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Review

Trafficking of Xylan to Plant Cell Walls

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Abstract: Plant cell walls are classified as primary and secondary walls. The primary wall is necessary for plant morphogenesis and supports cell growth and expansion. Once the growth and expansion ceases, specialized cells form secondary walls in order to give strength and rigidity to the plant. Secondary cell walls are the main constituent of woody biomass. This biomass is raw material for industrial products, food, and biomaterials. Recently, there are an increasing number of studies using biomass for biofuel production and this area has gained importance. However, there are still many unknowns regarding the synthesis and structure of complex polysaccharides forming biomass. Cellulose, being one of the main components of the cell wall, is synthesized at the plasma membrane by cellulose synthase complexes and does not require transportation. On the other hand, pectin and hemicelluloses are synthesized by enzymes located in the Golgi apparatus. Therefore, they need to be transported to the plasma membrane. Even though this transport mechanism is very important, it is one of the least understood parts of the endomembrane system. Xylan is the major hemicellulose in many biomasses and is important for renewable material production. There is limited knowledge about the cellular trafficking of xylan. In this review, we cover the current information and what we know about the vesicular transport of xylan to the cell wall.

Keywords: xylan; cell wall; hemicellulose; vesicular trafficking; Golgi apparatus

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1. Introduction

Many cells in plants have different sizes and shapes, which give each tissue or cell group its unique functions, and also enable the morphological diversity among plants. Plant cells are surrounded by a cell wall that determines their size and shape. A cell wall is a dynamic structure formed by a large number of complex macromolecules [1]. This complex structure plays a critical role in the realization of vital activities such as cell integrity, signal transduction, defense and turgor pressure, and is essential for many cellular functions [2].

Typically, the cell wall is divided into a primary and secondary wall. The primary wall is essential for plant morphogenesis and supports the growth and expansion of cells. When growth and expansion stops, a secondary wall is formed in differentiated cells to provide support and strength to the plant. Although the composition of cell walls can change, in general, about 90% of the dry weight consists of polysaccharides in primary cell walls. The remaining part is composed of structural glycoproteins, phenolic esters, minerals, and enzymes [3]. In the primary wall, the main polysaccharides are cellulose, pectin, and hemicellulose. The primary walls of commelinoid monocotyledonous plants contain 20–30% cellulose, 5% pectin, and 50–60% hemicellulose (mostly xylan and mixed linkage glucans), while the primary walls of dicotyledonous plants contain 15–30% cellulose, 30–40% pectin, and 20–30% hemicellulose (mostly xyloglucans) [1,4]. In the cells forming the secondary wall, there are mainly cellulose, xylan, and glucomannan polysaccharides, the latter two replacing pectin and xyloglucans, respectively [5]. In addition, lignin, a phenolic molecule, also participates in the secondary wall structure. The composition of the secondary wall differs in different plant species and even in different cell types of the same plant. For example, the secondary wall of a gymnosperm plant (*Pinus strobus*) contains 41% cellulose, 9% glucuronoxylan, 18% glucomannan, and 29% lignin, while the secondary wall

of an angiosperm plant (*Populus tremuloides*) contains 48% cellulose, 24% glucuronoxylan, 3% mannan, and 21% lignin [6]. Therefore, although the amount of glucomannan is high in the secondary wall of angiosperm plants, the main hemicellulose in the secondary wall of angiosperm plants is xylan.

The secondary wall makes up a large part of the biomass of woody plants, which is a source of raw materials for many products that we use in our daily lives such as industrial products, feed, and biomaterials [7]. Additionally, there has been a tendency to move away from fossil fuels due to global warming and the interest in more environmentally friendly fuels. In this perspective, the use of biomass as a lignocellulosic raw material for biofuel production has gained importance and studies have increased in this direction. However, there are still many unknown facts about the formation and structure of biomass, which has a complex nature. Therefore, studies aimed at better understanding this complex structure are of great importance.

