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Short communication

Occurrence of fucosylated and non-fucosylated xyloglucans in the cell walls of monocotyledons: An immunofluorescence study

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ABSTRACT

The xyloglucans of monocotyledons are known to vary in the abundance of fucosylated side chains, with most commelinid monocotyledons having xyloglucans with lower proportions than non-commelinid monocotyledons. In many commelinid species, and some non-commelinid species that have lower proportions of fucosylated side chains, these side chains have been shown to be cell-type specific. To determine whether it is just the fucosylated side chains that are cell-type specific, or whether xyloglucan is cell-type specific in these species, we used the monoclonal antibody LM15 in conjunction with immmunofluorescence microscopy. We examined the distribution of cell-wall labelling among cell types in these species. The primary walls of all cell types were shown to contain xyloglucans in all species that had cell-type specific distributions of fucosylated side chains. This indicates that it is the fucosylated side chains of xyloglucans that is cell-type specific. Although the functional significance of xyloglucan fucosylation remains unknown, such cell-type specificity supports hypotheses that the fucosylated side chains may indeed have a functional role within the cell wall.

1. Introduction

Xyloglucans are a family of polysaccharides that occur in primary cell walls throughout the land plants, although the proportions vary considerably with phylogenetic position (Hsieh et al., 2009; Pauly and Keegstra, 2016). For example, in the angiosperms, the primary walls of most eudicotyledons usually contain high proportions, whereas the primary walls of species in the monocotyledon family Poaceae (the grasses) contain small proportions (Hsieh and Harris, 2009). The xyloglucan content of Poaceae cell walls has been estimated as 2–5% of the dry cell wall mass (Fry, 1989).

Structurally, xyloglucans have a linear backbone of (1 → 4)-linked β-D-glucosyl residues substituted with α-D-xylosyl residues in some positions; the xylosyl residues in turn can be further substituted with other glycosyl residues. The side chains can be described by letters (Fry et al., 1993), where "G" refers to an unsubstituted glucosyl residue, "X" to α-D-Xyl$_p$-(1 → 6)-β-D-Glc$_p$, "L" to β-D-Gal$_p$-(1 → 2)-α-D-Xyl$_p$-(1 → 6)-β-D-Glc$_p$ and "F" to α-L-Fuc$_p$-(1 → 2)-β-D-Gal$_p$-(1 → 2)-α-D-Xyl$_p$-(1 → 6)-β-D-Glc$_p$. Many other side chains structures have also been described (Schultink et al., 2014; Tuomivaara et al., 2015). The xyloglucans in the primary walls of most eudicotyledons have a repeating XXXG core structure with L and F side chains, and are known as fucogalactoxyloglucans. Treatment of these with a xyloglucan-specific *endo*-(1 → 4)-β-glucanase yields the following oligosaccharides: XXXG, XXFG and XLFG, which are usually in the highest proportions, and XXLG, XLXG and XLLG (Hsieh and Harris, 2009). However, other types of xyloglucans occur in the eudicotyledons. For example, xyloglucans in the cell walls of family Solanaceae, which includes tobacco (*Nicotiana tabacum*) and tomato (*Solanum lycopersicum)*, have a repeating XXGG core structure, with S side chains (α-L-Ara$_f$-(1 → 2)-α-D-Xyl$_p$-(1 → 6)-β-D-Glc$_p$), some L, but usually no F (Hsieh et al., 2009).

In monocotyledons, two groups are recognized, the commelinid and non-commelinid monocotyledons (APG IV, 2016). Most non-commelinid monocotyledons have fucogalactoxyloglucans, but the structures of the xyloglucans in the commelinid monocotyledons vary depending on the phylogenic position (Hsieh and Harris, 2009). In the family Poaceae (grasses), the xyloglucans mostly have a repeating XXG$_n$ core structure, where "*n*" = 1–5, with L, but usually not F side chains. However, there have been some instances of F sidechains reported in *Oryza sativa* (Liu et al., 2015), suspension-cultured cells of *Festuca arundinacea* (McDougall and Fry, 1994), and *Lolium* spp. (Brennan and Harris, 2011). Some commelinid monocotyledon families examined were shown to have xyloglucans with the repeating XXGn core structure, others with the repeating XXXG core structure and others with

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Abbreviations: CCRC-M1, Carbohydrate Research Centre – Monoclonal 1; LM15, Leeds Monoclonal 15

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both. Where both core structures are present, it is not known if they can occur in the same molecule. The proportions of total xyloglucan oligosaccharides released from commelinid monocotyledon cell-wall preparations by a xyloglucan-specific (1 → 4)-β-glucanase that contain F side chains has been shown to vary from zero to 51.8% (Hsieh and Harris, 2009).

