BOOK OF PROCEEDINGS

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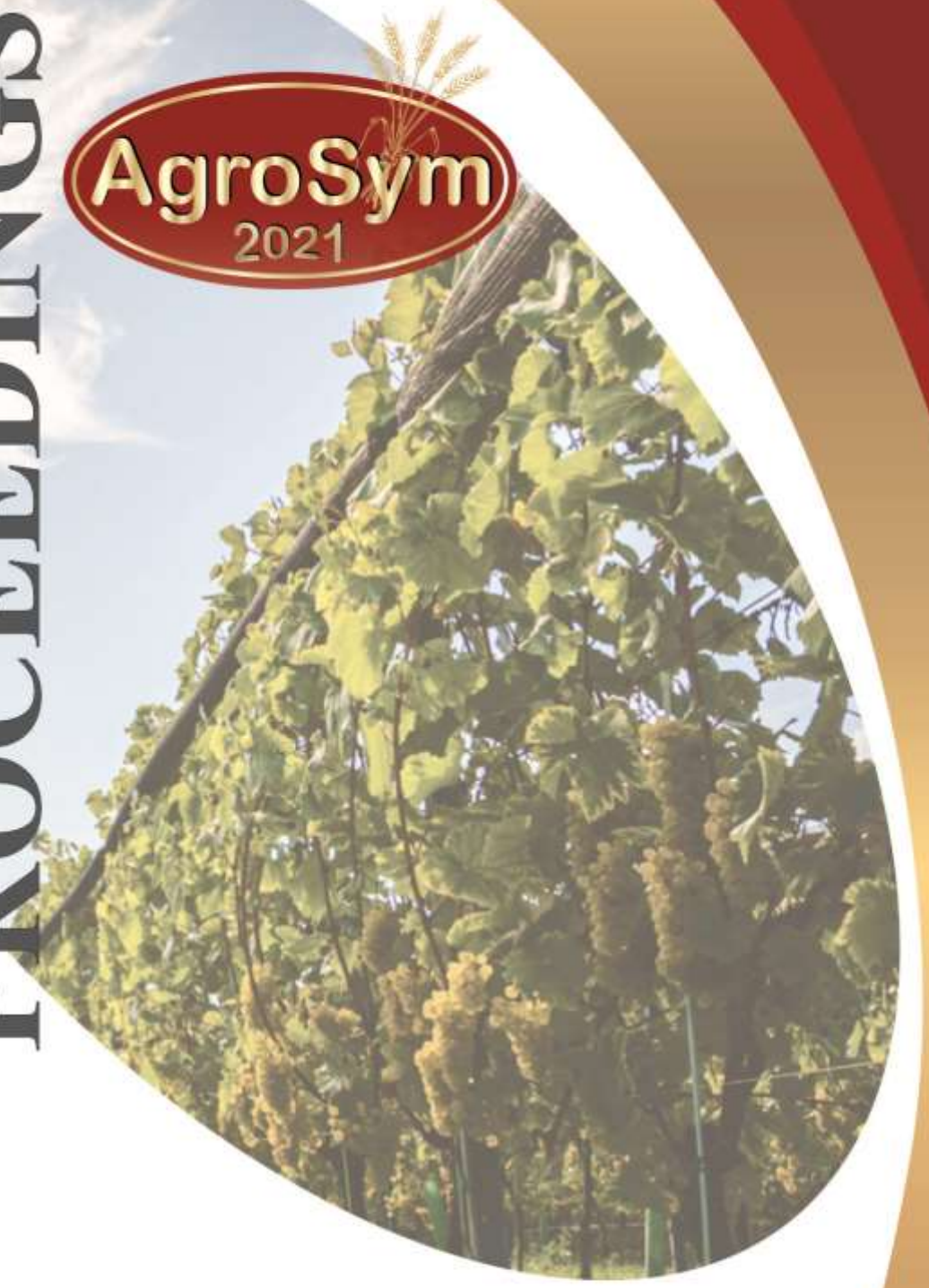

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COMPARISON OF CELL WALL STRUCTURE OF DIFFERENT WESTERN BALKAN PLANT SPECIES AS A SOURCE FOR BIOFUELS

