Functional conservation and divergence of *Miscanthus lutarioriparius* GT43 gene family in xylan biosynthesis

Xiaoyu Wang¹,², Qi Tang¹, Xin Zhao¹,², Chunlin Jia³, Xuanwen Yang¹, Guo He¹, Aimin Wu⁴, Yingzhen Kong⁵, Ruibo Hu*¹ and Gongke Zhou*¹

**Abstract**

**Background:** Xylan is the most abundant un-cellulosic polysaccharides of plant cell walls. Much progress in xylan biosynthesis has been gained in the model plant species *Arabidopsis*. Two homologous pairs *Irregular Xylem 9* (*IRX9*)/9L and *IRX14*/14L from glycosyltransferase (GT) family 43 have been proved to play crucial roles in xylan backbone biosynthesis. However, xylan biosynthesis in grass such as *Miscanthus* remains poorly understood.

**Results:** We characterized seven GT43 members in *M. lutarioriparius*, a promising bioenergy crop. Quantitative real-time RT-PCR (qRT-PCR) analysis revealed that the expression of *MlGT43* genes was ubiquitously detected in the tissues examined. In-situ hybridization demonstrated that *MlGT43A-B* and *MlGT43F-G* were specifically expressed in sclerenchyma, while *MlGT43C-E* were expressed in both sclerenchyma and parenchyma. All seven *MlGT43* proteins were localized to Golgi apparatus. Overexpression of *MlGT43A-E* but not *MlGT43F* and *MlGT43G* in *Arabidopsis irx9* fully or partially rescued the mutant defects, including morphological changes, collapsed xylem and increased xylan contents, whereas overexpression of *MlGT43F* and *MlGT43G* but not *MlGT43A-E* complemented the defects of *irx14*, indicating that *MlGT43A-E* are functional orthologues of *IRX9* while *MlGT43F* and *MlGT43G* are functional orthologues of *IRX14*. Overexpression of all seven *MlGT43* genes could not rescue the mucilage defects of *irx14* seeds. Furthermore, transient transactivation analyses of *MlGT43A-E* reporters demonstrated that *MlGT43A* and *MlGT43B* were differentially activated by *MlSND1*, *MlMYB46* or *MlVND7*.

**Conclusion:** The results demonstrated that all seven *MlGT43* s are functionally conserved in xylan biosynthesis during secondary cell wall formation but diversify in seed coat mucilage xylan biosynthesis. The results obtained provide deeper insight into xylan biosynthesis in grass, which lay the foundation for genetic modification of grass cell wall components and structure to better suit for next-generation biofuel production.

**Keywords:** *Miscanthus lutarioriparius*, Glycosyltransferase family 43, Xylan biosynthesis, Secondary cell wall, Seed coat mucilage

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**Highlight**

The functional roles of *M. lutarioriparius* GT43 family genes are conserved and diversified in xylan biosynthesis.

**Background**

Plant cell walls are complex and dynamic structures composed mainly of polysaccharides (cellulose, hemicellulose and pectin), phenolic compounds (lignin) and glycoproteins [1]. Xylans are the major hemicellulosic saccharides in the primary cell walls of grasses and the secondary cell walls of grasses and dicots, ranking as the second most abundant polysaccharides in nature [2]. Xylans are mainly composed of a linear backbone of β-(1,4)-linked D-xylosyl residues with various sidechains that vary among different plant species and tissue types [3]. Based on the sidechain substitutions, xylans can generally be classified as (methyl)glucuronoxylan (GX), arabinoxylan (AX), and glucuronorabinoxylan (GAX) [3]. As the major xylan in
dicot plants, GX is usually decorated with α,1,2-linked glucuronic acid (GlcA) or 4-O-methylglucuronic acid (MeGlcA), and acetylated at C-2 or C-3 [3, 4]. AX has α,1,3-linked arabinose (Ara) sidechains, and presents as typical hemicellulose components in starchy endosperm of cereal grains [3]. GAX is the predominant hemicellulose in grass cell walls, and has sidechains of α,1,2 or α,1,3-linked arabinose (Ara) and GlcA residues [3]. In addition, GX in angiosperm and GAX in several gymnosperm species contain a tetrasaccharide sequence [β-D-Xyl(1,3)-α-L-Rha-(1,2) -α-D-GalA-(1,4)-D-Xyl] at the reducing end [5–7]. However, no such oligosaccharide has yet been identified for xylans in grasses [8, 9]. It is still in controversy whether this oligosaccharide functions as a primer or as a terminator in xylan backbone biosynthesis [10].

Several xylan-related mutants named as irregular xylem (irx) due to secondary cell wall deficiencies have been identified in Arabidopsis by reverse genetics approaches [11, 12]. Most of these identified genes encode putative glycosyltransferases (GT) that are involved in the biosynthesis of xylan. IRX9/IRX9L and IRX14/IRX14L from GT43 family as well as IRX10/IRX10L from GT47 family are responsible for the biosynthesis of xylan backbone [13–19]. IRX9, IRX10 and IRX14 play dominant roles in xylan backbone biosynthesis, and mutations in each gene lead to reduced xylan content and growth defect. By contrast, IRX9L, IRX10L and IRX14L seem to perform partially redundant roles together with their close homologues, as loss-function of these genes have no observable phenotypes and they only partially complement the phenotypes of irx9, irx10 and irx14 mutants. In addition, double mutations in each gene pairs dramatically enhance the phenotypes of the single mutant [13, 14, 18, 19]. However, a recent study proposed that these gene pairs play equivalent roles in xylan biosynthesis [20]. Furthermore, two members of DUF579 domain-containing proteins, IRX15 and IRX15L, are essential for the normal elongation of xylan backbone [21, 22]. IRX7/IRX7L from GT47 family, IRX8 and PARVUS from GT8 family are required for the biosynthesis of the reducing end oligosaccharide [5, 23–26]. Mutations in these genes lead to almost entirely loss of the tetrasaccharide accompanied with reduced xylan contents, while the xylan backbone elongation activity is not disturbed [5, 23–26].

Recently biochemical and genetic studies have also led to the identification of several genes that are required for the sidechain modifications of xylan. For instance, GLUCURONIC ACID SUBSTITUTION OF XYLAN (GUX) 1, GUX2, GUX4 and GUX5 from GT8 family are proposed to catalyze the addition of GlcA and MeGlcA sidechains to GX backbone [20, 27–29]. GLUCURONOXYLAN METHYLTRANSFERASE (GXMT) 1, a DUF579 domain protein, has been revealed to be responsible for the 4-O-methylation of GlcA residues in GX [30]. In addition, ESKIMO1/TRICHOME BIREFRINGENCE-LIKE (TBL) 29, a DUF231 domain protein, is required for the O-acetylation of xylan backbone [31, 32]. Moreover, several XYLAN ARABINOSYLTRANSFERASE (XAT), members of GT61 family proteins from rice and wheat, are responsible for transferring the Ara residues onto xylan backbone [33, 34]. XYLOSYL ARABINOSYL SUBSTITUTION OF XYLAN (XAX) 1, another member from GT61 family in rice, is involved in transferring the Xyl residues in β-Xylp-(1→2)-α-Araf-(1→3) sidechain [34].