The structures of monocotyledon xyloglucans have mostly been determined using cell-wall preparations obtained from whole organs, but the structures and amounts of xyloglucans can vary with cell type. To examine the occurrence of xyloglucan F side chains in the walls of different cell types in vegetative organs (mostly leaves) of a range of monocotyledon species, these were examined by immunofluorescence microscopy with the monoclonal antibody CCRC-M1, which recognises the epitope α-L-Fuc$_p$-(1 → 2)-β-D-Gal$_p$ (Brennan and Harris, 2011). This showed that in most species of non-commelinid monocotyledons examined, F side chains were detected in all non-lignified, primary cell walls. In contrast, in many of the commelinid monocotyledons, F side chains were often present in the walls of only certain cell types, particularly phloem cells, but also stomatal guard and subsidiary cells, and raphide idioblasts. Where F side chains occur in the walls of only particular cell types, it is unknown if the walls of the other cell types contain non-fucosylated xyloglucans, or contain no xyloglucans at all.

In the present study, we re-examined the same monocotyledon species in which we had previously found xyloglucan F side chains in the walls of only particular cell types. This was to determine if the walls of the cell types that contain no xyloglucan F side chains contain either non-fucosylated xyloglucans or no xyloglucans at all. To do this we carried out immunofluorescence microscopy with the monoclonal antibody LM15. Although LM15 was generated using the oligosaccharide XXXG coupled to bovine serum albumen (Marcus et al., 2008), it also binds to the xyloglucans of tobacco and grasses (Christensen et al., 2010; Marcus et al., 2008; Wilson et al., 2015), which is consistent with the results of epitope mapping studies using synthetic oligosaccharides (Ruprecht et al., 2017). To add context to the results, we also included results from our earlier immunofluorescence microscopy study using CCRC-M1.

2. Methods

2.1. Fixation, embedding and sectioning

The plant material was a subset of that examined by Brennan and Harris (2011); the sources and organs used are shown in Table S1. Leaves were used, except for the three species *Cyperus papyrus*, *Elegia capensis* and *Juncus inflexus*, which have only scale-like leaves and therefore culms were examined. Briefly, segments (~2 mm long) were fixed for 1 h in 100 mM Na-PIPES buffer (pH 7.2) containing 2% (w/v) paraformaldehyde and 0.1% (w/v) glutaraldehyde. Fixed segments were dehydrated in 30, 50, 70, 90, 95, 100 and 100% ethanol for 15 min before infiltration with LR White resin (London Resin Co. Ltd, Basingstoke, UK). Resin infiltration was done in resin:ethanol ratios of 1:2 (v/v) for 1 h, 2:1 (v/v) for 1 h and pure resin for 18 h. The resin was then polymerised for 24 h at 55 °C. Sections (200 nm thick) of resin embedded material were cut using an ultramicrotome (model EM UC6 Leica Microsystems, Wetzlar, Germany), transferred to poly-L-lysine coated slides (Biolab Scientific, Auckland) and dried at 55 °C for 30 min before immunolabelling. Sections 1 μm thick were cut for histochemical detection of primary and lignified secondary walls; these were dried onto glass slides at 60 °C for 5 min, stained with 1% (w/v) tolduidine blue O in 1% sodium borate for 30 s and rinsed for 30 s in running water. Non-lignified walls stain purple and lignified walls stain blue-green.

2.2. Immunofluorescence microscopy

Non-specific antibody binding sites were blocked by incubation with phosphate-buffered saline (9.3 mM sodium phosphate buffer containing 140 mM NaCl) (pH 7.2) with 0.1% (v/v) Tween-20 (PBS-T), and 0.1% (v/v) BSA-c™ (Aurion, Wageningen, Netherland) (PBS-T/BSA-c) for 45 min at room temperature. Sections were then incubated with the rat monoclonal antibody LM15 (Marcus et al., 2008) diluted 1:5 (v/v) in PBS-T/BSA-C for 18 h at 4 °C, followed by washing three times for 5 min each wash in PBS-T. Sections were then incubated with secondary antibody (anti-rat IgG) labelled with the fluorescent probe AlexaFluor 546 (Molecular Probes Inc., Eugene, OR, USA) diluted 1:600 (v/v) in PBS-T/BSA-C for 1 h at room temperature. Sections were washed as above, and then twice with distilled water before mounting in Citifluor™ anti-fade reagent (ProsciTech, Kirwan QLD 4817, Australia). The following controls were done: omission of the primary antibody (LM15) to detect any non-specific binding of the secondary antibody and omission of both the primary (LM15) and secondary antibody to determine if cell-wall autofluorescence was visible using the filter set described below for the secondary antibody. Histochemistry was examined by bright-field microscopy, and immunomicroscopy by fluorescence microscopy using a Carl Zeiss Axioplan 2 microscope (Oberkochen, Germany). Immunolabelled sections were examined using Zeiss filter set 15 (excitation filter BP546/12, chromatic beam splitter FT 580 and barrier filter LP 590). All images were taken with an exposure time of 1000 ms.

