The Arabidopsis Family GT43 Glycosyltransferases Form Two Functionally Nonredundant Groups Essential for the Elongation of Glucuronoxylan Backbone

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There exist four members of family GT43 glycosyltransferases in the Arabidopsis (Arabidopsis thaliana) genome, and mutations of two of them, IRX9 and IRX14, have previously been shown to cause a defect in glucuronoxylan (GX) biosynthesis. However, it is currently unknown whether IRX9 and IRX14 perform the same biochemical function and whether the other two GT43 members are also involved in GX biosynthesis. In this report, we performed comprehensive genetic analysis of the functional roles of the four Arabidopsis GT43 members in GX biosynthesis. The I9H (IRX9 homolog) and I14H (IRX14 homolog) genes were shown to be specifically expressed in cells undergoing secondary wall thickening, and their encoded proteins were targeted to the Golgi, where GX is synthesized. Overexpression of I9H but not IRX14 or I14H rescued the GX defects conferred by the irx9 mutation, whereas overexpression of I14H but not IRX9 or I9H complemented the GX defects caused by the irx14 mutation. Double mutant analyses revealed that I9H functioned redundantly with IRX9 and that I14H was redundant with IRX14 in their functions. In addition, double mutations of IRX9 and IRX14 were shown to cause a loss of secondary wall thickening in fibers and a much more severe reduction in GX amount than their single mutants. Together, these results provide genetic evidence demonstrating that all four Arabidopsis GT43 members are involved in GX biosynthesis and suggest that they form two functionally nonredundant groups essential for the normal elongation of GX backbone.

Secondary walls constitute the bulk of cellulosic biomass produced by vascular plants. Cellulosic biomass in the form of fibers and wood is an important raw material for a myriad of industrial uses, such as timber, pulping, papermaking, and textiles. Due to the dwindling of nonrenewable fossil fuels and the detrimental effects of burning fossil fuels on the global environment, there has been an urgent call to develop alternative renewable energy sources, and the lignocellulosic biomass from plants is considered to be an attractive renewable source for biofuel production (Somerville, 2006). However, lignocellulosic biomass is recalcitrant to the enzymatic conversion of cellulose into sugars, because cellulose is embedded in a complex mixture of polysaccharides and lignin polymers that block the accessibility of degrading enzymes. It has been shown that reduction of lignin and xylan by chemical or enzymatic treatment or by the transgenic approach reduces the recalcitrance of the lignocellulosic biomass to saccharification (Chen and Dixon, 2007; Himmel et al., 2007; Lee et al., 2009a). Therefore, a complete understanding of how individual components of lignocellulosic biomass are biosynthesized will potentially allow us to design novel strategies for genetic modification of cell wall composition and, hence, reduction in biomass recalcitrance to biofuel production.

Xylan is the main hemicellulose that cross-links with cellulose in the secondary walls of dicot plants (Carpita and McCann, 2000). It is made of a linear backbone of β-(1,4)-linked xylosyl residues, about 10% of which are attached with side chains of single residues of glucuronic acid (GlcA) and/or 4-O-methylglucuronic acid (MeGlcA) via α-(1,2)-linkages. The backbone xylosyl residues may also be substituted with the arabinosyl group and acetylated. Based on the nature of the side chains, xylan is generally grouped as (methyl)glucuronoxylan (GX), which is the main hemicellulose in dicots, and arabinoxylan and glucuronoarabinoxylan, which are the most abundant hemicelluloses in grass cell walls (Ebringerová and Heinze, 2000). In addition to the xylosyl backbone, the reducing end of xylan from birch (Betula verrucosa), spruce (Picea abies), Arabidopsis (Arabidopsis thaliana), and poplar (Populus alba × Populus tremula) contains a unique tetrasaccharide sequence β-D-Xylp-(1→3)-α-L-Rhap-(1→2)-α-D-GalpA-(1→4)-D-Xylp (Shimizu et al., 1976; Johansson...
The biosynthesis of xylan requires multiple glycosyltransferases and other modifying enzymes. Early biochemical studies revealed the activities of xylosyltransferases, glucuronosyltransferases, arabinosyltransferases, methyltransferases, and acetyltransferases that are likely involved in the biosynthesis of xylan (Baydoun et al., 1983, 1989; Kuroyama and Tsumuraya, 2001; Gregory et al., 2002; Porchia et al., 2002; Urahara et al., 2004; Zeng et al., 2008). However, none of the genes corresponding to these xylan biosynthetic enzymes have been identified. Recent molecular and genetic studies in Arabidopsis and poplar have led to the identification of a number of glycosyltransferases that are essential for GX biosynthesis. Among them, several members of the families GT47 and GT8 from Arabidopsis (FRA8, F8H, IRX8, and PARVUS) and poplar (GT47C, GT8D, and GT8E/8F) are implicated in the biosynthesis of the GX reducing end sequence (Aspeborg et al., 2005; Brown et al., 2005, 2007; Zhong et al., 2005; Zhou et al., 2006, 2007; Lee et al., 2007b, 2009b, 2009c; Peña et al., 2007; Persson et al., 2007). These glycosyltransferase genes are specifically expressed in vessels and fibers, and their encoded proteins are targeted to Golgi, where GX is synthesized, except for PARVUS and GT8E/8F, which are predominantly located in the endoplasmic reticulum (Lee et al., 2007b, 2009c). Mutations of the Arabidopsis FRA8, IRX8, and PARVUS genes all led to a near loss of the reducing end tetrasaccharide sequence and a reduction in GX amount (Brown et al., 2007; Lee et al., 2007b; Peña et al., 2007), indicating their essential roles in the biosynthesis of the GX reducing end sequence, although their exact enzymatic activities are still unknown.

The genetic studies have also identified roles of two members of family GT43 glycosyltransferases, IRX9 and IRX14, from Arabidopsis and GT43B from poplar in the biosynthesis of the GX xylosyl backbone (Brown et al., 2007; Peña et al., 2007; Zhou et al., 2007). The expression of IRX9 has been shown to be associated with cells undergoing secondary wall biosynthesis, and its encoded protein is targeted to the Golgi. Mutation of the IRX9 gene causes a drastic reduction in xylan xylosyltransferase activity (Brown et al., 2007; Lee et al., 2007a) and concomitantly a substantial decrease in the GX chain length and GX amount (Peña et al., 2007). Mutation of IRX14 was shown to result in a reduction in the GX level and the xylosyltransferase activity (Brown et al., 2007). In addition, two functionally redundant glycosyltransferases, IRX10 and IRX10-like, which belong to family GT47, were also demonstrated to be required for the normal GX level and xylan xylosyltransferase activity, suggesting their involvement in the biosynthesis of the GX xylosyl backbone (Brown et al., 2009; Wu et al., 2009).