Grass xylans have several unique features compared to those from dicots. GX is the most abundant hemicellulose in dicots, while grass xylans usually contain many Ara residue substitutions and thus are termed as GAX or AX [3]. Even though there are clear differences in xylan structure between grasses and dicots, accumulating evidence implicates that GT43 members are functionally conserved in xylan biosynthesis between dicots and monocots. For example, four rice IRX9 orthologues OsGT43A, OsGT43C, OsGT43E and OsGT43F can fully or partially rescue the xylan defect phenotype of irx9, while OsGT43J is able to complement the xylan defect phenotype of irx14 in Arabidopsis [35, 36]. Three poplar IRX9 orthologues PtrGT43A, PtrGT43B and PtrGT43E are capable of rescuing the defects of irx9, whereas the other two IRX14 orthologues PtrGT43C and PtrGT43D are able to complement the phenotypes of irx14 [37]. Furthermore, it has been demonstrated that rice and poplar GT43 family proteins are evolved to retain two functionally non-redundant groups involved in xylan backbone biosynthesis [36–38]. Additionally, two GT43 members GhGT43A1 and GhGT43C1 from cotton have been revealed to be functional orthologues of Arabidopsis IRX9 and IRX14, respectively, and have been shown to participate in xylan backbone biosynthesis during fiber development [39].

Miscanthus is a perennial rhizomatous grass with superior characteristics as a bioenergy plant such as high photosynthetic efficiency, low fertilizer and water demand, wide adaptability and high biomass yield. It has attracted increasing attention and concern worldwide as an ideal lignocellulosic feedstock for next-generation bioenergy production [40–42]. Hemicelluloses account for 29–42 % of the Miscanthus cell walls [43], and the most abundant hemicellulosic polysaccharide is AX [43, 44], which is also the typical xylan in grass cell walls [45]. It has been shown that hemicellulose exerts dominant and positive effects on biomass digestibility by affecting cellulose crystallinity after pre-treatment with alkali or acid [46]. Although much progress has been gained in the understanding of xylan biosynthesis in the model plant Arabidopsis thaliana, relatively less is known about xylan biosynthesis in grasses. To the best of our knowledge, none of GTs responsible for the
biosynthesis of xylan has been isolated and characterized in Miscanthus as yet.

To provide insight into xylan biosynthesis in Miscanthus, we identified seven GT43 genes in M. lutarioriparius and characterized their functional roles in xylan biosynthesis. Complementation assay including plant height, irregular xylem cells in stem cross sections and xylose content measurements revealed that MIGT43 genes have evolved into two distinct functional groups, in which MIGT43A-E are orthologous to IRX9, while MIGT43F and MIGT43G are orthologous to IRX14. Furthermore, our results indicated that substantial divergence has occurred in the functional roles of MIGT43s during xylan biosynthesis especially in seed coat mucilage. The results presented deepened our understanding of xylan biosynthesis in grasses and may lay the foundation for future genetic manipulation of Miscanthus cell wall structure and components.

Results
Isolation of GT43 genes in M. lutarioriparius
To identify the GT43 family in M. lutarioriparius, the amino acid sequences of four Arabidopsis GT43 members were used as query baits to BLAST against the draft genome sequences of M. lutarioriparius, and seven GT43 orthologous genes were identified. Specific primers were designed and seven candidate genes encoding putative GT43 proteins designated as MIGT43A to MIGT43G were obtained by PCR in M. lutarioriparius. As indicated in Fig. 1a, all seven proteins had a conserved structure and ranged in size from 358 to 451 amino acids. Pairwise comparison of the amino acid sequences showed that MIGT43C and MIGT43D shared the highest sequence similarity (75.3 %), while MIGT43D and MIGT43G shared the lowest sequence similarity (43.3 %) (Fig. 1b).

Deduced MIGT43A and MIGT43B amino acid sequences shared the highest sequence identities with Arabidopsis IRX9 (37 and 41 %), and MIGT43C-E shared relatively higher sequence identities with IRX9L (42, 48 and 53 %) than with IRX14 or IRX14L. By contrast, MIGT43F and MIGT43G proteins had the highest sequence identities with IRX14 and IRX14L (59 and 37 %) than with IRX9 (Additional file 1: Table S1).

Furthermore, the gene structure of each MIGT43 was obtained through the alignment of their coding sequences and genomic sequences (Fig. 1c). All MIGT43 genes

![Fig. 1](image-url)
shared very similar gene structure in terms of intron number and exon length. They all contained three exons and two introns. In addition, the intron phases with respect to codons were well conserved among different MIGT43 genes.

**Phylogenetic analysis of GT43 members from *M. lutarioriparius* and other plant species**

To gain insight into the origin and evolutionary history of the GT43 family, we further identified GT43 proteins from nine other currently sequenced genomes that cover a wide spectrum of plant taxonomic groups including moss (*Physcomitrella patens*), spikemoss (*Selaginella moellendorffii*), the monocot angiosperms (*Oryza sativa*, *Brachypodium distachyon* and *Sorghum bicolor*), and the dicot angiosperms (*Arabidopsis thaliana*, *Populus trichocarpa*, *Medicago truncatula* and *Vitis vinifera*). Totally 57 GT43 proteins were identified from these nine plant species (Additional file 2) and a phylogenetic tree was constructed with these GT43 proteins (Fig. 2a). The phylogenetic tree separated all GT43 proteins into three distinct subfamilies designated as IRX9, IRX9L and IRX14/IRX14L, which was similar to the previous studies [13, 38]. The seven GT43 proteins from *Miscanthus* were classified into the three subfamilies. MIGT43A and MIGT43B were clustered into the IRX9 subfamily, MIGT43C-E were classified into the IRX9L subfamily, while MIGT43F and MIGT43G were distributed into the IRX14/IRX14L subfamily.

The distribution of the three subgroups among the ten plant species varied within each subfamily (Fig. 2b). It is noteworthy that the number of GT43 proteins in the monocot species seems to be higher than that of the dicot species, at least it is the case for the selected plant species. For example, there were 10, 10, 10 and 7 members in the monocot species *O. sativa*, *B. distachyon*, *S. bicolor* and *M. lutarioriparius*, whereas the number of GT43 in the dicot species *A. thaliana*, *P. trichocarpa*, *M. truncatula* and *V. vinifera* were 4, 7, 4 and 4, respectively. In addition, the members of IRX9 and IRX9L subfamilies in the monocot angiosperms were generally higher than those of the dicot species. For instance, the IRX9 subfamily accounted for 40, 40, 40 and 28 % in the monocot species *O. sativa*, *B. distachyon*, *S. bicolor* and *M. lutarioriparius*, respectively, whereas the percentages of the IRX9 subfamily in the dicot species *A. thaliana*, *P. trichocarpa*, *M. truncatula* and *V. vinifera* were 25, 25, 28 and 25 %, respectively. Noticeably, no IRX9 subfamily members were present in *P. patens* and *S. moellendorffii*.

