Changes in Cell Wall Biomechanical Properties in the Xyloglucan-Deficient xxt1/xxt2 Mutant of Arabidopsis1[W][OA]

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The main load-bearing network in the primary cell wall of most land plants is commonly depicted as a scaffold of cellulose microfibrils tethered by xyloglucans. However, a xyloglucan-deficient mutant \((xylxylosyltransferase1/xylxylosyltransferase2 \text{ [xxt1/xxt2]})\) was recently developed that was smaller than the wild type but otherwise nearly normal in its development, casting doubt on xyloglucan’s role in wall structure. To assess xyloglucan function in the Arabidopsis \((Arabidopsis thaliana)\) wall, we compared the behavior of petiole cell walls from \textit{xxt1}/\textit{xxt2} and wild-type plants using creep, stress relaxation, and stress/strain assays, in combination with reagents that cut or solubilize specific components of the wall matrix. Stress/strain assays showed \textit{xxt1}/\textit{xxt2} walls to be more extensible than wild-type walls (supporting a reinforcing role for xyloglucan) but less extensible in creep and stress relaxation processes mediated by \(\alpha\)-expansins. Fusidicin-induced “acid growth” was likewise reduced in \textit{xxt1}/\textit{xxt2} petioles. The results show that xyloglucan is important for wall loosening by \(\alpha\)-expansin, and the smaller size of the \textit{xxt1}/\textit{xxt2} mutant may stem from the reduced effectiveness of \(\alpha\)-expansins in the absence of xyloglucan. Loosening agents that act on xylans and pectins elicited greater extension in creep assays of \textit{xxt1}/\textit{xxt2} cell walls compared with wild-type walls, consistent with a larger mechanical role for these matrix polymers in the absence of xyloglucan. Our results illustrate the need for multiple biomechanical assays to evaluate wall properties and indicate that the common depiction of a cellulose-xyloglucan network as the major load-bearing structure is in need of revision.

The primary cell wall plays important roles in cell growth, tissue mechanics, pathogen resistance, water relations, mineral uptake, and numerous other aspects of plant development and physiology. For insights into these processes, a molecular description of cell wall architecture is important (Carpita and Gibeaut, 1993; Cosgrove, 2005; Burton et al., 2010; Scheller and Ulvskov, 2010). The first detailed molecular model of the primary cell wall by Keegstra et al. (1973) was based primarily on biochemical considerations and hypothesized a structure in which all matrix polymers (hemicelluloses, pectins, and structural proteins) were covalently linked to form a continuous macromolecular structure analogous to bacterial peptidoglycan; this macromolecule was hypothesized to be hydrogen bonded to the surface of cellulose microfibrils via xyloglucan (XyG). According to this model, cell wall enlargement could be induced by slippage of the XyG-cellulose complex or by cutting (and possibly religating) components of the XyG-pectin-protein complex. Despite its elegance, this model was abandoned when key hypothetical linkages between matrix components could not be confirmed.

Subsequent work led to a very different model of primary cell wall architecture (Fry, 1989; Hayashi, 1989), which we call the “tethered network model.” Here, XyGs are envisioned as polymers that coat cellulose surfaces to minimize direct cellulose-cellulose contacts and simultaneously function as tethers to cross-link adjacent cellulose microfibrils, forming a complex load-bearing network embedded in an independent pectin gel (Albersheim et al., 2010). With this model, modification of XyGs or the XyG-cellulose interface is seen as the principal means for controlling cell wall enlargement, potentially through the action of \(\alpha\)-expansins, XyG endotransglucosylase, or \(\beta\)-1,4-endoglucanases (Fry et al., 1992; Fanutti et al., 1993; Rose et al., 2002; Chaliand et al., 2004; Cosgrove, 2005). Other arrangements of the cell wall polymers have been suggested (Talbott and Ray, 1992; Ha et al., 1997; Zykwinska et al., 2005), but there is little in the way of compelling evidence to decide which cell wall model comes closer to reality. Structures resembling microfibril cross-links were detected by electron microscopy of onion \((Allium cepa)\) parenchyma walls (McCann et al., 1990) but were not seen in elongating cell walls of pea \((Pisum sativum)\) epicotyls (Fujino et al., 2000), nor were they evident in some primary walls imaged by atomic force microscopy (Thimm et al., 2000; Ding and Himmel, 2006). NMR studies have not found evidence for extensive XyG-cellulose interactions in...
muro (Bootten et al., 2004; Dick-Pérez et al., 2011), as postulated in this model, so the extent and nature of XyG-cellulose interactions in the wall remain unsettled questions.

Despite these reservations, the tethered network model has dominated the cell wall field for the past two decades (McCann and Roberts, 1991; Carpita and Gibeaut, 1993; Somerville et al., 2004; Albersheim et al., 2010), so it came as a shock when Cavalier et al. (2008) reported that a XyG-deficient mutant of Arabidopsis (Arabidopsis thaliana) displayed a relatively minor phenotype. This line was generated by combining mutations in two xylosyltransferase genes (XYLOSYLTRANSFERASEI [XXT1] and XXT2) involved in XyG synthesis, resulting in seedlings without detectable XyG. Root hair growth was abnormal in the mutant and the mature plants were reduced in size, but otherwise, plant development appeared normal.

The results of Cavalier et al. (2008) were viewed as a challenge to the conventional model of the primary cell wall. Hypocotyl walls were 20% to 50% weaker in xxt1/xxt2 seedlings, suggesting that XyG plays a strengthening role for the cell wall. It was speculated that other matrix polysaccharides replaced XyG’s function in the cell walls of the mutant, but compositional analysis of the xxt1/xxt2 cell walls did not yield any strong clues on this point. NMR analysis confirmed the absence of XyG in the mutant (Dick-Pérez et al., 2011) and pointed to potential interactions of cellulose with other matrix polysaccharides, but their significance for wall mechanical behavior was not tested. The basis for the reduced growth was also unexplained, since one might expect that weaker (more extensible) walls would lead to more cell expansion rather than less (Darley et al., 2001).

To address some of these issues, we compared the biomechanical responses of cell wall specimens from wild-type and xxt1/xxt2 plants, using a suite of treatments to loosen selective components of the cell wall in order to assess which, if any, of the matrix polymers have assumed a greater mechanical role in the XyG-deficient walls. The results demonstrate that pectins and xylans take on a larger role in cell wall biomechanics when XyG is missing, and they also indicate that the growth reduction in xxt1/xxt2 plants likely arises from the absence of the native target for cell wall loosening by α-expansins.

