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Evolution and diversity of green plant cell walls

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Plant cells are surrounded by a dynamic cell wall that performs many essential biological roles, including regulation of cell expansion, the control of tissue cohesion, ion-exchange and defence against microbes. Recent evidence shows that the suite of polysaccharides and wall proteins from which the plant cell wall is composed shows variation between monophyletic plant taxa. This is likely to have been generated during the evolution of plant groups in response to environmental stress. Understanding the natural variation and diversity that exists between cell walls from different taxa is key to facilitating their future exploitation and manipulation, for example by increasing lignocellulosic content or reducing its recalcitrance for use in biofuel generation.

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Introduction

Cellulose-rich cell walls, one of the defining characteristics of plants, are of fundamental importance for normal plant growth and development. Despite recognition that distinct differences exist in wall chemistry between Angiosperm taxa [1,2], the main focus of cell wall research has been on Angiosperm, primarily crop, species. Recently, however, there has been increasing interest in the cell wall biochemistry of non-Angiosperm plants [3••,4•,5–8,9•,10–14], and evidence has emerged to support the hypothesis [15] that changes in cell wall composition are fundamentally involved with plant evolution and diversification (Figure 1).

Interest in non-Angiosperm cell wall composition is not only of intrinsic interest, but the increasing use of *Physcomitrella patens* (Hedw.) B.S.G., a moss, as a tool for investigating Angiosperm cell wall biochemistry [16] demands more complete characterisation of bryophyte walls. The main cell wall components, polysaccharides, represent a major and renewable source of photosynthetically fixed carbon and are of importance as a source of biomass for the emerging biofuel industry. Cell walls have been used as a source of fuel for millennia, for example in the form of wood, derived from dicotyledonous trees, and peat, derived from *Sphagnum* moss. Algae, while being among the most efficient photosynthetic organisms and of interest for biofuel generation, will not be specifically discussed within this review because their greatest potential is as a source of oils (localised in the vacuole) for use in bio-diesel production, rather than cell wall biomass. This review will focus on the natural variation and diversity that exists within land plant cell wall composition that is associated with land plant phylogeny (Table 1).

A more comprehensive investigation of diverse land plant cell walls not only is essential to facilitate wall modification enabling production of biomass with improved qualities, for example an increased cellulose concentration; but may also suggest the potential of novel crop plants.

Types of cell wall

Cells walls are composed of three types of layers: the middle lamella, the primary cell wall and the secondary cell wall. The middle lamella is deposited soon after mitosis creating a boundary between the two daughter nuclei and, once the cell plate is complete, the primary cell wall is deposited and continues to be deposited throughout cell growth and expansion. Therefore, although the primary cell wall is typically only 0.1–10 μm thick, its composition is of importance for biomass accumulation through controlling cell growth.

