SUNLIBB

Sustainable Liquid Biofuels from Biomass Biorefining

Grant Agreement no. 251132

Collaborative Project
EU 7th Framework Programme
ENERGY

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Deliverable 3.6

"Five maize or Miscanthus mutant lines with altered cell wall polysaccharides characterised"

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Workpackage: **3**
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Dissemination Level: **PU**

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SUNLIBB deliverables

D3.6	Deliverable Name: Five maize or Miscanthus mutant lines with altered cell wall polysaccharides characterised			
WP3	**Lead partner:** P9	**Dissemination level:** PU	**Delivery date:** Month 48	**Actual delivery date:** Month 50

Objective:

Hemicellulose is one of the main components of the matrix surrounding the cellulose; it is one of the main factors limiting saccharification. Xylan is the main hemicellulose in grasses. Therefore finding grasses mutants with altered xylan composition could lead to the improvement of saccharification yield. The aim was to identify xylan synthesis mutants in maize, with altered patterns of substitution and/or substitution quantity leading to improved saccharification.

Results:

P4 (Biogemma) has generated and provided maize transposon mutants in the selected gene family (figure 1).

Gene name	Screening Season	Expected BC2S1	FST location
GT61_Group2-6	2009	April 2012	promoter
GT47 like 1	2010	April 2013	first exon
GT47 like 2	2010	April 2013	first intron
GT47 like 7	2010	April 2013	first intron
GT43 like 1	2010	April 2013	first intron
GT61_1_la	2011	April2014	3rd and last exon
GT61_2_8	2011	April2014	first intron
GT61_2_8a	2011	April 2014	2nd exon
GT61_2_l0a	2011	April 2014	3rd exon
GT61_2_9	2011	April 2014	promoter
GT61_3_12	2011	April 2014	first exon?
GT61_3_12a	2011	April2014	exon

Figure 1 Maize mutants generated

The candidate families of genes targeted are the GT43, GT47 and GT61 families. The GT43 and GT47 families are involved in the synthesis of the xylan backbone and the GT61 family has recently been characterised by P9 as adding the arabino-furanose residues onto the xylan backbone (D3.5) (Anders *et al*., 2012). The maize transposon mutants, in the selected genes, have been backcrossed two or three times and selfed once. Internode WT and mutant siblings were sampled, sent to P1 for milling and saccharification assays and then sent to P9 for hemicellulose analysis. Where the mutants showed a changed phenotype they were sent to P6 for NIRS and lignin analysis.

In 2012 one GT61 mutant was sent for analysis. In 2013 one GT43 and three GT47 mutants were sent for analysis. The last batch of seven GT61 mutants was dispatched to P1 during August 2014, was milled and received by P9 in September 2014, just before the completion of SUNLIBB.

Until now, none of the saccharification analyses carried by P1 revealed a differential between the twelve mutants (figures 2.1, 2.2 & 2.3). This suggests there are no major changes in structure of the walls in the cell wall mutants that impact on saccharification.

