Supporting Information

Held *et al*. 10.1073/pnas.0809408105

SI Methods

Plant Growth Conditions—*Hordeum vulgare* cv. Black Hulless plants were grown 5–7 d in BM-7 soil (Berger), supplemented with Osmocote (Scotts) 14-14-14 slow release fertilizer (1.8 g/L), Marathon (Olympic) granular insecticide (1 g/L), and Turface (MVP) (25%, vol/vol), in a Conviron PGR15 growth chamber under high intensity discharge lamps (450–700 μmol m^{-2} sec^{-1}) programmed for a 16-h photoperiod (25 °C day temp, 20 °C night temp). Lengths of the second and third leaf of 10 plants were measured from soil surface to leaf tip before harvest. Coleoptiles were from caryopses soaked and germinated in wet filter paper and incubated 2.5 d in darkness, as described earlier (1). Third leaves from ≥10 plants were pooled and collected into liquid nitrogen at daily intervals; 10–15-d after germination. These time points coincide with third leaf blade elongation from blade emergence from the sheath 10 d after germination to cessation of elongation 15 d after germination.

Analysis of Developing Barley Third Leaves. Barley seeds were preincubated to coleoptile stage and then sown in soil and grown as described above. The pooled leaf material was pulverized in liquid nitrogen from which total RNA was prepared in triplicate for each time point by using TriZol (Invitrogen). Time course RNA was used for strand-specific PCR (1 μg per time point), real-time PCR, and ribonuclease protection assays as described below. Cell walls were prepared from the remaining pulverized leaf tissue (2). Cellulose biosynthesis in developing barley third leaves was estimated by weight remaining after acetic-nitric hydrolysis of the purified cell walls. β-Glucan in the cell walls of the developing barley third-leaf tissues was quantified by HPAEC (described below) by comparison with control digests of authentic barley (1 → 3),(1 → 4)-β-D-glucan standards (Sigma).

Extraction of RNA and First-Strand cDNA Synthesis. Total barley RNA was extracted from 100–250 mg of leaf tissue by using TriZol reagent (Invitrogen) following the manufacturer's protocol, quantified by A_{260}, and assessed for quality by agarose gel electrophoresis. RNA from 2.5-d-old etiolated coleoptiles was similarly prepared. Before real-time PCR, genomic DNA contamination was removed from total RNA (10 μg) by Turbo DNA-free DNase treatment (Ambion). The RNA samples were again assayed for quality and quantity by agarose gel electrophoresis. First strand cDNA was synthesized from total RNA (1 μg per reaction) by using iScript first strand cDNA synthesis kit (Bio-Rad) and subsequently diluted (1:5) with nuclease-free water.

Detection of Antisense Transcripts by Strand-Specific RT-PCR. First-strand cDNA was synthesized from 1.7 μg total RNA extracted from late-expanded barley third leaves with the SuperScript III kit (Invitrogen), by using oligo dT as well as the gene specific primers: A6-sense-(l), 5′-CAA CGA GTT CGT CAT GAT CC-3′; A6-sense-(m), 5′-AAA ACC CGC ATG ATG AAG AG-3′; A6-sense-(s), 5′-CAA CAG CGG TTA CCA ATC CT-3′; A6-antisense-1, 5′-GAG ATG GAG GAT CAC CCA GA-3′; and A6-antisense-2, 5′-TGA AAG GGT CGA TCT TCA CC-3′. Control reactions lacking RT were also performed for all templates to ensure amplification was from the cDNA template.

First-strand cDNA synthesized by using the A6-sense-(s) strand-specific primer was subjected to PCR by using the primer pair A6-sense-(s), 5′-CAA CAG CGG TTA CCA ATC CT-3′, and A6-antisense-2, 5′-TGA AAG GGT CGA TCT TCA CC-3′. cDNA synthesized by using the A6-sense-(m) strand-specific primer was subjected to PCR by using the primer pair A6-sense-(m), 5′-AAA ACC CGC ATG ATG AAG AG-3′, and A6-antisense-1, 5′-GAG ATG GAG GAT CAC CCA GA-3′. cDNA synthesized by using the A6-sense-(l) strand-specific primer was subjected to PCR by using the primer pair A6-sense-(l), 5′-CAA CGA GTT CGT CAT GAT CC-3′, and *A6*-antisense-2, 5′-TGA AAG GGT CGA TCT TCA CC-3′. All primer pairs were used to amplify cDNA synthesized from oligo dT primers. PCRs (25 μl) assembled on ice contained 0.5-μl dNTPs (10 mM each; Invitrogen), 1 μl of each primer (10-μM dilution), 1-μl cDNA template, and 1 unit of GoTaq (Promega) in 1× GoTaq green buffer (Promega). Cycling conditions included 2 min of activation at 95 °C, followed by 35 cycles of 95 °C for 30 s, 51 °C for 30 s, and 72 °C for 1 min. Equal volumes (16 μl) of PCR products were assessed by agarose gel electrophoresis.

