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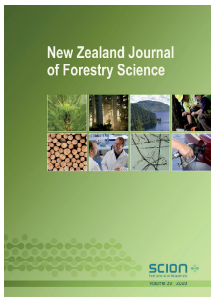


# Structural Diversity, Functions and Biosynthesis of Xyloglucans in Angiosperm Cell Walls†

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## Abstract

Xyloglucans occur in the primary cell walls of all angiosperms where they are thought to have a structural role and be involved in the control of cell expansion. They also occur in the thick, non-lignified secondary cell walls of seeds of some species of eudicotyledons where they serve as reserve carbohydrates. The xyloglucans in the primary cell walls of most eudicotyledons have similar structures with side chains containing galactose and fucose. However, in one group of eudicotyledons, the asterids, there is considerable structural variability in the xyloglucans, with many containing arabinose. There is also variability in the xyloglucans of the monocotyledons, with those in the family Poaceae containing no fucose and only small proportions of galactose. The xyloglucans of the thick, secondary cell walls of seeds are similar to those in the primary walls of most eudicotyledons, but contain no fucose. The functional significance of these structural variations is unknown. A number of genes and their encoded glycosyltransferases have been identified as being involved in the biosynthesis of the xyloglucans of the model eudicotyledon *Arabidopsis thaliana*.

**Keywords:** cell expansion; eudicotyledons; grasses and cereals (Poaceae); monocotyledons; primary cell walls; seed cell walls; xyloglucan biosynthesis.

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## Introduction

Xyloglucans are a family of non-cellulosic polysaccharides that occur in the cell walls of all land plants, including the angiosperms (flowering plants), and have been intensively studied, particularly over the last two decades (Fry, 1989; Hayashi, 1989; Vincken et al., 1997; O'Neill & York, 2003; Popper & Fry, 2003; Harris, 2005a, b; Hoffman et al., 2005; Harris & Stone, 2008). Xyloglucans usually comprise 20-25% of the dry weight of the primary walls of eudicotyledons, but only 2-5% of the primary walls of the commelinid monocotyledon family Poaceae (grasses and cereals). Xyloglucans also occur as the major component of thick, non-lignified secondary walls in the cotyledons or endosperms of seeds in some species of the Fabaceae subfamily Caesalpinioidae and in some other eudicotyledon families, but not in monocotyledons (Kooiman, 1960; Buckeridge et al., 2000; Harris, 2005a). These seed xyloglucans were originally referred to as amyloids because they stain blue with solutions of iodine and potassium iodide. Those from the seeds of some species, including tamarind (*Tamarindus indica*, Fabaceae) and



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*Detarium senegalense* (Fabaceae), are of significant economic importance and have, for example, been used as thickening, stabilising and gelling agents in foods (Nishinari et al., 2000; Urakawa et al., 2002; Harris & Smith, 2006). In this short review, we outline some of the structural diversity in xyloglucans, their possible functions and indicate recent progress that has been made in understanding their biosynthesis.

## Structural diversity of xyloglucans

Structurally, xyloglucans are composed of a backbone of (1→4)-linked β-D-Glc*p* residues substituted at O-6 with α-D-Xyl*p* residues, with other substituents present on some of the latter residues (Figures 1 & 2). To help describe the structures of xyloglucans, Fry et al., (1993) developed an unambiguous nomenclature with the letters G, X, S, L and F referring to the following structures: G = unsubstituted β-Glc*p*; X = α-D-Xyl*p*-(1→6)-β-D-Glc*p*; S and L = X with α-L-Ara*f*-(1→2)- and β-D-Gal*p*-(1→2)- attached to the xylosyl residue, respectively; and F = L with α-L-Fuc*p*-(1→2)- attached to the galactosyl residue.