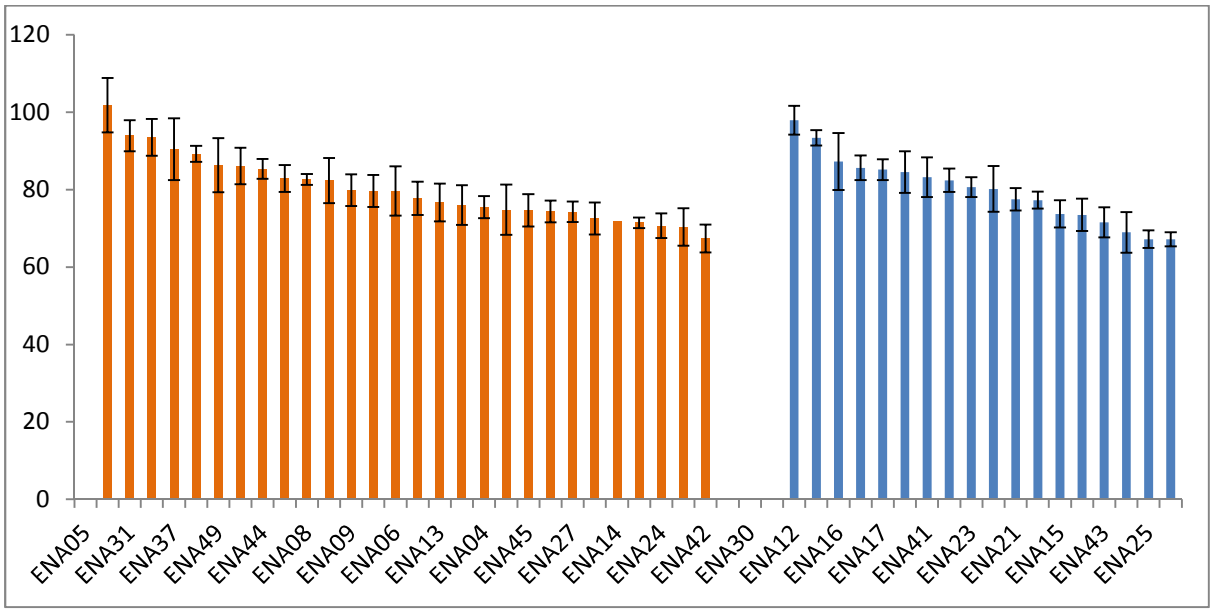

Figure 2.1 GT61-2.6 Internode A saccharification

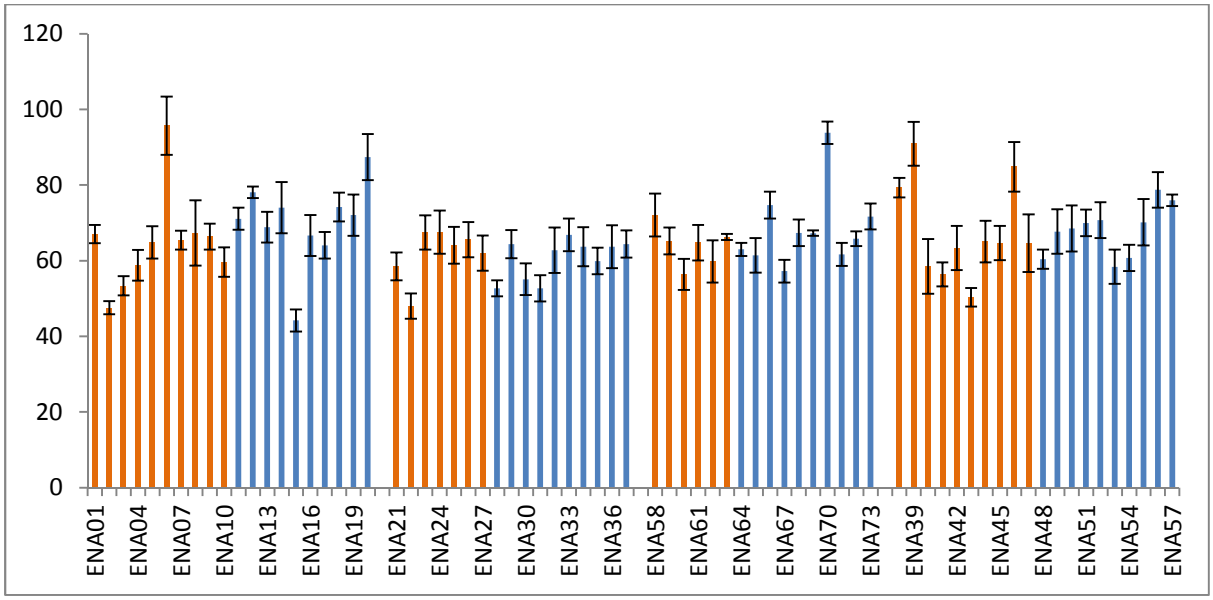

Figure 2.2 GT 43/47 Internode A saccharification

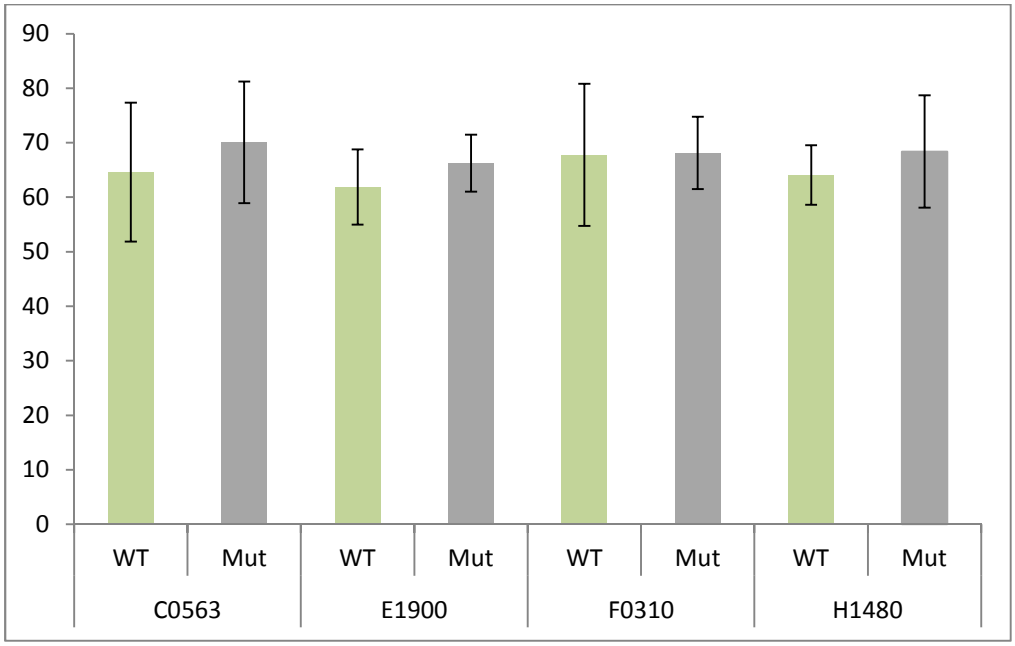

Figure 2.3 Maize GT61-2.6 mutant digested with GH11

P9 carried an analysis of the hemicellulose using the DASH technique (DNA sequencer-Assisted Saccharide analysis in High throughput). This allowed generation of a profile of the oligosaccharides released by a xylanase enzymatic digest (figure 3).

