AtBGAL10 Is the Main Xyloglucan β-Galactosidase in Arabidopsis, and Its Absence Results in Unusual Xyloglucan Subunits and Growth Defects1[W][OA]

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In growing cells, xyloglucan is thought to connect cellulose microfibrils and regulate their separation during wall extension. In Arabidopsis (Arabidopsis thaliana), a significant proportion of xyloglucan side chains contain β-galactose linked to α-xylose at O2. In this work, we identified AtBGAL10 (At5g63810) as the gene responsible for the majority of β-galactosidase activity against xyloglucan. Xyloglucan from bgal10 insertion mutants was found to contain a large proportion of unusual subunits, such as GLG and GLLG. These subunits were not detected in a bgal10 xyl1 double mutant, deficient in both β-galactosidase and α-xylosidase. Xyloglucan from bgal10 xyl1 plants was enriched instead in XXLG/XLXG and XLLG subunits. In both cases, changes in xyloglucan composition were larger in the endoglucanase-accessible fraction. These results suggest that glycosidases acting on nonreducing ends digest large amounts of xyloglucan in wild-type plants, while plants deficient in any of these activities accumulate partly digested subunits. In both bgal10 and bgal10 xyl1, siliques and sepals were shorter, a phenotype that could be explained by an excess of nonreducing ends leading to a reinforced xyloglucan network. Additionally, AtBGAL10 expression was examined with a promoter-reporter construct. Expression was high in many cell types undergoing wall extension or remodeling, such as young stems, abscission zones, or developing vasculature, showing good correlation with α-xylosidase expression.

The growing cells of plants are surrounded by a primary wall that is able to extend in response to turgor pressure. This extension is not spatially uniform, leading to an array of different cell shapes (Baskin, 2005). Primary cell walls contain rigid cellulose microfibrils embedded in a matrix of more flexible polysaccharides (Geitmann and Ortega, 2009). One of these polysaccharides is xyloglucan, which can form hydrogen bonds with cellulose and has chains long enough to connect neighboring microfibrils. All these characteristics make xyloglucan a good candidate to influence the rate of cell expansion (Obel et al., 2007).

Xyloglucans are present in all groups of land plants, although with some differences in structure (Peña et al., 2008; Scheller and Ulvskov, 2010). What all these xyloglucans have in common is a backbone of (1→4)-linked β-D-glucopyranosyl residues, a large proportion of which are substituted with α-D-xylopyranosyl residues at O6. In many vascular plants, including Arabidopsis (Arabidopsis thaliana), only every fourth glucosyl residue is unsubstituted (Vincken et al., 1997). In the standard nomenclature for xyloglucan structures, these residues are represented by G, while X, L, and F indicate Glc residues substituted, respectively, with α-D-Xylp, β-D-Galp-(1→2)-α-D-Xylp, and α-L-Fucp-(1→2)-β-D-Galp-(1→2)-α-D-Xylp side chains (Fry et al., 1993). Conventionally, the reducing end of the molecule is positioned to the right. Treatment of Arabidopsis xyloglucan with an endoglucanase that attacks unsubstituted residues results in oligosaccharide mixtures that include XXG, GXXG, XXXG, XXLG, XLXG, XLG, XXFG, and XLFG, with some of the Gal residues O-acetylated (Madson et al., 2003; Obel et al., 2009).

In addition to covering the surface of cellulose microfibrils, the ends of xyloglucan chains could also be trapped inside (Pauly et al., 1999). There is also evidence for covalent linkages between xyloglucan and the pectic polysaccharide rhamnogalacturonan I, which could also strengthen the wall (Popper and Fry, 2008). Expansins are able to increase cell wall creep under tension, and it has been proposed that they achieve this by detaching xyloglucan chains from cellulose (Cosgrove, 2005). Xyloglucan molecules can also be severed by enzymes in the xyloglucan transglycosylase/hydrolase (XTH) family (Nishitani and Vissenberg, 2007). These enzymes hydrolyze the bond between the anomeric carbon of an unsubstituted Glc and the next residue in the backbone. They form an enzyme-donor complex with the unsubstituted Glc,
which they can later reattach to the nonreducing end of another xyloglucan chain (Johansson et al., 2004). In addition to xyloglucan endotransglucosylases (XETs), the family includes some xyloglucan endohydrolases that can use water as the final acceptor, but they seem to be in the minority (Baumann et al., 2007).

There are many strands of evidence in favor of a role for xyloglucan in the regulation of microfibril separation and cell wall extension. Xyloglucan oligosaccharides, by acting as XET acceptors, can shorten xyloglucan chains and stimulate growth (Takeda et al., 2002). Similarly, the overexpression of xyloglucanase in poplar (Populus spp.) results in a reduction in xyloglucan content and a concomitant increase in stem length (Park et al., 2004). By contrast, the incorporation of polymeric xyloglucan to pea (Pisum sativum) stem segments is enough to slow down cell elongation (Takeda et al., 2002). Arabidopsis mutants with no \( \alpha \)-xylosidase activity against xyloglucan show a reduction in the elongation of several organs that could be linked to an excess of xyloglucan chains (Sampedro et al., 2010; Günl and Pauly, 2011). Despite these results, it appears that Arabidopsis plants with no detectable xyloglucan can still regulate cell expansion, although these plants are significantly smaller and they have irregularly spaced microfibrils, more extensible walls, and a reduced response to expansins (Cavalier et al., 2008; Anderson et al., 2010; Park and Cosgrove, 2012).

In addition to its role in primary walls, xyloglucan is a reserve polysaccharide in many seeds, and the degradation mechanism of storage xyloglucan has been extensively studied (Buckeridge, 2010). In both seeds and primary walls, specific glycosidase activities remove the different sugar residues in xyloglucan in a stepwise manner (Crombie et al., 1998; Iglesias et al., 2006). \( \alpha \)-Xylosidase can only remove unsubstituted Xyl from the nonreducing ends (Fanutti et al., 1991; Sampedro et al., 2001). A \( \beta \)-glucosidase is then required to remove the unsubstituted Glc before \( \alpha \)-xylosidase can act again (Crombie et al., 1998). \( \beta \)-Galactosidases involved in the mobilization of reserve xyloglucan have been purified from nasturtium (Tropaeolum majus; Edwards et al., 1988), Copaifera langsdorffii (de Alcântara et al., 1999), and Hymenaea courbaril (de Alcântara et al., 2006). The sequence of the nasturtium enzyme has been included in a patent application, but it has not been published otherwise (Chengappa et al., 1996). Fuc, which is not present in reserve xyloglucan, is removed from side chains by another glycosidase (Léonard et al., 2008; Günl et al., 2011). In addition to completing the digestion of oligosaccharide fragments, indirect evidence suggests that glycosidase activities can act on the nonreducing ends of polymeric xyloglucan (Sampedro et al., 2010; Günl et al., 2011).

