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Xylan Metabolism in Primary Cell Walls†

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Abstract

Xylans are hemicellulosic polysaccharides consisting of a (1→4)-β-D-xylan backbone, which is substituted in all land plants with short side chains (usually monosaccharide) of α-L-arabinose and/or α-D-glucuronic acid residues. The glucuronic acid residues are frequently 4-*O*-methylated. Xylans are found in the primary cell walls of all higher plants, and are a major component of secondary cell walls. Highly substituted glucuronoarabinoxylans are the major cross-linking structural hemicellulose in the primary walls of monocots of the commelinoid group, except in growing tissues of the grasses (Poales) where their function is temporarily replaced by a mixed linkage (1→3),(1→4)-β-D-glucan. In non-commelinoid monocots and dicots, glucuronoarabinoxylans are a minor component of primary cell walls, and xyloglucan is the major structural hemicellulose. During cell growth in grasses, the degree of substitution of the xylan backbone declines and xylans become increasingly firmly attached into the wall by ester and ether links. In dicots, a proportion of the xylan may become increasingly associated with pectin and xyloglucan in complexes as growth ceases. In both grasses and dicots, evidence for depolymerisation of the xylan backbone during the wall loosening associated with growth is lacking. In cereal grains, germination is accompanied by complete degradation of arabinoxylan-rich primary cell walls, involving substantial cytosolic endo-(1→4)-β-xylanase activity. This is, however, a specialised case of programmed cell death rather than cell wall loosening for growth. Nevertheless, *Arabidopsis* possesses four putative endo-(1→4)-β-xylanase proteins with a predicted signal sequence for secretion to the apoplast, suggesting that xylan depolymerisation is involved in some aspects of plant development. A large increase in endo-(1→4)-β-xylanase activity accompanies fruit ripening in papaya, indicating that in some species xylan depolymerisation may be a component of the wall disassembly leading to fruit softening.

Keywords: arabinoxylan; cell expansion; endo-(1→4)-β-xylanase; fruit softening; glucuronoarabinoxylan; glucuronoxylan.

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Introduction

Plant cell walls vary in composition and structure between tissue types and between species, and particularly between primary and secondary walls. In all cases, however, wall architecture has as its basis a lattice of cellulose microfibrils tethered by hemicelluloses. The hemicelluloses, or cross-linking glycans, are a group of polysaccharides composed largely of neutral sugars whose role is to cross-link cellulose microfibrils into a strong network and thus provide rigidity and mechanical strength to the wall (Talbott & Ray, 1992a; Carpita & Gibeaut 1993) to allow it to resist tensile and compressive forces. In the primary cell walls of gymnosperms and dicotyledonous and the non-commelinoid monocotyledonous angiosperms, the most abundant hemicellulose is xyloglucan; this is believed to carry out most of the cross linking (Fry, 1989; Hayashi, 1989; Carpita & Gibeaut, 1993), together with smaller amounts of various xylans and glucomannans (McNeil et al., 1975; Whitney et al., 1998). In the primary walls of commelinoid monocots, xyloglucan is a minor component and a cross-linking role is carried out mainly by heteroxylan (Carpita & Gibeaut, 1993; Carpita, 1996; Vogel, 2008). Xylan is also the main cross-linking polysaccharide in algal cell walls, where it is linked to charged glycoproteins (Deniaud et al., 2003). As will be discussed in the following sections, the primary wall is a dynamic structure whose components are modified to allow cell growth and development. Such modifications occur principally to the hemicelluloses, although the polymers involved may vary with species, tissue and developmental stage.

Xylans are found at high abundance in secondary cell walls, where they are a major component of secondary thickening (Ebringerová & Heinze, 2000). Glucuronoxylans play an important structural role in secondary walls, since they form tight attachments to cellulose (Awano et al., 2002) and become covalently bonded to lignin (Iiyama et al., 1990, 1994; Hatfield et al., 1999). Due to their abundance in the secondary walls of hardwoods and softwoods, where they form up to 20-30% of the biomass, xylans are probably the second most abundant organic biopolymer on earth after cellulose (Ebringerová & Heinze, 2000). Xylans appear to be absent in non-vascular land plants (liverworts and mosses) with the exception of specific cells of the sporophyte of hornworts, a group closely related to the tracheophytes (Carafa et al., 2005). The ubiquitous occurrence of xylans in the secondary cell walls of all present-living species of tracheophytes, but not in bryophytes, suggests that the development of xylan may have been a pivotal event for the evolution of the vascular tissues that allowed the development of large plants able to live in water-limited habitats (Carafa et al., 2005).

Although the structure of xylans from various sources has been well studied, the emphasis has been on the abundant xylans found in the secondary walls of dicot hardwoods, monocot grains and various gums and mucilages (Ebringerová & Heinze, 2000; Ebringerová et al., 2005). Secondary cell wall xylans are abundant and structurally important to the mechanical strength of the wall, but when extensively cross-linked by lignins (Iiyama et al., 1994) are essentially inert. The structure and properties of primary cell wall xylans are less well known, particularly in dicots, as are the potential modifications that occur during cell wall loosening to allow growth and during cell wall disassembly to enable fruit softening. This review will survey some of the limited data available concerning primary wall xylans involved in these processes. Readers interested in the details of xylan structure or biosynthesis should consult excellent recent reviews by Ebringerová et al. (2005), and York and O'Neill (2008), respectively.

Structure of Xylans

Xylans are a divergent group of polymers possessing a backbone of β-linked D-xylopyranosyl (Xyl) residues (Table 1). Although homoxylans do exist, as (1→3)-β-D-xylan or a mixed linkage (1→3),(1→4)-β-D-xylan in some seaweeds (Deniaud et al., 2003; Ebringerová et al., 2005), in land plants xylans are present as heteroxylans consisting of a backbone of (1→4)-β-D-xylan, substituted to some extent usually with short side chains of either α-L-arabinose (Ara) or α-D-glucuronic acid (GlcA) residues, or both, and are often partially *O*-acetylated (Ebringerová et al., 2005). Five examples of xylan structure are shown in Figure 1. Glucuronoxylans have monosaccharide (1→2)-α-D-GlcA residues attached to the main chain Xyl units, and this α-D-GlcA side chain can be either non-methylated or 4-*O*-methylated (Figure 1a). A 4-*O*-methyl-D-glucurono-β-D-xylan is the main cellulose-linking hemicellulose in the secondary walls of gymnosperms and dicot hardwoods. Such 4-*O*-methyl-D-glucurono-β-D-xylans are usually acetylated on the Xyl residues at O-2 or O-3, and the degree of acetylation varies considerably with species and tissue but in hardwoods ranges from 0.3 to over 0.6 (Teleman et al., 2002; Evtuguin et al., 2003; Goncalves et al., 2008). Acetylation of xylan may reduce polymer solubility and water content of the wood, increasing the hydrophobic character of secondary walls (Grondahl et al., 2003).