3. Results

3.1. Fucosylated and non-fucosylated cell walls in non-commelinid monocotyledons

In our previous immunofluorescence study using the monoclonal antibody CCRC-M1, the primary cell walls of some of the cell types in three non-commelinid species were unlabelled (see Table 1). However, using the monoclonal antibody LM15, all the primary walls in all of the cell types of these species were labelled, although to different extents (Table 1). In *Z. muelleri*, only the primary walls of phloem cells were labelled with CCRC-M1, whereas all primary walls were labelled with LM15 (Fig. 1A and B). In the aquatic species *Landoltia punctata*, the walls of the mesophyll parenchyma cells were labelled by LM15 (Fig. 1C and D) but not CCRC-M1 (Fig. 1E). In *Vanilla pompona*, the walls of the ground parenchyma and xylem parenchyma cells were labelled with LM15 (Fig. 1F and G) but were not labelled with CCRC-M1 (Fig. 1H).

3.2. Fucosylated and non-fucosylated cell walls in commelinid monocotyledons

In our previous immunofluorescence study using the monoclonal antibody CCRC-M1, the primary cell walls of some of the cell types in 14 commelinid species were unlabelled (see Table 1). However, using the monoclonal antibody LM15, all the primary walls in all of the cell types of these species were labelled. Although the labelling intensity varied with cell type and species, the phloem cell walls were usually strongly labelled (Table 1). Lignified secondary walls of sclerenchyma fibres and xylem vessels were not labelled by LM15, but some labelling was observed in the primary wall layer of cells with lignified secondary walls such as in sclerenchyma fibres of *Musa* sp. (Fig. 2A and B, arrow), *Ananas comosus* (Fig. 2D and E, arrow) and *Juncus inflexus* (Fig. 2G and H, arrow). In the vascular bundles of these species, only some cell types with primary walls were labelled with CCRC-M1 (Fig. 2C, F and I, respectively).

Within the Commelinales, in *Tradescantia virginiana* (Commelinaceae), the primary walls of no cell types were labelled by CCRC-M1, although *T. virginiana* is closely related to *Tradescantia fluminensis*, which is known to contain fucosylated xyloglucans (Hsieh and Harris, 2009). However, the primary walls of all cell types were labelled by LM15 (Fig. 2J and K). In *Pontederia cordata* (Pontederiaceae), walls

Table 1
Occurrence of xyloglucans labelled with the monoclonal antibodies LM15 and CCRC-M1 in the walls of different cell types in relation to monocotyledon phylogeny.

Order, family and species[a]	Cell Type											
	Epidermal cells: guard cells		Epidermal cells: subsidiary		Other epidermal cells		Parenchyma cells: mesophyll and/or ground		Phloem cells[b]		Xylem parenchyma	
	LM15	CCRC-M1	LM15	CCRC-M1	LM15	CCRC-M1	LM15	CCRC-M1	LM15	CCRC-M1	LM15	CCRC-M1
Non-commelinid monocotyledons												
Alismatales												
Araceae												
Landoltia punctata	+ + +	+	/	/	+ + +	+	+ + +	− to + +[d]	+ + +	+ + +	+ + +	+ +
Zosteraceae												
Zostera muelleri	+ + +	–	/	/	+	–	+	–	+	+	+	–
Asparagales												
Orchidaceae												
Vanilla pompona	+ +	+ +	+ +	+ +	+ +	+ +	+	–	+ + +	+ +[f]	+	–
Commelinid monocotyledons												
Commelinales												
Commelinaceae												
Tradescantia virginiana	+ + +	–	+ +	–	+ +	–	+ +	–	+ + +	–	+ +	–
Pontederiaceae												
Pontederia cordata	+ +	+ +	+ +	+ +	+ +	+ +	+ +	–[g]	+ +	+ +[f]	+ +	–
Zingiberales												
Musaceae												
Musa sp.	+ + +	+ +	+ + +	–	+ + +	–	+ + +	–	+ + +	+ + +[f]	+ + +	–
Cannaceae												
Canna indica	+ + +	+	+ + +	–	+ + +	–	+ + +	–	+ + +	+[f]	+ + +	–
Zingiberaceae												
Hedychium greenii	+ + +	–	+ + +	–	+ + +	–	+ + +	–	+ + +	+[f]	+ + +	–
Poales												
Typhaceae												
Typha orientalis	+ + +	+ + +	+ + +	+ + +	+ + +	+	+ +	–	+ + +	+ + +	+ +	+ +
Bromeliaceae												
Ananas comosus	+ +	+	+ +	+	+	–	+ +	–	+ + +	+ + +	+ +	+ +
Juncaceae												
Juncus inflexus	+ + +	–	+ + +	–	+ + +	–	+ +	–	+ + +	+ + +	+ + +	+ +[h]
Cyperaceae												
Cyperus papyrus	+ +	–	+ +	–	+ +	–	+ +	–	+ + +	+ +	+ +	+[i]
Restionaceae												
Elegia capensis	+ +	+ +	+ +	+ +	+ +	+ +	+ +	− & + +[j]	+ + +	+ + +	+ + +	+ +
Flagellariaceae												
Flagellaria indica	+ + +	+	+ + +	+	+ +	+	+	–	+ + +	+ + +	+	–
Joinvilleaceae												
Joinvillea ascendens	+ + +	–	+ +	–	+ +	− to + +[k]	+ +	–	+ + +	+	+ +	+[i]
Poaceae												
Lolium multiflorum	+ +	–	+ +	–	+ +	–	+ +	–	+ + +	+ +	+ +	–
Zea mays	+	–	+	–	+	–	+	–	+	–	+	–

