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**Original article**

# Radial Variation of Microfibril Angle and Cell Wall Thickness in *Eucalyptus camaldulensis* Clones

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## ABSTRACT

Five superior clones and seedlings of *Eucalyptus camaldulensis* at a site in the Sakaew plantation in eastern Thailand were selected for determining the radial variation of the microfibril angle and cell wall thickness. The results revealed that the microfibril angle across the radial gradient of every sample slightly decreased with fluctuation from the pith to the bark but on the other hand, the cell wall thickness steadily increased from the pith to the bark. Secondly, the mean microfibril angle of all clones was smaller than in the seedlings, indicating that the wood properties of clones were better than those of seedlings. Thirdly, the thinner cell wall and the larger microfibril angle were found in the region of all samples which was nearer the pith than the bark.

**Keywords:** radial variation**,** microfibril angle, cell wall thickness, *Eucalyptus camaldulensis*

## INTRODUCTION

*Eucalyptus camaldulensis* is one of the potential plantation species in Thailand. Seedlings or young plants that are used to establish plantations are generally obtained from seeds or cuttings. The mass production of young plants through tissue culture technology was begun in 1989 by a group in the Faculty of Forestry, Kasetsart University. In 1992, they succeeded in establishing young plants from tissue culture, after naturalizing and planting them at Sakaew plantation (Siripatanadilok and Thaiutsa, 1992).

The main purpose of *E. camaldulensis* plantations in Thailand is to provide raw material for the pulp and paper industry and for the wood composite board industry. However, they are also expected to provide lumber wood in the future. Therefore, information on the wood properties of *E. camaldulensis* cloned from tissue culture is needed.

The mean microfibril angle in the $S_2$ layer of the secondary wall is one of the basic structural characteristics of the wood cell-wall that influences the physical properties of wood such as dimensional stability (Yamamoto *et al.*, 1993). Meylan and Probine (1969) and Cave (1997) reported that the mean orientation of the cellulose microfibril in wood tissue is a fundamental parameter of the wood cell-wall structure that has major relevance to the mechanical properties of wood and wood products. Stuart and Evans (1995); Sahlberg *et al.*(1997) and Reiterer *et al.* (1998) reported that the fibrillar orientation in the $S_2$ layer to a great extent determines the mechanical properties of the wood fiber, with regard to the strength, stiffness and swelling properties. These properties influence the properties of paper products.

The microfibril angle can be measured by a wide range of techniques, such as the angle of the slit pits, polarizing microscopy, electron microscopy, fluorescent microscopy, iodine staining, and X-ray diffraction. All these techniques (except X-ray diffraction) are tedious and time consuming because measurements have to be made on a large number of individual cells to give a meaningful average. X-ray diffraction, on the other hand, is simple, and fairly quick as it involves little observer time and for a beam of 0.25 mm diameter and a 1 mm specimen, it gives a measure of the mean angle of a few hundred cells (Cave, 1966, 1997; Meylan, 1967; Andersson *et al.*, 2000).

There are many studies on the microfibril angle in the genus *Eucalyptus* by the X-ray diffraction technique. Boyd (1980) studied the relationship between growth strain and the microfibril angle of *E. regnans*; Yoshida *et al.* (1992) investigated the growth stresses of branches and the microfibril angle in *E. rubida*; Stuart and Evans (1995) investigated variation of the microfibril angle in *E. nitens*; and Wimmer *et al.* (2002) determined variation of the microfibril angle in *E. nitens* grown under different irrigation regimes. All of these studies measured the microfibril angle using X-ray diffraction. However, investigation of the microfibril angle in *E. camaldulensis* clones has not been reported.

In this report, radial variation of the microfibril angle in *E. camaldulensis* clones and a seedling were compared and the data were analyzed with regard to the cell wall thickness.

## MATERIALS AND METHODS

Five superior clones of five-year-old *E. camaldulensis* at a plantation site in Sakaew province in eastern Thailand were selected as sample trees. The name of the clones were T5, Kitti, S9, Y2, and K2 and they were coded in this report as clones 1, 2, 3, 4 and 5, respectively. A nine-year-old seedling in the same plantation was selected for comparison. The average diameter at breast height over bark (dbh) of clones 1, 2, 3, 4, 5, and the seedling were 17.67, 16.88, 17.40, 17.27, 18.43, and 19.46 cm, respectively. Five-cm-thick disks were cut from each sample tree at 50 cm above the ground level.