A common way of analysing xyloglucan structures is to treat them with either a cellulase [*endo*-(1→4)-β-glucanase], or more recently with a xyloglucan-specific *endo*-(1→4)-β-glucanase, and characterise the oligosaccharides released using a variety of techniques including high performance anion exchange chromatography with pulsed-amperometric detection (HPAEC-PAD), matrix-assisted laser-desorption ionisation time-of-flight mass spectrometry (MALDI-TOF MS) (Lerouxel et al., 2002) and nuclear magnetic resonance (NMR) spectroscopy (York et al., 1996; Hoffman et al., 2005).

### Xyloglucans in primary walls

#### *Most eudicotyledons*

The xyloglucans in the primary walls of most eudicotyledons analysed so far have a XXXG core structure and are substituted with galactose and fucose (Figure 1A). They are often referred to as fucogalactoxyloglucans. Treating these xyloglucans

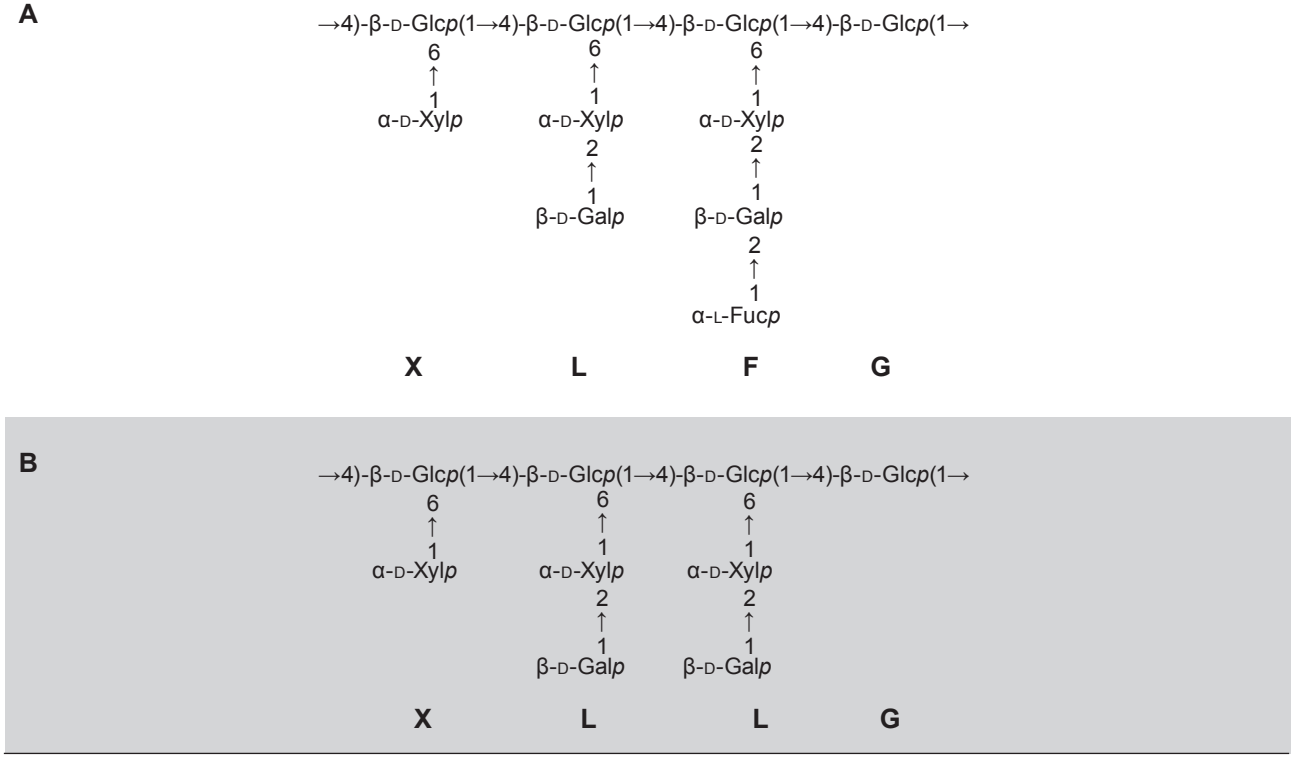


FIGURE 1: A- A common oligosaccharide unit (XLFG) from the most abundant structural type of xyloglucan in the primary walls of eudicotyledons. This xyloglucan has a XXXG core structure with L and F side chains and is often referred to as a fucogalactoxyloglucan.