2. Xylan

Hemicelluloses are polysaccharides that bind to cellulose microfibrils and can be isolated from the wall in an alkaline solution. Xyloglucan, xylan, and glucomannan are common hemicelluloses. Xyloglucans have a β-1,4 glucan main chain and are the main hemicellulosic polysaccharide in the primary walls of dicotyledonous plants. About 75% of this main chain is branched, and xylose, galactose, and fucose monosaccharides can be found in these side chains [1]. Xyloglucans are replaced by xylans in the secondary wall. Xylans have β-(1,4)-D-xylose main chain and may contain arabinose, and glucuronic acid monosaccharides in their side chains. Mannans are also hemicellulosic polysaccharides. Mannan has a backbone of β-1,4-linked mannosyl residues while glucomannan contains β-1,4-linked glucosyl and mannosyl residues in the backbone. If there is a α-1,6-linked galactosyl residue substitution on backbone mannosyl residues, they are called galactomannan and galactoglucomannan, respectively [1]. Among these hemicellulosic polysaccharides, xylans are more abundant in the secondary walls, and in the walls of the Poaceae family of monocotyledonous plants [6].

Figure 1 demonstrates the xylan structures that can be found on xylan in monocots and dicots. In xylan, approximately 100–200 xylose monosaccharides are linked to each other by β-(1,4) bonds, forming the structure of the xylan backbone. Monosaccharides such as glucuronic acid (α-1,2), 4-O-methylglucuronic acid (α-1,2), or arabinose (α-1,2 and or α-1,3) can be attached to the backbone by the sidechains (Figure 1) [7]. Xylan could be acetylated at the O-2 and/or O-3 positions. Some arabinosyl sidechains can be decorated with ferulic acid esters in monocots. According to the nature of the sidechains, xylans can have names such as glucuronoxylan, methylglucuronoxylan, and arabinoxylan. Glucuronoxylan is the dominant xylan in the secondary walls of dicotyledonous plants [6].

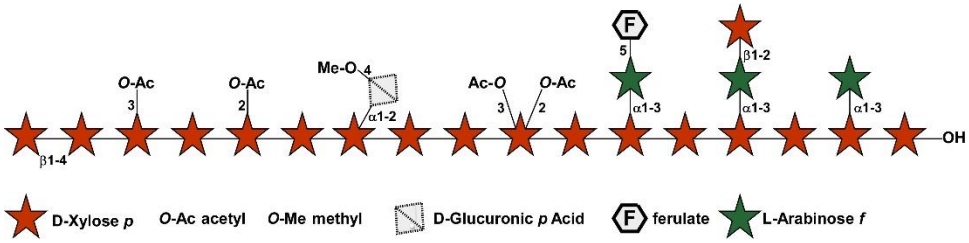

Figure 1. Representation of xylan structures found in monocots and dicots, modified from [8].

Xylan and lignin interact with the cellulose microfibrils, which provides durability to the secondary wall. In addition, lignin is a complex phenolic polymer, imparting water-repellent (hydrophobic) properties to the wall, which is an important property for the efficiency of water transport through the xylem.