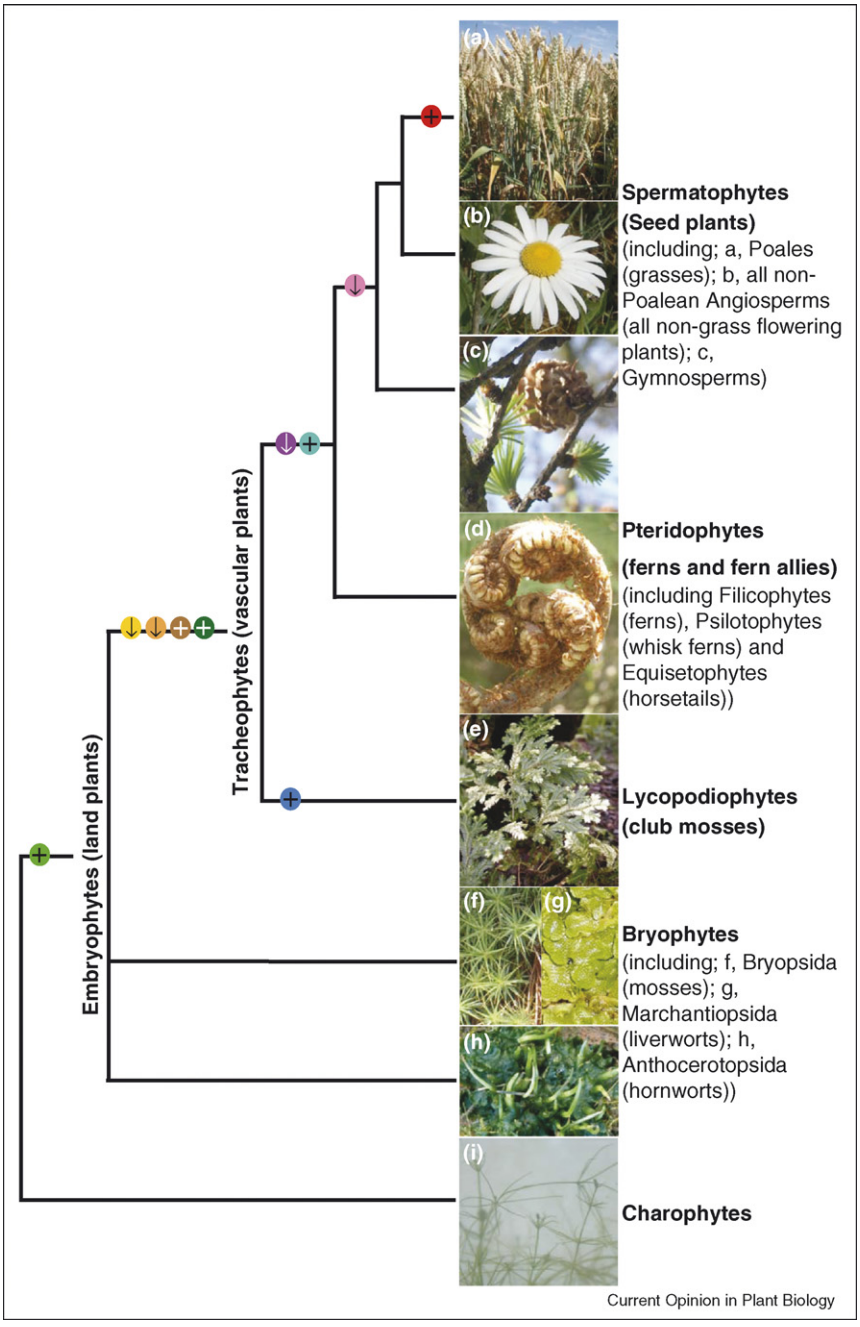
The secondary cell wall is deposited internally to the primary cell wall at the onset of differentiation, once cell growth has ceased. However, secondary cell walls are not present in all cell types, parenchyma and collenchyma frequently have only a primary cell wall, or in all plant taxa. In spermatophytes, secondary cell wall composition is known to vary from one cell type and reflects cell function. For example, many secondary cell walls, particularly xylem cells, contain lignin that increases wall strength.

Primary cell wall polysaccharides

The majority of research regarding cell wall composition in diverse plant groups has concentrated on the primary cell wall owing to the existence of cell type specific variation between secondary cell walls.

The primary cell wall is composed of cellulose microfibrils embedded in a gel-like matrix of non-cellulosic polysaccharides and glycoproteins. The cellulose–hemicellulose

