Supporting Information

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SI Materials and Methods

Growth of Plants. Rice plants were grown in the summer at 30 °C in day and 24 °C at night, 60–80% relative humidity in the University of California, Davis greenhouses. T-DNA insertion lines were obtained from the Postech rice T-DNA insertion library in Korea. Mutant lines were genotyped using primers designed to be just outside each insertion site as well as an insertion internal primer. *Nicotiana benthamiana* plants were grown for 4 wk at 24 °C with 12 h light (70 μmol photons $m^{-2}{\cdot}s^{-1}$) and 60% humidity.

Expression Analysis. Total RNA was extracted using the RNeasy plant mini kit (Qiagen) following manufacturer's instructions. RNA preparations were treated with DNase1 (Qiagen) to remove traces of DNA contamination. One microgram of RNA was used for reverse transcription with the Transcriptor high-fidelity cDNA synthesis kit (Roche) and oligo dT primers. After synthesis, the cDNA reaction was diluted four times in RNase-free water, and 2 μL was used for PCR using the Fast SYBR Green master mix (Applied Biosystems) and gene-specific primers in a StepONE plus Q-PCR machine (Applied Biosystems).

Cell Wall Isolation and Monosaccharide Composition Analysis. For rice mutants, 5-wk-old leaf tissue was collected, frozen in liquid nitrogen, and freeze-dried overnight using a lyophilizer. Alcohol insoluble residue (AIR) preparation and destarching was done according to methods described in ref. 1. For monosaccharide composition analysis, 5 mg was hydrolyzed in 2 M trifluoroacetic acid (TFA) at 120 °C for 1 h. For mild TFA hydrolysis, 5 mg AIR was hydrolyzed with 0.1 M TFA at 120 °C for 1 h (2). The released monosaccharides were separated by HPAEC on a Dionex ICS3000 system equipped with a pulsed amperometric detector (PAD) as described (3).

Sequential Extraction of Xylan. Xylan was extracted from AIR as previously described (4). AIR (5 mg) was suspended in 0.5 mL 0.05 M CDTA (pH 6.5) for 24 h at room temperature on a thermomixer. The suspension was centrifuged at 48,000 × *g* at 4 °C and the pellet washed twice with deionized water. The pellet was subsequently extracted using 0.05 M Na_2CO_3 containing 0.01 M $NaBH_4$ for 24 h at 4 °C and washed twice with deionized water. For the xylan-rich fractions, 1 M KOH and 4 M KOH were used for 24 h each at room temperature with shaking on a thermomixer. The 1 M KOH and 4 M KOH fractions were similarly centrifuged at 48,000 × *g* at 4 °C. The KOH fractions were adjusted to pH 5 with glacial acetic acid. The fraction was further dialyzed against deionized water and lyophilized. For the linkage analysis and xylan fingerprinting, the 4 M KOH fraction was used directly following pectic extraction (without a 1 M KOH extraction).

Extraction, Separation, and Quantification of Hydroxycinnamates. For hydroxycinnamate extraction of plant material, 5 mg destarched AIR was saponified in 2 M sodium hydroxide at 22 °C for 24 h in 10-mL Teflon tubes at room temperature on a rocking agitator. After acidification with 0.8 mL concentrated hydrochloric acid, samples were extracted three times in ethyl acetate. The combined supernatants were vacuum dried and solubilized in 50% (vol/vol) methanol. HPLC separation was done as described (5) with a gradient of solvent A (0.2% TFA) and solvent B (acetonitrile): 0–5 min, 10% B isocratic; 5–25 min, 10–30% B linear; 25–40 min, 30% B isocratic; 40–45 min, 30–35% B linear; 45–46 min, 35–100% B linear; 46–51 min, 100% B isocratic; 51–53 min, 100–10% B linear; and 53–60 min, 10% B isocratic.

Saccharification Assay. To determine the amount of sugars released from plant material, water was added to 5 mg destarched AIR and the mixture was autoclaved at 120 °C for 1 h. A 500-μL enzyme mixture containing 50 mM citrate buffer pH 6.2, 1.6% (wt/vol) tetracycline, 2 μL Ctec2 enzyme mixture (Novozymes), which contains cellulases, β-glucosidases, and hemicellulases, was added and incubated at 50 °C for 24 h with shaking. After enzyme treatment, samples were pelleted, and released sugars in the supernatant were measured using DNS reagent [1% (wt/vol) 3,5-dinitrosalicylic acid, 30% (wt/vol) potassium sodium tartrate, 400 mM NaOH] reading absorbance at 540 nm.

Liquid Chromatography (LC)/TOF Mass Spectrometry of "Peak 1." The separation of metabolites was conducted on a fermentation monitoring HPX-87H column with 8% cross linkage (150 mm length, 7.8 mm internal diameter, and 9 μm particle size; Bio-Rad) using an Agilent Technologies 1100 Series HPLC system. A sample injection volume of 10 μL was used throughout. The temperature of the sample tray was maintained at 4 °C by an Agilent FC/ALS thermostat. The column compartment was set to 50 °C. Metabolites were eluted isocratically with a mobile phase composition 0.1% formic acid. A flow rate of 0.5 mL min^{-1} was used throughout. Xylobiose, xylotriose, xylotetraose, xylopentaose, and xylohexaose (Megazyme) were run as standards.

