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Review

Research review

Modern mannan: a hemicellulose's journey

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Summary

Hemicellulosic polysaccharides built of β-1,4-linked mannose units have been found throughout the plant kingdom and have numerous industrial applications. Here, I review recent advances in the biosynthesis and modification of plant β-mannans. These matrix polymers can associate with cellulose bundles to impact the mechanical properties of plant fibers or biocomposites. In certain algae, mannan microfibrils even replace cellulose as the dominant structural component of the cell wall. Conversely, patterned galactoglucomannan found in *Arabidopsis thaliana* seed mucilage significantly modulates cell wall architecture and abiotic stress tolerance despite its relatively low content. I also discuss the subcellular requirements for β-mannan biosynthesis, the increasing number of carbohydrate-active enzymes involved in this process, and the players that continue to be puzzling. I discuss how cellulose synthase-like enzymes elongate (gluco)mannans in orthogonal hosts and highlight the discoveries of plant enzymes that add specific galactosyl or acetyl decorations. Hydrolytic enzymes such as endo-β-1,4-mannanases have recently been involved in a wide range of biological contexts including seed germination, wood formation, heavy metal tolerance, and defense responses. Synthetic biology tools now provide faster tracks to modulate the increasingly-relevant mannan structures for improved plant traits and bioproducts.

Introduction

Plant cells secrete carbohydrate-rich polymers to enable their survival in a wide range of aqueous and terrestrial environments. This extracellular matrix represents the bulk of plant biomass and provides the most abundant source of renewable materials on the planet (Pauly & Keegstra, 2008). Plant cell walls contain three major classes of polysaccharides: cellulose, hemicelluloses, and, to a lesser extent, pectin. While cellulose microfibrils are built of insoluble β-1,4-linked glucose (Glc) chains, hemicelluloses are typically alkali-soluble and feature β-1,4-Glc, mannose (Man), or xylose backbones with frequent branches (Scheller & Ulvskov, 2010). Here, I review advances in our understanding of β-1,4-linked homomannans and heteromannans, an ancient class of hemicelluloses that is highly conserved through plant evolution (Scheller & Ulvskov, 2010; Pauly *et al.*, 2013). For simplicity, I use the term plant 'mannans' to encompass all types of β-1,4-Man-containing polysaccharides. Although the study of plant mannans commenced in the 1960s and was spearheaded by luminaries such as J.S. Grant Reid in the 1980s (Reid, 1985), molecular insights lagged behind other polymers until recently. Nevertheless, plant mannans have enjoyed widespread industrial uses (see food review by Singh *et al.*, 2018) and have emerging biotechnological applications (e.g. for the production of sustainable biofuels and bioproducts). Even though plant β-mannans are structurally distinct from α-linked mannans that are abundant in fungal cell walls (Pauly *et al.*, 2019), they have the potential to be introduced in health-promoting foods, where yeast cell wall derivatives are already commercialized (Yamabhai *et al.*, 2014).

Structure and properties of β-mannans

Despite the distribution of mannan polysaccharides throughout the plant kingdom, their structure and abundance vary dramatically between species, tissues, as well as developmental stages. Based on their carbohydrate composition, four categories of mannan

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polysaccharides accumulate in certain plant tissues (Fig. 1a). Homomannan contains primarily (> 90%) of β-1,4-Man, while glucomannan backbones also include β-1,4-Glc residues (Fig. 1a). Pure β-mannans are rich in palm seeds, including ivory nuts that serve as carving materials. Glucomannan accumulates as a storage polysaccharide in *Amorphophallus konjac* corms akin to starch in potato tubers (Gille *et al.*, 2011) and is the dominant hemicellulose in orchid stems (e.g. *Dendrobium officinale*; He *et al.*, 2017). (Gluco)mannan chains can be thousands of units long, but their molecular weight is challenging to measure because even ivory nut mannan, containing only 15–20 residues (Megazyme Ltd, Bray, Ireland), forms insoluble crystals in water. Since unbranched Man units can form intermolecular hydrogen bonds, their substitution with carbohydrates or other molecules can alter their conformation and interactions.

Galactomannan and galactoglucomannan (GGM; Fig. 1a), containing α-galactose (Gal) side chains at the carbon 6 (C-6) of Man units, are abundant in legume seeds and in gymnosperm stems (softwood), respectively (Pauly & Keegstra, 2008). For a long time, the arrangement of glycosyl residues in β-mannan polysaccharides has been thought to lack a regular pattern (Scheller & Ulvskov, 2010). However, this hypothesis needs to be reassessed because *Arabidopsis thaliana* seed mucilage was found to contain GGM polymers with Glc-Man repeating units and Gal branches on evenly spaced Man units (Fig. 1b; Yu *et al.*, 2018). While pectin makes > 90% of Arabidopsis seed mucilage (Voiniciuc *et al.*, 2018), this specialized extracellular matrix also contains small amounts of GGM and branched xylan typical of secondary cell walls (Voiniciuc *et al.*, 2015b). Furthermore, natural Arabidopsis ecotypes produce mucilage with the varying Man content (Fig. 1b; Voiniciuc *et al.*, 2016). Man units can also feature decorations such as sulfation in algae (Fernández *et al.*, 2012) and O-linked acetylation in land plants (Fig. 1a; Zhong *et al.*, 2018). Proton-nuclear magnetic resonance (^{1}H-NMR) spectroscopy of bryophytes and conifers showed that 22–34% of Man residues are O-acetylated (Zhong *et al.*, 2019), predominantly at the C-2 and C-3 positions. While native acetylation of GGM does not impair the enzymatic cleavage of β-1,4-Man units, a high degree of chemical acetylation increases the recalcitrance of mannan substrates (Arnling Bååth *et al.*, 2018) and can thus modulate polysaccharide properties for industrial applications.

Plant β-mannan extracts from various species have been evaluated as potential food hydrocolloids via physicochemical characterization (Singh *et al.*, 2018). In general, the physical properties of polysaccharides are determined by their monosaccharide composition, backbone length, and their degree of substitution. Changes in one or more of these parameters can shape β-mannan conformation and, for instance, convert crystalline microfibrils into water-soluble polymers. GGM makes up to a quarter of softwood dry weight and solid-state nuclear magnetic resonance (ssNMR) indicates that this polymer forms a flattened ribbon when bound to the surface of cellulose microfibrils in spruce softwood (Terrett *et al.*, 2019). Molecular dynamics simulations of the GGM found in Arabidopsis seed mucilage (Fig. 1b) also suggest that the backbone and even substitution pattern of this polymer promotes its binding to cellulose microfibrils (Yu *et al.*, 2018). Bacterial cellulose hydrogels containing wood glucomannans were shown to have an increased elastic modulus in compression (Berglund *et al.*, 2020). Therefore, β-mannans can associate with cellulose bundles to impact the mechanical properties of plant fibers or composites.

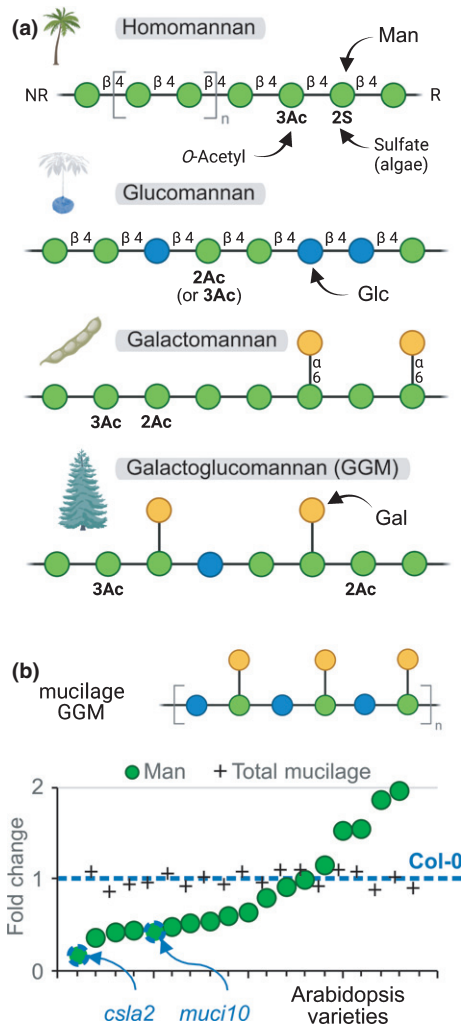

Fig. 1 Overview of β-mannan categories and seed mucilage pattern. (a) Four major classifications of homomannans and heteromannans, with a representative plant example. Numbers indicate the carbon position. This figure was partly drawn using BioRender (BioRender.com). (b) Patterned galactoglucomannan (GGM) structure identified by Yu *et al*. (2018) in Arabidopsis seed mucilage and the distribution of mucilage carbohydrates (mannose and total sugars) in a selection of 16 Arabidopsis natural accessions and two insertional mutants (*csla2* and *muci10*). Data was extracted from Voiniciuc *et al*. (2016), and are relative to Col-0 wild-type (represented by the dashed line).