In this report, we performed comprehensive molecular and genetic studies of the roles of all members of the Arabidopsis family GT43 glycosyltransferases in GX biosynthesis. We show that, like IRX9, the other three GT43 members, I9H (IRX9 homolog), IRX14, and I14H (IRX14 homolog), are expressed in secondary wall-containing cells and that their encoded proteins are targeted to the Golgi. We have found that the GX defects in the irx9 mutant can be rescued by overexpression of I9H but not IRX14 and I14H. Similarly, overexpression of I14H but not IRX9 and I9H is able to complement the GX defects caused by the irx14 mutation. Furthermore, genetic analysis of an array of double mutants revealed redundant and nonredundant roles of GT43 members in GX biosynthesis. Our findings demonstrate that the Arabidopsis family GT43 glycosyltransferases form two functionally nonredundant groups essential for the normal elongation of GX backbone.

RESULTS

Family GT43 Glycosyltransferase Genes Are Expressed in Cells Undergoing Secondary Wall Thickening

The previous findings that two members of the Arabidopsis family GT43 glycosyltransferases are involved in GX biosynthesis raised the question of whether other GT43 members perform a similar function. There exist four GT43 members, IRX9 (At2g37090), I9H (IRX9 homolog; At1g27600), IRX14 (At4g36890), and I14H (IRX14 homolog; At5g67230), in the Arabidopsis genome. Among them, IRX14 and I14H share the highest sequence similarity (81.1%), and I9H shares a relatively higher sequence similarity with IRX9 than with IRX14 and I14H (Fig. 1, A and B). Phylogenetic analysis further indicates that IRX14 and I14H are more closely related to each other than to IRX9 and I9H, and vice versa (Fig. 1C).

IRX9 was previously shown to be specifically expressed in vessels and fibers (Peña et al., 2007), but the expression patterns of other GT43 members are not known. Quantitative PCR analysis showed that I9H and I14H were ubiquitously expressed in most of the organs examined, whereas IRX9 and IRX14 were preferentially expressed in the inflorescence stems (Fig. 1D). Further expression analysis in the isolated cells from stems revealed that IRX9, 19H, IRX14, and I14H were preferentially expressed in xylem and/or interfascicular fibers, the cell types undergoing secondary wall thickening (Fig. 1E). We next tested whether the expression of IRX9, I9H, IRX14, and I14H was regulated by SND1, a master transcriptional switch activating the biosynthetic pathways of xylan, cellulose, and lignin (Zhong et al., 2006, 2007). It was found that their expression was reduced in the SND1/NST1 RNA interference line and that SND1 overexpression induced the expression of IRX9, I9H, and IRX14 (Fig. 1F).

We further examined the expression patterns of I9H, IRX14, and I14H in transgenic Arabidopsis plants using the GUS reporter gene. Consistent with the quantitative PCR analysis, these genes were found to
Figure 1. Sequence comparison and expression analysis of family GT43 members. A, Amino acid sequence alignment of the four Arabidopsis GT43 proteins. The numbers shown at the left of each sequence are the positions of amino acid residues in the corresponding proteins. Identical and similar residues are shaded with black and gray, respectively. B, Percentage of identity and similarity of GT43 proteins. C, Phylogenetic relationship of Arabidopsis GT43 members. The GT43 sequences were aligned using the ClustalW program (Thompson et al., 1994), and the phylogenetic tree was displayed using the TREVIEW program (Page, 1996). The 0.1 scale denotes 10% change, and the bootstrap values are presented in percentages at the nodes. D,
be specifically expressed in interfascicular fibers and xylem cells in the inflorescence stems (Fig. 2, A, B, D, E, G, and H). In the roots, IRX14 and I14H were expressed in both developing vessels and xylary fibers in the secondary xylem (Fig. 2, F and I), whereas I9H expression was only evident in developing vessels but not in xylary fibers (Fig. 2C). These expression studies demonstrate a close association of I9H and I14H with cells undergoing secondary wall thickening, suggesting their possible involvement in secondary wall biosynthesis.

Family GT43 Glycosyltransferases Are Targeted to the Golgi

IRX9 was previously demonstrated to reside in the Golgi (Peña et al., 2007), but the subcellular locations of other GT43 members have not yet been examined. Sequence analysis of I9H, IRX14, and I14H using the TMHMM2.0 program for the prediction of transmembrane helices (http://www.cbs.dtu.dk/service/TMHMM-2.0/) predicts that they are membrane proteins with a single (I9H and IRX14) or double (I14H) transmembrane helices (Fig. 3, A–C). To study their actual subcellular locations, yellow fluorescent protein (YFP)-tagged GT43 members were coexpressed with cyan fluorescent protein (CFP)-tagged FRA8 in carrot (Daucus carota) cells. Examination of the fluorescent signals revealed that I9H, IRX14, and I14H exhibited a punctate distribution, a pattern matched with FRA8 (Fig. 3, F–Q), which is known to be localized in the Golgi (Zhong et al., 2005). The control cells expressing YFP alone showed signals throughout the cytoplasm (Fig. 3, D and E). These results demonstrate that I9H, IRX14, and I14H are Golgi-localized proteins.

Overexpression of I9H But Not IRX14 and I14H Complements the irx9 Mutant Defects

Previous genetic studies showed that mutation of either IRX9 or IRX14 caused a partial reduction in the xylosyltransferase activity and GX content (Brown et al., 2007; Lee et al., 2007a; Peña et al., 2007). Because all the GT43 members are expressed in cells undergoing secondary wall thickening, we reasoned that they might be functionally redundant and that the partial defects in GX biosynthesis in irx9 or irx14 could be due

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**Figure 1.** (Continued.)
Quantitative PCR analysis of the expression of IRX9, I9H, IRX14, and I14H in different Arabidopsis organs. The lowest expression level among the organs analyzed was set to 1. E, Quantitative PCR analysis of the expression of IRX9, I9H, IRX14, and I14H in pith cells, xylem cells, and interfascicular fibers that were laser microdissected from wild-type Arabidopsis stems. The expression level in pith cells was taken as 1. F, Quantitative PCR analysis of the expression of IRX9, I9H, IRX14, and I14H in the SND1 overexpressor (SND1-OE; left) and the SND1/NST1 RNA interference line (right). The expression of each gene in the wild type was used as a control. Error bars in D to F denote s of three biological replicates.
Figure 3. Subcellular localization of I9H, IRX14, and I14H. Fluorescent protein-tagged fusion proteins were expressed in carrot protoplasts, and the signals were visualized with a laser confocal microscope. A to C, I9H, IRX14, and I14H are membrane proteins as predicted by the TMHMM2.0 program. Inside indicates the cytoplasmic side of the membrane, and outside indicates the noncytoplasmic side of the membrane. D and E, A carrot protoplast (D; differential interference contrast image) expressing YFP alone showing the fluorescent signals throughout the cytoplasm (E). F to I, A carrot protoplast (F) coexpressing I9H-YFP (G) and the Golgi-localized FRA8-CFP (H). J to M, A carrot protoplast (J) coexpressing IRX14-YFP (K) and FRA8-CFP (L). N to Q, A carrot protoplast (N) coexpressing I14H-YFP (O) and FRA8-CFP (P). Note the colocalization of I9H-YFP (I), IRX14-YFP (M), and I14H-YFP (Q) with the Golgi marker FRA8-CFP.
to the partial compensation by other GT43 members. To test this possibility, we investigated whether the defects caused by either irx9 or irx14 could be rescued by overexpression of GT43 members driven by the cauliflower mosaic virus (CaMV) 35S promoter. One of the prominent phenotypes of irx9 was the weakened

