

Comparison of the characteristics of cellulose microfibril aggregates isolated from fiber and parenchyma cells of Moso bamboo (*Phyllostachys pubescens*)

Kentaro Abe · Hiroyuki Yano

Received: 10 September 2009 / Accepted: 11 November 2009 / Published online: 27 November 2009
© Springer Science+Business Media B.V. 2009

Abstract This study examined the relationship between the functions of plant cells and the characteristics of cellulose microfibril aggregates in the cell walls. For this purpose, the mature bamboo (*Phyllostachys pubescens*) culm was separated into fiber and parenchyma cells, and then the morphological and physical properties of the cellulose microfibril aggregates isolated from both cells were compared. SEM observations revealed that both fiber and parenchyma cells consist of similar microfibril aggregates approximately 15–20 nm in width. Moreover, X-ray analysis and the tensile tests of the sheets prepared from the microfibril aggregates showed that the cellulose microfibrils isolated from fiber and parenchyma cells had almost the same cellulose crystallinity and longitudinal Young's modulus in the dry state. These results suggest that all the cellulose microfibrils synthesized in the same individual exhibit the same characteristics in the dry state regardless of cell function.

Keywords Cellulose microfibril · Moso bamboo · Cellulose crystallinity · Tensile property

Introduction

Currently, it is recognized that the tallest living tree in the world is the coast redwood (*Sequoia sempervirens*), known as “Hyperion” in Northern California, measuring 115.5 m. Softwoods like Sequoia are composed mostly of tracheid fibers, and the oriented assembly of such fiber cells supports the huge body. Naturally, superior physical properties are required from the cell walls.

In the case of wood cell walls, the aggregates of crystalline cellulose microfibrils behave as a rigid framework and are embedded in soft matrix substances such as hemicelluloses and lignin with a tight interface. Such structural features in wood cell walls are analogous to the requirements for composites to be stiff and tough (Fratzl et al. 2004); consequently, stress can transfer efficiently from the matrix to the microfibril aggregates. Therefore, the high rigidity of cellulose microfibrils in the cell walls of tracheids is crucial.

However, not all types of plant cells need constant mechanical support like tracheids do. For example, parenchyma cells' primary function is to store nutrients. Is the high rigidity of microfibrils really important for the walls of parenchyma cells? In this study, we have attempted to shed some light on this question by focusing on the relationship between the functions of plant cells and the characteristics of the cellulose microfibril aggregates.

We have chosen a mature bamboo for this purpose because the bamboo culm has many parenchyma

K. Abe (✉) · H. Yano
Research Institute for Sustainable Humanosphere,
Kyoto University, Gokasho, Uji, Kyoto 611-0011, Japan
e-mail: abekentaro@rish.kyoto-u.ac.jp

cells and fiber cells in vascular bundle sheaths, whose functions are distinctly different. Bamboo samples were separated into single cells by the removal of lignin, and then sieved into fiber and parenchyma cells. The microfibril aggregates from each cell were isolated and their morphological and physical properties were compared.

In recent studies (Abe et al. 2007; Abe and Yano 2009; Iwamoto et al. 2008), we have been trying to isolate cellulose nanofibers from wood using a simple mechanical treatment. The obtained nanofibers were approximately 15 nm in width and at least a few micrometers in length, and corresponded to cellulose microfibril aggregates in wood cell walls (Fahlén and Salmén 2005; Donaldson 2007). Moreover, because in these studies the nanofibers were isolated by only a single grinding treatment, the mechanical damage to the isolated fibers remains minimal (Abe and Yano 2009; Iwamoto et al. 2007), suggesting that we can compare the characteristics of the microfibril aggregates in almost their natural state.

Materials

Plant material

Mature culms of Moso bamboo (*Phyllostachys pubescens*), aged 5 years, were collected from Kyoto Prefecture, Japan, in October 2008. After the removal of epidermal and endodermal layers from the culm, small sections 30 mm long and 5 mm wide were cut from the middle portion. The sections were dewaxed in a Soxhlet apparatus with a 2:1 (v/v) mixture of toluene/ethanol for 6 h.