− = no labelling; + = weak labelling; + + = moderate labelling; + + + = strong labelling;/= cell type not present.

[c] In protoxylem elements with helical or annular lignified wall thickenings, non-lignified walls between the thickenings were labelled.

[e] The walls of some epidermal cells appeared to be labelled more heavily than others. Comparison with sections stained with toluidine blue O suggests that this is due to these walls being thicker than other epidermal walls.

[a] Classification according to APG IV (2016).

[b] Includes sieve tube elements, companion cells and phloem parenchyma.

[d] The walls of the ground parenchyma cells were labelled, but the walls of the specialised mesophyll parenchyma cells were not.

[f] Only the walls of the developing phloem sieve tube elements were labelled.

[g] Only the walls of raphide idioblasts with the mesophyll were labelled.

[h] The walls of the xylem parenchyma were only labelled in the medullary ring of vascular bundles, and were not labelled in the outer ring of vascular bundles.

[i] Walls of the xylem parenchyma adjacent to protoxylem lacunae in large vascular bundles were labelled.

[j] Walls of chlorenchyma cells were unlabelled, but walls of the ground parenchyma were labelled.

[k] The epidermal cells at the inner fold of the leaf surface were the only epidermal cells with labelled walls.

of the xylem parenchyma cells and mesophyll parenchyma cells were labelled with LM15 (Fig. 2L and M), although they were not labelled with CCRC-M1 (Fig. 2N). However, the walls of raphide idioblasts that occur within the mesophyll parenchyma were labelled by both antibodies (Brennan and Harris, 2011).

Within the order Zingiberales, in *Canna indica* (Cannaceae) the primary walls of all cell types labelled with LM15 (Fig. 2O and P), whereas only the walls of stomatal guard cells and phloem sieve tube elements labelled with CCRC-M1 (Fig. 2Q). In *Musa* sp. (Musaceae) the walls of the stomatal guard cells and phloem sieve tube elements and, as in *P. cordata* (see above), raphide idioblasts that occur within the mesophyll also labelled with both antibodies. In *Hedychium greenii* (Zingiberaceae) the primary walls of all cell types labelled with LM15 (Fig. 2R and S), whereas only the walls of the stomatal guard and subsidiary cells were labelled by CCRC-M1 (Fig. 2T).

Within the order Poales, in *Elegia capensis* (Restionaceae) (Fig. 3A and B) and *Flagellaria indica* (Flagellariaceae) (Fig. 3D and E) all the primary walls were labelled with LM15, and also CCRC-M1 except those