3. Xylan Biosynthesis

Xylan biosynthesis has been demonstrated to require over a dozen proteins that work together in Golgi. It is difficult to mention all the research on xylan biosynthesis in detail in this review. Therefore, there are excellent reviews on xylan biosynthesis that can be sourced for further information [9–14]. Briefly, over the years, many research groups have worked on glycosyltransferases (GTs) that are responsible for xylan biosynthesis. Initial work on Arabidopsis irregular xylem (***irx***) mutants, displaying collapsed or irregular xylem with stunted phenotype, opened a way to isolate GTs that are responsible for xylan biosynthesis. IRX9/IRX9L, IRX14/IRX14L from glycosyltransferase family (GT) 43, and IRX10/IRX10L from GT 47, are the main proteins on xylan backbone synthesis in monocots and dicots [15–18]. However, it is unknown how these proteins play a role in a larger xylan synthase complex [19]. IRX10 and its homologs are recently shown to synthesize nanocompartments *de novo* in plant vessel wall patterning [20]. In terms of side chain substitutions, GT8 family members, GUX1, GUX2, and GUX3 are shown to be involved in GlcA (O-2 and O-3 positions) and 4-O-MeGlcA (O-2 position) substitutions since mutants showed reduced side chains [21]. 4-O-methylation of GlcA sidechains is accomplished by Gluruconoxylan Methyl Transferase (GXMT) proteins [22]. Proteins in the GT61 family are responsible for arabinosylation of the backbone [23]. Some of the arabinosyl sidechains can be decorated with ferulic acid esters in monocots even though the exact mechanism is yet to be characterized fully [24]. O-Acetylation is another important modification in xylan that could be established by Reduced Wall Acetylation (RWA) proteins, Trichome Birefringence-Like (TBL) proteins, the Altered XYloglucan 9 (AXY9) protein, and GDSL acetylesterases of xylan [25]. Xylan acetylation is also related to biomass recalcitrance, thus, these proteins are possible targets to reduce recalcitrance [26,27]. The substrate for xylan backbone synthesis, UDP-xylose, is synthesized in the cytosol and transported by the Golgi-localized UDP-xylose transporters such as UXT1 that are thought to be involved in the transport process [28]. Finally, especially monocot grass xylans contain fewer side chains in older tissues, implicating that enzymes such as trans-β-xylanase, xylosidase, and arabinosidase could be involved in modifying xylan substitutions as the plant ages [29], which is still unclear for what purpose plants modify xylan in their cell walls during development.

4. Cellular Trafficking of Xylan

Especially in the last two decades, many enzymes that play a role in the biosynthesis of wall polysaccharides such as hemicellulose and pectin have been revealed. For example, it has been shown that the biosynthesis of the xylan polysaccharide requires enzymes such as xylosyltransferases, glucuronyltransferases, methyltransferases, arabinosyltransferases, and these enzymes are located in the Golgi [9]. However, there is no comprehensive information about the transport mechanisms of most polysaccharides stemming from Golgi [6]. If the transport mechanisms of the molecules that make up the cell wall are well understood, the dynamic organization of the cell wall can be explained more clearly, and detailed models can be revealed accordingly.

The Golgi is part of the cell endomembrane system. The cell endomembrane system is a very important and sensitive system that ensures the timing and regular delivery of cargo molecules necessary for the cell and other vital activities. Cellulose, an important component of the cell wall, is synthesized by cellulose synthase complexes in the plasma membrane and does not require transport in the endomembrane system. On the other hand, pectin and hemicelluloses are synthesized by enzymes in the Golgi apparatus. Therefore, they must be transported from the Golgi to the plasma membrane. Although this transport mechanism is important, it constitutes one of the least understood parts of the endomembrane system [6].

The Trans Golgi Network (TGN) (Figure 2) is responsible for directing the molecules exiting the Golgi to the vacuoles or plasma membrane with the necessary vesicles or endomembrane transport structures in the system [30]. Compared to other eukaryotes, the Golgi apparatus and the Trans-Golgi Network have a specialized function in plants. This

task is the biosynthesis of biosynthetic enzymes, structural proteins, and various matrix polysaccharides (such as hemicellulose and pectin) required for the cell wall, and their cargo and delivery to the cell wall [31,32]. The transport of polysaccharides by the Golgi apparatus to the cell wall happens via vesicles. The electron microscopy image in Figure 2 shows vesicles leaving Golgi (arrows) and vesicles (asterisk) being transported to the cell wall through TGN. In this process, vesicles fuse with the plasma membrane and release their content into extracellular space. However, information on the types of vesicles and which cargo polysaccharides they carry is very limited.

