



SUNLIBB

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Deliverable 3.2

“Detailed characterisation of maize matrix polysaccharides”

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Workpackage: **3**

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SUNLIBB deliverables

Del No: D3.2	Deliverable Name: Detailed characterisation of maize matrix polysaccharides.			
WP3	Lead partner: P9 UCAM	Dissemination level: PU	Delivery date : Project month 18	Actual delivery date: Project month 18

Objective:

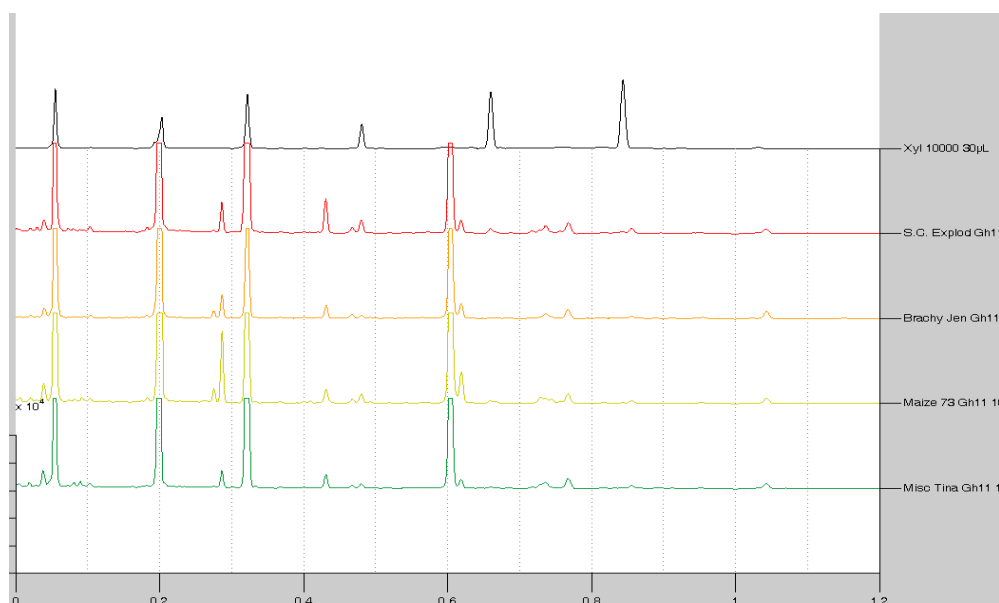
The objective of this deliverable was to provide the structural composition of the xylan present in maize stems, since xylan is the main matrix polysaccharide.

To achieve this, we extracted xylan using a cell wall preparation protocol allowing isolation of Alcohol Insoluble Residues (AIR). This AIR was treated with 4M NaOH, before being digested with various xylanases and other types of enzymes combinations. This digestate was then analysed using a High-Throughput method (DASH) (Li *et al.*, 2013). This gave us a fingerprint of the oligosaccharides released by the enzymes. Meanwhile, the oligosaccharides produced were processed by an HILIC method, coupled off-line to a MALDI-ToF-MS, in order to separate and identify structural isomers.

Results:

We have characterised by DASH several grasses: maize, Miscanthus, Brachypodium and sugar cane. Different digests by xylanases GH10 and GH11 of the stem cell wall all gave a similar pattern for all grasses, indicating some structural similarities (Fig1 and Fig2).

Fig 1: DASH analysis of Miscanthus, maize, Brachypodium and sugar cane after GH11 digest.



The main difference observable between grass species is the relative intensity of the oligosaccharide peaks, which means that the oligosaccharides “blocs” released by the xylanase digests are all present in those for grasses, but in variable amount. The polysaccharide matrix of those grasses is therefore similar, and the structural identifications are transposable from one to the other.

As the DASH fingerprinting is similar for maize and Miscanthus, we know that the xylan matrix is similar. All the oligosaccharides identified in Miscanthus are similar in maize. We have characterised all the characterisable oligosaccharides released by GH10 and GH11 (Table 1 and D3.3).

Fig2: DASH analysis of Miscanthus, maize, Brachypodium and sugar cane after GH10 digest.