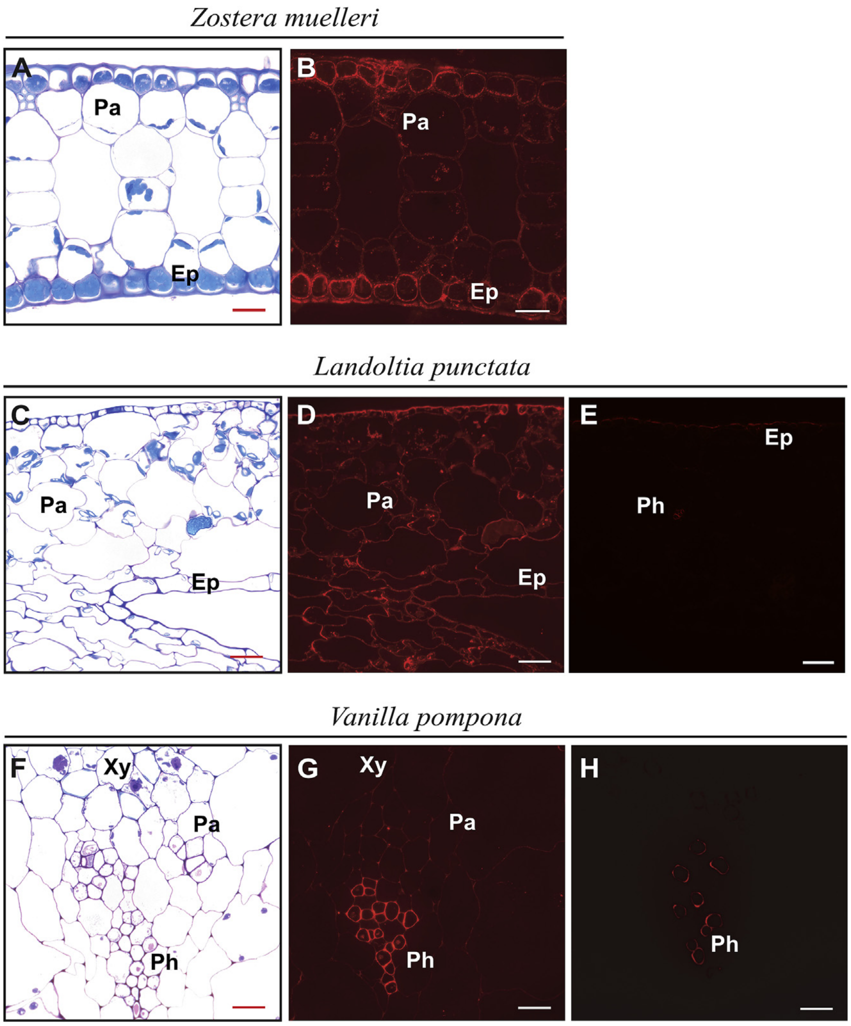

Fig. 1. Micrographs of sections from non-commelinid monocotyledon species with primary walls of some cell types that do not contain fucosylated xyloglucans. (A), (C) and (F) are bright-field micrographs of sections stained with Toluidine blue O showing the anatomy. (B), (D) and (G) are immunofluorescence micrographs of sections labelled with the LM15 monoclonal antibody, showing labelling in the primary walls of all cell types. (E) and (H) are immunofluorescence micrographs of sections labelled with the CCRC-M1 monoclonal antibody, showing comparative labelling in the walls of only some cell types, often the walls of phloem cells. Ep, epidermal cells; Pa, parenchyma cells; Ph, phloem sieve tube elements and companion cells; Xy, xylem parenchyma. Scale bars = 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

of chlorenchyma cells in *E. capensis* (Fig. 3C) and xylem parenchyma cells in *F. indica* (Fig. 3F). *Typha orientalis* (Typhaceae) and *Ananas comosus* (Bromeliaceae) showed a similar labelling pattern with both LM15 (Fig. 3G and H) and CCRC-M1 (Fig. 3I) except the only cell walls labelled in the mesophyll of these species by CCRC-M1 were those of raphide idioblasts similar to those described above. Additionally in *Ananas comosus* the only epidermal cell walls labelled by CCRC-M1 were those of the stomatal guard and subsidiary cells. In *Zea mays* (Poaceae), the primary walls of all cells were labelled by LM15 (Fig. 3J and K), but no cell walls were labelled by CCRC-M1. Similarly, in *Lolium multiflorum* (Poaceae) the primary walls of all cells were labelled by LM15 (Fig. 3L and M), but only the walls of phloem cells were labelled by CCRC-M1 (Fig. 3N). *Juncus inflexus* (Juncaceae) (Fig 3O, P and Q), *Cyperus papyrus* (Cyperaceae) (Fig 3R, S and T) and *Joinvillea ascendens* (Joinvilleaceae) (Fig 3U, V and W) had similar labelling patterns to *L. multiflorum* except the walls for the xylem parenchyma cells were also labelled by CCRC-M1.

In control experiments for both non-commelinid and commelinid monocotyledons, in which the primary antibody or the primary and secondary antibodies were omitted, no cell walls were labelled.

4. Discussion

The finding that the primary walls of all cell types in the monocotyledons investigated contain xyloglucans indicates that the fucosylated xyloglucans in the primary walls of specific cell types results from the regulation of xyloglucan biosynthesis during cell differentiation. We cannot rule out that it is the proportion of fucosylated xyloglucan, rather than the presence, that is downregulated so far as to be undetectable by the immunofluorescence methodology used here. Only xyloglucans with a repeating XXXG core have F side chains. Thus, where the xyloglucans have only the repeating XXXG core, as in the stem cell walls of *Juncus inflexus* (Hsieh and Harris, 2009), this involves only a regulation of the xyloglucan fucosyl tranferase adding the terminal fucosyl residue. However, where the xyloglucans have a repeating XXGn and XXXG core, such as in the leaf cell walls of *Canna indica* (Hsieh and Harris, 2009), then cell-specific regulation of xyloglucan biosynthesis involves both the repeating core as well as the xyloglucan fucosyl transferase.