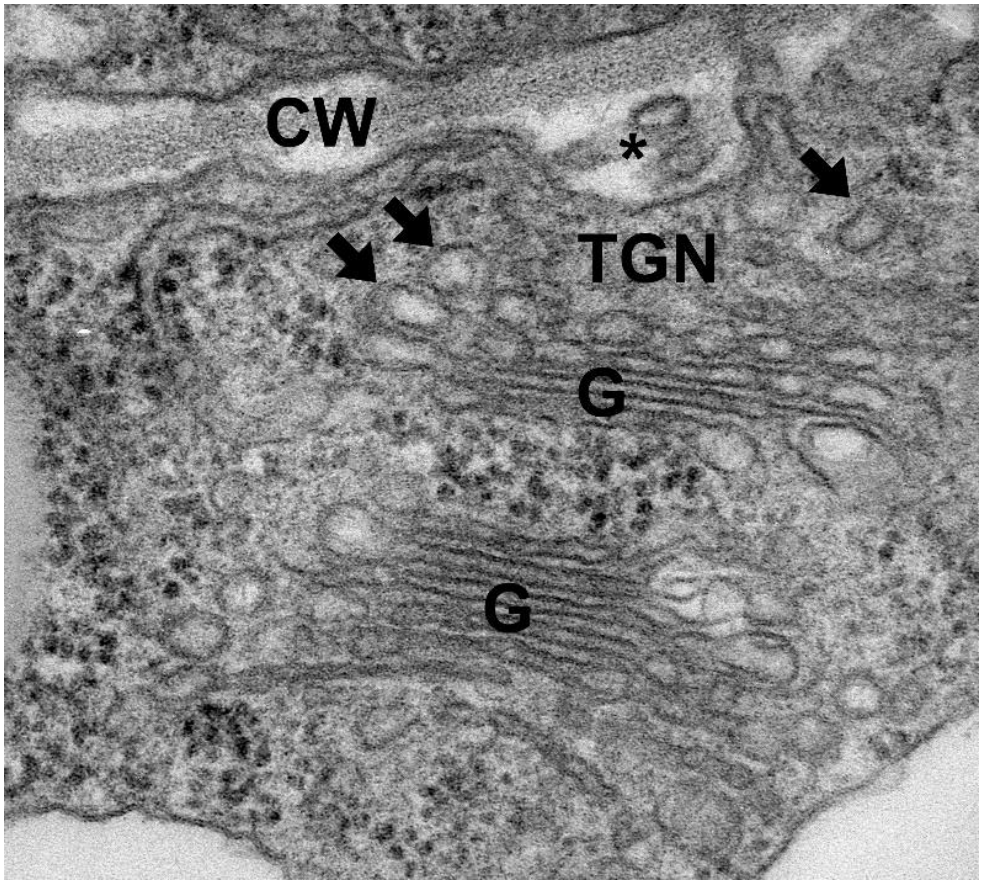

Figure 2. Cellular trafficking of molecules by endomembrane system in plants. Two Golgi apparatus (G) are located close to each other, producing vesicles (arrows). Their cargo is delivered to cell wall (CW) via vesicles (asterisk) through trans Golgi network (TGN).

There is limited literature information on the trafficking of structural polysaccharides from the Golgi to the plasma membrane. This information has been obtained from several immunoelectron microscopy (immuno-EM) studies. Immuno-EM studies have made use of antibodies that recognize cargo vesicles, and glycan directed cell wall antibodies that recognize epitopes on cell wall polysaccharides.

In a study with a protein (Rab GTPase) known to be involved in post-Golgi cargo mechanisms in Arabidopsis meristem cells, it was observed that an antibody recognizing this protein labeled certain vesicles [33]. The same study used an antibody (CCRC-M1) that recognizes fucosylated xyloglucans, and it was demonstrated by immuno-EM that this antibody was in the same vesicles with the cargo protein. This study was carried out in the primary wall-forming meristem cells of the Arabidopsis plant and immunogold labeling was performed with only one cell wall-specific antibody [33]. However, it was visual proof that fucosylated xyloglucans are carried in vesicles containing Rab GTPases. Whether the other type of xyloglucans uses the same vesicles remains a question to be answered. In another study, cells in Arabidopsis seed coat were labeled with two antibodies that