RESULTS

We used cell wall specimens prepared from growing petioles of 28-d-old plants, as such specimens proved amenable to biomechanical analysis in previous work (Cho and Cosgrove, 2000). The rosettes of xxt1/xxt2 plants were appreciably smaller than those of wild-type plants, with shorter petioles and smaller leaf blades (Fig. 1). The petioles used in this study were from the fifth to eighth rosette leaves (not counting cotyledons) and were similar in diameter and cross-sectional area in wild-type and xxt1/xxt2 plants (Supplemental Fig. S1, A and B). Dry weight per unit length of petiole, which is used as a surrogate for cell wall cross-sectional area (or, more strictly, polymer area in cross-section), was similar in xxt1/xxt2 and wild-type petioles (Supplemental Fig. S1C). Thus, the biomechanical behavior of wall specimens from xxt1/xxt2 and wild-type petioles could be compared directly without the need to correct for differences in wall cross-section. Such differences, if present, would give rise to differences in wall stresses in the biomechanical assays and would complicate the interpretation of the results because of nonlinear stress/strain responses.

We first assessed XyG content in xxt1/xxt2 petioles. Although Cavalier et al. (2008) did not detect XyG in young seedlings and etiolated hypocotyls, it was possible that XyG might be present in petioles at later stages of development (i.e. if other genes in the XXT family were active during petiole growth). We used three assessment methods. First, XyG was extracted from whole aerial tissues and digested with xyloglucan-specific endoglucanase (XEG; Pauly et al., 1999) to release xyloglucan oligosaccharides (XGOs) that were analyzed by matrix-assisted laser-desorption ionization time of flight (MALDI-TOF) mass spectrometry. No XGOs were detected in xxt1/xxt2 samples, whereas they were readily detected in the wild type (Fig. 2A). Second, we used high-performance anion-exchange chromatography-pulsed amperometric detection (HPAEC-PAD) analysis of the XEG digest to quantify XGOs. Consistent with the MALDI-TOF results, XGOs were readily detected in wild-type samples but not in xxt1/xxt2 samples (Fig. 2B). Third, we used three monoclonal anti-
bodies (CCRC-M1, CCRC-M39, and CCRC-M87) that reportedly bind to XyG epitopes (Cavalier et al., 2008) to probe for XyG in cell wall extracts of wild-type and xxt1/xxt2 petioles. All three antibodies gave a strong signal with the alkali-soluble fraction of wild-type walls, and these signals were reduced by predigestion with XEG, confirming that the antibodies detected XyG (Fig. 2C). XEG digestion completely eliminated the signal with the CCRC-M39 antibody, whereas some signal remained with the other two antibodies. We conclude that CCRC-M39 is more specific for XyG than are CCRC-M1 and CCRC-M87, which apparently cross-react weakly with non-XyG epitopes present in the wall extract. For the xxt1/xxt2 samples, no binding of CCRC-M39 above background was detected. Weak signals were observed with the CCRC-M1 or CCRC-M87 antibody, but they were not reduced by XEG treatment, so we conclude that these weak signals were not due to XyG. These results indicate that xxt1/xxt2 petioles lack alkali-extractable XyG. To put this on a more quantitative basis, we used serial dilutions of the wild-type extracts to assess the lower limit of detection by CCRC-M39 in this immunoblot assay (Fig. 2D). The result indicated that XyG is at least 500-fold less abundant in xxt1/xxt2 petioles compared with the wild type. Thus, all three methods indicated that xxt1/xxt2 petioles lack XyG, whereas wild-type petioles contain it. In principle, a highly aberrant XyG that was alkali insoluble, XEG resistant, and lacking CCRC-M39 epitopes might be present, but this is not likely.

Stress/Stain Assays

A technique commonly used to assess cell wall mechanical properties is a stress/strain measurement in which wall specimens are extended to a predetermined force in two cycles. The second extension is reversible, and its slope (force/extension \( \sim \) stress/strain) is taken as the elastic compliance (=reciprocal of elastic modulus). The first extension includes both reversible (elastic) and irreversible (plastic) components, so the plastic compliance is calculated from the difference between the two extension curves. The compliances, or extensibilities, measured by this method are sensitive to the properties of the matrix and its binding to cellulose. We found that walls from xxt1/xxt2 petioles were more extensible than wild-type walls, with 53% higher elastic compliance and 164% higher plastic compliance compared with the wild type (Fig. 3). This result is consistent with the results of Cavalier et al. (2008), who measured etiolated hypocotyls, and is consistent with a wall-strengthening role for XyG.

The more extensible walls seem at odds with the reduced-growth phenotype of the xxt1/xxt2 line; that is, one might expect the greater extensibilities of xxt1/xxt2 walls to result in more growth, not reduced
growth. The resolution of this conundrum comes from the results of wall creep assays.

**XyG-Deficient Walls Have Diminished Creep Responses to Acid Buffers and \( \alpha \)-Expansins**

Wall creep measurements (long-term, irreversible extension at constant force) offer an alternative to rapid stress/strain assays for assessing the biomechanical properties of cell walls. This method was used to detect expansin action (McQueen-Mason et al., 1992; McQueen-Mason and Cosgrove, 1995) and has the virtue of being a close mimic of the slow creep of cell walls during cell expansion.

First, we measured the acid-induced creep responses of native walls. In dicot cell walls, this extension is primarily the result of wall loosening by endogenous \( \alpha \)-expansins, which are hypothesized to act on a XyG-cellulose junction in the cell wall (Cosgrove, 2000). For these measurements, petioles were frozen and thawed to kill the cells, and the cuticle was abraded to facilitate the penetration of buffer and reagents into the wall space. In this first set of measurements, nothing was done to inactivate endogenous expansins or other proteins bound to the wall. The specimens were clamped at constant force (10 g) in pH 6.8 buffer, which was exchanged at 30 min for a pH 4.5 buffer. The walls from wild-type plants responded to acidic buffer with rapid extension, whereas the walls from \( xxt1/xxt2 \) plants gave only a feeble response, about 25% of that of wild-type walls (Fig. 4A). This result showed that acid-induced extension, presumably mediated by endogenous \( \alpha \)-expansins bound to the wall, was greatly diminished in the XyG-deficient walls.