The HPLC system was coupled to an Agilent Technologies 6210 time-of-flight mass spectrometer (LC/TOF MS), by a 1/5 post-column split (Agilent Technologies). Nitrogen gas was used as both the nebulizing and drying gases to facilitate the production of gas-phase ions. The drying and nebulizing gases were set to 12 L/min and 30 psi, respectively, and a drying gas temperature of 330 °C was used throughout. Electrospray ionization (ESI) was conducted in the positive ion mode and a capillary voltage of 3,500 V was used. MS experiments were carried out in the full scan mode, at 0.86 spectra/s, for the detection of $[M + Na]^+$ ions. The instrument was tuned for a range of 50–1,700 *m/z*. Before LC/TOF MS analysis, the TOF MS was calibrated via an ESI-L-low concentration tuning mix (Agilent Technologies). Data acquisition and processing were performed by the MassHunter software package.

Preparative Chromatography of Peak 1. The xylanase treatment as described in the main text was scaled up to 100 mg of AIR. The digest was fractionated by size-exclusion chromatography on a Sephadex peptide column (GE Healthcare) equilibrated and eluted with 50 mM ammonium formate pH = 5 at 0.6 mL/min. Eluting oligosaccharides were detected with an in-line refractive index detector (Shodex). Peaks corresponding to oligosaccharides with degree of polymerization 4–6 and enriched in *m/z* 833 were collected and lyophilized, redissolved in water, and lyophilized again. The sample was resuspended in water and aliquots further fractionated by several runs of HPAEC on a semiprep CarboPac P100 column (9 × 250 mm) as described in the main text with the modification of a 2-mL/min flow rate. Peak 1 was collected in all of the runs and combined for subsequent NMR analysis.

NMR Spectroscopy. The content of peak 1 was freeze dried, deuterium (D) exchanged, and dissolved in D_2O (0.5 mL, 99.99% D). ^{1}H-NMR spectra were recorded at 298 K on a Bruker AVANCE 600 MHz NMR spectrometer equipped with an inverse (proton coils closest to the sample) gradient 5-mm TXI $^1H/^{13}C/^{15}N$ CryoProbe. A 2D total correlation spectroscopy (TOCSY) NMR experiment was recorded using standard Bruker pulse program "dipsi2ph." The following parameters were applied in the TOCSY

experiment: 2,048 data points in F2 (^{1}H) dimension and 256 data points in F1 (^{1}H) dimension; scan number (SN) of 64; dummy scans (DS) 4; interscan delay (D1) of 1.5 s; prescan delay (DW) 69.6 μs and mixing time (D9) 80 ms. All chemical shifts were referenced relative to DSS-D_6 (0.00 ppm for ^{1}H). The NMR data processing and analysis were performed using Bruker's Topspin 3.1 software.

Analysis of NMR Results. Peak 1 was collected and subjected to mass spectrometry indicating the presence of a hexapentose [$(M+Na^+)^+$ = 833 Da]. The anomeric protons of the hexasaccharide were established based on ^{1}H-NMR (Fig. S5*B*) and all nonanomeric signals (H2–H5) were assigned to each monosaccharide based on the TOCSY experiment (Fig. S5*C*). These NMR data confirmed the presence of a hexapentose. Most of the proton signals could be assigned to specific sugar residues based on published chemical shifts of the xylan-hexasaccharide $XB^{2,3}XX$ (fraction 3) (6, 7). The published data indicated that the hexapentose in peak 1 contains a xylotetraose backbone; all units β-(1→4) linked. In addition, the hexapentose also contained a 2-α-L-Ara*f* residue linked to the O-3 position of the third xylose from the reducing end. However, in contrast to the published assignments of $XB^{2,3}XX$, no resonances corresponding to a T-α-Araf were found [apart from a minor oligosaccharide contamination (<5%), anomeric signal at 5.4 ppm, Fig. S5*B*]. Instead, an additional monosaccharide moiety was present with ^{1}H resonances consistent with another T-D-Xyl*p* residue (table in Fig. S5*C*; see similarity between resonances of Xyl_4 and Xyl_5). The coupling constant of this monosaccharide ($J_{1,2}$ 8 Hz) is indicative of a β-linkage. Thus, it can be concluded that the oligosaccharide present in peak 1 is $XD^{2,3}XX$ containing a β-D-Xyl*p*-(1→2)-α-L-Ara*f*-(1→3) sidechain.

Transient Expression of XAX1 in *N. benthamiana*. Infiltration of 4 wk-old *N. benthamiana* leaves was done using *Agrobacterium tumefasciens* strain C58 (OD = 1), following the method described in ref. 1. For details on the plasmid constructs, see *SI Materials and Methods*. Leaves were collected 5 d postinfiltration for protein isolation.

Microsomal Extraction of *N. benthamiana* Leaves. For protein isolation, leaves were flash frozen, ground in a mortar and pestle in 15 mL of buffer [50 mM Hepes pH 7.0, 400 mM sucrose, 1 mM PMSF, 1% (wt/vol) PVPP, protease inhibitor mixture]. The plant material in buffer was filtered through Miracloth mesh and centrifuged at 3,000 × *g* for 10 min. The supernatant was centrifuged at 50,000 × *g* for 1 h. (Beckman Ultracentrifuge). The pellets containing the microsomes were resuspended in a 50-mM Hepes (pH 7.0), 400-mM sucrose buffer and stored at −80 °C. Protein concentration was determined using the Bradford method.