Methods

Separation into fiber and parenchyma cells

The sections were delignified according to Wise et al. (Wise et al. 1946) with vigorous stirring in order to separate single cells by removing lignin in the middle lamella. The individualized cells were collected every 3 h and then were rinsed with distilled water until their pH was neutral. This process was repeated for the residual sections until the sections were

almost completely disassembled into individual cells. Water suspension of the single cells was repeatedly sieved using two kinds of mesh in order to separate fiber from parenchyma cells. Using 200 mesh (aperture of 75 μm), only short cells like parenchyma cells were obtained. Because some short cells remained although most of the residual suspension consisted of long cells like fiber cells, only long cells were collected by repeated sieving through 30 mesh (aperture of 500 μm).

Isolation of cellulose microfibril aggregates

Both samples were separately purified by the iteration of a set of acidified sodium chlorite (NaClO_2) treatments at 70 °C for 3 h according to the method by Wise et al. (Wise et al. 1946) and an alkaline treatment with 5 wt% potassium hydroxide (KOH) at 80 °C for 2 h. The iterations of these chemical treatments were performed two and four times for fiber cells and parenchyma cells, respectively. After each chemical treatment, the samples were filtered and rinsed with distilled water until the residues were neutralized. The water slurry with a 1 wt% undried purified sample was passed once through a grinder (MKCA6-3; Masuko Sangyo Co., Ltd., Saitama, Japan) at 1,500 rpm (Abe et al. 2007; Abe and Yano 2009; Iwamoto et al. 2008). The grinding treatment was performed with a clearance gauge of -6 (corresponding to 0.6 mm shift) from a zero position, which was determined by the point of slight contact between the grinding stones.

Field-emission scanning electron microscope (FE-SEM)

Prior to observation using FE-SEM, the separated fiber and parenchyma cells were freeze-dried for 1 day. The purified samples before the grinding treatment and the fibril slurries after the grinding treatment were diluted with more than 10 times the volume of ethanol, cast on Teflon petri dishes, and then dried at 105 °C. All the samples were coated with platinum by an ion sputter coater and were observed under FE-SEM (JSM-6700F; JEOL Ltd., Tokyo, Japan) operating at 1.5 kV. Although the coating thickness was approximately 2 nm in this condition, we confirmed that the coating did not

change the lateral dimension of fibrils significantly. The diameter range of isolated microfibril aggregates was estimated from the diameter of 30 microfibril aggregates obtained by manual measurement.

X-ray diffraction

The purified samples and the fibril samples after the grinding treatment were subjected to X-ray diffraction measurement by the transmission method. The purified samples dried at 105 °C were pressed into pellets. For the fibril slurries, water slurry at a fiber content of 0.2 wt% was prepared, then converted by filtration into thin sheets 80 µm in thickness and dried at 55 °C. Eight-layered sheets were used for the measurement. Equatorial diffraction profiles were obtained with Ni-filtered CuK α ($\lambda = 0.154$ nm) from an X-ray generator (UltraX 18HF; Rigaku Corp., Tokyo, Japan) operating at 30 kV and 100 mA. The diffraction profile was detected using an X-ray goniometer scanning from 5 to 40°. Five samples of each were subjected to the measurement. The relative degree of cellulose crystallinity was calculated according to the Segal method (Segal et al. 1959). The average values of cellulose crystallinity were calculated from the five samples.

Tensile test

Thin sheets of the fibril samples prepared for the X-ray diffraction measurement were also used for a tensile test using a universal materials testing machine (model 3365; Instron Corp., Canton, MA) at a crosshead speed of 1 mm/min with a gauge length of 10 mm. The load cell capacity was 5 kN. The dimensions of the sheet strips were 20 mm in length by 3 mm in width by 80 µm in thickness. The ends of each specimen were covered with aluminum and clasped with serrated chucks in order to avoid damage to the specimens. The specific modulus and the specific strength were defined by dividing the experimental values of the Young's modulus and the tensile strength by the bulk density of the sheet, respectively. The average values were calculated from the five samples. The bulk density was calculated by measuring the average thickness at nine points and air-dried weight.

Results

Separation into fiber and parenchyma cells

Figure 1 shows transverse and longitudinal images of bamboo sections with a thickness of 20 µm stained with safranin using a light microscopy. Bamboo culms, except epidermal and endodermal layers, are nearly occupied by fiber cells in vascular bundle sheaths (hereinafter called fiber cells) and parenchyma cells. When the delignification treatment was performed with vigorous stirring in order to separate both cells, a number of single cells were obtained from bamboo culm as shown in Fig. 2a and b. The single cells were clearly distinguished into two different types: fibrous cells approximately 2–3 cm long and 8–20 µm wide and rectangular cells approximately 60–120 µm long and 30–50 µm wide, which were regarded mostly as fiber and parenchyma cells, respectively. Because of the notable difference in form, the cell types could be separated from each other by sieving as shown in Fig. 2c and d. Although the fragments of fiber cells were slightly observed in the group of parenchyma cells (Fig. 2d), these