Distribution of β-mannans from water to land

Various reports suggest that Man-rich polysaccharides are more abundant in algae and bryophytes than in vascular plants. Yet the abundance and composition of β-mannans in most nonvascular plant cell walls remains ambiguous due to the lack of comprehensive monosaccharide and/or glycosidic linkage analyses. Although antibody-based profiling indicates the presence of β-mannan epitopes in alkaline-solubilized material from 10 *Charophyceae*

green algae (Sørensen *et al.*, 2011), 4-linked Man represented < 2% of the total glycosyl linkages in most of these samples. Even in commonly studied bryophytes such as the moss *Physcomitrium* (previously *Physcomitrella*) *patens*, 4-linked Man and 4,6-Man accounted for only 7% of glycosidic linkages in protonemata cell walls (Moller *et al.*, 2007). Hence, antibodies assays overestimated the content of β-mannan in some plants.

Nevertheless, species with unusually high content of β-mannans exist throughout the plant kingdom. Linear β-1,4-mannans replace cellulose as the dominant structural polymer in certain algae, such as the green seaweed *Codium vermilara* (Fernández *et al.*, 2012). This algal mannan is mostly fibrillar, with 2-linked sulfate on 23% of Man units resulting in partially soluble chains that could help to maintain amorphous cell wall regions. Another Man-rich polymer, likely GGM, is the predominant hemicellulose in the gametophytic stem of the umbrella moss *Hypnodendron menziesii* (Chavan *et al.*, 2021). The β-mannan epitopes in this moss species and in Arabidopsis (Marcus *et al.*, 2010) are partially masked by O-acetyl groups and pectic polymers. The labeling of *H. menziesii* cell walls with the LM21 and LM22 monoclonal antibodies was strongest after pre-treatment with both sodium carbonate and pectate lyase to remove pectin (Chavan *et al.*, 2021). Due to the restricted access of exogenous proteins to mannans in plant cell walls, future studies must apply comprehensive biochemical analyses and/or novel imaging probes with fewer compromises. In contrast to previously described angiosperms, the fern-like *Psilotum nudum* was found to be mannan-rich in both primary and secondary walls (Chernova *et al.*, 2020). Therefore, investigating how mannans are distributed in the plant kingdom will likely reveal further surprises.

Biosynthesis of β-mannan polysaccharides

Similar to most hemicelluloses (Scheller & Ulvskov, 2010; Pauly *et al.*, 2013), the metabolic pathway for β-mannans spans the cytosol, the secretory system, and the extracellular space (Fig. 2). Hemicelluloses are synthesized in the Golgi apparatus using activated sugar donors, which are typically linked to uridine diphosphate (UDP) or guanidine diphosphate (GDP) and interconverted in the cytosol (Bar-Peled & O'Neill, 2011). While β-mannans are primarily destined for the cell wall, they also accumulate as storage polysaccharides in membrane-bound granules in specialized tissues such as *Dendrobium* orchid stems (He *et al.*, 2017), the legume endosperm, and the konjac corm (Gille *et al.*, 2011). The *in vitro* synthesis of mannans using plant enzymes depends on the presence of GDP-Man, while glucomannan elongation requires the addition of GDP-Glc (Elbein, 1969). Although it remains unclear how it is generated in plant cells, GDP-Glc cannot be replaced with UDP-Glc (Elbein, 1969), the precursor for cellulose synthesis and other β-1,4-glucans (Liepman *et al.*, 2005; Yang *et al.*, 2020). In contrast, plant pyrophosphorylase (PPases) are known to convert Man-1-phosphate (Man-1-P) to GDP-Man for β-mannan elongation, ascorbate biosynthesis and protein N-glycosylation (Conklin *et al.*, 1999; Lukowitz *et al.*, 2001). VITAMIN C DEFECTIVE1 (VTC1; also known as GMP1/CYT1) is the major PPase producing GDP-Man in Arabidopsis (Table 1) and its activity is enhanced via physical interactions with the KONJAC1 (KJC1) and KJC2 protein cofactors (Sawake *et al.*, 2015). Genetically reducing GDP-Man availability in *vtc1* and *kjc1* mutant seeds severely reduced GGM content in Arabidopsis mucilage (Nishigaki *et al.*, 2021). However, the *kjc1* mucilage displayed a surprisingly normal gel electrophoresis profile of GGM oligosaccharides (Fig. 1b). This suggests that, at least in certain cell types, heteromannan composition is primarily specified by glycosyltransferases (GTs) instead of nucleotide sugar availability.

Elongation of (gluco)mannan backbones

The elongation of β-mannans is catalyzed by membrane-bound CELLULOSE SYNTHASE-LIKE A (CSLA) enzymes from the

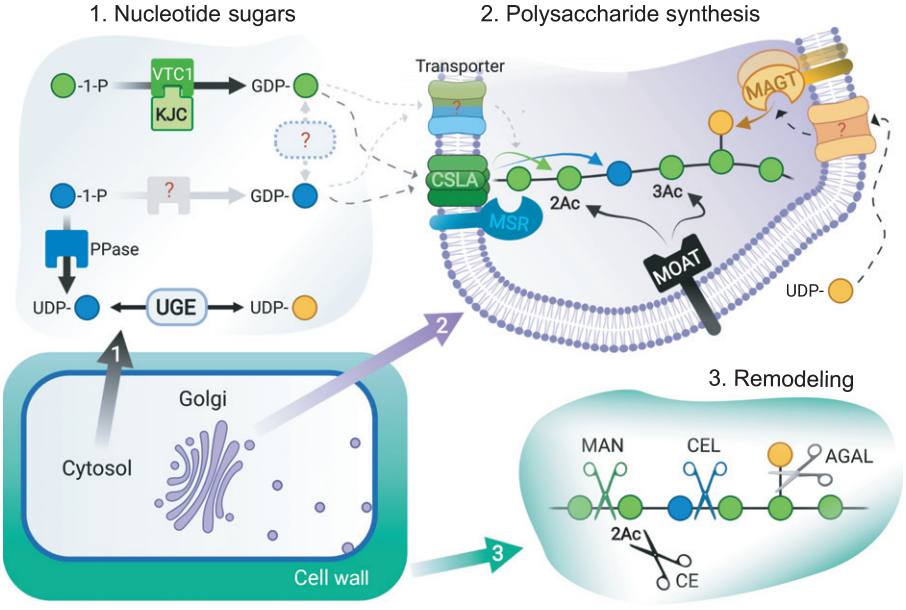

Fig. 2 Major proteins involved in β-mannan biosynthesis and remodeling. Schematic of major biochemical steps occurring in different compartments of a plant cell. Dashed lines indicated hypothetical routes, and question marks indicate proteins that have not been identified in plants. All the labeled proteins are described in detail in the main text. The model was drawn using BioRender (BioRender.com). Sugars and related enzymes are colored as in Fig. 1: green (mannose), blue (glucose), orange (mannose). AGAL, α-galactosidase; CE, carbohydrate esterase; CEL, cellulase; CSLA, Cellulose Synthase-Like A; GDP, guanidine diphosphate; KJC, KONJAC cofactors; MAGT, mannan galactosyltransferase; MAN, mannanase; MOAT, mannan O-acetyltransferase; MSR, mannan synthesis-related; P, phosphate; PPase, pyrophorylase; UDP, uridine diphosphate; UGE, UDP-glucose 4-epimerase; VTC1, VITAMIN C DEFECTIVE1.