Detection of siRNAs by Ribonuclease Protection Assays (RPA). Primers were designed for cloning a 465-bp fragment from the 3′-end of the *HvCesA6*-coding region by Primer 3 software (http://fokker.wi.mit.edu/primer3/input.htm; forward, 5′-GTG TTC AAG TGG ACC AGT CT-3′ and reverse, 5′-GGG GCC AAT AAA TTT TAG AT-3′). The fragment was amplified by RT-PCR from cDNA prepared from barley third-leaf RNA. The PCR product was sequenced and then cloned into the pGEM-T easy vector kit (Promega). α-^{32}P-UTP (Amersham) radiolabeled probes were prepared from linearized plasmid templates having 5′ overhanging ends with either T7 or SP6 RNA polymerase by using the MAXIscript Kit (Ambion) to produce the *HvCesA6* antisense riboprobe (522 nt) and the *HvCesA6* sense riboprobe (214 nt), respectively. A portion of the *HvCesA6* sense riboprobe was derived from the *pGEM-T easy* vector, therefore a short *Vector* sense probe (99 nt) was similarly prepared as a negative control. Riboprobes for the detection of siRNAs derived from the 5′ portion of the *HvCesA6* gene, *HvGT-8A*, and *HvGT-8D* genes were generated similarly by using primers: A6-240 forward, 5′-ATG GCG GCC AAC CGG GGG ATG G-3′; A6-240 reverse, 5′-GAC CCC ATC CTT GCG CTC GTA CTC-3′; GT-8A-698 forward, 5′-TGA ATG GTG GTG AGC AGA AG-3′; 8A-698 reverse, 5′-GTG GAA CAG CAA GCT CAC AA-3′; 8D-869 forward, 5′-GCA AGG CCA CTA TCC ATG TT-3′; and 8D-869 reverse, 5′-CGA TGC TTG GGT TGT ATC CT-3′, respectively.

Ribonuclease protection assays were performed by using the RPAIII kit (Ambion). Labeled riboprobes were gel-purified by 5% PAGE containing 8 M Urea in 1× TBE buffer according to kit instructions and hybridized with 10–20 μg total RNA from either barley, yeast, or mouse for 16–18 h at 42 °C. Reaction mixtures were digested with RNase A/T1 (1:100) for 30 min at 37 °C, then stopped with inactivation buffer (Ambion) and protected fragments were precipitated by using 10 μg yeast RNA as a carrier. The protected fragments were separated by 12.5% PAGE containing 8 M Urea in 1× TBE buffer. γ-^{32}ATP (Amersham) end-labeled Decade Marker (Ambion), prepared according to manufacturer's protocol, served as size standards. The protected HvCesA6 nat-siRNAs were reproduced across ≥3 independent protection assays.

Measurement of Gene Expression by Quantitative Real-Time PCR. With the exception of *CslF* and *CesA*, primer sets were designed within consensus regions to measure the expression of as many gene family members as possible in uninfected and VIGS-treated

plants (Table S5). *CslF* expression was measured by using primers specific for the *HvCslF6* gene (Table S5; ref 3), and *CesA* gene expression was measured by using either the *CesA-CR2* cloning primers (listed above) for *BSMV/CesA-CR1-PDS* and *BSMV/CesA-6-PDS* VIGS plants or primers specific for the *HvCesA6* gene in *BSMV/CesA-CR2-PDS* VIGS plants (Table S5). Reactions (25 μl) consisted of 2.5 μl of diluted cDNA template (25 ng), 0.25 μl of each 10 μM forward and reverse primer (100 nM), and 12.5 μl of (2×) iTaq SYBR reagent kit (Bio-Rad). Technical replicates were performed in quadruplicate. Standard curves covering 3 orders of magnitude, and notemplate controls were performed in triplicate for each primer set. Relative gene expression of VIGS plants and controls from 3–5 separate third leaf samples was assessed by quantitative real-time PCR by using *ubiquitin10* expression as the normalizing target and *BSMV/V-PDS* (BSMV/PDS4as) as a calibrator (4). *Ubiquitin 10* was selected as the best normalizing target after a systematic survey of 4 housekeeping gene candidates (data not shown, primers are listed in Table S5). Numerous independent experiments were performed and the data presented represent typical values obtained across experiments. Reactions were performed on an MX3000P thermocycler (Stratagene). Cycling conditions included 2 min of activation at 95 °C, followed by 40–45 cycles of 95 °C for 15 s, 52 °C for 30 s, and 72 °C for 30 s followed by a dissociation curve cycle of 95 °C for 1 min, 52 °C for 30 s, then 95 °C for 30 s.

Design and Construction of BSMV Vectors for CesA/Csl VIGS. Targets for the suppression of the barley (*Hordeum vulgare*) *HvCesA* gene family, the *HvCslH* gene family, and the *HvCslF* gene family were first designed by aligning full-length rice cDNA sequences of each gene family member by using Clustal W (5). VIGS targets were selected on the basis that they possessed at least 85% nucleotide similarity, contained at least one 21-nt stretch of 100% nucleotide identity toward prospective targets, and that they did not meet these criteria with other nontarget genes, especially not the other *Csl*s genes. A portion of the "Class-Specific Region" (CSR) was selected as a VIGS fragment to target the *HvCesA6* gene as a single isoform. The CSR of this *CesA* was selected as the target, because this region is unique to each *CesA* gene (6). Potential VIGS target sequences garnered from rice were then aligned with available barley *CesA* and *Csl* EST sequences to identify the homologous regions, verify desired specificity, and design primers for cloning.

Amplification of the VIGS-targeting fragments by reverse transcriptase PCR of total barley RNA was performed by using the following primers: *CslH* (148 bp) forward, 5′-GAC ATT TTG ACC GGA CAA CGG; A-3′ reverse, 5′-TCT CCA GAA CCC CTG TTG CCA T-3′; *CslF* (170 bp) forward, 5′-GTT CGC GCT CGG GAT AAT; reverse, 5′-ATG CAG CAT TAC TTG ACA AA-3′; *CesA-CR*1 (110 bp) forward, 5′-TGG TGA TGT CTT TGT CGC CT, reverse, 5′-GAA CTC GGG GGC TAC CTT TGA; *CesA-CR2* (187 bp) forward, 5′-CCC TAG GGA CCA TCC TGG-3′; reverse, 5′-GGC ACC ATT TGT CAA CAC-3′; *CesA-6* (200 bp) forward, 5′-TGT ATG GCT ATG ATC CTG TAT TAA CTG AG-3′; and reverse, 5′-CCT TTC ATC CTC ATA ACC TTC TAT ACC C-3′. PCR products were cloned bidirectionally into the pGEM T-easy vector (Promega). Plasmids were sequenced, and VIGS targets were then subcloned into plasmids harboring the γ-portion of the BSMV genome (designated pBSMV-γ) bidirectionally as a *Not*I fragment (7, 8). The inserts were sequenced, and plasmids having a *CslH* fragment in the antisense orientation (designated pBSMV-γ-CslH-as), a *CslF* fragment in the sense orientation (designated pBSMV-γ-CslFs), a *CesA* fragment in the sense orientation (designated pBSMV-γ-CesA-CR1-s), another *CesA* fragment in the sense orientation (designated pBSMV-γ-CesA-CR2-s), and a *CesA6* fragment in the antisense orientation (designated pBSMV-γ-CesA-6-as) were used as templates for in vitro transcription and subsequent plant inoculations.