B- A common oligosaccharide unit (XLLG) from the xyloglucan in the thick, secondary walls of some eudicotyledon seeds. This xyloglucan has a XXXG core structure, with L, but no F side chains, and is referred to as a galactoxyloglucan.

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with a cellulase or xyloglucan-specific *endo*-(1→4)-β-glucanase yields XXXG, XXFG and XLFG as the three major oligosaccharide subunits. For example, treatment of the xyloglucan from the leaves of the model eudicotyledon plant *Arabidopsis thaliana* with cellulase yielded XXXG (45%), XXFG (24%), XLFG (16%), XXLG (8%), XLLG (4%) and XLXG (3%) (Vanzin et al., 2002). One or two *O*-acetyl groups frequently occur on the galactosyl residues in F units (Kiefer et al., 1989; Pauly et al., 2001). However, not all eudicotyledons that have been examined have fucogalactoxyloglucans in their primary walls.

***Asterid eudicotyledons***

The known variation in xyloglucan structure is most evident in the asterid clade as defined by the Angiosperm Phylogeny Group (2003). This major clade comprises four subclades: the Cornales, Ericales, campanulids (euasterids II) and lamiids (euasterids I). As far as we are aware, there are no published accounts of the structures of xyloglucans in the Cornales and all species examined in the campanulids have fucogalactoxyloglucans as defined above. In the Ericales, the xyloglucans of two species, the argan tree (*Argania spinosa*, Sapotaceae) (Ray et al., 2004) and bilberry (*Vaccinium myrtillus,* Ericaceae) (Hilz et al., 2007), have been investigated. These xyloglucans contain all the units of fucogalactoxyloglucans, but in addition contain units with the unusual side chain β-D-Xyl*p*-(1→2)-α-D-Xyl*p*-(1→6)-β-D-Glc*p*, known as U, for example XUFG.

However, most known variation occurs in the xyloglucans of the fourth subclade, the lamiids (Harris, 2005a; Hoffman et al., 2005). The xyloglucan of oleander (*Nerium oleander*, Apocynaceae) differs from fucogalactoxyloglucans in also containing XXSG and XLSG units. Olive (*Olea europea*, Oleaceae) xyloglucan is similar in having a XXXG core structure with S and L side chains, but differs in having no F side chains; XXXG, XXSG and XLSG are the major units (Vierhuis et al., 2001).

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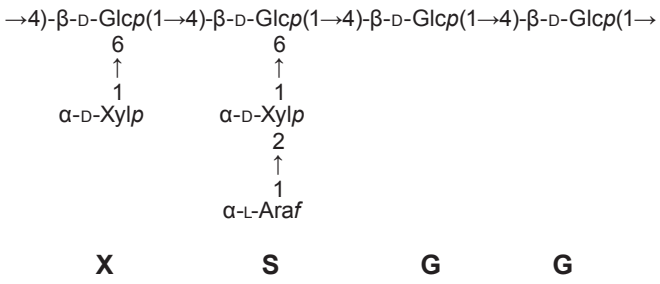


**B**

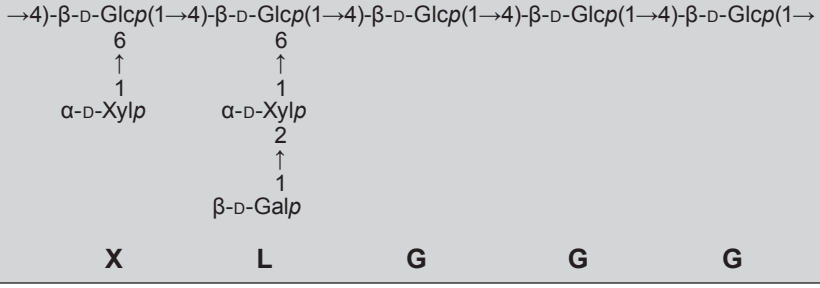


FIGURE 2: A- A common oligosaccharide unit (XSGG) from the xyloglucans in the primary walls of solanaceous plants (family Solanaceae). These xyloglucans have a XXGG core structure, with S and, in some species, L side chains.

