A Galacturonic Acid–Containing Xyloglucan Is Involved in Arabidopsis Root Hair Tip Growth

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Root hairs provide a model system to study plant cell growth, yet little is known about the polysaccharide compositions of their walls or the role of these polysaccharides in wall expansion. We report that Arabidopsis thaliana root hair walls contain a previously unidentified xyloglucan that is composed of both neutral and galacturonic acid–containing subunits, the latter containing the \( \beta-O\)galactosyluronic acid\((1\rightarrow2)\)\(\alpha-O\)xylosyl\((1\rightarrow\) and/or \(\alpha-L\)fucosyl\((1\rightarrow2)\)\(\beta-O\)galactosyluronic acid\((1\rightarrow2)\)\(\alpha-O\)xylosyl\((1\rightarrow\) side chains. Arabidopsis mutants lacking root hairs have no acidic xyloglucan. A loss-of-function mutation in At1g63450, a root hair–specific gene encoding a family GT47 glycosyltransferase, results in the synthesis of xyloglucan that lacks galacturonic acid. The root hairs of this mutant are shorter than those of the wild type. This mutant phenotype and the absence of galacturonic acid in the root xyloglucan are complemented by At1g63450. The leaf and stem cell walls of wild-type Arabidopsis contain no acidic xyloglucan. However, overexpression of At1g63450 led to the synthesis of galacturonic acid–containing xyloglucan in these tissues. We propose that At1g63450 encodes XYLOGLUCAN–SPECIFIC GALACTURONOSYLTRANSFERASE1, which catalyzes the formation of the galactosyluronic acid\((1\rightarrow2)\)\(\alpha-O\)xylopyranosyl linkage and that the acidic xyloglucan is present only in root hair cell walls. The role of the acidic xyloglucan in root hair tip growth is discussed.

INTRODUCTION

The primary wall that surrounds all growing plant cells must be sufficiently resilient to prevent the cell from bursting due to turgor pressure yet allow the cell to expand and grow in a controlled and oriented manner (Cosgrove, 2000; O’Neill and York, 2003). Most growing plant cells have the potential to expand along any axis, allowing the cell to control its morphological development (Szymanski and Cosgrove, 2009). By contrast, growth occurs exclusively at the cell apex in pollen tubes and root hairs, which exhibit an elongated morphology as the result of so-called tip growth (Shaw et al., 2000; Hepler et al., 2001).

The Arabidopsis thaliana root hair provides a model system to study the molecular mechanisms that control and localize expansion to a single region of the cell wall (Galway, 2006; Emons and Ketelaar, 2009; Nielsen, 2009). Many of the genes and transcription factors involved in root hair initiation and development have been identified (Dolan et al., 1993; Bibikova and Gilroy, 2003; Grierson and Schiefelbein, 2009; Benfey et al., 2010). In addition, some of the factors that affect root hair development, including ion gradients (Bibikova and Gilroy, 2003), pH and reactive oxygen species (Carol and Dolan, 2006; Monsma et al., 2007), and the availability of nutrients (Bibikova and Gilroy, 2009; Grierson and Schiefelbein, 2009), have also been identified. By contrast, the function of the primary cell wall and its constituent polysaccharides in root hair initiation and growth is not understood.

Root hair walls (Mort and Grover, 1988; Galway, 2006; Nielsen, 2009) and the walls of other growing plant cells (O’Neill and York, 2003) are believed to consist of cellulose microfibrils embedded in a matrix composed primarily of pectins and hemicelluloses together with smaller amounts of glycoproteins and minerals. Current models of the root hair wall predict that the organization of the glycan in the walls determines where the cell can expand. Thus, the random orientation of cellulose microfibrils in the primary wall at the root hair tip facilitates expansion in this region (Dumais et al., 2006; Akkerman et al., 2012), whereas lateral expansion of the root hair side walls is restricted by the deposition of a secondary wall containing cellulose microfibrils with a helicoidal orientation (Shaw et al., 2000; Galway, 2006; Emons and Ketelaar, 2009; Nielsen, 2009). These primary and secondary wall layers have also been proposed to contain distinct noncellulosic polysaccharides, although the nature of these differences and their role in tip growth has not been established (Emons and Ketelaar, 2009; Nielsen, 2009). A recent study has also reported that Hyp-rich glycoproteins are required for assembly of the root hair wall and for normal root hair growth, although the role of these glycoproteins in wall assembly is not understood (Velásquez et al., 2011).

Xyloglucan is the most abundant hemicellulose in the primary cell walls of dicotyledonous plants (Hoffman et al., 2005). This polysaccharide has been proposed to interact with cellulose microfibrils in the primary wall to form a cellulose-xyloglucan network (Hanus and Mazeau, 2006) whose enzymatic restructuring is required for wall expansion and cell growth (Nishitani, 1998; Takeda et al., 2002; Fry, 2004; Park et al., 2004; Szymanski and Cosgrove, 2009). However, the role of xyloglucan in cell growth became a subject of debate when it was shown that the aerial portions of an Arabidopsis mutant (xxt1 xxt2), which lacks discernible amounts of...
xyloglucan, are almost indistinguishable from wild-type plants (Cavalier et al., 2008). Interestingly, this mutant has root hairs that have swollen bases and are much shorter than their wild-type counterparts. Plants carrying mutations in other xyloglucan-specific xylosyltransferase (XXT) genes also have root hairs that are shorter than those of the wild type (Zabotina et al., 2012). Thus, xyloglucan is required for normal expansion in tip-growing root hairs (Cavalier et al., 2008), although the molecular mechanisms underlying this requirement remain to be determined (Galway et al., 2011).

*Arabidopsis* produces a XXXG-type xyloglucan (Vrincken et al., 1997) in which three consecutive (1→4)-linked β-D-glucopyranosyl backbone residues are substituted at O-6 with a glycosyl side chain (Perrin et al., 2003). Treating such a xyloglucan with a xyloglucan-specific endoglucanase (XEG) fragments the backbone at the unbranched glucosyl residues (Pauly et al., 1999). The structures of the resulting oligosaccharide subunits, which differ in the types of side chain they contain, are readily determined using matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF MS) and 1H nuclear magnetic resonance (NMR) spectroscopy (Hoffman et al., 2005; Peña et al., 2008). The structures of the xyloglucan side chains and their distribution along the glucan backbone are described using a single-letter nomenclature (Fry et al., 1993). The letter G denotes (1→4)-β-D-glucosyl, X denotes α-D-xylosyl-(1→6)-β-D-glucosyl, L denotes β-D-galactosyl-(1→2)-α-D-xylosyl-(1→6)-β-D-glucosyl, and F denotes α-L-fucosyl-(1→2)-β-D-galactosyl-(1→2)-α-D-xylosyl-(1→6)-β-D-glucosyl (Figures 1A to 1C).