To confirm and extend this result, we measured the creep response of heat-inactivated cell walls to endogenous \( \alpha \)-expansin (McQueen-Mason et al., 1992). For these and subsequent creep experiments, the walls were briefly heated to inactivate endogenous expansins and other wall-loosening proteins. The extension response of \( xxt1/xxt2 \) walls to exogenous \( \alpha \)-expansin was diminished compared with wild-type walls (Fig. 4B), although not as markedly as seen in acid-extension responses. Quantitative differences between the two experiments may stem from heat pretreatment of the walls and/or the use of an \( \alpha \)-expansin preparation from cucumber (Cucumis sativus) hypocotyls. Initial responses (first 5 min) to \( \alpha \)-expansin were similar in both walls, but the creep rate soon declined to background rates in \( xxt1/xxt2 \) walls, whereas creep was maintained at a moderate rate in the wild-type walls. These results support the conclusions that (1) XyG is an important substrate for cell wall loosening by \( \alpha \)-expansin or generates wall structures that are the target of \( \alpha \)-expansins (for elaboration of this point, see “Discussion”) and (2) XyG-deficient walls are diminished in their acid-induced extension response by virtue of their reduced response to \( \alpha \)-expansin. The results also indicate that \( \alpha \)-expansin can act on a wall polymer other than XyG, most likely cellulose (see “Discussion”). The diminished responsiveness to “acid growth” and to

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**Figure 3.** Elastic and plastic compliances of wild-type (WT) and \( xxt1/xxt2 \) petiole walls. Error bars represent SE. * Statistically different from the wild type at \( P < 0.05 \) by Student’s two-tailed \( t \) test (13 \( \leq n \leq 15 \)).

**Figure 4.** Acid-induced and \( \alpha \)-expansin-induced wall extension of wild-type (WT) and \( xxt1/xxt2 \) walls. A, Extension of native walls in response to acidic buffer (pH 4.5). Extension of wild-type walls in pH 6.8 buffer is also shown. Each curve is the average of 10 responses. B, Extension of heat-inactivated wild-type and \( xxt1/xxt2 \) walls in pH 4.5 buffer upon the addition of cucumber \( \alpha \)-expansin. These curves are averages of eight to 14 individual responses. The wild-type control without the addition of \( \alpha \)-expansin is also shown. Arrows indicate when the incubation buffer was switched (A) and \( \alpha \)-expansin was added (B). In both A and B, the total extension (30–150 min) of wild-type walls was significantly greater than that of \( xxt1/xxt2 \) walls at \( P < 0.015 \) by Student’s one-tailed \( t \) test. The initial length of samples was 5 mm.
α-expansins provides a likely basis for the reduced growth of xxt1/xxt2 plants.

**XyG-Deficient Walls Display Reduced Stress Relaxation and Fusicoccin-Induced Growth**

Complementary to creep assays (see above) are stress relaxation assays, which provide a means to assess the time-dependent mobility of load-bearing polymer networks. In these assays, walls are rapidly extended to a defined force and then held at constant length while the subsequent decay in holding force is monitored in time. In “normal” walls containing XyG, α-expansins contribute a major component of wall stress relaxation, accounting for approximately 30% of the stress relaxation in the time range 0.1 to 100 s (McQueen-Mason and Cosgrove, 1994, 1995; Keller and Cosgrove, 1995). Since xxt1/xxt2 walls were less responsive to endogenous and exogenous α-expansins, we hypothesized that they would display less stress relaxation than wild-type walls. Consistent with this hypothesis, xxt1/xxt2 walls indeed relaxed more slowly than wild-type walls (Fig. 5). We take this as additional evidence, albeit indirect, that wall loosening by α-expansin involves XyG and that the xxt1/xxt2 mutant undergoes less wall loosening by α-expansin.

To test this idea in yet another way, we measured fusicoccin-induced growth in excised (living) petioles. Fusicoccin stimulates a plasma membrane proton pump, resulting in cell wall acidification and acid growth (Rayle and Cleland, 1992). The result shows that fusicoccin-induced growth in xxt1/xxt2 petioles was approximately half that of wild-type petioles (Fig. 6), a further indication that xxt1/xxt2 plants have diminished acid growth and α-expansin response.

**Xylans and Pectins Play a Larger Biomechanical Role in XyG-Deficient Walls**

In the absence of XyG, other matrix polymers might assume a greater biomechanical role in xxt1/xxt2 walls (Cavalier et al., 2008; Dick-Pérez et al., 2011). To test this possibility, we compared the ability of wild-type and xxt1/xxt2 wall specimens to undergo creep in response to a series of potential wall-loosening treatments with differing targets of action.

Since arabinoxylan is the second most abundant hemicellulose in Arabidopsis cell walls after XyG (Zablackis et al., 1995), we measured the creep response of heat-inactivated wall specimens to several wall-loosening treatments selective for arabinoxylans. An initial test showed that xxt1/xxt2 walls extended more rapidly (2-fold increase over wild-type walls) in response to an unfractionated extract from maize (*Zea mays*) pollen (Fig. 7A). The extract contains β-expansins and xylanase, both of which are selective for arabinoxylans (Li et al., 2003; Yennawar et al., 2006; Suen and Huang, 2007). Hence, this result suggested that arabinoxylans make a relatively larger contribution to the load-bearing network in the xxt1/xxt2 cell walls. To confirm and refine this test, we used a purified β-expansin (EXPB) fraction from maize pollen, primarily containing ZmEXB1, which has selectivity for arabinoxylans (Yennawar et al., 2006; Tabuchi et al., 2011). The XyG-deficient walls yielded much greater response to β-expansin compared with wild-type walls (Fig. 7A). A similar result was obtained with the use of the bacterial expansin BsEXLX1 (Fig. 7A), which likewise shows higher activity toward cell walls enriched in arabinoxylans (Georgelis et al., 2011). Finally, a microbial xylanase (M4; from Megazyme), which cuts xylans with a low/moderate degree of arabinosyl substitution (Tabuchi et al., 2011), induced about three times greater wall extension in xxt1/xxt2 walls compared with the wild type (Fig. 7A). The extension responses in all of these cases were small relative to the acid-induced extension of wild-type walls; this could mean that arabinoxylans have not fully replaced XyG in the xxt1/xxt2 walls or that they are relatively inaccessible to enzyme attack. Nevertheless, the results do indicate that arabinoxylans bear a
greater share of the mechanical load in \( \text{xxt1/xxt2} \) walls compared with wild-type walls.

Pectins constitute about 42% of the primary cell wall of Arabidopsis leaves (Zablackis et al., 1995), so we tested the effect of various pectin-loosening treatments on the creep responses of petiole walls. Compared with the wild type, the \( \text{xxt1/xxt2} \) walls were three times more responsive to polygalacturonase (PGase) and two times more responsive to pectate lyase (Fig. 7B). Both enzymes cut the homogalacturonan backbone. For pectate lyase, the extension was very transient, decaying in 10 to 15 min, whereas the creep response was much less than that induced by PGase.