Figure 1



Key transitions in cell wall components mapped onto a land plant phylogeny (adapted from references [44,45]). Changes in composition are symbolised as: (+) appearance or substantial increase in occurrence; (↓) either a reduction or loss; (●) (1 → 3), (1 → 4)-β-D-glucan [18]; (●) xyloglucan [13]; (●) mannose; (●) 3-*O*-Methylgalactose [13]; (●) 3-*O*-Methylrhamnose [13]; (●) galacturonic acid; (●) glucuronic acid; (●) tannins [12]; (●) branched 4-linked xylan [6]; (●), rhamnogalacturonan II [11]. Representatives of taxonomic groups shown are **(a)** Poales, wheat (*Triticum aestivum* L.); **(b)** Angiosperms (excluding Poales), Ox-eye Daisy (*Leucanthemum vulgare* Lam.); **(c)** Gymnosperms, Larch (*Larix decidua* L.); **(d)** Pteridophytes, Tree fern; **(e)** Lycopodiophytes, Selaginella (*Selaginella martensii* Spring.); **(f)** Bryopsida, mosses (*Polytrichum* sp.); **(g)** Marchantiopsida, Liverworts (*Lunularia cruciata* (L.) Lindb.); **(h)** Anthocerotopsida, hornworts (*Phaeoceros carolinanus* (Michx.) Prosk.); **(i)** Charophytes, (*Nitella* sp.)

network co-exists with a network consisting of pectic polysaccharides. A covalent linkage between xyloglucan and pectin, interconnecting the two networks, appears to be widespread among Angiosperm taxa [17].

Although relatively few detailed structural analyses have been performed on cell walls from non-Angiosperm taxa, it appears that seed plants have cell walls with similar, though not identical, compositions. The quantitatively

Table 1

Occurrence of cell wall components shown to vary, with phylogenetic significance, between land plant taxa

Plant group	Monosaccharides			Polysaccharides						Proteins	
	3-*O*-MeRha	3-*O*-MeGal	Uronic acids	Xylan	Mannan	Xyloglucan[a]	RGII	Pectin	(1 → 3), (1 → 4)-β-D-glucan	Expansins	Ces/Csl
Charophytes	+	−	+	−	+	−	±	++	−		
Hornwort	+	−	+	+	+	++	±	+++	−		
Liverworts and basal mosses	+	−	+	−	+	++	±	++	−		
Advanced mosses	+	−	±	+	+	++	±	++	−	EXPA (conserved function) and EXPB	CesA, CslA, CslC, CslD[b]
Homosporous lycopodiophytes	+	+	±	+	+	++	+	+	−		
Heterosporous lycopodiophytes	+	+	±	+	+	++	+	+	−		
Eusporangiate ferns	−	±	±	+	+	++	+	+	−		
Leptosporangiate ferns	−	±	±	+	±	++	+	+	−		
Gymnosperms	−	±	±	+	±	++	+	+	−		
Dicotyledonous Angiosperms	−	±	±	+	±	++	+	+	−	EXLA, EXLB, EXPA, EXPB	CesA, CslA, CslB, CslC, CslD, CslE, CslG
Poales members of monocotyledonous Angiosperms	−	±	±	+	±	+	+	+	+		CesA, CslA, CslC, CslD, CslE, CslF, CslH

−, not detectable; ±, trace; +, present at low concentration; ++, present at moderate concentration; +++, present at high concentration.
[a] Xyloglucan shows diversity in glycosyl composition.
[b] CslD is highly represented among *P. patens* ESTs that may reflect their involvement in tip growth of moss protonemata [16].

predominant monosaccharides present in the walls are D-glucose (Glc), D-galactose (Gal), D-mannose (Man), D-xylose (Xyl), L-arabinose (Ara), L-fucose (Fuc), L-rhamnose (Rha) and D-galacturonic acid (GalA). Poales walls contain more Xyl and less GalA, Gal and Fuc than other Angiosperms and Gymnosperms contain more Man residues. Differences in cell wall composition are correlated with diversification of specific plant taxa. The lycopodiophytes form a distinctive, basal, monophyletic clade within extant vascular plants whose cell walls uniquely contain the unusual monosaccharide residue 3-*O*-methylgalatose [12] that is likely to be a component of many lycopodiophyte primary cell wall polysaccharides including xyloglucan (MA O'Neill, personal communication).

