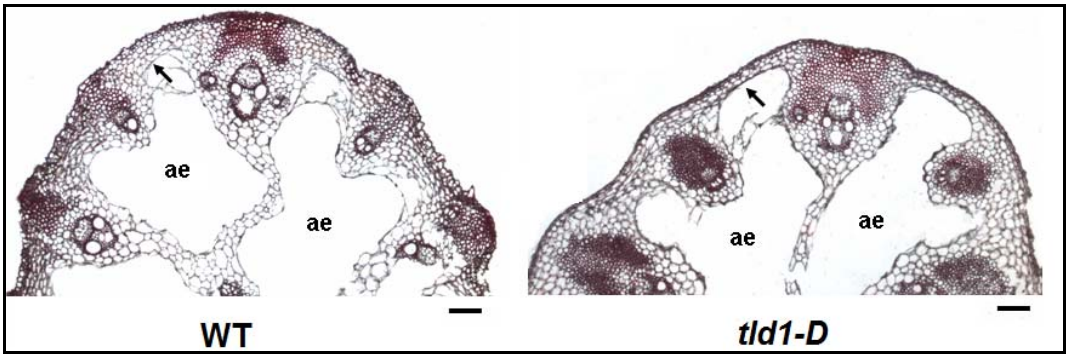


Supplemental Figure S4 Alignment of the deduced primary sequences of TLD1 and TLD2, together with members of the GH3 families in rice (*Oryza sativa*) and *Arabidopsis*. The gene accession numbers for each homolog are as follows: AtGH3.3 (At2g23170), AtGH3.6 (At5g54510), AtGH3.5 (At4g27260), AtGH3.2 (At4g37390), AtGH3.17 (At1g28130), OsGH3.1 (Os01g57610), and OsGH3.8 (Os07g40290). Three conserved motifs are underlined.

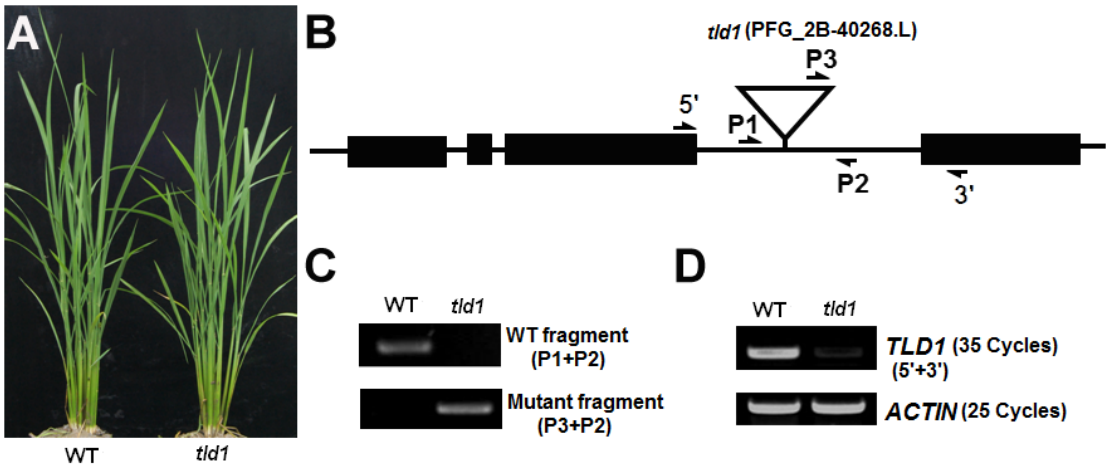


Supplemental Figure S5 Comparison of culm and root from WT and *tld1-D* plants.

One-week-old *tld1-D* seedlings grown on 0.5X MS agar medium (A) and soil-grown *tld1-D* plants at the tillering stage (B) display fewer lateral roots and adventitious roots compared to WT seedlings. (C) Comparison of culm cross-sections prepared in (B).