Here, we provide chemical, biochemical, and genetic evidence for the presence of a unique acidic xyloglucan in *Arabidopsis* root hair cells. This acidic xyloglucan contains the previously unidentified galacturonic acid (GalA)-containing side chains: the disaccharide β-D-galacturonic acid-(1→2)-α-D-xylosyl-(1→+), and the trisaccharide α-L-fucosyl-(1→2)-β-D-galactosyluronic acid-(1→2)-α-D-xylosyl-(1→+), to which we have assigned the letters Y and Z, respectively, in addition to neutral subunits (Figures 1A to 1E). We also provide data indicating that a gene (Atg63450), previously referred to as *ROOT HAIR SPECIFIC8* (RHS8) (Won et al., 2009), encodes a CA2y family GT47 (Cantarel et al., 2009) xyloglucan-specific galacturonosyltransferase (XUT1) that forms the β-D-galactosyluronic acid-(1→2)-α-D-xylosyl linkage. An *Arabidopsis* mutant with a loss-of-function *RHS8/XUT1* lacks acidic xyloglucan and has short root hairs, suggesting that this polysaccharide has a key role in normal expansion in these tip-growing cells.

**RESULTS**

**An Acidic Xyloglucan Is Present Only in Arabidopsis Root Hair Cells**

To gain insight into xyloglucan structure and function in root hairs, we first structurally characterized the xyloglucan from the roots of four *Arabidopsis* mutants that overproduce root hairs (*glabrous2* [gt2] [Lee and Schiefelbein, 2001], *transparent testa glabra1* [ttg1] [Galway et al., 1994], *proscutea* [proc] [Desnos et al., 1996], and *werewolf* [wer] [Lee and Schiefelbein, 1999]), two *Arabidopsis* mutants that produce no or few root hairs (*capricene triptychon* [cpc try]) [Schellmann et al., 2002] and *root hair defective6* [rhd6] [Masucci and Schiefelbein, 1994]), and their wild-type progenitors (Columbia-0 [Col-0], Landsberg erecta-0 [Ler-0] and Wassilewskija-2 [Ws-2]; Figure 2A). Somewhat unexpectedly, these analyses revealed the existence of a unique acidic xyloglucan that was present only in plants that form root hairs.

Most of the xyloglucan present in root cell walls is solubilized by 4 N KOH (Peng et al., 2000; Perrin et al., 2003). Thus, the 4 N KOH-soluble xyloglucan from the alcohol-insoluble residue (AIR) of each plant was fragmented with XEG (Pauly et al., 1999) and the mixture of xyloglucan oligosaccharides (XyGOs) generated isolated by size-exclusion chromatography (SEC; see Supplemental Figure 1A online). As expected, the 1H-NMR spectra of the XyGOs from Ler-0, wer, and *cpc try* (Figure 2B) contained signals characteristic of the X, L, and F side chains (Hoffman et al., 2005). However, the Ler-0 and wer spectra contained an additional resonance at δ 5.250 that was assigned, using one- and two-dimensional 1H-NMR spectroscopy and by reference to published data (Peña et al., 2008), to H-1 of α-D-xylopyranosyl (Xylp) residues bearing a β-D-galactopyranosyluronic acid (GalpA) substituent (Figures 3A, 3C, and 4). This signal (δ 5.250; Figure 2B, middle spectrum) was more intense in the wer XyGOs than in the Ler-0 XyGOs, suggesting that the XyGO with this unusual structure originates from the root hair wall. Indeed, the signal at δ 5.250 was absent in the NMR spectrum of the XyGOs of the *cpc try* mutant that lacks root hairs (Figure 2B, bottom spectrum).

Additional evidence that the GalA-containing XyGOs originated from an acidic xyloglucan present in root hair cell walls was obtained by MALDI-TOF MS of the XyGOs from Ws-2 and Col-0 and two mutants (*ttg1* and *proc*) that overproduce root hairs (Figure 2C). All the spectra contained ions corresponding to a subunit that contains a residue with an incremental mass (176 atomic mass units) corresponding to a hexuronosyl residue (Figure 2B) in addition to neutral subunits. The mass of the predominant acidic subunit (mass-to-charge ratio [m/z] 1283) corresponds to the [M – H + 2Na+] ion, which is consistent with its assignment as a hexuronic acid–containing oligosaccharide, since an acidic oligosaccharide can exist as a salt form (Na+–RCOONa). These data together with the results of NMR analyses are consistent with an XXXG core substituted with a single GalA residue. No ions corresponding to this acidic subunit were present in the MALDI-TOF mass spectrum of the XyGOs from the *rhd6* mutant that forms no root hairs (Figure 2C). We conclude that root hair cell walls contain an acidic xyloglucan and that this xyloglucan is not present in the walls of other root cells.

**The Glycosyl Sequence of the Predominant GalA-Containing Subunit**

Partial characterization of the XyGOs from wild-type and root hair mutant plants provides strong evidence that an acidic xyloglucan is present only in root hair walls but does not establish the locations and structures of the GalA-containing side chains. To obtain this information, the acidic XyGOs were isolated (material eluting between 15 and 22 min; see Supplemental Figure 1B online) and then structurally characterized. The MALDI-TOF mass spectrum of these acidic XyGOs (Figure 3E) was dominated by the [M + Na]+ and the [M- H + 2Na+] ions at m/z 1261 and 1283, respectively. These ions support the 1H-NMR
data (Figure 3C) and are consistent with an XXXG core substituted with a GalA residue. As expected, the neutral fraction (material eluting between 31 and 34 min; see Supplemental Figure 1B online) contained the XXXG, XXLG/XLXG, XXFG, and XLFG subunits (Figures 3B and 3D) previously identified in Arabidopsis leaf and root xyloglucan (Perrin et al., 2003).