Arabinoxylans possess monosaccharide α-L-Ara residue side chains, which can be linked (1→2) or (1→3) to the main chain Xyl units, or both (Figure 1c). This disubstitution can allow quite high rates of substitution with Ara, which in rye grain can be as high as 1.3 Ara per Xyl (Nilsson et al., 1996). Arabinoxylans are typical of grasses and cereal grains such as wheat, barley, rye and maize, with α-L-Ara residues attached by (1→3) linkages rather than the (1→2) linkage found in dicots (Carpita, 1996).

Xylans can also possess substitution by both α-L-Ara

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TABLE 1: Structure and occurrence of xylans in plants

Polysaccharide	Backbone	Backbone substitution	Function and occurrence	Features
Homoxylan	(1→3)-β-D-Xyl or (1→3),(1→4)-β-D-Xyl residues	none	Structural in some algae.	Backbone not linear due to (1→3)-β-linkage.
Arabinoxylan	(1→4)-β-D-Xyl residues	(1→2) and/ or (1→3)-α-L-Ara residues	Structural/storage polysaccharide in seeds of grasses and cereal grains. Some sporophyte cells of hornworts.	Can be disubstituted (both (1→2)- and (1→3)-linked Ara on same Xyl). Ferulic acid or coumaric acid can be *O*-esterified to some Ara residues.
Glucuronoxylan	(1→4)-β-D-Xyl residues	(1→2)-α-D-GlcA residues	Secondary walls of hardwoods (methylated form). Various dicot seeds and fibres (methylated form).	Backbone often *O*-acetylated. GlcA residues can be 4-*O*-methylated
Glucuronoarabino-xylan (GAX)[1] and **Arabinoglucurono-xylan (AGX)[1]**	(1→4)-β-D-Xyl residues	(1→2)- and/ or (1→3)-α-L-Ara residues and (1→2)-α-D-GlcA residues.	GAX is a minor component of dicot primary walls and main hemicellulose in grasses. AGX in conifers and lignified grass tissues.	Ara residues mainly (1→2)-linked in dicots but mainly (1→3)-linked in grasses (some also feruloylated). GlcA residues can be 4-*O*-methylated.
Complex heteroxylans	(1→4)-β-D-Xyl residues	Ara and GlcA, plus complex side chains including: Gal-Ara-Gal-Xyl-Ara-Ara-Xyl-GlcA-Ara-Ara-Xyl-Ara-	Mainly found in seeds, gum exudates and mucilages. Corn bran has complex heteroxylan.	Structure characteristic of species/tissue. Side chains (1→2)- or (1→3)-linked. Soluble and highly viscous polymers. Some can form gels.

[1] *named with minor substituent first*

and α-D-GlcA residues, and these are referred to as arabinoglucuronoxylan or glucuronoarabinoxylan, depending on which is the dominant substituent. In both cases the side chains are almost always monosaccharides, either (1→2)-linked α-D-GlcA or 4-*O*-methylated α-D-GlcA residues, or α-L-Ara residues linked (1→2) or (1→3) to the main chain Xyl units. Arabinoglucuronoxylan with 4-*O*-methylated (1→2)-α-D-GlcA and (1→3)-α-L-Ara is found in coniferous softwoods. In primary walls of dicots and non-commelinoid monocots, the glucuronoxylan has α-L-Ara residues attached mostly by (1→2) linkages but occasionally by (1→3) linkages, and less frequently α-D-GlcA attached exclusively by (1→2)linkages (Carpita & McCann, 2000) (Figure 1d). In the commelinoid monocots, which includes the Poales (grass family) such as maize, the main hemicellulose in the growing primary cell wall of coleoptiles is glucuronoarabinoxylan with α-L-Ara residues attached by (1→3) linkages and underivatised α-D-GlcA residues attached by (1→2)

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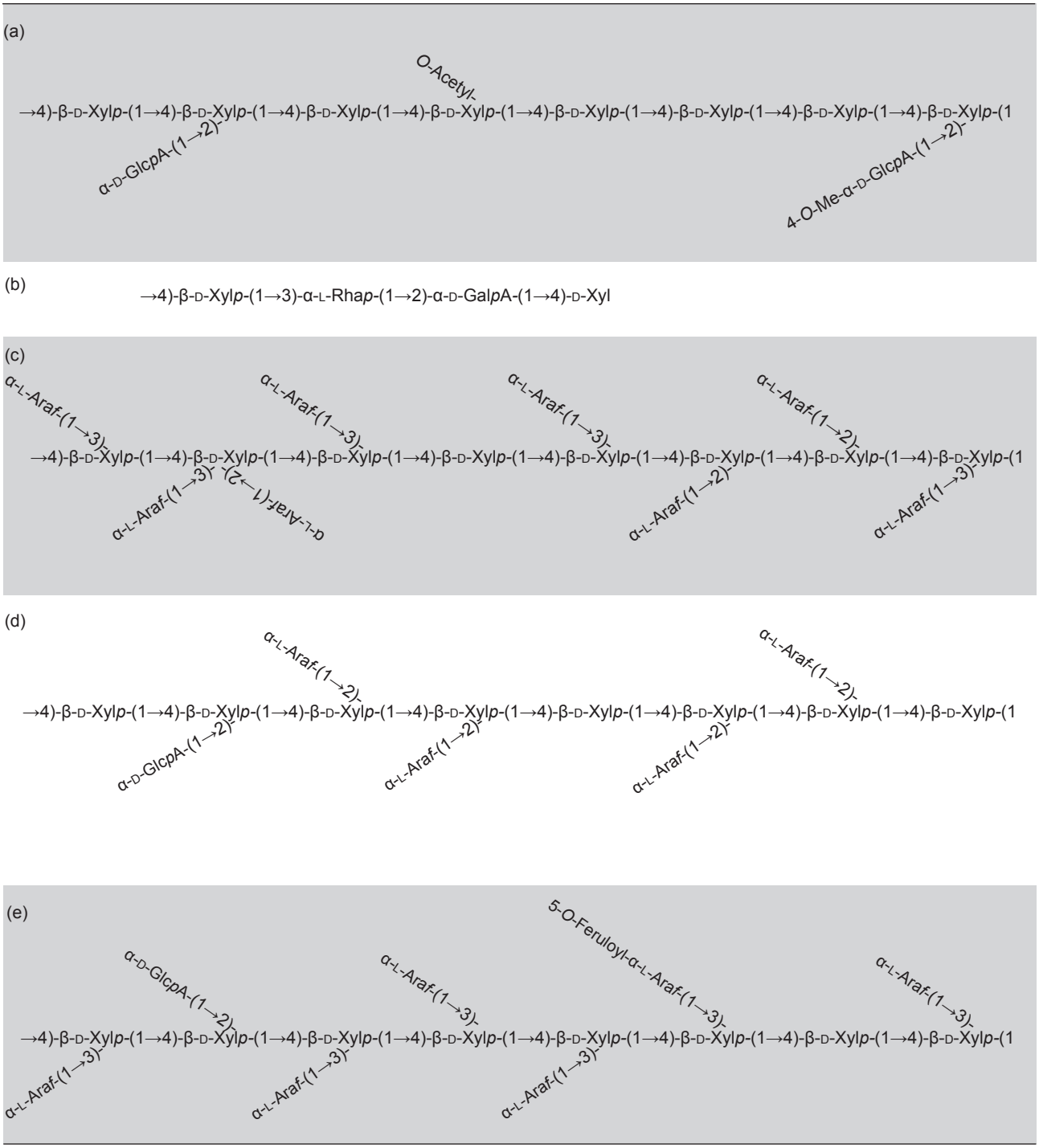