This work identifies \( \text{AtBGAL}10 \) as the gene responsible for the large majority of \( \beta \)-galactosidase activity against xyloglucan in Arabidopsis. Disruptions of this gene cause a strong alteration in xyloglucan composition that can be explained by a blockage in xyloglucan digestion and is associated with shorter siliques and sepals.

**RESULTS**

**\( \text{Atbgal}10 \) Mutants Are Deficient in \( \beta \)-Galactosidase Activity**

Xyloglucan composition was investigated in a number of Arabidopsis mutants with T-DNA insertions in different genes of family 35 of glycosyl hydrolases (\( \text{BGAL}1, -2, -4, -6, -8, -9, -10, -11, -12, -15, -16, \) and -17). Changes in xyloglucan composition were only observed in homozygous \( \text{bgal}10-1 \) plants (SAIL 735F06). A second line with an independent T-DNA insertion in the same gene, \( \text{bgal}10-2 \) (SALK 039100), showed similar changes in xyloglucan composition. These insertions are situated in exons 9 and 17 of the \( \text{AtBGAL}10 \) (At5g63810) gene, respectively (Fig. 1A). Both lines are derived from a Columbia background, which was used as the wild type for all comparisons.

In both mutants, \( \beta \)-galactosidase activity against XLLG was reduced more than 90% in rosette leaves, in comparison with wild-type plants (Fig. 1B). A similar level of residual \( \beta \)-galactosidase activity was detected in rosette leaves of \( \text{bgal}10-1 \) xy1-2 double mutants (data not shown) as well as in a mix of \( \text{bgal}10-1 \) flowers and developing siliques (Supplemental Fig. S1A). On the other hand, \( \alpha \)-xylosidase and \( \beta \)-glucosidase activities against xyloglucan oligosaccharides did not seem to be affected in \( \text{bgal}10 \) lines (Fig. 1, C and D). When we analyzed the liquid medium in which \( \text{bgal}10-1 \) or \( \text{bgal}10-2 \) seedlings had been grown for 7 d, we were not able to detect xyloglucan oligosaccharides (data not shown). This result stands in contrast to a similar experiment performed with mutants deficient in \( \alpha \)-xylosidase activity, in which oligosaccharides were detected at micromolar levels (Sampedro et al., 2010).

To characterize its activity, \( \text{AtBGAL}10 \) was expressed in Pichia pastoris fused to the \( \alpha \)-factor signal sequence. \( \beta \)-Galactosidase activity against XLLG was present in the growth medium of 19 out of 20 clones transformed with \( \text{AtBGAL}10 \), but it was not detectable in four clones transformed with empty vector (Supplemental Fig. S1B). In a larger scale experiment, \( \beta \)-galactosidase activity measured with 1.5 mM XLLG as substrate ranged from 468 to 1,173 pkat mg\(^{-1}\) in three clones transformed with \( \text{AtBGAL}10 \), while no activity was detected in control clones (Fig. 1E). On the other hand, \( \beta \)-galactosidase activity against 3.3 mM polymeric xyloglucan, corresponding to a similar concentration of XLLG subunits, although replicable, was close to the detection limit (Fig. 1E). Extensive digestion of XLLG with heterologously expressed \( \text{AtBGAL}10 \) resulted in complete loss of a single Gal from this substrate, with only a very small amount of XXXG produced (Fig. 1F). In contrast, XLLG was left unmodified by proteins from control clones, with the amount of XXLG/XLXG staying at the same low level present in the substrate (Fig. 1F). The fragmentation of the major product of \( \text{AtBGAL}10 \) activity allowed us to identify it as largely XXLG (Supplemental Fig. S1C). The diagnostic peak for this oligosaccharide, with a
mass-to-charge ratio (m/z) of 659, was much higher than the 773 m/z peak characteristic of XLXG (Madson et al., 2003; Tine´ et al., 2006). AtBGAL10 protein fused to α-factor signal sequence is expected to have a size of 95 kD. Two protein bands of approximately 100 kD were detected on electrophoresis gels of medium from Pichia clones transformed with AtBGAL10, possibly corresponding to different glycosylation states or the removal of the signal peptide (Supplemental Fig. S1D).

When XLG was digested with Arabidopsis wild-type protein extracts, the Gal closest to the nonreducing end was also lost very quickly to produce XXLG, as shown by fragmentation analysis, while XXXG never accumulated (Supplemental Fig. S1, E and F). When the same XLG was digested with bgal10-1 protein extracts, the first product to accumulate was GLLG, detected as an ion with an m/z of 1,277, indicating the loss of a pentose. After extensive digestion, smaller amounts of other products were detected (Fig. 1G).

The identity of the m/z 1,277 ion was confirmed through matrix-assisted laser-desorption ionization tandem time of flight (MALDI-TOF/TOF) fragmentation (Supplemental Fig. S1G). Similarly, the fragmentation pattern of the ion with an m/z of 821 allowed us to identify the major component as GLG (Supplemental Fig. S1H). The ion at 1,541 m/z units (Fig. 1G) is not present in the substrate, and it is most likely a product of transglycosylation by α-xylosidase (Sampedro et al., 2010).

Most of the Sequenced Angiosperm Genomes Have a Single AtBGAL10 Ortholog

A phylogenetic tree was obtained from an alignment of all the members in family 35 of glycosyl hydrolases for both Arabidopsis and rice (Oryza sativa) as well as the three closest AtBGAL10 homologs from an additional 21 plant proteomes (Supplemental Fig. S2). AtBGAL10 appears in a well-defined clade with members from all species, with the exceptions of Physcomitrella patens, Selaginella moellendorfii, Carica papaya, and Manihot esculenta (Fig. 2). In the remaining 19 species, there is a single representative of this clade, with the exceptions of Glycine max, Eucalyptus grandis, Citrus sinensis, and Citrus clementina.