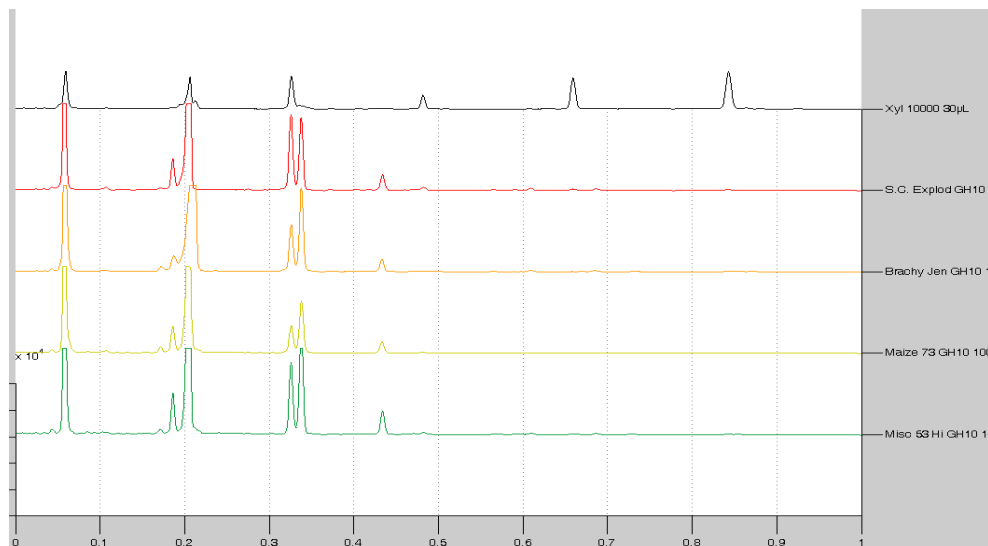
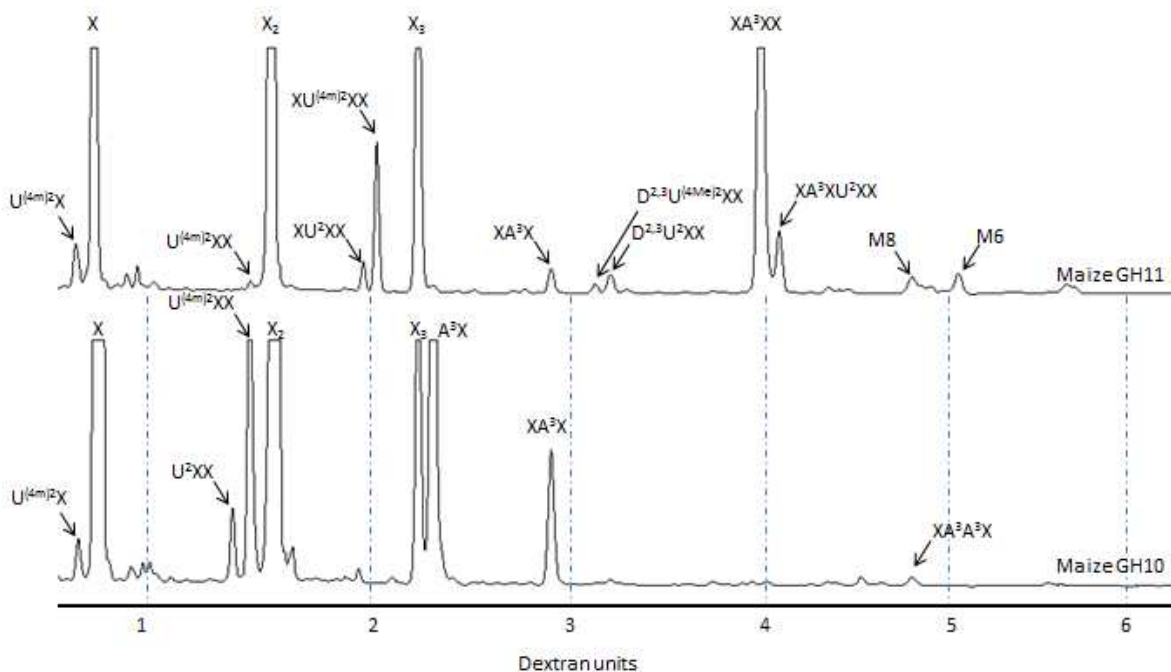


Table 1: Maize GH10 and GH11 oligosaccharide products. Single letter code according to Faure *et al.* 2009.

	GH10	GH11
Oligosaccharide name	Dextran position	Dextran position
U ² X	N/A	
U ^{(4m)2} X	0.79	0.79
X	0.87	0.87
U ² XX	1.44	
U ^{(4m)2} XX	1.51	1.51
X ₂	1.6	1.6
XU ² XX		2.03
XU ^{(4m)2} XX		2.07
X ₃	2.24	2.24
A ³ X	2.31	
A ³ U ^{(4m)2} XX		2.75
XA ³ X	2.89	2.89
XA ³ U ^{(4m)2} XX		3.11
D ²⁻³ X	3.15	
D ²⁻³ U ² XX		3.19
A ³ A ³ X	3.73	
A ³ XXX	3.89	
XA ³ XX		3.96
D ²⁻³ XX	4.01	
XA ³ XU ² XX		4.05
XA ³ A ³ X	4.52	

We annotated the DASH traces produced by Maize AIR digested by GH10 and GH11 xylanases using the analogy with Miscanthus identified oligosaccharides.

Fig 3: Maize GH10 and GH11 oligosaccharide products



The oligosaccharides of the xylan maize matrix are the same as in Miscanthus; only their relative abundance varies.

Discussion /Conclusion:

The goal of characterising the detailed composition of the xylan polysaccharide matrix in maize is now achieved. We know the elementary blocs composing the xylan released by those xylanases.

We intend to address the layout of those blocs. For example, are the different structures clustered in the polysaccharide? Is there any regular pattern? We also need to investigate to what extent the digested products are representative of the whole xylan matrix.

References

Faure, R. *et al.* (2009) A brief and informationally rich naming system for oligosaccharide motifs of heteroxylans found in plant cell walls. *Australian J. of Chem.* 62(6):533-7.

Li X, Jackson P, Rubtsov DV, Faria-Blanc N, Mortimer JC, Turner SR, Krogh KB, Johansen KS, Dupree P. (2013) Development and application of a high throughput carbohydrate profiling technique for analyzing plant cell wall polysaccharides and carbohydrate active enzymes. *Biotechnol Biofuels.* 6:94.