FIGURE 1: Examples of heteroxylans found in higher plants. The backbone consists of (1→4)-β-D-xylopyranosyl residues, a linkage that requires consecutive xylosyl residues to be orientated approximately 180° from each other. (a): glucuronoxylan of hardwood secondary walls. Approximately 1 xylosyl residue in 10 is glucuronylated; (b): the tetrasaccharide found at the reducing end of dicot glucuronoxylan; (c): the highly substituted arabinoxylan found in cereal grains (note disubstitution on some xylosyl backbone residues); (d): dicot primary wall glucuronoarabinoxylan. α-L-Ara is linked mainly to the O-2 position of the xylosyl units of the backbone, and α-D-GlcA exclusively to the O-2 position; and (e): commelinoid primary wall glucuronoarabinoxylan. α-L-Ara is linked exclusively to the O-3 position of the xylosyl units of the backbone, and α-D-GlcA to the O-2 position.

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linkages (Carpita, 1983, 1996; Carpita et al., 2001) (Figure 1e). In contrast, the predominant hemicellulose in lignified secondary cell walls of grasses is arabinoglucuronoxylan with a much-reduced degree of arabinosyl substitution and 4-*O*-methylated α-D-GlcA residues (Ebringerová et al., 1992).

More structurally complex xylans possessing disaccharide or larger side chains containing galactose (β-D-Gal), β-D-Xyl, α-L-Ara and α-D-GlcA residues are found in some gum exudates and mucilages (Ebringerová et al., 2005).

Biosynthesis of Xylan

Xylan synthase (UDP-D-Xyl:(1→4)-β-D-xylan xylosyltransferase) activity has been detected *in vitro* using membrane preparations from tissues of several species undergoing primary or secondary wall synthesis (e.g. Baydoun et al., 1989a; Kuroyama & Tsumuraya, 2001; Urahara et al., 2004). Recently, the study of mutants defective in xylem development has identified several genes involved in the biosynthesis of the glucuronoxylan that forms a major component of secondary cell walls. Five *Arabidopsis thaliana* (L.) Heynh. T-DNA knockout lines where xylem-specific glycosyltransferases (GT) had been disrupted all displayed an irregular xylem (*irx*) or fragile fiber (*fra*) mutant phenotype, combined with reduced xylan content of the walls (Brown et al., 2005, 2007; Zhong et al., 2005; Persson et al., 2007). Mutant *fra8* (also called *irx7*) was disrupted in a putative glycosyltransferase of family GT47, *irx8* and *parvus-3* in glycosyltransferases of family GT8, and *irx9* and *irx14* in glycosyltransferases of family GT43 (Brown et al., 2007). Recent re-discovery by Peña et al. (2007) that the reducing end of glucuronoxylan consists of the tetrasaccharide sequence →4)-β-D-Xyl*p*-(1→3)-α-L-Rha*p*-(1→2)-α-D-Gal*p*A-(1→4)-D-Xyl (Figure 1b) indicated that the biosynthesis of glucuronoxylan is more complex than originally perceived, and increased the number of glycosyltransferases required to synthesise this polymer to a minimum of six (possibly up to nine when considering that side chains need to be added and modified, and that arabinoxylan and glucuronoarabinoxylan may also need to be produced during development). Whether the tetrasaccharide acts as a primer for xylan synthesis (which is then extended from the non-reducing end) or is added to the reducing end of a chain to terminate elongation has not been resolved (York & O'Neill, 2008).

The decreased incidence of this tetrasaccharide sequence at the reducing end of xylan from *fra8*, *irx8* and *parvus-3* mutants indicates that the glycosyltransferases corresponding to FRA8, IRX8 and PARVUS-3 are involved in its synthesis (Brown et al., 2007; Lee et al., 2007a; Peña et al., 2007). PARVUS-3 has an endoplasmic reticulum location, and may be involved in the initiation of the oligosaccharide onto an unknown acceptor (Lee et al., 2007a). IRX8 and FRA8 are localised in the Golgi apparatus (Zhong et al., 2005; Peña et al., 2007), and IRX8 may be a glucuronyltransferase involved in transferring a α-D-GalA residue onto the O-4 of the reducing Xyl residue at the tetrasaccharide reducing end (Peña et al., 2007). FRA8 may transfer either β-D-Xyl to O-3 of Rha or α-L-Rha to O-2 of GalA during tetrasaccharide synthesis (Peña et al., 2007).

The tetrasaccharide was present in xylan from *irx9* and *irx14* mutants, indicating that the corresponding glycosyltransferases IRX9 and IRX14 are involved in other aspects of xylan synthesis (Brown et al., 2007; Peña et al., 2007). Xylan in the *irx9* mutant had a greatly reduced degree of polymerisation (Peña et al., 2007) and, in the presence of xylooligosaccharide acceptors, microsomes from *irx9* mutant plants were impaired in xylosyltransferase activity relative to wild-type (Brown et al., 2007; Lee et al., 2007b). These findings strongly suggest that IRX9 is a xylan xylosyltransferase involved in elongation of the xylan backbone. IRX14, which like IRX9 is in the GT43 family, may also be a xylan xylosyltransferase, and it is possible that IRX9 and IRX14 act as a complex to synthesise the xylan backbone (Brown et al., 2007).

Less is known about the side chain additions to xylan, but xylan arabinosyltransferase activity (Porchia et al., 2002) and glucuronyltransferase activity (Waldron & Brett, 1983; Baydoun et al., 1989a; Baydoun & Brett, 1997; Zeng et al., 2008) have been detected. It has been suggested that synthesis of the xylan backbone and the addition of α-D-GlcA side chains occur concurrently in the Golgi apparatus, and probably co-operatively (Baydoun et al., 1989a; Zeng et al., 2008). α-D-GlcA appears to be methylated after it is transferred onto the glucuronoxylan backbone (Baydoun et al., 1989b), with the efficiency of this process determining the ratio of GlcA to 4-*O*-methyl-GlcA sidechains (Peña et al., 2007).