For in vitro transcription of VIGS plasmids, capped viral RNAs were prepared from linearized plasmids (1 μg per reaction) by using the mMessage mMachine T7 in vitro transcription kit (Ambion). Plasmid linearization and in vitro transcription was monitored by agarose gel electrophoresis.

Viral Inoculation of Barley Plants. Plant inoculations were carried out as described previously (7, 8). Briefly, in vitro transcripts of the *pBSMV/α*, *pBSMV/β*, *pBSMV/γ-PDSas*, and the appropriate experimental gene silencing construct (either *BSMV/γ*, *BSMV/γ-CslH-as*, *BSMV/γ-CslF-s*, *BSMV/γ-CesA-CR1-s*, *BSMV/γ-CesA-CR2-s*, or *BSMV/γ-CesA-6-as*) were combined in a 1:1:0.5:0.5 ratio by volume, respectively. For plant inoculations, 3 μl of the mixture of α, β, γ-PDS, and respective γ-RNA transcripts were added to 22.5 μl of FES and applied to 6–7-day-old seedlings by rub-inoculating the first leaf of each plant 2–3 times. The *γ-PDSas* transcripts were coinoculated with all plants to serve as a visual marker for gene silencing. Infected plants were grown under the same conditions until viral symptoms were visually evident in the third leaves, typically 6–7 d later.

In vivo Labeling of VIGS Barley with [U-^{14}C]-Glucose and Cell Wall Characterization. Young, expanding third leaves of plants (3–5 each) infected with either *BSMV/V-PDS* alone, *BSMV/CesA-CR1-PDS*, *BSMV/CesA-CR2-PDS*, *BSMV/CesA-6-PDS*, *BSMV/CslH-PDS*, or *BSMV/CslF-PDS* were harvested under 0.01% (vol/vol) Tween 20 (aq) and pulse labeled in vivo with D-[U-^{14}C]-glucose (Amersham Pharmacia) for 2–3 h at ambient temp under fluorescent lights. Labeled leaf tissue was collected into liquid nitrogen from which cell walls were prepared as described earlier (2).

Isoxaben and DCB Treatment and In Vivo Labeling With $^{14}CO_2$ (g). Barley seedlings were grown 11 d in a growth chamber, as described above, in autoclaved Redi-earth (Sun Gro) potting soil. Twelve plants each were sprayed (≈5 ml/plant) with 0.1% (vol/vol) Tween 20 (aq), and 0.1% (vol/vol) DMSO containing no inhibitor (mock), 1 μM isoxaben or 100 μM DCB. Plants were incubated under fluorescent lights for 3–6 h at ambient temp. Plants were pulse-labeled with $^{14}CO_2$, liberated by adding a few drops of 1 M H_2SO_4 to a dish containing 10 μCi per plant of $NaH^{14}CO_3$ (aq) (2.04 GBq mmol^{-1}; MP Biomedicals) in a sealed Plexiglas chamber. The plants were incubated in the presence of the radiolabel under fluorescent lighting for 1 h. The third leaves for each treatment were pooled and harvested into liquid nitrogen. Cell walls and total RNA were prepared as described above.

Cellulose Determination. The ability to incorporate radiolabeled carbon into cellulose was assayed by acetic-nitric hydrolysis (9). Briefly, isolated cell walls (2) were hydrolyzed in acetic acid, nitric acid, and water (8:1:2, vol/vol/vol) at 100 °C for 90 min. Samples were centrifuged at 2,500 × *g* for 5 min and the supernatant was removed. Pellets containing pure cellulose were washed with water, then acetone, and then briefly dried. Pulse-labeled cellulose pellets were dissolved in 11.9 M sulfuric acid (aq) for 1 h at ambient temp. Neutralized aliquots of the supernatant and soluble pellet were then assayed for radioactivity. The ability to incorporate label into cellulose in these plants was expressed as the percentage of insoluble radioactivity to the total radioactivity in the isolated walls.

$(1\rightarrow3),(1\rightarrow4)$-$\beta$-Glucan Profile Determination. The ^{14}C-labeled VIGS cell walls were digested at 37 °C for 3 h with *B. subtilis* $(1\rightarrow4)$ endo-β-D-glucanase, which specifically cleaves a

β-(1 → 4)-linkage, only if it is preceded by a β-(1 → 3) linkage (10), as described previously (11). The reactions were stopped by boiling for 5 min then centrifuged at 10,000 × g for 10 min. Radioactivity in the pellet was recorded by scintillation counting. (1 → 3),(1 → 4)-β-D-Glucan oligosaccharides released into the supernatant were separated by HPAEC-PAD (11). Fractions were collected at 0.5–1 min intervals, neutralized, and assayed for radioactivity. The ability to incorporate label into (1 → 3),(1 → 4)-β-glucan was expressed as the ratio of the sum of the radioactivity possessed by the cellotriose and cellotetraose β-glucan oligomers to the total radioactivity (oligomers plus pellet).

