

## Branched mannan and xyloglucan as a dynamic duo in plant cell walls

Annika Grieb-Osowski<sup>a,b</sup>, Cătălin Voiniciuc<sup>a,c,\*</sup>

<sup>a</sup> Independent Junior Research Group–Designer Glycans, Leibniz Institute of Plant Biochemistry, 06120 Halle (Saale), Germany

<sup>b</sup> Department of Biological Data Science, Heinrich-Heine-University Düsseldorf, Universitätsstraße 1, 40225 Düsseldorf, Germany

<sup>c</sup> Horticultural Sciences Department, University of Florida, Gainesville, FL 32611, United States

### ARTICLE INFO

#### Keywords:

Plant cell wall  
Hemicelluloses  
Galactoglucomannan  
Xyloglucan  
Arabidopsis

Plant surfaces are encased in cellulose, hemicelluloses, pectin, glycoproteins and additional classes of molecules such as lignin and cuticular waxes. While the actual composition of the extracellular matrix varies remarkably between different plant organs and developmental stages, a complex network of cellulose and hemicelluloses is generally responsible for providing strength as well as guiding the growth of plant cells. Cellulose microfibrils are synthesized directly at the plasma membrane, yet their sustained deposition and organization also depend on the intracellular synthesis and delivery of hemicelluloses such as xyloglucan (XyG), mannan and xylan (Zhang et al., 2021). The substitution of these matrix polysaccharides can directly influence their ability to cross-link cellulose microfibrils (Khodayari et al., 2021). (Gluco)mannan backbones are built of  $\beta$ -1,4-mannosyl (Man) units, sometimes interspersed with  $\beta$ -1,4-glucosyl (Glc) units, while XyG backbone is built exclusively of  $\beta$ -1,4-Glc. The biosynthesis of conserved XyG branches has been extensively investigated by several research groups (reviewed by Julian and Zabolina, 2022), but plant  $\beta$ -mannans appeared to be composed of irregularly distributed glycosidic residues until recently (reviewed by Voiniciuc, 2022). Subsequently, Yu et al. (2022) identified a patterned galactoglucomannan (GGM) containing repeating Glc-Man backbone units and regularly spaced  $\beta$ -Gal- $\alpha$ -Gal disaccharide side chains. Oligosaccharide profiling of primary cell wall (PCW) extracts from several eudicot species, including important crops such as tomato, suggests that  $\beta$ -mannans with conserved molecular patterns are widespread. In this update, we summarize newfound

relationships between mannan and XyG decorated with galactose (Gal) and discuss their impact on PCW function. The roles of Gal substitutions on mannans and XyG require further investigation but likely facilitate interactions with other cell wall polymers such as cellulose. Notably, the synthesis of these two classes of hemicelluloses relies on a common set of Carbohydrate-Active Enzyme (CAZY; <https://www.cazy.org/>) families (Table 1).

### Mannan and XyG elongation

While  $\beta$ -mannans likely represent the most ancient hemicellulose in the plant kingdom, XyG emerged as the dominant hemicellulose in the PCWs of most angiosperms (Scheller and Ulvskov, 2010). Nevertheless, in some walls such as the softwood of gymnosperms, GGM accumulates as the most abundant hemicellulose after cell expansion is completed (Voiniciuc, 2022). In *Arabidopsis thaliana* (Arabidopsis hereafter), five CELLULOSE SYNTHASE-LIKE C (CSLC) genes are functionally redundant for the elongation of XyG (Kim et al., 2020), while at least four of the nine CELLULOSE SYNTHASE-LIKE A (CSLA) genes encode (gluco) mannan synthases (Voiniciuc, 2022). Plant  $\beta$ -1,4-glucan or mannan polymers can be elongated in yeast using CSLC (Cocuron et al., 2007; Robert et al., 2021) or CSLA expression (Robert et al., 2021; Voiniciuc et al., 2019), respectively. One notable difference between these integral Golgi enzymes is that CSLAs use GDP-Man and GDP-Glc as sugar donors, while CSLCs utilize UDP-Glc. Furthermore, their catalytic domains were

**Abbreviations:** XyG, xyloglucan; GGM, galactoglucomannan; CSLA, CELLULOSE SYNTHASE-LIKE A; CSLC, CELLULOSE SYNTHASE-LIKE C; GT, glycosyltransferase.