Arrangement of Xylan in the Wall

The first model of primary cell wall structure proposed that cellulose microfibrils were tethered together by being hydrogen bonded to a complex matrix of hemicellulosic and pectic polysaccharides that were themselves covalently linked to one another (Keegstra et al., 1973). This proposal has been superseded by models in which the matrix polysaccharides interact mainly through non-covalent linkages. Two models of primary cell wall structure are commonly accepted. In the sticky network model (Fry, 1989; Hayashi, 1989; McCann et al., 1990; Carpita & Gibeaut, 1993), cellulose microfibrils are coated with hemicelluloses, which span between them and thus tether (or separate) the microfibrils. Pectin forms a co-extensive, entangled but largely separate network interspersed with the cellulose-xyloglucan network. In the multi-coat

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model (Talbott & Ray, 1992a), cellulose microfibrils are also coated with xyloglucan, but these are embedded in layers of less tightly bound hemicellulose and finally pectin molecules. The multi-coat model thus differs from the sticky network model in that the microfibrils are coated in layers of polysaccharides of decreasingly firm attachment, and connections between adjacent microfibrils are not accomplished by single molecules but by a series of interconnected molecules of different types. In this model, xyloglucan acts more like an 'adaptor', facilitating interaction between microfibrils and other polysaccharides.

Xyloglucan interacts strongly with cellulose *in vitro* (Hayashi et al., 1987; Atalla et al., 1993; Whitney et al., 1995), and in dicots, non-commelinoid monocots and gymnosperms is believed to be the major cross-linking hemicellulose (Hayashi & Maclachlan, 1984; Hayashi et al., 1987; Hayashi, 1989; McCann et al., 1990; Carpita & Gibeaut, 1993). This has been termed the Type I wall (Carpita & Gibeaut, 1993). Xylan is a minor component of the Type I wall, but xylan has also been reported to hydrogen bond to cellulose *in vitro* in a manner similar to, but weaker than, binding by xyloglucan (McNeil et al., 1975; Carpita, 1983; Hayashi et al., 1987). Although xylan has a linear (1→4)-β-linked backbone similar to the glucan backbone of cellulose and xyloglucan, xylose is a pentapyranose in which all the carbons are part of the heterocyclic ring. The necessary absence of 6-hydroxyls and their associated intra- and inter-chain hydrogen bonds means that xylans have a much reduced propensity for forming crystalline structures. The α-L-Ara residues present as side chains also reduce chain interactions, due to their highly-hydrated furanose conformation. Indeed, not all xylans will bind to cellulose. Glucuronoarabinoxylan from oat coleoptiles did not bind to cellulose powder *in vitro* (Labavitch & Ray, 1978), and glucuronoxylan from aspen secondary cell walls showed minimal interaction with bacterial cellulose in artificial composites (Dammström et al., 2005). It is, therefore, possible that only some forms of xylan (perhaps containing regions relatively low in substitution) can coat cellulose microfibrils and potentially form inter-microfibril cross links additional to those provided by xyloglucan. However, glucuronoxylan may bind to other hemicelluloses in the wall (Brett et al., 1997).

In the Type II cell wall of the commelinoid monocots (Poales and various other groups, including gingers and bromeliads such as pineapple), xyloglucan and pectin are minor wall components and glucuronoarabinoxylan is believed to provide the major load-bearing connections between cellulose microfibrils (Carpita & Gibeaut, 1993; Carpita, 1996; Harris et al., 1997; Vogel, 2008). In the special case of growing tissues of the Poales, an unbranched (1→3),(1→4)-β-D-glucan, present only during growth, may be the most important cross-linking polysaccharide (Carpita, 1996). Non-lignified primary cell walls contain aromatic substances that cross-link glucuronoarabinoxylans through ester and ether links (Carpita, 1996). At intervals, feruloyl groups and sometimes other hydroxycinnamic acids are esterified to the O-5 position of the α-L-Ara side chain units (Figure 1e). In newly-secreted arabinoxylans of the primary wall of maize cell suspension cultures, 1 in ~50 sugar residues possessed a ferulate residue, of which at least 30% were dimerised or bridged by larger coupling products (Fry et al., 2000). This shows that a proportion of arabinoxylan coupling by phenolics occurs intra-protoplasmically, and may be responsible for the creation of arabinoxylan complexes. Further cross-linking of feruloyl arabinoxylan occurred in the cell wall, probably mediated by apoplastic peroxidase (Encina & Fry, 2005), and was much more extensive in older cells than in younger cells (Fry et al., 2000; Lindsay & Fry, 2008). These phenolic cross-links help stabilise the Type II wall (Carpita & Gibeaut, 1993), perhaps being necessary due to the weaker interaction between glucuronoarabinoxylan and cellulose, and may be responsible for tightening the wall as growth ceases by locking microfibrils together.

Xylan Metabolism in Commelinoid Monocot Vegetative Tissues

The commelinoid monocots include the Poales, which contains many economically important cereals (such as barley, maize, wheat, rye and rice) and so has been well studied. In maize internodes or leaves, immunostaining with two anti-xylan antibodies showed that highly substituted glucuronoarabinoxylan was localised evenly in primary cell walls, in all tissues, and immunolabelling was particularly strong in phloem unlignified secondary cell walls (Suzuki et al., 2000; Carafa et al., 2005). Highly substituted glucuronoarabinoxylan was much less abundant in lignified secondary cell walls. In contrast, a low substitution xylan was abundant in all lignified tissue types, including leaf, and its presence correlated with lignification (Suzuki et al., 2000). These findings suggest that two different types of xylan, differing in substitution pattern, function in primary and lignified secondary cell walls of commelinoid monocots. It is possible that different suites of genes are responsible for their synthesis, as is the case for cellulose synthesis in primary and secondary walls (Somerville, 2006).

Most studies on primary cell wall metabolism and wall loosening in commelinoid monocots have been carried out using the coleoptile, the rapidly growing sheath for the developing first leaf. Despite being a specialised tissue found only in grasses, the coleoptile is an attractive model system due to its striking auxin-stimulated growth. In the Type II wall of the grasses, only approximately 2% of the dry weight of the primary wall is xyloglucan (McNeil et al., 1984; Vogel, 2008). In oat coleoptiles, glucuronoarabinoxylan provided at least 65% of the matrix (non-cellulosic component) of

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the wall (Wada & Ray, 1978). Its apparent molecular weight was estimated by sedimentation and intrinsic viscosity as ~200 kDa (Wada & Ray, 1978). In maize coleoptiles, glucuronoarabinoxylan was approximately 44% of the non-cellulosic cell wall polysaccharide in mesophyll cells and approximately 48% in the cellulose-rich epidermal cells (Carpita et al., 2001). Maize coleoptile glucuronoarabinoxylan could be fractionated into three groups depending on the strength of dilute alkali required for extraction (Carpita, 1983). The most loosely attached glucuronoarabinoxylan was highly substituted with α-L-Ara residues (Ara:Xyl ratio of ~0.85:1), whereas the most tightly bound fraction was less substituted and would bind to cellulose *in vitro*, presumably because of the presence of stretches of unbranched xylan available for hydrogen bonding (Carpita, 1983). A small proportion of the α-L-Ara residues possessed ferulic acid esters that were available to dimerise with other feruloyl groups to cross-link the glucuronoarabinoxylan molecules into a network (Carpita, 1996). Glucuronoarabinoxylans with a low degree of α-L-Ara residue substitution were found to be co-extensive with (1→3),(1→4)-β-D-glucans and glucomannans, in tight association with cellulose (Carpita et al., 2001). Glucuronoarabinoxylans with a high degree of α-L-Ara substitution (plus additional pectin and glucomannan) formed an interstitial domain that interconnected the coated microfibrils.

