TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Review Article

Turk J Bot
(2016) 40: 561-565

doi:10.3906/bot-1603-24

Plant biomass: insights from imaging cell walls using molecular probes

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Received: 15.03.2016 • **Accepted/Published Online:** 10.06.2016 • **Final Version:** 06.12.2016

Abstract: Cell walls are an important and growing subject of research in plant biology. In the last decade, there has been an increasing interest in using cell walls as feedstock for the production of second generation biofuels. This has resulted in an expansion in the number of cell wall studies and has highlighted the need for a better understanding of cell wall structure and function. Cell walls are composed of polymers with complex and dynamic structures that vary between cell types and developmental stages as well as between taxonomic groups of plants. To address the diversity of glycans that form the cell wall, it is important to have tools that image these glycans at the cellular level. Antibodies that recognize specific cell wall components are currently one of the most effective and specific molecular probes available for determining the location and distribution of polymers in plant cell walls in situ. Here, emphasis is given to the use of cell-wall–directed antibodies in the context of bioenergy research and the development of cost-effective strategies and effective bioengineering processes to efficiently deconstruct biomass into fermentable sugars.

Key words: Antibody, biomass, biotechnology, cell wall, imaging, microscopy

1. Introduction

Plant cells have different sizes and shapes. These morphological differences contribute to the functions of a cell or a tissue and are also responsible for the richness of plant diversity. The shape and size of plant cells are largely determined by the cell wall, which is composed predominantly of complex carbohydrates. Cellulose, the major and most abundant organic molecule in the wall, is embedded in a matrix containing pectic and hemicellulosic polysaccharides. Different meshworks of matrix polysaccharides embedded in the wall play critical roles in many mechanisms that are vital for plant development and survival (O'Neill and York, 2003).

Plants synthesize primary cell walls during growth and secondary cell walls (only in certain cell types) after growth ceases (Cosgrove and Jarvis, 2012). The main components of primary cell walls are cellulose, pectic polysaccharides (homogalacturonan and rhamnogalacturonan I and II), and hemicellulosic polysaccharides (xyloglucans, arabinoxylans, and mixed-linkage glucans) with structural proteins (O'Neill and York, 2003; Keegstra, 2010). Compared to primary cell walls, in secondary cell walls, xyloglucans and pectins are mostly replaced by xylan, glucomannan, and lignins (Cosgrove and Jarvis, 2012; Kumar et al., 2016), which gives the wall tensile and compression strength. Lignin, providing the compression strength, is a phenolic complex polymer and is mainly composed of *p*-hydroxylphenyl (H), guaiacyl (G), and syringyl (S) units (Kumar et al., 2016). All these components assemble together to form a functional cell wall. Secondary cell walls are the main constituents of plant biomass for second generation biofuel production. We know the molecular structures of most cell wall glycans in some detail. However, there is still a lack of information about how these polysaccharides interact with each other and how they are modified as a plant grows and develops.

Several models describing the organization of polysaccharides in plant cell walls have been proposed (McCann and Roberts, 1991; Carpita and Gibeaut, 1993; Carpita, 1996; Cosgrove, 1997, 2016; Somerville et al., 2004). However, one important feature that is missing from these models is that they do not capture the dynamic nature of plant cell walls. Cell walls change throughout plant development and new polymers are continually incorporated into existing walls. Plants, including those that are being considered biomass feedstocks (e.g., poplar and switchgrass), are composed of cells with different wall composition and architecture. Moreover, there may be distinct domains with different glycan composition within the wall of an individual cell. Such heterogeneity is typically lost in biochemical studies designed to understand plant cell wall structure and function in detail.

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Therefore, there is a need for new tools to determine the location and distribution of individual wall components as a plant develops. Such tools will facilitate the development of effective bioengineering processes to efficiently deconstruct biomass.

2. Probes for plant cell wall glycans

Cell-wall–directed probes are tools that are increasingly used by scientists to investigate the diversity and dynamic nature of plant cell walls. These probes include low molecular-weight fluorescent molecules (Wallace and Anderson, 2012), proteins with carbohydrate-binding modules (CBMs), and monoclonal or polyclonal antibodies (Knox, 2008; Pattathil et al., 2010, 2015; Lee et al., 2011). In this review, emphasis is given to cell-wall–directed antibodies, which have become one of the most commonly used probes.

