Probing the role of xylan in irreversible deformation of stems of *Arabidopsis thaliana*

Darshil Shah[1*], Thomas Reynolds[1], Marta Busse-Wicher[2], Li Yu[2], Paul Dupree[2], Michael Ramage[1]

[1] Centre for Natural Material Innovation, Department of Architecture, University of Cambridge, UK
[2] Department of Biochemistry, University of Cambridge, Cambridge, UK
*dus20@cam.ac.uk

Introduction

Plant stems exhibit a combination of elastic and plastic deformation under external loading. The biomechanical processes behind the plastic component of deformation are not yet well understood. Keckes et al. (2003) proposed a stick-slip mechanism analogous to Velcro, in which a permanent shear deformation of the lignin-hemicellulose matrix around the cellulose microfibrils is possible whilst retaining the strength and elastic stiffness of the undeformed cell. Under cycles of load, this plastic behaviour may present itself as viscoelasticity, with hysteretic energy dissipation by the plastic component of the deformation. Köhler and Spatz (2002) showed the role of lignin in this viscoelasticity, using chemically modified *Aristolochia macrophylla*, noting a great increase in viscoelasticity under the first cycle of load in the delignified version of the latter. This suggests a greater irreversible slip of the microfibrils due to a given load in the absence of lignin. Busse-Wicher et al. (2014) showed the potential for xylan to form the link between the hydrophobic lignin and hydrophilic faces of the cellulose chains, making it possible that it plays a role in this stick-slip mechanism. Tests on genetically modified *Arabidopsis thaliana* have given some evidence for the influence of various cell-wall compounds on strength and stiffness of secondary cell walls (Goubet et al. 2009). These stems are of particular interest because of the similarity between the secondary cell walls of *Arabidopsis thaliana* and those in dicot trees, so that they may give some insight into the nature of viscoelasticity and plastic behaviour in wood.

Through mechanical testing of *Arabidopsis Thaliana* stems, we investigate the role of xylan in forming physical bonds between the cellulose microfibrils and the lignin, and therefore its role in the stick-slip mechanism of irreversible deformation in secondary cell walls of plants.

Methodology

Basal stem sections (approximately 30 to 50 mm in length) were obtained from wild type (WT), and xylan-deficient (*irx9*) xylem mutants and cellulose-deficient (*bah*) mutants of Col-0 ecotype of Arabidopsis plants. Plant material and growth conditions are the same as those described elsewhere (Goubet et al. 2009).

For mechanical testing, stem sections were mounted onto thick cardboard frames using a slow-setting precision Araldite epoxy resin (Fig. 1). The laser-cut frames had 1 mm wide grooves running along the centreline, within which the stems were placed, to protect specimen

ends from crushing during specimen loading. A gauge length of 10 mm was used, with an embedded length of at least 10 mm either side.

Mechanical testing involved loading the frames onto an Instron tensile testing machine, equipped with screw-action grips and a 500N load cell. A loading rate of 0.5 mm/min was used. Strain was monitored from cross-head displacement, as well as using a laser extensometer. The latter relied on measuring the relative displacement of retroflective tapes that were placed on the gauge-length section of stems (Fig. 1). To convert applied load into stress, stem cross-section areas were determined from optical microscopy of failed specimens that were cast into solid resin blocks and polished to 4000 grit sand paper (particle size of 3 μm). Image analysis was conducted on Image-J freeware.

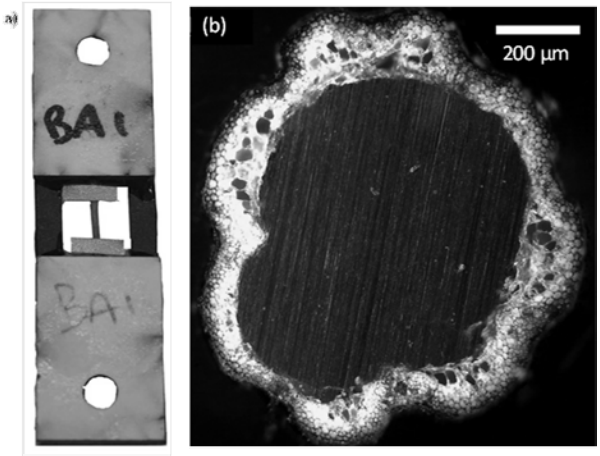

Fig. 1 : a) Specimen mounted in laser-cut cardboard frame for tensile testing. Retroflective tapes are used for laser extensometry. b) Cross-section area of the stems was determined using optical microscopy.