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Abstract

Understanding of composition and connections between the building macromolecules of plant biomass, such as cellulose, hemicellulose and lignin, is main key for their better utilization in biofuels industry. We compared four different plant species which are abundant in the region of the Western Balkans. We investigated the structure of the cell walls, as the main constituent of plant biomass, isolated from branches of softwood (*Picea omorika* (Pancic) Purkine), hardwood (*Acer platanoides L.*), maize stem (*Zea mays L.*) as examples of crop species, and *Paulownia tomentosa* tree as a fast-growing species with a huge biomass yield. For our investigation, we combined Fluorescence-detected linear dichroism (FDLD) method and X-ray Diffraction. We obtained data for anisotropy and crystallography which are a base for prediction of the best and appropriate plant species for easy deconstruction of its biomass. Our results show that Acer branch as a hardwood shows the highest anisotropy and the lowest crystallinity compared to the other species while *Picea Omorika* needles show opposite results as the lowest anisotropy and the higher crystallinity. The results for maize show that the stems are easier for utilization than leaves. The isolated cell walls from leaves of *Paulownia tomentosa* show similar results and good correlation between anisotropy and crystallinity, thus we can conclude that this plant is easy to use in biofuel industries.

Keywords: *cell wall, biofuels, anisotropy, crystallinity.*

Introduction

Plant cell walls are the most abundant renewable and biodegradable composite material on the Earth. The secondary cell walls are rich in biopolymers such as cellulose, hemicellulose and lignin, which are of great importance in the biofuel industry.

The chemical composition and interconnection of the polymers in plant cell walls vary between different plant species and directly affects their application and accessibility for use (Pauly and Keegstra, 2008; Ragauskas et al., 2006). Cellulose consists of linear chains of glucose units linked by β-1,4-glycosidic bonds and is the most abundant component in plant cell walls. A large amount of hydroxyl groups in cellulose is involved in complex intra- and inter-molecular hydrogen bonds and so directly form high-order (crystalline) or low-order (amorphous) regions (Kondo, 2004; Wang, 2008). Amorphous regions in cellulose interact with hemicellulose chains (Atalla et al., 1993; Cosgrove, 2005). Hydroxyl group distribution of the cellulose surface affects a hydrophilic character of this molecule (Perez and Mazeau, 2005). Different hemicelluloses interact with cellulose and lignin, and the strength of the structure depends on this interaction

(Atalla et al., 1993; Barakat et al., 2007; Rueland Joseleau, 2005; Scheller and Ulvskov, 2010). Lignin is the second most abundant polymer on the Earth. It has a complex polymer structure composed of polyphenolic units (coniferyl, syringyl, coumaryl). Each plant species has a specific lignin composition. Trees like softwood and hardwood have lignin content from 30% to 34%, the higher content being in the softwood species. Lignin in herbaceous species is rich in syringil units. Lignin is a major source of large-scale biomass utility.

In our study, we compared the structure of four different plant species: spruce wood (*Picea omorika (Pančić) Purkine*) as an example of softwood, maple wood (*Acer platanoides L*.) and *Paulownia tomentosa* wood as a hardwood species, and maize stems (*Zea mays* L.) as a herbaceous plant and widely used agricultural plant.

In this investigation we measured Fluorescence-detected linear dichroism (FDLD) of cellulose labeled with Congo Red by using differential polarization laser scanning microscope (DP-LSM), which provides information of structural order of cellulose fibers. We also measured cellulose crystallinity in the isolated cell walls from branches and leaves by X-ray diffraction (XRD) method. These two methods have not been combined before for structural characterization of biomass. Thus cell wall structural characteristics and polymer interactions were monitored through structural order of cellulose fibers.

Materials and methods

Plant material: Four different plant species were used. The cell walls were isolated from branches of spruce (*Picea omorika* (Pančić) *Purkyne,* Gymnospermae, conifer), maple (*Acer platanoides L.*, Angiospermae, Dicotyledones, deciduous species), *Paulownia tomentosa* wood, and from maize stems (*Zea mays L.*, Angiospermae, Monocotyledones, grass family).