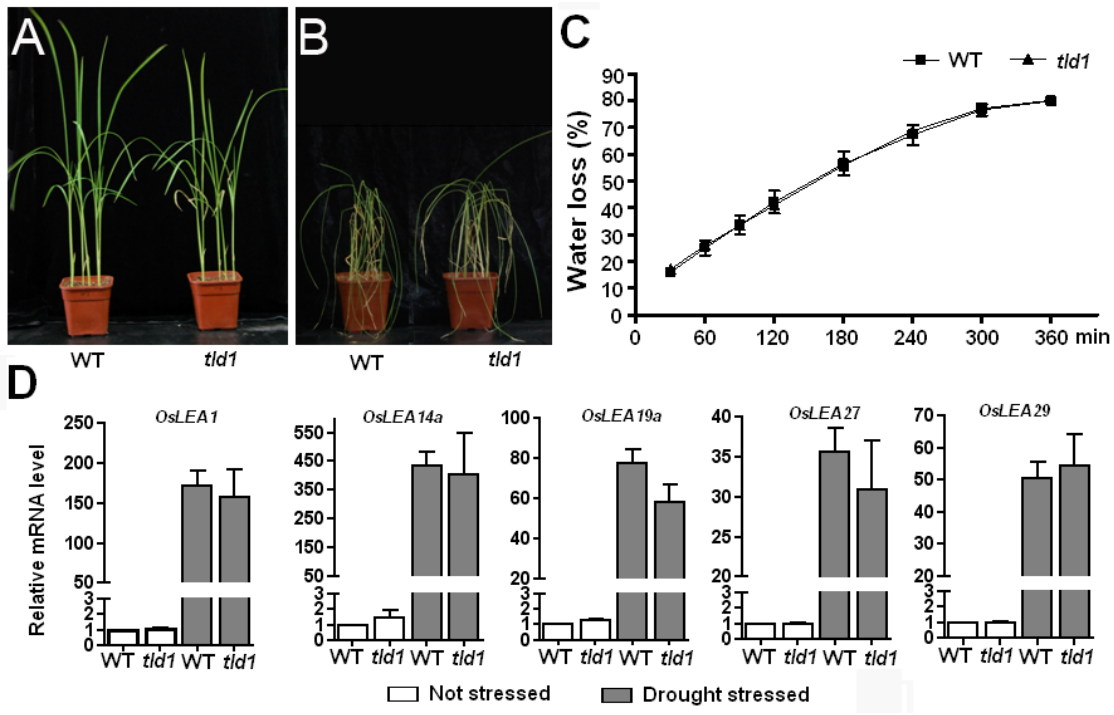


Supplemental Figure S6 Comparison of the lamina joint in cross-section between WT and *tld1-D*. The reduced sclerenchymatous cell layers in *tld1-D* (arrow) are shown. ae: lysigenous aerenchyma. Bar, 10 μm.