The glycosyl sequence of the XXXG core substituted with a GalA residue (Figure 1D) was established by one- and two-dimensional 1H-NMR spectroscopy (Figure 4; see Supplemental Table 1 online). The two-dimensional gradient correlation spectroscopy (gCOSY) spectrum of the root acidic XyGOs (Figure 4) contained the resonances of an isolated spin system that was identified as a nonreducing terminal \( \beta - \text{Gal}A \) residue. Interglycosidic cross-peaks in the nuclear Overhauser effect spectroscopy (NOESY) spectrum indicated that the \( \beta - \text{Gal}A \) residue is linked to O-2 of an \( \alpha - \text{D-Xyl}p \) residue, which itself is linked to O-6 of a glucopyranosyl (Glc) residue (see Supplemental Table 1 online). We assigned the letter \( Y \) to this acidic side chain \( [\beta - \text{Gal}A-(1 \rightarrow 2) - \alpha - \text{D-Xyl}p-(1 \rightarrow 6) - \beta - \text{D-Glc}p; \) Figures 1D and 1E].

The low intensity of the signal at \( \delta 4.94 \) (indicated by the unlabeled bold arrow in Figure 3C), which is diagnostic of a terminal \( \alpha - \text{D-Xyl}p \) residue linked to O-6 of the Glc residue at the nonreducing end of a XyGO (Hoffman et al., 2005), together with an analysis of scalar and dipolar interactions observed in the two-dimensional spectra suggested that the \( Y \) side chain is located predominantly at the nonreducing end of the XyGO (Figures 1D and 1E). To confirm the position of this \( Y \) side chain, we used the xyloglucan reducing end–specific cellobiohydrolase (OXG-RCBH) from Geotrichum (Yaoi and Mitsuishi, 2002). This enzyme releases substituted cellobiosyl units from the reducing end of XyGOs that have no Glc attached to the middle Xyl. Thus, XXLG is hydrolyzed to give XX and LG, whereas XLXG is not hydrolyzed at all. The oligosaccharide containing the \( Y \) side chain (\( m/z 1261 \) and 1283; Figure 5A) was completely hydrolyzed by OXG-RCBH to fragments (\( m/z \) 497 [XG + Na]+, 805 [YX + Na]+, and 827 [YX \( H + 2 \text{Na}]+; \) Figure 5B), which are the products expected when YXXG is hydrolyzed by the cellobiohydrolase. Thus, we conclude that YXXG is the major acidic subunit in the xyloglucan (Figure 1D).
α-L-Fuc Is Linked to Some of the GalA Residues

Two-dimensional NMR spectroscopic analyses of the acidic XyGOs indicated the presence of lower abundance XyGOs that contain another acidic side chain. The gCOSY (Figure 4) and total correlation spectroscopy spectra contained resonances (see Supplemental Table 1 online) of three isolated spin systems that were assigned to a terminal α-L-fucopyranosyl (Fucp), a 2-linked β-D-GalpA, and a 2-linked α-D-Xylp residue. Cross-peaks in the NOESY spectra indicated that the side chain, to which we assigned the letter Z, has the sequence α-L-Fucp-(1→2)-β-D-GalpA-(1→2)-α-D-Xylp-(1→ (Figure 1F). This NMR analysis is in agreement with the MALDI-TOF MS data, which also indicated the presence of subunits containing the Z side chain (Figure 3E).

The low-intensity ions corresponding to XXXG substituted with GalA and Fuc (m/z 1407 and 1429), with Gal and GalA (m/z 1445), and with two GalAs (m/z 1459 and 1481) disappeared upon treatment with OXG-RCBH (Figure 5B). Thus, none of these subunits contain a substituent on the middle Xyl. On the basis of this result and the masses of the OXG-RCBH-generated fragments, we propose the sequences XXZG (m/z 1407 and 1429), YXLG (m/z 1445), and YXYG (m/z 1459 and 1481). The nearly complete disappearance of the ions at m/z 1569 and 1591 upon OXG-RCBH treatment suggests that YXFG is much more abundant in the mixture than its isomer XLZG, which is resistant to hydrolysis by OXG-RCBH. Together, these spectroscopic data provide evidence that the root hair acidic xyloglucan contains small amounts of the YXFG, XXZG, YXYG, and YXLG subunits (Figures 1E and 1F).

The Root Hair Acidic Xyloglucan Contains Acidic and Neutral Subunits

Spectroscopic characterization of the products formed by XEG and OXG-RCBH demonstrates the existence of both acidic and neutral XyGO subunits but does not establish if these subunits are linked to one another or if root hair walls contain a xyloglucan composed entirely of acidic subunits. Thus, the 4 n KOH-soluble material from Col-0 root AIR was fractionated on a Q-Sepharose anion-exchange column to obtain neutral and acidic xyloglucan. Most (85%) of the xyloglucan did not bind to the column and was composed exclusively of neutral subunits (XXXG, XXLG, and XLFG subunits (Figures 1A to 1C). The acidic subunits account for at least 30% of the total subunits present in the acidic xyloglucan.

We next generated a fraction enriched in acidic XyGOs with an XXXGXXXG core from Ler-0 root xyloglucan to determine if there
are blocks of consecutive acidic subunits or if acidic and neutral subunits are linked to one another. Such S2 fragments are formed in small amounts by incomplete XEG fragmentation of xyloglucan. The MALDI-TOF mass spectrum of the S2-enriched fraction (Figure 7A) contained an abundant ion at \( m/z \) 2635 that corresponds to an S2 core with one GalA, one Gal, and one Fuc residue. XEG treatment showed that this S2 oligosaccharide is composed of one YXG and one XFG subunit (Figure 7B). The removal of XG from the reducing end of the oligosaccharide upon OXG-RCBH treatment (\( m/z \) 2179; Figure 7C) established the sequence XFGYXXG. Small amounts of S2 structures composed of combinations of XXYG, XXZG, XLFG, and YXFG also likely exist (Figure 7B), although no unique glycosyl sequences could be deduced. No evidence was obtained for XyGOs composed of two acidic S1 subunits, suggesting that blocks of acidic subunits are rare or do not exist in Arabidopsis root hair cell xyloglucan.