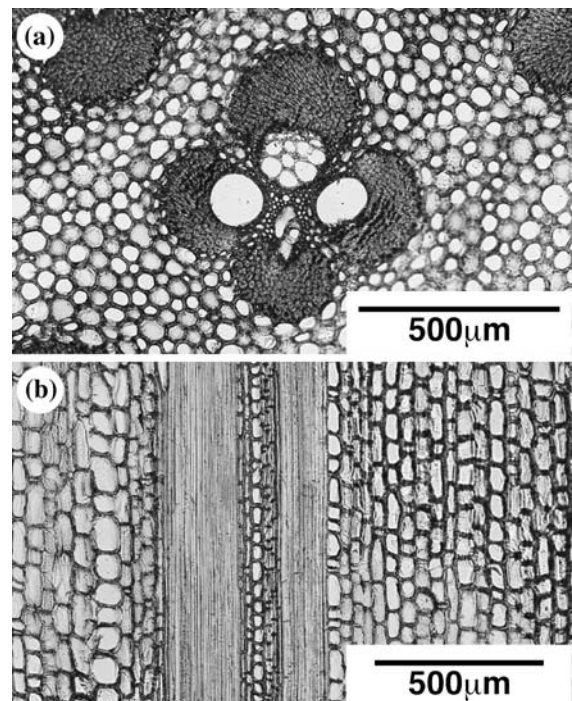
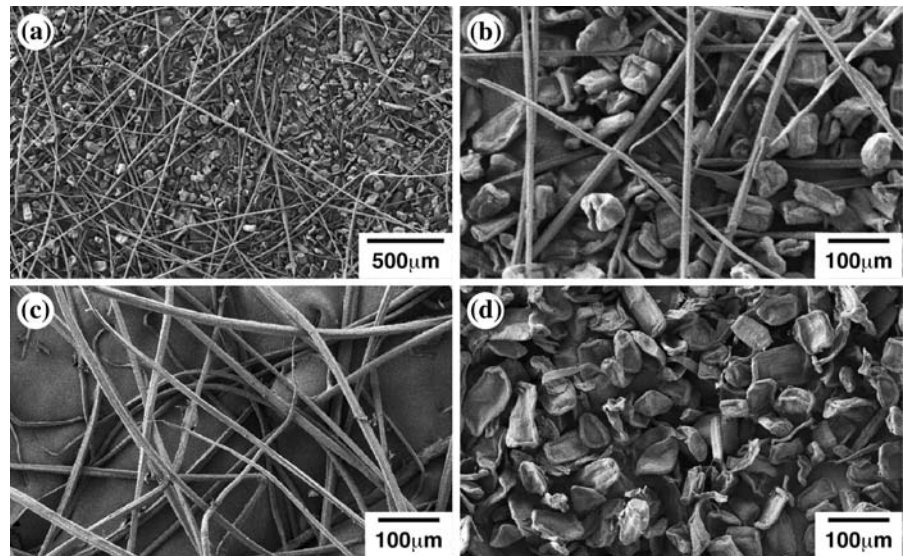


Fig. 1 Light microscopic images of **a** transverse and **b** longitudinal images of bamboo sections

Fig. 2 FE-SEM images of (a and b) single cells obtained from bamboo culm, and c fiber and d parenchyma cells separated by sieving



observations confirmed the distinct separation into fiber and parenchyma cells from bamboo culm.

SEM observations

The morphologies of the cellulose microfibril aggregates in the purified cell wall (before the grinding treatment) were compared between fiber and parenchyma cells (Fig. 3a and b). FE-SEM images showed

the similar appearance of continuous microfibril aggregates approximately 15–20 nm wide in fiber and parenchyma cells. The grinding treatment eliminated the cell wall structures and enabled the isolation of individualized microfibril aggregates from fiber and parenchyma cells. These forms of microfibril aggregates in both cell types were maintained even after the grinding treatment, as shown in Fig. 3c and d.

