03-05: Combined bioorthogonal labeling, Raman spectroscopy and fluorescence histochemistry provide detailed spatial information on lignification in plant cell walls

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Lignin is the second most abundant plant biopolymer after cellulose on Earth. It is a major constituent of the cell wall in certain specialized plant tissues where it plays a vital role in providing mechanical support, facilitating water transport and enhancing protection against pathogens [1]. The quantity of lignin, as well as its chemical composition and the existence of covalent bonds to other cell wall polymers also have an important effect on different economically important plant (lignocellulose) biomass properties e.g. cell wall degradability for biofuel/biorefinery; mechanical resistance for timber etc. A better understanding of how the lignin polymer can i) affect the industrial processing of lignocellulose biomass, ii) influence the development of the plant during growth, and iii) modify soil microbiota during the carbon cycle depends upon the availability of appropriate analytical tools allowing scientists to characterize the structure of this complex polymer at the multi-scale level in a wide range of samples. Many chemical/physical techniques that are currently available to quantify and/or characterize lignin are unable to provide an in-depth picture of the spatial distribution of this polymer at the cell wall level. In contrast, the use of Raman spectroscopy and the more recent development of chemical reporter techniques are now allowing scientists to analyze the heterogeneity and dynamics of lignin formation *in situ* at the cell wall level [2,3,4].

In this communication we report the development of an original, *in vivo* triple bioorthogonal labeling technique for visualizing the incorporation of the three main lignin monomers (H, G and S units) into lignin [5]. This multiple labeling approach allowed us to study lignification dynamics in several model plant species (flax, arabidopsis, tobacco, poplar) by confocal fluorescence microscopy. In an ongoing project we are currently combining this triple chemical reporter approach with Raman spectroscopy and ratiometric safranin-based fluorescent microscopy to provide a highly detailed overview of changes in lignin and other cell wall polymers in the flax EMS mutant *lbf1* [6]. This study shows how different high resolution imaging techniques can be combined to provide more complete information on cell wall structure in plants.

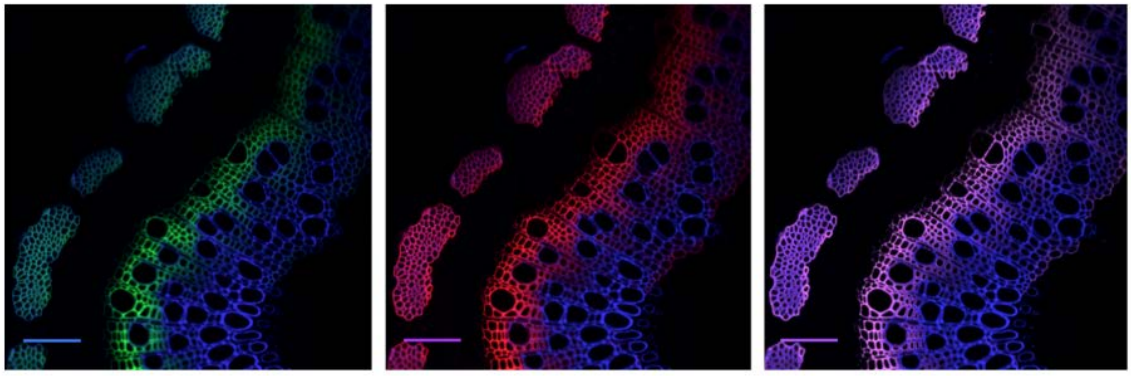

Fig. 1 Triple incorporation of H (left), G (middle) and S (right) lignin chemical reporters in fibers and xylem tissues in a poplar stem cross section

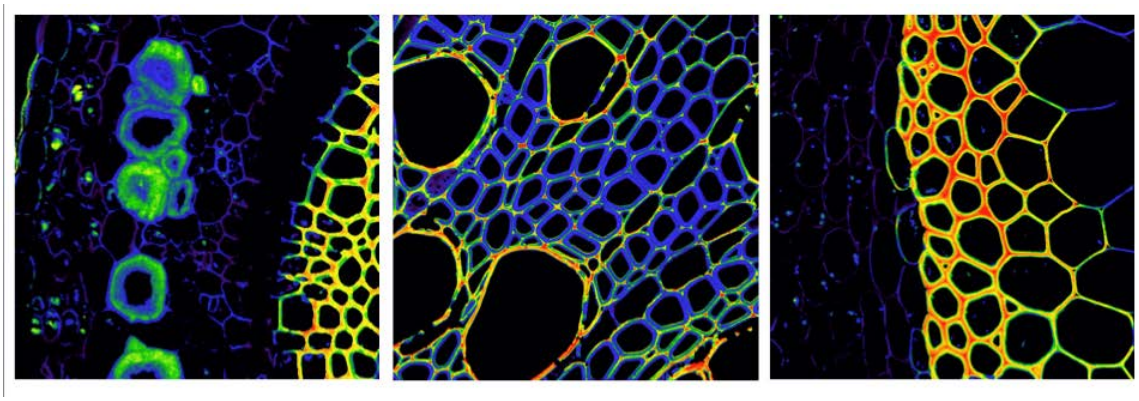

Fig. 2 Ratiometric safranin fluorescent microscopy in flax (left), poplar (middle) and arabidopsis (right) stems.

References

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