The xyloglucan-specific β-galactosidase from nasturtium (Chengappa et al., 1996) also seems to be an ortholog of AtBGAL10 (TmBGAL1 in Fig. 2). A few

Figure 1. Enzymatic activity of AtBGAL10. A, Gene model of AtBGAL10 showing the locations of the T-DNA insertions. Exons in the coding region are represented by rectangles. The six nucleotides preceding the insertions are shown. B, β-Galactosidase activity against XLG in rosette leaves. Two replicate protein extractions were obtained from each wild-type (Columbia [Col]; black bars) and mutant (white bars) line. Error bars show so. C, α-Xylosidase activity against XXXG. Error bars show so. D, β-Glucosidase activity against XXXG. Error bars show so. E, β-Galactosidase activity against XLG (white bars) and xyloglucan (black bars). Proteins secreted by three independent P. pastoris clones transformed with empty vector or AtBGAL10 were washed and concentrated. Error bars show so. F, MALDI-TOF spectrum of XLG digested for 24 h with proteins secreted by Pichia transformed with an empty vector or AtBGAL10. The arrowhead indicates a peak identified through MALDI-TOF/TOF analysis. G, MALDI-TOF spectrum of XLG digested for 24 h with bgal10-1 protein extract. The arrowheads indicate peaks identified through MALDI-TOF/TOF analyses.
members of this clade that have been studied previously were found in the literature (Fig. 2). TOMATO β-GALACTOSIDASE7 from tomato (*Solanum lycopersicum*) is expressed in young fruits and stems (Smith and Gross, 2000). *OsBGAL4* from rice is most highly expressed in imbibed seeds (Tanthanuch et al., 2008). Finally, *PpGAL3* is expressed in pear (*Pyrus communis*) during fruit growth (Mwaniki et al., 2005).

Mutants Deficient in β-Galactosidase Activity Accumulate Novel Xyloglucan Subunits

When xyloglucan from *bgal10-1* or *bgal10-2* plants was analyzed through MALDI-TOF, seven ions were detected at significant levels that were absent or very rare in Columbia plants (Fig. 3; Supplemental Fig. S3, A and B). These ions had *m/z* values of 659, 821, 953, 1,115, 1,157, 1,277, and 1,319. In the endoglucanase-accessible fraction of stems, these ions represented less than 1% of the total xyloglucan peak area in Columbia but increased to 15% and 17% in *bgal10-1* and *bgal10-2*, respectively (Supplemental Fig. S3A). When the analysis was performed in *bgal10-1* mature leaves, these subunits accounted for 31% of the total xyloglucan peak area (Fig. 3). The same subunits were also found in *bgal10-1* silicles (Supplemental Fig. S3B).

We performed a preliminary characterization of some of these subunits by obtaining MALDI-TOF/TOF spectra of selected ions in *bgal10-1* leaf samples (Supplemental Fig. S4). The fragmentation of the *m/z* 1,277 ion (six hexoses and two pentoses) was identical to the GLLG oligosaccharide obtained from digestions of XLLG (Supplemental Figs. S1G and S4A). In both cases, the spectrum was dominated by glycosidic cleavages, and the absence of a peak for the loss of a pentose confirmed that both Xyl residues were substituted with Gal. Furthermore, all the strongest signals corresponded to fragments that can be obtained from GLLG by a single cleavage, but not from LLGG, the only reasonable alternative. An acetylated version of GLLG could explain the ion with an *m/z* of 1,319.

The MALDI-TOF/TOF spectrum for the ion with an *m/z* of 1,115 (five hexoses and two pentoses) showed a strong peak at 983 *m/z* units, corresponding to the loss of a pentose, suggesting that at least one Xyl was unsubstituted (Supplemental Fig. S4B). The lack of a peak for the loss of two pentoses, together with the strong peak for the loss of a hexose with a pentose (*m/z* 821), indicated a substituted Xyl. An intense signal for the ion at 641 *m/z* units (loss of reducing-end XG) pointed toward GLXG, but the presence of an intense *m/z* 659 fragment (loss of nonreducing-end GX or L) suggested either GXLG or LLG. The *m/z* 1,157 ion, and possibly its acetylated version at *m/z* 1,159, could correspond to a mixture of GLXG and GXLG or LLG.

The MALDI-TOF/TOF spectrum of the *m/z* 953 ion (four hexoses and one pentose) also suggested a mixture of oligosaccharides that could include XLG, LXG, and GLXG (Supplemental Fig. S4C). The ion with an *m/z* of 851 (four hexoses and one pentose) was identified as mostly GLG based on a weak signal for the loss of a pentose and the similarity of its fragmentation to the oligosaccharide of the same size observed in

![Figure 2. Phylogenetic tree of AtBGAL10 and putative orthologs from other plants. For proteins with no published reference, species names are indicated (annotations can be found in Supplemental Fig. S2).](image)

![Figure 3. Xyloglucan composition in *bgal10-1* and *bgal10-1 xyl1-2* mutants. Alcohol-insoluble cell wall residues were obtained from two samples of mature leaves from wild-type (black bars), *bgal10-1* (white bars), or *bgal10-1 xyl1-2* (gray bars) plants. Xyloglucan was first extracted with endoglucanase, and peak areas from three MALDI-TOF spectra were quantified to estimate SD, presented as error bars. The proportion of acetylated subunits corresponds to the area above the horizontal lines. After enzyme extraction, samples were treated with 6 M NaOH. Released xyloglucan was digested with endoglucanase and analyzed by MALDI-TOF.](image)
digested of XLLG with bgal10-1 proteins (Supplementary Figs. S1H and S4D). Finally, the \textit{m/z} 659 ion was identified as mainly LG, since it also showed a weak signal for the cleavage of a pentose (Supplementary Fig. S4E).

When endoglucanase digestions of bgal10-1 xyloglucan were stopped after 2 h, additional peaks at 1,571, 1,613, and 1,759 \textit{m/z} units were observed (Supplementary Fig. S3C). These peaks could correspond to XLLGG, acetylated XLLGG, and acetylated XLFGG. Their disappearance in prolonged digestions could be explained by the removal of the reducing-end Glc by endoglucanase, as has been suggested for XXGG subunits in monocot xyloglucan (Hsieh and Harris, 2009). Other possible subunits of this type, such as XXXGG or XXLGG, would have similar sizes to more common subunits.

Xyloglucan from mature leaves of the double mutant bgal10-1 xyl1-2 was analyzed in the same experiment, and the unusual bgal10-1 subunits were as rare as in Columbia. The double mutant was enriched instead in XXLG/XXLG and XLXG, which accounted for 56% of the xyloglucan signal (Fig. 3). In both bgal10 and bgal10 xyl1, the proportion of fucosylated oligosaccharides was strongly reduced. In stems, the percentage of fucosylation decreased from 57% of total peak area in Columbia to 42% on average in bgal10 mutants, while in leaves, it went down from 70% in Columbia to 41% in bgal10-1 and just 25% in bgal10-1 xyl1-2. The percentage of acetylated subunits in xyloglucan was also lower in the mutants, particularly in leaves, where it went from 62% in Columbia to 43% in bgal10-1 and 34% in bgal10-1 xyl1-2.