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Table 1 Known roles of Arabidopsis proteins that influence β-mannan content or structure.

Protein	Gene ID	*In vivo* or *in vitro* function	Reference
CSLA2	At5g22740	(Gluco)mannan synthesis (*in vitro* and *in vivo*)	Liepman *et al*. (2005); Voiniciuc *et al*. (2019)
CSLA3	At1g23480	(Gluco)mannan synthesis in stems; no heterologous data (poor expression in insect cells)	Liepman *et al*. (2007); Goubet *et al*. (2009)
CSLA7	At2g35650	Mannan synthase (*in vitro* and *in vivo*); embryo-essential	Liepman *et al*. (2005); Goubet *et al*. (2009); Voiniciuc *et al*. (2019)
CSLA9	At5g03760	(Gluco)mannan synthesis (*in vitro* and *in vivo*)	Liepman *et al*. (2005); Goubet *et al*. (2009); Wang *et al*. (2012)
MAGT1/MUCI10	At2g22900	Glucomannan α-galactosylation (*in vitro* and *in vivo*)	Voiniciuc *et al*. (2015a); Yu *et al*. (2018)
MSR1	At3g21190	Enhances *in vivo* (gluco)mannan elongation by CSLAs	Voiniciuc *et al*. (2019)
MSR2	At1g51630	Similar to AtMSR1 *in planta*	Wang *et al*. (2013)
VTC1	At2g39770	PPase that makes GDP-Man (*in vitro* and *in vivo*)	Sawake *et al*. (2015); Nishigaki *et al*. (2021)
KJC1	At1g75910	Binds to and enhances VTC1 (*in vitro* and *in vivo*)	Sawake *et al*. (2015)
KJC2	At2g04650		
MOAT1	At4g11090	Mannan O-acetylation, low transferase activity *in vitro*	Zhong *et al*. (2018)
MOAT2	At4g23790		
MOAT3	At1g01430	Mannan O-acetylation, high transferase activity *in vitro*	
MOAT4	At4g01080		
AGAL2	At5g08370	*In vitro* α-galactosidase activity, but unclear roles *in planta*	Imaizumi *et al*. (2017)
AGAL3	At3g56310		
MAN1	At1g02310	*In vitro* β-1,4-mannanase activity, unclear role *in planta*	Wang *et al*. (2014)
MAN2	At2g20680		Wang *et al*. (2015)
MAN3	At3g10890	β-1,4-mannanase activity (*in vitro* and *in vivo*), Man signaling	Chen *et al*. (2015); Yan *et al*. (2021)

The biochemical activity of MSRs, which are distantly related to animal protein O-fucosyltransferases (Wang *et al*., 2013; Voiniciuc *et al*., 2019) as well as plant UDP-rhamnosyltransferases (Takenaka *et al*., 2018), has not been determined. Carbohydrate or enzyme assays have not been performed for MAN5, MAN6 and MAN7 (Fig. 4), which are influence seed germination.

GT2 family (Dhugga *et al*., 2004). Around a dozen CSLA proteins from land plants have been demonstrated to produce (gluco)mannan *in vitro* using microsomes (Liepman *et al*., 2005, 2007; Suzuki *et al*., 2006) or *in vivo* using *Pichia pastoris* yeast cells (also known as *Komagataella phaffii*; Voiniciuc *et al*., 2019; Verhertbruggen *et al*., 2021). In addition to (gluco)mannan synthesis by CSLAs, yeast-expressed CELLULOSE SYNTHASE (CESA; Purushotham *et al*., 2016), CSLC (Cocuron *et al*., 2007), and CSLD (Yang *et al*., 2020) proteins were shown to be sufficient for β-1,4-glucan synthesis. These related groups of enzymes, together with mixed-linkage β-(1,3;1,4)-glucan synthases from the CSLF clade (Jobling, 2015), have multiple transmembrane (TM) domains that are important for polysaccharide synthesis. CSLA (Fig. 3; typically five to six TMs) and CSLC sequences (typically seven to eight TMs) are noticeably shorter and have fewer membrane spans compared to CESAs and CSLD proteins (Schwacke *et al*., 2003; Robert *et al*., 2021). Using yeast for recombinant protein expression, Arabidopsis CSLD3 and CESA6 were unambiguously shown to synthesize β-1,4-glucans *in vitro* using UDP-Glc (Yang *et al*., 2020). In the same assay, AtCSLA9 produced β-1,4-mannan using GDP-Man and could not utilize UDP-Glc. Moreover, AtCSLD3 and AtCESA6 were demonstrated to be functionally equivalent *in planta* following catalytic domain swaps (Yang *et al*., 2020). *CSLA* and *CSLC* genes (required for xyloglucan elongation) have evolutionarily diverged from the other *CESA* superfamily clades and appear to be absent in the genomes of the first sequenced algae (Mikkelsen *et al*., 2014). However, algae encode single-copy *CSLK* genes with putative roles in polysaccharide synthesis that might resemble those of terrestrial *CSLA* or *CSLC* sequences.

Thus far, only CSLAs enzymes have been shown to have *bona fide* (gluco)mannan synthase activity. In Arabidopsis (see Fig. 4), *CSLA2*, *CSLA3*, *CSLA7* and *CSLA9* were demonstrated to elongate (gluco)mannans based on genetic and/or heterologous expression experiments (Table 1). CSLA enzymes display varying preference for Glc incorporation *in vitro* (Liepman *et al*., 2005, 2007). Membrane-bound plant GTs can be challenging to express in heterologous systems and more than half of the Arabidopsis CSLAs have unclear functions (Fig. 4). For example, insect cell microsomes containing AtCSLA1 showed very weak mannan synthase activity *in vitro* even when the protein was expressed above the detection level (Liepman *et al*., 2005, 2007). In addition, the topology of active CSLAs with five predicted TM domains (Fig. 3) in the endomembrane system remains puzzling. A set of *in vitro* protease digestion experiments indicated that the AtCSLA9 active site faces the lumen of *Pichia* microsomes (Davis *et al*., 2010). CSLAs with a cytosolic N-terminus and an TM domain prior to the GT2 catalytic region (Fig. 3) would require nucleotide sugars to be present inside the Golgi lumen (Fig. 2). However, despite several promising candidates, none of the known GDP-sugar transporter mutants in plants have altered β-mannan content (Jing *et al*., 2021). The GOLGI-LOCALIZED NUCLEOTIDE SUGAR TRANSPORTER 1 (GONST1) can uptake GDP-Man and GDP-Glc with equal preference *in vitro*, but only affects sphingolipid mannosylation in Arabidopsis (Mortimer *et al*., 2013). Therefore,

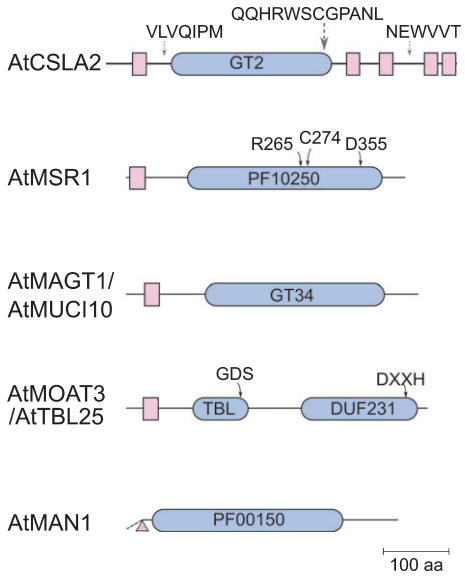

Fig. 3 Features of five major classes of proteins active on mannan backbones. Conserved CSLA amino acid (aa) sequences were previously used as border regions for (gluco)mannan synthase domain swaps (Robert *et al*., 2021). Highly conserved residues that are functionally important for AtMSR1 (Voiniciuc *et al*., 2019) and AtMOAT3 (Zhong *et al*., 2018) are based on site-directed mutagenesis. Pink features, denoting consensus transmembrane domains, the cleavable signal peptide of AtMSR1, and conserved protein family (PF) domains were annotated using ARAMEMNON (Schwacke *et al*., 2003). AtMOAT3 domain annotations are based on Zhong *et al*. (2018). All proteins are drawn to the same scale.