Although phloem cell walls were labelled by CCRC-M1 in the leaves of the grass *Lolium multiflorum* (Brennan and Harris, 2011) no fucosylated xyloglucans or repeating XXXG core were found analytically (Hsieh and Harris, 2009) suggesting they were present in only very small proportions. It is known that small proportions of the CCRC-M1 epitope occur in the pectic polysaccharide rhamnogalacturonan-I (RG-I) when isolated from sycamore (*Acer pseudoplatanus*) walls (Puhlmann et al., 1994), and it is possible that the epitope is present in RG-I in Poaceae samples of the present study contributing to the unexpected labelling. However, in maize (*Zea mays*) leaves, phloem cell walls were not labelled with CCRC-M1 and no fucosylated xyloglucans were detected analytically, but some repeating XXXG core was found (Hsieh and Harris, 2009). Both repeating cores were also found analytically in the xyloglucans of young rice (*Orzya sativa*) roots, but additionally XXFG was released, and the walls of the outer layer of the root

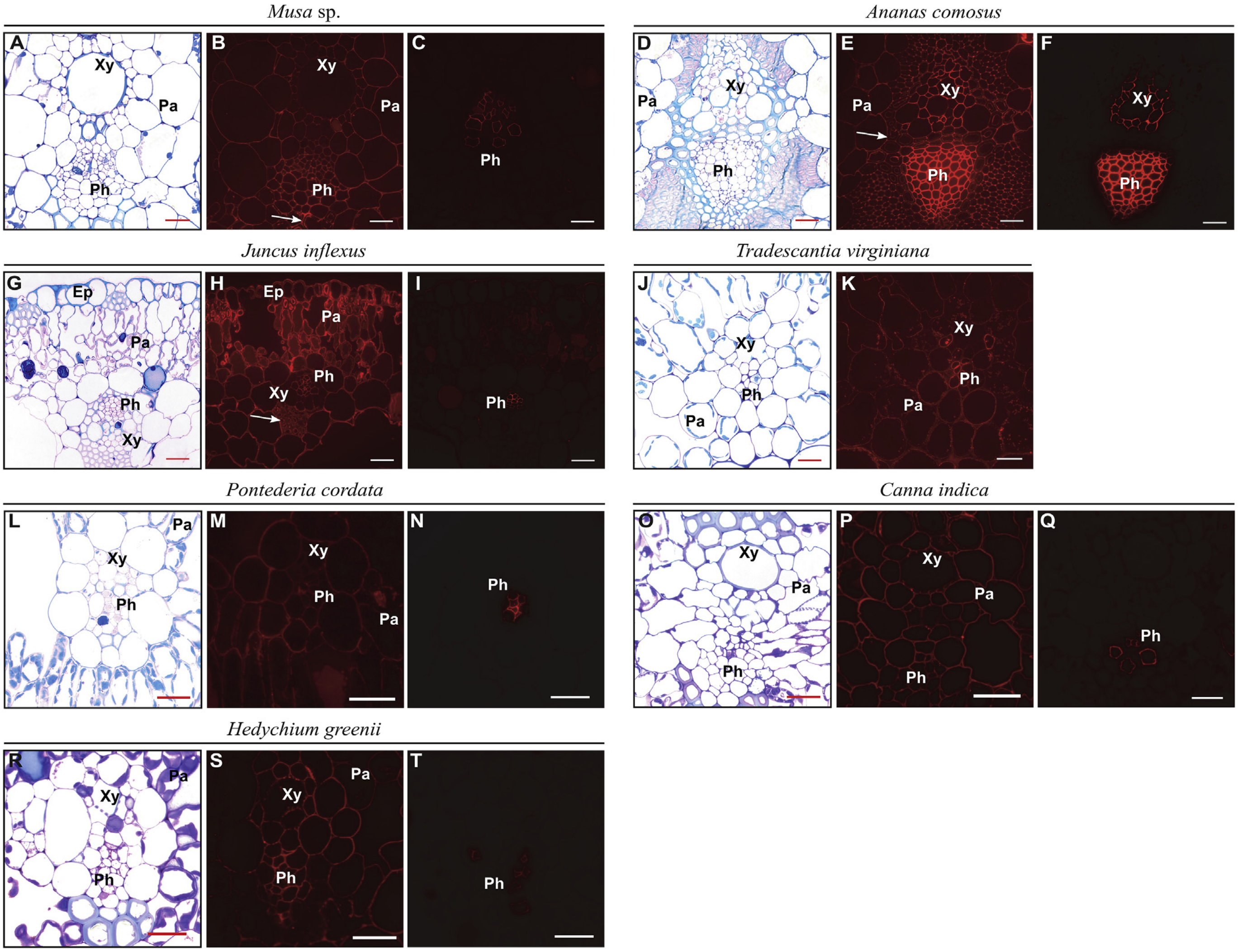

Fig. 2. Micrographs of sections from commelinid monocotyledon species with primary walls of some cell types that do not contain fucosylated xyloglucans. (A), (D), (G), (J), (L), (O) and (R) are bright-field micrographs of sections stained with Toluidine blue O showing the anatomy. (B), (E), (H), (K), (M), (P) and (S) are immunofluorescence micrographs of sections labelled with the LM15 monoclonal antibody, showing labelling in the primary walls of all cell types. (C), (F), (I), (N), (Q) and (T) are immunofluorescence micrographs of sections labelled with the CCRC-M1 monoclonal antibody, showing comparative labelling in the walls of only some cell types. Ep, epidermal cells; Pa, parenchyma cells; Ph, phloem sieve tube elements and companion cells; Xy, xylem parenchyma. Scale bars = 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