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10. Anderson MA, Stone BA (1975) A new substrate for investigating the specificity of β-glucan hydrolases. *FEBS Lett* 52:202–207.
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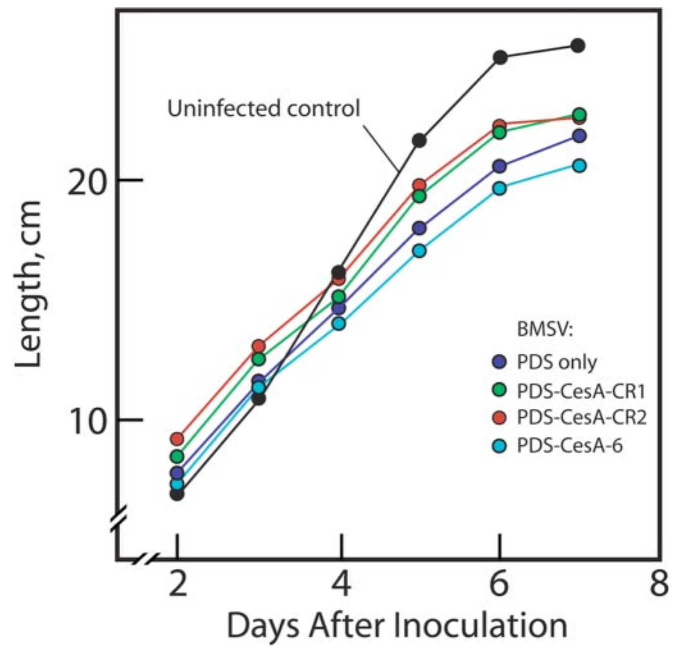

Fig. S1. Comparison of leaf lengths after inoculation with BSMV/PDS alone or BMSV/PDS and *CesA* VIGS constructs. Second leaf lengths (cm) were recorded at daily intervals from 10 plants after inoculation with respective VIGS constructs. Data are represented as the average lengths of 10 biological replicates.

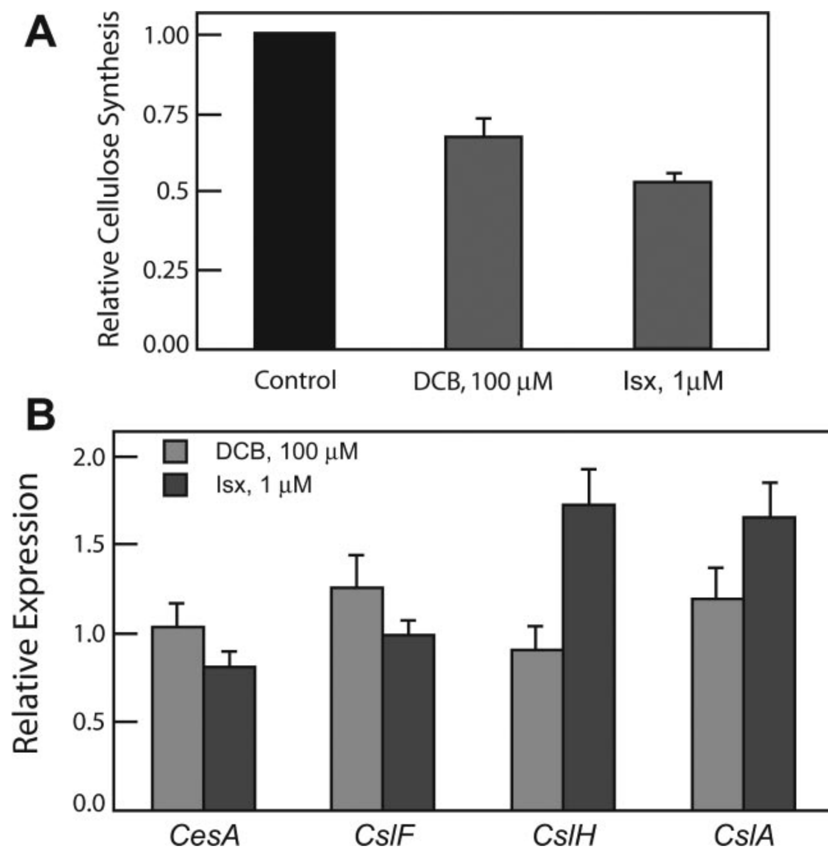

Fig. S2. Barley third leaves treated with chemical inhibitors of cellulose biosynthesis do not exhibit the same gene response as VIGS treatment. Barley third leaves were treated with either 100 μM DCB or 1 μM isoxaben (Isx), and compared with control plants sprayed with buffer containing no inhibitor. (*A*) Cellulose determinations and (*B*) comparative quantitative PCR data were collected from 10–12 pooled biological replicates. Values are the mean ± SD relative to plants infected with empty vector-PDS control plants (BSMV/PDS expression = 1) and normalized to *ubiquitin10* gene expression.

Fig. S3. Alignment of *OsCslF6* (Os08g06380) with *OsCesA* gene superfamily members. A rice glucan synthase gene OsCslF6 shares short regions of high complementarity with nearly all CesA genes in rice.