**Plants Carrying a Loss-of-Function Mutation in At1g63450 Lack Acidic Xyloglucan and Have Shorter Root Hairs Than Those of the Wild Type**

The well-characterized *MURUS3* (*MUR3*) and *XYLOGLUCAN L-SIDE CHAIN GALACTOSYLTRANSFERASE POSITION2* (*XLT2*) genes encode family GT47 glycosyltransferases that catalyze the regiospecific transfer of a \( \beta-\)D-Galp residue to O-2 of the \( \alpha-\)D-Xylp side chains of xyloglucan to form the L side chain (Madson et al., 2003; Jensen et al., 2012). This L side chain is structurally homologous to the acidic Y side chain described herein (cf. Figures 1D to 1F). A BLAST search allowed us to identify the Arabidopsis locus At1g63450 as a gene encoding a MUR3 homolog. This gene encodes a protein with 664 amino acids that shares \( \approx \)39% amino acid sequence identity and \( \approx \)64% sequence similarity with MUR3 and \( \approx \)38% amino acid sequence identity and \( \approx \)60% sequence similarity with XLT2 (see Supplemental Figure 2 online). Transcript analyses for At1g63450 (Brady et al., 2007; Won et al., 2009; Lin et al., 2011; Bruex et al., 2012) have shown that this gene is expressed specifically in root hairs (see Supplemental...
Moreover, a study of the Arabidopsis root proteome has shown that the protein encoded by the At1g63450 locus is present only in root hairs (Petricka et al., 2012). Together, these data led us to hypothesize that At1g63450 encodes a glycosyltransferase that catalyzes the transfer of β-D-GalA residues to O-2 of the α-D-Xyl side chains of root hair xyloglucan. Based on evidence described in the following paragraphs, we named this gene XUT1.

We detected no T-DNA inserts in XUT1 in the two lines annotated as SALK_039544 and SALK_028024 available from the Salk Insertion Sequence Database (Alonso et al., 2003). However, we did identify an Arabidopsis gene trap line (GT11805) in the Cold Spring Harbor collection (Sundaresan et al., 1995) that interrupts the XUT gene locus. This mutant has a Ds insert located 69 bp downstream of the predicted XUT1 start codon (Figure 8A). No full-length transcripts corresponding to XUT1 were obtained by RT-PCR using total RNA prepared from homozygous xut1 mutant plant roots (Figure 8B), indicating this line is a null allele of XUT1.

The aerial portions of xut1 and Ler-0 plants were visibly indistinguishable. However, the root hairs of the xut1 mutant were shorter than their wild-type counterparts when the seedlings were grown on nutrient agar (Figures 8C and 8D). The growth rate of the mutant root hairs was decreased by ~50% compared with wild-type hairs (Figure 8D). The short root hair phenotype was rescued when xut1 was transformed with a wild-type copy of the XUT1 gene driven by its native regulatory and promoter regions 2020 bp upstream of the start codon (Figure 8C). 1H-NMR spectroscopy established that the xut1 mutant root xyloglucan lacks subunits that contain β-α-GalA (Figure 9A). The β-α-GalA-containing subunits are rescued in the XyGOS isolated from the roots of complemented xut1 (Figure 9B). These data provide evidence that XUT1 is required for the addition of GalA to root hair xyloglucan. Although our data do not eliminate the possibility that the xut1 mutation affects the structure of other cell wall polysaccharides, including rhamnogalacturonan II, that are known to contain a β-linked GalA (O’Neill and York, 2003), they are consistent with the acidic xyloglucan having a key role in the normal growth of root hairs.

Expressing Recombinant XUT1 in Leaves and Stems Results in the Synthesis of Acidic Xyloglucan

We were unable to establish an in vitro root microsome assay to demonstrate transfer of GalA from UDP-GalA onto xyloglucan or...
XyGO acceptors. As no acidic xyloglucan is discernible in the cell walls of Col-0 leaves (Figure 10A; see Supplemental Figure 4A online) or stems (see Supplemental Figure 4C online), we obtained evidence for xyloglucan-specific GalA transferase activity by overexpressing XUT1 under the control of a 35S promoter in Col-0 (see Supplemental Figure 5 online).

The β-D-GalA–containing YXXG subunit was shown by 1H NMR (Figure 10B) and MALDI-TOF MS (see Supplemental Figures 4B and 4D online) analyses to be present in the XyGOs generated from 35Spro:XUT1 leaf xyloglucan. We also identified small amounts of the subunits that contain the Z side chain as well as S2 subunits with the glycosyl sequences XXFGYXXG and XLFGYXXG in the XyGOs generated from 35Spro:XUT1 leaf and stem xyloglucan (see Supplemental Figure 6 online). The presence of acidic xyloglucan in the leaf and stem cell walls had no discernible effect on the growth and development of 35Spro:XUT1 plants. Moreover, the number and length of root hairs of 35Spro:XUT1 and wild-type plants were also comparable. Based on these data, we propose that At1g63450 encodes a xyloglucan-specific galacturonosyltransferase (XUT1) that catalyzes the formation of the β-D-GalA-(1→2)-α-D-Xyl linkag.

Arabidopsis fut1 Mutant Acidic Xyloglucan Lacks the α-L-Fuc-(1→2)-β-D-Gal Linkage

Arabidopsis FUT1 is a xyloglucan-specific fucosyltransferase that catalyzes the formation of the α-L-Fuc-(1→2)-β-D-Gal linkage (Perrin et al., 1999). To determine if this transferase is involved in the formation of the acidic Z side chain, we structurally characterized the acidic XyGOs generated from the root cell wall xyloglucan of the fut1-3 mutant. The root hairs of this mutant and wild-type plants have comparable morphologies. Signals corresponding to the fucosyl residues of acidic side chains were readily discernible in the gCOSY spectrum of the XyGOs from wild-type roots (Figure 11A). By contrast, these signals were absent in the corresponding spectrum of acidic XyGOs generated from fut1 root xyloglucan (Figure 11B). The MALDI-TOF mass spectrum of the fut1 acidic XyGOs confirmed that none of the subunits were fucosylated (see Supplemental Figure 7A online). Together, these data suggest that FUT1 fucosylates the GalA residue present in the acidic xyloglucan of Arabidopsis root hair cell walls.