The enhanced responsiveness of the XyG-deficient walls to these pectin-degrading enzymes suggests that pectic polysaccharides make a larger contribution to the biomechanics of \( \text{xxt1/xxt2} \) walls. Another possibility is that the absence of XyG renders the pectins more accessible to enzymatic attack (i.e. if XyG limited enzyme penetration into the walls). This seems unlikely, because pectins are thought to be the most accessible of wall components as well as the major determinants of cell wall porosity. As an additional test with fewer concerns about penetration, we used the calcium chelator EDTA, which can solubilize pectins cross-linked by calcium bridges (Caffall and Mohnen, 2009). Since EDTA is a small molecule, its penetration is less likely to be hindered by XyGs. In these experiments, the loosening effect of 5 mM EDTA was two times greater in \( \text{xxt1/xxt2} \) walls compared with wild-type walls (Fig. 7B), thus confirming the results with pectin-degrading enzymes and supporting the conclusion that pectins assume a substantially greater biomechanical role in the XyG-deficient walls.

Additionally, we tested wall creep responses to 60 mM NaOH, which breaks ester bonds (potentially cross-linking some pectic polysaccharides), dissociates hydrogen-bonded complexes (both pectins and weakly held hemicelluloses), and may initiate the degradation of labile pectins and hemicelluloses by \( \beta \)-elimination reactions (Whistler and Bemiller, 1958; Fry, 1989). The walls from the \( \text{xxt1/xxt2} \) mutant showed a greater creep response to NaOH (Fig. 7B), indicating a greater susceptibility to this treatment that disrupts weak interactions and labile polysaccharides.

Finally, we tested the effect of Pronase on cell wall creep to test the possibility that cell wall structural proteins might become a significant biomechanical component when XyG is absent. Pronase is a mixture of aggressive proteases from \textit{Streptomyces griseus} capable of digesting most proteins and glycoproteins. It had a negligible effect on cell wall creep in both wild-type and \( \text{xxt1/xxt2} \) walls (Supplemental Fig. S3), indicating a negligible role for wall structural proteins in cell wall mechanics in both cases. One caveat with this result is that glycosylation may protect wall structural proteins against proteolysis.

To summarize, cell walls from the \( \text{xxt1/xxt2} \) mutant gave greater creep responses to agents that degrade or solubilize pectic polysaccharides and arabinoxylans, indicating that both of these wall matrix components assumed a larger role in the biomechanics of XyG-deficient cell walls.

**DISCUSSION**

XyG has been hypothesized to play a key structural role in the primary cell wall of land plants; consequently, its placement and binding to other components in the wall are viewed as central to the control of cell wall extensibility, cell enlargement, and cell wall mechanics (Hayashi, 1989; Carpita and Gibeaut, 1993; Obel et al., 2006; McCann and Knox, 2011). Hence, the existence of an Arabidopsis mutant that grows more or less normally without XyG in its walls poses a significant challenge to current views of primary cell wall architecture and offers new opportunities to elucidate the role of XyG in cell wall biomechanics, cell enlargement, and other physiological and developmental
Biomechanics of Xyloglucan-Deficient Cell Walls

XyG’s Role in Cell Wall Architecture and Biomechanics

XyG is commonly depicted as an extended polymer coating the surface of cellulose in long stretches and simultaneously tethering cellulose microfibrils together (Fry, 1989; Hayashi, 1989; Somerville et al., 2004), with the implication that the tethers limit the movement of cellulose microfibrils and thereby control wall extensibility. The increased elastic and plastic extensibilities of xxt1/xxt2 walls, observed here with petiole walls and by Cavalier et al. (2008) with hypocotyl walls, are thus consistent with a tethering role for XyG. However, these results do not exclude other possible explanations of this mechanical effect. For instance, if XyG acted as a rigidifying element in the matrix, without direct tethering between microfibrils, its absence could result in increased extensibilities in this assay. Such an effect on cell wall mechanics was evidently observed with the Arabidopsis qua2 mutant (Abasolo et al., 2009), whose pectin contains half the homogalacturonan content of wild-type pectin, resulting in a more flexible pectic complex (Rafel et al., 2008). Thus, the greater mechanical extensibility of xxt1/xxt2 walls supports a reinforcing role for XyG but does not necessarily prove that it tethers microfibrils, as commonly depicted.

XyG has also been pictured as coating the surface of cellulose microfibrils, potentially enforcing a minimum distance between microfibrils and preventing direct contacts, which could be friction points that limit microfibril motions. Evidence for this role may be seen in the collapse of wall fibril networks after treatment with strong alkali to extract xyloglucans from onion parenchyma walls (McCann et al., 1990) and the increased bundling of wall fibrils observed in xxt1/xxt2 roots by confocal microscopy (Anderson et al., 2010). Also consistent with this notion, cellulosic pellicles of Acetobacter grown so as to incorporate XyG into the pellicles were much more extensible than those grown without XyG (Whitney et al., 1999; Chanliaud et al., 2002). Based on these results, one might expect the lack of XyG in Arabidopsis walls to lead to more condensed, less extensible walls because of increased interactions and friction between microfibrils, yet just the opposite result was found in stress/strain assays (Fig. 3; Cavalier et al., 2008). Other results based on 13C solid-state NMR (Bootten et al., 2004, 2009; Dick-Pérez et al., 2011) likewise raise doubts that much of the cellulose surface is coated with XyG, as commonly depicted. Thus, this aspect of xyloglucan function in wall architecture is not well supported and needs further testing and refinement.

Xyloglucan and α-Expansin Action

Our results also provide new evidence that XyG is an important wall component for efficient wall loosening by α-expansins, and this dependency offers an explanation for the reduced growth phenotype of the xxt1/xxt2 line. These conclusions are supported by the reduced acid-induced extension of xxt1/xxt2 walls (mediated primarily by endogenous α-expansins), the reduced response of xxt1/xxt2 walls to exogenous α-expansins, and the diminished growth response of excised petioles to fusicoccin, which induces a strong acid-growth response (Rayle and Cleland, 1992). Previous work also implicated a link between α-expansin and XyG based on in vitro binding (McQueen-Mason and Cosgrove, 1995) and on the loosening action of α-expansin with Acetobacter pellicles (Whitney et al., 2000). However, these are rather indirect indicators of endogenous XyG function, and our results here provide stronger evidence that wall loosening by α-expansins indeed involves XyGs.