During rapid (auxin-stimulated) growth of coleoptiles, the deposition then degradation of (1→3),(1→4)-β-D-glucan, and the correlation of this with growth, has been well documented (Loescher & Nevins, 1972; Sakurai & Masuda, 1977; Carpita, 1984a; Inouhe & Nevins, 1991; Chen et al., 1999). This polymer is believed to provide temporary cross-linking of cellulose via hydrogen bonds during rapid growth, and is replaced as growth ceases by cross linking with arabinoxylan, which forms a more rigid network due to its di-ferulate dimers (Carpita & Gibeaut, 1993). In barley coleoptiles the proportion of arabinoxylan in the wall increased during growth (Sakurai & Masuda, 1978), and in oat coleoptiles there was still an increase in the Ara and Xyl content of the wall when exogenous glucose was not supplied, although auxin only slightly enhanced these increases (Loescher & Nevins, 1972). In maize coleoptiles and leaves, the amounts of highly substituted glucuronoarabinoxylan increased during early elongation (Carpita, 1984a; Gibeaut & Carpita, 1991). During subsequent tissue development the degree of substitution of the xylan chain with α-L-Ara and α-D-GlcA residues decreased markedly, resulting in a greater proportion of unbranched xylan (Darvill et al., 1978; Carpita, 1984a, b; Nishitani & Nevins, 1990; Gibeaut & Carpita, 1991). Similar observations have been made in wheat seedlings and barley coleoptiles (Obel et al., 2002; Gibeaut et al., 2005). These findings indicate that glucuronoarabinoxylan is synthesised in a highly branched form that is subsequently modified by loss of Ara (and possibly GlcA) residues. This presumably has the effect of reducing the solubility of the polymer, and increasing the potential for hydrogen bond-mediated cross-linking to other polysaccharides. Correlations between reduced substitution of glucuronoarabinoxylan and increased wall strength have been observed *in vivo*, presumably due to increased cellulose cross-linking (Ceusters et al., 2008).

Debranching of arabinoxylan is probably accomplished by α-arabinosidases. cDNAs encoding arabinoxylan α-arabinosidases (AXAH-I and AXAH-II) have been cloned from developing barley seedlings (Lee et al., 2001). These enzymes are in Glycosyl Hydrolase family 51 (GH51), and had activity against (1→2)-α- and (1→3)-α-Ara substituents of arabinoxylan, as well as against the (1→5)-α-Ara found in pectic arabinans. Barley also contains a bifunctional α-L-arabinosidase/β-D-xylosidase (ARA-I) and a β-D-xylosidase (XYL), both of the GH3 family (Lee et al., 2003). These latter enzymes were weakly active against wheat flour arabinoxylan, but showed greater activity against xylopentaose and against arabinoxylan oligosaccharides produced by endo-(1→4)-β-D-xylanase action. *ARA-I* mRNA was detected at low levels in developing grains, roots, coleoptiles and leaves, whereas *XYL* mRNA was present at high abundance in all tissues examined. All these enzymes possess signal sequences predicting an apoplastic localisation, and would be expected to be active in reducing the substitution of primary cell wall arabinoxylan or in the degradation of arabinoxylan fragments after the action of endo-(1→4)-β-D-xylanase.

Glucuronoarabinoxylans with long stretches of unsubstituted Xyl residues may be capable of hydrogen bonding to one another, cellulose and possibly other wall hemicelluloses. Although the loss of α-L-Ara residue side chains could decrease the cross-linking due to ferulic acid esters (which are attached to the α-L-Ara residues), the proportion of ferulic acid increased as growth rate declined (Nishitani & Nevins, 1990), and the proportion of ferulic acid dimers to ferulic acid increased (Obel et al., 2002). This may have helped stabilise the wall after the (1→3),(1→4)-β-D-glucan had been degraded. Treatment of maize coleoptile cell walls with chlorite to oxidise aromatic cross links followed by sodium hydroxide removed large amounts of glucuronoarabinoxylan from the wall, suggesting that glucuronoarabinoxylans are largely interconnected by aromatic residues (Carpita et al., 2001). As growth ceased, an increasing proportion of glucuronoarabinoxylan molecules appeared to become firmly enmeshed in the wall via etherified aromatics (Carpita, 1986). The increased amounts of ferulic acid and *p*-coumaric acid esters present as growth ceased and lignification began (Obel et al., 2002) perhaps reflect the changing nature of the wall since lignin cross-links to the ferulic acid (Iiyama et al., 1990, 1994; Grabber et al., 2000).

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In addition to the depolymerisation of (1→3),(1→4)-β-D-glucan, the xyloglucan of oat coleoptile cell walls also showed depolymerisation during auxin-induced growth (Inouhe et al., 1984). However, the size distribution of Ara- and Xyl-containing polysaccharides (putative arabinoxylan) did not change during the auxin-induced growth of oat coleoptiles or during the rapid submergence-induced growth of rice coleoptiles (Sakurai et al., 1979; Revilla & Zarra, 1987). Growth-associated cell wall loosening in coleoptiles thus appears to be associated with a debranching of glucuronoarabinoxylan, but not with depolymerisation of the xylan backbone.

Xylan Metabolism in Cereal Grains

In the grains of cereals, arabinoxylans are significant components of the cell walls. Arabinoxylan is the major component (65% by dry weight) of the thick primary cell walls of the aleurone layer in wheat and barley, while the thinner cell walls of the starchy endosperm contain approximately 20% arabinoxylan and a greater proportion of (1→3),(1→4)-β-D-glucan (Fincher, 1989). Immunostaining with monoclonal antibodies to xylans showed that in wheat and barley grains arabinoxylan was deposited late in endosperm development (Phillipe et al., 2006; Wilson et al., 2006), and that in mature grains highly substituted arabinoxylan was present in all aleurone and endosperm cell walls, whereas low substitution xylan was found in the outer cell walls of the aleurone and inner seed coat only (McCartney et al., 2005). During germination, the walls of the starchy endosperm completely disappear, together with most of the walls of the aleurone layer and their component sugars are mobilised to fuel germination (Fincher, 1989). Arabinoxylan is solubilised and degraded by endo-(1→4)-β-xylanase, α-arabinosidase and (1→4)-β-xylan exohydrolase (Hrmova et al., 1997) as the walls of the aleurone layer are disassembled to allow enzymes involved in reserve mobilisation to move from their site of synthesis in the aleurone to the endosperm (Fincher, 1989). In barley, the majority of the endo-(1→4)-β-xylanase is secreted late in germination. These endo-(1→4)-β-xylanases are cytoplasmic enzymes, and their release coincides with the disintegration of the aleurone cells (Caspers et al., 2001). This is, therefore, an example of programmed cell death (apoptosis) rather than cell wall loosening as described above for growth.