XUT1 Orthologs Exist in Other Land Plants

XUT1 clusters with several other GT47 proteins from selected dicots (see Supplemental Figure 8 and Supplemental Data Set 1 online). These proteins as well as proteins from other dicots cataloged in the Phytozome database (www.phytozome.net) have between 60 and 84% sequence identity and 75 and 91% similarity with XUT1. Thus, other dicots may contain xyloglucan-specific GalA transferases. The currently available genomic data indicate that grasses contain proteins with an overall relatively low sequence identity (~40%) and similarity (~60%) with XUT1. Many of these proteins have higher amino acid sequence similarity (~53%) and identity (~70%) with MUR3, suggesting they are xyloglucan-specific galactosyltransferases. XUT1 also clusters with several GT47 proteins from the lycophyte Selaginella moellendorffii and from the moss Physcomitrella patens (see Supplemental Figure 8 online). These proteins have ~48% sequence identity and 66% sequence similarity with XUT1. P. patens is known to synthesize xyloglucan that contains GalA (Peña et al., 2008); thus, the possibility cannot be discounted that some of these genes have the same function as XUT1.

DISCUSSION

In this study, we have shown that root tissue cell walls contain two types of xyloglucan: a fucogalactoxyloglucan composed of neutral subunits and a previously unidentified xyloglucan composed of neutral and acidic subunits. In Arabidopsis, the acidic
xyloglucan is only detected in root hair cell walls and has at least two different GalA-containing side chain structures (Figures 1D to 1F). Our identification of a mutant unable to add GalA to root xyloglucan and the demonstration of GalA-containing xyloglucan upon overexpression of XUT1 in non-root tissue provides strong evidence that At1g63450 encodes a GalA transferase (XUT1), which catalyzes the addition of \( \beta-D\text{-G}al\) at \( O-2 \) of the \( \alpha-D\text{-Xyl} \) substituents of xyloglucan. In the absence of this acidic xyloglucan, root hairs are shorter than their wild-type counterparts. XUT1 Is Involved in the Synthesis of the Acidic Side Chains of Xyloglucan

XUT1 encodes a protein that is a member of subgroup A of family GT47 (Li et al., 2004), which includes the xyloglucan-specific \( \beta-(1\rightarrow2)-D\text{-galactosyltransferases} \) MUR3 (Madson et al., 2003) and XLT2 (Jensen et al., 2012). MUR3 and XLT2 catalyze the regiospecific transfer of Gal from UDP-Gal to \( O-2 \) of the Xyl adjacent to the unbranched Glc of the backbone and to \( O-2 \) of the second Xyl residue after the unbranched Glc, respectively. The glycosyl linkages formed by MUR3 and XLT2 (\( \beta-D\text{-Gal-1}\rightarrow2-\alpha-D\text{-Xyl} \)) and by XUT1 (\( \beta-D\text{-GalA-1}\rightarrow2-\alpha-D\text{-Xyl} \)) are structurally homologous; thus, it is not unexpected that an enzyme related to MUR3 and XLT2 catalyzes the transfer of GalA residues in root hair xyloglucan.

Xyloglucans containing \( \beta-D\text{-GalA} \) residues have been identified in \( P. patens \) (Peña et al., 2008), which contains several cell types that expand by tip growth (Eklund et al., 2010). However, the GalA-containing side chains of moss and \( Arabidopsis \) root hair xyloglucans are structurally distinct. In \( P. patens \), a terminal GalA is linked to \( O-2 \) of a Xyl that has a \( \beta-D\text{-Gal} \) substituent at \( O-4 \) (P side chain). The GalA is itself substituted at \( O-2 \) with a \( \beta-D\text{-Gal} \) in the Q side chain (Peña et al., 2008). No acidic subunits have been identified in the xyloglucan present in the walls of tippgrowing \( Arabidopsis \) pollen tubes (Dardelle et al., 2010). However, some of the xyloglucan in these walls is highly \( O\)-acylated and may have a role in tip growth. Such data support the proposal that

Figure 8. A Loss-of-Function Mutation in a Gene (At1g63450) Encoding a Putative Xyloglucan-Specific Galactosyluronic Acid Transferase (XUT1) Results in Short Root Hairs.

(A) Gene structure of the intronless At1g63450 locus. The exon and untranslated regions are shown in black and white, respectively. The gene trap Dissociation-glucuronidase (Ds-GUS) element insertion (inverted triangle) is located 69 bp downstream of the predicted ATG start codon. (B) RT-PCR analysis of XUT1 transcript in roots from Ler-0 and xut1 plants. Actin2 was used as a control. The experiment was conducted on three different pools of 10-d-old seedlings (15 to 20 seedlings per pool) from each line, and the typical result of an ethidium bromide–stained agarose gel is shown. WT, the wild type. (C) The root hair phenotypes of Ler-0, xut1, and complemented xut1 seedlings grown on nutrient agar. Bar = 1 mm. (D) Root hair lengths of Ler-0 and xut1 seedlings grown on nutrient agar. The values reported are the means ± se obtained from 160 to 300 root hairs of five to 10 seedlings. The slopes (y) indicate that the growth rate of the mutant root hairs is decreased by ~50% compared with that of wild-type root hairs.

Figure 9. The xut1 Mutant Xyloglucan Lacks Acidic Subunits.

The \( 4\% \) KOH-soluble material from the root AIR of Ler-0 and xut1 plants grown in liquid culture was treated with XEG, and the XyGOs formed were isolated by SEC. The anomeric region of the \( ^1H\text{-NMR} \) spectrum of the XyGOs generated from xut1 root xyloglucan (A) and from complemented xut1 root xyloglucan (B). The resonances corresponding to the GalA residue are not discernible in the spectrum of the xut1 mutant but are present in the xut1 complemented XyGOs.
differences in the branching patterns, side chain structures, and O-acetylation of xyloglucans provide plants with alternative mechanisms to modulate xyloglucan–cellulose interactions (Peña et al., 2008).

A protein, referred to as glycosyltransferase 16 (GT16) with no known function was identified as a MUR3 homolog in a previous study (Li et al., 2004). This protein and XUT1 have the same amino acid sequences (see Supplemental Figure 2 online). In the same study, two additional MUR3 homologs (GT18 and GT20) were also identified. GT18 is the xyloglucan-specific galactosyltransferase (XLT2) that adds a β-D-Gal to the middle Xyl (Jensen et al., 2012). GT20, XUT1, MUR3, and XLT2 all have a predicted cytosolic domain, a transmembrane domain, a variable domain, and a large conserved C-terminal domain that includes the exostosin domain (see Supplemental Figure 9A online). The conserved C-terminal domain that likely harbors the catalytic regions of GT20 and XUT1 have 73% sequence identity but have a considerably lower sequence identity (38 to 40%) with the corresponding regions of MUR3 and XLT2 (see Supplemental Figures 9B to 9D online). XUT1 and GT20 differ predominantly in the length of the variable region between the putative transmembrane and catalytic domains (see Supplemental Figure 10 online). XUT1 contains 191 amino acids and GT20 contains 128 amino acids with 30% sequence identity in this region. It is not known if these regions are required for enzymatic activity or serve another function. Irrespective of the function of this variable region, GT20 is unlikely to be a root hair xyloglucan-specific GaIA transferase, as no acidic xyloglucan is formed by the xut1 mutant. Nevertheless, the possibility cannot be discounted that GT20 catalyzes the formation of a β-GaIA linkage or an as yet unidentified linkage in a xyloglucan located in the cell walls of tissues other than roots, leaves, and stems or that GT20 catalyzes the formation of a β-GaIA linkage in another glycan.