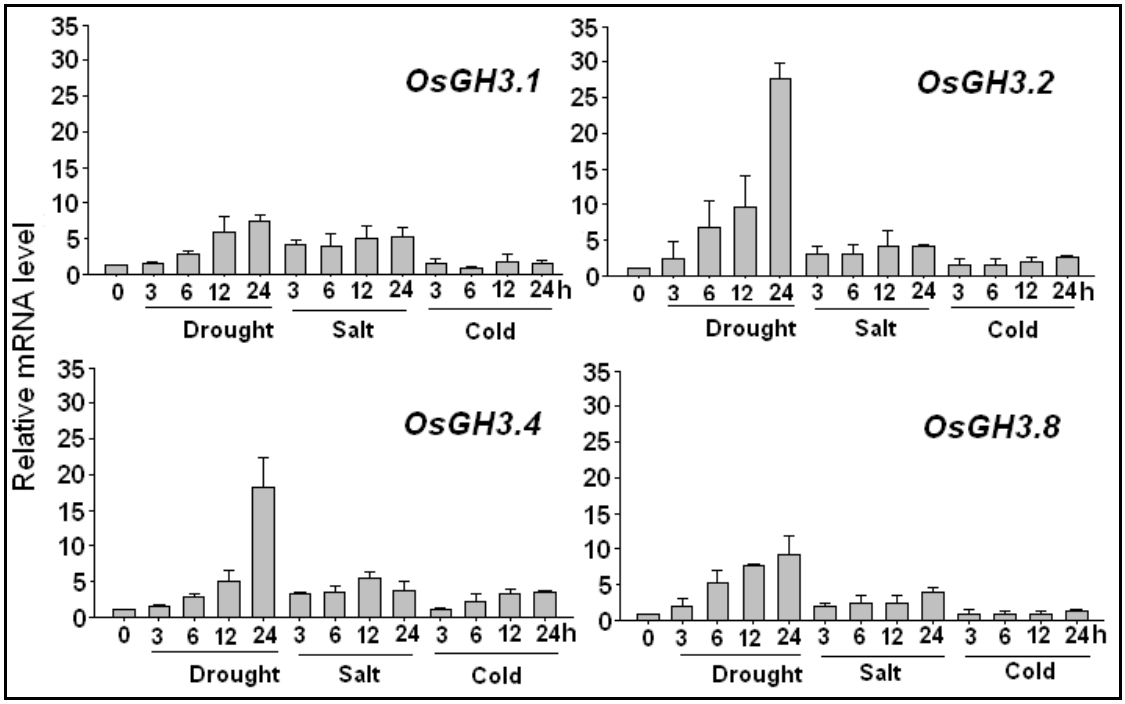


Supplemental Figure S 7　Characterization of *TLD1* loss-of-function mutant.

(A)No obvious difference between the wild-type (*Hwayoung*, left) *tld1* mutant (right) at tillering stage. *tld1* (PFG_2B-40268.L) is in *Hwayoung* background. (B) Schematic representation of the T-DNA insertion site of *tld1*. P1, P2, and P3 are primers used for DNA identification, 5’ and 3’ are primers used for RNA identification (for primer sequence see Supplemental Table S5). (C) Genotyping of *tld1* homozygous. (D) Identification of *TLD1* transcripts in *tld1*mutant by RT-PCR, RNA was isolated from roots of WT and *tld1* homozygous. *OsACTIN* was used as an internal control.



Supplemental Figure S 8 No difference between WT and *tld1* under drought stress. Three-week-old WT and *tld1* seedlings were grown in soil before (A) and after (B) drought stress for 7 days. (C) Water loss from three-week-old *tld1* and WT seedlings. (D) *LEA* expression in WT and *tld1*mutant before and after 6 hours drought stress by qRT-PCR.



Supplemental Figure S9 Differential expression of several *OsGH3* members under salt, cold, and drought stress. Total RNA was extracted from 14-day-old WT rice seedlings; the fold-changes in mRNA expression were determined by qRT-PCR.

2. Supplemental Tables

Supplemental Table S1 Optimal parameters for the MRM detection of plant hormones by LC-MS/MS.

Compound	Transition (*m/z*)	Retention time (min)	Collision energy (eV)
IAA	176.00/130.47	6.15	13
D_2-IAA	182.05/135.92	6.15	18
IAA-Ala	247.09/130.17	5.70	29
IAA-Asp	291.10/130.10	4.99	30
IAA-Leu	289.10/130.10	7.53	30
IAA-Phe	323.14/130.86	7.60	33
IAA-Glu	305.10/130.10	4.96	32
ABA	263.08/153.06	6.81	14

Supplemental Table S2 Primers used for gene cloning.

