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Differential Polarization Laser Scanning Microscopy (DP-LSM) -Technique for Rapid Screening of Cell Walls of Different Plant Species

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Plant cell walls represent the most abundant, renewable and biodegradable composite on Earth. It forms a large part of the plant body and defines its characteristics. The specific form and function of the cell walls and interaction with the environment are based on variation in its chemical composition and connections between the building macromolecules. The secondary cell walls are rich in biopolymers such as cellulose, hemicellulose and lignin, but the most abundant component is cellulose [1-3].

DP-LS microscopy is a useful and easily applicable technique for rapid screening of cell wall structural order, by mapping the linear dichroism of cellulose fibrils [4]. It allows screening and quantification of the alignment of cellulose fibrils, relative to one another, in cell walls [5]. The goal was to provide new information about structural characteristics of the isolated cell walls among species which may be a basis for their possible applications, such as biofuel and biomaterial industry, but also to understand what plant response to stress is.

We applied DP-LS microscopy to compare alignment of cellulose fibrils in cell walls of spruce, maize and liana (*D.balcanica*).

The confocal fluorescence intensity images were recorded on a Zeiss LSM 410 laser scanning microscope (Carl Zeiss Jena, Jena, Germany) equipped with a differential polarization (DP) attachment [6, 7]. The images were in resolution of 512x512 dots, covering the area of 50x50 and 64x64 μm . Each image consists of two channels: FDL channel and fluorescence emission channel. FDL values for dipoles

oriented along the Y axis correspond to 1, while the values of dipoles oriented along the X axis correspond to -1. The FDL values are in the range from -1 to +1 due to the definition: $FDL = (I1-I2)/(I1+I2)$. (I1 fluorescence intensity was measured with vertically, I2 with horizontally polarized excitation). The acquired 8-bit TIFF images were set to have a color scale from blue (#0000FF) to yellow (#FFFF00) for the FDL channel. The samples of isolated cell walls were stained with freshly prepared 2 % (w/v) solution of Congo Red for 1h, followed by rinsing in distilled water three times. Samples were excited at 488 nm, and fluorescence emission was observed above 560 nm.

Distribution of linear dichroism was analyzed on images of the cell wall (Fig. 1). Width of obtained distribution curves indicates degree of linear order in the analyzed structures. The distributions show a quite similar orientation for the three species. However, the narrowest is for maize, corresponding to simpler structure and more regular packing (observed parallel linear structures) of cellulose molecules than in the wood species.

The DP-LSM method is shown to be a simple and rapid way to check the structural complexity in the cell wall through cellulose labeling.

References:

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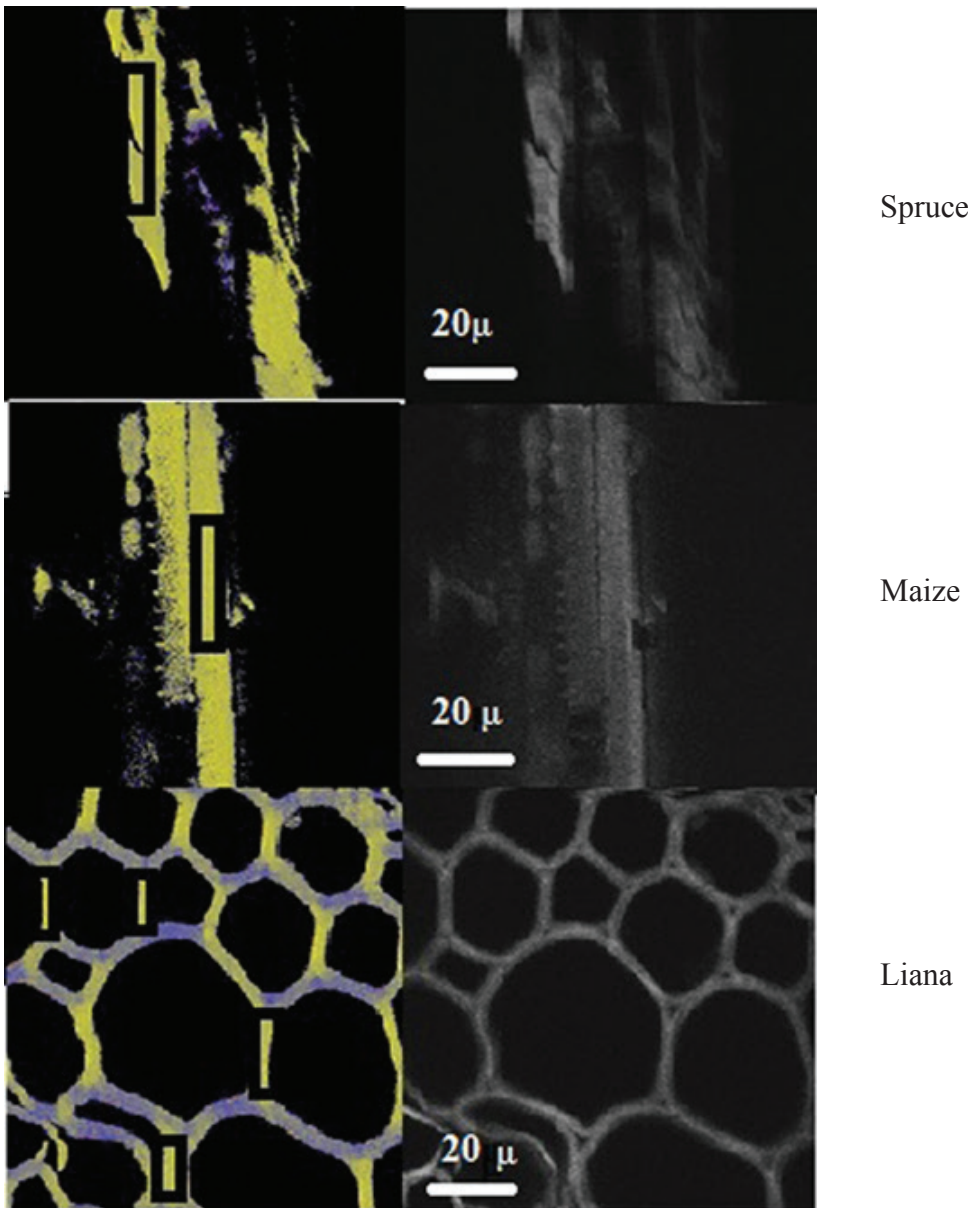


Figure 1. Confocal fluorescence-detected linear dichroism (FDLD) measurements (left panel), confocal fluorescence intensity (right panel) of the isolated cell walls of spruce, maize and liana stained with Congo Red.