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Microanalysis of Plant Cell Wall Polysaccharides

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ABSTRACT Oligosaccharide Mass Profiling (OLIMP) allows a fast and sensitive assessment of cell wall polymer structure when coupled with Matrix Assisted Laser Desorption Ionisation Time Of Flight Mass Spectrometry (MALDI–TOF MS). The short time required for sample preparation and analysis makes possible the study of a wide range of plant organs, revealing a high degree of heterogeneity in the substitution pattern of wall polymers such as the cross-linking glycan xyloglucan and the pectic polysaccharide homogalacturonan. The high sensitivity of MALDI–TOF allows the use of small amounts of samples, thus making it possible to investigate the wall structure of single cell types when material is collected by such methods as laser micro-dissection. As an example, the analysis of the xyloglucan structure in the leaf cell types outer epidermis layer, entire epidermis cell layer, palisade mesophyll cells, and vascular bundles were investigated. OLIMP is amenable to *in situ* wall analysis, where wall polymers are analyzed on unprepared plant tissue itself without first isolating cell walls. In addition, OLIMP enables analysis of wall polymers in Golgi-enriched fractions, the location of nascent matrix polysaccharide biosynthesis, enabling separation of the processes of wall biosynthesis versus post-deposition apoplastic metabolism. These new tools will make possible a semi-quantitative analysis of the cell wall at an unprecedented level.

Key words: Carbohydrate metabolism; xyloglucan; mass spectrometry; cell expansion; cell walls; *Arabidopsis*; laser microdissection.

INTRODUCTION

Plant cells are surrounded by a cell wall that consists of complex heteroglycans, structural and catalytic proteins as well as polyphenolics (Carpita and McCann, 2000). This complex structure not only is responsible for the structural integrity of the cell, but it also represents the plant's outer barrier against the environment, thus protecting the plant against biotic and abiotic stresses. The wall is a highly dynamic entity that undergoes reorganization and metabolism during the essential processes of cell elongation and differentiation (Carpita and Gibeaut, 1993; Cosgrove, 2005).

Even though an enormous wealth of information has been obtained about the polysaccharide structures present in plant walls in general (Carpita and Gibeaut, 1993), very little is known about how the various components contribute to the diverse functions of the wall (Somerville et al., 2004). Major contributions have come from the model system *Arabidopsis thaliana*, which allowed the identification and characterization of wall mutants (Reiter et al., 1997). Moreover, the unique genetic resources available for *Arabidopsis* allowed reverse genetics approaches encompassing the identification of genes that are involved in wall biosynthesis and metabolism (Lerouxel et al., 2006), genetic manipulation of their expression levels (e.g. by obtaining insertional knockout mutants), and subsequent characterization of those mutants (Cavalier et al., 2008; Jensen et al., 2008; Madson et al., 2003). With advances in such approaches, the molecular mechanisms of wall polysaccharide biosynthesis and metabolism are emerging (Farrokhi et al., 2006). Although the structure of the walls of *Arabidopsis* resembles that of many other dicots (Zablackis et al., 1995), one disadvantage of using *Arabidopsis* as a wall model system is its size and thus the small amount of rather heterogenous plant material. *Arabidopsis*, like other higher plants, consists of up to 40 functionally different cell types, most of which are identifiable by their wall structure (Carpita and McCann, 2000). Even a monosaccharide compositional analysis of various plant organs demonstrates the overall

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variance in wall composition (Richmond and Somerville, 2001). Such crude analyses as these demonstrate that techniques are urgently needed to analyze wall structure in detail in a cellular rather than organ or whole-plant context.

One technique that enables us to look at wall structure on a cellular level is the labeling of tissue sections with polysaccharide-specific antibodies (Willats et al., 2000). Unfortunately, few antibodies towards defined wall epitopes are available to date. Moreover, the failure to detect an epitope by an antibody does not indicate an absence of the epitope, as it could also be masked by further substituents, polymer aggregation, or conformational changes.

Another technique that can be used for elucidating changes in cell wall architecture is FTIR microscopy, but assigning observed changes to a specific polymer or linkage type remains elusive (McCann et al., 2001).