CSLAs might (1) require noncanonical transporters, or (2) might utilize nucleotide sugars directly from the cytosol like CESAs and β-glucan synthases (Fig. 2; Jing *et al*., 2021).

Yeast as a testbed for plant β-mannan synthesis

Expression of membrane-bound proteins or complexes thereof in surrogate hosts such as *Pichia* provides a testbed to produce and modify plant cell wall polysaccharides (Pauly *et al*., 2019). Since yeast cells natively contain trace amounts of 4-Man (Pauly *et al*., 2019), they provide a clean background for the bioengineering of β-1,4-mannan biosynthesis and modification. The konjac AkCSLA3 protein produces copious amounts of unbranched glucomannan in *Pichia* cells (Voiniciuc *et al*., 2019), resembling with its natural product (Gille *et al*., 2011). This synthetic biology strategy bypasses the tedious protein purification steps and expensive substrates required for *in vitro* assays, by re-wiring yeast to produce plant polysaccharides from affordable carbon sources. Yeast therefore provides a convenient eukaryotic system to screen for the products of GTs sourced from various plants or of new-to-nature enzyme variants. While glucomannan production by AkCSLA3 reduced *Pichia* growth by *c.* 30% and resulted in smaller cells, mannan-producing AtCSLA2 did not affect biomass accumulation (Robert *et al*., 2021). Modular domain swaps of these two enzyme sequences (borders marked in Fig. 3) led to the discovery of three chimeric CSLAs that produced as much or more (gluco)mannan than the parental controls but reduced yeast toxicity (Robert *et al*., 2021). Therefore, further domain swaps or mutagenesis of CSLA subregions provide exciting avenues to fine-tune β-mannan synthases.

Additional genes co-expressed with CSLAs were identified by several transcriptomic studies in the last decade, starting with the profiling of konjac corms (Gille *et al*., 2011), fenugreek seeds (Wang *et al*., 2012) and coffee seeds (Joët *et al*., 2014). The fenugreek study found a putative *GT65-RELATED* (*GT65R*) gene called *MANNAN-SYNTHESIS RELATED* (*MSR*), whose Arabidopsis homologs (*AtMSR1* and *AtMSR2*; Table 1) were subsequently knocked out to reduce glucomannan content (Wang *et al*., 2013). While the functions of MSRs in the Golgi remained challenging to elucidate *in planta*, the use of the *Pichia* cells to study β-mannan synthesis revealed that the expression of AtMSR1 alone did not alter the yeast cell wall composition (Voiniciuc *et al*., 2019). However, the co-expression of MSR1 with CSLAs in *Pichia* cells could: (1) convert mannan synthesis into glucomannan synthesis (e.g. AtCSLA2 + AtMSR1); (2) boost glucomannan synthesis (e.g. AkCSLA3 + AtMSR1); or (3) activate an otherwise nonfunctional coffee CSLA (e.g. *Coffea canephora* CcMANS1 mannan synthase + CcMSR1). Close MSR homologs are only found in land plants (Wang *et al*., 2013) and their effects are specific to certain CSLAs, because AtMSR1 was incompatible with AtCSLA7 or CcMANS1 (Voiniciuc *et al*., 2019). MSRs may be indispensable for the activity of additional CSLAs, which might explain the lack of *in vitro* activity for PtCSLA5 (84% sequence homology with AtCSLA2), encoded by the highest expressed *CSL* gene during poplar wood formation (Suzuki *et al*., 2006). While the AtMSR1 requires conserved motifs involved in GDP-sugar binding by animal protein O-fucosyltransferase 1 (POFUT1) enzymes (Fig. 3; Voiniciuc *et al*., 2019), several related proteins from the large plant GT65R family (re-classified as GT106) act as UDP-rhamnosyltransferases in pectin elongation (Takenaka *et al*., 2018). In light of these recent discoveries, the activities of MSR proteins on carbohydrate substrates and/or CSLA enzymes remain particularly intriguing to explore.

β-Mannan substitution: dress for success

The substitution of hemicelluloses typically increases their gelling ability and is thought to be essential for preventing their aggregation in the endomembrane system (Scheller & Ulvskov, 2010). Indeed, homomannans can form crystalline allomorphs that are insoluble in water. Carbohydrate branches can be added by α-1,6-galactosyltransferases (GalTs) from the GT34 family using UDP-Gal as a donor sugar. While a *Trigonella foenum-graecum* (fenugreek) galactomannan GalT (TfGMGT) accepts only homomannan oligosaccharides (Edwards *et al*., 1999), the Arabidopsis *MUCILAGE-RELATED10* (*AtMUCI10*) encodes a strict glucomannan α-GalT1 (MAGT1; Table 1). TfGMGT enzyme could be efficiently secreted from *Pichia* cells without its TM domain and transferred Gal onto oligosaccharides with at least five Man units (Edwards *et al*., 1999). Subsequently, detergent-solubilized fenugreek membrane extracts showed that GMGT activity is geared towards the transfer of Gal to the third Man residue from the nonreducing end of mannohexaose (Man_6) sequences (Edwards *et al*., 2002). In contrast, MAGT1/MUCI10,

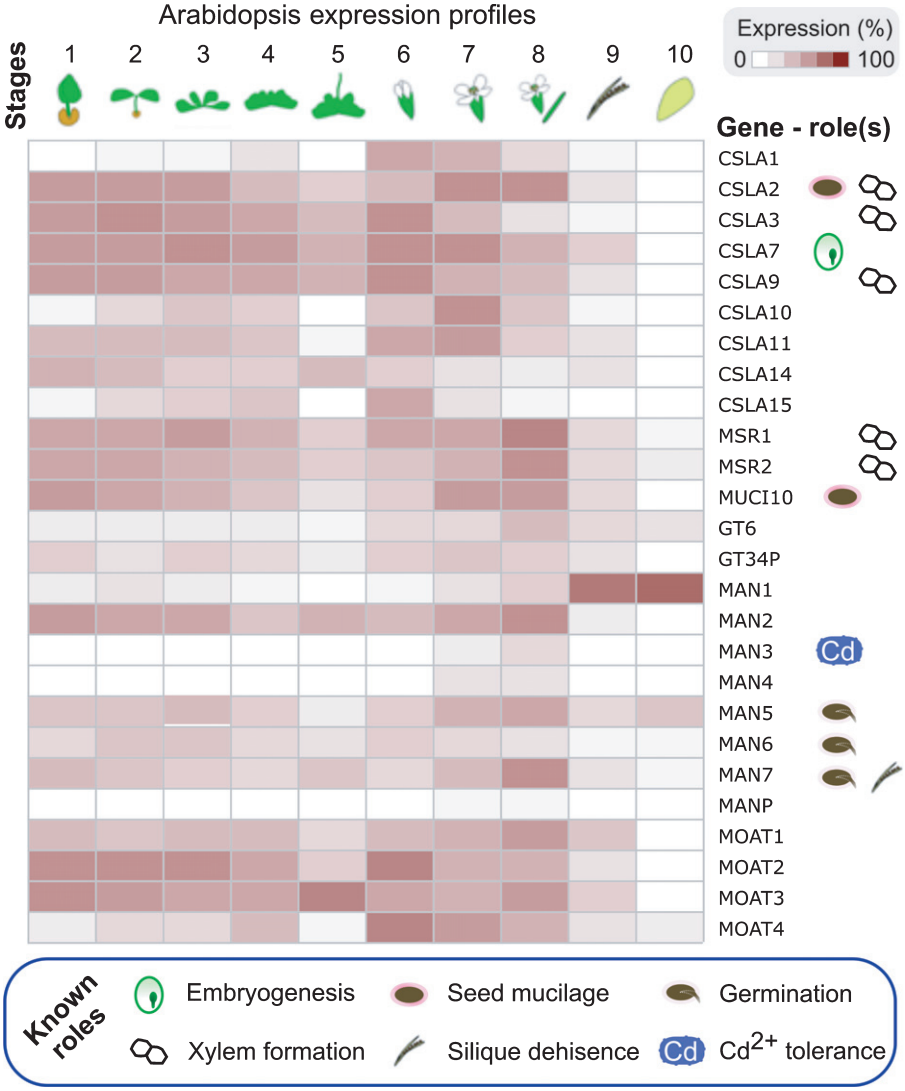

Fig. 4 Expression of mannan-related genes in Arabidopsis and known biological roles. Transcriptional heatmap across 10 developmental stages was visualized using Genevestigator 9.0 (mRNAseq wild-type data; Hruz *et al*., 2008). The developmental stages are: (1) germinated seed; (2) seedling; (3) young rosette; (4) developed rosette; (5) bolting; (6) young flower; (7) developed flower; (8) flowers and siliques; (9) mature siliques; (10) senescence. *GT34P* and *MANP* are likely pseudogenes. Schematics on the right side of the genes indicate the biological processes that they are associated with based on mutant analyses.