stem strength due to the reduced secondary wall thickness (Fig. 4, A and B). Overexpression of I9H and IRX9 in the irx9 mutant effectively restored the stem breaking strength and the secondary wall thickness of fibers to the wild-type levels (Fig. 4, A, C, D, and G), indicating that I9H is a functional homolog of

![Figure 4](image-url)
IRX9. In addition, overexpression of I9H and IRX9 rescued the collapsed vessel phenotype caused by the irx9 mutation (Supplemental Fig. S2, A–C). It was noted that not all I9H-complemented irx9 plants exhibited the same breaking strength as that of the wild type, which is likely due to the variation of the degree of complementation among different individuals. In contrast, overexpression of IRX14 and I14H did not complement the defects in stem strength, secondary wall thickness, and vessel morphology conferred by irx9 (Fig. 4, A, E, and F; Supplemental Fig. S2, D and E).

The reduced secondary wall thickness in irx9 was previously found to be caused by GX defects, including a decrease in GX level, a loss of nonmethylated GlcA, and a reduced GX chain length (Penña et al., 2007). Analysis of the GX content revealed that I9H overexpression in the irx9 mutant restored the cell wall Xyl level to that of the wild type (Table I), indicating that excess I9H could complement the loss of IRX9 to restore the GX level. It should be noted that similar to fra8, irx9 and irx14 cause not only a reduction in Xyl but also a reduction in Glc, which might be attributed to an indirect impediment of cellulose synthesis caused by a reduction in GX (Zhong et al., 2005). The observed increase in the levels of some other cell wall sugars might be simply due to the relative decrease in the levels of cellulose and GX or an increased synthesis of other wall polysaccharides in response to the reduced level of cellulose, a phenotype commonly observed in cellulose-deficient mutants (Fagard et al., 2000; His et al., 2001). Overexpression of GT43 members in irx9 also resulted in a change in the levels of other sugars in addition to Xyl, which might reflect the complexity of cell wall metabolism. The structure of GX in the complemented plants was examined by analyzing the acidic xylooligosaccharides using matrix-assisted laser-desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). Acidic xylooligosaccharides from the wild type contain two prominent peak masses [M+Na]⁺ at mass-to-charge ratios (m/z) of 745 and 759, which correspond to xylotetrasaccharides substituted with one GlcA and one MeGlcA, respectively (Fig. 5A). The loss of GlcA side chains caused by the irx9 mutation (Fig. 5B) was rescued by overexpression of I9H and IRX9 (Fig. 5, C and D) but not by IRX14 and I14H (Fig. 5, E and F).

The structure of GX from the complemented plants was further analyzed using NMR spectroscopy. The resonances that are assigned to the GX reducing end tetrasaccharide sequence, β-D-Xyl-(1→3)-α-L-Rha-(1→2)-α-D-GalA-(1→4)-D-Xyl, include H1 of α-D-GalA, H1 of α-L-Rha, H1 of 3-linked β-D-Xyl, H4 of α-D-GalA, and H2 of α-L-Rha (Penña et al., 2007). It was previously found that the resonances for the GX reducing end tetrasaccharide sequence in irx9 were highly elevated compared with those of the wild type, which is caused by a significant reduction in the GX chain length and thereby a relative increase in the ratio of the GX reducing end sequence over the backbone xylosyl residues (Penña et al., 2007). It was evident that overexpression of I9H but not IRX14 and I14H in irx9 restored the resonance intensity for the GX reducing end tetrasaccharide sequence to the wild-type level (Fig. 6; Table III), indicating that excess I9H is sufficient to complement the GX chain length defect conferred by irx9. Consistent with the MALDI-TOF MS results showing that I9H overexpression restores the α-D-GlcA side chains in GX (Fig. 5), the resonances assigned to H1 of α-D-GlcA residues, which were absent in irx9, were restored to the wild-type level in the I9H-complemented irx9 plants (Fig. 6).

The restoration of GX level and structure in I9H-complemented irx9 plants was accompanied by a partial rescue of the GX xylosyltransferase activity (Fig. 7A). Overexpression of IRX14 and I14H did not restore the xylosyltransferase activity in the irx9 mutant. It was interesting that overexpression of IRX9 in irx9 led to an increased xylosyltransferase activity compared with the wild type. Together, these results suggest that I9H can perform the same biochemical function as IRX9 in the biosynthesis of GX backbone and that they are functionally distinct from IRX14 and I14H.

### Table I. Monosaccharide composition of cell walls from inflorescence stems of the wild type, irx9, irx14, and the irx9 and irx14 plants overexpressing GT43 members