recognize a pectic polysaccharide (CCRC-M36) and xyloglucan (α-XG), and immuno-EM results revealed that these two polysaccharides were observed in vesicles around the Golgi apparatus [34]. In a study performed on the root tip and leaf tissues of a red clover plant, two antibodies that could recognize a pectic polysaccharide and xyloglucan were localized in the Golgi vesicles [35]. In immuno-EM studies performed on suspension culture cells obtained from sycamore plant, the labeling with pectin antibodies included cis, medial and trans Golgi, whereas fucosylated xyloglucan (CCRC-M1) was found only in trans Golgi [36]. As can be seen, studies have been carried out on primary cell walls and mostly using pectin and xyloglucan specific antibodies. In these studies, during the transport of polysaccharides from the Golgi apparatus to the cell wall, the transport is carried out by a type of vesicle, which is defined as secretory vesicles [33]. However, it is known that clathrin-coated vesicles and multiple vesicular bodies (multi vesicular bodies) are used for Golgi transport for different purposes [37]. Whether clathrin-coated vesicles or multi vesicular bodies are ever used in the trafficking of cell wall polysaccharides also remains a question.

In addition to the above-mentioned immuno-EM studies, there is a recent study on the isolation and detection of Golgi vesicles using antibodies [38]. In this study, the isolated vesicles were tested with cell wall-specific antibodies using the ELISA method, and many polysaccharides were found in these vesicles. However, the results of this study were made with Golgi vesicles obtained from cells in the root of the Arabidopsis plant and gave a general result. In other words, no explanatory result has been revealed as to which type of vesicles there are, and whether polysaccharides are found in the same type of vesicles or in different types of vesicles. Meents et al. [39] also used a xylan-specific antibody (CCRC-M138) to show vesicles containing a particular xylan epitope leaving Golgi, but did not mention the type of vesicle either. Therefore, there is no detailed study found on immunolocalization of wall transport of xylan polysaccharides in the literature.

In order to answer some of the questions about the type of vesicles, double immuno-EM staining could be carried out by using cell wall directed antibodies and antibodies against different types of vesicles. There are many cell wall directed antibodies with known epitopes for xylan [40]. For example, LM10 recognizes the xylose monosaccharide at the non-reducing end of the xylan main chain. CCRC-M150 recognizes glucuronoxylan structure. CCRC-M154 recognizes arabinoxylan structure, CCRC-M146 recognizes methyl-glucuronoxylan structure. CCRC-M148 only recognizes the main chain and requires at least 5 xylose monosaccharides side by side and no side chains. LM11, on the other hand, can recognize the xylan main chain, which may have different side chains. Diverse epitopes can be studied by using these antibodies. In terms of vesicle specific antibodies, RabA4b (antibody to mark secretory vesicles) and RabF2a (antibody to mark multiple vesicular structures) were successfully used [33]. The anti-clathrin antibody for labeling clathrin-coated vesicles is also available commercially [41]. A study using such a comprehensive range of antibodies to analyze cell wall trafficking in detail by immuno EM would be very informative.

5. Conclusions, Challenges, and Future Perspectives

Xylan is the second most abundant polysaccharide after cellulose in plant biomass. Therefore, a detailed understanding regarding how xylan is transported to the cell wall is vital to better utilize biomass. Co-expression and microscopy analysis showed that xylan synthase complex in the wheat assembly in the endoplasmic reticulum and accumulate in the Golgi [42]. However, there is almost no detailed research about the vesicles carrying various xylan polysaccharides and there are many questions to be addressed. For example, it would be important to know whether different xylan polysaccharides are transported with only one type of vesicle or with different vesicular structures. It would be important to know whether various polysaccharides such as xylan, pectin, and xyloglucan are transported together in the same vesicles or in different vesicles. It would also be important to know whether there are specific plasma membrane domains for xylan to be delivered.

Therefore, ultrastructural studies making use of various antibodies are key to answering such questions in the future.