which homodimerizes when expressed in tobacco microsomes (Fig. 2), recognizes acceptors that contain repeating Glc-Man disaccharides (Yu *et al*., 2018) instead of Man_6 (Voiniciuc *et al*., 2015). The Arabidopsis GT34 family includes a closely related AtGT6 and GT34P (Fig. 4), a potential pseudogene without a TM domain. Since *muci10 gt6* double mutant seeds phenocopied the *muci10* mucilage defects (Voiniciuc *et al*., 2015a), the biochemical activity of GT6 and the roles of GT34 proteins beyond the seed coat remain to be determined. The UDP-Gal required for heteromannan synthesis is likely produced by cytosolic UDP-Glc 4-epimerases (UGEs; Barber *et al*., 2006; Rösti *et al*., 2007), which must then be transported across the Golgi membrane by specific transporters (Bar-Peled & O'Neill, 2011). As an alternative to UGE activity, UDP-Gal could be produced from Gal-1-P by a UDP-SUGAR PYROPHOSPHORYLASE (USP, At5g52560) with broad substrate specificity (Kotake *et al*., 2004; Litterer *et al*., 2006). However, the actual genes that supply UDP-Gal for heteromannan substitution are not obvious based on their expression profiles and will have to be determined empirically.

In addition to carbohydrate substitutions, (gluco)mannans can be O-acetylated by specific members of the TRICHOME-BIREFRINGENCE-LIKE (TBL) family, which target distinct classes of matrix polysaccharides. *In vitro* assays using Man_6 and radiolabeled acetyl-CoA demonstrated that four TBLs (TBL23 to TBL26) in Arabidopsis act as mannan *O*-acetyltransferases (MOAT1 to MOAT4; Fig. 2; Zhong *et al*., 2018). AtMOAT3 (AtTBL25) and AtMOAT4 (AtTBL26) had the highest activities and added O-acetyl groups to the C-2 and C-3 of Man_6, consistent with the acetylated glucomannan in Arabidopsis stems. Site-directed mutagenesis experiments showed that GDS and DXXH motifs are indispensable for AtMOAT3 activity (Fig. 3; Zhong *et al*., 2018). Furthermore, the evolution of MOATs has been recently explored by examining the *in vitro* activities of orthologs from two algae, moss, *Selaginella*, pine, spruce, rice and poplar (Zhong *et al*., 2019). Acetyltransferase activity on Man_6 was detected for the tested isoforms of land plants, but not for the two DUF231 proteins identified in a green alga (*Klebsormidium nitens*). Therefore, mannan acetylation by TBLs may have evolved with the emergence of bryophytes (Zhong *et al*., 2019).

Extracellular β-mannan modification and signaling

In addition to interacting with cellulose microfibrils in plant cell walls, mannans play specialized roles in a variety of developmental scenarios that are indispensable for survival. For example, galactomannans accumulate in the thick endosperm of legume seeds to store carbon akin to starch in cereal grains (Meier & Reid, 1982). Once synthesized and secreted, β-mannans can be modified or degraded via a suite of glycosyl hydrolases, transglycosylases and carbohydrate esterases (Fig. 2) such as acetylesterases. To mobilize energy reserves from legume seeds, galactomannans must be first debranched by α-galactosidases (Fig. 2) to then be cleaved into smaller fragments by endo-β-1,4-mannanases (MAN) that soften the endosperm and nourish the embryo (Rodríguez-Gacio *et al.*, 2012). In addition to hydrolytic activity, certain MAN enzymes (e.g. *Lycopersicon esculentum* LeMAN4a from ripening tomatoes) show transglycosylation activity, suggesting that the extracellular remodeling of heteromannan crosslinks are particularly important for the cell wall structure of developing fruits (Schröder *et al.*, 2009). Nevertheless, the roles of most mannan-modifying enzymes remain poorly understood and are challenging to study *in vivo*. For example, *Pichia*-expressed AGAL2 and AGAL3 show α-galactosidase activity *in vitro* (Table 1; Imaizumi *et al.*, 2017), but their native substrates and biological roles in Arabidopsis remain unclear. Furthermore, the players required for the de-acetylation of Man residues in plants remain unknown and their discovery could have important consequences of industrial applications.

MAN genes encode the best studied group of mannan hydrolytic enzymes, but even they have been discovered to play unexpected roles in the past decade (Fig. 4). Classically, MAN enzymes have been involved in the softening and degradation of mannan-enriched cell walls (Rodríguez-Gacio *et al.*, 2012). Based on transcriptional and mutant analyses, *MAN5*, *MAN6* and *MAN7* encode three putative MANs with partially overlapping roles in promoting Arabidopsis seed germination (Iglesias-Fernández *et al.*, 2011). Together with *CELLULASE6* (*CEL6*), *MAN7* also contributes to silique dehiscence (He *et al.*, 2018) and might act on glucomannans (Fig. 2). In addition to structural cell wall alterations, MAN activity may also release oligosaccharides that trigger a variety of signaling cascades. During *Populus* wood formation, *PtrMAN6* negatively regulates secondary cell wall deposition (Zhao *et al.*, 2013). Secondary cell walls were thicker when *PtrMAN6* was suppressed, but thinner in overexpression lines. The exogenous application of GGM oligosaccharides also stimulates xylogenic *Zinnia elegans* cultures by increasing cell density and altering secondary wall patterning (Benová-Kákosová *et al.*, 2006). In addition, an Arabidopsis forward genetic screen for heavy metal tolerance identified an unexpected mechanism requiring MAN3 hydrolytic activity. *MAN3* expression enhances plant tolerance to cadmium (Chen *et al.*, 2015), a toxic soil pollutant, by releasing Man in the apoplast. Exogenous Man supplementation also boosts plant growth during cadmium stress and is perceived by a Man-binding protein that may function as a signaling receptor (Yan *et al.*, 2021). Finally, in two recent studies on plant–microbe interactions, Man_2 to Man_6 application was sufficient activate defense responses (Zang *et al.*, 2019) and changes in mannan epitopes correlated with altered pathogen resistance in a variety of plant cell wall mutants (Molina *et al.*, 2021).

Manning the wall: vital roles of β-mannans in plants and beyond

There are several indications that mannans may be important for the formation of new cell walls. A comprehensive study of cell wall synthases in the Arabidopsis apical meristem (Yang *et al.*, 2016), found that antibody-labeled β-mannans were restricted to anticlinal walls of the upper cell layers. Since *MUCI10* is one of the highest expressed GTs in the shoot apical meristem (Yang *et al.*, 2016), small amounts of galactosylated heteromannans might play foundational roles in the initial deposition as well as the re-generation of primary cell walls. Indeed, super-resolution micrographs of dividing cells showed increasing deposits of β-mannans during the first stages of cell plate formation (Peaucelle *et al.*, 2020). Mannans labeled with the PDM antibody were distributed in small clusters that correlated with callose deposition but were largely independent from cellulose labeled with CBM3a.

Despite their relatively low abundance, mannans are indispensable for Arabidopsis seed development. In contrast to other hemicellulose-deficient mutants, the *csla7* mutant is arrested during the early stages of embryo formation and can only by partially rescued by overexpressing AtCSLA2 or AtCSLA9 (Goubet *et al.*, 2009). In heterologous systems, AtCSLA7 produces only homomannan and has a lower activity than the AtCSLA2 and AtCSLA9 (Liepman *et al.*, 2005; Voiniciuc *et al.*, 2019). Surprisingly, the *csla239* triple mutant develops normally despite accumulating only trace amounts of β-mannans in its stems (Goubet *et al.*, 2009). However, young *csla239* seedlings still express other CSLA paralogs (Fig. 4) and thus produce up to 70% of the wild-type Man content (Yang *et al.*, 2021). Since higher-order mutants (e.g. *csla2379*) needed to eliminate co-expressed *CSLA* genes are expected to be lethal, additional biological roles of β-mannans likely remain hidden.