Primer name	5'→3'
TLD1 promoter-F	gtcgacGAAGCTAGTAGAGAAATGCTAG
TLD1 promoter-R	gaattcGTTAGATGATCCATGCATGCATG
Used to clone the *TLD1* promoter	
TLD1-FL-F/P1f	tctagaAATGACGTCCACGTCGTCTG
TLD1-FL-R/P1r	ggatccCTGGTCGCTGCCGGTGGC
TLD2-FL-R/P2r	ggatccGCCGTTGATTTTGCGATCGG
TLD1-FL-F/P1f and TLD1-FL-R/P1r were used to clone *TLD1*. TLD1-FL-F/P1f and TLD2-FL-R/P2r were used to clone *TLD2*.	
GST-TLD1,2-F	cgggatccATGACGTCCACGTCGTCTG
GST-TLD1-R	cggaattcCTGGTCGCTGCCGGTGGC
GST-TLD2-R	cggaattcGCCGTTGATTTTGCGATCG
Used for expression of TLD1 and TLD2	

Supplemental Table S3 Primers used for the RT-PCR.

Gene name	Primer name	5'→3'
Os11g32510	RT-Os11g32510-F	GGTTGTACCGGTACAGGGTG
	RT-Os11g32510-R	GTGTGCCACCTTGTCCTTGTC
Os11g32520	RT-Os11g32520-F	AATGACGTCCACGTCGTCTG
	RT-Os11g32520-R	GCCGTTGATTTTGCGATCGG
Os11g32530	RT-Os11g32530-F	ATGCCAACTCATCACCTTAC
	RT-Os11g32530-R	TTACGCATCGCGCTGACG
Os11g32540	RT-Os11g32540-F	TTCAGTGCAGCGCACGTCGAT
	RT-Os11g32540-R	AGTAACATCGTCTCGATGGAG
ACTIN	RT-ACTIN-F	CCTCGTCTCGACCTTGCTGGG
	RT-ACTIN-R	GAGAACAAGCAGGAGGACGGC
18S	RT-18S-F	CCACAT CCA AGG AAG GCA GC
	RT-18S-R	CAC CAG ACT TGC CCT CCA ATG

Supplemental Table S4 Primers used for qRT-PCR.

Gene name（Accession No）	Primer name	5'→3'
TLD1/OsGH3.13	q TLD1-F	TGTGTAATGTCAAACGTTGCTCAT
	q TLD1-R	TGATTCATAAAGAACACTGCTCGTATT
OsGH3.1	q-GH3.1-F	CGGGAACAAGCAATGGAACA
	q-GH3.1-R	CAGATCATCACCCTCTAGCTTCAA
OsGH3.2	q-GH3.2-F	TCATGCCCGTCATGAACTTG
	q-GH3.2-R	TCGTCTCCGACTTGATGAACAG
OsGH3.4	q-GH3.4-F	CGCCTCCTCCGAGTGCTA
	q-GH3.4-R	ACATTGTTGGGATGAGGGTGTAG
OsGH3.8	q-GH3.8-F	TTGGACCGTGTCCAAGAATCT
	q-GH3.8-R	TCTTGCCACTAACTGACAGAGTTGA
OsIAA1	q-IAA1-F	GCCGCTCAATGAGGCATT
	q-IAA1-R	GCTTCCACTTTCTTTCAATCCAA
OsIAA9	q-IAA9-F	AAGAAAATGGCCAATGATGATCA
	q-IAA9-R	CCCATCACCATCCTCGTAGGT
OsIAA20	q-IAA20-F	TTGTACGTGAACGGGATTATTTTG
	q-IAA20-R	CATGCTTATGAAATTGCTGAAACA
OsLEA14a	q-OsLEA14a-F	TCGGGATGTCAGGCGATAA
	q-OsLEA14a-R	GCTTGTAGGTGCTGGTGTCCTT
OsLEA1	q-OsLEA1-F	GTCGCAAGTCGAAGCACAAA
	q-OsLEA1-R	TGCGTTGCGTATCAGTGTGA
OsLEA19a	q-OsLEA19a-F	TGTTTTTGTGTCGTTTTGAGTCTGT
	q-OsLEA19a-R	CCACACCCGTCAGAAATCCT
OsLEA8a	q-OsLEA8a-F	GCTTGATGGACAAGGCGAAA
	q-OsLEA8a-R	GGCTCATCCCCTTGAACGA
OsLEA18	q-OsLEA18-F	CGGTTCAGTTCTTTTGGTTGAAT
	q-OsLEA18-R	AAGTTCCTTTCCATTACAGTTACAGTCTT
OsLEA27	q-OsLEA27-F	CCCGGCCAGCACTAAATAAG
	q-OsLEA27-R	AAACTGCACGTACATCACGACAT
OsLEA28	q-OsLEA28-F	GTGTATAGCGTCCTCGAAGAGAATAGT
	q-OsLEA28-R	CACAAGCTATAGGTACAACCCAACA
OsLEA29	q-OsLEA29-F	CGAGCGCAATAAAAGGAAAAA
	q-OsLEA29-R	AGACACGGTCCGTACTGGAGAA
OsLEA23	q-OsLEA23-F	ACAAGAGCAGCGCTTAATTGG
	q-OsLEA23-R	GCTGCAGTGCAGAAAAAGCA
OsLEA24	q-OsLEA24-F	ATGCAGTGATCTGTCTCATGCAT
	q-OsLEA24-R	GCACCGTGCAGCCATTATTA
OsACTIN 1	q-ACTIN-F	TGGCATCTCTCAGCACATTCC
	q-ACTIN-R	TGCACAATGGATGGGTCAGA