**MIGT43 genes are ubiquitously expressed and have specific expressions in stem cells**

To investigate the expression patterns of MIGT43 genes, we first used the quantitative real-time RT-PCR (qRT-PCR) to examine their expressions across seven different tissues. As shown in Fig. 3a, all seven MIGT43 genes were ubiquitously expressed in seven different tissues examined, but their relative expression levels differed significantly. For example, MIGT43A, MIGT43D and MIGT43E genes shared similar expression patterns with predominant expressions in leaf, whereas the expressions of MIGT43B and MIGT43G genes were relatively lower. MIGT43C and MIGT43F genes were broadly expressed in the majority of the tissues, while especially higher expressions were detected in the basal stem. Furthermore, all MIGT43 genes except MIGT43B exhibited higher expressions in the basal stem than in the upper stem.

To obtain more detailed expression patterns of MIGT43 genes in specific cell types, we further performed the in situ hybridization analysis to examine their expressions in the 11th internode of the stem. For all seven genes, intense hybridization signals were observed in sclerenchyma cells and vascular bundle fiber cells, the cell types undergoing secondary wall thickening (Fig. 3b-h). Moreover, relatively weak hybridization signals were also observed for MIGT43C-E in parenchyma cells. By contrast, the control hybridized with sense probes did not show any signals in vascular bundle or sclerenchyma cells (Fig. 3g). These results suggest that MIGT43 genes may participate in diverse plant development processes especially in the secondary cell wall formation.

**MIGT43 members are targeted to Golgi apparatus**

To investigate the subcellular localization of MIGT43 proteins, we constructed fluorescently tagged fusion proteins by fusing Yellow Fluorescent Protein (YFP) to the C terminus of each MIGT43 protein. The recombinant constructs were transiently co-expressed in *Nicotiana benthamiana* leaf epidermal cells with the Golgi marker Man49-mCherry [47]. Examination of the fluorescent signals revealed that seven YFP-tagged MIGT43s all exhibited a punctate distribution, and the pattern perfectly matched with that of Man49-mCherry (Fig. 4), whereas the YFP control protein had signals throughout the cytoplasm and the nucleus (data not shown). The co-localization of MIGT43 proteins with the Golgi marker indicate that MIGT43s are Golgi-localized proteins.

**MIGT43 genes rescue the morphological defects of irx9 or irx14**

To reveal whether MIGT43 genes perform the same functions as *IRX9* and *IRX14* orthologues in *Arabidopsis*, we examined their abilities to rescue the morphological defects of *irx9* and *irx14*. Due to the severely dwarfed plant stature and poor fertility of homozygous *irx9* plants [5], we used the heterozygous line for the transformation with the 35S:MIGT43s constructs. Positive
transgenic lines for each construct were tested for the presence of MIGT43 genes in homozygous irx9 and irx14 background by semi-quantitative RT-PCR (Fig. 5a). Homozygous T2 plants from at least two independent transformants with higher expressions were used for the phenotypic analyses.

The growth of the irx9 plants was characterized by the dwarf stature, smaller rosette size and dark-green leaves under our growth conditions, which is similar to the previous reports [5, 12]. Overexpression of MIGT43A-E genes in irx9 displayed an intermediate growth phenotype between the mutant and the wild type (WT) in
terms of rosette size and inflorescence height. The rosette diameters of the complemented plants increased by two- to three-fold, and the inflorescence stems were two- to four-fold taller compared to the irx9 plants after four weeks of growth (Fig. 4b, d), suggesting that the irx phenotype may be partially complemented in these transformants. By contrast, transformants of MlGT43F or MlGT43G overexpression in irx9 mutant exhibited a morphology resembled of the irx9 mutant, indicating that MIGT43F and MIGT43G were unable to complement the irx9 phenotypes (Fig. 4b, d, f).

The growth of irx14 mutant did not show any other obvious phenotypes except for a slight reduction in plant height compared to WT (Fig. 4c, e) as described previously [14]. The height of all MIGT43 complemented irx14 plants was indistinguishable from that of

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**Fig. 3** Expression patterns of MIGT43 genes. **a** Expression analysis of MIGT43 genes by qRT-PCR. Relative expression levels in seven tissues were normalized using MlACT11 as the reference gene. For each gene, the tissues with the lowest expression level are set to 1. Data are the means ± SE of three biological replicates. **b** In situ localization of MIGT43 genes in Miscanthus stem. Cross-sections of stems were hybridized with digoxigenin-labeled antisense MIGT43A (b), MIGT43B (c), MIGT43C (d), MIGT43D (e), MIGT43E (f), MIGT43F (g), MIGT43G (h), or sense (i) RNA probes, and the hybridization signals were detected with alkaline phosphatase-conjugated antibody and were shown as purple color. pv, pitted vessel; x, xylem; ph, phloem; pa, parenchyma; sc, sclerenchyma. Bar = 100 μm.
irx14 or WT plants, thus it is hard to evaluate the ability of seven MIGT43 genes to complement the irx14 mutant merely judged from their growth phenotypes. Subsequently, xylem morphology, xylan immunolocalization and cell wall monosaccharide compositions will be further examined in the transgenic plants to determine the abilities of MIGT43s to complement the irx14 phenotypes.

Microscopic analysis of the secondary cell wall
To demonstrate whether the morphological complementation by MIGT43 genes could be accompanied with the rescue of xylem morphology, the basal inflorescence stems of each complemented line were sectioned and observed by light and transmission electron microscopy. Toluidine blue O (TBO) staining was performed on stem sections of WT, irx9, irx14 and complemented
Fig. 5 Expression of seven MlGT43 genes in Arabidopsis irx9 or irx14 mutants. 

**a** RT-PCR detection of the MlGT43 transcripts in the complemented irx9 or irx14 plants. The Arabidopsis UBQ10 gene was used as a reference. 

**b**, **d**, **f** Phenotype of four-, six- and eight-week-old soil-grown WT, irx9 and MlGT43s complemented irx9 plants. 

**c**, **e**, **g** Phenotype of four-, six- and eight-week-old soil-grown WT, irx14 and MlGT43s complemented irx14 plants. 