Additional variation of cell wall composition exists at the polysaccharide level, (1 → 3), (1 → 4)-β-D-linked glucans are restricted to the Poales [18] and xyloglucan is present in the cell walls of all land plants but appears to be absent from the charophytes, their closest extant ancestors [13]. Structural diversity is also seen within specific polysaccharides. Bryophyte and charophyte cell walls are rich in uronic acids [13]. In xyloglucans the presence of the α-L-Fuc*p*-(1-2)-β-D-Gal*p*-(1-2)-α-D-Xyl*p* side-chain appears to be conserved, but novel side-chains have been characterised including uronic acid-containing side-chains in bryophytes, equisetophytes and lycopodiophytes (MJ Peña *et al.*, abstract in *Physiol Plant* 2007, 130:18).

Rhamnogalacturonan II (RGII) is required for growth and development in Angiosperms, and comparable amounts occur in members of the most primitive extant plants lycopodiophytes, equisetophytes, psilotophytes and pteridophytes. By contrast, gametophytes of bryophytes contain only ~1% of the amounts present in vascular plants. In addition the glycosyl sequence of RGII appears to be conserved with the exception that the non-reducing Rha residue present on the aceric acid containing side-chain of RGII is replaced by 3-*O*-methylrhamnose (3-*O*-MeRha) in some lycopodiophytes and pteridophytes [11]. RGII is linked with the ability to form upright stem and formation of lignified cell walls, which correlates well with its increased concentration in vascular plants.

Secondary cell wall polysaccharides

Variation in secondary cell wall composition is perhaps the most pertinent in the context of biomass production. Secondary cell walls are composed of cellulose, xylan and lignin and, in Gymnosperms, glucomannan.

Cellulose is the most abundant biopolymer on the planet, and cellulose microfibril structure is largely determined by cellulase synthase catalytic subunits encoded by CesA genes. Despite conserved regions of CesA genes in plants and bacteria, mosses lack vascular tissue appear to lack secondary cell wall specific CesA orthologues [19]. True secondary cell walls are perhaps, therefore, restricted to vascular plants.

Xylan, composed of a (1 → 4)-linked β-D-xylopyranose backbone substituted with α-glucuronic acid or 4-*O*-methyl-α-D-glucuronic acid, is the second most abundant polysaccharide in dicot wood, and xylan containing the epitopes recognised by LM10 (unsubstituted) and LM11 (substituted) are present in the secondary cell walls of all extant vascular plants. However, xylans were not detected, even in thickened cell walls, from both the sporophyte and gametophyte generation of bryophytes, with the exception of specific cell wall layers in hornwort pseudoelators and spores [6]. The ubiquitous occurrence of xylans in vascular plants suggests that it may have provided a pre-adaptive advantage enabling the evolution of highly efficient vascular and mechanical tissues and allowing vascular plants attain greater size and to colonise water-limited environments.

Galactoglucomannans are major components of the cell walls of the woody tissues of both Angiosperms and Gymnosperms, and also occur in fern and moss cell walls suggesting that the polysaccharide evolved before divergence of vascular plants and may have played an important role in providing tensile strength in bryophyte cell walls [20••].

Palaeobotanical evidence of tracheids with degradation-resistant possibly lignified walls indicates that the secondary cell wall had evolved by the early Devonian (~415 million yeas ago) [21], and the evolution of tracheids is associated with a burst of structural diversity. Although lignans are found in bryophytes, lignin is limited to vascular plants [7,22]. In association with cellulose lignin provides strength to walls under tension. Variation exists in lignin composition between Angiosperms and Gymnosperms. Gymnosperm lignins are composed primarily of guaiacyl units, whereas Angiosperm lignins are composed of both guaiacyl and syringyl units. However, peroxidases extracted from Gymnosperms were found to be capable of oxidising syringyl moieties, and homologies exist between peroxidases extracted from bryophyte species, which lack xylem and lignin, and eudicot syringyl peroxidases [23•].

Cell wall enzymes

The proportion of genes encoding carbohydrate acting enzymes in plants is likely to be higher than in any other group of organisms [24]. A complete description of enzymes involved in cell wall biosynthesis and modification is therefore a huge task and has not yet been obtained. Therefore, only enzyme families whose function has been at least partially elucidated, and for which there is known variation between taxa, will be discussed.