B- A common oligosaccharide unit (XLGGG) from the xyloglucans in the primary walls of grasses and cereals (family Poaceae). These xyloglucans have a $XXG_n$ core structure (where n = ~1–5) and L, but no F side chains. This is also the most common oligosaccharide unit in xyloglucans from the primary walls of the lamiids plantain (*Plantago major*) and basil (*Ocimum basilicum*) (Hoffman et al., 2005).

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In contrast to the xyloglucan structures discussed above, the economically important family Solanaceae, which includes potato (*Solanum tuberosum*), tobacco (*Nicotiana tabacum*) and tomato (*Solanum lycopersicum*), have xyloglucans with a XXGG core structure and S and sometimes L, but not F side chains. For example, the oligosaccharides released on treating potato xyloglucan with cellulase included XXGG, XSGG, XLGG, and LSGG (Vincken et al., 1996; Harris, 2009). These solanaceous xyloglucans (Figure 2A) have been referred to as arabinoxyloglucans. In addition to the structures of solanaceous xyloglucans from primary walls, the structures of xyloglucans secreted into the medium of cell-suspension cultures of tomato and tobacco (*N. plumbaginifolia* and *N. tabacum*) have been examined (Sims et al., 1996; York et al., 1996; Jia et al., 2003, 2005). Their structures are similar, but more complex. For example, secreted tomato xyloglucan contains the unusual side chain, β-L-Ara*f*-(1→3)-α-L-Ara*f*-(1→2)-α-D-Xyl*p*-(1→6)-β-D-Glc*p*, known as T (York et al., 1996; Jia et al., 2003), but this is absent from the xyloglucan in primary walls of leaves (Hoffman et al., 2005).

A third core structure, XXGGG, occurs in the xyloglucans of morning glory (*Ipomoea purpurea*, Convolvulaceae), plantain (*Plantago major*, Plantaginaceae) and basil (*Ocimum basilicum*, Lamiaceae) (Hoffman et al., 2005). In morning glory xyloglucan, XSGGG is the major unit and no L or F side chains occur, but in plantain and basil xyloglucans, XLGGG is the major unit with no S or F side chains (Figure 2B).

The xyloglucans in asterid primary walls are also *O*-acetylated, with *O*-acetyl groups occurring on the galactosyl residue in F side chains, and sometimes on glucosyl residues of the backbone or arabinosyl residues of S side chains.

### *Other eudicotyledons*

Although most of the known variation in xyloglucan structures in primary walls of eudicotyldons is in the asterids, some other variation has been reported. For example, the xyloglucan in the primary walls in the cotyledons of jojoba (*Simmondsia chinensis*, Simmondsiaceae) seeds (Rost et al., 1977) differs from a fucogalactoxyloglucan in also containing units with unusual J side chains that differ from F side chains in having α-L-Gal*p* rather than α-L-Fuc*p* residues (Hantus et al., 1997). These J side chains also occur in the xyloglucan in primary walls from aerial parts of the *mur1* mutant of *A. thaliana*, which is unable to make GDP-fucose, used in the biosynthesis of F side chains (see below) (Zablackis et al., 1996; Freshour et al., 2003).