These data are consistent with the idea that α-expansin operates on a cellulose-xyloglucan complex that links adjacent cellulose microfibrils together (McQueen-Mason and Cosgrove, 1995). Alternatively, it is possible that the presence of XyG modifies the cellulose network structure such that it is more efficiently loosened by α-expansin. Previous work showed that α-expansin (McQueen-Mason and Cosgrove, 1994), as well as bacterial expansin (Georgelis et al., 2011), can weaken pure cellulose networks (filter paper), indicating that XyG presence is not an absolute requirement for expansin’s loosening actions. The strong initial but transient extension observed in the xxt1/xxt2 walls upon treatment with exogenous α-expansin (Fig. 4B) may result from rapid loosening of cellulose-cellulose junctions in the wall, with the loosening of a cellulose-XyG structure coming later in time, but other considerations may also come into play. The xxt1/xxt2 walls were reduced in their responses to both acidic buffer and exogenous α-expansin (Fig. 4), but not to the same degree. It is difficult to compare the two results quantitatively, because the two assays differ in important ways. For the assays involving exogenous α-expansin, the walls were briefly heated to inactivate endogenous expansins, potentially changing the physical state of the cell wall. Additionally, the cucumber α-expansin preparation used in the assay differed in its composition (type and amount of expansins as well as potentially other wall proteins that might modify the creep response) from the endogenous, wall-bound Arabidopsis expansins that mediate the response to acidic buffer in the acid-extension assays. Despite these differences in conditions, the results are consistent in implicating XyG as an important,
albeit not essential, player in the process of wall loosening by α-expansins.

In a similar vein, we interpret the diminished stress relaxation of xxt1/xxt2 walls (Fig. 5) as the result of diminished α-expansin action in the XyG-deficient walls. The difference in relaxation, spanning quite a range of log time, is remarkably similar to the results found with heat-inactivated walls with or without α-expansin from tomato (Solanum lycopersicum) and cucumber seedlings (Keller and Cosgrove, 1995; McQueen-Mason and Cosgrove, 1995). The long time span indicates that the polymer rearrangement by α-expansin is a relatively slow process and probably involves large-scale polymer motions, which is consistent with the slow time course of cell wall creep induced by expansin. With our results, there is an additional technical issue to consider, namely that the more extensible xxt1/xxt2 walls must have undergone greater elastic and plastic strain at the start of the stress relaxation measurement compared with wild-type walls. Could the greater strain and/or higher extensibility have directly caused the slower subsequent relaxation? This alternative seems unlikely in view of previous experiments, where cucumber walls were digested with an endoglucanase prior to the stress relaxation assay (Yuan et al., 2001). The enzyme-treated walls had increased plastic and elastic extensibilities (compliances) and, like the xxt1/xxt2 walls, underwent larger strains during the initial phase of the relaxation measurement, but stress relaxation was not affected in the time span where α-expansins act. Thus, we conclude that the slower relaxation of xxt1/xxt2 walls stems from reduced α-expansin action.

What Replaces XyG?

Our results also indicate that in the absence of XyG, other matrix components assume a larger share of the mechanical load of the wall, with pectic polysaccharides and arabinoxylans being the prime candidates in this regard, based on the relatively larger responses of xxt1/xxt2 walls to treatments that affect these wall components. However, the effectiveness of these different treatments for cutting or solubilizing matrix components is unlikely to be equal, so one cannot use these results to draw quantitative conclusions about the relative importance of pectins versus arabinoxylans. Nevertheless, our results support a different picture of the primary cell wall than what is commonly discussed. Instead of a cellulose network cross-linked by XyG tethers, our results point to a shared load-bearing function by XyG, pectins, and arabinoxylan. Related concepts have recently been discussed by others, based on in vitro binding (Zykwinska et al., 2005) and on 13C solid-state NMR (Dick-Pérez et al., 2011). Other studies have pointed to examples of primary cell walls with low XyG contents, such as celery (Apium graveolens) parenchyma (Thimm et al., 2002), leading to questions about the role of XyG. The value of the biomechanical tests used here is that they directly demonstrate mechanical significance for these wall components, whereas binding assays and NMR results merely admit the potential for biomechanical significance but do not actually demonstrate it. We did not find evidence that wall structural proteins contribute to cell wall mechanics in this tissue, but other recent results indicate a role for glycoproteins in early wall formation. An Arabidopsis mutation in a hydroxyproline-rich glycoprotein (HRGP) was found to be embryo lethal, evidently due to a failure of cell plate formation (Cannon et al., 2008), indicating a role in stabilizing the nascent wall, perhaps via pectin interactions. Such a function is also supported by results indicating that O-glycosylation of HRGPs is crucial for wall assembly in root hairs (Velasquez et al., 2011). A defect in the glycosylation of HRGPs was also implicated in the longer hypocotyl phenotype of a glycosyltransferase mutant (Gille et al., 2009). If this phenotype were due to reduced cross-linking by HRGPs, one would predict weaker walls in the mutant, but this prediction was not borne out in stress/strain tests of mutant hypocotyls (I. Burgert, personal communication).

Finally, our results point out the advantage of using multiple biomechanical assays to dissect the complex notion of wall extensibility. In the case of xxt1/xxt2 plants, the cell walls are mechanically weaker in assays of breaking strength and tensile modulus but stronger (less extensible) in other assays of their ability to undergo the more complex processes of wall creep, stress relaxation, and extension growth. The concept of wall extensibility thus has multiple dimensions and is not captured in the results of a single type of assay.

MATERIALS AND METHODS

Plant Growth and Petiole Measurements

The Arabidopsis (Arabidopsis italiana) xxt1/xxt2 double mutant seeds were obtained from the Arabidopsis Biological Resource Center (stock no. CS16349) deposited by Cavalier et al. (2008). After 4 d of cold treatment at 4°C, wild-type (ecotype Columbia) and mutant seedlings were grown on 1× MS medium (Murashige and Skoog, 1962) containing 1% Suc for 1 week, transferred to soil, and grown for 3 more weeks (day/night, 16/8 h; temperature, 22°C/16°C). Petioles from fifth to eighth leaves were harvested and stored at −80°C. To measure petiole geometry, free-hand cross-sections of fresh petioles were made from the middle of the petiole, photographed with a light microscope (Zeiss), and cross-sectional area was measured from digital photographs using the Scion Image tool (NIH Image).