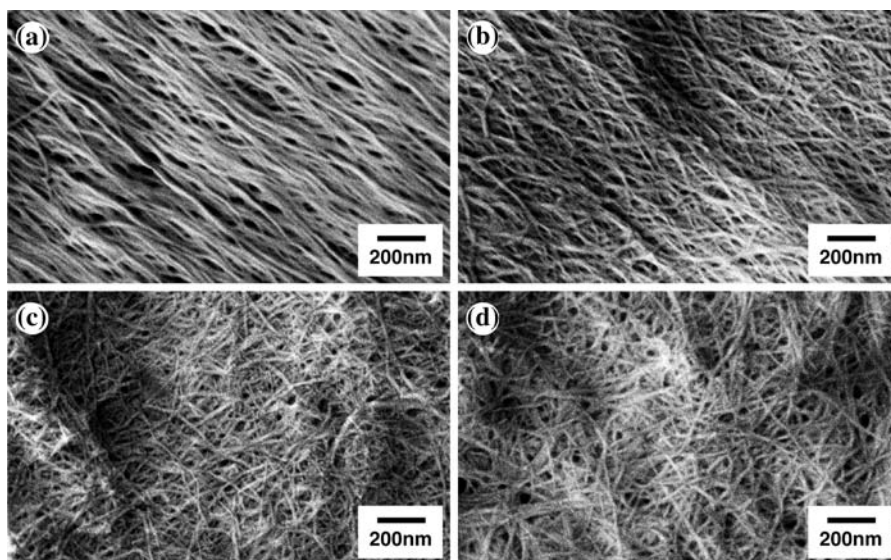


Fig. 3 FE-SEM images of the microfibril aggregates in a purified samples before isolation from a fiber and b parenchyma cells and the isolated microfibril aggregates from c fiber and d parenchyma cells

X-ray analysis

The purified samples from fiber and parenchyma cells indicated very similar α -cellulose content, which was determined to be approximately 86% by extraction with 17.5% NaOH. Therefore, X-ray diffraction measurement can fairly compare the cellulose crystallinity of the microfibril aggregate between fiber and parenchyma cells without considering the influence of amorphous noncellulosic components. Figure 4a and b show the diffraction profiles of the purified samples and the isolated microfibril aggregates, respectively. All the profiles indicated the typical Cellulose I pattern with the alkaline treatment showing little effect. Both in the purified samples and the isolated microfibril aggregates, the profiles from fiber cells were virtually identical to those from parenchyma cells. In the relative degree of cellulose crystallinity, there was also no significant difference between fiber and parenchyma cells (Table 1). In

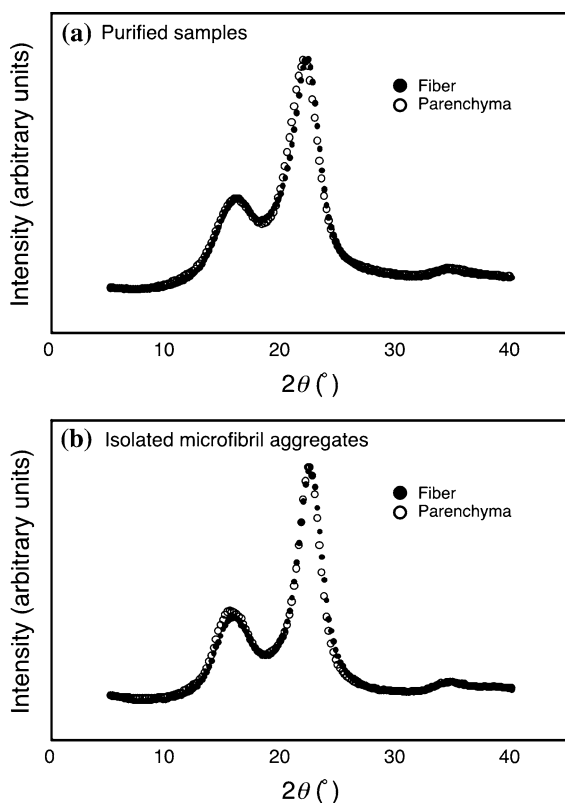


Fig. 4 Equatorial X-ray diffraction profiles of **a** purified samples and **b** isolated microfibril aggregates from fiber cells (black) and parenchyma cells (white)

Table 1 Average relative degrees of cellulose crystallinity of purified samples and isolated microfibril aggregates from fiber and parenchyma cells

	Relative crystallinity (%)	
	Purified samples	Isolated microfibril
Fiber	73 (3)	81.8 (0.7)
Parenchyma	73 (3)	84 (2)

Note: Standard deviations are given in parentheses ($n = 5$)

addition, the relative crystallinity of the isolated microfibril aggregates was higher than that of the purified samples before the grinding treatment.