### *Monocotyledons*

Most research has been focussed on the xyloglucans in the primary walls of the economically important commelinid monocotyledon family Poaceae (grasses and cereals). These xyloglucans are less branched than the fucogalactoxyloglucans of most eudicotyledons and contain less xylose, much less galactose and no fucose (Fry, 1989; Vincken et al., 1997). Commonly occurring core structures are XXGG, XXGGG and XXGGGG, together with small proportions of XXG and XXGGGGG (Kato et al., 1981; Fry, 1989; Kato et al., 2004; Gibeaut et al., 2005). We thus refer to the core structure as $XXG_n$, where n = ~ 1–5 (Figure 2B). Oligosaccharide units containing galactosyl residues such as XLGGG and LXGGG have been reported (Kato et al., 2004). The xyloglucans are acetylated, with the acetyl groups probably occurring at O-6 of non-reducing, unbranched glucosyl residues (Gibeaut et al., 2005).

However, not all monocotyledon xyloglucans are similar to those of the Poaceae. The non-commelinid monocotyledons onion (*Allium cepa*, Alliaceae) and garlic (*A. sativa*) have fucogalactoxyloglucans identical to those in the primary walls of most eudicotyledons (Ohsumi & Hayashi, 1994). Furthermore, a recent survey has identified considerable other diversity in the structures of monocotyledon xyloglucans, especially in the commelinids (Hsieh & Harris, 2009).

## Xyloglucans in secondary walls of seeds

Unlike the xyloglucans in primary cell walls, which can be extracted only by using a solution of an alkali, for example 4 M potassium hydroxide, the xyloglucans in the thick, non-lignified cell walls of seeds can be extracted with water. The structures of these seed xyloglucans have been investigated for a number of species (Buckeridge et al., 1992; Buckeridge et al., 2000; Harris, 2005a). All were found to be similar to the fucogalactoxyloglucans in having XXXG core structures, but they lack fucose and are thus often referred to as galactoxyloglucans (Figure 1B). Some of the most detailed studies have been done on the xyloglucans extracted from the cotyledon walls of tamarind (*Tamarindus indica*, Fabaceae) seeds (Gidley et al., 1991; Buckeridge et al., 1992; York et al., 1993; Marry et al., 2003). Treatment of tamarind xyloglucan with *endo*-(1→4)-β-glucanase released four types of oligosaccharides: XXXG, XLXG, XXLG and XLLG (Buckeridge et al., 1992; Marry et al., 2003). However, an interesting structural variation has been reported in the xyloglucan from the seeds of *Hymenaea coubaril* (Fabaceae) (Buckeridge et al., 1997; Tiné et al., 2006). In this xyloglucan, in addition to the xylocellotetraosyl (XXXG) core units,

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xylocellopentaosyl (XXXXG) and xylocellohexaosyl (XXXXXG) core units were present in the ratio 2 : 1 : 0.2, respectively. As far as we are aware, there are no reports indicating that the xyloglucans from the thick, secondary walls of seeds are acetylated.

## Functions of xyloglucans

### Xyloglucans in primary walls

Xyloglucans are considered to play an important role in the architecture and mechanical properties of the primary cell wall of eudicotyledons and non-commelinid monocotyledons (Bootten et al., 2004; Harris & Stone, 2008). Current models of the architecture of these walls are often referred to as "tethered or sticky network models" (Cosgrove, 2001; 2005). It is postulated that xyloglucan molecules hydrogen bond to cellulose microfibrils and may cross-link adjacent microfibrils to form a cellulose-xyloglucan network, which is thought to be the main load-bearing structure of the wall. This network is often considered to be coextensive with, but independent of, a second network composed of the pectic polysaccharides. However, evidence is accumulating that indicates that at least some of the xyloglucans in primary cell walls are covalently linked to pectic polysaccharides via the neutral side chains of the rhamnogalacturonan I (RG I) domain, although the exact structure of this linkage is unknown (Popper & Fry, 2008). It is also often assumed in these wall models that as well as cross-linking the cellulose microfibrils, the xyloglucans completely coat the microfibril surfaces. However, using solid-state $^{13}C$ NMR spectroscopy, it was shown that in the primary walls of mung beans (*Vigna radiata*) a maximum of only 8% of the surfaces of the microfibrils had adsorbed xyloglucans (Bootten et al., 2004). Furthermore, the primary walls of celery (*Apium graveolens*) contain only ~2% xyloglucans. This proportion was considered too small to coat completely the cellulose microfibrils, but sufficient for the formation of at least some cross-links (Thimm et al., 2002).