Endogenous Activity Assay. Microsomes corresponding to 100 μg protein were incubated with 50 mM Hepes pH 7.0, 400 mM sucrose, 5 mM $MnCl_2$, 740 Bq UDP-[^{14}C]xylose (American Radiolabeled Chemicals) in a total reaction volume of 50 μL. After incubation at 24 °C for various times, reaction was stopped by adding 70% ice-cold ethanol and 100 μg dextran as a carrier. The products were precipitated with ice-cold 70% ethanol, washed with 70% ethanol, sonicated in a sonicator bath for 30 min at 30 °C to resuspend pellet, and precipitated at −20 °C for 1 h. Washes were repeated until no counts were found in the wash solution. The pellet was resuspended in water, an equal volume of scintillation fluid was added (National Diagnostics), and the amount of activity was determined using a scintillation counter set to measure ^{14}C counts for 2 min (Beckman; LS 6500).

Subcellular Localization of Fluorescent-Tagged Proteins. For the onion bombardments, the XAX1 *pENTR* clone without a stop codon was recombined into pBullet GW-YFP G-CFP, a customized bombardment vector containing a Gateway recombination site fused to a C-terminal YFP and a *GmMan1::CFP*, the α-mannosidae I *cis*-Golgi marker from *Glycine max* (8). Vectors were used to bombard onion peels using a PDS-1000 particle bombardment system (Bio-Rad). Before bombardment, onions peels were placed on agar plates containing 0.5× Murashige and Skoog medium and 2% (wt/vol) sucrose for 1 h at 22 °C to recover. Approximately 1 μg of plasmid DNA was used to coat 50 μL of 60 mg/mL, 1 μm gold particles (Bio-Rad) in the presence of 20 mM $CaCl_2$ and 0.8 mM spermidine for 3 min with mild vortexing. Particles were dehydrated with three ethanol washes and transferred onto plastic discs to dry. Particles were accelerated into onion peels by a burst of helium at 1,100 psi under 28 inHg vacuum. Plates containing bombarded onions were covered in foil and incubated at 22 °C for 22 h before being visualized by confocal microscopy. Transformed samples were analyzed using a Zeiss LSM710 meta (QUASAR detector) equipped with a 408-nm diode, argon, and an In Tune laser. Images were taken using the inverted scope with a 1.30 NA oil 40× objective. Samples were fixed with formaldehyde and imaged sequentially (CFP: excitation, 405 nm; emission, 454–515 nm and YFP: excitation, 514 nm; emission, 519–621 nm). The Zen software package (Carl Zeiss) was used for image acquisition and image analysis. The individual frames from 20 images were *Z* stacked.

Glycosidic Linkage Analysis. Xylan was extracted from destarched AIR (5 mg) as described above in the sequential extraction of xylan method. The 4 M KOH fraction directly following pectic extraction was used for this analysis. The methylation procedure was modified from the method described by Ciucanu and Kerek (9). In brief, the pellet was partially dissolved in a suspension of dry DMSO and NaOH (12.5 mg/mL) and 100 μL methyliodide was added twice, with 3 h of stirring following the first addition and 1 h following the second. Water (2 mL) was added to quench the reactions and dichloromethane (2 mL) was used to extract the partially methylated carbohydrates. The residue remaining after evaporation of the dichloromethane was hydrolyzed with 2 M TFA at 121 °C for 90 min. The TFA was removed by evaporation under nitrogen and the pellet was reduced and acetylated as described (10). The residue was extracted with 1.2 mL ethyl acetate and 5 mL water. The organic fraction was dried and the partially methylated alditol acetates were dissolved in acetone for GC/MS analysis with a 7890A GC system and a 5975C MS detector (Agilent) in EI mode. An SP-2380 column (Supelco) was used for the separation.

Mass Distribution Analysis. Destarched AIR was incubated in 1 M KOH, 1% $NaBH_4$ (30 mg/mL) for 3 h with 1,400 rpm shaking at RT. The supernatant was collected after centrifugation for 10 min at 12,000 × *g*. The supernatant was neutralized using glacial acetic acid. After neutralization the supernatant was centrifuged for 10 min at 12,000 × *g* and the mass distribution resulting supernatant was analyzed as described (5). Equal amounts of sugar were loaded onto the SEC column.

Construction of GT61 Phylogeny. GT61 family sequences in rice and *Arabidopsis* were determined on the basis of a sequence possessing a DUF563 domain as well as a predicted transmembrane domain. To identify other family members, the completed genomes of four grass species (*Brachypodium distachyon*, *Oryza sativa*, *Sorghum bicolor*, and *Setaria italica*), 13 dicot species (*Aquilegia coerulea*, *Arabidopsis lyrata*, *Arabidopsis thaliana*, *Carica papaya*, *Cannabis sativa*, *Glycine max*, *Manihot esculenta*, *Mimulus guttatus*, *Medicago truncatula*, *Prunus persica*, *Populus trichocarpa*, *Ricinus communis*, and *Vitis vinifera*), the moss *Physcomitrella patens*, and lycophyte *Selaginella moellendorffii* were clustered to the GT61 sequences from *Arabidopsis* and rice at 50% sequence identity using the program uCLUST (11). An alignment of all family members was constructed with MUSCLE (12), and this alignment was used to

construct a maximum likelihood phylogeny with PhyML3 (13). The resulting tree was visualized using the tools available from the online resource interactive Tree Of Life (iTOL) (14). Clades were named according to ref. 15.