Rice and *Arabidopsis* have a similar number of cell wall related gene families and members within each family even though rice has a far greater number of genes than *Arabidopsis*. For example rice has 52 xyloglucan endotransglycosylase/hydrolase (XTH) genes compared with 40 in *Arabidopsis*, despite a far lower concentration of xyloglucan in grass cell walls [25–27]. This implies that similar numbers of genes are required for wall construction and maintenance, at least among Angiosperms [28].

Proteins that have a role in cellulose synthesis are encoded by a large family of cellulose synthase (CesA) genes. CesAs, highly expressed in loblolly pine (gymnosperm) xylem development have been shown to be orthologous to those in Angiosperms [29].

A superfamily of cellulose synthase-like (Csl) genes has also been described with each subfamily indicated in synthesis of a specific hemicellulose, for example CslA is involved in glucomannan synthesis [20••,30,31] and appears to have diversified subsequent to the divergence of monocots and eudicots. By contrast, CslF that is involved in synthesis of (1 → 3), (1 → 4)-β-D-glucan [32••] occurs uniquely in grasses, and is therefore closely correlated with the reported occurrence of this polysaccharide [18]. Genomic sequences from *P. patens* have been interrogated to identify genes with a high degree of sequence similarity to vascular plant CesA and Csl genes. Four of the gene families identified in vascular plants (CesA, CslA, CslC and CslD) were present in mosses (Table 1), and phylogenetic analysis suggests that mosses and vascular plants diverged before CesAs diversified and specialised to have distinct roles in the primary and secondary cell wall [16].

XTHs are a class of enzymes that transglycosylate xyloglucan allowing expansive cell growth. Some XTHs, for example Tm-NXG1 and Tm-NXG2 from *Tropaeolum majus* are hydrolytic. This is suggested to be a gain of function in an ancestral XTH that had endotransglucosylase activity [33]. In *Arabidopsis* there are at least 33 XTHs genes that exhibit temporal and spatial expression patterns. XTH appears to be highly conserved in land plants, XTH sequenced from *Selaginella kraussiana* (lycopodiophyte) shows strong conservation of the xyloglucan endotransglucosylase (XET) domain of higher plants with catalytic site variation of only one amino acid [4•]. XET activity is associated with sites of growth and elongation and has been identified in all land plants [3••,34] and perhaps more surprisingly, given their apparent lack of xyloglucan [13], within the green algae *Chara* and *Ulva* [3••,4•]. Owing to the control of tracheary element elongation by an XTH [35] and because XET activity has been detected in sporophyte and gametophyte tissues [34] it is likely that divergence of XTH genes played a role in evolution of vascular plants.

Cell wall associated proteins

Cell wall associated proteins may directly influence plant morphogenesis that, in plants, results from differential growth of the organs at the level of cell walls.

Histological and immunocytological evidence suggests that the cell wall associated proteins known as arabinogalactan proteins (AGPs) occur in all land plants and their charophyte ancestors (A McCann *et al.*, abstract in *Physiol Plant* 2007, 130:125). AGPs exhibit structural and functional diversity, and it is likely that subtle function differences may be brought about through structural heterogeneity, largely within the carbohydrate domain [36]. 3-*O*-MeRha has been detected in the primary cell walls of Charophytes, Bryophytes, Pteridophytes and Gymnosperms, but not Angiosperms [5,11–13]. Recent analysis of the carbohydrate portion of AGPs extracted from *P. patens* has shown that they contain relatively high levels of terminal 3-*O*-MeRha; this monosaccharide has not been detected in Angiosperm AGPs [37]. It is hypothesised that the relatively non-polar nature of 3-*O*-MeRha may alter arabinogalactan protein polarity and enable function through hydrophobic interactions [37]. Similarities also exist between Angiosperm and non-Angiosperm derived AGPs. ESTs encoding putative AGP core proteins have been identified from *P. patens* that show 29% amino acid identity to sequences from *Arabidopsis*, *Brassica napus* and *Oryza sativa* [8]. In addition, LM6, an anti-(1 → 5)-α-L-arabinan monoclonal antibody that was raised against oligosaccharides from an Angiosperm-derived source, is able to recognise and bind to epitopes present in *P. patens* that have a role in regulating the extension of protonemal cells with apical tip growth [8]. AGPs and, in particular, their carbohydrate domain, may be of importance for defining land plant body plans and thus instrumental in the evolution of major groups of land plants.