epidermal cells and of the root hairs were labelled with CCRC-M1 using immunofluorescence microscopy (Liu et al., 2015). Whether the walls of cell types with the fucosylated xyloglucans also contain xyloglucans with the repeating XXGn core is unknown. Fucosylated xyloglucans have also been found in pollen-tube walls of several species in the eudicotyledon family Solanaceae. Pollen tubes, like root hairs grow at their tips. The xyloglucans in this family usually have a repeating XXGG core, but in the walls of the pollen tubes of the ornamental tobacco *Nicotiana alata*, only a repeating XXXG core occurs. XXFG and XLFG oligosaccharides were released by an *endo*-(1 → 4)-β-D-glucanase, and the walls labelled with CCRC-M1 (Lampugnani et al., 2013). Xyloglucans in pollen-tube walls of tobacco (*N. tabacum*) and three tomato, *Solanum* species, were similar, but some repeating XXGG core was present (Dardelle et al., 2015).

Despite information about the occurrence of fucosylated xyloglucans, little is known about the functions of the F side chains or how they may relate to the growth and functions of the cells. However, the fucosylated oligosaccharide XXFG, which can be enzymatically released from fucosylated xyloglucans, has been shown to have a number of biological activities (Côté and Hahn, 1994). These include inhibiting the elongation of segments of pea stems induced by 2,4-dichlorophenoxyacetic acid; the oligosaccharide XXLG, which lacks the fucosyl residue, is inactive. Another possible function of xyloglucan fucosylation is in protection against aluminium toxicity. The degree of xyloglucan fucosylation has recently been shown to influence the binding capacity of aluminium, with an *Arabidopsis thaliana* mutant containing xyloglucans with fewer fucosyl substituents being more sensitive to aluminium toxicity (Wan et al., 2018). If such a function was important, the presence of fucosyl residues specifically in the phloem cell walls would be advantageous. Nevertheless, mutants of *Arabidopsis thaliana* with only small proportions or no fucosyl residues on xyloglucan (*mur2* and *atfut1* respectively) have no visible phenotypes, except for small changes in the trichomes of *mur2* plants (Perrin et al., 2003; Vanzin et al., 2002).

One hypothesis about the function of F side chains of xyloglucans is that they favour the binding, via hydrogen bonding, of xyloglucans to cellulose. However, *in vitro* experiments have shown equal or better binding occurs when the xyloglucan has been selectively de-fucosylated (Chambat et al., 2005; Park and Cosgrove, 2015). Extensive binding of xyloglucans to cellulose microfibrils formed the basis of models of primary cell walls of eudicotyledons and non-commelinid monocotyledons for many years, with xyloglucans envisaged to form tethers