While *csla239* triple mutant stems have normal morphology and strength in three-point bending tests despite having only trace amounts of Man (Goubet *et al.*, 2009), *csla2* and *muci10* mutants significantly impair seed mucilage architecture (Voiniciuc *et al.*, 2015a). In the past six years, Arabidopsis seed mucilage has emerged as an attractive model to investigate the synthesis and roles of hemicelluloses, despite their relatively low abundance in this pectin-rich wall (Voiniciuc *et al.*, 2015b, 2018). Both *csla2* and *muci10* seeds significantly reduce the mucilage Man content (Fig. 1b) and release denser mucilage capsules with impaired cellulose organization (Yu *et al.*, 2014; Voiniciuc *et al.*, 2015a). GGM must be an important pre-requisite for the deposition of crystalline microfibrils in mucilage, because its loss reduced cellulose content more than mutations in *TRM4*, which directly affects CESA-microtubule organization (Yang *et al.*, 2019). In addition, a genome-wide association study (GWAS) linked *MUCI10* to natural variation in Man content (Fig. 1b; Voiniciuc *et al.*, 2016). The Gal, Glc and Man deficiency of the Ema-1 Arabidopsis ecotype (originating from East Malling, UK) was

rescued by the constitutive expression of MUCI10-sYFP proteins (Voiniciuc *et al.*, 2016). GGM composition as well as content could influence seed physiology in adverse environments, since *muci10* seeds are found to enhance germination and early growth in saline solutions (Yang *et al.*, 2021).

Beyond plants, β-mannan polysaccharides can have important impacts for the bioenergy and hydrocolloid markets. Increasing the hexose to pentose ratio of lignocellulosic biomass (e.g. by replacing xylans with mannans) would enhance saccharification efficiency (Pauly & Keegstra, 2008), but has been challenging to engineer *in planta*. The introduction of *CSLA* transgenes alone may have unintended consequences on plant metabolism, or may not be sufficient to achieve desirable traits. Nevertheless, galactomannans and glucomannans extracted from natural sources have gained importance in the Western world as food ingredients and nutritional supplements with potential benefits in the treatment of lifestyle diseases such as Type 2 diabetes. Plant mannans represent an excellent source of dietary fiber and can be utilized by the beneficial human gut bacterium *Roseburia intestinalis*, whose metabolic loci were recently identified in a multi-omic study (La Rosa *et al.*, 2019). Therefore, recent insights into mannan-related enzymes and in synthetic biology are paving the path to re-design polysaccharide structures for improved plant traits and bioproducts.

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Author contribution

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References

Arnling Bååth J, Martínez-Abad A, Berglund J, Larsbrink J, Vilaplana F, Olsson L. 2018. Mannanase hydrolysis of spruce galactoglucomannan focusing on the influence of acetylation on enzymatic mannan degradation. *Biotechnology for Biofuels* **11**: 114.

Barber C, Rösti J, Rawat A, Findlay K, Roberts K, Seifert GJ. 2006. Distinct properties of the five UDP-D-glucose/UDP-D-galactose 4-epimerase isoforms of *Arabidopsis thaliana*. *Journal of Biological Chemistry* **281**: 17276–17285.

Bar-Peled M, O'Neill MA. 2011. Plant nucleotide sugar formation, interconversion, and salvage by sugar recycling. *Annual Review of Plant Biology* **62**: 127–155.

Beňová-Kákošová A, Digonnet C, Goubet F, Ranocha P, Jauneau A, Pesquet E, Barbier O, Zhang Z, Capek P, Dupree P *et al.* 2006. Galactoglucomannans increase cell population density and alter the protoxylem/metaxylem tracheary element ratio in xylogenic cultures of Zinnia. *Plant Physiology* **142**: 696–709.

Berglund J, Mikkelsen D, Flanagan BM, Dhital S, Gaunitz S, Henriksson G, Lindström ME, Yakubov GE, Gidley MJ, Vilaplana F. 2020. Wood hemicelluloses exert distinct biomechanical contributions to cellulose fibrillar networks. *Nature Communications* **11**: 4692.

Chavan RR, Singh AP, Azizan A, Harris PJ. 2021. Heteromannans are the predominant hemicelluloses in the gametophytic stem of the umbrella moss *Hypnodendron menziesii* and occur in the walls of all cell types. *Planta* **254**: 2.

Chen J, Yang L, Gu J, Bai X, Ren Y, Fan T, Han Y, Jiang L, Xiao F, Liu Y *et al.* 2015. *MAN3* gene regulates cadmium tolerance through the glutathione-dependent pathway in *Arabidopsis thaliana*. *New Phytologist* **205**: 570–582.

Chernova T, Ageeva M, Mikshina P, Trofimova O, Kozlova L, Lev-Yadun S, Gorshkova T. 2020. The living fossil psilotum nudum has cortical fibers with mannan-based cell wall matrix. *Frontiers in Plant Science* **11**: 488.

Cocuron J-C, Lerouxel O, Drakakaki G, Alonso AP, Liepman AH, Keegstra K, Raikhel N, Wilkerson CG. 2007. A gene from the cellulose synthase-like C family encodes a β-1,4 glucan synthase. *Proceedings of the National Academy of Sciences, USA* **104**: 8550–8555.

Conklin PL, Norris SR, Wheeler GL, Williams EH, Smirnoff N, Last RL. 1999. Genetic evidence for the role of GDP-mannose in plant ascorbic acid (vitamin C) biosynthesis. *Proceedings of the National Academy of Sciences, USA* **96**: 4198–4203.

Davis J, Brandizzi F, Liepman AH, Keegstra K. 2010. Arabidopsis mannan synthase CSLA9 and glucan synthase CSLC4 have opposite orientations in the Golgi membrane. *The Plant Journal* **64**: 1028–1037.

Dhugga KS, Barreiro R, Whitten B, Stecca K, Hazebroek J, Randhawa GS, Dolan M, Kinney AJ, Tomes D, Nichols S *et al.* 2004. Guar seed beta-mannan synthase is a member of the cellulose synthase super gene family. *Science* **303**: 363–366.

Edwards ME, Dickson CA, Chengappa S, Sidebottom C, Gidley MJ, Reid JSG. 1999. Molecular characterisation of a membrane-bound galactosyltransferase of plant cell wall matrix polysaccharide biosynthesis. *The Plant Journal* **19**: 691–697.

Edwards ME, Marshall E, Gidley MJ, Reid JSG. 2002. Transfer specificity of detergent-solubilized fenugreek galactomannan galactosyltransferase. *Plant Physiology* **129**: 1391–1397.

Elbein AD. 1969. Biosynthesis of a cell wall glucomannan in mung bean seedlings. *Journal of Biological Chemistry* **244**: 1608–1616.

Fernández PV, Estevez JM, Cerezo AS, Ciancia M. 2012. Sulfated β-D-mannan from green seaweed *Codium vermilara*. *Carbohydrate Polymers* **87**: 916–919.

Gille S, Cheng K, Skinner ME, Liepman AH, Wilkerson CG, Pauly M. 2011. Deep sequencing of voodoo lily (*Amorphophallus konjac*): an approach to identify relevant genes involved in the synthesis of the hemicellulose glucomannan. *Planta* **234**: 515–526.

Goubet F, Barton CJ, Mortimer JC, Yu X, Zhang Z, Miles GP, Richens J, Liepman AH, Seffen K, Dupree P. 2009. Cell wall glucomannan in Arabidopsis is synthesised by CSLA glycosyltransferases, and influences the progression of embryogenesis. *The Plant Journal* **60**: 527–538.

He C, Wu K, Zhang J, Liu X, Zeng S, Yu Z, Zhang X, Teixeira da Silva JA, Deng R, Tan J *et al.* 2017. Cytochemical localization of polysaccharides in *Dendrobium officinale* and the involvement of DoCSLA6 in the synthesis of mannan polysaccharides. *Frontiers in Plant Science* **8**: 173.