**h** Stem height of the WT, irx9 and MlGT43s complemented irx9 plants through 40, 47, 57 days of growth. 

**i** Stem height of the WT, irx14 and MlGT43s complemented irx14 plants through 40, 47, 57 days of growth. Data are means ± SD from at least twelve plants for each background. Two homozygous T3 lines of MlGT43s complemented irx9 or irx14 were used in the analysis.
plants to examine the morphology of secondary cell walls. As shown in Fig. 6, all $MIGT43A-E$ complemented $irx9$ plants exhibited dramatically thickened cell walls in interfascicular fibers compared to $irx9$. The majority of xylem vessels in $MIGT43A$ and $MIGT43B$ complemented $irx9$ plants were characterized by large open round cells comparable to those in WT plants (Fig. 6C1, D1, L1, M1). In addition, the xylem vessels of $MIGT43C$, $MIGT43D$ or $MIGT43E$ complemented $irx9$ plants were usually smaller in size with occasionally irregular shapes, probably due to the not fully thickened cell walls compared to WT (Fig. 6E1-G1, N1-P1). By contrast, overexpression of $MIGT43F$ or $MIGT43G$ in $irx9$ could not restore the collapsed vessels and the weakly thickened interfascicular fibers in $irx9$ (Fig. 6 H1, I1, Q1, R1), which is in consistency with their growth phenotypes (Fig. 5b, d).

The homozygous $irx14$ plants also showed collapsed xylem vessels and thinner secondary cell walls, which is consistent with the previous study [15]. Overexpression of either $MIGT43F$ or $MIGT43G$ could almost fully rescue the $irx$ phenotype of $irx14$ as witnessed by a relatively less irregular vessel cells compared to $irx14$. However, the

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**Fig. 6** Morphology of xylem and interfascicular fibers of WT, $irx9$, $irx14$ and $MIGT43$ complemented plants. Stems of eight-week-old plants were sectioned (8 μm-thick) and stained with TBO for examination of the morphology of vessels, xylary fibers and interfascicular fibers. A1-I1, interfascicular fibers for WT, $irx9$ and $MIGT43$ complemented $irx9$ plants. A2-I2, interfascicular fibers for WT, $irx14$ and $MIGT43$ complemented $irx14$ plants. J1-R1, xylary fibers and vessels for WT, $irx9$ and $MIGT43$ complemented $irx9$ plants. J2-R2, xylary fibers and vessels for WT, $irx14$ and $MIGT43$ complemented $irx14$ plants. At least two homozygous T3 lines of $MIGT43$s complemented $irx9$ or $irx14$ were used in the analysis. Images for each tissue are set as the same magnification. Bar = 50 μm
complemented lines still retained relatively thinner cell walls in both interfascicular fibers and xylem vessels compared to WT (Fig. 6 H2, I2, Q2, R2). By contrast, overexpression of MIGT43A-E in irx14 displayed a collapsed xylem vessel and thinner fiber cell wall phenotype that was indistinguishable from the irx14 mutant (Fig. 6 C2-G2, L2-P2), indicating that MIGT43A-E genes could not rescue the defects of irx14.

Transmission electron microscopy confirmed that the thickness of interfascicular fiber cell walls of the MIGT43A-E complemented irx9 plants was intermediate between irx9 and WT (Fig. 7a and Table 1). Meanwhile, the wall thickness of xyllary fibers and vessels in MIGT43A-E complemented irx9 lines was also significantly increased but not restored to the WT level. By contrast, the wall thickness of interfascicular fibers, xyllary fibers and vessels of MIGT43F or MIGT43G complemented irx9 plants was similar to that of the irx9 mutant (Fig. 7a and Table 1). The wall thickness of interfascicular fibers, xyllary fibers and vessels for MIGT43F or MIGT43G complemented irx14 plants was intermediate between irx14 and WT, while the wall thickness for MIGT43A-E complemented irx14 lines was similar to that of irx14 (Fig. 7b and Table 1). Together, these results indicate that MIGT43A-E can fully or partially rescue the irx9 but not the irx14 phenotypes, while MIGT43F and MIGT43G can complement the irx14 but not the irx9 defects.

**Immunolocalization of xylan in MIGT43s complemented lines**

To investigate whether the phenotypes of the complemented plants are correlated with xylan deposition in secondary cell walls, we performed immunolocalization of xylan using the xylan-directed monoclonal antibody LM10, which recognizes unsubstituted or low-substituted xylan [48], to examine the distribution of xylan in the cell walls. As indicated in Fig. 8, strong fluorescence signals were present in the cell walls of interfascicular fibers and xylem cells in the WT stem, however, relatively weaker signals were detected in the corresponding tissues of the irx9 plants, although the overall pattern of labeling was unchanged compared with the WT plants (Fig. 8 A1, B1). In MIGT43A and MIGT43B complemented irx9 lines, the intensity of fluorescence signals was almost restored to the WT level, and the overall pattern of labeling was almost identical to that of WT, indicating that the GX content in interfascicular fibers and xylem cells was nearly restored to the WT level (Fig. 8 C1, D1). The LM10 signals in the MIGT43C-E complemented irx9 plants were intermediate between irx9 and WT plants (Fig. 8 E1-G1). By contrast, the LM10 signals for MIGT43F and MIGT43G complemented irx9 lines were relatively weaker compared with the others, and the intensity was comparable to that of the irx9 mutant (Fig. 8 H1, I1). As for the irx14 background, the intensity of fluorescence signals of MIGT43F and MIGT43G complemented lines was comparable to that of WT in xylem cells and interfascicular fibers (Fig. 8 H2, I2). By contrast, MIGT43A-E complemented irx14 lines exhibited nearly equal signal intensity to the irx14 mutant (Fig. 8 C2-G2). These results indicate that MIGT43A-E can partially rescue the xylan biosynthesis defect in irx9, whereas MIGT43F and MIGT43G share a conserved biochemical function with IRX9, thus leading to a restoration of normal xylan synthesis in their complemented plants.

**Analysis of cell wall composition**

To determine whether the complementation of xylem morphology and xylan deposition is correlated with the restoration of chemical composition, we measured the monosaccharide composition, cellulose and lignin contents of the transgenic lines. Monosaccharide composition analysis was performed on cell wall preparations from eight-week-old inflorescence stems of WT, irx9, irx14 and MIGT43 complemented lines (Fig. 9). The xyl content in irx14 was decreased by 40 % compared to WT, whereas it was decreased more dramatically in irx9, with only 21 % of the WT. The transgenic plants overexpressing MIGT43A and MIGT43B in irx9 significantly increased the content of xyl to 73 and 82 % of the WT level, respectively. A modest increase was also observed in the MIGT43C-E complemented irx9 lines. However, no significant increases in xyl content were observed in MIGT43F or MIGT43G complemented irx9 lines compared to irx9. Overexpression of MIGT43F and MIGT43G in irx14 restored the xyl content to 92 and 83 % of the WT, respectively. The xyl content of MIGT43A-E complemented irx14 plants was individually increased by approximately 5 to 10 % compared to irx14.

In addition, mutations of irx9 and irx14 caused significant reductions in cellulose and lignin contents compared to WT. Not unexpectedly, overexpression of MIGT43A-E but not MIGT43F and MIGT43G in irx9 restored the contents of cellulose and lignin almost to the WT level. Similarly, overexpression of MIGT43F and MIGT43G but not MIGT43A-E in irx14 recovered the levels of cellulose and lignin nearly to the WT level (Additional file 3: Figure S1). These results further indicate that MIGT43A-E but not MIGT43F-G can partially restore the xylan biosynthesis in irx9, while MIGT43F-G but not MIGT43A-E are able to rescue the xylan biosynthesis defect in irx14, suggesting that MIGT43A-E are orthologous to IRX9, while MIGT43F and MIGT43G are orthologous to IRX14.