| Sample         | Xyl   | Glc   | Man  | Gal  | Ara  | Rha  |
|----------------|-------|-------|------|------|------|------|
| Wild type      | 108.7 ± 4.6 | 412.1 ± 4.7 | 18.9 ± 1.3 | 17.5 ± 1.3 | 12.6 ± 2.2 | 11.1 ± 1.2 |
| irx9           | 60.8 ± 0.3  | 337.9 ± 0.4   | 39.0 ± 0.1  | 38.4 ± 1.3  | 28.6 ± 0.2  | 16.4 ± 0.6  |
| irx9 + IRX9    | 91.6 ± 3.1  | 370.4 ± 3.0   | 19.1 ± 3.2  | 16.7 ± 0.8  | 16.7 ± 0.9  | 14.9 ± 0.9  |
| irx9 + I9H     | 107.4 ± 2.8 | 446.3 ± 3.8   | 24.1 ± 0.9  | 18.2 ± 0.8  | 18.2 ± 0.6  | 12.7 ± 0.6  |
| irx9 + IRX14   | 60.2 ± 5.9  | 366.9 ± 35.0  | 44.2 ± 5.0  | 25.8 ± 3.4  | 28.8 ± 3.2  | 19.3 ± 2.9  |
| irx9 + I14H    | 52.7 ± 11.6 | 393.7 ± 42.2  | 43.1 ± 5.6  | 24.9 ± 3.6  | 23.9 ± 2.2  | 16.4 ± 1.2  |
| irx14          | 49.4 ± 5.0  | 334.8 ± 8.4   | 23.6 ± 0.7  | 32.9 ± 2.9  | 12.0 ± 1.5  | 12.2 ± 0.9  |
| irx14 + IRX9   | 45.9 ± 3.9  | 338.5 ± 11.7  | 23.6 ± 2.2  | 28.6 ± 0.8  | 13.7 ± 1.3  | 17.1 ± 0.8  |
| irx14 + I9H    | 56.0 ± 2.0  | 372.1 ± 9.6   | 31.5 ± 2.6  | 29.1 ± 0.2  | 14.6 ± 0.1  | 18.2 ± 0.9  |
| irx14 + IRX14  | 98.5 ± 4.3  | 385.4 ± 3.8   | 27.7 ± 1.3  | 24.9 ± 1.4  | 13.9 ± 0.2  | 12.7 ± 0.8  |
| irx14 + I14H   | 96.9 ± 1.2  | 397.5 ± 6.0   | 18.0 ± 1.3  | 22.7 ± 3.3  | 11.5 ± 0.1  | 15.2 ± 0.2  |
Overexpression of I14H But Not IRX9 and I9H Complements the irx14 Mutant Defects

Our finding that overexpression of IRX14 and I14H does not complement the irx9 mutant defects prompted us to test whether IRX14 and I14H form another pair of functional homologs involved in GX backbone biosynthesis. It was found that overexpression of I14H and IRX14 but not IRX9 and I9H in the irx14 mutant rescued the defects in the stem strength, secondary wall thickness, vessel morphology, the GX level, the GlcA side chains, and the GX xylosyltransferase activity (Figs. 4, A and H–M, 5, G–L, and 7; Table I; Supplemental Fig. S2, G–L). The irx14 mutation was previously shown to cause a reduction in the GX level (Brown et al., 2007), but it is unknown whether the GX chain length is affected in the mutant. NMR analysis showed that similar to those in irx9, the resonances for the GX tetrasaccharide reducing end sequence in irx14 were highly increased compared with those in the wild type (Fig. 6), indicating that the GX chain length in irx14 is significantly reduced (Table III). Overexpression of I14H in irx14 apparently restored the resonance signals for the GX tetrasaccharide reducing end sequence to the wild-type level, indicating its ability to complement the GX chain length defect conferred by irx14 (Fig. 6; Table III). These results demonstrate that I14H and IRX14 form another group of functional homologs involved in GX biosynthesis.

Double Mutant Analysis Reveals Redundant Roles of GT43 Members in GX Biosynthesis

The findings that I9H is expressed in the same cell types as IRX9 and excess I9H is able to complement the irx9 mutant defects indicate that they function redundantly in GX biosynthesis. Likewise, I14H might function redundantly with IRX14 in GX biosynthesis. To test these hypotheses, we generated an array of double mutants with all possible combinations of the GT43 members (Fig. 8; Supplemental Fig. S1) and examined their effects on plant growth and GX biosynthesis. Although the irx9 or irx14 mutant exhibited reductions in stem strength, secondary wall thickness, and GX level (Brown et al., 2007; Peña et al., 2007), T-DNA insertion mutation of I9H or I14H alone did not have any discernible effects (Figs. 8 and 9; Table II; Supplemental Fig. S3). However, homozygous double mutations of IRX9 and I9H led to a strong retardation in plant growth (Fig. 8D). Even after prolonged growth (4 months), no inflorescence stems grew out from the rosettes, which prevented us from further analysis of the double homozygous mutants. The double mutant that is homozygous for irx9 and heterozygous for i9h (+/−) did grow out short inflorescence stems after prolonged growth. Examination of the irx9 i9h (+/−) inflorescence stems revealed that the interfascicular fibers had thinner walls (Figs. 8J and 9F) than did irx9 (Figs. 8F and 9B) and that the xylem vessels were severely collapsed (Supplemental Fig. S3F). In addition, cell wall composition analysis showed that the Xyl content in irx9 i9h (+/−) was more severely reduced than that in irx9 (Table II). These results demonstrate that IRX9 and I9H play redundant roles in GX biosynthesis and secondary wall thickening.

Similar to irx9 i9h, double homozygous mutations of IRX14 and I14H cause a much stronger effect on plant growth compared with irx14 (Fig. 8D). The irx14 i14h grew out short inflorescence stems, allowing us to examine the secondary wall thickening and the GX level. The irx14 i14h stems had little secondary wall thickening in the interfascicular fibers (Figs. 8O and

Figure 5. MALDI-TOF mass spectra of acidic xylooligosaccharides generated by β-endoxylanase digestion of GX from the stems of the wild type, irx9, irx14, and the mutants overexpressing GT43 members. The ions at m/z 745 and 759 correspond to xylooligosaccharides bearing a GlcA residue (XG) or a methylated GlcA residue (XM). The ion at m/z 781 corresponds to the doubly sodiated XM. Note that the missing ion at m/z 745 corresponding to XG in irx9 was restored by overexpression of IRX9 or I9H and that in irx14 was restored by overexpression of IRX14 or I14H.
a severely collapsed vessel morphology (Supplemental Fig. S3K), and a much lower Xyl content (Table II) than those of irx14 (Figs. 8H and 9D). The double mutant that is homozygous for irx14 and heterozygous for i14h (+/2) also had a more severe retardation in plant growth compared with irx14 (Fig. 8D).

We next examined the plant growth and secondary wall thickening in the double mutants irx9 i14h, i14h irx9, and i9h i14h. The plant growth (Fig. 8D), secondary wall thickness (Figs. 8 and 9), and vessel morphology (Supplemental Fig. S3) of the irx9 i14h and the i9h i14h double mutants were similar to those of irx9 and irx14, respectively. Cell wall composition analysis revealed that the Xyl content in irx9 i14h had a slight reduction compared with irx9, whereas the Xyl content in i9h irx14 was similar to that in irx14 (Table II). Although no significant alterations in plant growth, secondary wall thickness, and vessel morphology were seen in the i9h i14h double mutant (Figs. 8, D and N, and 9; Supplemental Fig. S3) compared with the wild type (Figs. 8E and 9A; Supplemental Fig. S3A), the Xyl content in i9h i14h was slightly reduced (Table II).