Tensile test

Although it is quite difficult to perform the tensile tests on a single microfibril aggregate or tiny plant cell walls, the sheet preparation from the isolated microfibril aggregates enables a rough comparison of the tensile properties of the microfibril aggregates between fiber and parenchyma cells. However, because the sheet density varies somewhat regardless of the type of sample, causing a variation of the Young's modulus and strength, their sheet properties were compared using the specific modulus and the specific tensile strength given in Table 2. As for the specific modulus, there were no significant differences between fiber and parenchyma cells. This is also supported by Fig. 5, which shows the selected specific stress–strain curves of the microfibril sheets from fiber and parenchyma cells. The curve from fiber cells was almost coincident with that from parenchyma cells until the sheets from fiber cells fractured. On the other hand, the tensile strain on the sheets from the fiber cells was higher than that on the parenchyma cell sheet. As a result, the sheets from fiber cells exhibited higher specific strength than that from parenchyma cells.

Discussion

In the purified fiber and parenchyma cell walls, microfibril aggregates with similar widths, approximately 15–20 nm, were observed (Fig. 3a and b). However, these images show only the surface layer of the cell walls. The SEM observations in Fig. 3c and d show that the one-time grinding treatment in an

Table 2 Average mechanical properties of microfibril aggregates sheets from fiber and parenchyma cells

	Density (g/cm ³)	Young's modulus (GPa)	Specific modulus (GPa)	Tensile strength (MPa)	Specific strength (MPa)
Fiber	1.26	11.0 (0.7)	8.7 (0.5)	200 (10)	160 (10)
Parenchyma	1.35	12.2 (0.7)	9.0 (0.5)	198 (5)	147 (3)

Note: Standard deviations are given in parentheses ($n = 5$)

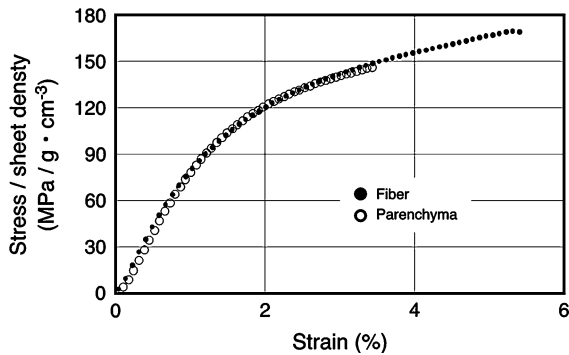


Fig. 5 Selected specific stress–strain curves of the microfibril aggregate sheets from fiber (black) and parenchyma cells (white)

undried state uniformly isolated the microfibril aggregates from the fiber and parenchyma cell walls and that both the cell walls consist entirely of the microfibril aggregates with a similar width of 15–20 nm. These values agree rather well with those obtained by Crow and Murphy (2000). In our previous paper (Abe and Yano 2009), the thicker aggregates of 35–55 nm in thickness were observed in the cell walls of rice straw and potato tuber, but bamboo culms did not have such thick aggregates.

Cellulose crystallinity is particularly valuable in determining the mechanical properties of the microfibril aggregates in cell walls. For both the purified cells and the isolated microfibril aggregates, the crystallinity was almost the same between fiber and parenchyma cells (Table 1). Considering the very similar α -cellulose contents in both the cell types, these results suggest that the cellulose microfibrils synthesized in fiber and parenchyma cell walls exhibit much the same cellulose crystallinity in the dry state. The higher crystallinity of the isolated microfibril aggregates is most likely caused by the fact that the filtration for sheet preparation eliminated the fraction produced by the grinding treatment. Accordingly, this increment does not mean the

microfibrils themselves have higher crystallinity. Furthermore, this result and the reports by Iwamoto et al. (Iwamoto et al. 2007) and in our previous paper (Abe and Yano 2009) suggest that the one-time grinding treatment minimizes the deterioration of and damage to the microfibrils in cell walls. This suggestion is corroborated by the similar results between tracheids of softwood and parenchyma cells of potato tubers in our previous paper (Abe and Yano 2009). However, it does not necessarily mean that the undried microfibrils in the natural state show the same characteristics between fiber and parenchyma cells.