Expansins are wall proteins that modify the mechanical properties of cell walls enabling turgor-driven cell enlargement. They are encoded by a superfamily of genes that can be grouped into four families; EXLA, EXLB (which have unknown function), EXPA (α-expansins) and EXPB (β-expansins). EXLA and EXLB are absent from *P. patens*, and sequence analysis suggests a divergence of EXPB function between bryophytes and Angiosperms [38]. Among Angiosperms EXPBs are present at much higher concentrations in members of the monocotyledonous Poales than they are in dicotyledonous Angiosperms. However, EXPA function is probably preserved in all land plant taxa [38]. Vascular plant evolution may be closely associated with that of expansins as they are thought to play a role in xylem development.

Conclusions and future perspectives

Although bryophytes and Angiosperms diverged more than 400 million years ago, the level of similarity in wall composition [11,13] enables the use of *P. patens* as a model for primary cell wall structure and function. *P. patens* has a unique combination of features, including high levels of gene targeting [39••] that make it powerful tool for comparative genomics across land plants. This in combination with high throughput and low tissue demanding techniques (e.g. glycoarrays [40], Fourier-transformed infrared spectroscopy of whole plant tissues [41] and oligosaccharide mass profiling [42]) will fuel increased understanding of the diversity of cell walls, for example enabling analyses of differences between plant populations and between gametophyte and sporophyte generations, which will facilitate their future manipulation. The development of an increased number of cell wall specific monoclonal antibodies will also enable better understanding of cell wall composition at the tissue level enabling an enhanced view of plant morphology and perhaps adaptive processes; recently wall-directed immunocytochemistry clarified that intercellular pectic protuberances in ferns do not originate solely from the middle lamella [43••].

Note added in proof

Although occurrence of (1 → 3), (1 → 4)-β-D-glucan was thought to be delimited, within vascular plants, to members of the order Poales [18] two independent studies have shown that the polysaccharide, albeit with some structural differences, is a major component of *Equisetum* (horsetail) cell walls [46••,47••]. It would be of interest to determine whether CslF, which is responsible for (1 → 3), (1 → 4)-β-D-glucan synthesis in the Poales [32••], is present in *Equisetum*. The importance of (1 → 3), (→ 4)-β-D-glucan to *Equisetum* wall architecture is perhaps indicated by the high activity of a novel enzyme isolated from *Equisetum* walls which transglucosylates (1 → 3), (1 → 4)-β-D-glucan to xyloglucan [48••]. No other land plants, including other Pteridophytes, were found to contain (1 → 3), (1 → 4)-β-D-glucan [46••,47••] and distribution of the novel enzyme activity appears to be limited to *Equisetum* and charophytic algae [48••]. Therefore, *Equisetum*, a phylogenetically isolated genus, clearly has a unique wall architecture [46••,47••,48••] emphasising the influence of evolution on cell wall composition and the diversity that exists in wall composition among land plants.

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The polysaccharide (1 → 3), (1 → 4)-β-D-glucan, previously only found in the Poales, was shown to be a quantitatively significant component of *Equisetum* cell walls. This was concurrently discovered by independent researchers [47••]. Structural characterisation determined differences between *Equisetum* and Poales (1 → 3), (1 → 4)-β-D-glucan.

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Activity of a novel enzyme, isolated and characterised from *Equisetum*, indicates that (1 → 3), 1 → 4)-β-D-glucan, only recently discovered to occur in *Equisetum* [46••,47••], is important for *Equisetum* wall properties.