We further investigated the effects of double mutations of IRX9 and IRX14 on plant growth and GX biosynthesis. Both irx9 irx14 and irx9 irx14 (+/−) exhibited strong retardation in plant growth (Fig. 8D). The irx9 irx14 plants grew out small inflorescence stems after 6 months of growth, and examination of these stems revealed the absence of discernible secondary walls in the interfascicular fibers (Figs. 8K and 9G) and collapsed vessels in the xylem (Supplemental Fig. S3G). These phenotypes were much more severe than those in the irx9 (Fig. 8F; Supplemental Fig. S3B) or irx14 (Fig. 8H; Supplemental Fig. S3D) single mutant. Further cell wall composition analysis of irx9 irx14 stems revealed a drastic reduction in the Xyl content (Table II), indicating a more severe effect on GX biosynthesis than the irx9 or irx14 mutation alone.

The results from these comprehensive genetic analyses demonstrate that all four GT43 members are
involved in GX biosynthesis, although IRX9 and IRX14 play major roles. Together with the mutant complementation studies, our findings suggest that the family GT43 glycosyltransferases form two functionally non-redundant groups, IRX9 together with I9H and IRX14 together with I14H, both of which are essential for GX biosynthesis.

DISCUSSION
IRX9 and IRX14 Function Nonredundantly in the Elongation of GX Backbone

Our findings that IRX9 cannot functionally complement the loss of IRX14 and vice versa suggest that IRX9 and IRX14 are two functionally nonredundant GT43 members essential for GX biosynthesis. Considering the fact that both IRX9 and IRX14 are required for the elongation of GX backbone (Peña et al., 2007; Fig. 6), it is reasonable to propose that IRX9 and IRX14 function cooperatively in the elongation of GX backbone. Such a cooperative function might have important implications in understanding the biochemical mechanism of GX backbone biosynthesis. The GX backbone is composed of β-(1,4)-linked xylosyl residues, and its biosynthesis needs to overcome a steric problem because each xylosyl residue is flipped nearly 180° with respect to its neighboring residues. Similar to a model proposed for cellulose biosynthesis, in which two glycosyltransferase activities operate cooperatively from opposite sites to add cellobiosyl units to the β-(1,4)-glucan chain (Carpita and Vergara, 1998), the biosynthesis of GX backbone might also require two enzyme activities that operate in such a mechanism. Although their exact biochemical functions await further investigation, IRX9 and IRX14 are strong candidates for such two glycosyltransferase activities involved in the elongation of the β-(1,4)-xylan chain. This hypothesis is consistent with the facts that mutation of either irx9 or irx14 results in a defect in the GX chain length and a reduction in the xylosyltransferase activity and that IRX9 and IRX14 are nonredundant in their functions.

The chemical analysis of GX showed that the irx9 and irx14 mutants exhibited a similar severity of defects in the amount and the degree of polymerization (DP) of GX (Tables II and III), which is congruent with their proposed cooperative functions in the elongation of GX backbone. However, the irx9 mutant appears to have a more severe retardation in plant growth than the irx14 mutant. It has been demonstrated that a deformation in vessels in cell wall mutants could lead to a strong retardation in plant growth (Lee et al., 2009b; McCarthy et al., 2009), which is likely due to defects in solute transport. It is possible that the GX defect in the vessels in irx9 is more severe than that in irx14, thereby causing a stronger effect on plant growth. However, chemical analysis did not reveal a significant difference in the amount and the DP of GX between irx9 and irx14. This could be due to the fact that the total cell walls used for analysis are from stems in which fibers are the most abundant secondary wall-containing cells; therefore, any possible difference of GX defects in the vessels between irx9 and irx14 could not be detected.

Redundancy of Family GT43 Genes in GX Biosynthesis

We have demonstrated that I9H and I14H are redundant in function with IRX9 and IRX14, respectively, based on the following lines of evidence. First, all four GT43 members are expressed in the same cell types that undergo secondary wall thickening (Fig. 2), and their proteins are targeted into the Golgi (Fig. 3), where GX is synthesized. Second, overexpression of I9H and I14H can effectively rescue the GX defects,
Figure 8. Effects of single and double mutations of GT43 members on plant growth and fiber wall thickening. A, Diagrams showing the sites of T-DNA insertions in the I9H and I14H genes. B, PCR identification of homozygous i9h and i14h mutants. T-DNA indicates the amplified DNA fragment with a T-DNA left border primer and an I9H or I14H primer flanking the T-DNA insertion site. Endogenous indicates the amplified I9H or I14H DNA fragment with primers spanning the T-DNA insertion site. WT, Wild type. C, Breaking strength measurement showing that the stem strength of i9h and i14h is similar to that of the wild type. Each bar represents the breaking force of the inflorescence stem of individual plants. D, Morphology of 10-week-old single and double GT43 mutants. Insets show the enlarged images of the rosette plants of irx9 i9h and irx9 i14h. E to O, Cross sections of stems showing the interfascicular fibers in single and double GT43 mutants. Note the extremely thin fiber walls in irx9 i14h and i14h i14h. The bottom parts of inflorescence stems of 10-week-old plants except for irx9 i9h (+/−) and irx9 i14h, which were from 4- and 6-month-old plants, respectively, were sectioned and stained with toluidine blue for anatomy. if, Interfascicular fiber. Bar in E = 93 mm for E to O.
including the GX chain length, caused by the mutations of IRX9 and IRX14, respectively (Figs. 4–6; Tables I and III). Third, double mutation of IRX9 and I9H results in a more severe defect in the GX level and a strong retardation of plant growth, and likewise, double mutation of IRX14 and I14H leads to similar phenotypes (Fig. 8; Table II). Together, these results suggest that IRX9 and I9H are a pair of functional homologs and IRX14 and I14H are another pair of functional homologs. This finding explains the early observations that the irx9 or irx14 mutant only exhibits partial reductions in GX content and secondary wall thickness (Brown et al., 2005, 2007; Peña et al., 2007). However, the i9h or i14h mutation alone does not cause any observable GX defects, indicating that IRX9 and IRX14 are functionally dominant in GX biosynthesis in vivo. The functional dominance of IRX9 and IRX14 could be caused by their higher expression levels or their higher specific enzymatic activities. It should be pointed out that although all the GT43 members are expressed in cells undergoing secondary wall biosynthesis, there are some differences in their expression.

