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Kyoto University
Synchrotron X-ray Diffraction Analysis of Cellulose in Developing Xylem Cell Walls from Cryptomeria japonica*1

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Synchrotron radiation available today is such a powerful source for X-ray diffraction analysis that a micrometer size probe can be used to obtain diffraction diagrams from nano-gram objects because of its high flux and coherency1-3). In this study, resin-embedded semi-thin sections of wood cells were subjected to this method in order to evaluate the local change of cellulose structure during development of xylem cells.

A small wood block trimmed from a 9 year-old Cryptomeria japonica D. Don was fixed with 3% GA and embedded in epoxy resin. Oblique-radial sections of 20 µm thick tilted approximately 5° were cut and mounted on electron microscope grids. Sections were carefully observed by in-line video microscope, and the area including differentiating xylem cells was line-scanned by X-ray beam at the positional resolution of 2 µm (Fig. 1). All the measurement was performed at ID 13 beam line, European Synchrotron Radiation Facility.

Diffraction diagram at each position was radially integrated to measure the diffraction intensity profile, from which the crystallographic parameters, such as d-spacing, intensity, line-breadth were estimated. The integrated intensity of the strongest equatorial reflection 200 in each profile was first examined as a measure of cellulose content in the actual illuminated area of the sample. The cellulose content of the cell walls increased upon further development of the tracheid cell walls. However, in the first 100 µm of developing xylem tissue from cambium there are few diffraction signal of cellulose detectable. The primary wall was further investigated by electron diffraction as shown in Fig. 2, but in the diagram, only the meridional 004 reflection appears clearly, the other characteristic equatorial reflections of cellulose were not detectable. This implies that the cellulose is well organized along the chain but considerably distorted in lateral direction, which is in good agreement with previous diffraction data from the primary walls4,5). The result was somewhat controversial to the previous FT-IR microscopy investigation6,7) in that primary wall region was reported as containing better crystalline cellulose than the secondary wall and equal amount of crystalline allomorphs of Iα and Iβ.

The 200 equatorial reflection of secondary wall cellulose was further analyzed in terms of the radial reflection width, which can be related to a microfibril diameter by Scherrer formula. It should be noted that also other effects may lead to a line broadening, thus, the estimated apparent

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Fig. 3. Estimated crystallite size from SR X-ray profiles. Arrow shows start point of S₂ deposition. S₁ beginning region gives slightly smaller values than matured S₂ dominant region.

Particle size is just a lower limit for the real dimension. In Fig. 3 data from the start of S₁ formation to maturation of S₃ were presented. Although there is a considerable mean deviation of the values due to uncertainties of the profile fits, the average shows a tendency of larger microfibril size with further development of the secondary cell walls.

References