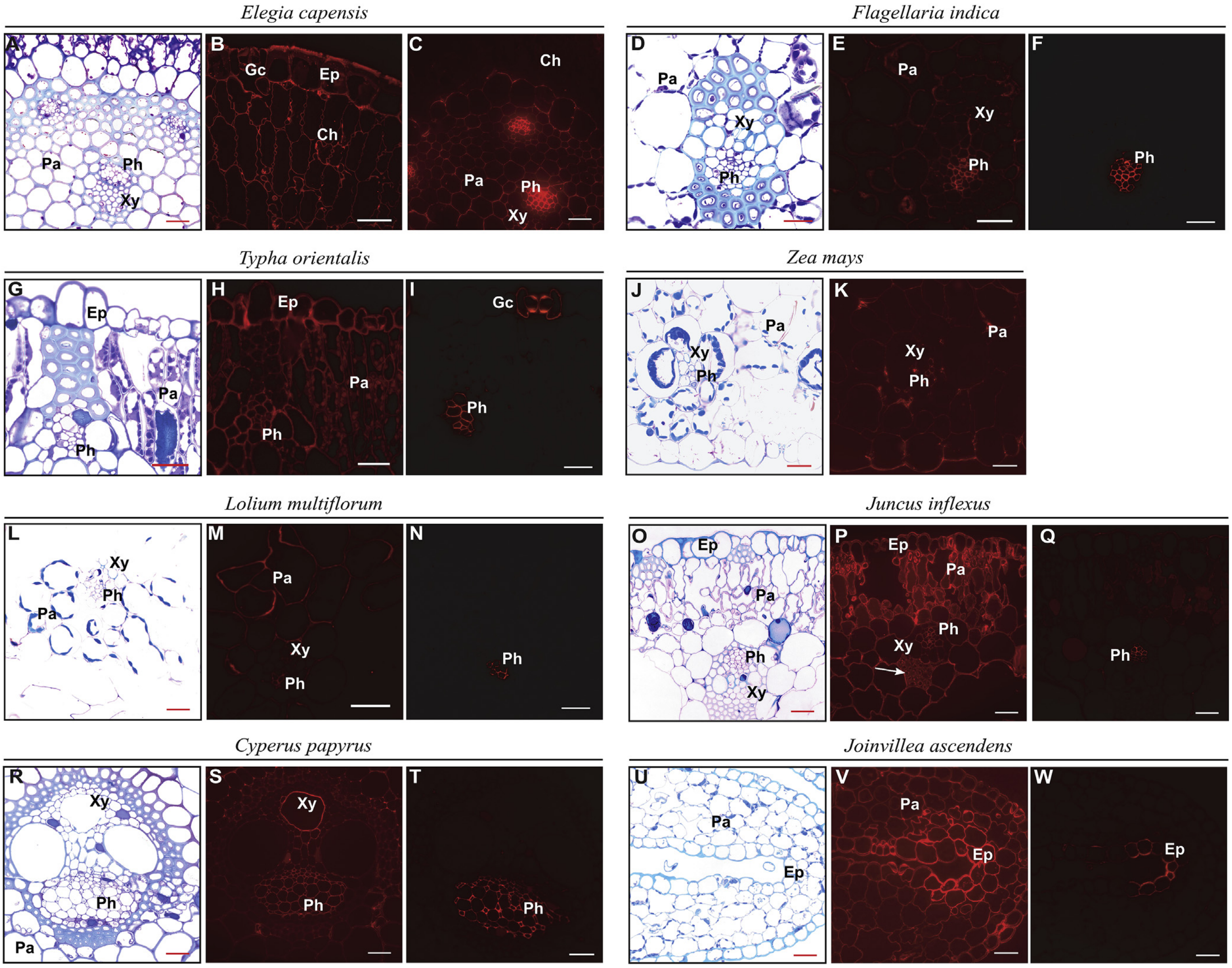

Fig. 3. Micrographs of sections from species within the Poales with primary walls that do not contain fucosylated xyloglucans. (A), (D), (G), (J), (L), (O) (R) and (U) are bright-field micrographs of sections stained with Toluidine blue O showing the anatomy. (B), (E), (H), (K), (M), (P) (S) and (V) are immunofluorescence micrographs of sections labelled with the LM15 monoclonal antibody, showing labelling in the primary walls of all cell types. (C), (F), (I), (N), (Q) (T) and (W) are immunofluorescence micrographs of sections labelled with the CCRC-M1 monoclonal antibody, showing comparative labelling in the walls of only some cell types. Ch, chlorenchyma cells; Ep, epidermal cells; Gc, stomatal guard cells; Pa, parenchyma cells; Ph, phloem sieve tube elements and companion cells; Xy, xylem parenchyma. Scale bars = 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

between the microfibrils and control wall expansion. This view was challenged by the finding that an *Arabidopsis thaliana* mutant containing no detectable xyloglucan showed only minor morphological differences from the wildtype (Cavalier et al., 2008), and by nuclear magnetic resonance spectroscopy, indicating that only a small portion of the microfibril surface had bound xyloglucans (Bootten et al., 2004; Dick-Pérez et al., 2011). More recent evidence indicates that only a minor component of the xyloglucans in such walls is mechanically important (Park and Cosgrove, 2015). Whatever the functions of the F side chains of xyloglucans, they occur widely within the walls of land plants, including the hornworts (Peña et al., 2004). Their presence on xyloglucans in the walls of specific cell types in the monocotyledons, especially the commelinid monocotyledons suggests that they are advantageous.

5. Conclusion

The occurrence of xyloglucan F side chains was previously shown to be manifest in the primary walls of only some cell types within some species of monocotyledons, particularly commelinid monocotyledons, where F side chains often occur only in the walls of phloem cells, specialised epidermal cells and sometimes raphide idioblasts. Here we have shown by using immunofluorescence labelling in conjunction with the LM15 monoclonal antibody, which recognises xyloglucans with XXXG and XXG_n core structures, the primary walls of all cell types in these species do contain xyloglucans. This indicates that the cell-type-specific labelling found with CCRC-M1 was indeed due to regulation of the localisation of the fucosyl residue within xyloglucans, rather than due to cell-type-specific localisation of the xyloglucan polysaccharide. Although the functional significance of xyloglucan fucosylation remains unknown, such cell-type specificity supports hypotheses that the fucosylated side chains may indeed have a functional role within the cell wall.

Declarations of interest

None.

Contributions

MB and PJH conceived the study, MB, PJH and DF designed the research, MB and DF carried out the experiments. All authors analysed the results, contributed to the writing of the manuscript and approved the final version.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.04.005.

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