These xyloglucan cross-links are believed to constrain cell enlargement and must be broken to allow "wall loosening" and cell expansion. Several mechanisms have been postulated for "wall loosening", including mechanisms involving the actions of either expansin or xyloglucan endotransglucosylase/hydrolase (XTH) proteins (Cosgrove, 2001, 2005; Rose et al., 2002; Fry, 2001, 2004). Expansin proteins may act by catalysing the breakage of the hydrogen bonds holding the cross-linking xyloglucans to cellulose microfibrils. The xyloglucan endotransglucosylase (XET) activity of XTH proteins may break and rejoin cross-links by a transglycosylation reaction. Briefly, a xyloglucan cross-link may be cleaved by the enzyme, which forms a xyloglucan-enzyme complex. After wall expansion has occurred, the xyloglucan part of this complex is then reattached to the non-reducing end of a different xyloglucan molecule.

Whatever the mechanism of "wall loosening", the functional significance of variation in xyloglucan structure is also unknown. The primary walls of those angiosperms in which the xyloglucans are structurally different from those of most eudicotyledons, apparently function normally. Furthermore, tensile strength measurements on the *A. thaliana* mutants *mur2* and *mur3*, which have primary walls with altered xyloglucan compositions, showed significant differences from the wild type only in the seedling hypocotyl (Peña et al., 2004). As well as playing a structural role, xyloglucans in primary walls are also the source of biologically active oligosaccharides, or oligosaccharins, some of which are able to regulate plant cell expansion (Aldington & Fry, 1993; Côté & Hahn, 1994; Fry, 2001).

However, one of the most surprising recent findings concerning xyloglucan function in primary walls comes from a double T-DNA insertion mutant of *A. thaliana* in which two genes involved in xyloglucan biosynthesis, *XXT1* and *XXT2*, were not expressed (Cavalier et al., 2008). This mutant had walls that contained no detectable xyloglucan, but despite this, there was no catastrophic effect on wall integrity and, compared with the wild type, the mutant plants were only slightly smaller and had abnormal root hairs. It is possible that in these walls, the pectic polysaccharide network between the cellulose microfibrils is able to function alone in maintaining wall structure and functionality (Harris, 2009).

### Xyloglucans in secondary walls of seeds

The xyloglucans that occur in the thick, non-lignified secondary walls of some seeds function as reserve carbohydrates and are mobilised during germination (Buckeridge et al., 2000). The xyloglucans are degraded by the simultaneous actions of four enzymes, which have been particularly well characterised in the seeds of nasturtium (*Tropaeolum majus*, Tropaeolaceae): a β-galactosidase, an α-xylosidase, a β-glucosidase and a XTH protein showing xyloglucan endohydrolase (XEH) activity (Fanutti et al., 1993: Rose et al., 2002). Any functional significance in the lack of fucosyl residues on the xyloglucans of seed secondary walls is unknown.

## Biosynthesis of xyloglucans

Xyloglucans are synthesised within the Golgi apparatus by membrane-bound glycosyltransferases from nucleotide sugars (Fry, 2004; Lerouxel et

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al., 2006). The synthesised xyloglucans are then moved in Golgi-derived secretory vesicles to the plasma membrane, and into the wall by exocytosis. The newly-secreted xyloglucans may be 'grafted' into the wall by XTH proteins showing xyloglucan endotransglucosylase activity (Fry, 2004). Recently, considerable progress has been made in identifying and characterising a number of genes and their encoded membrane-bound glycosyltransferases that are involved in the biosynthesis of *Arabidopsis thaliana* fucogalactoxyloglucans. The enzymes comprise an α-fucosyltransferase, two β-galactosyltransferases, three α-xylosyltransferases and a (1→4)-β-glucan synthase.