Here, we describe the optimization and use of a cell wall analytical technique, oligosaccharide mass profiling (OLIMP, Lerouxel et al., 2002; Obel et al., 2006), which allows wall analysis even at cell type and organelle levels, or on plant tissue itself. We focus on the analysis of xyloglucan (XyG), the dominant cross-linking glycan in *Arabidopsis*, whose metabolism is thought to play a major role in cell elongation (Pauly et al., 2001). XyG sidechain structures can be quite diverse and are typically described using a single-letter nomenclature to represent the substitution pattern of each backbone glucosyl residue (Fry et al., 1993). For XyG occurring in *Arabidopsis*, the letters **G** and **X** denote an unbranched Glc*p* residue and α--DXyl*p*-(1→6)-β-D-Glc*p*, respectively. The xylosyl residue may be substituted with a β-D-Gal*p* (**L** sidechain), which is often further substituted with an α-L-Fuc*p* residue to form the **F** sidechain. The Galp residue can also be O-acetylated indicated by underlining the one-code-letter (Hoffman et al., 2005). However, the precise distribution and modification of the sidechains in XyG is not known, presenting a major impediment to understanding the XyG structure and function during plant cell development.

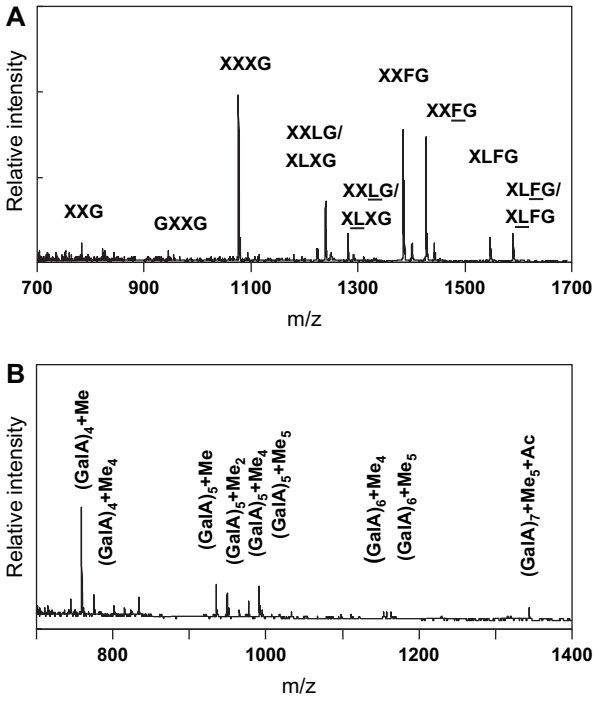

Figure 1. OLIMP Spectra of Wall Material Derived from 8-Week-Old Rosette Leaves.

(A) Spectrum of solubilized XyG oligosaccharides (XyGOs) facilitating a xyloglucanase (XEG). The structures of the various ion signals are shown using the nomenclature described by Fry et al. (1993). Underlined structures represent O-acetylated sidechains.

(B) Spectrum of solubilized oligogalacturonides using a combination of endopolygalacturonase (endoPG) and pectinmethylesterase (PME). Putative structures are shown. GalA, galacturonic acid; Me, methylester; Ac, O-acetyl substituent.

RESULTS

Polysaccharide Analysis of Plant Organs

OLIMP takes advantage of the specificity of glycosylhydrolases to solubilize specific polysaccharides from wall materials in the form of oligosaccharides, which are then analyzed by mass spectrometry (MS) (Lerouxel et al., 2002). OLIMP has been successfully performed on cell wall material for the analysis of XyG and the pectic polysaccharide homogalacturonan (HG; Obel et al., 2006). For XyG analysis, cell wall material is digested with xyloglucan-specific endo-glucanase (XEG), resulting in a detailed XyG oligosaccharide (XyGO) composition of the tissue including detailed sidechain distribution patterns (Figure 1A). When the pectins present in wall materials are digested with polygalacturonase (endoPG) together with pectin methylesterase (PME), OLIMP profiles can be generated for homogalacturonan (HG), the major component of the pectic polysaccharides (Figure 1B). The analysis results in a profile that includes the occurrence and distribution of the remaining methylesters and/or O-acetyl-esters. The peak areas of individual ion signals of the spectrum can be used to calculate the relative abundance of each structure.

OLIMP on XyG was generated from tissue material derived from 10 different plant organs harvested, whereas pectin spectra could be obtained only from rosette leaves, various stem segments, petioles, and cauline leaves. The spectra looked qualitatively as shown in Figure 1—namely the same ions were observed—but the spectra varied in the quantities of the various ions. To highlight differences, we performed a principal component analysis (PCA) using the mean abundance of the various XyGOs and HG oligosaccharides (HGOs) generated from individual growth experiments (Figure 2). For XyG, principal components 1 and 2 represented 93.7% of the total variance. The loading indicated that component 1 represented mainly an abundance of the structures XXXG and XXFG, whereas component 2 represented mainly O-acetylated XXFG. The greatest variation in XyGO composition was observed between trichomes and the upper part of the stem, but there was