The cell wall residue remaining after digestion of the leaf samples with endoglucanase was treated with 6 M NaOH to release xyloglucan inaccessible to enzymes. This second xyloglucan fraction was also digested and analyzed through MALDI-TOF (Fig. 3). In wild-type plants, the composition of the xyloglucan fractions accessible and inaccessible to enzymes was quite similar. In the case of bgal10-1, there was a strong reduction in the proportion of unusual subunits in the inaccessible domain, where they only accounted for 8% of the xyloglucan signal. The composition of the inaccessible fraction, therefore, was very similar in bgal10-1 and Columbia plants. In contrast in bgal10-1 xyl1-2, this fraction still showed a considerable enrichment in XLLG (4%–20%) and XLXG/XXLG (9%–15%) when compared with Columbia. The difference, however, was less pronounced than in the enzyme-accessible fraction.

**Blockage of Xyloglucan Digestion Is Linked to Shorter Siliques and Sepals**

Both bgal10 lines had a similar morphological phenotype, with an average reduction in silique length of 17% at 11 d after anthesis (Fig. 4A). This phenotype was the result of slower growth during the phase of rapid elongation. Silique width appeared to be slightly

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**Figure 4.** Morphological phenotypes of bgal10 and bgal10 xyl1 plants. A. Silique elongation in galactosidase-deficient mutants. Siliques from eight wild-type (black squares), bgal10-1 (white triangles), and bgal10-2 (white circles) plants were measured using a scanner. Error bars indicate ss. Numbers indicate the mutant allele whose length is different from the wild type with 99.95% confidence at each age. B, Silique width in bgal10 plants. Symbols are as in A, except that numbers indicate differences that are significant with 95% confidence. C, Elongated siliques from Columbia, bgal10-1, and bgal10-1 xyl1-2 plants. D, Flowers from Columbia, bgal10-1, and bgal10-1 xyl1-2 plants.
higher in galactosidase-deficient lines, but the difference was only significant in older siliques (Fig. 4B). A very similar phenotype was observed in bgal10-1 xyl1-2 (Fig. 4C). This same phenotype had previously been described in xyl1 single mutants (Sampedro et al., 2010; Günl and Pauly, 2011). In both bgal10 and bgal10-1 xyl1-2, the reduction in length was also noticeable in the sepals (Fig. 4D), as was the case for xylosidase-deficient lines. This phenotype was already noticeable before anthesis in the form of rounder flower buds (data not shown).

**AtBGAL10 Has a Pattern of Expression Similar to AtXYL1**

A 1.5-kb fragment upstream of the *AtBGAL10* start codon was cloned and used to drive the expression of the GUS gene. The pattern of expression was followed by histological staining during the life cycle of T2 plants. In roots, GUS expression under the control of the *AtBGAL10* promoter was very strong in columella cells (Fig. 5A). There was also strong staining in the distal elongation zone that weakened as cells elongated (Fig. 5A). Reporter expression in older parts of the root was mostly limited to the vascular cylinder (Fig. 5B). In lateral roots, expression only started after they emerged from the main root (Fig. 5B). Staining in leaves was strong in developing vasculature, trichomes, and socket cells as well as in guard cells, particularly at early stages of differentiation (Fig. 5, C–E). Reporter expression was also high in the hydathodes and stipulae (Fig. 5, C, F, and G). In young leaves, there was strong staining in the petiole, but in older leaves, it seemed limited to the main vein (Fig. 5, C and G).

Reporter expression was high in developing flower buds, particularly in the sepals. As the flower opened, expression in sepals became limited to vasculature and stomata (Fig. 5). At this stage, there was usually some expression in petals together with strong expression in elongating anther filaments and the style region of the gynoecium (Fig. 5). Expression in the abscission zones started before abscission (Fig. 5H), but it persisted well after (Fig. 5I). As the siliques elongated, expression in the style weakened and expression in the valves became stronger (Fig. 5K). Finally, there was strong staining in the youngest part of the stem and the elongating pedicels (Fig. 5M) that later became limited to the branching point (Fig. 5L), where the vestigial abscission zones are located (Stenvik et al., 2006).

**DISCUSSION**

Insertions in *AtBGAL10* reduce the level of β-galactosidase activity against XLLG more than 90% (Fig. 1B). Introducing this gene in *P. pastoris* results in high levels of β-galactosidase activity against XLLG, an activity that is not present in yeast transformed with an empty vector (Fig. 1, E and F; Supplemental Fig. S1B). Furthermore, a probable...
ortholog in nasturtium (Fig. 2) was identified in a patent application as responsible for the β-galactosidase activity involved in the mobilization of reserve xyloglucan (Chengappa et al., 1996). No other members of the distinct clade that includes both AtBGAL10 and the nasturtium enzyme have been functionally characterized (Fig. 2). It is thus reasonable to conclude that AtBGAL10 is the main Arabidopsis β-galactosidase acting on xyloglucan and that other members of this clade are likely to have a similar function. It has been proposed that a β-galactosidase gene cloned from germinating Sampedro et al. gene cloned from germinating Arabidopsis seedlings might act on storage xyloglucan, but its substrate is currently unknown and its sequence is closely related to those of β-galactosidases that degrade pectins (Brandão et al., 2009).

The residual β-galactosidase activity detectable in bgal10 lines (Fig. 1, B and G) could be due to one or more of the other 17 members of family 35 of glycosyl hydrolases (Ahn et al., 2007). A rice ortholog of AtBGAL8 had activity against both xyloglucan and galactans (Kaneko and Kobayashi, 2003). Similarly, a pea ortholog of AtBGAL16 could remove Gal from both xyloglucan and pectic polysaccharides (Dwevedi and Kayasta, 2009). Unspecific β-galactosidases could thus account for the residual activity in bgal10 lines. Xyloglucan oligosaccharides accumulate in the growth medium of xylosidase-deficient plants (Sampedro et al., 2010) but are not detectable in bgal10 lines. This indicates that at least part of this residual β-galactosidase activity could be secreted to the apoplast. However, the changes in xyloglucan composition in bgal10 suggest that this additional activity has at best a small role in xyloglucan metabolism (Fig. 3).

When expressed in Pichia, AtBGAL10 protein shows a strong preference for removing the Gal closest to the nonreducing end of XLG to produce mainly XXG, with XXXG accumulating at a much slower rate (Fig. 1F; Supplemental Fig. S1C). The β-galactosidase activity in Arabidopsis extracts shows a similar specificity (Supplemental Fig. S1F). Xyloglucan β-galactosidases from nasturtium and C. langsordfii were shown to remove Gal residues from XLG, but less efficiently or not at all from XXL (Crombie et al., 1998; de Alcântara et al., 1999). The same specificity has been found for Lepidium sativum β-galactosidase and is likely to be conserved in many other plant species (Franková and Fry, 2011). The low level of activity of AtBGAL10 on polymeric xyloglucan (Fig. 1E) is consistent with activity limited to the nonreducing end of the chain, although we cannot exclude the presence of some endoglucanase activity in the medium, and is similar to that of xyloglucan β-galactosidases purified from Copaifera and Hymenaea (de Alcântara et al., 1999, 2006). Only nasturtium β-galactosidase has been observed to remove significant amounts of Gal by acting on midchain positions (Edwards et al., 1988; Reid et al., 1988).

