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Prof. Dr Snežana Šerbula

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STRUCTURAL CHARACTERISATION AND ORIENTATION OF CELL WALL POLYMERS IN MAIZE LEAVES

Jasna Simonović Radosavljević[1], Nevena Pantić[1*], Jasna Stevanić[2], Daniela Đikanović[1], Aleksandra Lj. Mitrović[1], Lennart Salmén[2], Ksenija Radotić[1]

[1]University of Belgrade, Institute for multidisciplinary research, Kneza Višeslava 1, 11000 Belgrade, SERBIA

[2]*RISE/* Innventia, Box 5604, SE-114 86 Stockholm, SWEDEN

[*]nevena.pantic@imsi.rs

Abstract

Cell wall can be considered as a nano-composite in which cellulose, lignin and hemicelluloses are interconnected in a specific manner. Mechanical and physical propreties of plant fibres are dependent on the orientation of constituent polymers (cellulose, hemicellulose, lignin). Fourier transform infrared (FTIR) microscopy was used to examine the orientation of the main plant polymers in transversal and longitudinal direction of the isolated cell wall of the maize leaves. Polarised FTIR measurements indicated an anisotropy, i.e. orientation of the cellulose microfibrils that was more or less parallel to the longitudinal axis of the cell wall. Xylan has parallel orientation with regard to the orientation of cellulose, as well as lignin.

Keywords: cell wall, cellulose, xylan, lignin, anisotropy

INTRODUCTION

Plant cell walls (CW) are the most abundant, renewable and biodegradable composite on Earth. 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. Cell wall can also be considered as a nano-composite in which cellulose, lignin and hemicelluloses are interconnected in a specific manner. Biopolymers such as cellulose, hemicellulose and lignin, have wide applications in different industries, especially for biofuels and biomaterials [1,2].

Understanding structural organisation of the cell wall and related polymers is important for understanding mechanical properties of a plant, which has implications in plant response to stress, but also in possible applications of corn as a source of new biomaterials. By using imaging FT-IR microscopy, run in transmission mode and at different polarisation modes (from 0° to 90°), it is possible to follow chemical variability and orientation of cell wall polymers [3]. The orientation of cellulose, xylan and lignin, as essential components of plants, were analysed by iFTIR with regard to the sample axis.

MATERIALS AND METHODS

Cell wall isolation

To obtain extractive free cell walls, 1 g of maize leaves were homogenized in 10 mL of 80% methanol in 50 mL Big Clean tubes filled with a stainless steel matrix for 45 s at a speed of 4.5 m/s, using a FastPrep-24 apparatus (MP Biomedicals, Santa Ana, CA, USA). After stirring for 5 min at room temperature, the sample was again subjected to FastPrep homogenization at the same speed, which is a preferred technique in the case of plant material (Melton and Smith 2001). The homogenate was stirred at room temperature for 1 h and centrifuged at 1500 x g for 5 min. The pellet obtained was re-extracted twice, using 10 mL of 80% methanol. In order to remove the extractives, the pellet that had been obtained was then subjected to washing steps: 1M sodium chloride, 1% Triton X-100, distilled water, methanol, acetone [4].

FTIR microscopy

FTIR microscopy measurements were carried out using a Spectrum Spotlight 400 FTIR Imaging System (Perkin Elmer Inc, Shelton, CT, USA). The area of interest was first displayed, using a visible CCD camera to locate the cell wall area, which was then irradiated using mid-IR light. The scanning was carried out in imaging mode using an array detector, providing a pixel resolution of 6.25 µm x 6.25 µm, a spectral resolution of 4 cm^{-1} and a spectral range from 1,800 to 720 cm^{-1}. Polarisation: the incident IR radiation was polarised by a gold wire grid polariser from 0° to 90° polarisation in relation to the fibre orientation with intervals of 5°. The sample was mounted on the sample stage as parallel as possible to the orientation of the 0° polarisation. The IR spectra were processed by the software Spotlight 1.5.1, HyperView 3.2 and Spectrum 6.2.0 (Perkin Elmer Inc., Shelton, CT, USA) [3].

RESULTS AND DISCUSSION

From the in-depth study of polymer orientation, three areas from the sample (corn leaf) were selected. The transmission spectra were recorded from 0° to 90° polarisation modes. Figure 1 shows FTIR spectum of cell walls of maize leaves in the region 800–1800 cm^{-1}. Spectral signals related to absorptions from cellulose, xylan and lignin can be identified.