**Transactivation assay for MIGT43 genes**

SN1 (SECONDARY WALL-ASSOCIATED NAC DOMAIN PROTEIN 1), VND7 (VASCULAR-RELATED
Fig. 7 Transmission electron micrographs of stem sections of WT, irx9, irx14 and MIGT43 complemented plants. Stems of eight-week-old plants were cut into 70 nm-thick sections and observed with transmission electron microscope, indicating increased fiber and vessel wall thickness by expression of MIGT43 genes. a, Transmission electron micrographs of stem sections of MIGT43 complemented irx9 lines. b, Transmission electron micrographs of stem sections of MIGT43 complemented irx14 lines. At least two homozygous lines of MIGT43 complemented irx9 or irx14 were used in the analysis. ve, vessels; xf, xylary fibers. Bar = 5 μm
NAC-DOMAIN 7) and MYB46 have been shown to act as the master switches in the regulatory network of secondary cell wall biosynthesis [49]. To better understand the underlying regulatory mechanism of MIGT43 genes, we isolated the orthologues of SND1, VND7 and MYB46 in M. lutarioriparius and analyzed their transactivation abilities on proMIGT43A-E:GUS reporters using a transient transactivation assay (Fig. 10). The results showed that MIGT43A was transactivated by MISND1, MIMYB46a, MIMYB46b and MIVND7. MIGT43B was also transactivated by MISND1, MIMYB46a, but not by MIMYB46b and MIVND7. By contrast, MIGT43C-E were not transactivated by any effectors examined. These results indicate that MIGT43A and MIGT43B genes are differentially regulated by SND1, MYB46 and VND7 orthologues and there probably exist other transcriptional factors regulating the expression of MIGT43C-E genes besides the above effectors examined.

None of MIGT43 genes could rescue the mucilage defects of irx14 seeds
Since IRX14 has been shown to be responsible for the synthesis of xylan in seed coat mucilage and mutations in IRX14 lead to a defect in mucilage cohesiveness property [50, 51], we sought to examine whether MIGT43 genes could rescue the mucilage defect of irx14. The seeds of MIGT43 complemented lines in irx14 background were examined by ruthenium red staining (Additional file 4: Figure S2). When seeds were imbibed in water and subjected to gentle shaking, the seeds of seven MIGT43 complemented irx14 lines all exhibited a thin layer of mucilage phenotype similar to that of the irx14 seeds. By contrast, the WT seeds have a much thicker mucilage layer tightly attached to the seed. This result indicated that none of MIGT43 genes could rescue the mucilage defect of irx14.

We further determined the monosaccharide composition of seed mucilage for each complemented line. The xyl content was dramatically reduced in irx14 mucilage as

### Table 1 Cell wall thickness of fiber and vessel cells in the stems of WT, ix9, irx14, and MIGT43s complemented plants

|                   | Interfascicular fiber (μm) | Vessel (μm) | Xyary fiber (μm) |
|-------------------|---------------------------|-------------|------------------|
| WT                | 1.98 ± 0.11               | 1.35 ± 0.26 | 1.46 ± 0.28      |
| ix9               | 1.15 ± 0.23               | 0.47 ± 0.10 | 0.59 ± 0.18      |
| ix9 + MIGT43A     | 1.66 ± 0.19               | 1.21 ± 0.10 | 1.23 ± 0.10      |
| ix9 + MIGT43B     | 1.68 ± 0.33               | 1.19 ± 0.14 | 1.23 ± 0.20      |
| ix9 + MIGT43C     | 1.62 ± 0.25               | 0.97 ± 0.05 | 1.07 ± 0.15      |
| ix9 + MIGT43D     | 1.36 ± 0.29               | 0.90 ± 0.08 | 0.97 ± 0.20      |
| ix9 + MIGT43E     | 1.40 ± 0.18               | 0.95 ± 0.19 | 0.93 ± 0.14      |
| ix9 + MIGT43F     | 1.26 ± 0.18               | 0.62 ± 0.14 | 0.63 ± 0.17      |
| ix9 + MIGT43G     | 1.23 ± 0.26               | 0.59 ± 0.12 | 0.65 ± 0.11      |
| ix14              | 1.49 ± 0.25               | 0.98 ± 0.08 | 1.01 ± 0.22      |
| ix14 + MIGT43A    | 1.46 ± 0.30               | 0.97 ± 0.07 | 1.00 ± 0.19      |
| ix14 + MIGT43B    | 1.47 ± 0.19               | 0.93 ± 0.30 | 0.95 ± 0.10      |
| ix14 + MIGT43C    | 1.50 ± 0.13               | 0.96 ± 0.11 | 0.96 ± 0.15      |
| ix14 + MIGT43D    | 1.46 ± 0.24               | 0.95 ± 0.13 | 0.97 ± 0.17      |
| ix14 + MIGT43E    | 1.48 ± 0.21               | 0.97 ± 0.14 | 0.99 ± 0.13      |
| ix14 + MIGT43F    | 1.53 ± 0.13               | 1.04 ± 0.16 | 1.12 ± 0.12      |
| ix14 + MIGT43G    | 1.58 ± 0.11               | 1.10 ± 0.17 | 1.20 ± 0.11      |

At least two independent transgenic lines for each construct were used for measurement. WT, ix9, and ix14 were included for comparison. Eight-week-old plants for each background were used for analysis. Wall thickness was measured from transmission electron micrographs of fibers and vessels. Data are means (μm) ± SE from 20 cells.

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**Fig. 8** Immunolocalization of xylan using the monoclonal antibody LM10. Labelling was carried out on 8 μm-thick transverse sections from stem tissues of eight-week-old plants. A1-I1: xylan immunolocalization in WT, ix9 and MIGT43 complemented ix9 lines. A2-T2: xylan immunolocalization in WT, ix14 and MIGT43 complemented ix14 lines. Signals were detected with Alexa Fluor488-conjugated secondary antibody and observed with a BX51 fluorescence microscope (OLYMPUS). Bar = 50 μm
Not surprisingly, the xyl content in seven complemented lines was comparable to that of irx14 and not restored to the WT level (Additional file 5: Figure S3), suggesting that none of MlGT43s could synthesize the xylan in the seed coat mucilage.