2.1. Cell-wall–directed antibodies

Both monoclonal and polyclonal antibodies that recognize specific substructures or epitopes present in cell wall matrix polysaccharides have been generated. Monoclonal antibodies (mAbs) have several advantages over polyclonal antibodies (Pattathil, 2015). MAbs are available as hybridoma culture supernatants, and their supply is not limited since the cryopreserved cell lines can be grown repeatedly. Newly produced mAbs retain the binding affinity and specificity. Another advantage of mAbs is that they are monospecific in terms of the epitope they recognize, as they are produced from a single clonal cell line, which makes them highly specific and sensitive probes.

Approximately 200 wall-directed mAbs have been produced over the years in different laboratories. MAbs that specifically recognize glycan epitopes on xyloglucan, xylan, mannan, arabinogalactan, homogalacturonan, or rhamnogalacturonan I are available (Knox, 2008; Pattathil et al., 2010). Many of these mAbs can be purchased from stock centers such as CarboSource Services, Complex Carbohydrate Research Center, University of Georgia, USA (http://www.ccrc.uga.edu/~carbosource/CSS_home.html); Biosupplies, Australia (http://www.biosupplies.com.au); PlantProbes, University of Leeds, UK (http://www.plantprobes.net); or obtained from the individual laboratories where the antibody was produced.

When interpreting the results obtained from the use of cell-wall–directed antibodies, it is important to keep in mind the following points. The oligosaccharides that decorate polysaccharide backbones often have side chains with common structural features. For example, α-L-Fuc-(1,2)-β-D-Galp exists in XyG and RG-I (Pattathil et al., 2015). Cell-wall–directed antibodies are epitope specific, and binding of a specific antibody does not necessarily pinpoint a specific polysaccharide, but rather a specific structural motif (Pattathil et al., 2015). Moreover, the absence of immunolocalization by a given mAb does not imply the absence of a cell-wall polysaccharide. The polysaccharide itself may be biochemically modified, making it inaccessible immunohistochemically (Avci et al., 2012). Therefore, it is better to use multiple antibodies against the polymer of interest. In addition, combined use of immunolocalization with high-throughput glycan profiling (Willats et al., 2002; Pattathil et al., 2015) can reveal inaccessible epitopes by making them readily available in extracted cell wall material.

3. Plant cell wall imaging by immunohistochemistry

Immunohistochemistry (also referred to as immunolocalization) is a powerful and relatively rapid technique that enables researchers to investigate the cellular and subcellular locations of antigens of interest. Currently, immunohistochemical techniques are one of the best approaches for determining the localization and distribution of cell wall glycans in situ within plant tissues (Knox, 2000; Avci et al., 2012). Interest in developing second generation biofuels has increased the need to develop a detailed understanding of plant cell wall structure and organization, as this material accounts for lignocellulosic biomass. Immunohistochemistry using mAbs provides a unique opportunity to study changes to the cell wall in the context of bioenergy research.

In one study, disrupting the expression of WRKY transcription factor (TF) genes in Arabidopsis and Medicago stems resulted in the ectopic deposition of xylan-rich secondary cell wall thickenings in pith cells, giving rise to about 50% increases in biomass density (Wang et al., 2010). Pith cells in wild-type plants normally have only primary walls, which are not labeled with xylan antibodies. The use of three xylan antibodies (CCRC-M149, CCRC-M138, and CCRC-M153) further demonstrated the accumulation of xylan in pith cells (Wang et al., 2010). The xylan-directed antibody, LM10, has been used to demonstrate that genetic engineering of xylan-deficient mutants can be used to reintroduce functional xylan biosynthesis genes specifically into xylem vessel cells (Petersen et al., 2012). This study showed that engineered plants may be used to produce more desirable biofuel feedstock with improved properties. Immunolocalization studies of rice mature culm internodes with the xylan-directed mAbs antibodies LM10 and LM11 supported data from mutant plants showing that a decrease in xylan content resulted in reduced recalcitrance and improved biomass saccharification (Chen et al., 2013).