The GalA residue is linked to at least two different xylosyl residues of the acidic subunits from Arabidopsis root hair xyloglucan (Figures 1D and 1E). Thus, we expected that different regiospecific GaIA transferases would be required for the synthesis of these acidic side chains. However, no GaIA-containing side chains were detected in the xyloglucan synthesized by the xut1 mutant. The Y and Z side chains were restored to levels comparable with the wild type by complementing xut1 with XUT1. The presence of both the Y and Z side chains in the acidic xyloglucan present in the stem and leaf cell walls of 35Spro:XUT1 plants also supports the notion that XUT1 catalyzes the formation of both GaIA linkages. Nevertheless, the possibility cannot be discounted that a second GaIA transferase exists, for example, GT20, but its activity requires the presence of a functional XUT1.

Figure 10. The Leaf Xyloglucan from 35Spro:XUT1 Col-0 Contains β-Linked GaIA.

The 4 n KOH-soluble materials from the leaves of 6-week-old Col-0 and 35Spro:XUT1 Col-0 plants grown in soil were treated with XEG and the XyGOs formed isolated by SEC. The anomeric region of the 1H-NMR spectrum of the XyGOs generated from Col-0 leaf xyloglucan (A) and from 35Spro:XUT1 Col-0 leaf xyloglucan (B). Resonances corresponding to acidic XyGOs are present only in the leaf XyGO generated form 35Spro:XUT1 plants.

Figure 11. Arabidopsis fut1 Mutant Root Xyloglucan Lacks Fucosylated Acidic Subunits.

The 4 n KOH-soluble material from the root AIR of Col-0 and Fut1 plants grown in liquid culture was treated with XEG and the XyGOs formed isolated by SEC. Portion of the gCOSY 1H-NMR spectrum of the acidic XyGOs generated from the root xyloglucan of Col-0 plants (A) and from the root xyloglucan of fut1-3 plants (B).
Most of the β-ol-GalpA in Arabidopsis root hair acidic xyloglucan is linked to the third xylosyl residue (XyXG) from the unbranched Glc in the backbone (Figure 1D). This xylosyl residue is rarely if ever substituted in other XyXG-type xyloglucans (Hoffman et al., 2005). The remaining GalpA is linked to the first xylosyl residue, and this is the only GalpA residue that is fucosylated (XXZG). Our results show that FUT1, the fucosyltransferase that adds a fucosyl residue to one of the Gal residues of xyloglucan in the cell walls of Arabidopsis aerial tissues (Perrin et al., 1999, 2003), is also responsible for the fucosylation of the GalpA in root hair xyloglucan. Interestingly, FUT1 can use GDP-β-Gal (a structural homolog of GDP-β-Fuc) as a sugar donor in xyloglucan biosynthesis (Zablackis et al., 1996). Thus, FUT1 may be much less specific than previously believed.

The Role of Xyloglucan in Root Hair Growth

The mechanisms underlying wall expansion in growing plant cells remain a subject of debate. Many wall models predict that xyloglucan interacts with cellulose microfibrils and with other cell wall components and that modification of these interactions during cell growth affects the orientation and distribution of cellulose microfibrils that ultimately control wall expansion (Thompson, 2005; Burgert and Fratzl, 2007; Szymanski and Cosgrove, 2009; Anderson et al., 2010; Winship et al., 2010; Dick-Pérez et al., 2011). Xyloglucan and other wall components have been proposed to be spacers that hold the cellulose microfibrils apart rather than tethers that cross-link them (Thompson, 2005). Alternatively, xyloglucan may be a tether in the longitudinal direction and a spacer in the lateral direction (Burgert and Fratzl, 2007). Interestingly, wall extensibility is increased in the Arabidopsis xxt1 xxt2 mutant that lacks xyloglucan, which led to the suggestion that xyloglucan increases wall stiffness, although this may not involve direct cross-linking of cellulose microfibrils (Park and Cosgrove, 2012).

Irrespective of the function of xyloglucan in the walls of growing plant cells, it is likely that the properties of neutral and acidic xyloglucans are different and that such differences will affect the interactions between xyloglucan and other wall components, including cellulose. Indeed, acidic xyloglucan generated in vitro by a combination of galactose oxidase and iodine treatments does not interact as strongly with cellulose when compared with neutral xyloglucan (Parikka et al., 2012). Thus, we propose that the presence of the acidic Y and Z side chains will affect the interactions between xyloglucan and cellulose microfibrils in the expanding tip wall of root hairs.

On the basis of our data and current knowledge of wall composition and organization in tip and diffuse growing cells (Emons and Ketelaar, 2009; Maris et al., 2009; Nielsen, 2009; Akkerman et al., 2012), we suggest that the acidic xyloglucan together with other wall components maintain the separation and/or random orientation of the cellulose microfibrils at the growing tip. This facilitates locally isotropic wall expansion in this region. In the xut1 mutant, reduced root hair tip growth is thus a consequence of the more ordered cellulose microfibrils in the absence of acidic xyloglucan.