Table II. Monosaccharide composition of cell walls from inflorescence stems of the wild type and GT43 mutants

| Sample           | Xyl (mg g⁻¹ dry cell wall) | Glc (mg g⁻¹ dry cell wall) | Man (mg g⁻¹ dry cell wall) | Gal (mg g⁻¹ dry cell wall) | Ara (mg g⁻¹ dry cell wall) | Rha (mg g⁻¹ dry cell wall) |
|------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|---------------------------|
| Wild type        | 110.4 ± 1.4               | 402.0 ± 0.2               | 18.0 ± 0.4               | 14.6 ± 0.2               | 11.0 ± 0.2               | 11.7 ± 0.4               |
| irx9             | 57.2 ± 0.3                | 336.9 ± 0.4               | 35.1 ± 0.1               | 39.9 ± 1.3               | 23.8 ± 0.2               | 13.9 ± 0.6               |
| i9H              | 106.6 ± 3.8               | 418.1 ± 4.5               | 17.5 ± 0.7               | 13.9 ± 0.6               | 9.0 ± 0.1                | 10.4 ± 0.1               |
| irx14            | 59.5 ± 2.1                | 346.5 ± 7.5               | 24.6 ± 2.5               | 16.2 ± 1.3               | 11.2 ± 0.1               | 10.9 ± 0.5               |
| i14h             | 102.3 ± 4.8               | 391.8 ± 7.6               | 17.9 ± 2.3               | 15.1 ± 1.1               | 10.3 ± 0.5               | 10.6 ± 0.5               |
| irx9 i9h (+/-)   | 40.2 ± 1.1                | 329.7 ± 8.4               | 40.4 ± 2.1               | 43.4 ± 1.4               | 35.8 ± 1.1               | 19.4 ± 2.0               |
| irx9 irx14       | 26.5 ± 0.5                | 292.4 ± 5.3               | 20.2 ± 4.6               | 49.3 ± 3.4               | 26.0 ± 1.9               | 12.1 ± 0.2               |
| irx9 i14h        | 49.2 ± 1.7                | 337.9 ± 13.4              | 30.6 ± 1.5               | 23.1 ± 1.8               | 15.2 ± 1.3               | 13.7 ± 0.9               |
| i9H irx14        | 59.3 ± 3.9                | 358.0 ± 15.7              | 25.7 ± 2.9               | 15.6 ± 1.7               | 11.2 ± 0.5               | 11.9 ± 0.3               |
| i9H i14h         | 94.2 ± 21.9               | 366.7 ± 24.3              | 12.6 ± 0.4               | 12.3 ± 1.0               | 8.9 ± 0.4                | 9.5 ± 0.5                |
| irx14 i14h       | 29.1 ± 1.8                | 251.7 ± 11.1              | 24.3 ± 1.8               | 26.9 ± 2.2               | 19.3 ± 1.1               | 13.9 ± 0.6               |

Figure 9. Transmission electron micrographs of the cell walls of interfascicular fibers in the stems of the wild type and GT43 mutants. The bottom parts of the inflorescence stems of 10-week-old plants except for irx9 i9h (+/-) and irx9 irx14, which were from 4- and 6-month-old plants, respectively, were examined for fiber wall thickness. Bar in A = 7.2 mm for A to K.
levels in different organs and secondary wall-forming cell types (Figs. 1 and 2). For example, I9H is specifically expressed in vessels in the secondary xylem of roots (Fig. 2C), and I14H has a relatively higher expression level in the xylem than in the interfascicular fibers in nonelongating stems (Fig. 2H). The differences in their expression patterns might account for the differential involvement of GT43 members in GX biosynthesis in different secondary wall-forming cell types.

The gene redundancy appears to be common in GX biosynthesis in Arabidopsis. For example, FRA8 and F8H are a pair of redundant genes required for GX reducing end sequence biosynthesis (Lee et al., 2009b), and IRX10 and IRX10-L are another pair involved in GX backbone biosynthesis (Brown et al., 2009; Wu et al., 2009). In both of these cases, single mutation of FRA8 or IRX10 results in a defect in GX biosynthesis but single mutation of its homolog does not. Double mutations of these genes, either FRA8 and F8H or IRX10 and IRX10-L, cause a strong retardation in plant growth, a phenotype that is also observed in the double mutants irx9 i9h and irx14 i14h. Since GX is a critical component required for cross-linking cellulose microfibrils in secondary walls, gene redundancy in GX biosynthesis likely presents an evolutionary advantage to safeguard the proper making of secondary walls, which are the key structure of vessels essential for water transport in the plant body.

It was noticed that the DP of GX in irx9 or irx14 was not completely rescued by overexpression of itself or its corresponding functional homolog (Table III). This could be attributed to the fact that the cell walls used for GX analysis were isolated from a pool of individual transgenic plants that likely had different degrees of complementation in the xylan backbone length. Therefore, the average DP of GX in the rescued mutants appears slightly shorter than that in the wild type. However, cell wall composition analysis showed a nearly complete restoration of the total Xyl content (Table I), secondary wall thickness (Fig. 4), and vessel morphology (Supplemental Fig. S2) in the rescued mutants compared with the wild type. This finding indicates that a substantial restoration of the DP of GX is sufficient to rescue the defects in GX level, leading to the restoration of secondary wall thickness and vessel morphology.

### Genes Involved in GX Backbone Biosynthesis

It was previously proposed that some of the cellulose synthase-like (Csl) genes might encode xylan synthase (Richmond and Somerville, 2001), which is congruent with the findings that several hemicellulose backbones, such as xyloglucan β-1,4-glucan (Cocuron et al., 2007), β-1,4-mannan (Dhugga et al., 2004; Liepman et al., 2005), and β-(1,3;1,4)-mix-linked glucan (Burton et al., 2006), are synthesized by members of the Csl gene families. However, no recombinant CSL proteins were shown to exhibit any xylan synthase activity (Liepman et al., 2005). Transcription profiling analysis of wood formation in poplar revealed no correlations between secondary wall synthesis and the expression of CSL genes with the exception of the CSLA (a mannan synthase) gene (Aspeborg et al., 2005; Suzuki et al., 2006). Although mutation of CSLD5 has been reported to cause a decrease in xylosyltransferase activity and xylan immunolabeling, the CSLD5 gene is not expressed in cells undergoing secondary wall thickening; thus, the observed phenotypes were considered to be indirect (Bernal et al., 2007). Therefore, genes responsible for the biosynthesis of GX backbone remain elusive. Because GT43 members are specifically expressed in cells undergoing secondary wall thickening and GX biosynthesis (Peña et al., 2007; Fig. 2) and IRX9 and IRX14 are required for the normal GX xylosyltransferase activity