Xyloglucan α-fucosyltransferase catalyses the transfer of fucosyl residues from the nucleotide sugar GDP-β-L-Fuc to the galactosyl residue on the L side chain adjacent to the unsubstituted Glc*p* residue (Figure 1). The gene encoding this enzyme (*MUR2*, *AtFUT1*) was identified from the ethyl methanesulfonate (EMS) mutant *mur2* (Reiter et al., 1997; Vanzin et al., 2002). This mutant has a lower proportion of fucosyl residues in its wall polysaccharides than the wild type due to a lesion in the *MUR2* (*AtFUT1*) gene.

Xyloglucan β-galactosyltransferase catalyses the transfer of galactosyl residues from the nucleotide sugar UDP-α-D-Gal to the third xylosyl residue of the XXXG core structure (Figure 1) (Madson et al., 2003). The gene encoding this enzyme (*MUR3*) was also identified from an EMS mutant, *mur3* (Reiter et al., 1997; Madson et al., 2003). Compared with the xyloglucan of the wild type, that of the mutant had no F side chains and the third xylosyl residue of the XXXG core structure was not galactosylated, but the second xylosyl residue had enhanced galactosylation. The *MUR3*-like gene *AtGT18* is a possible candidate for encoding the xyloglucan β-galactosyltransferase for transferring galactosyl residues to the second xylosyl residue (Li et al., 2004; Mouille et al., 2006).

Xyloglucan α-xylosyltransferase catalyses the transfer of xylosyl residues from the nucleotide sugar UDP-α-D-Xyl to glucosyl residues of the (1→4)-β-glucan backbone of xyloglucans. Two closely related genes, *XXT1* and *XXT2*, encoding two enzymes have been identified. When expressed heterologously, the genes yielded proteins with xyloglucan xylosyltransferase activity *in vitro*. Both enzymes were able to add two and even three xylosyl residues to cellohexaose, which is the preferred substrate (Faik et al., 2002; Cavalier & Keegstra, 2006; Fauré et al., 2007). A third related gene, *XXT5*, has also been found to encode a putative xyloglucan xylosyltransferase (Zabotina et al., 2008). However, the activity of either XXT1 or XXT2 may be required before XXT5 can act (Cavalier et al., 2008).

Xyloglucan (1→4)-β-glucan synthase catalyses the processive transfer of glucosyl residues from the nucleotide sugar UDP-α-D-Glc to the growing (1→4)-β-glucan backbone. The gene encoding this enzyme, *AtCSLC4*, is from the cellulose synthase-like C family (Cocuron et al., 2007). Heterologous expression of this gene in the yeast *Pichia pastoris* produced a (1→4)-β-glucan not present in control cells.

The glycosyltransferases that add glycosyl residues to the xyloglucan backbone are all type II membrane proteins each with a single predicted amino-terminal transmembrane domain. However, the xyloglucan (1→4)-β-glucan synthase that forms the backbone is a type III membrane protein with several predicted transmembrane domains (Lerouxel et al., 2006). These different enzymes may form xyloglucan-synthesising complexes within the Golgi apparatus.

## Conclusions

Over the last few years, considerable structural variation has been found in the xyloglucans in primary walls of angiosperms, particularly in the asterid group of eudicotyledons and in the monocotyledons. Undoubtedly, further structural variation will be revealed in the structures of these xyloglucans as only a small proportion of the some 271 500 species of angiosperms has been examined. Substantial progress has also been made in understanding the biosynthesis of the commonest structural type of xyloglucan, but nothing is known about the control of this process or about the biosynthesis of xyloglucans with other core structures and side chains. Nevertheless, one of the most important gaps in our current knowledge about xyloglucans concerns their exact functions in primary walls. The recent finding that a mutant of *A. thaliana* without detectable xyloglucan in its primary walls had a phenotype similar to the wild type indicates that the presence of xyloglucans is not essential for the growth of this plant.

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