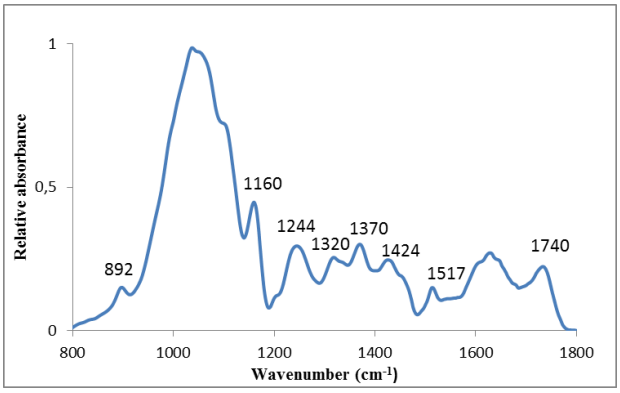

Figure 1 *Average absorbance spectrum of maize leaves cell wall*

Four distinct vibration bands that were related to cellulose, viz. the antisymmetric C–O–C bridge stretching vibration at 1160 cm^{-1}, CH_2 wagging vibration at 1320 cm^{-1}, the C–H bending vibration at 1370 cm^{-1} and the C–OH bending vibration of the CH_2–OH group at 1424 cm^{-1} were found [5–7]. The bands at 1244 cm^{-1} (the C–O stretching in the O=C–O group) and 1740 cm^{-1} (carbonyl group vibration, the C=O stretching vibrations in the O=C–OH group of the glucuronic acid units) are characteristic for xylan [5–7]. The band at 1517 cm^{-1} (aromatic skeletal vibrations) is characteristic for lignin [8,9].

The relative absorbance spectra are presented (Figure 2) as specific absorption peaks ($RA = (I_p - I_{min}) / (I_{max} - I_{min})$) where RA is relative absorbance, I_p is intensity of the absorbed IR radiation at a given angle of the polarisation, I_{max} is maximal intensity observed for a given vibration and I_{min} is minimal intensity observed for a given vibration. These relative absorbance values were presented in relation to the angle of the incident IR polarisation (from 0° to 90°).

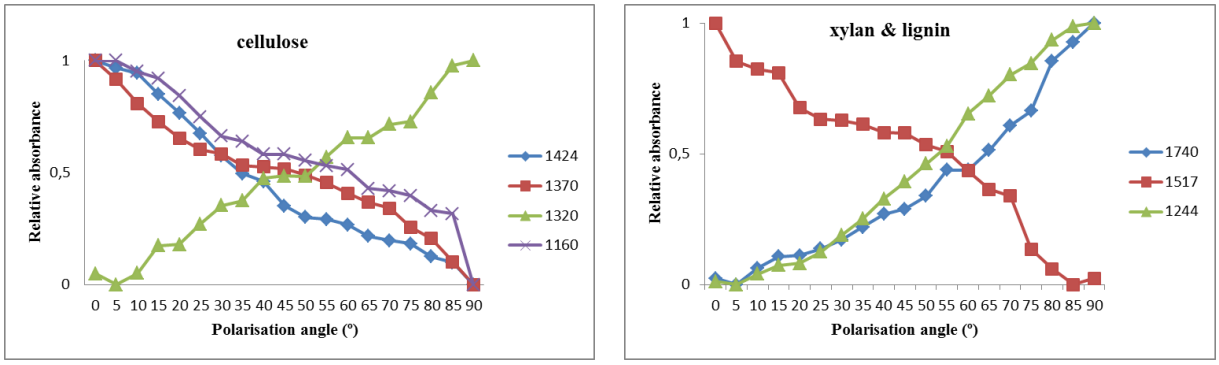

Figure 2 *The relative absorbance of IR specific absorption wavenumbers plotted against the polarisation angle for the different polymers for maize leaves*

It is evident (Figure 2 left) that the three cellulose peaks (1160 cm^{-1},1370 cm^{-1} and 1424 cm^{-1}) [3–5] had high absorption levels at low polarisation angles, which is a consequence of a more parallel orientation of the corresponding groups to the CW longitudinal axis. The fourth cellulose peak (the perpendicular signal at 1320 cm^{-1}) had the greatest intensity at a high polarisation angle, due to the perpendicular orientation of the corresponding group (Figure 2 left). For the xylan, the characteristic band signals (1244 cm^{-1}, 1740 cm^{-1}) [5–7] increased with an increase in the polarisation angle. Due to the parallel orientation of these side groups in xylan, an orientation parallel to the longitudinal CW axis is indicated (Figure 2 right). For the lignin, the characteristic band signal (1517 cm^{-1}) [8,9] decreased with an increase in the polarisation angle (Figure 2 right), indicating that lignin is organised in parallel with the longitudinal CW axis.

CONCLUSION

It has been shown that xylan is oriented in parallel to the cellulose and more or less parallel to the axis of a cell wall, in isolated CW fragments from maize leaves. There was also a clear indication of lignin orientation parallel to the longitudinal CW axis. This means that all of these components show strong anisotropic behaviour and organisation.

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