In this specialised aspect of development, endo-(1→4)-β-xylanase activity accumulates to relatively high levels, which has allowed purification and cloning of the enzymes involved (Slade et al., 1989; Banik et al., 1996). In barley, endo-(1→4)-β-xylanase activity appears to be the result of proteolytic processing of an inactive 61.5 kDa precursor protein to a 34 kDa active protein (Caspers et al., 2001). Examination of the deduced amino acid sequences of five endo-(1→4)-β-xylanase genes from barley, wheat, rice and maize found that all had primary translation products of 60-65 kDa (Simpson et al., 2003). Amino acid residues important in catalysis and substrate binding were identified based on conservation with microbial endo-(1→4)-β-xylanases, and two glutamic acid residues forming the catalytic acid/base and the catalytic nucleophile were totally conserved. Endo-(1→4)-β-xylanases all fall into the GH10 family, which cause hydrolysis with retention of anomeric configuration. The amino-terminal regions of the deduced proteins possessed domains showing homology to carbohydrate-binding modules, although residues important in substrate binding were not present. It appears that these domains may instead play an important role in ensuring correct protein folding prior to amino- and carboxyl-terminal protein processing and activation of the enzymes.

Xylan Metabolism in Dicot Vegetative Tissues

In dicot tissues, and monocots outside the commelinoid group, pectins are abundant in the wall and the major hemicellulose is xyloglucan (approximately 20-25% of the primary wall, McNeil et al., 1984; Vogel, 2008). Xylans are a minor hemicellulosic component comprising approximately 5% of the primary wall (Darvill et al., 1980). In leaves of *Arabidopsis*, xyloglucan formed 20% of the wall and glucuronoarabinoxylan 4% (Zablackis et al., 1995), although the whole leaves used would have included some secondarily thickened vasculature. In unlignified suspension-cultured cells (which contain atypically high contents of structural cell wall proteins), the matrix of primary cell walls was composed of approximately 10% xyloglucan and less than 1% glucuronoarabinoxylan in Douglas-fir (Thomas et al., 1987), and 13% xyloglucan and 3% arabinoxylan in 10-day old carrot cells (Shea et al., 1989). Immunostaining with an antibody raised to (1→4)-β-D-xylan showed that xylan was present in growing primary cell walls of bean root, although as expected much higher staining was detected in secondary walls where xylan is a major component (Northcote et al., 1989). Antibodies raised to unsubstituted (1→4)-β-D-xylan or low substitution arabinoxylan specifically recognised secondary cell walls (particularly xylem vessels and sclerenchyma fibres) in tobacco and pea stems, and did not bind to primary cell walls (McCartney et al., 2005, 2006). An antiserum raised against glucuronoxylan found the epitope to be present only in the secondary walls of xylem cells of Japanese beech, and not in primary walls or middle lamella (Awano et al., 1998). In *Pinus nigra* (Arnold), unsubstituted xylan was found in the xylem, transfusion tissue and sclerenchyma, but not in primary walls (Carafa et al., 2005). However, the distribution of xylan varies with species and tissue, and in flax and hemp xylan epitopes were present throughout secondary walls of xylem cells, but were detected in primary walls and not in secondary walls of phloem fibre cells (Blake et al., 2008).

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Although in dicots xyloglucan is believed to be the major cross-linking polysaccharide in the primary wall, both xylans and glucomannans are also capable (at least *in vitro*) of hydrogen bonding to cellulose and may also carry out this cross-linking function (McNeil et al., 1975; Whitney et al., 1998). However, in poplar cell suspension cultures, extraction of primary cell wall polysaccharides with 0.7 M potassium hydroxide (KOH) (which would solubilise xylan but not xyloglucan, Hayashi, 1989; Coimbra et al., 1994) only slightly affected the ordered spacing of microfibrils, whereas subsequent removal of xyloglucan with 4.3 M KOH collapsed the three-dimensional morphology of the cell walls (Itoh & Ogawa, 1993). Similar observations were made in pea epicotyl, where extraction of xylan with 0.7 M KOH did not remove inter-microfibril cross links, but these were destroyed by treatment with endo-(1→4)-β-glucanase which degraded xyloglucan (Fujino et al., 2000). In cucumber hypocotyls, treatment of inactivated cell walls with a fungal endoglucanase capable of degrading xyloglucan but not xylan or mannan resulted in major structural weakening of the wall (Yuan et al., 2001). These observations suggest that the contribution of xylan to the strength of the hemicellulose-microfibrillar network is relatively minor.

Covalent linkages between a proportion of xyloglucan molecules and pectin (presumed to be between the reducing end of xyloglucan and the arabinan/galactan side chains of rhamnogalacturonan-I [RG-I]) appear widespread amongst both dicots and monocots (Talmadge et al., 1973; Chambat et al., 1984; Thompson & Fry, 2000; Popper & Fry, 2005). Although these bonds have so far been detected only in suspension-cultured cells, if they exist widely *in planta* such linkages may interlock the two networks of the wall. Pectin may also contribute directly to cellulose cross linking, since arabinan and galactan side chains of RG-I bind to cellulose and different side chains of the same pectin molecule could bind to different microfibrils (Zykwinska et al., 2005, 2007). The tensile strength of dicot stems appears to depend on multiple components, including a xyloglucan-microfibril network and borate diester-linked complexes of RG-II (Ryden et al., 2003). Such a multiplicity of links may be more important than previously thought, since an *Arabidopsis* mutant in which two xyloglucan:xylosyltransferase genes had been disrupted lacked detectable xyloglucan in the wall yet exhibited no alterations in gross morphological phenotype (Cavalier et al., 2008). Whether the glucan backbone chain of xyloglucan (lacking the substitution with Xyl residues) was still synthesised, or if other cell wall polymers such as pectin or xylan provided compensatory cross linking, remains to be resolved. Although immunostaining by anti-xylan antibodies did not detect an increased content of xylan, a three-fold increase in 4-linked Xyl, indicative of xylan, was present in a 4 M KOH cell wall extract of the mutant.

Sequences of cDNA obtained from cereal grains were used to deduce the endo-(1→4)-β-xylanase gene family (GH10) of *Arabidopsis* (Simpson et al., 2003). The gene family may consist of 12 members (Henrissat et al., 2001), but of the 11 putative *Arabidopsis* sequences examined, three were larger than 60-65 kDa and possessed amino-terminal carbohydrate-binding modules (predicted to be functional) of different sizes, with deduced proteins of 84, 105 and 118 kDa. Seven of the gene products were predicted to be cytoplasmic. One of these is expressed in vascular bundles (Suzuki et al., 2002), and the others may also be involved in the programmed cell death that occurs during xylogenesis, or in that occurring in anther development or in the nucellus and suspensor cells in developing seeds. Endo-(1→4)-β-xylanase activity has also been found associated with the germination of dicot seeds (Mujer et al., 1991). The other four *Arabidopsis* endo-(1→4)-β-xylanases (none of which had the carbohydrate-binding module) had predicted ER-targeting signal peptides and may be secreted to the cell wall. The presence of these genes in the *Arabidopsis* genome implies that there are aspects of plant development that involve depolymerisation of cell wall xylan, although the role of these enzymes in plant development is unknown.