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References

1. O'Neill, M.; York, W.S. The composition and structure of primary cell walls. In *The Plant Cell Wall*; Rose, J.K.C., Ed.; Blackwell: Oxford, UK, 2003; pp. 1–54. [CrossRef]
2. Sinclair, R.; Rosquete, M.R.; Drakakaki, G. Post-Golgi Trafficking and Transport of Cell Wall Components. *Front. Plant Sci.* **2018**, *9*, 1784. [CrossRef] [PubMed]
3. Caffall, H.K.; Mohnen, D. The structure, function and biosynthesis of pectic polysaccharides in the context of the whole plant cell wall. *Carbohydr. Res.* **2009**, *344*, 1879–1900. [CrossRef] [PubMed]
4. Cosgrove, D.J.; Jarvis, M.C. Comparative structure and biomechanics of plant primary and secondary cell walls. *Front. Plant Sci.* **2012**, *3*, 204. [CrossRef] [PubMed]
5. Mellerowicz, E.J.; Sundberg, B. Wood cell walls: Biosynthesis, developmental dynamics and their implications for wood properties. *Curr. Opin. Plant Biol.* **2008**, *11*, 293–300. [CrossRef] [PubMed]
6. Zhong, R.; Ye, Z.H. Secondary cell walls: Biosynthesis, patterned deposition and transcriptional regulation. *Plant Cell Physiol.* **2015**, *56*, 195–214. [CrossRef] [PubMed]
7. Ebringerova, A.; Heinze, T. Xylan and xylan derivatives—biopolymers with valuable properties, 1. Naturally occurring xylans structures, isolation procedures and properties. *Macromol. Rapid Commun.* **2000**, *21*, 542–556. [CrossRef]
8. Smith, P.J.; Wang, H.T.; York, W.S.; Peña, M.J.; Urbanowicz, B.R. Designer biomass for next-generation biorefineries: Leveraging recent insights into xylan structure and biosynthesis. *Biotechnol. Biofuels* **2017**, *10*, 286. [CrossRef]
9. Rennie, E.A.; Scheller, H.V. Xylan biosynthesis. *Curr. Opin. Biotechnol.* **2014**, *26*, 100–117. [CrossRef]
10. York, W.S.; O'Neill, M.A. Biochemical control of xylan biosynthesis—Which end is up? *Curr. Opin. Plant Biol.* **2008**, *11*, 258–265. [CrossRef]
11. Doering, A.; Lathe, R.; Persson, S. An update on xylan synthesis. *Mol. Plant* **2012**, *5*, 769–771. [CrossRef]
12. Busse-Wicher, M.; Grantham, N.J.; Lyczakowski, J.J.; Nikolovski, N.; Dupree, P. Xylan decoration patterns and the plant secondary cell wall molecular architecture. *Biochem. Soc. Trans.* **2016**, *44*, 74–78. [CrossRef] [PubMed]
13. Wierzbicki, M.P.; Maloney, V.; Mizrachi, E.; Myburg, A.A. Xylan in the Middle: Understanding Xylan Biosynthesis and Its Metabolic Dependencies Toward Improving Wood Fiber for Industrial Processing. *Front. Plant Sci.* **2019**, *10*, 176. [CrossRef] [PubMed]
14. Zhong, R.; Cui, D.; Ye, Z.H. Secondary cell wall biosynthesis. *New Phytol.* **2019**, *221*, 1703–1723. [CrossRef] [PubMed]
15. Brown, D.M.; Goubet, F.; Wong, V.W.; Goodacre, R.; Stephens, E.; Dupree, P.; Turner, S.R. Comparison of five xylan synthesis mutants reveals new insight into the mechanisms of xylan synthesis. *Plant J.* **2007**, *52*, 1154–1168. [CrossRef]
16. Wu, A.M.; Hörnblad, E.; Voxeur, A.; Gerber, L.; Rihouey, C.; Lerouge, P.; Marchant, A. Analysis of the Arabidopsis IRX9/IRX9-L and IRX14/IRX14-L pairs of glycosyltransferase genes reveals critical contributions to biosynthesis of the hemicellulose glucuronoxylan. *Plant Physiol.* **2010**, *153*, 542–554. [CrossRef]
17. Lee, C.; Zhong, R.; Ye, Z.-H. Arabidopsis family GT43 members are xylan xylosyltransferases required for the elongation of the xylan backbone. *Plant Cell Physiol.* **2012**, *53*, 135–143. [CrossRef]
18. Chiniquy, D.; Varanasi, P.; Oh, T.; Harholt, J.; Katnelson, J.; Singh, S.; Auer, M.; Simmons, B.; Adams, P.D.; Scheller, H.V.; et al. Three novel rice genes closely related to the Arabidopsis IRX9, IRX9L, and IRX14 genes and their roles in xylan biosynthesis. *Front. Plant Sci.* **2013**, *4*, 83. [CrossRef]
19. Zeng, W.; Jiang, N.; Nadella, R.; Killen, T.L.; Nadella, V.; Faik, A. A glucurono(arabino)xylan synthase complex from wheat contains members of the GT43, GT47, and GT75 families and functions cooperatively. *Plant Physiol.* **2010**, *154*, 78–97. [CrossRef]
20. Wang, H.; Yang, H.; Wen, Z.; Gao, C.; Gao, Y.; Tian, Y.; Xu, Z.; Liu, X.; Persson, S.; Zhang, B.; et al. Xylan-based nanocompartments orchestrate plant vessel wall patterning. *Nat. Plants* **2022**, *8*, 295–306. [CrossRef]
21. Lee, C.; Teng, Q.; Zhong, R.; Ye, Z.H. Arabidopsis GUX proteins are glucuronyltransferases responsible for the addition of glucuronic acid side chains onto xylan. *Plant Cell Physiol.* **2012**, *53*, 1204–1216. [CrossRef]
22. Urbanowicz, B.R.; Peña, M.J.; Ratnaparkhe, S.; Avci, U.; Backe, J.; Steet, H.F.; Foston, M.; Li, H.; O'Neill, M.A.; Ragauskas, A.J.; et al. 4-O-methylation of glucuronic acid in Arabidopsis glucuronoxylan is catalyzed by a Domain of Unknown Function family 579 protein. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 14253–14258. [CrossRef] [PubMed]