Diverse pretreatment methods have been developed in an attempt to reduce the recalcitrance of biomass to conversion into fermentable sugar. In studies of different pretreatments, mAbs have been used to investigate

the effects of each pretreatment on the structure and composition of biomass from different plants (Brunecky et al., 2009; DeMartini et al., 2011; Holopainen-Mantila et al., 2013; Ma et al., 2015). Immunolabeling with LM10 has shown that xylan epitopes recognized by this mAb are reduced after dilute acid pretreatment of Miscanthus (*Miscanthus* × *giganteus*) biomass (Ji et al., 2015). These studies illustrate how antibodies can be used to better understand the effectiveness of pretreatment regimes and the changes to the cell wall they cause. Such information is of value to bioengineers and chemical engineers for optimizing the conditions for cost-effective conversion of biomass to fermentable sugar. To develop cost-effective pretreatment strategies and effective bioengineering processes to efficiently deconstruct biomass, it is important to know the glycan distribution and the architecture of cell walls in biomass. To this end, a set of cell-wall–directed monoclonal antibodies have been employed to show the heterogeneity in cell wall composition in stem tissues of different Miscanthus species (*Miscanthus* × *giganteus*, *M. sinensis*, and *M. sacchariflorus*) (Xue et al., 2013).

Lignin is the second most abundant natural polymer after cellulose and is one of the cell wall components that contribute to the recalcitrance of biomass. Antibodies produced against the most common types of lignin found in nature have been used to localize H-, G-, and S-type lignins in different plant species (Joselau and Ruel, 1997; Ruel et al., 2002; Kiyoto et al., 2013). There is still much to learn about the localization of lignin in the cell walls of different plant species. The increased availability of mAbs that recognize distinct epitopes of lignin has the potential to provide new insights into reducing or eliminating the recalcitrance of cell walls.

4. Diverse cell walls in biomass

All cells in plant tissues are surrounded by primary cell walls. Primary cell walls are further categorized into two groups in flowering plants (type I and type II walls) (Carpita, 1993). Type I walls are found in dicotyledonous and noncommelinoid monocotyledonous plants with about equal amounts of cellulose and xyloglucan forming a cellulose–xyloglucan framework embedded in a pectin matrix (Carpita, 1993). On the other hand, type II walls are found in only in commelinoid monocots and have much less xyloglucan and pectin than cellulose (Carpita, 1993, 1996). Apart from primary cell walls, specific cells such as vessel and tracheid cells in vascular tissue differentiate and form thickened secondary cell walls. Xylan is one of the major hemicellulosic components of these cell walls. In dicots, xylan is predominantly localized in secondary cell walls (McCartney et al., 2005; Pattathil et al., 2010). Based on biochemical analyses, the cell walls of grasses with type II walls differ from those of dicots in terms of the types and abundance of polysaccharides (Carpita, 1996; Vogel, 2008). Grass cell walls contain much less pectin and xyloglucan but more heteroxylan (Fincher, 2009) than dicot walls.

Switchgrass (*Panicum virgatum*) and poplar (*Populus* spp.) are two biomass feedstock crops. Switchgrass is a monocot with a typical type II cell wall (Carpita, 1996), whereas poplar is a woody dicot with a type I wall. An example of how different plants (switchgrass and poplar) can utilize similar polysaccharides to build cell walls that have different glycan epitope distribution patterns is shown in the Figure. Poplar and switchgrass stems were probed with three different mAbs. The epitope distributions of xylan (CCRC-M138), homogalacturonan (CCRC-M38), and xyloglucan (CCRC-M93) are very different in these two plants (Figure). Xylan epitopes are restricted to xylem cells (xy) in the poplar stem but are present in all cell walls in the switchgrass stem. The mAbs that recognize homogalacturonan and xyloglucan epitopes mainly label phloem (ph) cell walls in switchgrass stems, whereas almost all cell walls in the poplar stem are labeled. These differences in the walls of a dicot and a monocot must result from the differential expression of numerous genes that encode different enzymes involved in polysaccharide synthesis. Thus, determining which genes or transcriptional mechanisms are involved in establishing this diversity is an important research area for the near future.

5. Concluding remarks and future perspectives

Cell-wall–directed mAbs are selective molecular probes with high affinity that can be used in immunohistochemical approaches to localize cell wall glycans in situ. The main goal of sample fixation and processing in immunohistochemistry is to keep antigens in their native state as much as possible; minimal changes to structures can provide a native picture of cell wall glycans. Such information supplements and complements chemical and biochemical analyses aimed at improving the conversion of biomass to fermentable sugars. The generation of new antibodies with well-defined specificities will enable a more in-depth understanding of the dynamic nature of plant cell walls within and between plant species. There is a notable lack of mAbs and other probes against lignin, rhamnogalacturonan II, and cellulose. As there may still be unidentified or uncharacterized cell wall domains with important functions, discovering the identities and functions of these domains will be critical for a better understanding of how cell walls are constructed and how they change to enable plant growth and development.