\* Corresponding author at: Horticultural Sciences Department, University of Florida, Gainesville, FL 32611, United States.

E-mail address: [cvoinicu@ufl.edu](mailto:cvoinicu@ufl.edu) (C. Voiniciuc).

<https://doi.org/10.1016/j.tcs.2023.100098>

Received 1 January 2023; Received in revised form 21 January 2023; Accepted 23 January 2023

Available online 25 January 2023

2468-2330/© 2023 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

**Table 1**

Comparison of Arabidopsis GT enzymes for GGM and XyG biosynthesis. The list of characterized CAZy proteins is based on several publications (Julian and Zabolina, 2022; Pauly and Keegstra, 2016; Voiniciuc, 2022; Yu et al., 2022). For the GT34 and GT47 isoforms, the primary enzyme specificity is listed. New abbreviations: Fuc, fucose; GalA, galacturonic acid. Question marks indicate that no related enzymes have been identified for a particular polymer thus far.

	Xyloglucan (XyG)		Galactoglucomannan (GGM)	
	Enzyme	AGI ID	Enzyme	AGI ID
GT2	CSLC4	At3g28180	CSLA2	At5g22740
	CSLC5	At4g31590	CSLA3	At1g23480
	CSLC6	At3g07330	CSLA7	At2g35650
	CSLC8	At2g24630	CSLA9	At5g03760
	CSLC12	At4g07960		
GT34	XXT1 ( $\alpha$ -1,6-Xyl)XXT2	At3g62720	MAGT/MUCI10	At2g22900
	( $\alpha$ -1,6-Xyl)XXT5	At4g02500	( $\alpha$ -1,6-Gal)	
	( $\alpha$ -1,6-Xyl)	At1g74380		
GT37	MUR2 / FUT1 ( $\alpha$ -1,2-Fuc)	At2g03220	?	?
GT47	MUR3 ( $\beta$ -1,2-Gal)	At2g20370	MBGT1 ( $\beta$ -1,2-Gal)	At4g13990
	XLT2	At5g62220		
	( $\beta$ -1,2-Gal)XUT1 ( $\beta$ -1,2-GalA)	At1g63450		
GT106	?	?	MSR1	At3g21190
			MSR2	At1g51630

reported to have opposite orientations (Davis et al., 2010), which would influence nucleotide sugar accessibility and the need for product translocation. Structural prediction of CSLCs identified a “VET” amino acid motif instead of a “TED” sequence, important for product elongation and translocation by cellulose synthases (Julian and Zabolina, 2022). All active (gluco)mannan synthases share this VET motif with CSLCs and a number of other highly conserved motifs (Robert et al., 2021), suggesting that CSLAs and CSLCs could elongate polymers via similar mechanisms. While an initial catalytic domain swap of Arabidopsis CSLA2 with that of the nasturtium (*Tropaeolum majus*) CSLC4 was non-functional compared to the parental enzymes (Robert et al., 2021), structural data and/or detailed *in vitro* characterization are still needed to understand the biochemistry of CSLA/Cs.