The test regime involved progressive tensile loading to failure, with three loading-unloading cycles each from 4 N to 1 N, 8 N to 5 N and 8 N to 1 N (Fig. 2). From the tests, first, initial modulus E_0 was determined from the gradient of the stress-strain curve in the load range of 0 to 4 N applied load. To investigate the potential contribution of cell wall constituents (based on comparison between wild type and mutant stems), particularly xylan, to the stick-slip mechanism responsible for mediating irreversible deformation, residual moduli in the three loading-unloading regions of the stress-strain curve were measured from their gradients; these are referred to as E_1 (measured from 4 N to 1 N), E_2 (measured from 8 N to 5 N), E_3 (measured from 8 N to 1 N). Secondly, the residual plastic strain from unloading was also estimated for all cycles. This provided a measure of permanent deformation in the form of plastic displacement and visco-elastic creep. Thirdly, noting a characteristic yield point in the stress-strain curve for the stems, the stress at this yield point was recorded. Finally, for samples that failed in the gauge section, tensile stress at failure was recorded. Most of these measurements are illustrated on a simplified stress-strain profile for a typical sample (Fig. 2).

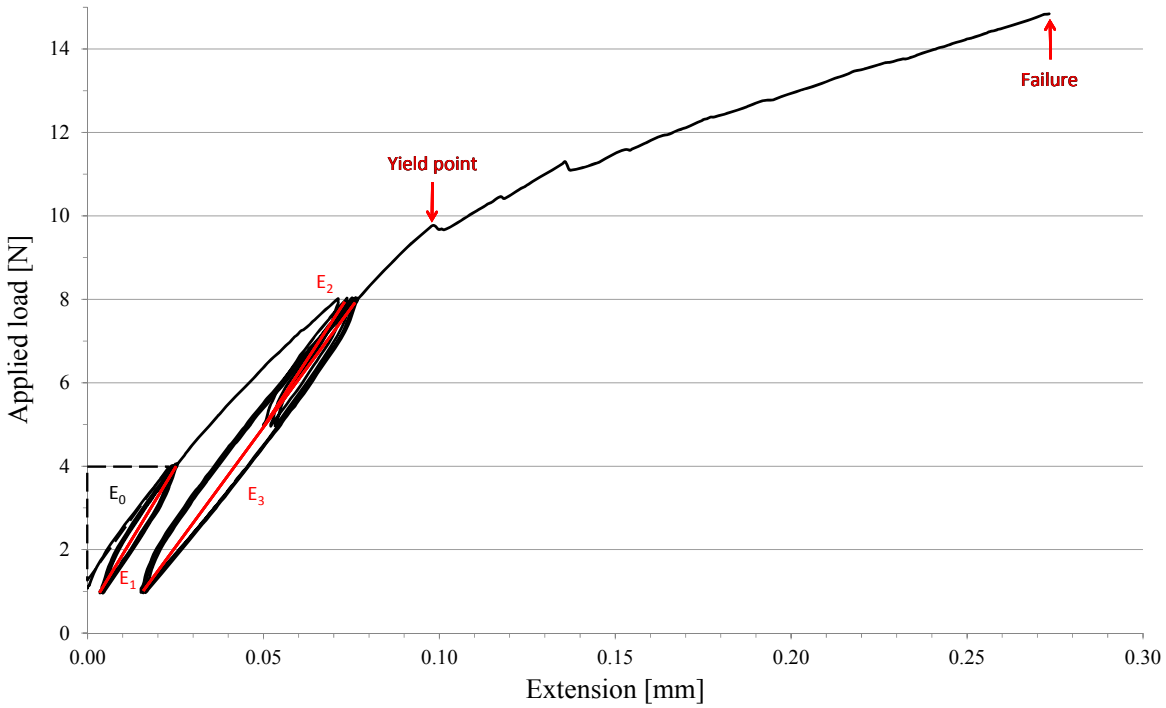

Fig. 2 : Example load-displacement curve, showing gradients measured for comparison of reversible and irreversible components of deformation, a characteristic yield point, and tensile stress at failure.

Results

Cross-section properties are key in accurately determining stem material properties such as elastic modulus and strength. The difference in the means of cross-section areas of WT stems to mutants stems was found to be statistically significant (two-tailed t-test, $p < 0.03$, $n = 8, 9$). WT stems were 22% larger in cross-section area than xylan-deficient *irx9* stems, and 57% larger than cellulose-deficient *bah* stems. The results are in accordance to visual observations that the mutants exhibit (severe, in the case of *bah*) dwarf growth phenotypes. The principal reason for this is attributed to xylem collapse and impediment of water/nutrient transport in the mutant stems.