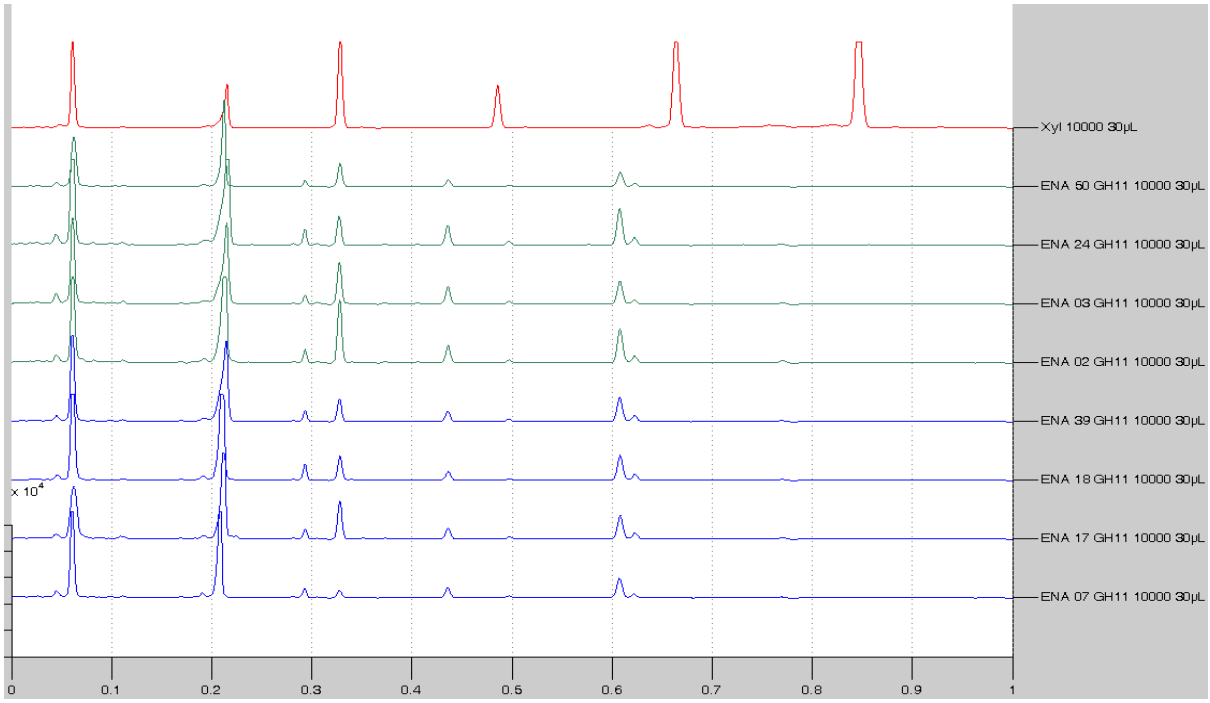

Figure 3 Maize GT61-2.6 mutant digested with GH11

The peaks could be identified as a result of progress made in D3.2 and D3.3, and quantified (figures 4.1 & 4.2). The peaks containing some arabinose substitution(s) were quantified for the GT61 mutants, and any modification of the pattern is assessed for the GT43 and GT47 mutants. This analysis did not reveal changes between the mutants and wild type analysed.