Interlinked cell wall matrix polysaccharides and their ordered deposition show differences during plant development through cell wall remodeling and also among different plant species. Current cell wall models and data

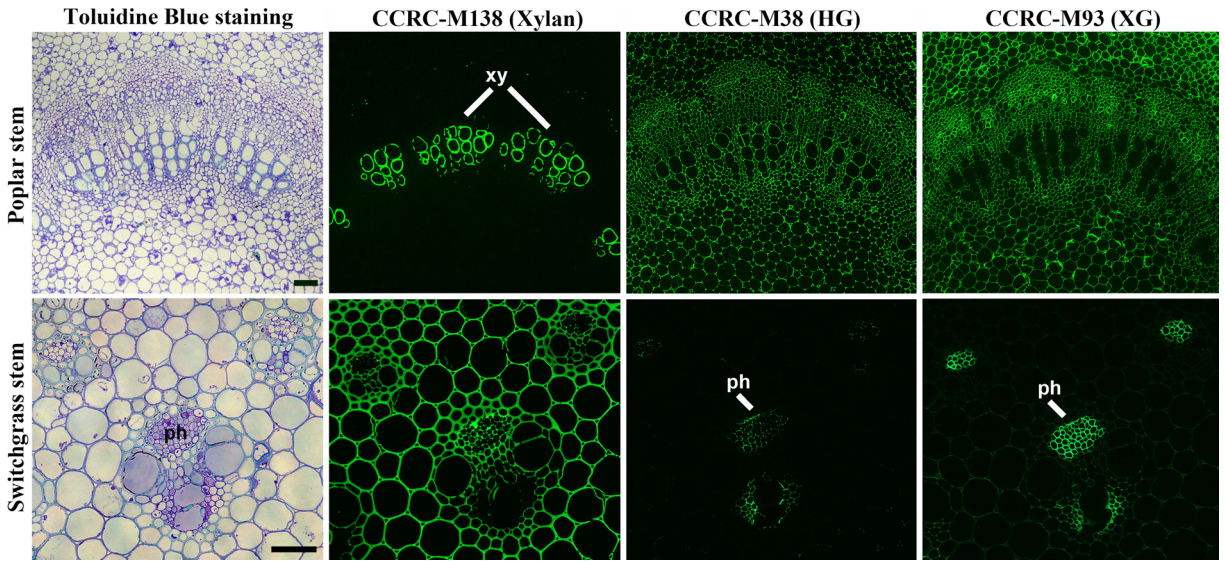

Figure. Comparison of immunolocalization patterns between poplar stem (upper images) and switchgrass stem (lower images) by CCRC-M138 (xylan), CCRC-M38 [homogalacturonan (HG)], and CCRC-M93 [xyloglucan (XG)] antibodies. Toluidine blue images show the general anatomy of stems in two plants. Bars = 50 µm and apply to all images in corresponding rows. Xylem and phloem are labeled xy and ph, respectively. Avci et al. (2012) describe detailed protocols for immunohistochemistry of cell wall glycans.

obtained from biochemical experiments cannot depict the heterogeneity of cell walls. Therefore, probes that enable us to visualize cell walls in situ are important for understanding cell wall synthesis and remodeling and will help us create better techniques or methods to break down biomass efficiently. Along with an improved set of molecular probes, newer biophysical and imaging techniques that have higher resolution and allow us more insight into the three-dimensional (3D) network within cell walls will also be important for future studies. Recently, several new microscopes and techniques have emerged to increase resolution at nanoscale, improve contrast, and allow long observation times of sensitive samples (Gonneau et al., 2012; Eggert et al., 2014). Stimulated emission depletion (STED) microscopy, photoactivated localization (PALM) microscopy, stochastic optical reconstruction (STORM) microscopy, and atomic force microscopy (AFM) are some examples of new high-resolution methods. Along with advances in sample preparation, labeling, and image processing algorithms, these microscopes will allow us to image cell walls in 3D at nanoscale and help us better understand the cell wall architecture of diverse plants and the functional relationships among their components.

Acknowledgments

The author would like to thank Malcolm O'Neill for his critical reading of the review. Funding for the author's postdoctorate research was from the BioEnergy Science Center (grant: DE-AC05-00OR22725) from the Office of Biological and Environmental Research, Office of Science, United States Department of Energy. The NSF Plant Genome Program grants DBI-0421683 and IOS-0923992 provided the funding to generate the CCRC series of plant cell wall glycan-directed monoclonal antibodies.

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