Discussion
Much progress has been gained in xylan biosynthesis mainly in the model species Arabidopsis. Several GT43 family proteins have been revealed to participate in xylan backbone biosynthesis in secondary cell walls [13, 19, 35–38]. By contrast, less knowledge regarding the biosynthesis of xylan is known in grass, despite that xylan especially arabinoxylan is the major hemicellulosic components in grass cell walls. In this study, we identified seven GT43 genes from M. lutarioriparius and revealed that they are functional orthologues of Arabidopsis IRX9 and IRX14. Phylogenetic analysis of GT43 proteins from nine representative plant species and Miscanthus revealed that these proteins were classified into three major clades, namely IRX9, IRX9L and IRX14/IRX14L (Fig. 2). Noteworthy, our results indicated that no IRX9 orthologues were present in the lower plant species moss (P. patens) and spikemoss (S. mellysellia). Moss has been demonstrated to be capable of synthesizing glucuronoxylans that are structurally similar to those present in the secondary cell walls of higher plants [52]. The glucuronoxylans are mainly located in primary cell walls in moss as no mechanical supporting tissues composed mainly of secondary cell walls have been evolved. As a basal vascular plant, spikemoss has evolved tissues containing secondary cell walls. Xylans have been shown to be one of the most abundant cell wall components in spikemoss [53]. Since IRX9 has been shown to be mainly responsible for the biosynthesis of xylans in secondary cell
walls [13, 19, 20, 35, 38, 54], the absence of xylans in secondary cell walls in moss may partially explain why no IRX9 orthologues are present in moss genome. Thus, it seems likely that vascular plants have evolved a specialized isoform of IRX9, which is responsible for xylan biosynthesis in secondary cell walls. However, this hypothesis seems somewhat implausible because IRX9 orthologues are also lacking in spikemoss. Together, these results indicate that the specialization of IRX9 for xylan biosynthesis in primary and secondary cell walls is not necessary for the evolution of vascular tissue.

Although the qRT-PCR analysis revealed that MIGT43A to MIGT43E in M. lutariopariparicus exhibited broad expression patterns across the tissues examined, the in situ hybridization analysis unambiguously indicated that Miscanthus IRX9 orthologues MIGT43A and MIGT43B were preferentially expressed in cells undergoing secondary wall thickening, while the IRX9L orthologues MIGT43C-E were expressed in both parenchymal cells and sclerenchyma cells (Fig. 3). In addition, IRX9 orthologues MIGT43A and MIGT43B were both transcriptionally regulated by MISND1, MMYB46a or MIVND7, three candidate transcriptional switches governing secondary cell wall biosynthesis. By contrast, the Miscanthus IRX9L orthologues (MIGT43C-E) were not significantly transactivated by these transcription factors (Fig. 10). Similar results were reported for IRX9 orthologues in Arabidopsis, rice (OsGT43A and OsGT43E) and poplar (PtrGT43A and PtrGT43B), which were shown to be highly expressed in tissues with abundant secondary cell walls [13, 35, 38]. In addition, poplar IRX9 orthologues (PtrGT43A and PtrGT43B) were transcriptionally regulated by PtxtMYB021 (MYB46 orthologue) and PNAC085 (SND1 orthologue), master transcriptional switches involved in secondary cell wall formation [38]. Together, these results indicated that IRX9 orthologues are mainly involved in secondary cell wall biosynthesis, and its role is highly conserved in angiosperm species.

In addition, the number of GT43 proteins in monocot species seems to be higher than that of dicot species, which was mainly due to a significantly expansion of IRX9 orthologues.
and IRX9L members in monocot species (Fig. 2b). In dicots, such as Arabidopsis and poplar, xylan is predominantly deposited in the secondary cell walls, whereas there is very limited amounts of xylan in the primary cell walls. By contrast, the monocot species including rice and Miscanthus have abundant amounts of xylan in both primary and secondary cell walls. This could partially explain why the number of IRX9 and IRX9L orthologues are over-presented in monocots compared with dicots.

Phylogenetic analysis also indicated that ancestral IRX9 orthologues emerged after the specification of the higher plants (Fig. 2a). In addition, IRX9 may possibly evolve from its IRX9L homologue through the duplication events during the evolutionary process as they share very high sequence identities [13, 38]. The functional diversification of IRX9 orthologues may be due to their expression specificities and their abilities to respond to the key transcriptional factors involved in secondary wall formation (Fig. 10). The different cis-regulatory elements present in the promoter of Miscanthus IRX9 and IRX9L orthologues may explain their functional divergences to some extent (Additional file 6: Table S2). In other words, Miscanthus IRX9 orthologues may have evolved to gain some key cis-regulatory elements, which confers their specific functions in xylan biosynthesis during secondary cell wall formation.

In Arabidopsis, IRX9 and IRX14 play independent roles in xylan biosynthesis, since the phenotypes of irx9 mutant cannot be rescued by the overexpression of IRX14 or IRX14L and vice versa [13, 19]. In addition, IRX9 and IRX14 are proposed to play dominant roles, whereas their homologues IRX9L and IRX14L are indicated to play partially redundant or minor roles in xylan backbone biosynthesis [13, 14, 19]. Contrary to this assumption, a recent study proposed that IRX9L and IRX14L play equally important roles with IRX9 and IRX14 in xylan biosynthesis [20]. The seven GT43 orthologues in Miscanthus were classified into three major subclades namely IRX9, IRX9L and IRX14/IRX14L. All five Miscanthus IRX9 and IRX9L orthologues (MlGT43A-E) could nearly fully or partially complement the phenotypes of irx9, while none of these genes could rescue the phenotypes of irx14. Similarly, two Miscanthus IRX14 and IRX14L orthologues (MlGT43F and MlGT43G) were able to rescue the phenotypes of irx14 but not irx9. These results indicated that GT43 genes have been evolved into two functional groups in Miscanthus, and the functions between the members in IRX9/IRX9L and IRX14/IRX14L groups have been diversified substantially. Likewise, the involvement of two distinctly functional groups of GT43 genes in xylan biosynthesis seems to be highly conserved in different plant species. For example, the rice orthologues of IRX9 (OsGT43A and OsGT43E) were able to rescue the phenotypes of irx9 but were not able to complement those of irx14. By contrast, the IRX14 orthologue OsGT43J was able to complement the irx14 phenotypes but unable to rescue those of irx9. Similarly, the poplar IRX9 orthologues (PtrGT43A, PtrGT43B and PtrGT43E) were able to rescue the xylan defects of irx9 but could not complement those of irx14, whereas the IRX14 orthologues (PtrGT43C and PtrGT43D) were capable of rescuing the defects of irx14 but not those of irx9.