Whether or not debranching forms part of the modification of dicot primary cell wall glucuronoarabinoxylan during development is unclear, although α-arabinosidases are present. In *Arabidopsis*, two GH51 α-arabinosidases showed differential expression, with *AtARAF1* being expressed at high levels in all tissues but particularly in the zones of cell division and expansion of the primary root apex, in emerging lateral roots, in differentiating leaves and in the vascular system (Fulton & Cobbett, 2003). *AtARAF2* expression was detected at low levels in the vasculature of older roots and stems, floral organs and abscission zones. ARAF1 showed both α-L-arabinosidase and β-D-xylosidase activity against a variety of plant arabinoxylans (Minic et al., 2004). Two β-D-xylosidases (XYL1 and XYL4, both of GH3) with high activity in young stems were also characterised, and while XYL1 showed effective bifunctional α-L-arabinosidase/β-D-xylosidase activity, XYL4 was a more specific β-D-xylosidase and liberated mainly D-Xyl from a range of plant arabinoxylans (Minic et al., 2004). However, no clear role for any of these gene products in primary wall metabolism has been shown. *XYL1* was highly expressed in cells undergoing secondary thickening, and antisense XYL1 plants showed reduced plant height when grown under dry conditions, consistent with the enzyme playing a role in some aspect of secondary wall modification occurring in vascular development (Goujon et al., 2003). The insertional mutant *araf1* did not show a visible phenotype, and although ectopic overexpression of *AtARAF1* resulted in an apparent increase in unsubstituted xylan, this occurred in secondary walls of cell types where *AtARAF1* is not normally expressed (Chávez Montes et al., 2008). It was concluded that pectic arabinans are the most likely target for AtARAF1

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rather than arabinoxylans (Chávez Montes et al., 2008).

In a purified alkali-soluble hemicellulose extract from cell walls of cultured cells of Douglas-fir, some of the xylan and xyloglucan appeared to be interacting or covalently attached, since treatment with endo-(1→4)-β-glucanase reduced the apparent molecular weight of the xylan (Thomas et al., 1987). The existence of xylan-pectin-xyloglucan complexes in alkali extracts from immature cabbage leaves and cauliflower stems has been reported (Stevens & Selvendran, 1984a; Femenia et al., 1999a). Treatment of the extracts with either endo-xylanase (which resulted in a decrease in molecular weight of some of the pectin and xyloglucan components) or endo-polygalacturonase (which resulted in a decrease in molecular weight of some of the xylan and xyloglucan components) was consistent with the linkage of these polymers into a complex (Femenia et al., 1999b). It should be noted that this finding assumes that the enzymes used were completely free of contaminating activities. The abundance of the complexes increased in maturing tissue that was becoming lignified, suggesting that xylan becomes increasingly covalently cross-linked into the wall as tissues mature. Similar descriptions of maturation-related xylan-pectic polysaccharide (-lignin) complexes have been made in maturing spears of asparagus (Waldron & Selvendran, 1992), a non-commelinoid monocot with a Type I cell wall. However, secondary walls generally lack pectin, which may indicate that the pectin-xylan complexes are the first carbohydrates involved in the onset of lignification at the edge of the primary wall (Waldron & Selvendran, 1992). As the secondary wall develops, it appears that cellulose of the developing secondary wall forms a scaffold upon which xylan is coated, into which large amounts of xylan together with lignin are then deposited (Awano et al., 2002).

Xylan Metabolism in Fruit Ripening

Fruit softening is known to involve the disassembly of the primary wall and the degradation of particular hemicellulose and pectin wall components, probably in a species-specific manner (Brummell, 2006). Xylan metabolism during fruit ripening is an interesting subject due to the presence of substantial levels of ripening-related endo- and/or exo-(1→4)-β-xylanase activity in many species, including Japanese pear, papaya, cucumber, avocado, carambola, pepper and banana (Yamaki & Kakiuch, 1979; Paull & Chen, 1983; Miller et al., 1989; Ronen et al., 1991; Priya Sethu et al., 1996; Prabha & Bhagyalakshmi, 1998; Chin et al., 1999; Chen & Paull, 2003).

The increase in endo-(1→4)-β-xylanase activity during papaya ripening was particularly large (Paull & Chen, 1983), and isolation of an endo-(1→4)-β-xylanase cDNA clone showed that the 65 kDa deduced protein possessed a secretory signal peptide, an amino-terminal carbohydrate-binding module and a carboxyl-terminal endo-xylanase catalytic domain (Chen & Paull, 2003). However, the active protein had a molecular weight of 32.5 kDa (Chen & Paull, 2003), indicating proteolytic processing similar to that occurring with the cereal grain endo-(1→4)-β-xylanases (Simpson et al., 2003). Endo-(1→4)-β-xylanase mRNA was not detected in immature fruit but accumulated during ripening, and the extent of accumulation of endo-(1→4)-β-xylanase mRNA, immunodetectable protein and activity correlated with the extent of softening in different varieties (Chen & Paull, 2003; Manenoi & Paull, 2007). Treatment of fruit with the ethylene antagonist 1-methylcyclopropene delayed fruit ripening and softening, and also suppressed the accumulation of endo-(1→4)-β-xylanase mRNA (Manenoi & Paull, 2007). However, the involvement of depolymerisation of (1→4)-β-xylan in the softening of papaya remains to be shown.

The walls of pear are known to be rich in Xyl (Ahmed & Labavitch, 1980; Martin-Cabrejas et al., 1994), presumably the majority of which is present as xylan, but much of this may be in secondarily-thickened stone cells. However, xylan has been found in the unlignified walls of fragile parenchymatous cells of guava that had been purified away from the lignified stone cells (Marcelin et al., 1993), and may be a significant component of the primary walls of some soft berries (Vicente et al., 2007a, b). In pineapple, a monocot fruit, glucuronoarabinoxylans (with a proportion of the α-L-Ara side branches ester linked to ferulic acid) were the major hemicellulose of the unlignified cell walls, although substantial amounts of xyloglucan were also present (Smith & Harris, 1995, 2001). In apple, tomato and olive, a small proportion of the xylan has been reported to be present in various complexes with pectins or xyloglucan (i.e. as xylan-pectin-xyloglucan or xylan-pectin or xylan-xyloglucan) (Stevens & Selvendran, 1984b; Seymour et al., 1990; Coimbra et al., 1994, 1995). Olive xylan-xyloglucan complex was reduced in molecular weight by treatment with purified endo-(1→4)-β-xylanase, without apparent degradation of the xyloglucan component, leading the authors to deduce a covalent attachment between xylan and xyloglucan (Coimbra et al., 1995). In olive it is possible that the majority of the xylan and most of such complexes may derive from secondarily thickened lignified sclereids in the pulp (Coimbra et al., 1994), but apple and tomato parenchyma possess very little lignified tissue.