Cellulose microfibrils have been shown to be more highly ordered along the side walls of the root hair than in the growing tip (Akkerman et al., 2012). We propose that these regions are enriched in neutral xyloglucan and contribute to the formation of the secondary wall that is deposited on the cytoplasmic side of the primary wall (Nielsen, 2009). This secondary wall limits growth by restricting both lateral and longitudinal extension of the root hair wall and thereby limits growth in that direction (Emons and Ketelaar, 2009; Akkerman et al., 2012). The mechanical strength of the side walls may also be increased by XTH-catalyzed restructuring of the xyloglucan (Maris et al., 2009). Two XTH genes, XTH12 and XTH14, are preferentially expressed in root hair cells and the abundance of their transcripts is reduced in the Arabidopsis cpc try mutant (Breux et al., 2012). This mutant does not form root hairs or synthesize acidic xyloglucan (Figure 2). Moreover, Arabidopsis mutants that produce no, or reduced amounts of, xyloglucan have root hairs that do not grow normally (Cavalier et al., 2008; Zabotina et al., 2008, 2012). Such results are in agreement with the proposal that root hair growth is dependent on cellulose–xyloglucan interactions in both the primary and secondary cell wall layers (Galway et al., 2011). Root hair growth has been reported to be correlated with extracellular pH oscillations that can modulate the activity of proteins, including expansins (Monshausen et al., 2007) and XTHs (Maris et al., 2009), which are involved in wall restructuring and wall expansion. Thus, root hair growth may involve cycles of wall extension and wall reinforcement with structurally distinct xyloglucans participating in each of these phases.

This study has demonstrated that the walls of the root hair contain a structurally unique acidic xyloglucan that appears to be required for normal tip growth. Other instances where specific polysaccharide structures are correlated with particular biological functions in vivo will likely be revealed by the development of sensitive techniques for structurally characterizing glycans (Mulloy et al., 2009) and the availability of new tools for localizing specific cell wall components (Lee et al., 2011). Such studies are necessary to resolve the long-standing enigma regarding the complexity and structural diversity of primary cell walls in land plants.

METHODS

Arabidopsis thaliana Accessions and Mutant Lines

Arabidopsis (Col-0), fut1-3 (Salk_139678), and prc1c seeds were obtained from the ABRC at Ohio State University. Homozygous fut1-3 was identified by PCR. Seeds of cpc try, gl2, roh6, wer, and ttg1 were obtained from John Schiefelbein (University of Michigan). Arabidopsis (Ler-0 and Wass) seeds were obtained from LeHe Seeds.

The Arabidopsis gene trap line GT11805 containing the Ds insert in XUT1 was obtained from Trapper DB (Cold Spring Harbor Laboratory). Homozygous insertion lines of xut1 were identified by PCR with primer sequences (GT11805-F and GT11805-R) flanking the genomic T-DNA insert and with a T-DNA–specific (DS3-1) primer (see Supplemental Table 2 online). DNA sequencing of PCR-amplified fragments that spanned the insertion site established the position of the T-DNA insertion in the gene (Figure 8A). RT-PCR with primer sets along the 3′, middle, and 5′ regions of At1g63450 verified that no transcript was present in the homozygous mutant lines.

Growth of Plants

Surface-sterilized seeds were grown in liquid culture using six-well polystyrene culture plates (Corning). Each well contained one seed in Gamborgs B5 medium (5 mL; Sigma-Aldrich), pH 5.8, supplemented with...
1% Suc and 0.05% MES. The plates were sealed with micropore surgical tape (3M Health Care) to prevent evaporation of the growth media. The seeds were germinated and grown under continuous light at 22°C with shaking (70 rpm). Plants were collected after 14 to 16 d, washed with deionized water, and manually dissected to obtain root tissue that was then used to prepare cell walls.

To obtain soil-grown plants, surface-sterilized Arabidopsis seeds were first stratified for 48 h at 4°C and then germinated on 0.5× Murashige and Skoog medium (Sigma-Aldrich) containing 1% agar and 1% Suc. The plates were sealed with micropore surgical chamber (16 h light, 120 µmol m⁻² s⁻¹ and 8 h dark) at 19°C (light) and 15°C (dark). After 10 d, seedlings were transferred to soil and grown under the same conditions.

Root hair phenotypes were determined using stratified seeds germinated and grown on nutrient agar (Schieltelbein and Somerville, 1990). Root hairs were photographed with a stereoscopic microscope (Olympus SZH-ILLD) equipped with a Nikon D-RiI camera and their lengths measured using NIS-Elements Basic Research software. The values reported are the means ± SE obtained from 160 to 300 root hairs of five to 10 seedlings.

Plasmid Construction and Plant Transformation

To complement xut1, a 4595-bp genomic DNA fragment containing intronless XUT1 (including a 2020-bp 5’ upstream region, the 1995-bp exon sequence, and a 580-bp 3’ downstream region) was amplified by PCR using a pfX50 DNA polymerase (Invitrogen) with the gene-specific oligonucleotides XUT1-COMP-F and XUT1-COMP-R (see Supplemental Table 2 online) using Col-0 genomic DNA as the template. The amplified fragment was cloned into the vector pCam35tl:egfps2#4 (Pattathil et al., 2005) and confirmed by sequencing. To overexpress XUT1 in Col-0, the XUT1 coding sequence was PCR amplified with pfX50 DNA polymerase using primers XUT1-OE-F and XUT1-OE-R (see Supplemental Table 2 online). The coding sequence was inserted downstream of the cauliflower mosaic virus 35S promoter in the pCam35tl:egfps2#4 vector. The constructs harboring the genomic region or the XUT1 coding sequence were transformed into Agrobacterium tumefaciens strain GV3101, which were then used to transform (Clough and Bent, 1998) the xut1 mutant or Col-0, respectively. Transgenic seeds were collected and germinated on agar supplemented with 0.5× Murashige and Skoog medium containing hydrogymcin (15 mg/L). Plants that survived the antibiotic selection were enumerated, and the presence of the transgenes was confirmed by PCR using the gene-specific primer XUT1-F-910 paired with the pCambia35tl: egfps2#4 vector primer P1300-R (see Supplemental Table 2 online). Six independent xut1 mutant lines transformed with the wild-type 4595-bp genomic region of XUT1 were examined for genetic and chemotypic complementation. Three independent 35S:pro:XUT1 lines were examined for the presence of the acidic xyloglucan.

Preparation of Alcohol Insoluble Residues and Isolation of Xyloglucan

Cell walls from root, leaf, and stem tissues were prepared as their AIRs as described (Peña et al., 2008). The AIR (200 to 500 mg) was then treated at room temperature, with 50 mM ammonium oxalate (20 mL, 16 h), with 1 n KOH (10 mL, 16 h) containing 0.5% (w/v) NaBH₄, and finally with 4 n KOH (10 mL, 16 h) containing 0.5% (w/v) NaBH₄. The alkali extracts were adjusted to pH 5.0 with glacial acetic acid, dialyzed (3.5-kD molecular weight cutoff) against deionized water and then freeze dried.