Xylans are typically substituted with α-1-Araf residues at C2- and/or C3-position in arabinoxylans (AX) and less frequently with GlcpA and/or 4-O-Me-GlcpA side-chains at C2- position in glucuronoxylans (GAX) in grasses [3, 4]. AX is the major xylan in Miscanthus and the degree of Ara substitution positively affects the lignocellulose saccharification under various pretreatments [44, 45]. AX is also the major xylan of the seed mucilage in psyllium (Plantago ovata) [55]. During Arabidopsis seed differentiation, the seed coat epidermal cells synthesize and secrete large amounts of mucilage, which encapsulated the seed upon imbibition. Although the Arabidopsis seed coat mucilage are primarily composed of pectic RG I, minor amounts of xylan are also present in the mucilage and play an important role in maintaining the structure of seed coat mucilage [50, 51]. Unlike the typical xylan in dicot secondary cell walls, mucilage xylan has a unique structure with frequent substitutions with Xyl rather than with GlcA or Ara residues [50, 51]. IRX14 has been revealed to be responsible for the biosynthesis of xylan in Arabidopsis mucilage and loss function lead to a mucilage cohesiveness defect [50, 51]. It is noteworthy that none of the MlGT43 genes could be able to complement the irx14 mucilage defect (Additional file 4: Figure S2), suggesting that MlGT43s could not synthesize the mucilage xylan, which is involved in maintaining the structure of seed coat mucilage (Additional file 5: Figure S3). The reason might due to the fact that mucilage xylan is structurally different from that of the stem secondary walls, and the functions of Miscanthus GT43 proteins have diversified from those of Arabidopsis orthologues during the evolutionary process. Similarly, there is also lines of evidence highlighting that mucilage xylan biosynthesis is diversified in different plant species. For example, IRX10 but not IRX9 or IRX14 might be responsible for the synthesis of the xylan backbone in psyllium mucilage because IRX10 orthologues were highly presented in psyllium mucilage, while relatively very lower transcripts of IRX9 and IRX14 were detected in a transcriptome analysis [55].

**Conclusion**

In this study, we functionally identified seven GT43 genes from M. lutarioriparius. Our results provided the first line of genetic evidence demonstrating that
Miscanthus has evolved to retain two functionally non-redundant groups of GT43 genes involved in xylan biosynthesis. MIGT43A-E are functional orthologues of IRX9, while MIGT43F and MIGT43G are functional orthologues of IRX14. Nevertheless, functional divergence of IRX14 orthologues in M. lutarioriparius has occurred as none of MIGT43 genes could rescue the mucilage defects of irx14 seeds. Furthermore, MIGT43A-E were differentially regulated by SND1, MYB46 or VND7 orthologues, the putative key regulators in secondary cell wall formation. The results obtained deepen our understanding of xylan biosynthesis in Miscanthus. Understanding how xylan polymers are synthesized may lay a foundation for the genetic modification of Miscanthus to be better suited for various economically important applications, including the more efficient utilization of xylan for biofuel production.

Methods

Plant materials and growth conditions

The M. lutarioriparius used in this study was provided by Shanghai Institute for Biological Sciences of the Chinese Academy of Sciences. The plants were clonally propagated by young rhizomes in greenhouse under 16 h light/8 h dark photoperiod at 25–28 °C.

T-DNA insertion mutants irx9 (SALK_058238) and irx14 (SALK_038212) were obtained from the Arabidopsis Biological Resource Center (ABRC). Seeds were surface sterilized and sowed on 1/2 MS plate. After stratified at 4 °C for 3 d, the plates were transferred to the growth chamber and germinated at 21 °C under 16 h light/8h dark photoperiod. Homozygous T-DNA insertions were identified by PCR of genomic DNA. The primers are listed in Additional file 7: Table S3.

RNA isolation and Quantitative real-time RT-PCR (qRT-PCR) analysis

The total RNA was isolated from root, rhizome, stem, leaf and sheath of M. lutarioriparius using Trizol reagent (Invitrogen), then treated with RNase-free DNaseI (Promega) to remove genomic DNA contamination. First-strand cDNA was synthesized using M-MLV reverse transcriptase (TaKaRa, Japan) according to the manufacturer’s instructions. The cDNAs were used as templates for qRT-PCR with gene-specific primers (Additional file 7: Table S3). The qRT-PCR was carried out using LightCycler® 480 detection system (Roche) with SYBR® Premix Ex Taq II (TaKaRa). MIACII was used as an internal control.

Identification of MIGT43 genes

The Arabidopsis GT43 proteins (IRX9, IRX9L, IRX14 and IRX14L) were used as baits to search against the draft genome sequence of M. lutarioriparius (Lu et al., unpublished data). Specific primers were designed to isolate the full length MIGT43 cDNAs (Additional file 7: Table S3). The PCR products were purified, cloned into pMD19-T vector (TIANGEN) and sequenced. The exon/intron organization was illustrated with Gene Structure Display Server (GSDS) program (http://gsds.cbi.pku.edu.cn/) by alignment of the cDNAs with their corresponding genomic DNA sequences [56].

Phylogenetic analysis of GT43 family from other plant species

GT43 family protein sequences from nine other species including moss (P. patens), spikemoss (S. moellendorffii), monocot angiosperms (O. sativa, B. distachyon and S. bicolor), and dicot angiosperms (A. thaliana, P. trichocarpa, M. truncatula and V. vinifera) were obtained using BLASTP search against Phytodrome10 database (https://phytozome.jgi.doe.gov/). Phylogenetic analysis was performed with MEGA6.0 by the Neighbor-Joining (NJ) method with 1000 bootstrap replicates with default parameters [57].

In situ mRNA hybridization

For the synthesis of antisense and sense probes, ~200 bp fragments of MIGT43A-G were amplified by PCR with their corresponding primers (Additional file 7: Table S3) and cloned into the pGM-T vector (TIANGEN). The RNA probes were synthesized with the DIG RNA labelling kit (Roche) according to the manufacturer’s instructions.

Miscanthus stem segments from the 11th internode were fixed in FAA solution (70% ethanol, 5% formaldehyde and 5% acetic acid) at 4 °C overnight, followed by dehydration in gradient ethanol series (10% increments). The samples were embedded in paraplast and cut into 8 µm-thick sections. The sections were mounted onto slides, and hybridized with DIG-labeled antisense or sense RNA probes. Images were captured with the OLYMPUS BX51 microscope.

Subcellular localization

The co-localization of fluorescent protein-tagged MIGT43A-G with the Golgi marker was examined using tobacco leaf transient expression system [58]. The full-length MIGT43 genes without a terminator codon were amplified and fused with yellow fluorescent protein (YFP) in pEarleyGate101 vector [59] via LR recombination reactions (Invitrogen). The proteins generated thus encode fusion proteins of MIGT43s with YFP tagged at the C terminus. After 3 days post-co-infiltration of YFP fusion proteins and the Golgi marker into tobacco leaves, leaf epidermal cells were examined for yellow fluorescence signal using a FluoView FV1000 Laser Scanning confocal microscope (OLYMPUS) equipped with 488 nm argon laser.
Overexpression vector construction and complementation

The full-length cDNA sequence of MIGT43s were amplified by PCR and ligated to the pGWC-T as described previously [60]. The products were sequenced and then transferred into the pEarleyGate 100 vector [59] via LR recombination reaction (Invitrogen) to produce the 35S CaMV overexpression constructs. The constructs were introduced into Agrobacterium tumefaciens strain EHA105 by electroporation.