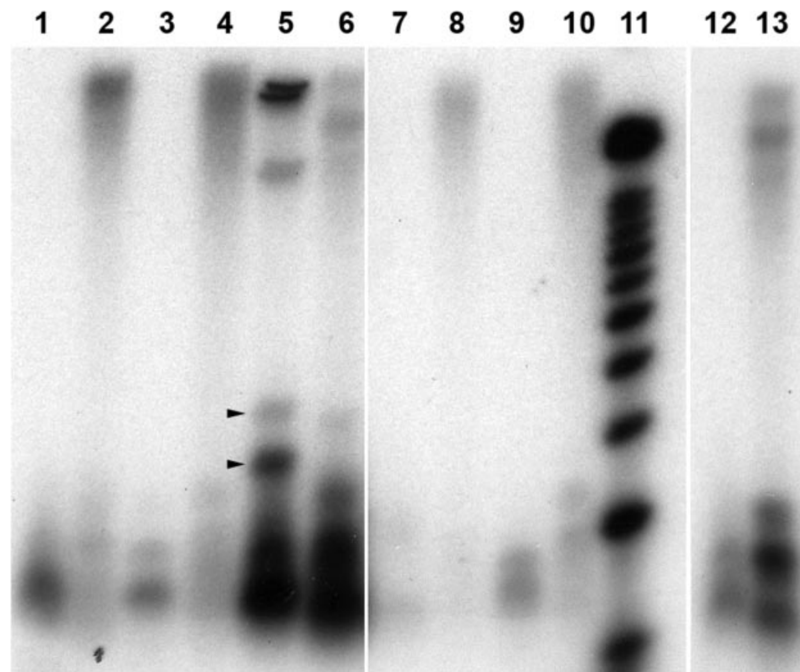

Fig. S4. Ribonuclease protection assays for *GT8* and *HvCesA6* upstream small RNAs. *Lanes*: *1*, Hv RNA (10 μg) probed with GT8A-sense probe; *2*, Hv RNA (10 μg) probed with GT8A-antisense probe; *3*, Hv RNA (10 μg) probed with GT8D-sense probe; *4*, Hv RNA (10 μg) probed with GT8D-antisense probe; *5*, Hv RNA (10 μg) probed with A6 upstream-sense probe; *6*, Hv RNA (10 μg) probed with A6 upstream-antisense probe; *7*, yeast RNA (10 μg) probed with GT8Asense probe; *8*, yeast RNA (10 μg) probed with GT8A-antisense probe; *9*, yeast RNA (10μg) probed with GT8D-sense probe; *10*, yeast RNA (10 μg) probed with GT8D-antisense probe; *11*, decade markers; *12*, yeast RNA (10 μg) probed with A6 upstream-sense probe; and *13*, yeast RNA (10 μg) probed with A6 upstream antisense probe. Arrowheads mark the 24-nt and 30-nt fragments in lane 5.

Table S1. Potential cis-NAT pairs and antisense sequences for Arabidopsis CesA genes

Name	Gene	Overlapping AS gene from TAIR*	No. AS tags, total/unique†	Genes hit for nonunique AS tags†
CesA1 (*RSW1*)	**At4 g32410**	At4 g32400 (sodium hypersensitive) close	12/9	**At2 g25540 (CesA10)**
CesA2	**At4 g39350**		6/5	At2 g23790 (transcription factor), **At5 g64740 (CesA6)**
CesA3 (ELI1) (CEV1)	At5 g05170	At5 g05160 (LRR) close	9/8	At4 g14800 (20 s proteasome β subunit 2)
CesA4 (IRX5)	At5 g44030	At5 g44040 (unknown)	5/4	At4 g35800 (RNA Pol2 LS), 1 sense small RNA
CesA5	**At5 g09870**		4/3	**At3 g03050 (CslD3), At5 g64740 (CesA6)**
CesA6 (PRC1)	**At5 g64740**		12/11	**At5 g09870 (CesA5), At4 g39350 (CesA2), At3 g03050 (CslD3)**
CesA7 (IRX3)	At5 g17420	At5 g17410 (tubulin family)	3/3	
CesA8 (IRX1)	At4 g18780	At4 g18770 (MYB98)	3/1	At3 g54340 (Apetala3); AT4 g28600 (no pollen germination related)
CesA9	At2 g21770		1/0	At1 g76190 (auxin response), At1 g36150 (lipid transfer protein)
CesA10	**At2 g25540**	At2 g25530 (ATPase-like) close	3/0	**At4 g32410 (CesA1)**

Bold text indicates ≥2 cell wall genes share antisense sequences.
*From the TAIR database (http://www.arabidopsis.org).
†From the MPSS database (http://mpss.udel.edu/).

Table S2. Potential cis-NAT pairs and antisense sequences for rice *CesA* genes

Name	Gene	Overlapping gene*	No. AS tags, total/unique†	Genes hit for nonunique AS tags†
CesA11	Os06 g39970		1/1	
CesA4	Os01 g54620		17/17	
CesA2	**Os03 g59340**	Os03 g59330 (polygalacturonase)	8/2	**Os07 g10770 × 3 (CesA8)**, Os05 g35630 (hypothetical), Os01 g05170 (expressed)
CesA8	**Os07 g10770**		19/10	**Os03 g59340 x3 (CesA2)**, **Os05 g08370 x2 (CesA1)**, Os05 g35630 (hypothetical)
CesA1	**Os05 g08370**		14/0	**Os07 g10770 x2 (CesA8)**
CesA3	**Os07 g24190**		16/12	**Os03 g62090 x2 (CesA5)**, Os02 g05670 (expressed)
CesA5	**Os03 g62090**		13/11	**Os07 g24190 x2 (CesA3)**
CesA6	Os07 g14850		8/6	Os05 g40820 (60 s ribosomal)
CesA9	Os09 g25490		11/11	
CesA7	Os10 g32980		11/10	Os04 g08240 (hypothetical)

Bold text indicates ≥2 cell wall genes share antisense sequences.
*From the TIGR database (http://rice.plantbiology.msu.edu/).
†From the MPSS database (http://mpss.udel.edu/).