Wall Extension (Creep) Assays

One-centimeter segments were cut from the middle of frozen petioles and abraded by rubbing the petiole surface 10 times with a carborundum slurry (300-mesh size; washed extensively before use) to facilitate buffer and protein penetration. The segments were pressed under a weight for 5 min to remove residual cell fluids and to flatten the wall sample, which was clamped (5 mm between clamps) in a constant-load extensometer under a constant tension of 10 g (Cosgrove, 1989; Durachko and Cosgrove, 2009). For acid-induced extension, the sample cuvette was initially filled with 250 μL of 50 mM HEPEs buffer (pH 6.8), and after 30 min, this was switched to 20 mM sodium acetate (pH 4.5). Also, wall creep responses to EDTA (5 mM) in 50 mM HEPEs (pH 6.8) and NaOH (60 mM) were tested under a constant force of 10 g. For studies of protein-induced creep, pressed wall specimens were heat inactivated in boiling water for 12 s immediately prior to clamping in the
extensometer under a constant force of 5 g. The sample cuvettes were filled with 250 μL of 20 mM sodium acetate (pH 4.5–5.5) including 5 mM dithiothreitol for 30 min, replaced with the same buffer containing a specific wall-loosening protein, crude cucumber (Cucumis sativus) α-expansins (500 μg mL⁻¹, pH 4.5), maize (Zea mays) pollen extracts, ZmEXPB1, bacterial expansin BeEXLX1 (all three delivered at 150 μg mL⁻¹, pH 5.5), xylanase M4 (from Aspergillus niger; Megazyme E-XYAN4; 80 μg mL⁻¹, pH 5.0), pectate lyase (catalytic domain from Colletbrosia japonicus); Megazyme E-PLYC); 20 μg mL⁻¹, 20 μs Tris-HCl, pH 8.5), arabinanase (from A. niger; Megazyme E-EARAB; 100 μg mL⁻¹, pH 5.0), and polygalacturonase (from Aspergillus japonicus; Sigma P3304; 2.6 μg mL⁻¹, pH 5.0). Protein concentrations were either based on supplier data or were measured with the protein quantification method of Bradford (1976) using bovine serum albumin as the protein standard.

Expansin Protein Preparation

α-Expansin

Wall proteins were extracted from cucumber hypocotyl walls as described (McQueen-Mason et al., 1992). The crude wall protein (ammonium sulfate pellet) was dissolved in 20 mM sodium acetate (pH 4.5) and was used at 500 μg mL⁻¹ protein concentration without further fractionation.

β-Expansin

Approximately 10 g of maize pollen was extracted at 4°C for 1 h in 30 mL of 50 mM sodium acetate (pH 4.5). The pollen extract was centrifuged at 17,000 × g at 4°C for 30 min to remove pollen debris, and the supernatant was filtered through a 0.45-μm filter. The supernatant was stored at 4°C. A concentrated sample (14% protein, pH 5.0) was used in the experiments, without further purification.

Bacterial Expansin

EXLX1 from Bacillus subtilis was expressed in Escherichia coli and purified as described (Kerf et al., 2008). It was used at 150 μg mL⁻¹ protein concentration in 20 mM sodium acetate (pH 5.5).

Stress/Strain and Stress Relaxation Assays

Stress/strain analysis was performed as described (Yuan et al., 2001). Frozen and thawed petiole specimens were kept on ice and incubated in 50 mM sodium acetate (pH 6.8) until the analysis was complete. Specimens were clamped with 5 mm between upper and lower clamps and reversibly extended in two cycles at 3 mm min⁻¹ until a force of 10 g was reached and immediately returned to zero force. For each of the two extensions, a second-degree polynomial was fitted to the stress/strain data, and the fitted polynomial was then used to calculate the slope (compliance) at the end of the cycle (Cosgrove, 1989).

For stress relaxation assays, petioles, prepared as above, were incubated at 4°C for 10 min in 50 mM sodium acetate buffer (pH 4.5), rapidly extended in a custom-made extensometer to a holding force of 10 g, and the subsequent decay in force (measured as gram force) was monitored for 5 min. The relaxation rates were calculated and graphed on a log time scale, which represents a first approximation to the relaxation spectrum (Cosgrove, 1989).

Fusicoccin-Induced Growth

Petioles from fifth to seventh leaves (not counting cotyledons) from 3-week-old plants were excised, and a 5-mm section from the mid region was cut. Segments were incubated at 27°C under room lighting in 0.5 mM Ca(OH)₂ and 2 mM MES with or without 2 μM fusicoccin (Sigma), with pH initially adjusted to 6.0 with KOH. The length of the excised petioles was measured with the aid of a dissecting microscope at 0, 2, 4, and 6 h. Petioles that became submerged during incubation were not used because cell growth strongly diminishes under anaerobic conditions (Hager, 2003). Fusicoccin was diluted with isopropanol to 1 mM stock concentration. A concentration of less than 0.5% isopropanol does not affect cell growth and elongation (Tode and Lüthen, 2001). The lengths of petiole segments were measured with image measuring software (SPOT Insight).

MALDI-TOF and HPAEC-PAD Analysis

Cell walls were prepared from aerial tissues of 28-d-old wild-type and xxt1/xxt2 plants as alcohol-insoluble residues (Zablackis et al., 1995; Tabuchi et al., 2001). Tissues of about 0.5 g fresh weight were boiled in 95% ethanol for 5 min, washed three times with 95% ethanol, then washed three times with ice-cold deionized distilled water, followed by homogenization by mortar and pestle with a minimal volume of deionized distilled water. Each homogenate was centrifuged at 7,000 × g at 4°C for 15 min and sequentially treated with 1.5% and 0.5% SDS to remove proteins. The recovered cell walls were consecutively washed with acetone, methanol:chloroform (1:1, v/v), 100% ethanol, and deionized distilled water three times in each solution. To measure the dry weight of wall residues (about 10 mg), each sample was dried overnight at 50°C. Petioles were extracted three times with 50 mM cyclohexane diamine tetracetic acid (CDTA; pH 6.8) for at least 3 h at room temperature. To extract XyG, the residue after pectin removal was extracted three times with 4 M NaOH containing 20 mM NaBH₄ for at least 2 h at room temperature. The NaOH-soluble fraction was neutralized with glacial acetic acid, dialyzed against deionized distilled water, and lyophilized. The lyophilized material was incubated with 100 mM NH₄OAc (pH 5.0) containing 10 μg mL⁻¹ XEG (Novozymes; Pauly et al., 1999) and 0.02% NaN₃ at 27°C for 16 h. The enzyme reaction was terminated by boiling for 15 s, and the supernatant was dried to collect XGOs, which were dissolved in 80% ethanol. After centrifugation at 10,000 rpm for 5 min, the supernatant was dried and the residue was resuspended in deionized distilled water. XGOs were analyzed by MALDI-TOF mass spectrometry (Lerouxel et al., 2002) or by Dionex HPAEC analysis using a CarboPac PA-200 column at a flow rate of 0.5 mL min⁻¹ with a linear gradient condition ramping over 40 min from 0.1 M NaOH to a 0.1 M sodium acetate and 0.1 M NaOH mixture (1:1, v/v). XGOS were detected with a pulsed amperometric detector.