1. Yin L, et al. (2011) The cooperative activities of CSLD2, CSLD3, and CSLD5 are required for normal Arabidopsis development. *Mol Plant* 4:1024–1037.
2. Fry S (1988) *The Growing Plant Cell Wall: Chemical and Metabolic Analysis* (Blackburn Press, Caldwell, NJ).
3. Brown DM, et al. (2007) Comparison of five xylan synthesis mutants reveals new insight into the mechanisms of xylan synthesis. *Plant J* 52:1154–1168.
4. Harholt J, et al. (2006) ARABINAN DEFICIENT 1 is a putative arabinosyltransferase involved in biosynthesis of pectic arabinan in Arabidopsis. *Plant Physiol* 140:49–58.
5. Harholt J, et al. (2010) Generation of transgenic wheat (Triticum aestivum L.) accumulating heterologous endo-xylanase or ferulic acid esterase in the endosperm. *Plant Biotechnol J* 8:351–362.
6. Mazumder K, York WS (2010) Structural analysis of arabinoxylans isolated from ball-milled switchgrass biomass. *Carbohydr Res* 345:2183–2193.
7. Höije A, et al. (2006) Evidence of the presence of 2-O-β-D-xylopyranosyl-α-l-arabinofuranose side chains in barley husk arabinoxylan. *Carbohydr Res* 341:2959–2966.
8. Nelson BK, Cai X, Nebenführ A (2007) A multicolored set of in vivo organelle markers for co-localization studies in Arabidopsis and other plants. *Plant J* 51:1126–1136.
9. Ciucanu I, Kerek F (1984) A simple and repaid method for the permethylation of carbohydrates. *Carbohydr Res* 131:209–217.
10. York WS, Darvill AG, McNeil M, Albersheim P (1985) 3-deoxy-d-manno-2-octulosonic acid (KDO) is a component of rhamnogalacturonan II, a pectic polysaccharide in the primary cell walls of plants. *Carbohydr Res* 135:109–126.
11. Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* 26:2460–2461.
12. Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* 32:1792–1797.
13. Guindon S, Gascuel O (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst Biol* 52:696–704.
14. Letunic I, Bork P (2007) Interactive Tree Of Life (iTOL): An online tool for phylogenetic tree display and annotation. *Bioinformatics* 23:127–128.
15. Vogel J, et al.; International Brachypodium Initiative (2010) Genome sequencing and analysis of the model grass Brachypodium distachyon. *Nature* 463:763–768.

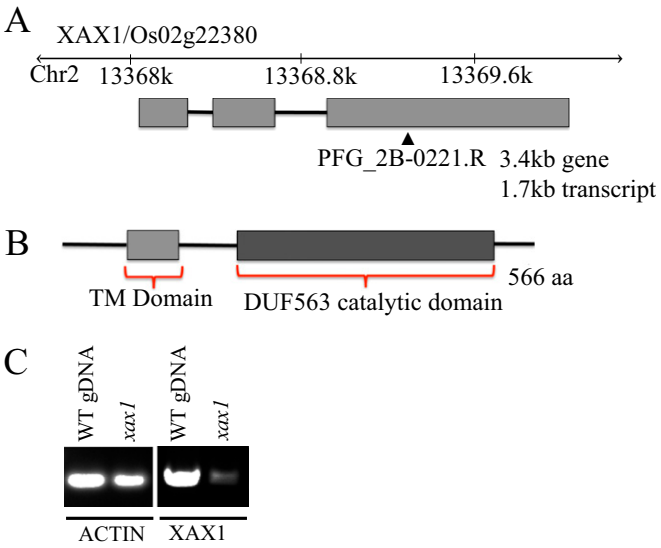


Fig. S1. XAX1 T-DNA insertional rice mutant. (*A*) Location of the T-DNA insertion. Image modified from RiceGE. (*B*) Protein structure of XAX1: N-terminal transmembrane domain and C-terminal DUF563 domain characteristic of GT61 family genes. (*C*) Confirmation of XAX1 knock out. Segregating wild-type plants were used as negative control.