In both wild-type and bgal10 extracts, more Glc than Xyl is released from XXXG (Fig. 1, C and D). This can be explained by the transglycosylating activity of Arabidopsis α-xylosidase, which can use XXXG as both acceptor and donor (Sampedro et al., 2010; Franková and Fry, 2011). The excess of β-glucosidase over α-xylosidase activity is inconsistent with the accumulation of GLG and GLG in digestions of XLLG with bgal10 extracts (Fig. 1E). This result can be explained if Arabidopsis xyloglucan β-glucosidase is blocked by the presence of a Gal side chain in the previous residue, as has been shown for the nasturtium β-glucosidase involved in the mobilization of storage xyloglucan (Crombie et al., 1998). It would appear that the substrate specificity is generally similar in glycosidases involved in the digestion of primary wall xyloglucan and those that mobilize xyloglucan reserves in seeds.

The clade that includes AtBGAL10 is present in the majority of sequenced angiosperms, and the apparent absence in C. papaya and M. esculenta could be due to the incomplete nature of the genome sequences (Fig. 2; Supplemental Fig. S2). A single AtBGAL10 ortholog was found in most cases, despite numerous rounds of genome duplication, suggesting limited potential for subfunctionalization of the xyloglucan digestion pathway (Soltis et al., 2009). More interesting is the fact that AtBGAL10 orthologs cannot be identified in P. patens and S. moellendorfii, since these species have Gal in their xyloglucan (Peña et al., 2008). A recent phylogenetic analysis of family 35 included, in addition to sequenced genomes, ESTs from a range of nonangiosperm plants (Del Bem and Vincentz, 2010). AtBGAL10 was placed in clade C, present in gymnosperms, but not in more basal groups. Only two of the 10 clades in the family were found in Physcomitrella and Selaginella. It is possible that a less specific β-galactosidase digests the xyloglucan of both species. The appearance of a xyloglucan-specific β-galactosidase could have happened after the divergence of these basal lineages, possibly linked to more intense xyloglucan recycling. This could also explain why nonseed plants have low β-galactosidase activity against xyloglucan (Franková and Fry, 2011).

Xyloglucan Composition

A deficiency in xyloglucan β-galactosidase results in the accumulation of unusual or novel xyloglucan subunits (Fig. 3; Supplemental Fig. S3). These subunits have been tentatively identified as LG, GLG, XLG/GXXG, GLXG/GXLG/LLG, and GLLG (Supplemental Fig. S4). The presence at early stages of xyloglucan digestion of products that could correspond to subunits with two Glc residues at the reducing end (XLLG or XLFGG) suggests that subunits such as LG, LXG, or LLG could actually be produced during digestion from GLG, GLXG, or GLLG. The presence of these subunits at midchain positions would result in two successive unsubstituted Glc residues. Endoglucanase attack on a XLFGGLG fragment could then result in either XLFG and GLG or XLFGG and LG subunits. XLFGG would then be trimmed to XLFG after extensive digestion. In any
case, the accumulation of unusual subunits in bgal10 xyloglucan requires α-xylosidase activity, since they are absent in bgal10 xyl1 xyloglucan, which instead shows an increase in the proportion of XLXG/XXLG and XLXLG subunits (Fig. 3). In bgal10, the action of α-xylosidase and β-glucosidase could trim these subunits to GLXG, GLG, and GLLG, respectively.

In previous studies, xyl1 mutants lacking α-xylosidase were shown to accumulate mainly XXXG and XXLG subunits, axy8 mutants deficient in α-fucosidase accumulated XFG, XFG, and GFG subunits, and xyl1 axy8 mutants deficient in both activities were enriched in XXXG and XXFG (Sampedro et al., 2010; Gün and Pauly, 2011; Gün et al., 2011). In all of these cases, the accumulating subunits can be explained as products of the activity of the remaining glycosidases on the xyloglucan subunits found in wild-type plants. For instance, XLFG could be digested to XLLG in bgal10 xyl1, to GLLG in bgal10 since β-glucosidase seems to be blocked by L side chains, to XXLG in xyl1 as a result of β-galactosidase specificity, to GFG in axy8, and finally to XXFG in xyl1 axy8. Formation of the subunits that accumulate in the different mutants through xyloglucan metabolism in muro would also explain why they are most abundant in the enzyme-accessible domain, as is the case also for bgal10 and bgal10 xyl1 (Fig. 3).

The magnitude of the changes observed in glycosidase-deficient mutants is difficult to explain by reincorporation through XET activity of partially digested oligosaccharides, since only the reducing-end subunit of each chain can have this origin. The accumulation of unusual subunits is easier to explain if glycosidases can digest the nonreducing ends of polymeric xyloglucan (Fig. 6). In glycosidase-deficient mutants, this process would stop at enzyme-resistant subunits. By acting as acceptors of endotransglycosylation reactions, these subunits would then move to midchain positions and their abundance could progressively increase in the enzyme-accessible fraction.

Direct evidence of glycosidase activity on polymeric xyloglucan is still limited, since these enzymes appear to attack mainly or exclusively the nonreducing end of the molecule and they can only remove a single residue in the absence of other glycosidases. Nasturtium α-xylosidase has been shown to release Xyl from oligosaccharides of at least eight Glc residues (Fanuttì et al., 1991, 1996). Additionally xyloglucan from mutants deficient in α-fucosidase activity has been found to include fragments such as GFGXXXG or GFGXXFG, which cannot be explained by the reincorporation of oligosaccharides (Gün et al., 2011). Glycosidase activity on xyloglucan ends, followed by endotransglycosylation, had also been proposed to explain the accumulation of GXXG and GXFG in the enzyme-accessible fraction of pea xyloglucan as well as the presence of GFGFG subunits in apple (Malus domestica) xyloglucan (Guillén et al., 1995; Spronk et al., 1997; Pauly et al., 2001).

