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Carbohydrate microarrays for measuring cell wall polysaccharides in relation to biomass conversion

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INTRODUCTION

Leaf to stem ratio

Wheat straw biomass (*Triticum spp.*) has the potential to be a sustainable resource for 2nd generation biofuel production. The wheat straw can be divided into two major anatomical fractions – leaves and stems, which account for 20-40% and 60-80% of the raw material respectively (Åman & Nordkvist,1983). Enzymatic convertibility of wheat straw biomass is linked to the overall chemical composition of the biomass, but also to the various anatomical fractions it consists of. The leaf fraction is more convertible than the stem material, but the leaf to stem ratio changes with the species, harvest and collection methods, which is why being able to measure the leaf to stem ratio in a high-throughput fashion is important (Zhang *et al.*, 2012, Lindedam *et al*., 2012).

High-throughput analysis wheat straw biomass

For this study the high-throughput technique Comprehensive Microarray Polymer Profiling (CoMPP) has been used to analyze the wheat straw material (Moller *et al.* 2007). In the Poaceae (grass) family, pectin is primarily found in the leaf fraction making it a good indicator of the leaf to stem ratio. Combining the CoMPP technique with pectin specific monoclonal antibodies (mAbs) we have developed a method that allows us to determine the leaf to stem ratio in wheat straw biomass by measuring the levels of pectin and various hemicelluloses.

MATERIALS AND METHODS

Poaceae polysaccharide mixture

A stock solution of the most abundant Poaceae polysaccharides was made and various amounts of pectin were added in order to establish the sensitivity of the pectin specific monoclonal antibodies (mAbs).

	Polymer	Specification	Ratio
Stock solution	Cellulose	Hydroxymethyl cellulose	3:5
	Mixed-linked glucans	β-D-glucan, barley	1:5
	(Glucorono)-arabinoxylans	Wheat arabinoxylan	1:5
Added	Citrus pectin	F43, Danisco	varies

CoMPP

The freshly harvested plant material was divided into leaf and stem fractions and homogenized using an automated plant material grinding and dispensing system (Marvin) manufactured by Labman Automation Ltd., UK.

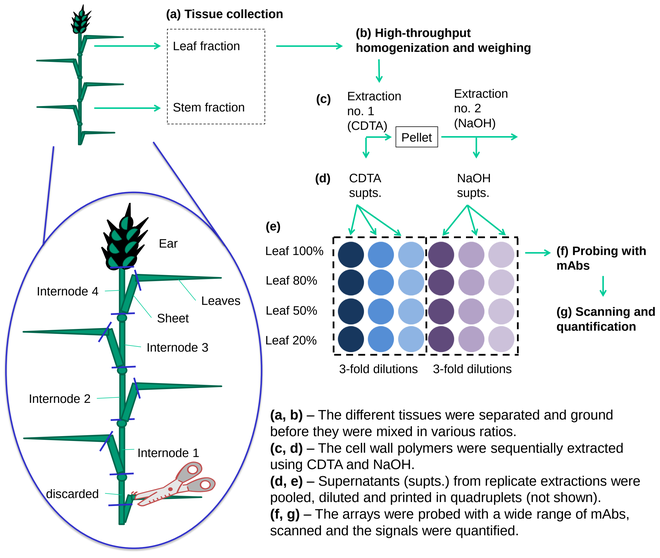
- Leaf fraction = leaf blade and sheet
- Stem fraction = internodes 1, 2, and 3
- Whole plant = ear, leaf blades and sheets, internodes 1, 2, 3 and 4

Four replicates of each sample were prepared for extractions.

- **Pectic polymers** were extracted with 50mM Trans-1,2 -diaminocyclohexane-N,N,N',N'-tetraacetic acid (CDTA)
- **Non-cellulosic polysaccharides** were extracted with 4M NaOH

Microarrays and antibodies

The extracted samples were printed on a nitrocellulose membrane (0.45 µm) using an Arrayjet Sprint (Arrayjet Ltd., UK) robotic printer, and developed using a wide range of mAbs in accordance to the procedures described by Moller *et al*. (2008).



(a, b) – The different tissues were separated and ground before they were mixed in various ratios.
(c, d) – The cell wall polymers were sequentially extracted using CDTA and NaOH.
(d, e) – Supernatants (supts.) from replicate extractions were pooled, diluted and printed in quadruplets (not shown).
(f, g) – The arrays were probed with a wide range of mAbs, scanned and the signals were quantified.