Immuno Dot-Blot Assay

This assay was carried out as described (Tabuchi et al., 2011). Wall matrix polysaccharides were extracted from a single petiole by incubation in 10 μL of 4 M NaOH containing 20 mM NaBH₄ overnight. The supernatant was collected and neutralized with 4 M of concentrated HCl. For XyG digestion, half the volume (7 μL) of each extract was digested with 1 μL of 200 μg mL⁻¹ XEG (Novozymes; Pauly et al., 1999) and 0.02% NaN₃ at 27°C for 16 h. The enzyme digestion was quenched using nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate (Sigma).

Supplemental Data

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

Supplemental Figure S1. Similar cross-sectional areas of wild-type and xxt1/xxt2 petioles.

Supplemental Figure S2. Creep responses of petiole walls to pectin-depolymerizing enzymes.

Supplemental Figure S3. Wall extension (creep) responses of wild-type and xxt1/xxt2 petioles to Pronase.

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LITERATURE CITED

Abasolo J, Eder M, Yamauchi K, Obel N, Reinecke A, Neumetzler L, Dunlop JW, Mouille G, Pauly M, Höfte H, et al (2009) Pectin may hinder the unfolding of xyloglucan chains during cell deformation: implications of the mechanical performance of Arabidopsis hypocotyls with pectin alterations. Mol Plant 2: 901–909

Albersheim P, Darvill A, Roberts K, Sederoff R, Staehelin A (2010) Plant Cell Walls. Garland Science, New York, pp 227–272

Anderson CT, Carroll A, Akhmetova L, Somerville C (2009) Real-time imaging of cellulosome reorientation during cell wall expansion in Arabidopsis roots. Plant Physiol 152: 787–796

Bootten TJ, Harris PJ, Melton LD, Newman RH (2004) Solid-state 13C-NMR spectroscopy shows that the xyloglucins in the primary cell walls of mung bean (Vigna radiata L.) occur in different domains: a new model for xyloglucan-cellulose interactions in the cell wall. J Exp Bot 55: 571–583

Bootten TJ, Harris PJ, Melton LD, Newman RH (2009) Solid-state 13C NMR study of a composite of tobacco xyloglucan and Glucanacetobacter xylinus cellulose: molecular interactions between the component polysaccharides. Biomacromolecules 10: 2961–2967

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254

Burton RA, Gidley MJ, Fincher GB (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15: 121–130

Carpita NC, Gibeaut DM (1995) Architecture of the primary cell wall. In CW Lloyd, ed, The Cytoskeletal Basis of Plant Growth and Form. Academic Press, London, pp 109–129

Cassab AP, DeSantis AR, Parry MCD, S zuiker R, Kieber JD (2006) Hemicelluloses and cell expansion. J Exp Bot 57: 1527–1537

Chen L, Wegenhart BL, Li LC, Bedinger PA, Volk C, Jones AD, Cosgrove DJ (2002) Rapid structural phenotyping of plant cell wall mutants by enzymatic oligosaccharide fingerprinting. Plant Physiol 130: 1754–1763

Chu HT, Cosgrove DJ (2000) Altered expression of expansin modulates leaf growth and pedicel abscission in Arabidopsis thaliana. Proc Natl Acad Sci USA 97: 7973–7978

Cosgrove DJ (1989) Characterization of long-term extension of isolated cell walls from growing cucumber hypocotyls. Plant Physiol 87: 121–130

Cosgrove DJ (2000) Loosening of plant cell walls by expansins. Nature 407: 321–326

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

Darley CP, Forrester AM, McQueen-Mason SJ (2001) The molecular basis of plant cell wall extension. Plant Mol Biol 47: 179–195

Dick-Pérez M, Zhang Y, Hayes J, Salazar A, Zabotina OA, Pauly M (2006) Structural and interactions of plant cell-wall polysaccharides by two- and three-dimensional magic-angle-spinning solid-state NMR. Biochemistry 50: 989–1000

Ding SY, Himmel ME (2006) The maize primary cell wall microfibril: a new model derived from direct visualization. J Agric Food Chem 54: 597–606

Durachko DM, Cosgrove DJ (2009) Measuring plant cell wall extension (creep) induced by acidic pH and by alpha-expansin. J Vis Exp 11: 1263

Fanutti C, Gidley MJ, Reid JS (1993) Action of a pure xyloglucan endotransglycosylase (formerly called xyloglucan-specific endo-(1→4)-beta-D-glucanase) from the cotyledons of germinated nasturtium seeds. Plant J 3: 1–10

Fry SC (1989) The structure and functions of xyloglucan. J Exp Bot 40: 1–11

Fry SC, Smith RC, Renwick KE, Martin DJ, Hedge SK, Matthews KJ (1992) Xyloglucan endotransglycosylase, a new wall-loosening enzyme activity from plants. Biochem J 282: 821–828

Fujino T, Sone Y, Mitsuishi Y, Itoh T (2000) Characterization of cross-links between cellulose microfibrils, and their occurrence during elongation growth in pea epicotyl. Plant Cell Physiol 41: 486–494

Georgelis N, Tabuchi A, Nikoladis N, Cosgrove DJ (2011) Structure-function analysis of the bacterial expansin EXLX1. J Biol Chem 286: 16814–16823

Gille S, Hänsel U, Ziemann M, Pauly M (2009) Identification of plant cell wall mutants by means of a forward chemical genetic approach using hydroxylases. Proc Natl Acad Sci USA 106: 14699–14704

Ha MA, Apperele DC, Jarvis MC (1997) Molecular rigidity in dry and hydrated onion cell walls. Plant Physiol 115: 593–598

Hager A (2003) Role of the plasma membrane H+-ATPase in auxin-induced elongation growth: historical and new aspects. J Plant Res 116: 483–505

Hayashi T (1989) Xyloglucans in the primary cell wall. Annu Rev Plant Physiol Plant Mol Biol 40: 139–168

Keegestra K, Talmadge KW, Bauer WD, Albersheim P (1973) The structure of plant cell walls. III. A model of the walls of suspension-cultured sycamore cells based on the interconnections of the macromolecular components. Plant Physiol 51: 188–197

Keller E, Cosgrove DJ (1995) Expansins in growing tomato leaves. Plant J 8: 795–802