### Table III. Relative abundance of the reducing end tetrasaccharide sequence and side chains, and the DP of GX from the wild type and GT43 mutants overexpressing various GT43 members

| Sample       | Frequency of Occurrence of GlcA and MeGlcA Side Chains* | Relative Abundance of the Reducing End Tetrasaccharide Sequenceb | Average DPc |
|--------------|----------------------------------------------------------|---------------------------------------------------------------|--------------|
| Wild type    | 21.6%                                                    | 100%                                                          | 93           |
| irx9         | 21.3%                                                    | 31.9%                                                         | 29           |
| irx9 + IRX9  | 20.3%                                                    | 88.1%                                                         | 84           |
| irx9 + I9H   | 19.0%                                                    | 79.2%                                                         | 72           |
| irx9 + IRX14 | 20.3%                                                    | 23.1%                                                         | 23           |
| irx9 + I14H  | 20.8%                                                    | 34.0%                                                         | 30           |
| irx14        | 22.0%                                                    | 37.6%                                                         | 36           |
| irx14 + IRX9 | 23.4%                                                    | 40.9%                                                         | 37           |
| irx14 + I9H  | 23.3%                                                    | 40.9%                                                         | 38           |
| irx14 + IRX14| 21.5%                                                    | 70.3%                                                         | 68           |
| irx14 + I14H | 20.4%                                                    | 82.3%                                                         | 77           |

*The frequency of occurrence of the GlcA and MeGlcA side chains was calculated from the ratios of the NMR resonance of the branched β-Xyl to that of total β-Xyl. bThe relative abundance of the reducing end tetrasaccharide sequence was determined from the ratios of the NMR resonance of the reducing end sequence to that of the branched β-Xyl. The abundance of the reducing end tetrasaccharide sequence in the wild type was taken as 100%. cThe average DP was calculated according to Peña et al. (2007).
and the normal elongation of GX chains (Brown et al., 2007; Lee et al., 2007a; Peña et al., 2007; Fig. 6). GT43 members are apparently strong candidates for GX xylosyltransferases or part of the complexes required for the xylosyltransferase activities. However, an attempt to assay the xylosyltransferase activity of recombinant IRX9 proteins was not successful (Peña et al., 2007). Our finding that IRX9 and IRX14 are nonredundant in function suggests that expression of both IRX9 and IRX14 would be required for the GX xylosyltransferase activity. Furthermore, the recent finding that IRX10 and IRX10-L are also required for the normal GX xylosyltransferase activity (Brown et al., 2009; Wu et al., 2009) indicates that the biosynthesis of GX backbone is much more complicated than expected. As suggested previously, a complex composed of multiple glycosyltransferases and other proteins might be involved in GX backbone synthesis (Peña et al., 2007). Further biochemical characterization of GT43 members together with other players will be necessary to unravel the biochemical process of GX biosynthesis.

**MATERIALS AND METHODS**

**Plant Growth Conditions**

Plants were grown in a greenhouse under 14-h-light/10-h-dark cycles. Common garden potting soil was used for growing plants with biweekly application of plant fertilizers.

**Gene Expression Analysis**

Total RNA from Arabidopsis (*Arabidopsis thaliana* ecotype Columbia) plants was isolated with a Qiagen RNA isolation kit. The organs used were from 7-week-old plants grown in a greenhouse. RNA from different cell types (interfascicular fibers, xylem, and pith cells) was isolated and amplified as described previously (Zhong et al., 2006). First-strand cDNA was synthesized from total RNA treated with DNase I and then used as a template for real-time quantitative PCR analysis with the QuantiTect SYBR Green PCR kit (Clontech). The PCR primers for *IRX9* are 5′-TGCCCAATGATGTTATAGCCT-3′ and 5′-CGTCTTAAACGGTCTTGTG-3′, those for *IRX10* are 5′-TCACGGACATCTATTAGACG-3′ and 5′-CTATTCCATAGTTATGAGACCTG-3′, those for *IRX14* are 5′-AAGATCAAATGCCATTATCCCAAG-3′ and 5′-TGGCCAATGGCATTGTTATCGGCT-3′. The PCR products were ligated between the CaMV 35S promoter and the nopaline synthase termina-

**Cell Wall Sugar Composition Analysis**

Inflorescence stems from at least 20 independent lines of Arabidopsis plants were used for cell wall isolation according to Zhong et al. (2005). Cell wall sugars (as alditol acetates) were determined following the procedure described by Hoebler et al. (1989). The alditol acetates of the hydrolyzed cell wall sugars were analyzed on an Agilent 6890N gas-liquid chromatograph equipped with a 30-m × 0.25-mm (i.d.) silica capillary column (DB 225; Altech Associates).

**MALDI-TOF MS**

Xylooligosaccharides were prepared by digestion of 4 N KOH solubilized wall preparations with β-xylanase M6 (Megazyme) as described (Zhong et al., 2005). The acidic xylooligosaccharides were analyzed using a MALDI-TOF MS device operated in the positive-ion mode with an accelerating voltage of 30 kV, an extractor voltage of 9 kV, and a source pressure of approximately 8 × 10⁻⁷ torr. The aqueous sample was mixed (1:1, v/v) with the MALDI matrix (0.2 M 2,5-dihydroxybenzoic acid and 0.06 M 1-hydroxyisoquinoline in 50% acetonitrile) and dried on the stainless steel target plate. Spectra are averages of 100 laser shots.

**1H NMR Spectroscopy**

NMR spectra of the acidic xylooligosaccharides from β-xylanase digestion were acquired at 20°C on a Varian Inova 600-MHz spectrometer (599.7 MHz, 1H) using a 5-mm cryogenic triple resonance probe (Varian). All NMR samples were prepared using 100% D₂O in 3-mm standard NMR tubes. 1H chemical shift values were referenced to residual water (4.79 ppm) in the water-suppressed spectra.
shifts were referenced to 2,2-dimethyl-2-silapentane-5-sulfonate sodium salt. For all experiments, 64 transients were collected using a spectral width of 6,000 Hz and an acquisition time of 5 s. The residual water resonance was suppressed by a 1-s presaturation pulse at a field strength of 40 Hz. One-dimensional spectra were processed using MestReC (MestReC Research) with 0.2-Hz apodization followed by zero filling to 128 k points. The 1H NMR assignments were done by comparison with the NMR spectra data for Arabidopsis acidic xylooligosaccharides (Zhong et al., 2005; Pen˜a et al., 2007).

Assay of Xylosyltransferase Activity

Microsomes were isolated from the inflorescence stems of at least four individual 8-week-old plants for each genotype following the procedure of Kuroyama and Tsumuraya (2001) and stored at 8°C for 5 months. The 1H NMR spectra were obtained using a Bruker Avance II 500 MHz spectrometer. For all experiments, 64 transients were collected using a spectral width of 8,000 Hz and an acquisition time of 5 s. The residual water resonance was suppressed by a 1-s presaturation pulse at a field strength of 40 Hz. One-dimensional spectra were processed using MestReC (MestReC Research) with 0.2-Hz apodization followed by zero filling to 128 k points. The 1H NMR assignments were done by comparison with the NMR spectra data for Arabidopsis acidic xylooligosaccharides (Zhong et al., 2005; Pen˜a et al., 2007).