The new oligosaccharide profiling of Arabidopsis mutants indicates that CSLA2 and CSLA9 are required for the synthesis of two different GGM motifs (Yu et al., 2022). Mannanase digestion released only trace amounts of carbohydrates from the *csla2 csla9* double mutant. This suggests that there may be a functional and tissue-dependent specialization within the CSLA family, but raises new questions about the roles of other CSLAs in Arabidopsis (Voiniciuc, 2022). The latest data show that CSLA9-dependent acetylated glucomannan motifs are found in PCW-rich tissues such as leaves (Yu et al., 2022), while CSLA2 is required for repeating Glc-Man domains regularly substituted Gal side chains (discussed in next section). However, it is still unclear how GGM backbone patterns are defined. Based on synthetic biology approaches in yeast, MANNAN SYNTHESIS-RELATED (MSR) proteins from the GT106 family modulate the activities of CSLAs to enhance the elongation of (gluco)mannan (Robert et al., 2021; Voiniciuc et al., 2019). Even when co-expressed with MSR1 to produce glucomannan in yeast cell walls, Arabidopsis CSLA2 was unable to generate glucomannan with the high Glc to Man molar ratio found in seed mucilage (Voiniciuc et al., 2015b; Yu et al., 2018). Therefore, additional proteins likely function together *in vivo* to define GGM patterns.

### Substitution of mannan and XyG

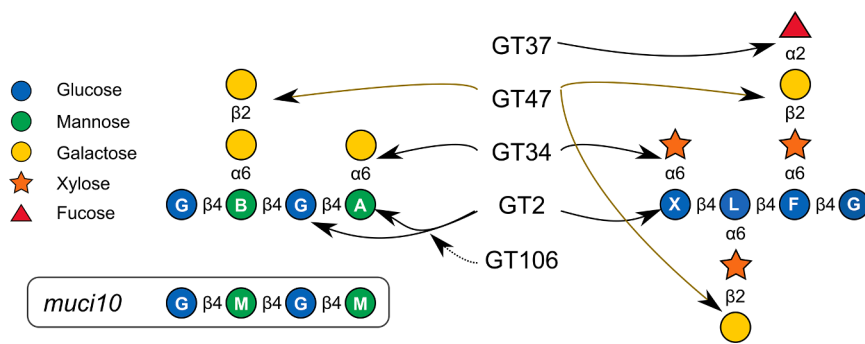
The backbones of mannans and XyG can be substituted by GT34 enzymes with  $\alpha$ -1,6-linked Gal or xylosyl (Xyl), respectively, or can be O-acetylated at the C-2 and C-3 positions (Pauly and Keegstra, 2016; Voiniciuc, 2022). Single  $\alpha$ -1,6-Gal units are attached to Man by MANNAN  $\alpha$ -GALACTOSYLTRANSFERASE1/MUCILAGE-RELATED 10

(MAGT1/MUCI10) using UDP-Gal (Voiniciuc et al., 2015b; Yu et al., 2018), while XyG Xyl TRANSFERASES (XXT) use UDP-Xyl as to add regular patterns *in vivo* (Culbertson et al., 2018). Interestingly, some XXTs can add additional Xyl residues on glucan oligosaccharides *in vitro* or even use other donor sugars such as UDP-Gal (Julian and Zabolina, 2022). This suggests that GT34 activity in plant cells is partly determined by donor substrate availability, and that the mannan-related enzyme specificities should be further explored. The Xyl side chains are usually further substituted with single  $\beta$ -1,2-Gal (abbreviated L) or  $\beta$ -1,2-Gal- $\alpha$ -1,2-Fucose (abbreviated F) units in Arabidopsis (Pauly and Keegstra, 2016). While XyG subunits as complex as XLFG are natively found in most Arabidopsis tissues (Fig. 1) and have been easy to identify based on characteristic mass spectra (Pauly and Keegstra, 2016), GGM oligosaccharides have been more challenging to fingerprint. Back in 2015, the significant reduction of terminal-Gal and 2-Gal linkages in mucilage extracted from *muci10* mutant seeds raised the hypothesis that  $\beta$ -1,2-Gal- $\alpha$ -1,6-Gal disaccharides may partly decorate glucomannan in Arabidopsis mucilage. Prompted by the previous evidence that  $\beta$ -galactosylation of  $\alpha$ -Gal residues on XyG is catalyzed by GT47 enzymes such as MURUS3 (MUR3; *murus* means wall in Latin) and XYLOGLUCAN L-SIDE CHAIN GALACTOSYLTRANSFERASE POSITION2 (XLT2; reviewed by Pauly and Keegstra, 2016), Yu et al., (2022) elegantly showed that MANNAN  $\beta$ -GALACTOSYLTRANSFERASE1 (MBGT1), belonging to an uncharacterized clade of this CAZY family, adds  $\beta$ -1,2-Gal onto GGM (Fig. 1). While these  $\beta$ -Gal side chains were not abundant enough to be detected by solid state NMR, the native expression of  $\beta$ -galactosidases could be reducing the frequency of  $\beta$ -Gal observed in some cell walls (Yu et al., 2022).