Table S3. Summary of MPSS tags and potential antisense sequences for Csl and GT8 gene families of Arabidopsis

Family	Gene	Overlapping AS gene from TAIR*	No. AS tags, total/unique†	Other genes targeted by shared AS tags†
CslA1	At4 g16590		4/3	Chr4, no gene
CslA2	At5 g22740		6/6	
CslA3	At1 g23480		1/1	
CslA7	At2 g35650		0/0	
CslA9	At5 g03760	At5 g03750 (C3HC4 RING finger) close	2/2	
CslA10	At1 g24070		0/0	
CslA11	At5 g16190	At5 g16180 (chloroplast splicing factor) close	1/1	
CslA14	At3 g56000	At3 g55990 (Eskimo1) close	0/0	
CslA15	At4 g13410		1/1	
CslB3	At2 g32530		0/0	
CslB4	At2 g32540		0/0	
CslB1	At2 g32610		0/0	
CslB2	At2 g32620		0/0	
CslB5	At4 g15290	At4 g15280 (UDP-glucuronosyl/glucosyl transferase)	1/1	
CslB6	At4 g15320		0/0	
CslC4	At3 g28180		4/3	At4 g26520 (fructose bisphosphate aldolase)
CslC5	At4 g31590		1/1	
CslC6	At3 g07330		4/4	
CslC8	At2 g24630		1/1	
CslC12	At4 g07960		2/2	
CslD1	At2 g33100		0/0	
CslD3	**At3 g03050**		3/2	**At5 g09870 (CesA5), At5 g64740 (CesA6)**
CslD4	At4 g38190		1/0	At4 g06692 (gypsy retrotransposon)
CslD5	**At1 g02730**	**At1 g02720 (GATL5) close**	6/4	Chr 1, no geneChr 1, no gene
CslD6	At1 g32180		2/2	
CslE1	At1 g55850	At1 g55860 (Ubiquitin-protein ligase 1)	2/2	
CslG3	**At4 g23990**		2/1	**At4 g2400 (CslG2), At4 g24010 (CslG1)**
CslG2	**At4 g24000**		3/1	**At4 g23990 (CslG3), At4 g24010 (CslG1),** DNA Mariner (retro element?)
CslG1	**At4 g24010**	At4 g24015 (RING-H2 zinc finger)	2/1	**At4 g2400 (CslG2), At4 g23990 (CslG3)**
GT8-A	At1 g08990	none	0/0	
GT8-A	At1 g54940	none	0/0	
GT8-A	At4 g33330	none	1/1	
GT8-A	At1 g77130	none	1/1	
GT8-A (PGS1P1)	At3 g18660	none	0/0	
GT8-B (GolS3)	At1 g09350	none	1/1	
GT8-B (GolS2)	At1 g56600	none	0/0	
GT8-B (GolS1)	At2 g47180	At2 g47170 (ADP-ribosylation factor)	2/2	
GT8-B	At1 g60450	none	0/0	
GT8-B	At1 g60470	At1 g60460 (unknown)	1/0	At5 g08620 (stress response suppressor 2)
GT8-B	At4 g26250	none	0/0	
GT8-B	**At5 g23790**	none	0/0	
GT8-B	At5 g30500	none	0/0	
GT8-C (GATL5)	**At1 g02720**	**At1 g02730 close (CslD5)**	2/2	
GT8-C (GATL6)	At4 g02130	At4 g02120 (CTP synthase) close	2/2	
GT8-C (GATL7)	**At3 g62660**	none	4/3	**At1 g24170 (GATL8)**
GT8-C (GATL8)	**At1 g24170**	none	3/1	**At3 g62660 (GATL7); At1 g70090 (GATL9)**
GT8-C (GATL9)	**At1 g70090**	none	3/2	**At1 g24170 (GATL8)**
GT8-C (GATL3)	At1 g13250	none	1/1	
GT8-C (GATL4)	At3 g06260	none	1/1	
GT8-C (GATL1, Parvis)	At1 g19300	none	1/1	
GT8-C (GATL2)	At3 g50760	none	1/1	
GT8-C (GATL10)	At3 g28340	none	2/2	
GT8-D (GAUT13)	At3 g01040	none	3/3	
GT8-D (GAUT14)	At5 g15470	none	2/2	
GT8-D (GAUT12)	At5 g54690	At5 g54680 (IAA-Leucine Resistant3)	0/0	
GT8-D (GAUT15)	**At3 g58790**	**At3 g58795 (5′ Natural Antisense)**	3/1	**At3 g25140 (Gaut8/QUA1)**; At1 g01080 (RNA binding), At3 g09980 (unknown), At4 g39750 (F-box protein), At5 g11390 (unknown), At5 g03660 (unknown)
GT8-D (GAUT2)	At2 g46480	none	0/0	

Family	Gene	Overlapping AS gene from TAIR*	No. AS tags, total/unique†	Other genes targeted by shared AS tags†
GT8-D (GAUT1)	At3 g61130	none	5/5	
GT8-D (GAUT3)	At4 g38270	At4 g38260 (in 5′ unknown)	0/0	
GT8-D (GAUT4)	At5 g47780	none	3/3	
GT8-D (GAUT9)	At3 g02350	none	2/2	
GT8-D (GAUT8, Qua1)	**At3 g25140**	At3 g25150 close (nuclear transport factor 2)	5/4	**At3 g58790 (GAUT15)**
GT8-D (GAUT11)	At1 g18580	none	0/0	
GT8-D (GAUT10)	At2 g20810	none	2/2	
GT8-D (GAUT6)	**At1 g06780**	none	2/2	
GT8-D (GAUT5)	**At2 g30575**	none	0/0	
GT8-D (GAUT7)	At2 g38650	none	2/1	At3 g49600 (ubiquitin specific protease 26)
GT8-E	At2 g35710	none	1/1	
GT8-E	At4 g16600	none	1/1	
GT8-E	At5 g18480	none	4/3	At3 g60150 (unknown)

Bold text indicates ≥2 cell wall genes may share antisense sequences.
*From the TAIR database (http://www.arabidopsis.org).
†From the MPSS database (http://mpss.udel.edu/).