Koepf F, Amoroso A, Herman R, Sauvage F, Petrella S, Filet P, Charlier P, Jones AD, Pauly M, et al. (2008) Crystal structure and activity of Bacillus subtilis Yol (EXLX1), a bacterial expansin that promotes root colonization. Proc Natl Acad Sci USA 105: 16876–16881

Lerouxel O, Choo TS, Sévénon M, Usadel B, Faye L, Lerouge P, Pauly M (2002) Rapid structural phenotyping of plant cell wall mutants by enzymatic oligosaccharide fingerprinting. Plant Physiol 130: 1754–1763

Li LG, Bellanger FA, Volk C, Jones AD, Cosgrove DJ (2003) Purification and characterization of four α-expansins (Ze a m isofoms) from maize pollen. Plant Physiol 132: 2073–2085

McCann MC, Knox JP (2011) Plant cell wall biology: polysaccharides in architectural and developmental contexts. Annu Rev Plant 41: 343–366

McCann MC, Roberts K (1991) Architecture of the primary cell wall. In CW Lloyd, ed, The Cytoskeletal Basis of Plant Growth and Form. Academic Press, London, pp 57–78

McCann MC, Wells B, Roberts K (1990) Direct visualization of cross-links in the primary cell wall. J Cell Sci 96: 323–334

McQueen-Mason S, Cosgrove DJ (1994) Disruption of hydrogen bonding between plant cell wall polymers by proteins that induce wall extension. Proc Natl Acad Sci USA 91: 6574–6578

McQueen-Mason S, Durachko DM, Cosgrove DJ (1992) Two endogenous proteins that induce cell wall extension in plants. Plant Cell 4: 1425–1433

McQueen-Mason SJ, Cosgrove DJ (1995) Expansin mode of action on cell walls: analysis of wall hydrolysis, stress relaxation, and binding. Plant Physiol 107: 87–100

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol Plant 15: 473–497

Obel N, Neumetzler L, Pauly M (2006) Hemicelluloses and cell expansion. In JP Verbelen, K Vissenberg, eds. The Expanding Cell. Springer, Berlin, pp 109–129

Pauly M, Andersen LN, Kauppinen S, Kofod LV, York WS, Albersheim P, Darvill A (1999) A xyloglucan-specific endo-beta-1,4-glucanase from Aspergillus aculeatus: expression cloning in yeast, purification and characterization of the recombinant enzyme. Glycobiology 9: 93–100

Raleff MC, Crépeau MJ, Lefebvre J, Mouille G, Höfte H, Thibault JF (2008) Reduced number of homogalacturanon domains in pectins of an Arabidopsis mutant enhances the flexibility of the polymer. Biomacromolecules 9: 1454–1460

Rayle DL, Cleland RE (1992) The acid growth theory of auxin-induced cell elongation is alive and well. Plant Physiol 99: 1271–1274

Rose JK, Braam J, Fry SC, Nishitani K (2002) The XTH family of enzymes involved in xyloglucan endotransglucosylation and endohydrolase: current perspectives and a new unifying nomenclature. Plant Cell Physiol 43: 1421–1435

Scheller HV, Ulvskov P (2010) Hemicelluloses. Annu Rev Plant Biol 61: 263–289

Somerville C, Bauer S, Brininstool G, Facette M, Hamann T, Milne J, Osborne E, Fareeda E, Persson S, Raab T, et al (2004) Toward a systems approach to understanding Arabidopsis cell walls. Science 306: 2220–2223

Suen DF, Huang AH (2007) Maize pollen coat xylanase facilitates pollen tube penetration into silk during sexual reproduction. J Biol Chem 282: 625–636

Tabuchi A, Li LC, Cosgrove DJ (2011) Matrix solubilization and cell wall

474

Plant Physiol. Vol. 158, 2012

Park and Cosgrove
weakening by β-expansin (group-1 allergen) from maize pollen. Plant J 68: 546–559

Tabuchi A, Mori H, Kamisaka S, Hoson T (2001) A new type of endo-xyloglucan transferase devoted to xyloglucan hydrolysis in the cell wall of azuki bean epicotyls. Plant Cell Physiol 42: 154–161

Talbott LD, Ray PM (1992) Molecular size and separability features of pea cell wall polysaccharides: implications for models of primary wall structure. Plant Physiol 98: 357–368

Thimm JC, Burritt DJ, Ducker WA, Melton LD (2000) Celery (Apium graveolens L.) parenchyma cell walls examined by atomic force microscopy: effect of dehydration on cellulose microfibrils. Planta 212: 25–32

Thimm JC, Burritt DJ, Sims IM, Newman RH, Ducker WA, Melton LD (2002) Celery (Apium graveolens) parenchyma cell walls: cell walls with minimal xyloglucan. Physiol Plant 116: 164–171

Tode K, Lüthen H (2001) Fusicoccin- and IAA-induced elongation growth share the same pattern of K" dependence. J Exp Bot 52: 251–255

Velásquez SM, Ricardi MM, Dorosz JG, Fernandez PV, Nadra AD, Pol-Fachin L, Egelund J, Harholt J, Ciancia M, et al (2011) O-Glycosylated cell wall proteins are essential in root hair growth. Science 332: 1401–1403

Whistler RL, Bemiller JN (1958) Alkaline degradation of polysaccharides. Adv Carbohydr Chem 13: 289–329

Whitney SE, Gidley MJ, McQueen-Mason SJ (2000) Probing expansin action using cellulose/hemicellulose composites. Plant J 22: 327–334

Whitney SE, Gothard MG, Mitchell JT, Gidley MJ (1999) Roles of cellulose and xyloglucan in determining the mechanical properties of primary plant cell walls. Plant Physiol 121: 657–664

Yennawar NH, Li LC, Dudzinski DM, Tabuchi A, Cosgrove DJ (2006) Crystal structure and activities of EXPB1 (Zea m 1), a beta-expansin and group-1 pollen allergen from maize. Proc Natl Acad Sci USA 103: 14664–14671

Yuan S, Wu Y, Cosgrove DJ (2001) A fungal endoglucanase with plant cell wall extension activity. Plant Physiol 127: 324–333

Zablackis E, Huang J, Müller B, Darvill AG, Albersheim P (1995) Characterization of the cell-wall polysaccharides of Arabidopsis thaliana leaves. Plant Physiol 107: 1129–1138

Zykwinska AW, Ralet MC, Garnier CD, Thibault JF (2005) Evidence for in vitro binding of pectin side chains to cellulose. Plant Physiol 139: 397–407

Zykwinska AW, Thibault JF, Ralet MC (2007) Organization of pectic arabinan and galactan side chains in association with cellulose microfibrils in primary cell walls and related models envisaged. J Exp Bot 58: 1795–1802