For complementation analysis, the overexpression constructs were transformed into the Arabidopsis irx9 heterozygous or irx14 homozygous mutant via the floral dip method [61]. Positive T0 and T1 generation plants were screened by spraying BASTA solution (50 mg/L) onto one-week-old seedlings in soil. For irx9 complemented lines, transformed seedlings were further genotyped with PCR to verify the homozygous T-DNA insertions. Homozygous T3 transgenic lines were used for further analysis.

Microscopy and immunolocalization analysis

Arabidopsis inflorescence stems were taken 0.5 cm above the rosette of eight-week-old plants. Samples were fixed in FAA solution, dehydrated via a series of ethanol gradients, and embedded in paraplast. For light microscopy, 8 µm-thick sections were stained with 0.5 % (w/v) toluidine blue O (Sigma-Aldrich) for 2 min and rinsed with water. The sections were photographed with a BX51 light microscope (OLYMPUS).

For the immunolabelling, sections were incubated with the LM10 antibody (1/20 dilution) for 2 h, then washed three times with phosphate-buffered saline, followed by incubation with rabbit anti-rat Alexa Fluor488-conjugated secondary antibody (1/100 dilution) in the dark for 1 h. Images were captured using a BX51 light microscope (OLYMPUS) equipped with fluorescent light.

For transmission electron microscopy, samples were embedded in Spurr’s resin. Ultra-thin sections (70 nm) were viewed by a H-7650 electron microscope (HITACHI). Cell wall thickness was measured in metaxylem vessels and interfascicular fibres using the software SmileView (JEOL). For each construct, at least three transgenic lines with the most severe phenotypes were examined.

Cell wall monosaccharide composition analysis

To prepare cell-wall alcohol-insoluble residues (AIR), eight-week-old inflorescence stems from at least 20 independent plants were collected, frozen in liquid nitrogen, and freeze-dried overnight using a lyophilizer. For monosaccharide composition analysis, AIR was hydrolyzed in 2 M trifluoroacetic acid for 2 h at 120 °C. The released monosaccharides were derived by 1-phenyl-3-methyl-5-pyrazolone (PMP) and the derivatives were separated on a Thermo ODS-2 C18 column (4.6 × 250 mm) connected to a Waters HPLC system. The absorbance was monitored at 245 nm. Cellulose content was assayed with the anthrone reagent according to Updegraff [62]. Lignin composition was determined using the acetyl bromide spectrophotometric method as described [63].

Transcriptional activation analysis

The pBI221 vector was used to produce both effector and reporter constructs. The MISND1, MIMYB46a/b and MIVND7 effector constructs were obtained by PCR using Miscanthus stem cDNA as the template (Additional file 7: Table S3). All effector constructs were individually ligated between the CaMV 35S promoter and the NOS terminator after removing GUS from the pBI221 vector. The MIGT43A-E promoters were cloned by hiTAIL-PCR [64] and ligated upstream of the GUS reporter gene after removing the 35S promoter region of pBI221 to create the reporter constructs.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The data supporting the results of this article are included as additional files. The MIGT43 gene and promoter sequences were deposited in the Genbank (https://www.ncbi.nlm.nih.gov/genbank) under accession numbers KX082754 to KX082765.

Additional files

- **Additional file 1: Table S1.** Sequence identity and similarity among seven MIGT43 proteins and their Arabidopsis orthologues. (DOCX 15 kb)
- **Additional file 2:** Protein sequences used for the phylogenetic analysis of GT43 family. (TXT 27 kb)
- **Additional file 3: Figure S1.** Cellulose and lignin contents in MIGT43 complemented lines. Cell walls were prepared from pooled inflorescence stems of six independent plants per genotype and used for measurement of the contents of cellulose (A) and lignin (B). The data are means ± SE of three independent assays. (TIF 522 kb)
- **Additional file 4: Figure S2.** None of MIGT43 genes could rescue the mucilage defect of irx14 seeds. Seeds of WT (A), irx14 (B) and MIGT43A-G complemented irx14 lines (B-I) were stained by ruthenium red with gentle shaking for 30 min. Bar = 200 µm. (TIF 14007 kb)
- **Additional file 5: Figure S3.** Mucilage weight and monosaccharide composition of WT, irx14 and MIGT43 complemented irx14 seeds. A, Mucilage weights from WT, irx14 and MIGT43 complemented irx14 lines. Water-soluble and adherent mucilage were sequentially extracted with water and 2 M NaOH. Error bars indicate SD (n = 3). B and C, Monosaccharide composition of water-soluble and adherent mucilage from WT, irx14 and MIGT43 complemented irx14 lines. (TIF 5994 kb)
Abbreviations
IRX: irregular xylem; GT: glycosyltransferase; qRT-PCR: quantitative real-time RT-PCR; GX: (methyl)glucuronic acid; AX: arabinoxylan; GAX: glucuronoarabinoxylan; GlcA: glucuronic acid; MeGlcA: methylglucuronic acid; Ara: arabinose; GXMT: glucuronoarabinoxylan methyltransferase; TBL: trichome birefringence-like; XAT: xylan arabinosyltransferase; XAX: xylotol arabinosyl substitution of xylan; Xyl: xylose; CDS: coding sequence; YFP: yellow fluorescent protein; WT: wild type; TBO: toluidine blue O; SN1D: secondary wall-associated NAC domain protein 1; VND7: vascular-related NAC-domain 7.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
XYW performed gene cloning, in-situ hybridization, qRT-PCR, plant transformation, histochemical assay, data processing and drafted the manuscript. QT cooperated with XYW in the histochemical assay, monosaccharide composition analysis, cellulose and lignin content measurement. XZ assisted in promotor cloning and sequence alignments. AMW assisted in the conception of the study, and discussion of the results. YZK assisted in the design of the study, data processing, and revision of the manuscript. XYW assisted in plant transformation and phenotypic analysis. GH performed phylogenetic analysis and sequence alignments. AMW assisted in the conception of the study, and discussion of the results. YZK assisted in the design of the study, discussion of the results and revision of the manuscript. RBH participated in the conception of the study, data analysis, discussion and draft of the manuscript. GYK conceived the study, designed the experiment, helped in interpretation of the results and revision of the manuscript. All authors have read and approved the final version of the manuscript.

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Author details
1Qingdao Institute of Bioenergy and Bioprocess Technology, Key Laboratory of Biofuels, Qingdao Engineering Research Center of Biomass Resources and Environment, Chinese Academy of Sciences, Qingdao 266101, PR China.
2University of Chinese Academy of Sciences, Beijing 100049, PR China.
3Shandong Institute of Agricultural Sustainable Development, Jinan 250100, PR China.
4State Key Laboratory for Conservation and Utilization of Subtropical Agroresources, South China Agricultural University, Guangzhou 510642, PR China.
5Tobacco Research Institute of Chinese Academy of Agricultural Sciences, Key laboratory of Tobacco Genetic Improvement and Biotechnology, Qingdao 266101, PR China.

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