Table S4. A summary of MPSS tags and potential antisense genes for the rice Csl and GT8 cell wall-related gene families

Family	TIGR gene name*	Antisense gene present	Distance	MPSS, total/unique†	Genes MPSS tags hit†
CslA5	**Os03G26050 (Os03 g26044)**	Os03 g26030 (expressed)	0.9 kb	7/5	**Os03 g07350 (CslA4)**, Os09 g26770 (expressed), Os03 g62670 (methyl transferase), Os10 g39320 chloroplast nucleotide DNA binding)
CslA6	Os02 g51060	Os02 g51050 (hypothetical)	1.0 kb	7/7	
CslA7	Os07G43710	Os97 g43720 (expressed)	0.1 kb	2/2	
CslA8	Os09 g39920	none		4/3	Os01 g40094 (phosphatase 2C ABI2), Os02 g43790 (ethylene responsive), Os03 g50740 (retrotransposon), Os07 g34260 (chalcone synthase), Os07 g47510 (stress related), Os10 g37850 (armadillo-repeat), Os12 g42610 (axial regulator YABBY2)
CslA9	Os06G42020	Os06 g42030 (potassium transporter)	overlapped	2/0	Os06 g42030 (potassium transporter)
CslA11	Os08G33740	Os08 g33750 (MYR1)	3.3 kb	10/10	
CslC1	Os01G56130	Os01 g56120 (RNA recognition)	5.4 kb	8/8	
CslC2	Os09G25900	Os09 g25890 (trehalose-6-phosphate synthase)	10.2 kb	8/7	Os10 g33660 (charged multivesicular body)
CslC3	Os08G15420	Os08 g15430 (hypothetical)	1.5 kb	4/3	over 130 genes
CslC7	Os05G43530	Os5 g43520 (BSD domain containing)	1.6 kb	8/7	Os06 g48300 (phosphatase 2C)
CslC9	Os03G56060	Os03 g56050 (ANT-like)	3.4 kb	8/8	
CslC10	Os07G03260	Os07 g03250 (AINTEGUMENTA)	1.0 kb	1/0	Os06 g01870 (expressed)
CslD1	**Os10G42750**	Os10 g42754 (expressed)	0.2 kb	8/6	Os02 g09480 (MYB transcription factor), **Os12 g36890 (CslD4)**, Os11 g29630 (expressed)
CslD2	Os06G02180	Os06 g02170 (heat shock)	1.1 kb	9/9	
CslD3	Os08G25710	Os08 g25700 (expressed)	0.1 kb	7/7	
CslD4	**Os12G36890**	Os12 g36880 (major pollen allergen)	1.6 kb	3/2	Os02 g09480 (MYB transcription factor), **Os10 g42750 (CslD1)**
CslD5	Os06G22980	none		2/2	
CslE1‡	Os09G30120	Os09 g30100 (rRNA methyl transferase)	6.9 kb	6/6	
CslE2	Os02G49330 (Os02 g49332)	Os02 g49340 (nitrate induced NOI)	0.8 kb	11/8	Os01 g54040 (ATP peptidase), Os06 g07923 (expressed), Os06 g08023 (flavonol synthase), Os08 g32510 (hypothetical), Os10 g29514 (expressed), Os01 g48260 (hypothetical), Os12 g42284 (hypothetical)
CslE6‡	Os09G30130	Os09 g30100 (rRNA methyl transferase)	14.2 kb	3/3	
CslF1	Os07G36700	Os07 g36710 (retrotransposon)	1.6 kb	0/0	
CslF2	Os07G36690	none		2/2	
CslF3	Os07G36750	none		1/1	
CslF4	Os07G36740	Os07 g36720 (retrotransposon)	3.8 kb	0/0	
CslF6	Os08G06380	Os08 g06370 (transcription factor)	8.7 kb	17/15	Os03 g33680 (retrotransposon), Os03 g51350 (expressed), Os11 g30230 (hypothetical)
CslF7	Os10G20260	none		5/4	Os05 g49290 (acyltransferase)
CslF8	**Os07G36630**	none		4/3	**Os07 g36610 (CslF9)**
CslF9	**Os07G36610**	none		6/4	**Os07 g36630 (CslF8)**, Os01 g62540 (hypothetical), Os10 g37590 (hypothetical)
CslH1	Os10G20090	none		9/8	Os02 g14870 (hypothetical)
CslH2	Os04G35020	Os04 g35010 (DNA binding)	overlapped	6/6	
CslH3	Os04G35030	none		0/0	
GT8A	Os01 g65780	Os01 g65790 (pectinesterase)	4.3 kb	12/12	
GT8A	Os05 g35200	Os05 g35190 (expressed)	0.4 kb	6/6	
GT8A	Os02 g35020	none		4/3	Os03 g58640 (FHA domain containing)
GT8A	Os03 g08600	none		6/4	Os02 g09690 (expressed), Os02 g09720 (multidrug resistance 13), Os04 g56120 (receptor-like kinase), Os04 g46040 (hypothetical)
GT8B	Os03 g20120	none		12/11	Os07 g08970 (expressed)
GT8B	Os07 g48830	Os07 g48820 (transcription factor)	2.9 kb	4/4	
GT8C	Os03 g18890	Os03 g18900 (mito DNA polymerase)	5.4 kb	9/9	