For the sheets of the microfibril aggregates isolated from fiber and parenchyma cells, both the tensile curves were closely matched (Fig. 5). In particular, this coincidence in the elastic region implies that the cellulose microfibrils in fiber and parenchyma cells have the same longitudinal Young's modulus in the dry state. However, because the sheet properties are greatly affected by the bonding pattern and strength in the sheets, the similar conclusion does not necessarily apply to the case of tensile strength of the single cellulose microfibrils. Actually, the sheets with lower density from fiber cells showed higher strain and tensile strength than that from parenchyma cells because of the structural elongation of the sheets (Fig. 5).

Conclusion

In this study, the cells of mature bamboo (*Phyllostachys pubescens*) were separated into fiber and parenchyma cells, and then the cellulose microfibril aggregates isolated from each type of cells were compared based on their morphological and physical properties. The results of SEM observations and X-ray analysis showed that the one-time grinding treatment provided nearly natural microfibril

aggregates of uniform width, 15–20 nm, from fiber and parenchyma cells. Moreover, the cellulose microfibrils isolated from fiber and parenchyma cells had almost the same morphology, cellulose crystallinity, and longitudinal Young's modulus. This suggests that the cellulose microfibrils synthesized in fiber and parenchyma cell walls are identical, and that the inherent characteristics of the cellulose microfibrils in the dry state correlate poorly with the cell functions in the same individual. This suggestion is corroborated by similar results in our previous paper (Abe and Yano 2009), between tracheids of softwood and parenchyma cells of potato tubers. However, it does not necessarily mean that the undried microfibrils in the natural state show the same characteristics between fiber and parenchyma cells. Given some indication that heat or drying treatments increase the crystallinity of wood cellulose (Bhuiyan et al. 2000; Rayirath et al. 2008; Yamamoto et al. 2005), it is quite likely that the ambient conditions, including moisture content, in cell walls control the crystallinity of the cellulose microfibril carrying out the cell functions.

Acknowledgments This work was supported by Grant-in-Aid from Research Fellowships of the Japan Society for the Promotion of Science for Young Scientists.

References

- Abe K, Yano H (2009) Comparison of the characteristics of cellulose microfibril aggregates of wood, rice straw and potato. *Cellulose* doi: [10.1007/s10570-009-9334-9](https://doi.org/10.1007/s10570-009-9334-9)
- Abe K, Iwamoto S, Yano H (2007) Obtaining cellulose nanofibers with a uniform width of 15 nm from wood. *Biomacromolecules* 8:3276–3278
- Bhuiyan MTR, Hirai N, Sobue N (2000) Changes of crystallinity in wood cellulose by heat treatment under dried and moist conditions. *J Wood Sci* 46:431–436
- Crow E, Murphy RJ (2000) Microfibril orientation in differentiating and maturing fibre and parenchyma cell walls in culms of bamboo (*Phy ZZostachys viridiglaucescens* (Cam.) Riv. & Riv.). *Bot J Linnean Society* 134:339–359
- Donaldson L (2007) Cellulose microfibril aggregates and their size variation with cell wall type. *Wood Sci Technol* 41:443–460
- Fahlén J, Salmén L (2005) Pore and matrix distribution in the fiber wall revealed by atomic force microscopy and image analysis. *Biomacromolecules* 6:433–438
- Fratzl P, Burgert I, Gupta HS (2004) On the role of interface polymers for the mechanics of natural polymeric composites. *Phys Chem Chem Phys* 6:5575–5579
- Iwamoto S, Nakagaito AN, Yano H (2007) Nano-fibrillation of pulp fibers for the processing of transparent nanocomposites. *Appl Phys A* 89:461–466
- Iwamoto S, Abe K, Yano H (2008) The effect of hemicelluloses on wood pulp nanofibrillation and nanofiber network characteristics. *Biomacromolecules* 9:1022–1026
- Rayirath P, Avramidis S, Mansfield SD (2008) The effect of wood drying on crystallinity and microfibril angle in black spruce (*Picea mariana*). *J Wood Chem Technol* 28: 167–179
- Segal L, Creely JJ, Martin AE, Conrad CM (1959) An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. *Textile Res J* 29:786–794
- Wise LE, Murphy M, D'Addieco AA (1946) Chlorite holo-cellulose, its fractionation and bearing on summative wood analysis and on studies on the hemicelluloses. *Paper Trade J* 122:35–43
- Yamamoto H, Abe K, Arakawa Y, Okuyama T, Gril J (2005) Role of the gelatinous layer (G-layer) on the origin of the physical properties of the tension wood of *Acer sieboldianum*. *J Wood Sci* 51:222–233