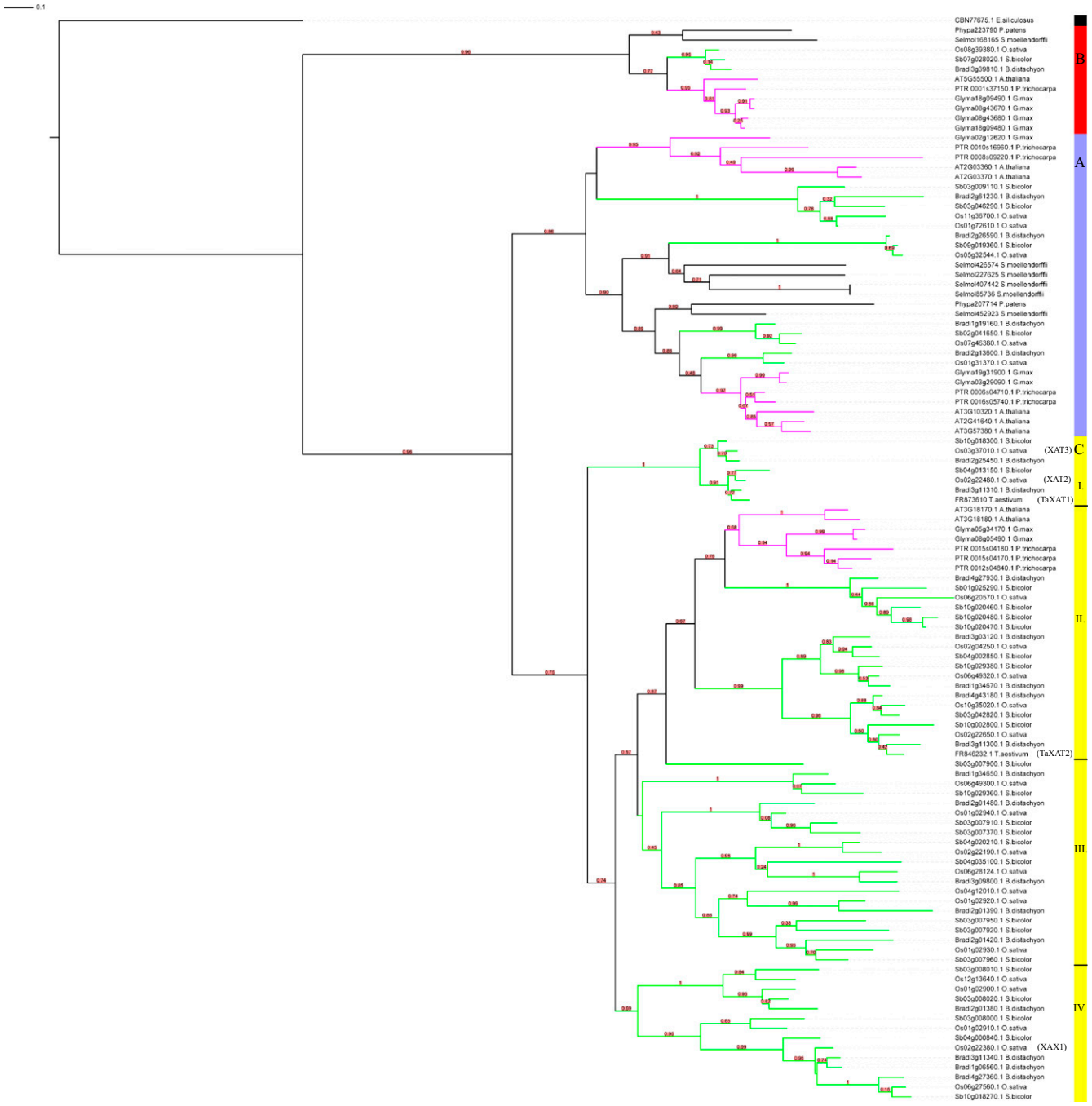


Fig. S2. Glycosyltransferase 61 tree. Color coding on tree arms: black represents basal plants (*Physcomitrella patens*, *Selaginella moellendorffii*), pink represents dicots (13 genomes included), and green represents grasses (4 genomes included). The color bar on the *Right* shows the three clades that GT61 is divided into (A, B, C: I, II, III, and IV). XAX1 is located in the grass-specific clade C.IV.