Although there is no assay specific for xylan, the amount of xylan can be estimated from sugar composition where linkage analysis has been carried out by taking the sum of 4-linked Xyl and 2,4-linked Xyl, plus the amount of terminal Ara and terminal GlcA equal to the 2,4-linked Xyl. Using this calculation, the content of xylan in grape was found to be low, but increased during ripening (Nunan et al., 1998). In tomato, this method was used to examine peaks

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of alkali-soluble hemicellulose separated by size exclusion chromatography (Tong & Gross, 1988). During ripening, the predominance of a high molecular weight peak decreased, while a lower molecular weight peak increased in prominence. Linkage analysis indicated that xyloglucan (2-linked Xyl) moved from peak 1 to peak 2 (was depolymerised), while xylan (4-linked and 2,4-linked Xyl) became an increasing proportion of peak 1 and a decreasing proportion of peak 2. This suggests that at least some cell wall xylan remains at high molecular weight during the ripening process that involves the selective depolymerisation of other wall components. Tomato contains ripening-related (1→4)-β-xylanase activity (Barka et al., 2000), although whether this is endo- or exo-acting activity was not determined. In kiwifruit, xylan was found as a heterogeneous population with a predominant size of 40 kDa (Redgwell et al., 1991). During ripening, no evidence was found for depolymerisation of xylans, although xyloglucan showed a decrease in molecular weight (Redgwell et al., 1991). However, in peach size exclusion chromatography of a 1 M KOH extract highly enriched in low molecular weight (<100 kDa) Xyl-containing polysaccharides (putative (1→4)-β-xylan) that were susceptible to digestion with exogenous endo-(1→4)-β-xylanase indicated that these polymers did exhibit some depolymerisation during ripening (Muramatsu et al., 2004).

Increased accumulation of β-D-xylosidase mRNA has also been detected during fruit ripening of several species (Itai et al., 1999, 2003; Martínez et al., 2004), and it is possible that the corresponding activities are involved in the degradation of the non-reducing ends of xylan fragments produced by the action of endo-(1→4)-β-xylanases. This, however, remains to be determined. A bifunctional α-L-arabinosidase/β-D-xylosidase of GH3 showed increased mRNA accumulation during ripening of Japanese pear (Tateishi et al., 2005), but unlike the enzymes from barley (Lee et al., 2003) and *Arabidopsis* (Minic et al., 2004) this enzyme did not release Xyl from xylan. The enzyme released Ara from pectic arabinan but not from arabinoxylan. An α-L-arabinosidase of a different family (GH51) is expressed in ripening tomato fruit, but its mRNA abundance declined during ripening and was negatively regulated by ethylene (Itai et al., 2003), making a role in ripening-related cell wall changes unlikely. Ripening-related α-L-arabinosidase activity (usually measured against artificial substrates) is found in many species (e.g. Sozzi et al., 2002; Brummell et al., 2004; Tateishi et al., 2005), but it is thought that debranching of pectin rather than arabinoxylan is their primary function. It is not possible to draw firm conclusions from the currently very limited data, but the evidence suggests that there may be differences between species in whether or not cell wall xylan is depolymerised during fruit ripening. Whether this has any significance for fruit softening will depend on the nature and importance of the xylan-microfibril or xylan-pectin-xyloglucan links.

Conclusions and Future Directions

Xylans appear to be a component of all primary cell walls, but with abundances varying between species. Glucuronoarabinoxylan plays an important structural role in the primary cell wall of commelinoid monocots. In the cell walls of grasses, a (1→3),(1→4)-β-D-glucan is a more predominant cross-linking hemicellulose than xylan during growth, and in non-commelinoid monocots and in dicots xyloglucan is more important. The metabolism during growth of (1→3),(1→4)-β-D-glucan in grasses (Carpita, 1996) and xyloglucan in dicots (Wakabayashi et al., 1991; Talbott & Ray, 1992b) is marked and well-characterised, but similar data for xylans is largely lacking. Current evidence suggests that a decrease in the arabinosyl substitution of xylan accompanies grass primary wall maturation, together with increasing phenolic coupling into the wall. Little information is available concerning the metabolism of xylan during dicot primary wall loosening, if indeed it occurs. During fruit softening in many species there is a considerable loss of Ara residues from the wall (Gross & Sams, 1984; Redgwell et al., 1997), probably due to increased α-L-arabinosidase activity during ripening (Sozzi et al., 2002; Tateishi et al., 2005). This loss of Ara is thought to occur mainly from the arabinan side chains of RG-I, but could also include the debranching of arabinoxylan. The reduced substitution of the xylan would make the xylan backbone more susceptible to hydrolysis by endo-(1→4)-β-xylanases, if present. Exhaustive digestion of substituted glucuronoarabinoxylan by purified endo-(1→4)-β-xylanase caused only limited depolymerisation (Darvill et al., 1980), suggesting that the α-L-Ara and α-D-GlcA residue side branches hinder the action of the enzyme. This implies that endo-(1→4)-β-xylanase activity *in muro* could require the previous action of an arabinosidase to reduce the level of backbone substitution.

The study of xylan localisation in different cell types *in situ* has been facilitated by the generation of antibodies recognising xylan with various substitution patterns (Northcote et al., 1989; Suzuki et al., 2000; Guillon et al., 2004; McCartney et al., 2005; Knox, 2008). Further advances are promised by the development of a suite of xylan-specific bacterial carbohydrate-binding modules, which due to the sensitivity of their binding appear to be able to detect which face of the xylan molecule is exposed when the polymer is bound to other polysaccharides in the wall (McCartney et al., 2006). Such *in situ* studies have illustrated the complexity with which xylan is embedded into the wall, and will reveal information on the diversity of cell wall structure and function that cannot be determined from cell wall extracts and polymer purification.

The endo-(1→3),(1→4)-β-D-glucanases responsible for glucan degradation in grasses have been cloned and their structure determined (Hrmova & Fincher, 2001), and the complex changes in

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xyloglucan molecular weight associated with cell expansion in growing tissues of dicots are thought to be accomplished by the hydrolytic and/or grafting actions of families of endo-(1→4)-β-glucanases and xyloglucan transglucosylases/hydrolases (Brummell et al., 1994; Rose et al., 2002; Libertini et al., 2004). In comparison, the study of higher plant endo-(1→4)-β-xylanases is in its infancy. Endo-(1→4)-β-xylanase activity has been most comprehensively studied in the specialised case of the degradation of the cell wall of the aleurone layer during the germination of cereal grains, and several grain endo-(1→4)-β-xylanases have been cloned (Simpson et al., 2003). The *Arabidopsis* genome possesses 12 predicted endo-(1→4)-β-xylanase genes, the deduced proteins of four of which are predicted to be targeted to the ER and possibly secreted (Simpson et al., 2003). The biochemical and physiological function of these enzymes remains to be established, but their existence implies that depolymerisation of xylan backbones is involved in some aspects of plant development. It is likely that other species will also possess endo-(1→4)-β-xylanase families containing multiple genes. Endo-(1→4)-β-xylanase activity appears to be a feature of fruit ripening in certain species (Chen & Paull, 2003), and this could indicate a further area of plant development where xylan metabolism plays a role. This may be particularly important in fruit such as pineapple, where glucuronoarabinoxylan is the most abundant hemicellulose present in the wall.

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