He H, Bai M, Tong P, Hu Y, Yang M, Wu H. 2018. CELLULASE6 and MANNANASE7 affect cell differentiation and silique dehiscence. *Plant Physiology* **176**: 2186–2201.

Hruz T, Laule O, Szabo G, Wessendorp F, Bleuler S, Oertle L, Widmayer P, Gruissem W, Zimmermann P. 2008. GENEVESTIGATOR v.3: a reference expression database for the meta-analysis of transcriptomes. *Advances in Bioinformatics* **2008**: 420747.

Iglesias-Fernández R, Rodríguez-Gacio MC, Barrero-Sicilia C, Carbonero P, Matilla A. 2011. Three endo-β-mannanase genes expressed in the micropylar endosperm and in the radicle influence germination of *Arabidopsis thaliana* seeds. *Planta* **233**: 25–36.

Imaizumi C, Tomatsu H, Kitazawa K, Yoshimi Y, Shibano S, Kikuchi K, Yamaguchi M, Kaneko S, Tsumuraya Y, Kotake T. 2017. Heterologous expression and characterization of an Arabidopsis β-L-arabinopyranosidase and α-D-galactosidases acting on β-L-arabinopyranosyl residues. *Journal of Experimental Botany* **68**: 4651–4661.

Jing B, Ishikawa T, Soltis N, Inada N, Liang Y, Murawska G, Fang L, Andeberhan F, Pidatala R, Yu X *et al.* 2021. The *Arabidopsis thaliana* nucleotide sugar

transporter GONST2 is a functional homolog of GONST1. *Plant Direct* **5**: e00309.

Jobling SA. 2015. Membrane pore architecture of the CslF6 protein controls (1-3,1-4)-β-glucan structure. *Science Advances* **1**: e1500069.

Joët T, Laffargue A, Salmona J, Doulbeau S, Descroix F, Bertrand B, Lashermes P, Dussert S. 2014. Regulation of galactomannan biosynthesis in coffee seeds. *Journal of Experimental Botany* **65**: 323–337.

Kotake T, Yamaguchi D, Ohzono H, Hojo S, Kaneko S, Ishida H, Tsumuraya Y. 2004. UDP-sugar pyrophosphorylase with broad substrate specificity toward various monosaccharide 1-phosphates from pea sprouts. *Journal of Biological Chemistry* **279**: 45728–45736.

La Rosa SL, Leth ML, Michalak L, Hansen ME, Pudlo NA, Glowacki R, Pereira G, Workman CT, Arntzen MØ, Pope PB *et al.* 2019. The human gut Firmicute *Roseburia intestinalis* is a primary degrader of dietary β-mannans. *Nature Communications* **10**: 905.

Liepman AH, Nairn CJ, Willats WGT, Sørensen I, Roberts AW, Keegstra K. 2007. Functional genomic analysis supports conservation of function among cellulose synthase-like a gene family members and suggests diverse roles of mannans in plants. *Plant Physiology* **143**: 1881–1893.

Liepman AH, Wilkerson CG, Keegstra K. 2005. Expression of cellulose synthase-like (Csl) genes in insect cells reveals that CslA family members encode mannan synthases. *Proceedings of the National Academy of Sciences, USA* **6**: 2221–2226.

Litterer LA, Schnurr JA, Plaisance KL, Storey KK, Gronwald JW, Somers DA. 2006. Characterization and expression of Arabidopsis UDP-sugar pyrophosphorylase. *Plant Physiology and Biochemistry* **44**: 171–180.

Lukowitz W, Nickle TC, Meinke DW, Last RL, Conklin PL, Somerville CR. 2001. Arabidopsis cyt1 mutants are deficient in a mannose-1-phosphate guanylyltransferase and point to a requirement of N-linked glycosylation for cellulose biosynthesis. *Proceedings of the National Academy of Sciences, USA* **98**: 2262–2267.

Marcus SE, Blake AW, Benians TAS, Lee KJD, Poyser C, Donaldson L, Leroux O, Rogowski A, Petersen HL, Boraston A *et al.* 2010. Restricted access of proteins to mannan polysaccharides in intact plant cell walls. *The Plant Journal* **64**: 191–203.

Meier H, Reid JSG. 1982. Reserve polysaccharides other than starch in higher plants. In: Loewus FA, Tanner W, eds. *Encyclopedia of plant physiology. Plant carbohydrates I: Intracellular carbohydrates*. Berlin/Heidelberg, Germany: Springer, 418–471.

Mikkelsen MD, Harholt J, Ulvskov P, Johansen IE, Fangel JU, Doblin MS, Bacic A, Willats WGTT. 2014. Evidence for land plant cell wall biosynthetic mechanisms in charophyte green algae. *Annals of Botany* **114**: 1217–1236.

Molina A, Miedes E, Bacete L, Rodríguez T, Mélida H, Denancé N, Sánchez-Vallet A, Rivière M-P, López G, Freydier A *et al.* 2021. Arabidopsis cell wall composition determines disease resistance specificity and fitness. *Proceedings of the National Academy of Sciences, USA* **118**: e2010243118.

Moller I, Sørensen I, Bernal AJ, Blaukopf C, Lee K, Øbro J, Pettolino F, Roberts A, Mikkelsen JD, Knox JP *et al.* 2007. High-throughput mapping of cell-wall polymers within and between plants using novel microarrays. *The Plant Journal* **50**: 1118–1128.

Mortimer JC, Yu X, Albrecht S, Sicilia F, Huichalaf M, Ampuero D, Michaelson LV, Murphy AM, Matsunaga T, Kurz S *et al.* 2013. Abnormal glycosphingolipid mannosylation triggers salicylic acid-mediated responses in Arabidopsis. *Plant Cell* **25**: 1881–1894.

Nishigaki N, Yoshimi Y, Kuki H, Kunieda T, Hara-Nishimura I, Tsumuraya Y, Takahashi D, Dupree P, Kotake T. 2021. Galactoglucomannan structure of Arabidopsis seed-coat mucilage in GDP-mannose synthesis impaired mutants. *Physiologia Plantarum* **173**: 1244–1252.

Pauly M, Gawenda N, Wagner C, Fischbach P, Ramírez V, Axmann IM, Voiniciuc C. 2019. The suitability of orthogonal hosts to study plant cell wall biosynthesis. *Plants* **8**: 516.

Pauly M, Gille S, Liu L, Mansoori N, de Souza A, Schultink A, Xiong G. 2013. Hemicellulose biosynthesis. *Planta* **238**: 627–642.

Pauly M, Keegstra K. 2008. Cell-wall carbohydrates and their modification as a resource for biofuels. *The Plant Journal* **54**: 559–568.

Peaucelle A, Wightman R, Haas KT. 2020. Multicolor 3D-dSTORM reveals native-state ultrastructure of polysaccharides' network during plant cell wall assembly. *iScience* **23**: 101862.

Purushotham P, Cho SH, Díaz-Moreno SM, Kumar M, Nixon BT, Bulone V, Zimmer J. 2016. A single heterologously expressed plant cellulose synthase isoform is sufficient for cellulose microfibril formation in vitro. *Proceedings of the National Academy of Sciences, USA* **113**: 11360–11365.

Reid JSG. 1985. Cell wall storage carbohydrates in seeds—biochemistry of the seed "gums" and "hemicelluloses". *Advances in Botanical Research* **11**: 125–155.

Robert M, Waldhauer J, Stritt F, Yang B, Pauly M, Voiniciuc C. 2021. Modular biosynthesis of plant hemicellulose and its impact on yeast cells. *Biotechnology for Biofuels* **14**: 140.

Rodríguez-Gacio MDC, Iglesias-Fernández R, Carbonero P, Matilla ÁJ. 2012. Softening-up mannan-rich cell walls. *Journal of Experimental Botany* **63**: 3975–3988.

Rösti J, Barton CJ, Albrecht S, Dupree P, Pauly M, Findlay K, Roberts K, Seifert GJ. 2007. UDP-glucose 4-epimerase isoforms UGE2 and UGE4 cooperate in providing UDP-galactose for cell wall biosynthesis and growth of *Arabidopsis thaliana*. *Plant Cell* **19**: 1565–1579.

