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Review

Improving the utilization of lignocellulosic biomass by polysaccharide modification

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ABSTRACT

Lignocellulosic biomass offers an economically sustainable renewable source of chemicals and fuels, but the development of efficient processes for lignocellulose utilization is challenging due to the complex and heterogeneous structure of plant cell walls. Transgenic plants expressing recombinant proteins that promote cell wall modification or deconstruction could facilitate the digestion of lignocellulose, and enzymes that facilitate the degradation of polysaccharides in particular could improve the industrial production of fibres, textiles, pulp, paper, chemicals and fuels. In this review we summarize what is known about plant cell wall architecture and discuss current strategies to improve plants by expressing recombinant proteins that facilitate the degradation of cell wall carbohydrates. We compare the ability of these different approaches to simplify the processing of lignocellulose and showcase particularly comprehensive approaches and the future perspectives of these technologies.

Contents

Abbreviations: AF, arabinofuranosidase; AGA, apiogalacturonan; CBH, cellobiohydrolase; CBM, carbohydrate-binding motif; CDH, cellulose dehydrogenase; CWDE, cell wall deconstructing enzyme; D-Dha, 3-deoxy-D-lyxo-2-heptulosaric acid; EG, endoglucanase; FAE, ferulic acid esterase; GAX, glucuronoarabinoxylan; GGM, galactoglucomannan; GH, glycosylhydrolase; GT, glycosyltransferase; HG, homogalacturonan; Kdo, 3-deoxy-D-manno-2-octulosonic acid; PAE, pectin acetylesterase; PG, polygalacturonase; PME, pectin methylesterase; RG-I, rhamnogalacturonan I; RG-II, rhamnogalacturonan II; RGL, rhamnogalacuronan lyase; UDP, uridine diphosphate; XEG, xyloglucan-specific endoglucanase; XET, xyloglucan endotransglucosylase; XGA, xylogalacturonan; XyG, xyloglucan.

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1. Introduction

Lignocellulose is an abundant and sustainable source of basic chemicals and energy carriers such as transportation fuels, but the development of economically feasible processes for the utilization of lignocellulose is challenging. Plants evolved the ability to synthesize lignocellulose as a means to resist abiotic and biotic stress, allowing the cell wall to be strengthened against pathogens while maintaining its dynamic architecture. This dual function is made possible by the heterogeneous composition of lignocellulose, which features a variety of chemical linkages within and between different polymers [1]. This structural and chemical complexity is the basis of biomass recalcitrance defined as collective resistance of plants to degradation by microbes and their enzymes [2] which subsequently increases the energy requirements, the cost and complexity of biorefinery operations, and/or reduces the recovery of biomass carbon into desired products [3]. This recalcitrance has to be overcome in order to develop an economically feasible conversion process [4]. Currently, efficient lignocellulose utilization requires biomass pre-treatment and fractionation steps which enhance subsequent processing without generating toxic by-products [5] as well as the cost-effective production of enzymes that deconstruct the complex and heterogeneous chemical linkages [6]. A greater understanding of plant cell wall architecture is necessary for the development of advanced processes for the fractionation and deconstruction of lignocellulose.

One promising way to overcome the recalcitrance of the plant cell wall is genetic engineering, which has already been used to modify the properties of crops [7–9]. Most strategies to improve biomass processing aim to increase the accessibility of specific cell wall polysaccharides [10]. This goal can be achieved by the genetic modification of lignin biosynthesis, which has been discussed elsewhere [10–12]. Furthermore, the modification of polysaccharides by the expression of carbohydrate active proteins (Fig. 1) has been shown to increase the yield, growth rate and porosity to improve biomass processability, even though the exact role of these proteins and their impact on cell wall biosynthesis is not yet fully understood [9,13]. The heterologous expression of cell wall deconstructing enzymes (CWDEs) can also increase the accessibility of cell walls during subsequent lignocellulose hydrolysis steps [7,8,14]. In both cases, the expression of CWDEs in crops is an emerging technology and further studies are needed to evaluate its full potential.

The following sections summarize our current understanding of the structure and composition of plant cell wall polysaccharides, discuss the *in vivo* and *post-harvest* modification strategies used in transgenic plants to achieve the cost-effective utilization of lignocellulose [15] and report on progress in the development of sustainable processes using transgenic crops for lignocellulose utilization.