Supplemental Table S5 Primers used for *tld1* identification

Primer name	**5'→3'**
tld1-K.out-P1	AGCTAACTGCAAGTCGATTGG
tld1-K.out-P3	CTTCCTTCATTTCACAATGTAAG
tld1-K.out-P2	GTAGGGAATCCCAAGCTATAAC
DNA level identification	
TLD1-RNA-5'	TCGTCGTCACCACCTTCACAG
TLD1-RNA-3'	GTGTGCCACCTTGTCCTTGT
RNA level identification	

3. Supplemental Methods

Quantification of IAA, its conjugates, and ABA by LC-MS/MS

The mobile phase was composed of 0.1% acetic acid in deionized water as solvent A and methanol as solvent B. A linear gradient was run at 200 μL/min, consisting of 20% solvent B at the start (t=0 min), increased to 90% solvent B at t=6 min, and then held at 90% solvent B for 2 min until t=8 min. The gradient was then returned to 20% solvent B at t=8.1 min, and stabilized at 20% solvent B for 1.9 min until t=10 min before the next injection. The atmospheric pressure ionization (API) source was operated in positive ESI mode. The TSQ quantum mass spectrometer was calibrated with a solution of polytyrosine-1, 3, 6 according to the manufacturer's instructions. The ESI source parameters were optimized for IAA at an LC flow of 200 μL/min based on preliminary results indicating that IAA was the analyte for which the method had the lowest sensitivity. The optimized source parameters were as follows: sheath gas pressure, 20 (arbitrary units); auxiliary gas flow, 30 (arbitrary units); spray voltage, 4,000 V; capillary temperature, 350 °C; and skimmer offset, 10 V. For quantification, the mass spectrometer was set to the data acquisition mode of multiple-reaction monitoring (MRM); the specified acquisition parameters are shown in Supplemental Table 1. Detection of the compounds was performed in the MRM mode with a single time segment containing seven events. The acquisition parameters common to all analytes were: scan width (m/z), 0.10; scan time, 0.05 s; peak width (FWHM), 0.4 for Q1 and 0.7 for Q3; and collision gas pressure, 1.5 mTorr. Data acquisition and analysis were accomplished with Xcalibur software v.1.4 (Thermo, San Jose, CA, USA).