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. RT-PCR detection of GT43 transcripts in various GT43 mutants.

Supplemental Figure S2. Complementation of the collapsed vessel phenotype in the irx9 and irx14 mutants by overexpression of GT43 members.

Supplemental Figure S3. Vessel morphology in single and double mutants of GT43 genes.

Supplemental Table S1. Primers used in this work.
GT43 Family and Glucuronoxylan Biosynthesis

Somerville C (2006) The billion-ton biofuel vision. Science 312: 1277
Suzuki S, Li L, Sun YH, Chiang VL (2006) The cellulose synthase gene superfamily and biochemical functions of xylem-specific cellulose synthase-like genes in Populus trichocarpa. Plant Physiol 142: 1233–1245
Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22: 4673–4680

McCarthy RL, Zhong R, Ye ZH
Lee C, Teng Q, Huang W, Zhong R, Ye ZH
Pen˜a MJ, Zhong R, Zhou GK, Richardson EA, O'Neill MA, Darvill AG, Page RDM

Lee C, Zhong R, Richardson EA, Himmelsbach DS, McPhail BT, Ye ZH
Shimizu K, Ishihara M, Ishihara T
Richmond TA, Somerville CR

(2009b) The F8H glycosyltransferase is a functional paralog of FRA8 involved in glucuronoxylan biosynthesis in Arabidopsis. Plant Cell Physiol 50: 812–827
(2009c) The poplar GT8E and GT8F glycosyltransferases are functional orthologs of Arabidopsis PARVUS involved in glucuronoxylan biosynthesis. Plant Cell Physiol 50: 1982–1987
(2007b) The PARVUS gene is expressed in cells undergoing secondary wall thickening and is essential for glucuronoxylan biosynthesis. Plant Cell Physiol 48: 1659–1672
Lipman AH, Wilkerson CG, Keegstra K (2005) Expression of cellulose synthase-like (Csl) genes in insect cells reveals that CslA family members encode mannan synthases. Proc Natl Acad Sci USA 102: 2221–2226
Liu ZB, Ulmasov T, Shi X, Hagen G, Guilfoyle TJ (1994) Soybean GH3 promoter contains multiple auxin-inducible elements. Plant Cell 6: 645–657
McCarthy RL, Zhong R, Ye ZH (2009) MYB83 is a direct target of SND1 and acts redundantly with MYB46 in the regulation of secondary cell wall biosynthesis in Arabidopsis. Plant Cell Physiol 50: 1950–1964
Page RDM (1996) TREEVIEW: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12: 357–358
Peña MJ, Zhong R, Zhou GK, Richardson EA, O’Neill MA, Darvill AG, York WS, Ye ZH (2007) Arabidopsis irregular xylem8 and irregular xylem9: implications for the complexity of glucuronoxylan biosynthesis. Plant Cell 19: 549–563
Persson S, Caffall KH, Freshoul G, Hilley MT, Bauer S, Poinexter P, Hahn MG, Mohnen D, Somerville C (2007) The Arabidopsis irregular xylem8 mutant is deficient in glucuronoxylan and homogalacturonan, which are essential for secondary cell wall integrity. Plant Cell 19: 237–255
Porchia AC, Sorensen SO, Scheller HV (2002) Arabinoxylan biosynthesis in wheat: characterization of arabinoxylantransferase activity in Golgi membranes. Plant Physiol 130: 432–441
Richmond TA, Somerville CR (2001) Integrative approaches to determining Csl function. Plant Mol Biol 47: 131–143
Shimizu K, Ishihara M, Ishihara T (1976) Hemicelluloses of brown rotting fungus, Tyromyces palustris. II. The oligosaccharides from the hydrolysate of a hardwood xylan by the intracellular xylanase. Mokuzai Gakkaishi 22: 618–625

Plants encode mannan synthases. Proc Natl Acad Sci USA 104: 2221–2226

content and an increased wood digestibility by cellulase. Plant Cell Physiol 50: 1079–1089
Lee C, Teng Q, Huang W, Zhong R, Ye ZH (2009b) The F8H glycosyltransferase is a functional paralog of FRA8 involved in glucuronoxylan biosynthesis in Arabidopsis. Plant Cell Physiol 50: 812–827
Lee C, Teng Q, Huang W, Zhong R, Ye ZH (2009c) The poplar GT8E and GT8F glycosyltransferases are functional orthologs of Arabidopsis PARVUS involved in glucuronoxylan biosynthesis. Plant Cell Physiol 50: 1982–1987
Lee C, Zhong R, Richardson EA, Himmelsbach DS, McPhail BT, Ye ZH (2007b) The PARVUS gene is expressed in cells undergoing secondary wall thickening and is essential for glucuronoxylan biosynthesis. Plant Cell Physiol 48: 1659–1672
Lipman AH, Wilkerson CG, Keegstra K (2005) Expression of cellulose synthase-like (Csl) genes in insect cells reveals that CslA family members encode mannan synthases. Proc Natl Acad Sci USA 102: 2221–2226
Liu ZB, Ulmasov T, Shi X, Hagen G, Guilfoyle TJ (1994) Soybean GH3 promoter contains multiple auxin-inducible elements. Plant Cell 6: 645–657
McCarthy RL, Zhong R, Ye ZH (2009) MYB83 is a direct target of SND1 and acts redundantly with MYB46 in the regulation of secondary cell wall biosynthesis in Arabidopsis. Plant Cell Physiol 50: 1950–1964
Page RDM (1996) TREEVIEW: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12: 357–358
Peña MJ, Zhong R, Zhou GK, Richardson EA, O’Neill MA, Darvill AG, York WS, Ye ZH (2007) Arabidopsis irregular xylem8 and irregular xylem9: implications for the complexity of glucuronoxylan biosynthesis. Plant Cell 19: 549–563
Persson S, Caffall KH, Freshoul G, Hilley MT, Bauer S, Poinexter P, Hahn MG, Mohnen D, Somerville C (2007) The Arabidopsis irregular xylem8 mutant is deficient in glucuronoxylan and homogalacturonan, which are essential for secondary cell wall integrity. Plant Cell 19: 237–255
Porchia AC, Sorensen SO, Scheller HV (2002) Arabinoxylan biosynthesis in wheat: characterization of arabinoxylantransferase activity in Golgi membranes. Plant Physiol 130: 432–441
Richmond TA, Somerville CR (2001) Integrative approaches to determining Csl function. Plant Mol Biol 47: 131–143
Shimizu K, Ishihara M, Ishihara T (1976) Hemicelluloses of brown rotting fungus, Tyromyces palustris. II. The oligosaccharides from the hydrolysate of a hardwood xylan by the intracellular xylanase. Mokuzai Gakkaishi 22: 618–625