The observed difference in cross-section area has several implications on the test results and interpretations. For example, as load-unload cycles were based on loads (e.g. 4 N to 1 N), the stem material in WT plants would experience lower stresses than the mutant plants. The higher stresses in the mutant stems (for the same load) would at least partly explain higher residual plastic strains. However, we note that while our cross-section area measurements account for the hollow nature of the stems (i.e. pith) and irregular cross-section shape, they don't account for the hollow nature of cells within stems (i.e. luminal porosity). Given that the stems of mutant plants have collapsed xylem tissue, luminal porosity may be less important in them, in comparison to stems of WT plants. A more accurate measurement technique for cross-section area of only solid cell wall material would enable more accurate interpretation of mechanical testing results.

The mechanical testing results are presented in Fig. 5. The initial stiffness E_0 of WT stems ranged between 0.45 to 0.55 GPa. This compared to *irx9* and *bah* stems with E_0 ranging between 0.38-0.60 GPa and 0.27-0.50 GPa. The unload-reload stiffness E_1 showed a similar

trend, with *irx9* stems exhibiting larger spread in properties with a mean comparable to the WT stems, and the *bah* stems having lower stiffness. The lower stiffness in *bah* was expected and was attributed to their cellulose-deficient composition, as cellulose is the principal structural polymer in the cell wall.

The comparable initial and unload-reload stiffness of *irx9* and WT stems was, however, not expected. As xylan is thought to be responsible for enabling the stick-slip mechanism in the cell wall, we had hypothesized that a reduction in xylan content and chain length (as is the case in *irx9*) would have increased the unload-reload stiffness by a lesser amount than the increase expected in a WT stem. Interestingly, we found that the ratio of E_0/E_1 was around 0.70-0.75 for the WT and the mutant plants we studied.

Cellulose-deficient *bah* mutant stems were the weakest, failing at loads lower than 8N (Fig. 3 d)), and therefore E_2 and E_3 could not be measured for them. WT stems did exhibit higher mean failure loads than *irx9* stems, however once cross-section areas were accounted for, the failure strengths were comparable if not slightly higher for *irx9* stems.