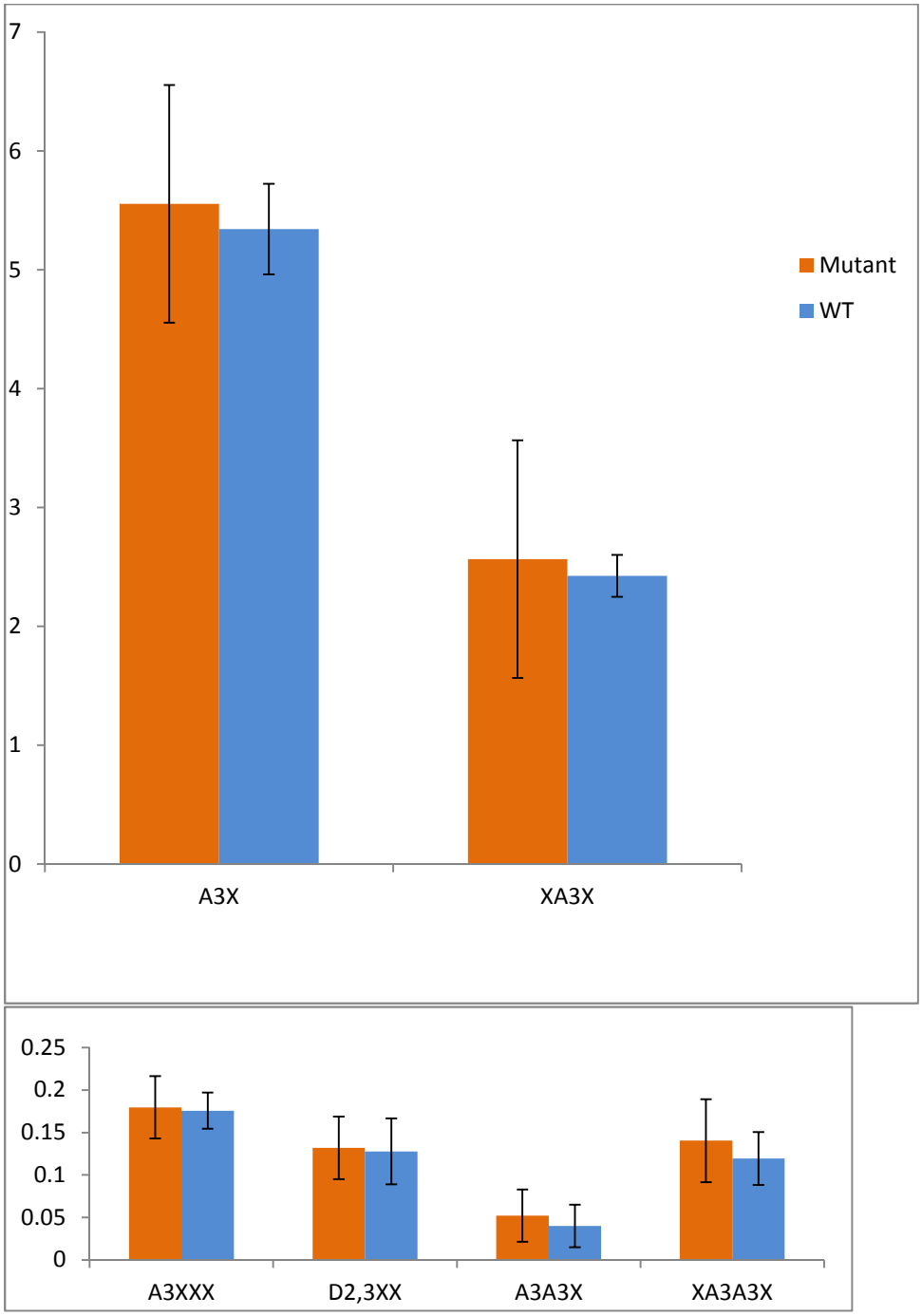

Figure 4.1 Quantification of peaks containing arabinose in the GT61-2.6 maize mutant. Inividual peak area quantification shows no difference between wild-type and mutant. (Vertical axis shows peak area % compared to total peak area)

Figures 4.2 Quantification of arabinose containing peaks different in the GT43 and GT47 maize mutants. Inividual peak area quantification showed no difference between wild-type and mutant.