Isolation of plant cell walls: The extractive-free cell wall material was obtained from the spruce, maple, paulownia and maize. Plant material (1g) was 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. Such obtained plant material was dried for 72 h at 80 C. Dry homogenates of plant material were ground into a fine powder. To obtain cell walls, 400 mg of powder was homogenized for 5–10 min in 10 mL 80 % methanol. The homogenate was slightly stirred for 1 h at room temperature and centrifuged for 5 min at 1500 x g. Further, the resulting precipitate was extracted twice with 10 mL 80 % methanol. The precipitate was subjected to the following washing steps, according to Strack et al. (1988) and Chen et al. (2000):1 x (1 M NaCl, 0.5 % Triton X-100), 2 x distilled water, 2 x 100 % methanol, 2 x 100 % acetone (each step in 20 mL, 30 min). In each washing step, the sample was homogenized in 20 mL of corresponding solvent and then subsequently stirred for 10 min at room temperature and then centrifuged at 1500 x g for 10 min. The supernatant was subsequently removed. A FastPrep-24 System (MP Biomedicals, Santa Ana, CA, USA) was used in each isolation step for more efficient extraction of the cell wall material. The FastPrep-24 instruments and matrix tubes provide rapid and thorough, automated disruption of plant cell walls, which are difficult to homogenize/lyse.

The X-ray measurements of dry isolated cell walls were carried out by Siemens D-500 powder diffractometer. CuKa radiation was used in conjunction with a CuKb nickel filter. Measurements were repeated for each type of sample (species), and representative diffractograms were shown. The percentage crystallinity of the samples was calculated from the ratio of the area under the

diffraction peaks to the total area under the whole diffraction pattern (Georget et al., 1999; Hermans and Weidinger, 1948). Amorphous background patterns were generated, fitted to and subtracted from each diffraction pattern by using OriginPro 7.5.
In the DP-LSM method, 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 (Garab et al., 2005; Steinbach et al., 2009). Briefly, the DP attachment modulates the polarization state of the excitation laser beam at 100 kHz between horizontally and vertically linear polarization, using a photoelastic modulator (PEM-90, Hinds Instruments). The FDLD signal, proportional to the fluorescence intensity difference, elicited by two orthogonally plane-polarized beams, was obtained from the demodulation circuit, and images were recorded on the LSM. Using the runtime calculation, the FDLD imaging needs only a single scan and it avoids all the artifacts from the multiple scans (such as sample and light intensity stability, bleaching). The provided pixel values are the average of more than ten cycles of modulation. The images were in resolution of 512 x 512 dots, covering the area of 50 x 50 and 64 x 64 lm. Each image consists of two channels: FDLD channel and fluorescence emission channel. FDLD 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 samples of isolated cell walls were stained with freshly prepared 2 % (w/v) solution of Congo Red (Merck, Darmstadt, Germany) for 30 min, followed by rinsing in distilled water three times. Congo Red has earlier been used to determine the mean cellulose fibril orientation in plants (Verbelen and Kerstens, 2000). The samples (isolated cell wall fibrillar fragments) stained with Congo Red were excited at 488 nm, and fluorescence emission was observed above 560 nm.

Results and Discussion

Cellulose crystallinity in the samples was followed by X-ray diffraction. Based on the data obtained from the diffraction patterns of the cell wall samples for acer, spruce, Paulownia and maize, by Nara & Komiya (1983); it is obvious that there is a difference in the overall crystallinity of the samples. Table 1 shows that crystallinity is nearly the same (42 %) in the cell walls of softwood and harwood species. Crystallinity of the cell wall of maize is much higher (58 %). The peaks obtained from the diffractograms are typical for cellulose I and are located at $2\Theta \approx 14.9^{o}$, 16.49^{o}, 22.84^{o} (Marchessault and Sundararajan 1983). The peak at $2\Theta \approx 23^{o}$ generally is described as "highly crystalline" region of cellulose, while a broad peak at $2\Theta \approx 16^{o}$ is characteristic of the less organized polysaccharide structure (graphs not shown) (Fig. 3). The crystallinity of maize is significantly higher because it contains a lower proportion of lignin compared to woody species, which results in a better packaging and a more ordered distribution of cellulose microfibrils. A percentage of crystallinity of approximately 42% was found in all trees, which corresponds to that calculated by Hulleman et al. (1994) for cellulose in cotton and wood of 40 - 48%.