23. Anders, N.; Wilkinson, M.D.; Lovegrove, A.; Freeman, J.; Tryfona, T.; Pellny, T.K.; Weimar, T.; Mortimer, J.C.; Stott, K.; Baker, J.M.; et al. Glycosyl transferases in family 61 mediate arabinofuranosyl transfer onto xylan in grasses. *Proc. Natl. Acad. Sci. USA* **2012**, *109*, 989–993. [CrossRef] [PubMed]
24. Saulnier, L.; Vigouroux, J.; Thibault, J.F. Isolation and partial characterization of feruloylated oligosaccharides from maize bran. *Carbohydr. Res.* **1995**, *272*, 241–253. [CrossRef]
25. Qaseem, M.F.; Wu, A.M. Balanced Xylan Acetylation is the Key Regulator of Plant Growth and Development, and Cell Wall Structure and for Industrial Utilization. *Int. J. Mol. Sci.* **2020**, *21*, 7875. [CrossRef]
26. Pawar, P.M.; Ratke, C.; Balasubramanian, V.K.; Chong, S.L.; Gandla, M.L.; Adriasola, M.; Sparrman, T.; Hedenström, M.; Szwaj, K.; Derba-Maceluch, M.; et al. Downregulation of RWA genes in hybrid aspen affects xylan acetylation and wood saccharification. *New Phytol.* **2017**, *214*, 1491–1505. [CrossRef]
27. Xiong, G.; Cheng, K.; Pauly, M. Xylan O-acetylation impacts xylem development and enzymatic recalcitrance as indicated by the Arabidopsis mutant tbl29. *Mol. Plant* **2013**, *6*, 1373–1375. [CrossRef] [PubMed]
28. Zhao, X.; Liu, N.; Shang, N.; Zeng, W.; Ebert, B.; Rautengarten, C.; Zeng, Q.Y.; Li, H.; Chen, X.; Beahan, C.; et al. Three UDP-xylose transporters participate in xylan biosynthesis by conveying cytosolic UDP-xylose into the Golgi lumen in Arabidopsis. *J. Exp. Bot.* **2018**, *69*, 1125–1134. [CrossRef] [PubMed]
29. Franková, L.; Fry, S.C. Phylogenetic variation in glycosidases and glycanases acting on plant cell wall polysaccharides, and the detection of transglycosidase and trans-β-xylanase activities. *Plant J. Cell Mol. Biol.* **2011**, *67*, 662–681. [CrossRef]
30. Rosquete, M.R.; Davis, D.J.; Drakakaki, G. The plant trans-golgi network: Not just a matter of distinction. *Plant Physiol.* **2018**, *176*, 187–198. [CrossRef]
31. Kim, S.J.; Brandizzi, F. The plant secretory pathway for the trafficking of cell wall polysaccharides and glycoproteins. *Glycobiology* **2016**, *26*, 940–949. [CrossRef]
32. Viotti, C.; Bubeck, J.; Stierhof, Y.D.; Krebs, M.; Langhans, M.; Van Den Berg, W. Endocytic and secretory traffic in Arabidopsis merge in the trans-Golgi network/early endosome, an independent and highly dynamic organelle. *Plant Cell* **2010**, *22*, 1344–1357. [CrossRef] [PubMed]