An interesting question is why partially digested subunits are not usually observed in wild-type Arabidopsis (Fig. 3). Two mechanisms could explain this observation. If the first step of backbone digestion (i.e. xylosidase attack on a four-Glc subunit) is rate limiting, few chains would be terminated by partially digested subunits. Additionally, the presence of these subunits at the nonreducing end could make xyloglucan chains poor acceptors in transglycosylation reactions. XET activities in pea extracts prefer XXXG to XXG as an acceptor and are unable to recognize GXG (Lorences and Fry, 1993). XXG is also a much poorer acceptor than XXXG or larger oligosaccharides for most of the Arabidopsis XTH proteins that have been characterized (Maris et al., 2009, 2011).

The precise acceptor requirements of XET activities are poorly understood. Pea XETs are unable to use GXG, but a nusturtium enzyme can utilize as acceptor GXXGXXXG (Lorences and Fry, 1993; Fanuttì et al., 1996). Binding studies have confirmed the importance of the Xyl residue at +2, but the importance of the Xyl at +1 is less clear (Ekloff and Brunner, 2010). The accumulation of subunits such as GLG or GLLG in bgal10 xyloglucan, possibly in midchain positions as discussed previously, suggests that some XET activities might use them as acceptors, at least when no other alternatives are available.

Expression Pattern

Expression of a promoter-reporter construct for AtBGAL10 is detectable in all organs, in most cases at
times and in cells where wall expansion or remodeling is taking place (Fig. 5). Examples include elongating roots, anther filaments and stems, as well as abscission zones, stomata, or developing vasculature. Our confidence that this pattern reflects that of the BgAL10 gene is increased by the similarity to the expression pattern of α-xylosidase (Sampedro et al., 2010). The coregulated expression of these two genes is also apparent in coexpression databases. Both genes are directly connected in the coexpression networks calculated by ATTED-II (http://atted.jp/data/locus/Af5g63810.shtml) and AraNet (http://aranet.mpimp-golm.mpg.de/aranet/a20098) using hundreds of microarray data (Mutwil et al., 2010; Obayashi et al., 2011).

The promoter-reporter constructs suggest some possible differences in the expression of β-galactosidase and α-xylosidase, such as higher expression of BgAL10 in the leaf midvein and weaker expression in the rest of the lamina (Fig. 5G) when compared with XYL1 (Sampedro et al., 2010). Similarly, during silique elongation, BgAL10 expression appears higher than XYL1 in the siliques valves and weaker in the style (Fig. 5, K and M). The expression pattern of FLUC95A/AXY8, the main xyloglucan α-fucosidase in Arabidopsis, is also quite similar to that of α-xylosidase and β-galactosidase, with strong staining in leaf vasculature and trichomes, as well as in the root elongation zone (Günl et al., 2011). However, there also appear to be some differences, such as stronger fucosidase expression in root buds and anthers. It would be interesting to investigate if these apparent differences in expression patterns have physiological consequences by modifying xyloglucan composition in different organs or at different developmental stages.

Phenotypic Effect

Silique length is significantly reduced in two independent bgal10 mutants as well as in bgal10 xyl1, while silique width is not affected or possibly increased (Fig. 4, A–C). This phenotype is associated with reduced β-galactosidase activity in flowers and developing siliques (Supplemental Fig. S1A) as well as the accumulation of unusual xyloglucan subunits in siliques (Supplemental Fig. S3B). The reduction in length is also noticeable in sepal (Fig. 4D). A similar phenotype was described previously in xyl1 plants deficient in xyloglucan α-xylosidase (Sampedro et al., 2010; Günl and Pauly, 2011). Xyloglucan composition in the three mutants is quite different. What they all share is a reduction in the proportion of fucosylated xyloglucan, but this is unlikely to be the main cause of the phenotype, since un46 has normal growth despite having no fucosylated xyloglucan (Madson et al., 2003).

On the other hand, glycosidase shortening of the nonreducing ends of xyloglucan chains should reduce their availability as acceptors of endotransglycosylation reactions. Since this process would be blocked in all three mutants, an abundance of nonreducing ends accessible to XET activities could facilitate the creation of additional xyloglucan connections among microfibrils, offering a possible explanation for the reduction in organ length. The proposed mechanism would work in a similar way to the addition of either purified XET or exogenous xyloglucan, both of which can result in reduced elongation, most likely by creating additional xyloglucan tethers (Takeda et al., 2002; Maris et al., 2009). Supporting this hypothesis, the amount of endoglucanase-accessible xyloglucan, which includes the chains that connect microfibrils, is three times higher in xyl1 mutants than in wild-type plants (Günl and Pauly, 2011; Günl et al., 2011). Plants deficient in xyloglucan α-fucosidase activity have a normal phenotype but only a 37% increase in accessible xyloglucan (Günl et al., 2011). Further study of the phenotype of glycosidase-deficient mutants could shed some light on the complex interplay between the synthesis, modification, and hydrolysis of xyloglucan and how these processes affect growing cell walls.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Atbgal10-1 (SAIL 735F06) from Arabidopsis (Arabidopsis thaliana) was obtained from the Syngenta Arabidopsis Insertion Library (Sessions et al., 2002). Atbgal10-2 (SALK 039200) was ordered from the collection at the Salk Institute Genomic Analysis Laboratory (Alonso et al., 2003). Homozygous plants for the T-DNA insertions were selected by PCR with the primer pairs detailed in Supplemental Table S1. Atbgal10-1 was manually crossed to Axy1-2 (Sampedro et al., 2010), and plants homozygous for both insertions were selected among the descendants. Mutants were compared with wild-type plants (CS60000) grown on the same trays in 16-h days at 22°C/18°C light/dark temperature and 60 μmol m−2 s−1 light intensity.

Phylogeny

Close homologs of AtBGAL10 were obtained from BLAST searches at the Phytozome version 7.0 database (www.phytozome.net). Phylogenetic analysis was done with MEGA5 (Tamura et al., 2011). Sequences were aligned with ClustalW, and phylogenetic trees were obtained by neighbor-joining with complete gap deletion, using the Poisson substitution model and 300 bootstrap replications.

Protein Extraction

Approximately 1.5 g of 11-d-old rosettes was homogenized in liquid N2. Proteins were extracted for 1 h at 4°C in 9 mL of 50 mM Na acetate buffer (pH 4.5), 1 M NaCl, 1.5% polyvinylpolypyrrolidone, and 0.1% (v/v) protease inhibitor cocktail for plant cell and tissue extracts (Sigma). After centrifugation, the supernatant was concentrated using Amicon ultra 30K (Millipore) and washed twice with 10 mL of 20 mM Na acetate buffer (pH 5.0). Protease inhibitor cocktail at 0.1% (v/v) was added to the second wash. Protein concentration was quantified with Coomassie Plus (Thermo Scientific).

