The Development of Better Biomimetic Substrates to Study Xylan-modifying Enzymes

Background

- Investigation of biocatalysts that synthesize carbohydrates in plant biomass has been hampered by a lack of pure substrates that closely represent structural features of the glycans under investigation. Xylan is the second most abundant biopolymer in Nature and is a key CBI valorization target.

Approach

- Here, chemo-enzymatic synthesis using glycosynthases was used to generate defined xylan oligosaccharides up to 12 monosaccharide units long. This is double the size of acceptor substrates that have previously used, and more closely mimic the xylan substrates found *in planta*. We used these xylan substrates to validate the activity of the enzymes involved in xylan sidechain substitution: xylan acetyltransferase (XOAT1), xylan α-1,3-arabinbofuranosyl-transferase (XAT3) enzymes from rice and switchgrass, and xylan α-1,2 glucuronosyltransferase (GUX3). The products were confirmed with MALDI-MS.

Results

- Our results suggest that GUX3 requires five unsubstituted xyloses adjacent to both sides of the glycosylation site to install a single GlcA residue, converting almost all of the acceptor to the glucuronodated form. This is in contrast to the activity of the XOAT and XATs, which added more than one substituent to the xylo-oligomer. This is the first time that the *in vitro* glycosylation pattern of GUX3 has been characterized and shown to be in agreement with the pattern proposed to be produced *in vivo*, suggesting that we have identified a more biosimilar substrate to study enzymes involved in xylan substitution.

Significance

- The amount and pattern of xylan substituents determines its conformation in the plant cell wall, influencing wall architecture at multiple length scales. Long xylan fragments were successfully used as acceptor substrates in assays with both glycosyl- and acetyltransferases, demonstrating the importance of the substrate's chain length in obtaining realistic data relating to sidechain patterning during synthesis.

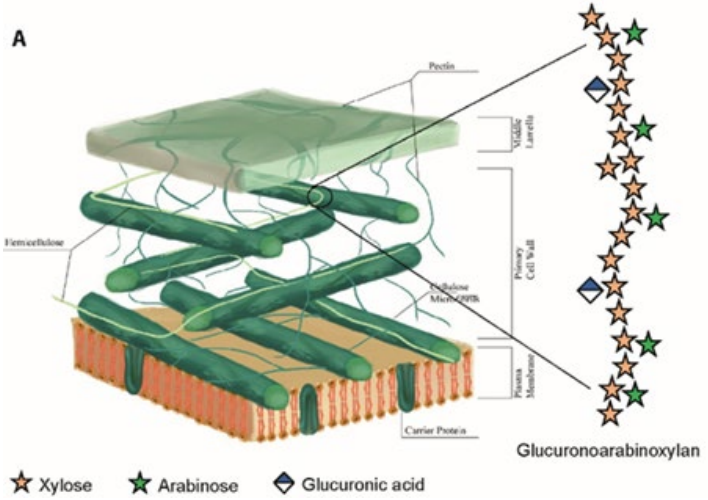

Figure 1. Schematic representation of a plant cell wall - highlighting the structure of the hemicellulose -- xylan.

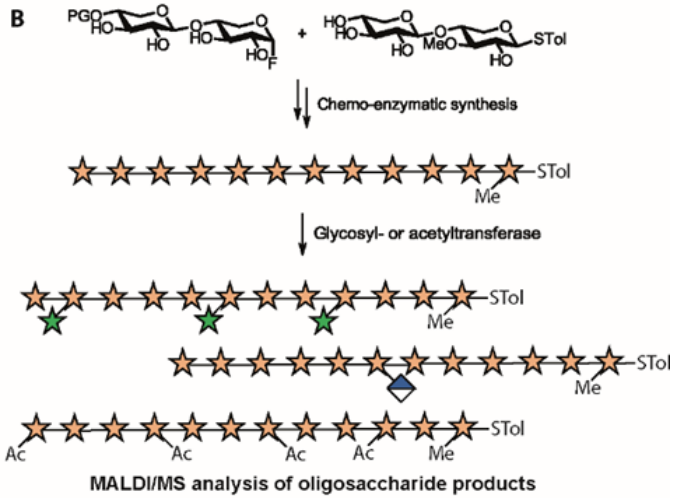

Figure 2. Overview of the chemo-enzymatic synthesis of long-chain xylan fragments for use as acceptor substrates. Structures of reaction products generated by glycosyl- and acetyl-transferases are cartoon representations and the distribution of substituent.

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