Scanning and quantification

The microarrays were scanned and imported into Photostudie 5.5 at a resolution of 1200dpi. The signals were quantified using ImaGene 6.0 microarray analysis software (BioDiscovery, USA) and averages were calculated for each sample before the dataset was normalized with a 5% cut off.

RESULTS

Pectin specific monoclonal antibodies

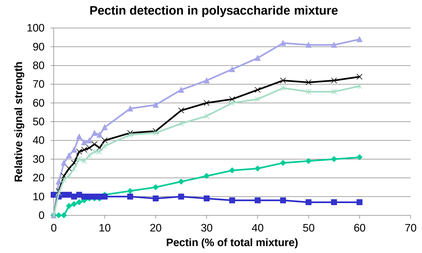
Pectin specific monoclonal antibodies (mAbs) could detect very subtle changes in pectin concentrations ranging from 0% to 60% in a Poaceae polysaccharide mixture. The lowest pectin concentration detected was 0.5% making the mAbs very suitable for the analysis of wheat straw material.

Leaf to stem ratio

Using the CoMPP technique pectin was also detected in wheat straw biomass containing 10% leaf material. Especially the mAb LM19 showed a good ability to detect changes in the leaf to stem ratio. Data obtained from whole plant samples supported the leaf to stem ratios found by Åman & Nordkvist (1983).

A comparison of the pectin signals from the Poaceae polysaccharide mixtures and the wheat straw CoMPP mixtures showed a change in the binding of mAbs LM18 and JIM7. This indicates a structural difference between the wheat straw pectin and the commercially available pectin (citrus origin). A blind testing of leaf to stem mixtures was also carried out with promising results (data not shown).

A



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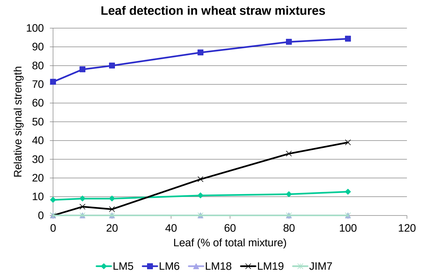


C

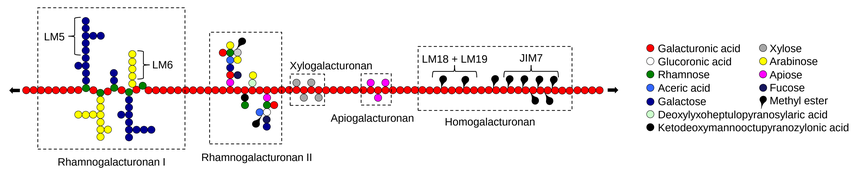
Sample	JIM5	JIM7	LM5	LM6	LM11	LM12	LM19	CBM3a	BS-400-2	BS-400-3	BS-400-4
Whole plant (CDTA)	0	0	0	8	0	0	3	0	0	0	0
Whole plant (NaOH)	0	0	6	40	81	0	2	33	0	84	15

Changes in relative signal strength for the different mAbs are shown in respect to the pectin **(A)** and leaf content **(B)**. The polysaccharide composition of whole plant samples are shown in the heatmap **(C)** . The highlighted cells are marking the mAbs also seen in A and B.
(D) The most relevant pectin mAbs and their specificity are shown along with a schematic drawing of the major domains found in pectins **(E)**.

D

Antibody	Specificity
LM5	(1→4)-β-D-galactan
LM6	(1→5)-α-L-arabinan
LM11	(1→4)-β-D-xylan/arabinoxylan
LM12	Feruloylated polymers
LM18	Partially methylesterified homogalacturonan
LM19	Partially methylesterified homogalacturonan
JIM7	Highly esterified homogalacturonan
BS-400-2	(1→4)-β-D-glucan

E



CONCLUSION

Can monoclonal antibodies detect subtle changes in polysaccharide composition of wheat straw material? → **YES** → Detection level of pectin by mAbs was 0.5% in the Poaceae polysaccharide mixture.

Can the leaf to stem ratio of wheat straw biomass be found using the CoMPP technique? → **YES** → LM19 was the most suitable mAb to detect changes in the leaf to stem ratio of wheat straw biomass.

Future prospects

- Optimizing the method
- Screen pre-treated wheat straw biomass
- Screen other wheat varieties
- Screen other types of biomass e.g. corn stover
- Use the microarrays for enzymatic screenings
- Validate the technique by other methods

References
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