Heterologous Expression

The coding region of AtBGAL10, excluding the signal peptide and the stop codon, was amplified from cDNA with primers BgAL10L1 and BgAL10R1, cloned into pENTR/D-TOPO (Invitrogen), and sequenced. The plasmid was digested with KpnI and NorI, and the A bgAL10 fragment was ligated with plasmid pGAPZa A (Invitrogen), previously digested with the same enzymes. The final construct as well as the empty vector were inserted in the genome of Pichia pastoris X-33 through electroporation. Transformed clones were screened for activity by growing them for 72 h at 30°C in culture tubes with
Xyl was quantified with a D-Xyl assay kit (Megazyme), Glc with a Glc assay concentrated using Amicon ultra 30K (Millipore) and washed twice with 10× supernatant was directly assayed for (VWR), repeatedly washed with 10 mM pyridine acetate buffer at pH 4.5, and acid. Extracted cell wall material was filtered through a 10K centrifugal filter. After centrifugation, the supernatant was neutralized with 450 L of 4.5 mM NaOH and 0.1% NaBH4.

Activity Assays

For each assay, 40 μL of protein extract (350–500 μg mL⁻¹ for plant extracts and 10–20 μg mL⁻¹ for yeast supernatants) was mixed with 20 μL of 4.5 mM XXG (Megazyme), 4.5 mM XLG (Megazyme), or 10 mM tamarindin (Tamarindus indica) xylroglin (a kind gift of Dr. Stephen Fry, University of Edinburgh). After a 16-h incubation at 37°C, samples were boiled for 5 min. Xyl was quantified with a β-Xyl assay kit (Megazyme), Glc with a Glc assay reagent (Sigma), and Gal with a β-Gal assay kit (Megazyme).

Xyloglucan Analysis

Stem xyloglucan was obtained from two replicates of each genotype consisting of eight mature green stems without the apical 8 cm. Leaf xyloglucan was obtained from two replicates consisting of approximately 1.5 g of 45-d-old leaves. Samples were homogenized in liquid nitrogen. The powder was boiled in 8 mL of ethanol for 10 min, washed six times with ethanol, twice with diethyl ether, and then dried. Five milligrams of cell wall residue was resuspended in 400 μL of 10 mM pyridine acetate buffer at pH 4.5, sonicated, and then washed three times. Accessible xyloglucan was extracted with 9 units of endocellulase from Trichoderma longibranchiatum (Megazyme) in an overnight digestion at 37°C. Undigested material was washed twice with water and then extracted for 24 h with 900 μL of 6 M NaOH and 0.1% NaBH4. After centrifugation, the supernatant was neutralized with 450 μL of acetic acid. Extracted cell wall material was filtered through a 10K centrifugal filter (VWR), repeatedly washed with 10 mM pyridine acetate buffer at pH 4.5, and digested overnight with 9 units of endocellulase. Reducing sugars were determined with the β-hydroxybenzoic acid hydrazide assay (Lever, 1972). Samples were finally dried and resuspended in 20 μL Na acetate buffer (pH 5.0) to a concentration of 3 μL reducing sugars.

Mass Spectrometry

Samples were mixed 1:1 or 1:3 (v/v) with 2,5-dihydroxybenzoic acid solution (10 mg mL⁻¹ in 70% acetonitrile). MALDI-TOF spectra were obtained with an UltraFlex mass spectrometer (Bruker Daltonics) equipped with a Smartbeam pulsed UV laser (337 nm) and operating in positive-ion mode. Ions were accelerated to a kinetic energy of 25 kV. Each spectrum was obtained with 1.5 g of 45-d-old leaves. Samples were homogenized in liquid nitrogen. The pattern of expression of the reporter gene was followed by histological staining during the life cycle of T2 plants for six selected lines. The promoter:GUS construct was introduced into Agrobacterium tumefaciens EHA105 by electroporation. Arabidopsis Columbia plants were transformed through floral dipping (Clough and Bent, 1998). A total of 15 transgenic plants were recovered in T1, and most showed a very similar expression pattern. Six lines were selected, and T2 plants were analyzed at different stages. GUS activity was detected by incubation in a solution of 1 μM 5-bromo-4-chloro-3-indolyl-β-D-Gal, 100 μM Na phosphate buffer (pH 7.0), 10 μM EDTA, 0.5 μM potassium ferrocyanide, 0.5 μM potassium ferricyanide, and 0.1% Triton X-100.

Supplemental Data

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

Supplemental Figure S1. Analysis of AtBGAL10 activity.

Supplemental Figure S2. Phylogenetic tree of AtBGAL10 and close homologs.

Supplemental Figure S3. Xyloglucan composition.

Supplemental Figure S4. Fragmentation of unusual subunits in lgal10-1 xylroglin.

Supplemental Table S1. Primers used in this work.

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LITERATURE CITED

Ahn YO, Zheng M, Bevan DR, Esen A, Shiu SH, Benson J, Peng HP, Miller JT, Cheng CL, Poultou JE, et al (2007) Functional genomic analysis of Arabidopsis thaliana glycosidase hydrodase family 35. Phytochemistry 68: 1510–1520

Alonso MJ, Stepanova AN, Leisse TJ, Kim CJ, Chen H, Shinn P, Stevenson DK, Zimmerman J, Barajas P, Cheuk R, et al (2003) Genome-wide inser
tional mutagenesis of Arabidopsis thaliana. Science 301: 653–657

Anderson CT, Carroll A, Akkemtova L, Somerville C (2010) Real-time imaging of cellulose reorientation during cell wall expansion in Arabidopsis roots. Plant Physiol 152: 787–796

Blanken T1 (2005) Anisotropic expansion of the plant cell wall. Annu Rev Cell Dev Biol 21: 203–222

Baumann MJ, Eklöf JM, Michel G, Kallas AM, Teeri TT, Czjzek M, Brumer H III (2007) Structural evidence for the evolution of xylroglin
canase activity from xylroglin endo-transglycosylases: biological implica
tions for cell wall metabolism. Plant Cell 19: 1947–1963

Brandão AD, Del Bem LE, Vinconza M, Buckeridge MS (2009) Expression pattern of four storage xylroglin mobilization-related genes during seedling development of the rain forest tree Hymenaea courbaril L. J Exp Bot 60: 1191–1206

Buckeridge MS (2010) Seed cell wall storage polysaccharides: models to understand cell wall biosynthesis and degradation. Plant Physiol 154: 1017–1023

Cavaler DM, Lerouxel O, Neumetzler L, Yamauthki K, Reinecke A, Freshour G, Zabotina OA, Hahn MG, Burgert I, Paul M, et al (2008) Disrupting two Arabidopsis thaliana xylosyltransferase genes results in plants deficient in xylroglin, a major primary cell wall component. Plant Cell 20: 1519–1537

