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Electron and Helium Ion Imaging of *Arabidopsis* Affected by Genetic Mutation and Thermochemical Treatment for Biofuel Applications

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Lignocellulosic biomass holds unlocked potential as biofuel feedstock. By enzymatic digestion or by pyrolysis, the biopolymers that compose plant cell walls are converted into liquid biofuels at the industrial scale. One strategy to increase the efficiency and lower the cost of these conversion processes is the use of genetically modified plants that produce biomass that is more amenable catalytic deconstruction. Lignin is a polymer found within plant cell walls that provides structural support and microbial defense, and has been shown to impede enzymatic conversion processes. In this study, we examined several *Arabidopsis thaliana* genetic variants that produce lignin polymers with different content and composition. Samples were characterized by enzymatic saccharification, confocal laser scanning microscopy (CLSM), contact resonance force microscopy (CRFM), scanning and transmission electron microscopy (TEM), and helium ion microscopy (HIM).[1]

To study the effect of content and composition on enzymatic digestibility, three *Arabidopsis* genetic variants were investigated: the wild type, a "high-G" type or *fah 1-2,* and a "high-S" type or C4H:F5H. The high-G and high-S terminology refer to the subunits of the lignin polymer, guaicyl(G) monomers and syringyl(S) monomers. Additionally, these three strains were either left untreated or were thermochemically treated for 10 min with maleic acid. The samples were ground and freeze-dried for microscopy.

Electron and ion microscopy revealed that changes in digestibility due to mutations or surface treatment were correlated to measureable changes in the structure of the bulk plant matter. HIM showed clear differences among the mutated and wild samples that were made even more pronounced with acid treatment. Although some samples were coated with a 7 nm Ir film, the electron flood gun on the HIM allowed for imaging of uncoated regions of samples where the Ir film was incomplete, as well as of samples that were never coated. By imaging with the flood gun, we were able to compensate for the charging behavior of the uncoated plant matter and collect high-resolution images of the *Arabidopsis* surface. HIM images at 1 μm to 5 μm fields of view revealed distinct changes in texture in all samples with acid treatment, and the exposure of nanoscale cellulose fibrils in the case of the wild type and high-S samples. Furthermore, it was shown that the high-G samples were the most recalcitrant to deconstruction during maleic acid treatment as well as enzymatic saccharification, and displayed little exposed cellulose. These results corresponded directly with enzymatic saccharification assays, where high-G samples had the least glucan and xylose conversion, high-S samples had the highest conversion, and all types experienced a boost in performance after acid treatment.

In addition to SEM and HIM imaging, TEM, CLSM, and CRFM were performed on cross sections of all six variants of *Arabidopsis* samples. In particular, TEM imaging revealed changes in the cell wall structure, including delamination of layers and the exposure of nanofibrils in high-S acid-treated samples. Imaging and characterization of these internal structural changes complemented the HIM observation of visible changes to the *Arabidopsis* surface and the performance variation measured in enzymatic saccharification assays. These studies provide a pathway to higher yield feedstocks for biofuel applications as well as suggesting the potential of maleic acid thermochemical treatment to improve the performance of existing feedstocks [2].

References:

[1] Ciesielski, P. N., et al. unpublished (submitted to Green Chemistry).
[2] This work is a contribution of NIST an agency of the U.S. government and as such is not subject to copyright in the United States.

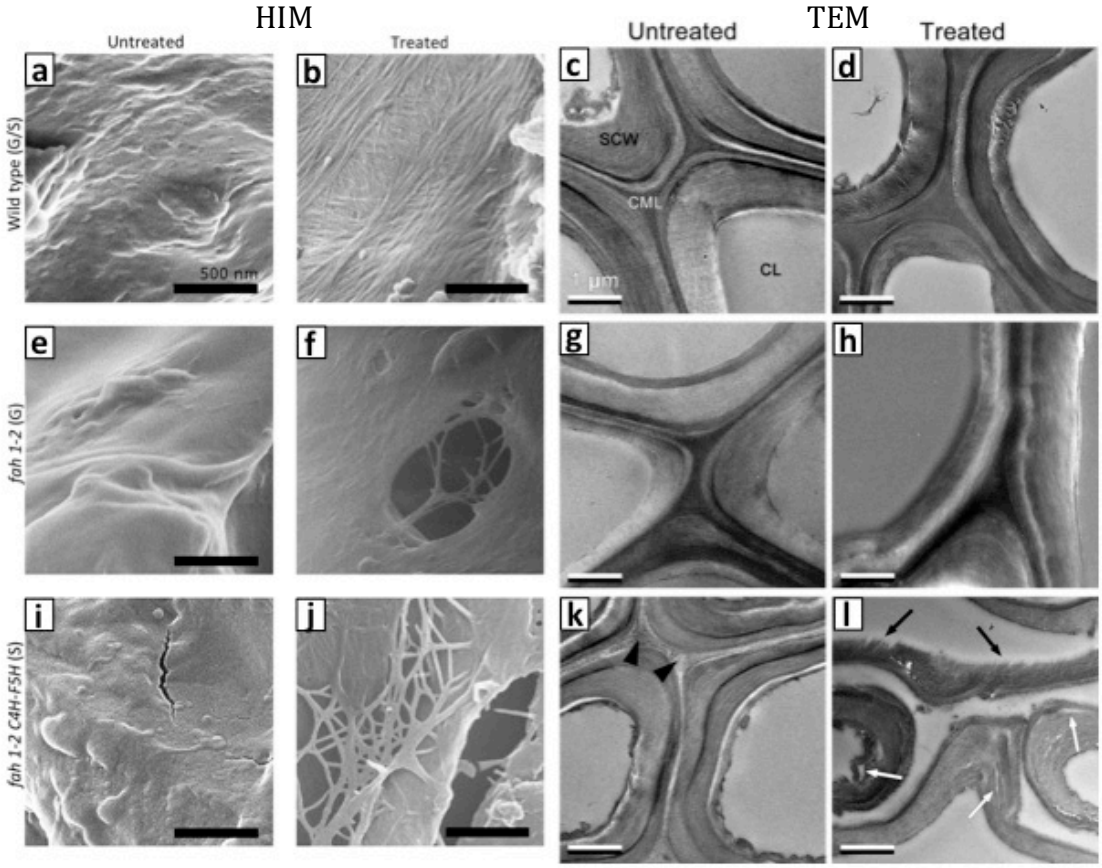

Fig 1: (a, b, e, f, i, j) High-resolution HIM was performed to investigate changes in surface texture caused by maleic acid pretreatment. All scale bars 500 nm. **(c, d, g, h, k, l)** TEM of the ultrastructure of the cell walls shows the varying effect of acid treatment on the three *Arabidopsis* strains, including pronounced delamination (white arrows) and deconstruction of the cell wall to expose nanofibrils (black arrows) in the high-S samples. All scale bars 1 µm.

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