Family	TIGR gene name*	Antisense gene present	Distance	MPSS, total/unique[†]	Genes MPSS tags hit[†]
GT8C	Os03 g24510	none		6/6	
GT8C	Os02 g50600	Os02 g50590 (hypothetical)	1.0 kb	6/6	
GT8C	Os06 g13760	Os06 g13770 (transposon)	4.3 kb	4/3	Os03 g52794 (phosphatidylinositol 3 & 4 kinase)
GT8C	Os07 g45260	none		12/11	Os08 g11720 (expressed), Os08 g11220 (expressed)
GT8C	Os04 g44850	none		6/6	
GT8C	Os03 g47530	none		11/10	Os04 g41280 (protein binding), Os05 g04660 (expressed)
GT8C	Os10 g31650	none		2/2	
GT8D	Os11 g37980	Os11 g37970 (win1 precursor)	1.8 kb	6/5	Os08 g04190 (homeobox), Os12 g25220 (retrotransposon)
GT8D	Os06 g49810	none		3/2	over 60 genes
GT8D	**Os09 g36190**	none		15/11	**Os09 g36180 (GT8D)**, **Os10 g21890 (GT8D)**, Os07 g34390 (protein binding), Os11 g38850 (protein kinase)
GT8D	**Os10 g21890**	none		11/6	30 genes
GT8D	Os04 g54360	Os04 g54370 (expressed)	0.1 kb	4/4	
GT8D	**Os02 g51130**	none		4/2	**Os06 g12280 (GT8D)**
GT8D	Os09 g30280	none		4/4	
GT8D	Os02 g29530	none		8/8	
GT8D	Os08 g38740	none		0/0	
GT8D	Os08 g23780	Os08 g23790 (exopolygalacturonase)	1.1 kb	7/7	
GT8D	Os06 g51160	Os06 g51150 (catalase isozyme B)	0.3 kb	3/2	**Os10 g21890 (GT8D)**
GT8D	Os03 g30000	none		9/8	over 30 genes, **Os10 g21890 (GT8D)**
GT8D	Os12 g38930	Os12 g38920 (heparanase-like)	0.2 kb	7/6	Os12 g29220 (MTN3)
GT8D	Os11 g03160	Os11 g03150 (hypothetical)	1.1 kb	0/0	
GT8D	Os12 g02910	Os12 g02900 (retrotransposon)	1.1 kb	0/0	
GT8D	Os01 g52710	none		2/2	
GT8D	Os03 g11330	Os03 g11320 (heparanase-like)	0.8 kb	7/7	
GT8D	**Os06 g12280**	Os06 g12270 (hypothetical)	0.8 kb	6/4	**Os02 g51130 (GT8D)**
GT8D	**Os09 g36180**	none		2/0	**Os09 g36190 (GT8D)**
GT8D	Os03 g21250	Os03 g21240 (PHR1)	7.4 kb	6/6	
GT8D	Os05 g40720	Oso5 g40710 (DELLA protein GAI)	1.2 kb	0/0	
GT8D	Os07 g48370	none		5/3	Os02 g26210 (flowering promoting factor), Os02 g26090 (anther-specific APG), Os03 g02050 (nonspecific lipid-transfer), Os04 g23550 (DNA binding), Os07 g06880 (gibberellin receptor), Os10 g41430 (nuc-1), Os12 g13800 (caffeic acid 3-O-methyltransferase), Os02 g36550 (phosphopantothenate-cysteine ligase), Os02 g48190 (expressed), Os03 g28090 (pectinesterase-2), Os03 g48480 (acyl-CoA thioesterase), Os03 g54000 (oligopeptide transporter 3), Os06 g04169 (catalytic/hydrolase), Os09 g04300 (expressed), Os09 g26240 (transposon)
GT8E	Os10 g40640	Os10 g40650 (holliday junction resolvase)	overlapped	17/12	Os10 g40650 (holliday junction resolvase)
GT8E	Os04 g46750	none		3/3	
GT8E	Os02 g41520	Os02 g41530 (hypothetical)	2.9 kb	4/4	
GT8E	Os04 g43700	none		8/8	

Bold text indicates ≥2 cell wall genes may share antisense sequences by MPSS tag data.
*From the TIGR database (http://rice.plantbiology.msu.edu/).
[†]From the MPSS database (http://mpss.udel.edu/).
[‡]CslE1 and CslE6 seem to have transcript assemblies suggesting readthrough of the rRNA methyl transferase.

Table S5. Real-time PCR primers

Real-time PCR primer	Sequence (5′ →>3′)	Product size, bp
Hv18 s-F	AAA CGG CTA CCA CAT CCA AG	154
Hv18 s-R	CCT CCA ATG GAT CCT CGT TA	
HvBActin-F	CTG CGA CAA TGG AAC CGG AAT G	150
HvBActin-R	TGC GCC TCA TCA CCA ACA TAA GC	
HvGA3PDH-F	GAC TCC AAG ACC CTT CTC TTC GGT G	106
HvGA3PDH-R	ACT CCA CAA CGT ACT CAG CAC CAG C	
HvUbi-F	CGC ACT CTG GCA GAC TAC AAC ATC C	137
HvUbi-R	GAG AAC ACC GAC AAC ACA AGA CCT G	
HvCesA-CR2-F	CCC TAG GGA CCA TCC TGG	187
HvCesA-CR2-R	GGC ACC ATT TGT CAA CAC	
HvCesA6-F	AAT GGC AAA GGT CCA GAG TG	158
HvCesA6-R	GTT GGG CTG CGG ATA GAG T	
HvCslA-F	CAG ATC CCC ATG TTC AAC GA	115
HvCslA-R	GGT CCG TGG AGT CGT CCA G	
HvCslC-F	GCC GGA GTC ATA TGA AGC AT	226
HvCslC-R	TGC TTC AGG AGC AAA CAT TG	
HvCslH-F	GCC AGC TTG ACC CAG TTC AAG AG	127
HvCslH-R	CGA TGA GAT AGC CAA GGC ATT GC	
HvGT8A-F	ACC CTC AAA CTG CAC ATT CC	231
HvGT8A-R	CCC AGG TAG TGG AGG ACG TA	
HvGT8D-F	GCC ACT CTT TCA GCA GGT TC	204
HvGT8D-R	AAA CAC TGC ACC ATT GAC CA	

Primers used for real-time PCR were designed using Primer 3 software. Oligonucleotide primers for *HvCesA6*, *HvCesA1*, *HvCesA8*, and *HvCslF6* genes were described previously [Burton RA, Shirley NJ, King BJ, Harvey AJ, Fincher GB (2004) The CesA gene family of barley. Quantitative analysis of transcripts reveals two groups of coexpressed genes. *Plant Physiol* 134:224–236].