Chengappa S, Hellyer A, de Silva J, Reid JSG (March 14, 1996) Xyloglucan composition, properties and character
zation of a cDNA clone. Plant J 15: 1519–1537

Clough SJ, Bent AF (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J 6: 735–743

Cosgrove DJS (2005) Growth of the plant cell wall. Nat Rev Mol Cell Biol 6: 850–861

Crombie HJ, Chengappa S, Hellyer A, Reid JSG (1998) A xylroglin oligosaccharide-active, transglycosylating beta-D-glucosidase from the cotyledons of nectarium (Tropaeolum majus L) seedlings: purification, properties and characterization of a cDNA clone. Plant J 15: 27–38

Curtis DJ, Hymenaea courbari MS (2007) A Gateway cloning vector set for high
troughput functional analysis of genes in planta. Plant Physiol 138: 462–469

de Alcantara PHN, Dietrich SMC, Buckeridge MS (1999) Xyloglucan mobili
sation and purification of a (XLG/XXG) specific β-galactosidase from cotyledons of Copaifera langsdorffii. Plant Physiol Biochem 37: 653–663

de Alcantara PHN, Martim L, Silva CO, Dietrich SMC, Buckeridge MS (2006) Purification of a beta-galactosidase from cotyledons of Hymenaea

β-Galactosidase Mutants with Altered Xyloglucan
c. 1972 A new reaction for colorimetric determination of carbohy-
drates. Anal Biochem 47: 273–279
Lorences EP, Fry SC (1993) Xylooligosaccharides with at least two
β-(1→4)-xylene residues act as acceptor substrates for xyloglucan endotrans-
glycosylase and promote the depolymerisation of xyloglucin. Plant Physiol
Plant Physiol 88: 105–112
Madson M, Dunand C, Li X, Verma R, Vanzin GE, Caplan J, Shoue DA, Carpita
NC, Reiter WD (2003) The MUR3 gene of Arabidopsis encodes a
xyloglucin endotransglycosylase that is evolutionarily related to animal
exostosins. Plant Cell 15: 1662–1670
Maris A, Kaewthai N, Eklöf JM, Miller JC, Brunner H, Fry SC, VerbeLEN
JP, Vissenberg K (2011) Differences in enzyme properties of five
recombinant xyloglucin endotransglycosylase/hydrolase (XTH) pro-
teins of Arabidopsis thaliana. J Exp Bot 62: 261–271
Maris A, Suslov D, Fry SC, VerbeLEN JP, Vissenberg K (2009) Enzymic
coloration of two recombinant xyloglucin endotransglycosylase/hydrolase
(XTH) proteins of Arabidopsis and their effect on root growth and
cell wall extension. J Exp Bot 60: 3959–3972
Muetwil M, Usadel B, Schütte M, Loriae E, EbenhöLl O, Peresson S (2010)
Assembly of an interactive correlation network for the Arabidopsis
genome using a novel heuristic clustering algorithm. Plant Physiol 152:
29–43
Mwaniki MW, Mathooko FM, Matsuzaki M, Hiwasa K, Tateishi A, Ushijima K, Nakano R, Inaba A, Kubo Y (2005) Expression character-
istics of seven members of the β-galactosidase gene family in 'La France'
pear (Pyrus communis L.) fruit during growth and their regulation by
1-methylcyclopentene during postharvest ripening. Postharvest Biol
Technol 36: 253–263
Nishitani K, Vissenberg K (2007) Roles of the XTH protein family in the
expanding cell. In JP Verbelen, K Vissenberg, eds, The Expanding Cell.
Springer-Verlag, Berlin, pp 89–116
Obel N, Erven V, Schwarz T, Köhnel S, Fodor A, Pauly M (2009)
Lack of β-galactosidase activity in tomato cell wall polysaccharides. Mol Cell 2: 922–932
Obel N, Neumetzler L, Pauly M (2007) Hemicelluloses and cell expansion. 
In JP Verbelen, K Vissenberg, eds, The Expanding Cell. Springer-Verlag, Berlin, pp 57–88
Park YW, Baba K, Furuta Y, Iida I, Sameshima K, Arai M, Hayashi T (2004)
Enhancement of growth and cellulose accumulation by overexpression
of xyloglucinase in poplar. FEBS Lett 564: 183–187
Park YB, Cosgrove DJ (2012) Changes in cell wall biomechanical properties in
the xyloglucin-deficient xtt1/xtt2 mutant of Arabidopsis. Plant Physiol 158: 465–475
Pauly M, Albersheim P, Darvill A, York WS (1999) Molecular domains of the
cellulose/xyloglucin network in the cell walls of higher plants. 
Plant J 20: 629–639
Pauly M, Qin Q, Greene H, Albersheim P, Darvill A, York WS (2001) Changes in the structure of xyloglucin during cell elongation. Planta 212: 842–850
Peña MJ, Darvill AG, Eberhard S, York WS, O’Neill MA (2008) Moss and
liverwort xyloglucins contain galacturonic acid and are structurally
distinct from the xyloglucins synthesized by hornworts and vascular
plants. Glycobiology 18: 891–904
Popper ZA, Fry SC (2008) Xyloglucin-peakin linkages are formed intra-
protoplasmically, contribute to wall-assembly, and remain stable in
the wall. Plant Physiol 127: 781–794
Reid JSG, Edwards M, Dea ICM (1988) Enzymatic modification of natural
seed gums. In Go Phillips, DJ Wedlock, PA Williams, eds, Gums and
Stabilisers for the Food Industry. IRL. Press, Oxford, pp 391–398
Sampado et al.
Sampado et al. 1156 Plant Physiol. Vol. 158, 2012
β-Galactosidase Mutants with Altered Xyloglucan

Takeda T, Furuta Y, Awano T, Mizuno K, Mitsuishi Y, Hayashi T (2002) Suppression and acceleration of cell elongation by integration of xyloglucans in pea stem segments. Proc Natl Acad Sci USA 99: 9055–9060

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28: 2731–2739

Tanthanuch W, Chantarangsee M, Maneesan J, Ketudat-Cairns J (2008) Genomic and expression analysis of glycosyl hydrolase family 35 genes from rice (Oryza sativa L.). BMC Plant Biol 8: 84

Tiné MAS, Silva CO, de Lima DU, Carpita NC, Buckeridge MS (2006) Fine structure of a mixed-oligomer storage xyloglucan from seeds of Hymenaea courbaril. Carbohydr Polym 66: 444–454

Vincken J-P, York WS, Beldman G, Voragen AGJ (1997) Two general branching patterns of xyloglucan, XXXG and XXGG. Plant Physiol 114: 9–13