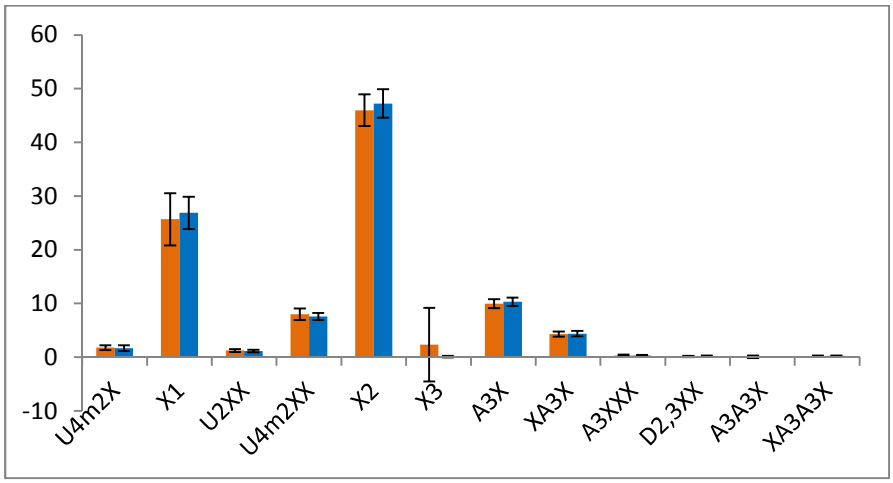

Figure 4.2 (a) GT47-C0563

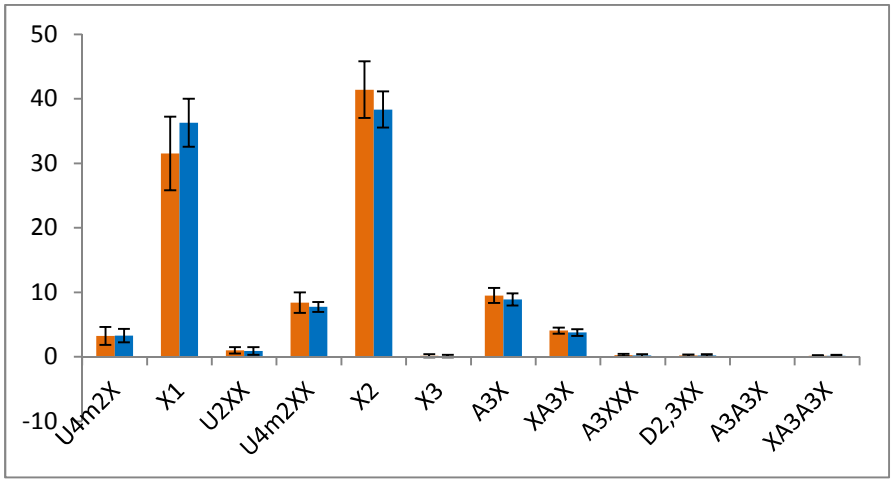

Figure 4.2 (b) GT43-F0310

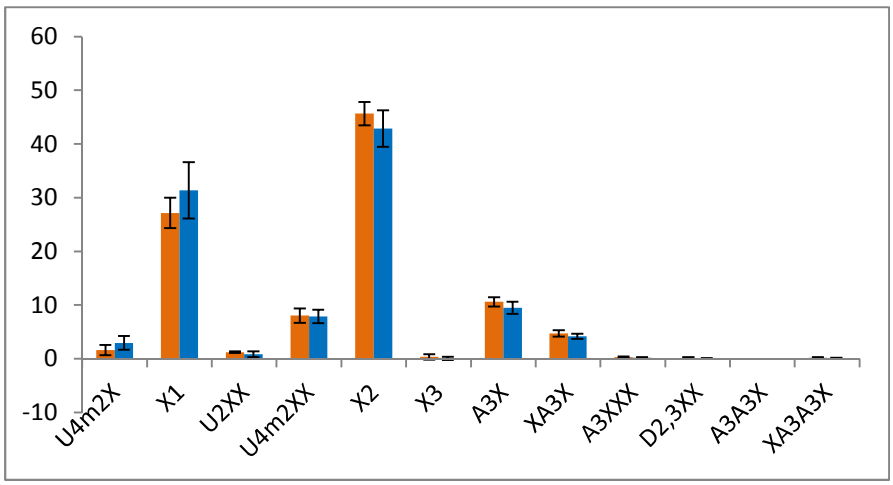

Figure 4.2 (c) GT47-E1900

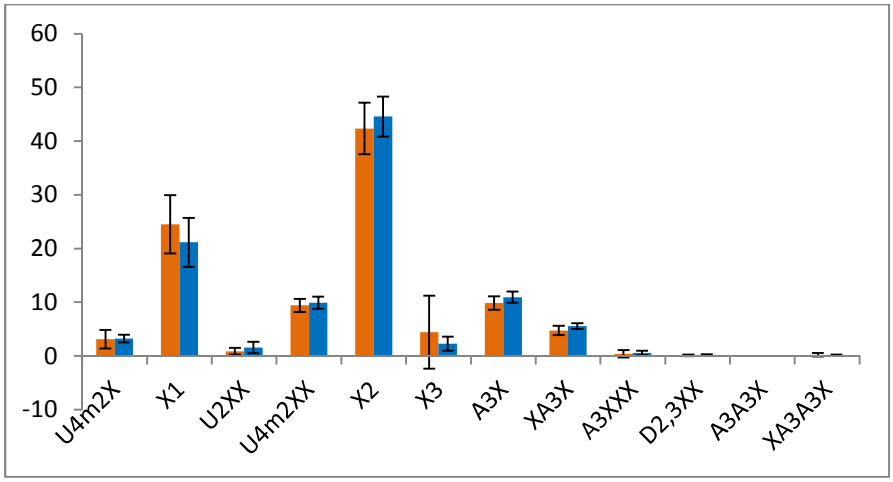

Figure 4.2 (d) GT47-H1480

Discussion /Conclusion:

We gained confidence that this approach of mutating xylan synthesis genes, finding changes in grass xylan structure, and consequently changing saccharification, is relevant as it has been fruitful for a *Brachypodium* putative *GT61* mutant (Marriot *et al*., in press), and a rice *GT61* mutant *xax1*. The absence of any changes seen to date in the samples suggests that the changes are small, and may be seen in replicates of the studies and more detailed analysis of xylan structures in the maize mutants, and this is underway. This work will be completed in maize by the end of February 2015.We also aim to make double mutants by crossing these maize plants, to increase the change in cell wall structure.

References:

Anders, N. *et al* (2012) Glycosyl transferases in family 61 mediate arabinofuranosyl transfer onto xylan in grasses. PNAS 109(3):989-993

Marriott, P.E. *et al* (2014) Range of cell wall alterations enhance saccharification in *Brachypodium distachyon* mutants. PNAS 111(40):14601-6