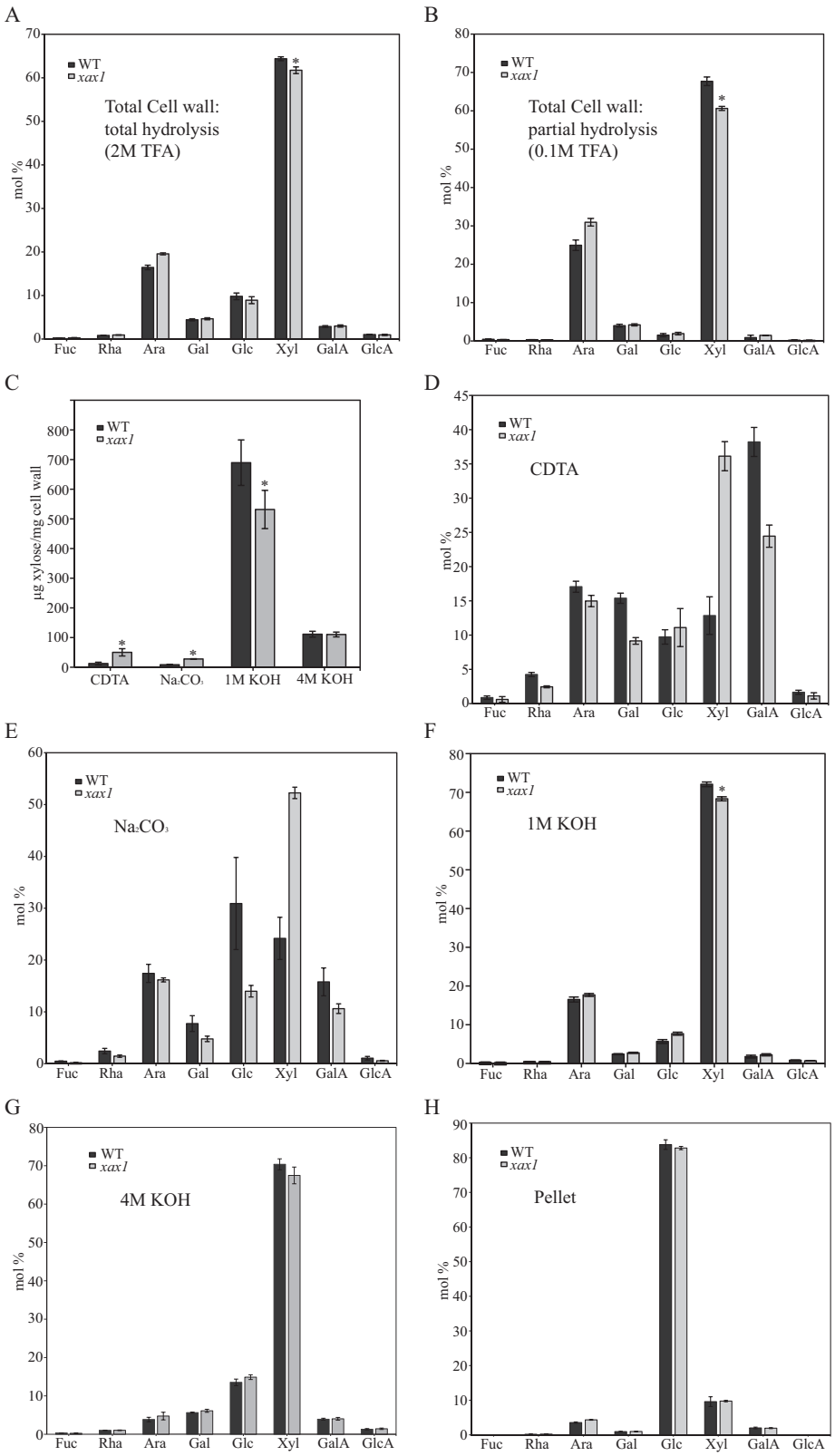


Fig. S3. Cell wall composition analysis. (*A*) Total sugar composition with total TFA hydrolysis with sugar separation and quantification on the HPAEC. (*B*) Total sugar composition with 0.1% TFA partial hydrolysis. (*C*) Xylose content of CDTA, Na_2CO_3, 1 M KOH, and 4 M KOH fractions by weight. By far, the largest change in xylose content was in the 1 M KOH fraction. (*D*) Cellular fractionation in mol %: CDTA fraction. (*E*) Na_2CO_3 fraction. (*F*) 1 M KOH fraction. (*G*) 4 M KOH fraction. (*H*) Sulfuric acid treatment of pellet. Error bars represent SD ($n = 4$). *Significantly different from WT, *t* test: $P < 0.05$.

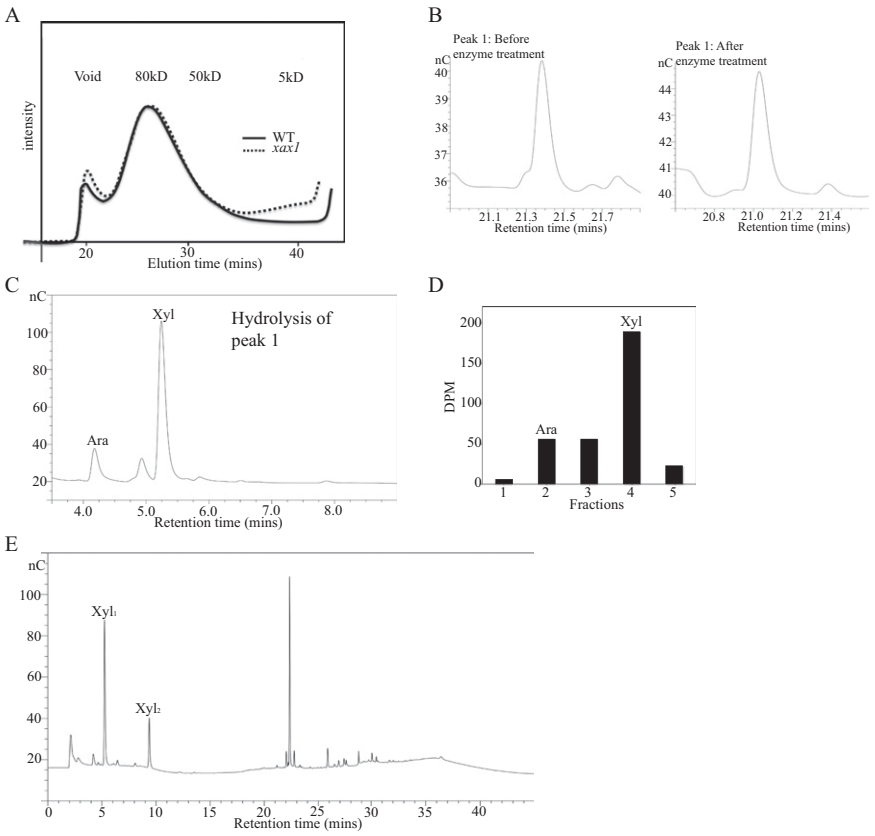
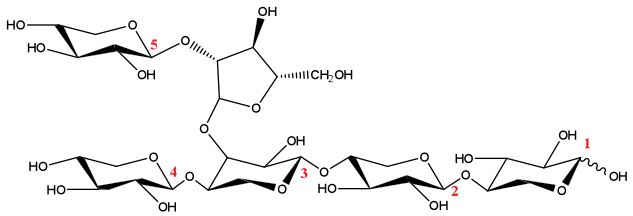


