Non-destructive imaging of lignin chemistry in plant cell wall

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Lignin is a major impediment in the deconstruction of plant biomass to its soluble monomeric constituents. Modification of the lignin biosynthetic pathway has proven to be an effective means of reducing biomass recalcitrance but can often result in impaired growth. As one of the successful examples of "designer" biomass, "Zip-lignin" has been generated in poplar to introduce ester linkages into the lignin backbone. The resulting plants grow normally and have improved biomass deconstructability, especially under mild alkaline conditions that can effectively cleave the uniquely introduced ester bonds. In order to further understand the structural and chemical features that may be associated with the observed improved processing efficiency, we used hyperspectral stimulated Raman scattering (hsSRS) to map the Zip-lignin *in planta* using lignin conjugated α–β carbon double bonds as the proxy. Such double bonds are presented in all types of lignin, but unique structures in Zip-lignins experience a ~20 cm^{-1} shift from 1650 cm^{-1} to 1630 cm^{-1} in their Raman spectra due to the conjugation between the double bond and an associated ester carbonyl group. Analysis of the hsSRS images has revealed that the Raman signal specifically representing Zip-lignin can be estimated. Moreover, it exhibits a distribution pattern in cell wall layers similar to that of the native lignin, but its intensity varies in different transgenic poplar lines. In addition, when the cell walls are imaged by atomic force microscopy and Simons' staining, we found that the poplar containing the Zip-lignin had increased accessible areas, which may be beneficial to the chemical penetration during pretreatment and substrate accessibility during enzymatic hydrolysis. Our study suggests that both physical and chemical modification of cell wall in Zip-lignin poplar could contribute to the observed improvements in sugar yields.