33. Kang, B.H.; Nielsen, E.; Preuss, M.L.; Mastronarde, D.; Staehelin, L.A. Electron tomography of RabA4b- and PI-4Kbeta1-labeled trans Golgi network compartments in Arabidopsis. *Traffic* **2011**, *12*, 313–329. [CrossRef] [PubMed]
34. Young, R.E.; Mcfarlane, H.E.; Hahn, M.G.; Western, T.L.; Haughn, G.W.; Samuels, A.L. Analysis of the Golgi apparatus in Arabidopsis seed coat cells during polarized secretion of pectin-rich mucilage. *Plant Cell* **2008**, *20*, 1623–1638. [CrossRef] [PubMed]
35. Moore, P.J.; Staehelin, L.A. Immunogold localization of the cell wall-matrix polysaccharides rhamnogalacturonan I and xyloglucan during cell expansion and cytokinesis in *Trifolium pratense* L.; implication for secretory pathways. *Planta* **1988**, *174*, 433–445. [CrossRef]
36. Zhang, G.F.; Staehelin, L.A. Functional compartmentation of the golgi apparatus of plant cells: Immunocytochemical analysis of high-pressure frozen and freeze-substituted sycamore maple suspension culture cells. *Plant Physiol.* **1992**, *99*, 1070–1083. [CrossRef]
37. Richter, S.; Voss, U.; Jurgens, G. Post-Golgi traffic in plants. *Traffic* **2009**, *10*, 819–828. [CrossRef]
38. Wilkop, T.; Pattathil, S.; Ren, G.; Davis, D.; Bao, W.; Duan, D.; Peralta, A.G.; Domozych, D.S.; Hahn, M.G.; Drakakaki, G. A Hybrid Approach Enabling Large-scale Glycomic Analysis of Post-Golgi Vesicles Reveals a Transport Route for Polysaccharides. *Plant Cell* **2019**, *31*, 627–644. [CrossRef]
39. Meents, M.J.; Motani, S.; Mansfield, S.D.; Samuels, A.L. Organization of Xylan Production in the Golgi during Secondary Cell Wall Biosynthesis. *Plant Physiol.* **2019**, *181*, 527–546. [CrossRef]
40. Ruprecht, C.; Bartetzko, M.P.; Senf, D.; Dallabernadina, P.; Boos, I.; Andersen, M.C.F.; Kotake, T.; Knox, J.P.; Hahn, M.G.; Clausen, M.H.; et al. Synthetic Glycan Microarray Enables Epitope Mapping of Plant Cell Wall Glycan-Directed Antibodies. *Plant Physiol.* **2017**, *175*, 1094–1104. [CrossRef]
41. Dhonukshe, P.; Aniento, F.; Hwang, I.; Robinson, D.G.; Mravec, J.; Stierhof, Y.D.; Friml, J. Clathrin-mediated constitutive endocytosis of PIN auxin efflux carriers in Arabidopsis. *Currrent Biol.* **2007**, *17*, 520–527. [CrossRef]
42. Jiang, N.; Wiemels, R.E.; Soya, A.; Whitley, R.; Held, M.; Faik, A. Composition, Assembly, and Trafficking of a Wheat Xylan Synthase Complex. *Plant Physiol.* **2016**, *170*, 1999–2023. [CrossRef] [PubMed]