Fig. S4. Xylan composition analysis. (*A*) Size-exclusion chromatography (SEC) profiles of wild type and mutant. Fractions soluble in 1 M KOH were analyzed by SEC using a Superose 12 column. The elution times of dextran molecular markers are indicated. Three biological replicates of both WT and mutant were analyzed and only insignificant differences could be observed. (*B*) HPAEC profile of arabinofuranosidase treatment of isolated peak 1 from wild-type rice showing no shift in retention time consistent with no arabinose release indicating that "peak 1" has no terminal arabinose. (*C*) HPAEC profile of TFA hydrolysis of isolated peak 1 from wild-type rice showing xylose and arabinose peaks. (*D*) Analysis of endogenous products after UDP-[^{14}C]xylose incubation with microsomes. TFA hydrolyzed product of activity assay (Fig. 3*C*), run on a TLC plate to separate sugars. Fraction 2 is where arabinose runs and fraction 4 is where xylose runs. Less than 22% of the counts are in the arabinose fraction, indicating that the epimerase activity is minimal. (*E*) HPAEC profile of xylan fingerprinting of *Selaginella* leaves after xylanase treatment showing lack of peaks that contain arabinose (as seen in Fig. 2*A*).

A



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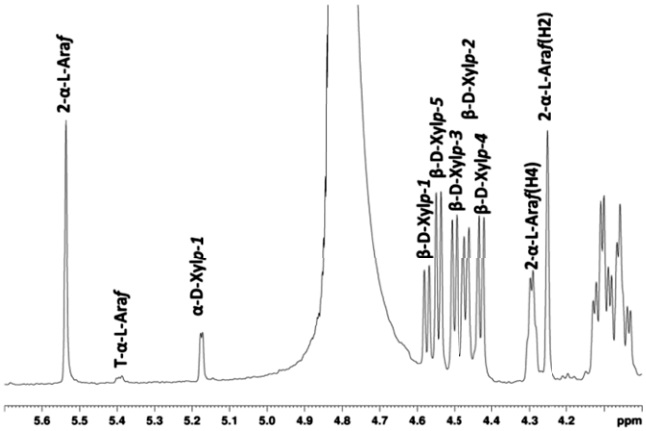

C

Sugar residues	H1	H2	H3	H4	$H5_{(eq)}$	$H5_{(ax)}$
2-α-L-Araf	5.540	4.271	4.074	4.301	3.893	3.724
β-D-Xylp^5	4.542	3.271	3.445	3.611	3.973	3.328
β-D-Xylp^4	4.427	3.234	3.413	3.602	3.915	3.278
β-D-Xylp^3	4.500	3.440	3.723	3.822	4.129	3.418
β-D-Xylp^2	4.468	3.295	3.554	3.779	4.092	3.364
β-D-Xylp^1	4.573	3.241	3.541	3.765	4.044	3.370
α-D-Xylp^1	5.185	3.545	3.747	ND	3.809	ND

ND: not determined

Fig. S5. ^{1}H-NMR spectroscopy of compound present in peak 1. (*A*) Proposed structure. Oligosaccharide nomenclature as in ref. 1. (*B*) Anomeric region of the ^{1}H-NMR spectrum of content of peak 1. (*C*) Table displaying assigned ^{1}H chemical shifts present in peak 1 based on ^{1}H, TOCSY, and NOESY spectra. For position of Xyl-residues, see *A*.

1. Faure R, et al. (2009) A brief and informationally rich naming system for oligosaccharide motifs of heteroxylans in plant cell walls. *Aust J Chem* 62:533–537.

Table S1. Rice T-DNA insertional mutants included in reverse genetics screen

Gene ID	Insertional line	First gen	Segregation			Second gen	Segregation			Third gen	Segregation		
		No. of plants	WT	Het	Hom	No. of plants	WT	Het	Hom	No. of plants	WT	Het	Hom
Os02g22380	PFG_2B-00221.R	6	2	3	1	20	6	10	4				
Os02g22380	PFG_3A-00858.L	7	7	0	0								
Os06g27560	RMD_04Z11LF36	4 LG	2	2	0	8	3	3	2	20	2	9	9
Os06g28124	PFG_3A-60924.L	4	4	0	0								
Os06g28124	RdSpm3886B_3.1	5	3	2	0	22	3	11	8				
Os02g04250	PFG_2B-20307.R	6	1	5	0								
Os01g31370	PFG_3A-50649.R	9	2	6	1								
Os03g37010	PFG_1B-11030.R	10	4	3	3								
Os03g37010	PFG_2D-40718.R	5	0	5	0								
Os01g02910	PFG_3A-06897.R	9	4	4	1								
Os01g02940	RMD_02Z15BI14	1 LG	1	0	0								
Os01g02940	PFG_1C-04714.L	6	6	0	0								
Os01g02940	PFG_3A-06293.R	7	7	0	0								
Os02g22190	PFG_2D-41191.R	8	6	1	1	18	6	7	5				
Os07g46380	PFG_3A-14284.R	5	1	0	4	S							
Os07g46380	PFG_1C-12733.L	NG											
Os12g13640	PFG_3C-00312.R	4 LG	2	1	1								
Os01g72610	PFG_3A-50023.L	4 LG	4	0	0								
Os01g72610	PFG_2D-40265.L	NG											
Os04g12010	PFG_3A-51973.L	6	0	0	6								
Os02g22650	PFG_3A-52621.R	2 LG	2	0	0								

Het, heterozygote; Hom, homozygote; LG, low germination; NG, no germination; S, sterile; WT, wild type.