Sawake S, Tajima N, Mortimer JC, Lao J, Ishikawa T, Yu X, Yamanashi Y, Yoshimi Y, Kawai-Yamada M, Dupree P *et al.* 2015. KONJAC1 and 2 are key factors for GDP-mannose generation and affect L-ascorbic acid and glucomannan biosynthesis in Arabidopsis. *Plant Cell* **27**: 3397–3409.

Scheller HV, Ulvskov P. 2010. Hemicelluloses. *Annual Review of Plant Biology* **61**: 263–289.

Schröder R, Atkinson RG, Redgwell RJ. 2009. Re-interpreting the role of endo-β-mannanases as mannan endotransglycosylase/hydrolases in the plant cell wall. *Annals of Botany* **104**: 197–204.

Schwacke R, Schneider A, van der Graaff E, Fischer K, Catoni E, Desimone M, Frommer WB, Flügge U-I, Kunze R. 2003. ARAMEMNON, a novel database for Arabidopsis integral membrane proteins. *Plant Physiology* **131**: 16–26.

Singh S, Singh G, Arya SK. 2018. Mannans: an overview of properties and application in food products. *International Journal of Biological Macromolecules* **119**: 79–95.

Sørensen I, Pettolino FA, Bacic A, Ralph J, Lu F, O'Neill MA, Fei Z, Rose JKC, Domozych DS, Willats WGT. 2011. The charophycean green algae provide insights into the early origins of plant cell walls. *The Plant Journal* **68**: 201–211.

Suzuki S, Li L, Sun Y-H, Chiang VL. 2006. The cellulose synthase gene superfamily and biochemical functions of xylem-specific cellulose synthase-like genes in *Populus trichocarpa*. *Plant Physiology* **142**: 1233–1245.

Takenaka Y, Kato K, Ogawa-Ohnishi M, Tsuruhama K, Kajiura H, Yagyu K, Takeda A, Takeda Y, Kunieda T, Hara-Nishimura I *et al.* 2018. Pectin RG-I rhamnosyltransferases represent a novel plant-specific glycosyltransferase family. *Nature Plants* **4**: 669–676.

Terrett OM, Lyczakowski JJ, Yu L, Iuga D, Franks WT, Brown SP, Dupree R, Dupree P. 2019. Molecular architecture of softwood revealed by solid-state NMR. *Nature Communications* **10**: 4978.

Verhertbruggen Y, Bouder A, Vigouroux J, Alvarado C, Geairon A, Guillon F, Wilkinson MD, Stritt F, Pauly M, Lee MY *et al.* 2021. The TaCslA12 gene expressed in the wheat grain endosperm synthesizes wheat-like mannan when expressed in yeast and Arabidopsis. *Plant Science* **302**: 110693.

Voiniciuc C, Dama M, Gawenda N, Stritt F, Pauly M. 2019. Mechanistic insights from plant heteromannan synthesis in yeast. *Proceedings of the National Academy of Sciences, USA* **116**: 522–527.

Voiniciuc C, Engle KA, Günl M, Dieluweit S, Schmidt MH-W, Yang J-Y, Moremen KW, Mohnen D, Usadel B. 2018. Identification of key enzymes for pectin synthesis in seed mucilage. *Plant Physiology* **178**: 1045–1064.

Voiniciuc C, Schmidt MH-W, Berger A, Yang B, Ebert B, Scheller HV, North HM, Usadel B, Günl M. 2015a. MUCILAGE-RELATED10 produces galactoglucomannan that maintains pectin and cellulose architecture in Arabidopsis seed mucilage. *Plant Physiology* **169**: 403–420.

Voiniciuc C, Yang B, Schmidt MH-W, Günl M, Usadel B. 2015b. Starting to gel: how Arabidopsis seed coat epidermal cells produce specialized secondary cell walls. *International Journal of Molecular Sciences* **16**: 3452–3473.

Voiniciuc C, Zimmermann E, Schmidt MH-W, Günl M, Fu L, North HM, Usadel B. 2016. Extensive natural variation in Arabidopsis seed mucilage structure. *Frontiers in Plant Science* **7**: 1–14.

Wang Y, Alonso AP, Wilkerson CG, Keegstra K. 2012. Deep EST profiling of developing fenugreek endosperm to investigate galactomannan biosynthesis and its regulation. *Plant Molecular Biology* **79**: 243–258.

Wang Y, Azhar S, Gandini R, Divne C, Ezcurra I, Aspeborg H. 2015. Biochemical characterization of the novel *endo*-β-mannanase *At*Man5-2 from *Arabidopsis thaliana*. *Plant Science* **241**: 151–163.

Wang Y, Mortimer JC, Davis J, Dupree P, Keegstra K. 2013. Identification of an additional protein involved in mannan biosynthesis. *The Plant Journal* **73**: 105–117.

Wang Y, Vilaplana F, Brumer H, Aspeborg H. 2014. Enzymatic characterization of a glycoside hydrolase family 5 subfamily 7 (GH5_7) mannanase from *Arabidopsis thaliana*. *Planta* **239**: 653–665.

Yamabhai M, Sak-Ubol S, Srila W, Haltrich D. 2014. Mannan biotechnology: from biofuels to health. *Critical Reviews in Biotechnology* **36**: 32–42.

Yan X, Huang Y, Song H, Chen F, Geng Q, Hu M, Zhang C, Wu X, Fan T, Cao S. 2021. A MYB4-MAN3-Mannose-MNB1 signaling cascade regulates cadmium tolerance in Arabidopsis. *PLoS Genetics* **17**: e1009636.

Yang B, Hofmann F, Usadel B, Voiniciuc C. 2020. Seed hemicelluloses tailor mucilage properties and salt tolerance. *New Phytologist* **229**: 1946–1954.

Yang B, Voiniciuc C, Fu L, Dieluweit S, Klose H, Usadel B. 2019. TRM4 is essential for cellulose deposition in Arabidopsis seed mucilage by maintaining cortical microtubule organization and interacting with CESA3. *New Phytologist* **221**: 881–895.

Yang J, Bak G, Burgin T, Barnes WJ, Mayes HB, Peña MJ, Urbanowicz BR, Nielsen E. 2020. Biochemical and genetic analysis identify CSLD3 as a beta-1, 4-glucan synthase that functions during plant cell wall synthesis. *Plant Cell* **32**: 1749–1767.

Yang W, Schuster C, Beahan CT, Charoensawan V, Peaucelle A, Bacic A, Doblin MS, Wightman R, Meyerowitz EM. 2016. Regulation of meristem morphogenesis by cell wall synthases in Arabidopsis. *Current Biology* **26**: 1404–1415.

Yu L, Lyczakowski JJ, Pereira CS, Kotake T, Yu X, Li A, Mogelsvang S, Skaf MS, Dupree P. 2018. The patterned structure of galactoglucomannan suggests it may bind to cellulose in seed mucilage. *Plant Physiology* **178**: 1011–1026.

Yu L, Shi D, Li J, Kong Y, Yu Y, Chai G, Hu R, Wang J, Hahn MG, Zhou G. 2014. CELLULOSE SYNTHASE-LIKE A2, a glucomannan synthase, is involved in maintaining adherent mucilage structure in Arabidopsis seed. *Plant Physiology* **164**: 1842–1856.

Zang H, Xie S, Zhu B, Yang X, Gu C, Hu B, Gao T, Chen Y, Gao X. 2019. Mannan oligosaccharides trigger multiple defence responses in rice and tobacco as a novel danger-associated molecular pattern. *Molecular Plant Pathology* **20**: 1067–1079.

Zhao Y, Song D, Sun J, Li L. 2013. Populus endo-beta-mannanase PtrMAN6 plays a role in coordinating cell wall remodeling with suppression of secondary wall thickening through generation of oligosaccharide signals. *The Plant Journal* **74**: 473–485.

Zhong R, Cui D, Ye Z-H. 2018. Members of the DUF231 family are O-acetyltransferases catalyzing 2-O- and 3-O-acetylation of mannan. *Plant and Cell Physiology* **59**: 2339–2349.

Zhong R, Cui D, Ye Z. 2019. Evolutionary origin of O-acetyltransferases responsible for glucomannan acetylation in land plants. *New Phytologist* **224**: 466–479.