### Unique and additive effects of mannan and XyG mutants

For the synthesis of GGM in the Arabidopsis seed mucilage, MAGT1/MUCI10 may function in a complex with CSLA2 to modulate its stability or activity because the glucomannan elongation is strongly reduced in *muci10* mutants. Similarly, the elongation of insoluble  $\beta$ -1,4-glucan by CSLC4 in yeast was boosted by XXT1 co-expression (Cocuron et al., 2007; Robert et al., 2021). The *csla2* and *muci10* increased the density of mucilage polysaccharides yet reduced the amount of crystalline cellulose relative to wild-type plants (Voiniciuc et al., 2015b). Biophysical changes associated with the loss of mannan branches have not been quantified, but we hypothesize the mutants such as *muci10* would enhance cell wall rigidity to mechanical stress, despite being more susceptible to enzymatic degradation by endo-mannanases and cellulases (Voiniciuc et al., 2015b). The effects of Indeed, GGM is more important for cellulose deposition than branched xylan (Voiniciuc et al., 2015a; Yang et al., 2020), another hemicellulose present in mucilage, while XyG-deficient *xtt1 xxt2 xxt5* triple mutant seeds have wild-type mucilage staining (Voiniciuc et al., 2015a). Even the loss of several microtubule-related proteins that guide cellulose synthase movement (Yang et al., 2022) caused less severe cellulose defects than GGM-deficient seeds. Therefore, mannans (akin to XyG in tip-growing cells; Kim et al., 2020) have unique roles in some tissues (Voiniciuc, 2022).

Although XyG is the most abundant hemicellulose in the PCW of eudicots, the lack of detectable XyG in quintuple *cslc* mutants (Kim et al., 2020) and triple *xtt* mutants (Zabolina et al., 2012) only results in minor growth defects. The hypothesis that other polysaccharides can compensate for the loss of XyG in these plants has lacked evidence until recently. While XyG-deficient mutants do not have clearly altered expression of CSLA genes (Kim et al., 2020; Sowinski et al., 2022), *xtt1 xxt2* double mutants have now been reported to have increased glucomannan and pectin content (Sowinski et al., 2022), potentially due to reduced polysaccharide turnover. Changes in glucomannan content were not detected by an independent team using oligosaccharide profiling (Yu et al., 2022), but a third group found significant changes in the mass distribution profiles of mannans labelled by the LM21 antibody in XyG-deficient mutants (Sathithnaitham et al., 2021). The higher order



**Fig. 1.** Schematic of GT families required for branched mannan and XyG biosynthesis. A highly branched motif found in Arabidopsis cell walls is shown for each type of polymer, and their biosynthesis requires related CAZy proteins. Models and single-letter nomenclature (Julian and Zabolina, 2022; Pauly and Keegstra, 2016; Yu et al., 2022) denotes the backbone unit and any associated branches, e.g. B for the disaccharide-decorated Man. Two different GT47 enzymes are required to add  $\beta$ -1,2-Gal to the different positions of XLFG. Characterized enzymes for each GT family are shown in Table 1.

mutants generated by Yu et al. (2022) provide the strongest evidence that  $\beta$ -galactosylated GGM and XyG have functionally overlapping roles in some PCWs. The *cls2a xxt1 xxt2* triple mutant reduced cell elongation and impaired cellulose organization, resulting in shorter plants and siliques compared to the respective mutants backgrounds (Yu et al., 2022). Furthermore, *mbgt1 mur3-3* double mutant was exacerbated the cabbage-like phenotype of the *mur3-3* single mutant, suggesting that  $\beta$ -galactosylated GGM partially compensated for the XyG deficiency during early growth (Yu et al., 2022). Further comparative studies of branched mannans and XyG are now needed to address several puzzles that remain at the cell surface. Exploring the biodiversity of proteins and their complexes that add or modify these patterned hemicelluloses will reveal new insights into their secretion and interaction with cellulose microfibrils.