Table S2. List of genotyping primers for rice mutants

Insertional line/target	Sequence (5′ to 3′) sense antisense	T-DNA specific internal primer
PFG_2B-00221.R	CAATGACAGCACTGTGGACCCCGTC ACCGTCACGTTGAAACCAAGCTCCGT	CATCACTTCCTGATTATTGACC
PFG_3A-00858.L	AGCCACATTCCCTCGAAAG AAAGGGAGCGGAAGAAAGAG	CATCACTTCCTGATTATTGACC
RMD_04Z11LF36	ATGTCGTCGACGGCGTACACGCGG CTATTCCTGCAATTCGTCGAGCGC	GAAACCCTTAGTATGTATTTG
PFG_3A-60924.L	TATTACCCCGTAATCAGCGCATAAAG CTAACAGGTCGCATATTGGCTTGCGT	CATCACTTCCTGATTATTGACC
RdSpm3886B_3.1	AACGATCATGAACGCTAGGCATCGA AACTGCAATGGCATTTAGCCATGAAG	GTGTGGGGTTTTGGCCGACAC
PFG_2B-20307.R	TTTGTCGGTGTCTGCTTGAGTACGAA AGCATGGTTAGGTTGTTGCCTGGTCA	CATCACTTCCTGATTATTGACC
PFG_3A-50649.R	TCGTGGTACTCGAGCATGACGAACAC CCAGTCCATCTCCAGTGGTCAGCGGC	CATCACTTCCTGATTATTGACC
PFG_1B-11030.R	CTTCGTCTTCGTTTCCAAGC GTGTACCAGCGAATGTGTGG	CATCACTTCCTGATTATTGACC
PFG_2D-40718.R	CCCTGACAAATCTGACGAGG TCCGCATCTTTGCTTTCTCTCTCT	CATCACTTCCTGATTATTGACC
PFG_3A-06897.R	TCGGCTTCTTCCTTGTTCTC CATGTGCTTCCGCGAGTAG	CATCACTTCCTGATTATTGACC
RMD_02Z15BI14	ATGGAGGGCGGTGGCAAGGCGGGG CTAGTTGTGTGTGGTGTTATTGTT	GAAACCCTTAGTATGTATTTG
PFG_1C-04714.L	ACATCTGGAAGTCGGACACC CAAAGGATCCTCCTGCTCAG	CATCACTTCCTGATTATTGACC
PFG_3A-06293.R	CAATCCTGCAATTCAAAGGG GACCTCTCTTGGCAAACAGC	CATCACTTCCTGATTATTGACC
PFG_2D-41191.R	CTAATCCGGCTTGGAATGTCATAAAA GTTTTAGCCTGCAGCCAGAGTAGCTA	CATCACTTCCTGATTATTGACC
PFG_3A-14284.R	ACTCGAGGATGACGAACACCACGCG GCTTCTCCAGAAACACGGAGATGATA	CATCACTTCCTGATTATTGACC
PFG_1C-12733.L	CTGTCCAAGTGTGTGATGGG CCTCGTCTGGTGATCGTGTC	CATCACTTCCTGATTATTGACC
PFG_3C-00312.R	TGCCTGCAACTACTTTGCAC TATGTGCGCCAATTGAGATC	CATCACTTCCTGATTATTGACC
PFG_3A-50023.L	CATGAGCAGGGTAGAGGAGG CGTCTATCCGCTCGAGAAAC	CATCACTTCCTGATTATTGACC
PFG_3A-51973.L	GATTCTTATGTTGAGGCGCG AATGTGATACTGTGGCACGC	CATCACTTCCTGATTATTGACC
PFG_3A-52621.R	CACGACTTGAAGCTGCTCAC CCTACTTGTGCTCTCCCTCG	CATCACTTCCTGATTATTGACC

Table S3. Glycosidic linkage analysis of 4 M KOH fraction in mol %

	WT	*xax1*
t-Araf	2.88 ± 1.16	3.22 ± 0.72
2-Araf	1.87 ± 0.10	0.82 ± 0.10
3-Araf	0.35 ± 0.01	0.48 ± 0.06
5-Araf	1.28 ± 0.07	1.50 ± 0.06
t-Xyl	1.96 ± 0.48	1.26 ± 0.33
4-Xyl (or 2-Xyl)	60.38 ± 2.75	56.61 ± 2.20
2,4 and 3,4 Xyl	16.53 ± 4.00	20.16 ± 0.74
2,3,4-Xyl	1.34 ± 0.25	1.80 ± 0.13
t-Gal	0.91 ± 0.08	1.13 ± 0.13
4-Glc	4.85 ± 0.72	6.04 ± 0.63
Total	92.34 ± 2.21	93.03 ± 0.69

Error is SD and represents four biological replicates.

Table S4. List of primers used for qPCR

Primer name	Orientation	Sequence (5′ to 3′)	Target
22380F	Sense	GGCAGCTCATGGCACACATC	qRT PCR amplicon *XAX1*
22380R	Antisense	TGTTCCTCGTCGTGTCGTCTC	qRT PCR amplicon *XAX1*
UBQ5 F	Sense	ATGGCCAACCACTTCGACCG	qRT PCR reference gene
UBQ5 R	Antisense	TAAGCCTGCTGGTTGTAGACGT	qRT PCR reference gene