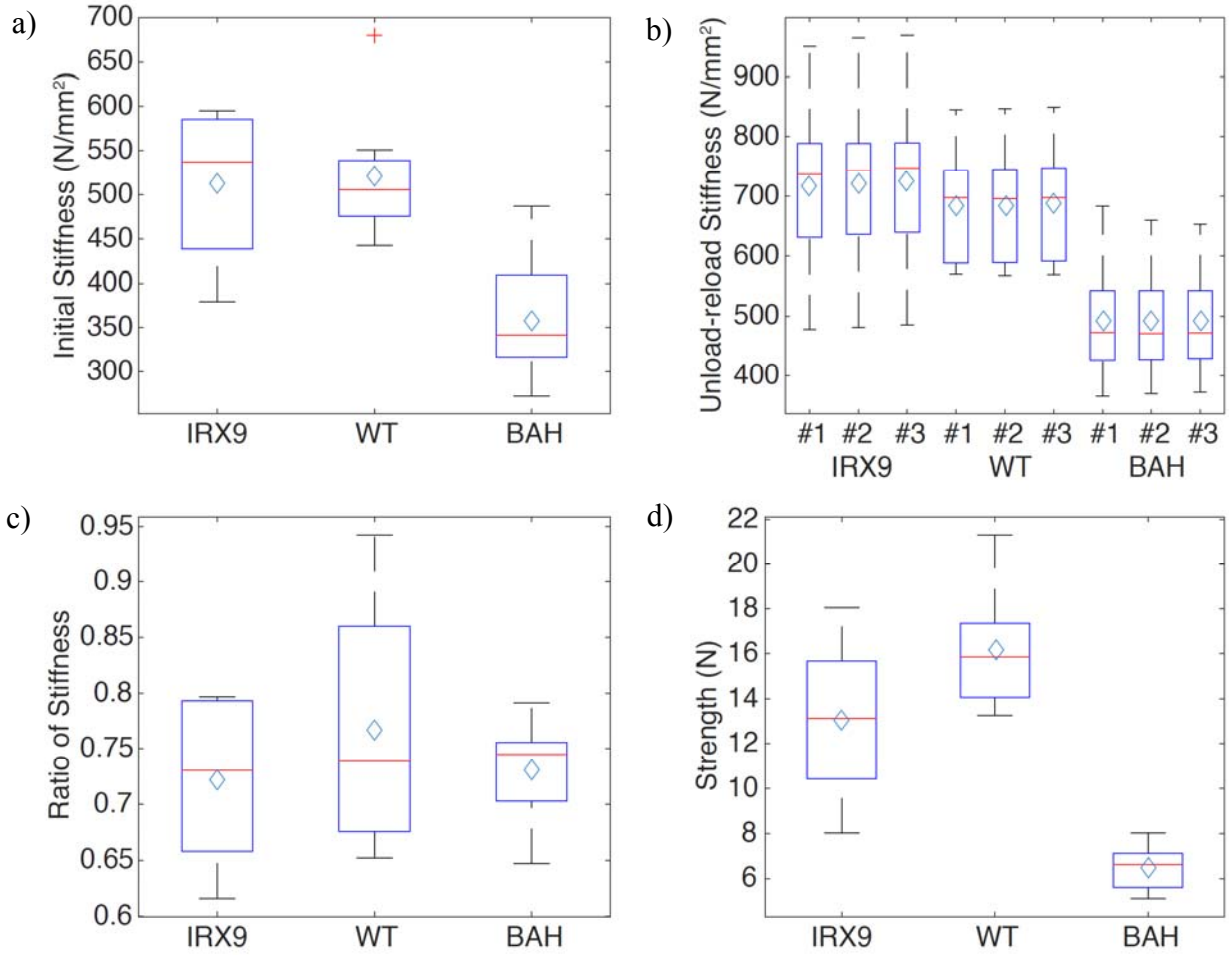

Fig. 3 : Box plots showing the variation in a) initial stiffness E_0, b) unload-reload stiffness E_2 for cycles 1, 2, and 3 (between 4 N and 1 N applied load), c) ratio of stiffness' E_0/E_1, and d) failure load, of stems from WT, *irx9* mutant and *bah* mutant plants. Box plots present the range as error bars, the upper 75% and lower 25% quartile as the blue box, the median as the red line and the diamond as the mean.

Summary and future work

We have developed a systematic testing methodology to measure the mechanical properties of plant stem materials to elucidate structure-property-function relations, ascertain the roles of the cell wall constituents (based on hypothesized molecular architecture), and to explain the behavior of wood by studying plant stems. To enable more accurate interpretation of results, we are looking into ways, such as measuring mass per unit length and cellular porosity through tomography, to appreciate the effects of luminal porosity on cross-section measurement.

Accounting for substantial differences in cross-section areas, our results clearly show that while cellulose-deficient *bah* stems have lower mechanical properties, and exhibit greater irreversibility than wild-type stems. However, no significant difference in properties of xylan-deficient *irx9* stems and wild-type stems was observed. This may be because although the content of xylan is reducing, how the xylan is interacting with the lignin and cellulosic microfibrils remains the same. For clarity on our current results, we will conduct mechanical tests on lignin mutants, as well as xylan mutants which do not exhibit severe dwarf growth phenotypes, and rather modify the way they link to the cellulose microfibrils (e.g. studying *gux-1,2* double mutants).

Encouraged by differences in residual plastic strain observed for the different stems, to further probe 'slippage' (in the stick-slip mechanism) and visco-elastic creep, we will be conducting stress relaxation tests on the stems to examine difference in fundamental material properties and creep-governing mechanisms, as measured by activation energies and volumes.

Acknowledgements

This work is part of a project funded by the Leverhulme Trust (Project title: 'Natural materials for sustainable living', PI: MHR). DUS also thanks the IOM3 for a travel grant to support participation at the conference.

References

Busse-Wicher, M., Gomes, T. C. F., Tryfona, T., Nikolovski, N., Stott, K., Grahtham, N. J., Bolam, D. N., Skaf, M. S., Dupree, P. (2014) The pattern of xylan acetylation suggests xylan may interact with cellulose microfibrils as a twofold helical screw in the secondary plant cell wall of Arabidopsis thaliana. The Plant Journal, 79(3), pp.492–506.

Goubet, F., Barton, C. J., Mortimer, J. C., Yu, X., Zhang, Z., Miles, G. P., Richens, J. Liepman, A. H., Seffen, K., Dupree, P. (2009) Cell wall glucomannan in Arabidopsis is synthesised by CSLA glycosyltransferases, and influences the progression of embryogenesis. The Plant Journal, 60(3), pp.527–538.

Keckes, J., Burgert, I., Fruhmann, K., Hamilton, M., Burghammer, M., Roth, S. V., Stanzl-Tschegg, S., Fratzl, P. (2003) Cell-wall recovery after irreversible deformation of wood. Nature Materials, 2(12), pp.810–813.

Köhler, L. & Spatz, H.-C. (2002) Micromechanics of plant tissues beyond the linear-elastic range. Planta, 215(1), pp.33–40.