Table 1. Calculated crystallinity of cellulose for the samples of four plant species

Acer branch %	42.39
Picea branch %	42.08
Maize stem %	57.80
Paulownia branch %	41.84

The FDLD observed by DP-LSM imaging is a suitable tool for mapping of the optical anisotropy of cellulose (marked by Congo red) in the cell wall, that corresponds to the cell wall linear dichroism (Steinbach et al., 2008). The anisotropy of each sample was calculated from the images by Image J program. Results are shown in Fig. 1. High anisotropy was obtained for maple branch, Paulownia wood and maize stems. We found a lower anisotropy for Picea branch. One can assume that high anisotropy indicates a simpler structure and more regular packing of cellulose molecules in hardwood species than in the softwood species.

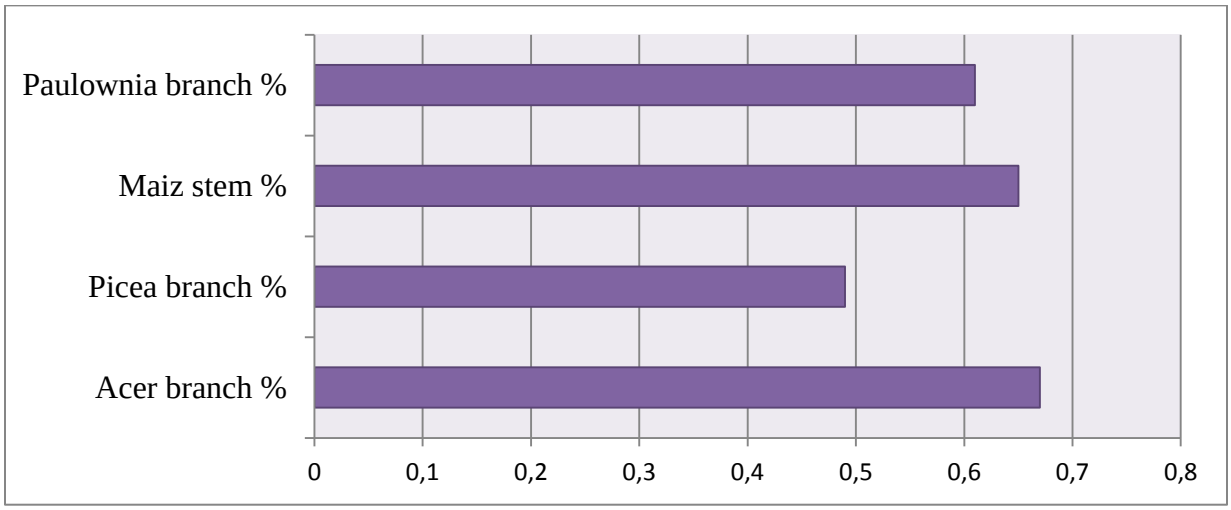

Figure 1. Anisotropy data of the cell walls isolated from the Acer branch, the spruce branch and maize stem calculated from DPLS images

Conclusions

All four different plant species have different chemical compositions and different interactions between the building macromolecules. Through microscopic visualization and X-ray diffraction, we obtained data for anisotropy and crystallography which are a base for prediction of the best and appropriate plant species for easy deconstruction of its biomass structure. Our results show that Acer branch as hardwood shows the highest anisotropy and the lowest crystallinity compared to the other species while spruce needles show opposite results as the lowest anisotropy and the highest crystallinity. The results for maize show that the stems are easy for utilization. The isolated cell walls from *Paulownia tomentosa* show similar results and good correlation between anisotropy and crystallinity, thus one can conclude that plants such as maize and hardwood species, especially Paulownia tree, may be suitable to use in biofuel industries.

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