2. Plant cell wall polysaccharides: structure and composition

The plant cell wall comprises two main groups of carbon-based polymers: lignin and the cell wall polysaccharides. The chemical composition and structure of lignocellulose must be understood to achieve targeted modification. Lignin is a heterogeneous and aromatic polymer composed of the phenylpropanoid building blocks paracoumaryl alcohol, coniferyl alcohol and sinapyl alcohol, which form a complex network with diverse chemical bonds [16,17]. Cell wall polysaccharides are assigned to two subgroups [18]. The stereo-regular homopolymer cellulose forms the micro fibrillary phase of the cell wall, whereas the stereo-irregular polysaccharides form a network associated with these micro fibrils, comprising structurally and compositionally distinct polysaccharides often described as hemicellulose and pectin [19]. These polymers are located in the matrix phase between the cellulose fibres where they cover the fibrils and may also be required to connect them to lignin in the secondary cell wall [20].

Pectic polysaccharides are complex and heterogeneous structures [21] that are most abundant in the primary cell walls of dicots and non-graminaceous monocot plant species. Some fruits, such as citrus and apples, also have a high content of pectic polysaccharides [21,22]. The pectic polysaccharides are assigned to three major classes: homogalacturonan (HG), rhamnogalacturonan I (RG-I) and rhamnogalacturonan II (RG-II). In some plants, there are additional substituted galacturonans such as apiogalacturonan (AGA) and xylogalacturonan (XGA) [21]. HG is the most abundant pectic polysaccharide and comprises α-1,4-linked galacturonic acid. It is often partially methyl esterified, but other cross-linking has been reported [22]. The RG-I backbone contains a disaccharide repeat of galacturonic acid linked to rhamnose which is abundantly decorated with other sugars and oligosaccharides [22]. RG-II is the most complex pectic polymer and has long been described as structurally highly conserved over plant evolution [19]. But recently, structural variations within the polysaccharides have been reported in *Vitis vinifera* [23]. RG-II is composed of 12 different sugars connected by more than 20 different types of linkages. It plays a key role in the growth and development in most plant species [21].

Pectins can be highly cross-linked. For example, RG-II in primary cell walls exists as a borate di-ester dimer [24] and non-esterified HG can interact via cross-linking calcium-ion salt bridges in the cell wall [25,26]. The biosynthesis of these heterogeneous polysaccharides takes place in the cisterna of the Golgi apparatus. The polysaccharides are synthesized from activated nucleotide sugars by glycosyltransferases (GTs) that produce a molecular backbone which subsequently can be decorated with other side chain sugars [1,27]. These precursors can be trimmed by glycoside hydrolases (GHs) before transfer to the apoplast and incorporation into the cell wall takes place [28,29].

Hemicelluloses can be categorized according to their backbone components [1,19]. The primary walls of spermatophytes (except for grasses) contain xyloglucan (XyG) as the most abundant hemicellulose, which comprises a β-1,4-linked glucan backbone decorated with xylosyl residues. In most species, these xylosyl groups are substituted further with other sugar residues forming a strong but extensible network cross-linking the cellulose micro fibrils [30]. XyG can also be covalently linked to pectin and lignin [20]. Xylans are the major form of hemicellulose found in the secondary cell walls of dicots and all cell walls in commelinid monocots including grasses [1,19]. Xylans are the major non-cellulosic polysaccharide in the primary walls of grasses, and they contain many arabinose residues attached to the xylose backbone. Therefore, they are named arabinoxylans and glucuronoarabinoxylans (GAXs). An important feature of GAX is the attachment of ferulic acid ester (FAE) to some of the arabinofuranosyl residues. FAE can cross-link in many different ways, *e.g.* they can convey the linkage between lignin and GAX which plays an important role in biomass recalcitrance [31]. Mannans or glucomannans are highly abundant in early land plants and are important storage polymers [32,33]. In Arabidopsis seeds, they help to determine the properties of mucilage [34]. Primary cell walls also contain mixed-linkage glucans that play a role in cell expansion and their abundance depends on the developmental stage of the plant [15,35].

Cellulose, the micro fibrillary phase, is usually the most abundant component of plant cell walls [36]. It consists of long unbranched chains of β-glucopyranose linked via β-1,4 glycosidic bonds. The sugar chains form a flat ribbon-like conformation which allows them to be packed into micro fibrils stabilized by hydrogen bonds and van der Waals interactions [18]. Native cellulose in living plants varies in structure from amorphous to crystalline depending

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