XEG Treatment

Solutions of the 4 n KOH-soluble material (1 to 10 mg) in 50 mM ammonium formate, pH 5, were treated with XEG (Novozymes) as described (Pauly et al., 1999). The mixture of acidic and neutral XyGOs formed was then isolated by SEC on a Superdex-75 HR10/30 SEC column (GE Healthcare) using a Dionex Ultimate 3000 HPLC equipped with a Shodex RI-101 refractive index detector. The column was eluted with 50 mM ammonium formate, pH 5, and the XyGO-containing fractions collected manually (see Supplemental Figure 1A online) and then freeze dried. Acidic and neutral XyGOs were separated by SEC on a Superdex 75 HR10/30 column eluted with water at 0.5 mL/min (Peña et al., 2008) (see Supplemental Figure 1B online). Fractions were collected manually and freeze dried.

Anion-Exchange Chromatography of the 4 n KOH-Soluble Material

A solution of the 4 n KOH-soluble material (~5 mg) from Col-0 AIR in 10 mM imidazole-HCl, pH 7.0 (1 mL), was applied to a Q-Sepharose fast-flow anion-exchange column (5 mL volume) equilibrated in 10 mM imidazole-HCl, pH 7. Column was washed with 10 mM imidazole-HCl, pH 7.0 (15 mL), and then with 1 M imidazole-HCl, pH 7.0 (15 mL), by gravity flow. The 10 mM and 1 M fractions were separately dialyzed against water (6- to 8.5-kD molecular weight cutoff) and then freeze dried. Portions (~0.5 mg) of each fraction were treated with XEG, and the resulting XyGOs were analyzed using MALDI-TOF MS.

Oligoxyloglucan Reducing End-Specific Cellobiohydrolase Treatment

Solutions of XyGOs (5 to 10 μg) in water (10 μL) were treated for 4 h at 22°C with 2 mM Units of OXG-RCBH (Yaoi and Mitsuishi, 2002) and the products formed then analyzed by MALDI-TOF MS. One unit of OXG-RCBH releases 1 μmol of glucose/min as reducing sugar from XXXG. This enzyme releases substituted cellobiosyl units from the reducing end of XyGOs that have no Glc attached to the middle Xyl. Thus, XXLG is hydrolyzed to give XX and LG, whereas XLXG is not hydrolyzed at all.

MALDI-TOF MS

MALDI-TOF MS was performed with a Bruker LT Microflex spectrometer operating in the positive ion mode. A portion (2 μL) of XyGOs (1 mg/mL in water) was mixed with an equal volume of 10 mM NaCl, and 1 μL of the mixture was added to dihydroxybenzoic acid (1 μL, 10 mg/mL in aqueous 50% acetonitrile) on the MALDI target plate. This mixture was concentrated to dryness under a flow of warm air. Data from at least 200 laser pulses were accumulated and averaged for each spectrum.

1H-NMR Spectroscopy

1H-NMR spectra of XyGOs (0.1 to 1 mg in D2O, 0.2 mL, 99.9%; Cambridge Isotope Laboratories) were recorded at 298K with a Varian Inova NMR spectrometer operating at 600 MHz for protons and equipped with...
Phylogenetic Analysis

Sequences were downloaded from Phytozome (http://www.phytozome.net). A local BLAST was performed using the Arabidopsis XUT1 protein as the query for the identification of the homologous genes from Physcomitrella patens, Selaginella moellendorffii, Glycine max, Cucumis sativus, Populus trichocarpa, and Onza sativa. Manual reannotation, if required, was performed using FGENESH (http://linux1.softberry.com/berry.pt.html). Multiple sequence alignments of the full-length protein sequences were performed using Clustal Omega (Sievers et al., 2011). The unrooted phylogenetic tree was constructed with MEGA 5.0 (Tamura et al., 2011) using the neighbor-joining method, a bootstrap test with 1000 replicates, the Poisson substitution model, uniform rates, and pairwise deletion.

Accession Numbers

The Arabidopsis Genome Initiative locus identifiers are as follows: At–XUT1, At1g63450.1; At–MUR3, At2g20370.1; At–GT18/At–XL72, At5g52220.1; At–GT20, At5g41250.1; At–ACTIN2, At3g18780.1; At–FUT1, At2g02320.1; At–PRC, At5g64740.1; At–GL2, At1g79840.1; At–RHD6, At1g66470.1; At–CPC, At2g46410.1; At–TRY, At5g53200.1; At–WER, At5g14750.1; and At–TTG1, At5g24520.1.

Supplemental Data

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

Supplemental Figure 1. Isolation of Xyloglucan Oligosaccharides by Size-Exclusion Chromatography.

Supplemental Figure 2. Amino Acid Sequence Alignments between XUT1, MUR3, XL72, and GT20 Proteins.

Supplemental Figure 3. Relative Levels of Expression in Root Cells of Selected Genes Encoding Glycosyltransferases Involved in Xyloglucan Biosynthesis.

Supplemental Figure 4. Galacturonic Acid–containing Subunits Are Present in the Xyloglucan Isolated from the Leaf and Stem Cell Walls of 35S–XUT1 Col-0.

Supplemental Figure 5. RT-PCR Analysis of XUT1 Transcripts in the Leaves and Stems of 35S–XUT1 Plants.

Supplemental Figure 6. The Glycosyl Sequences of the Two Major Acidic S5 Xyloglucan Oligosaccharides Generated from the Xyloglucan in the Stems of 35S–XUT1 Col-0.

Supplemental Figure 7. The Acidic Xyloglucan Oligosaccharides Generated from fut1 Root Xyloglucan Lack Fucosyl Residues.

Supplemental Figure 8. Unrooted, Bootstrapped Tree of Selected GT47 Proteins.

Supplemental Figure 9. The Domain Structure of XUT1, GT20, MUR3, and XL72.

Supplemental Figure 10. Amino Acid Alignments of the Region between the Putative Transmembrane and Catalytic Domains of XUT1 and GT20.

Supplemental Table 1. 1H-NMR Resonance Assignments for the Acidic Xyloglucan Oligosaccharides.

Supplemental Table 2. List of Primers Used in This Study.

Supplemental Data Set 1. Alignments Used to Generate the Phylogeny Presented in Supplemental Figure 8.

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AUTHOR CONTRIBUTIONS


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REFERENCES


Acidic Xyloglucan and Root Hair Growth


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