#### CRedit authorship contribution statement

**Annika Grieff-Osowski:** Writing – original draft, Writing – review & editing. **Cătălin Voiniciuc:** Writing – review & editing, Visualization.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

#### Acknowledgements

This work was supported by the Deutsche Forschungsgemeinschaft (DFG; German Research Foundation grant 414353267), the Leibniz Institute of Plant Biochemistry, and start-up funding from the University of Florida, Institute of Food and Agricultural Sciences (UF/IFAS) to Cătălin Voiniciuc.

#### References

- Cocuron, J.-C., Lerouxel, O., Drakakaki, G., Alonso, A.P., Liepman, A.H., Keegstra, K., Raikhel, N., Wilkerson, C.G., 2007. A gene from the cellulose synthase-like C family encodes a -1,4 glucan synthase. *Proc. Natl. Acad. Sci. USA* 104, 8550–8555. <https://doi.org/10.1073/pnas.0703133104>.
- Culbertson, A.T., Ehrlich, J.J., Choe, J.-Y., Honzatko, R.B., Zabolina, O.A., 2018. Structure of xyloglucan xylosyltransferase 1 reveals simple steric rules that define biological patterns of xyloglucan polymers. *Proc. Natl. Acad. Sci. USA* 115, 6064–6069. <https://doi.org/10.1073/pnas.1801105115>.
- Davis, J., Brandizzi, F., Liepman, A.H., Keegstra, K., 2010. Arabidopsis mannan synthase CSLA9 and glucan synthase CSLC4 have opposite orientations in the Golgi membrane. *Plant J.* 64, 1028–1037. <https://doi.org/10.1111/j.1365-313X.2010.04392.x>.
- Julian, J.D., Zabolina, O.A., 2022. Xyloglucan biosynthesis: from genes to proteins and their functions. *Front. rs in Plant Science* 13.

- Khodayari, A., Thielemans, W., Hirn, U., Van Vuure, A.W., Seveno, D., 2021. Cellulose-hemicellulose interactions – a nanoscale view. *Carbohydr. Polym.* 270, 118364. <https://doi.org/10.1016/j.carbpol.2021.118364>.
- Kim, S.-J., Chandrasekar, B., Rea, A.C., Danhof, L., Zemelis-Durfee, S., Thrower, N., Shepard, Z.S., Pauly, M., Brandizzi, F., Keegstra, K., 2020. The synthesis of xyloglucan, an abundant plant cell wall polysaccharide, requires CSLC function. *Proc. Natl. Acad. Sci. USA* 117, 20316–20324. <https://doi.org/10.1073/pnas.2007245117>.
- Pauly, M., Keegstra, K., 2016. Biosynthesis of the plant cell wall matrix polysaccharide xyloglucan. *Annu. Rev. Plant Biol.* 67, 235–259. <https://doi.org/10.1146/annurev-arplant-043015-112222>.
- Robert, M., Waldhauer, J., Stritt, F., Yang, B., Pauly, M., Voiniciuc, C., 2021. Modular biosynthesis of plant hemicellulose and its impact on yeast cells. *Biotechnol. Biofuels* 14, 140. <https://doi.org/10.1186/s13068-021-01985-z>.
- Sathitnaitam, S., Suttangkakul, A., Wonnapijit, P., McQueen-Mason, S.J., Vuttipongchaikij, S., 2021. GPC-ELISA method for systematic mass distribution profiling of plant cell wall matrix polysaccharides. *Plant J.* <https://doi.org/10.1111/tpl.15255>.
- Scheller, H.V., Ulvskov, P., 2010. Hemicelluloses. *Annu. Rev. Plant Biol.* 61, 263–289. <https://doi.org/10.1146/annurev-arplant-042809-112315>.
- Sowinski, E.E., Westman, B.M., Redmond, C.R., Kong, Y., Olek, A.T., Olek, J., McCann, M.C., Carpita, N.C., 2022. Lack of xyloglucan in the cell walls of the Arabidopsis *xtt1/xtt2* mutant results in specific increases in homogalacturonan and glucomannan. *Plant J.* 110, 212–227. <https://doi.org/10.1111/tpl.15666>.
- Voiniciuc, C., 2022. Modern mannan: a hemicellulose's journey. *New Phytol.* 234, 1175–1184. <https://doi.org/10.1111/nph.18091>.
- Voiniciuc, C., Günl, M., Schmidt, M.-H.-W., Usadel, B., Guenl, M., Schmidt, M.-H.-W., Usadel, B., 2015a. Highly branched xylan made by IRREGULAR XYLEM14 and MUCILAGE-RELATED21 links mucilage to arabidopsis seeds. *Plant Physiol.* 169, 2481–2495. <https://doi.org/10.1104/pp.15.01441>.
- Voiniciuc, C., Schmidt, M.-H.-W., Berger, A., Yang, B., Ebert, B., Scheller, H.V., North, H.M., Usadel, B., Günl, M., 2015b. MUCILAGE-RELATED10 produces galactoglucomannan that maintains pectin and cellulose architecture in arabidopsis seed mucilage. *Plant Physiol.* 169, 403–420. <https://doi.org/10.1104/pp.15.00851>.
- Voiniciuc, C., Dama, M., Gawenda, N., Stritt, F., Pauly, M., 2019. Mechanistic insights from plant heteromannan synthesis in yeast. *Proc. Natl. Acad. Sci. USA* 116, 522–527. <https://doi.org/10.1073/pnas.1814003116>.
- Yang, B., Hofmann, F., Usadel, B., Voiniciuc, C., 2020. Seed hemicelluloses tailor mucilage properties and salt tolerance. *New Phytol.* 229, 1946–1954. <https://doi.org/10.1111/nph.17056>.
- Yang, B., Stamm, G., Bürstenbinder, K., Voiniciuc, C., 2022. Microtubule-associated IQD9 orchestrates cellulose patterning in seed mucilage. *New Phytol.* 235, 1096–1110. <https://doi.org/10.1111/nph.18188>.
- Yu, L., Yoshimi, Y., Cresswell, R., Wightman, R., Lyczakowski, J.J., Wilson, L.F.L., Ishida, K., Stott, K., Yu, X., Charalambous, S., Wurman-Rodrich, J., Terrett, O.M., Brown, S.P., Dupree, R., Temple, H., Krogh, K.B.R.M., Dupree, P., 2022. Eudicot primary cell wall glucomannan is related in synthesis, structure and function to xyloglucan. *Plant Cell* koac238.
- Yu, L., Lyczakowski, J.J., Pereira, C.S., Kotake, T., Yu, X., Li, A., Mogelsvang, S., Skaf, M.S., Dupree, P., 2018. The patterned structure of galactoglucomannan suggests it may bind to cellulose in seed mucilage. *Plant Physiol.* 178, 1011–1026. <https://doi.org/10.1104/pp.18.00709>.
- Zabolina, O.A., Avci, U., Cavalier, D., Pattathil, S., Chou, Y.-H., Eberhard, S., Danhof, L., Keegstra, K., Hahn, M.G., 2012. Mutations in multiple XXT genes of Arabidopsis reveal the complexity of xyloglucan biosynthesis. *Plant Physiol.* 159, 1367–1384. <https://doi.org/10.1104/pp.112.198119>.
- Zhang, B., Gao, Y., Zhang, L., Zhou, Y., 2021. The Plant Cell Wall: biosynthesis, construction, and functions. *J. Integr. Plant Biol.* 63, 251–272. <https://doi.org/10.1111/jipb.13055>.