**Measurement of microfibril angle by X-ray diffraction**

A radial strip was divided into 1×1×1 cm blocks from the pith to the bark. Blocks were softened with boiling water and 200 μm tangential sections in thickness were cut using a sliding microtome. The sections were pressed using large paper clips and dried at 35°C for 1 hour in an oven. The tangential samples were analyzed with a Rigaku X-ray diffractometer. A point-focused X-ray beam (Cu-Kα X-ray, power 30 mA, 40 kV, beam diameter 1 mm) was applied to the tangential surface of each sample. Diffraction patterns were recorded on an imaging plate with an exposure time of 2 min. Data of the diffraction image were obtained by a Ringaku RAXIS II imaging plate reader (Nishiyama *et al*., 2000).

As shown in Figure 1, the angle T was obtained from a diffraction intensity distribution around the (002) arc. Cave's method gives the mean microfibril angle using the formula:

$$MFA = 0.6 \times \text{Angle T}$$

where:

MFA = microfibril angle (degrees)

Angle T = angle is predicted in Figure 1, which is determined in the (002) diffraction arc (degrees)

0.6 = conversion constant

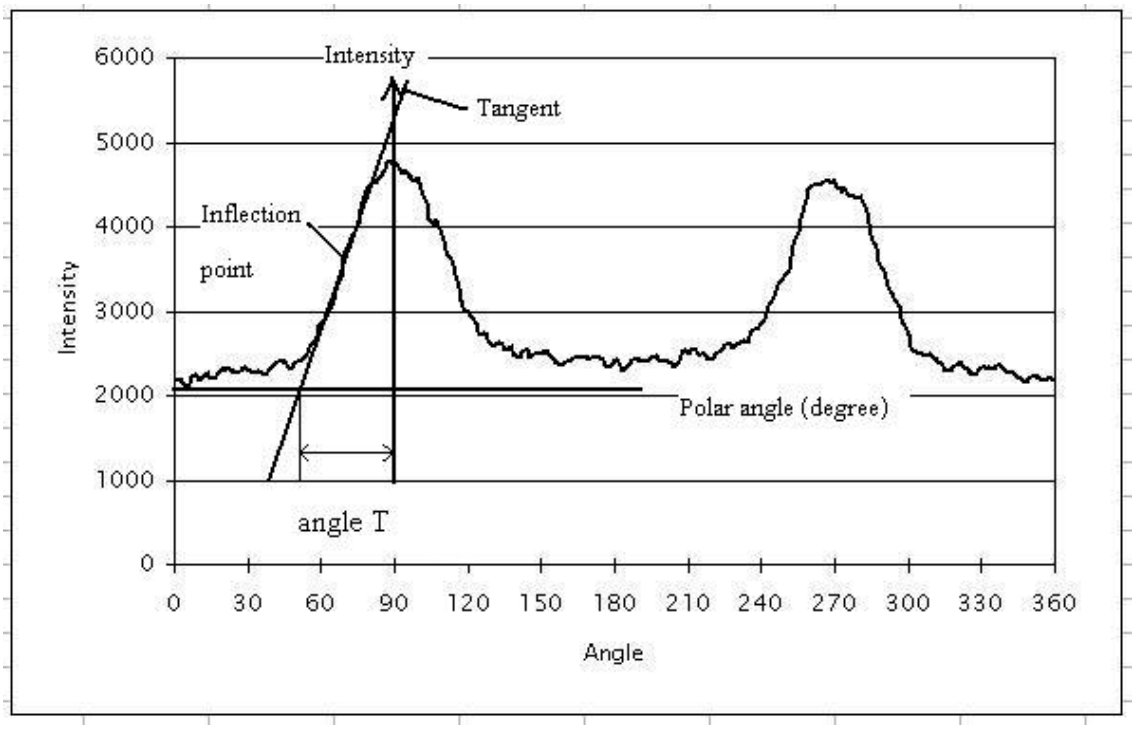


**Figure 1** Measurement procedure of angle T from a (002) reflection**.**

**Cell wall thickness**

To determine the cell wall thickness, wood specimens (T×R×L) 2×2×5 mm were sampled near the pith, in the middle between the pith and the bark, and near the bark. Wood specimens embedded in epoxy resin and 3 μm transverse sections in thickness were cut using a rotary microtome equipped with a glass knife. Sections were stained with 0.01% safranin in water and mounted as permanent slides with Canada balsam. The measurements were made at a magnification of 100×with a light microscope. The tangential cell wall thickness was determined by measuring the radial direction of the total width of the two common cell walls of three adjoining fibers and the intervening lumen (Figure 2). To obtain the thickness of a single cell wall, the lumen measurement was subtracted from the overall measurement and the difference was divided by 4. Fifty determinations were made at random over the entire width of sections (Hiller 1964).

From Figure 2, the cell wall thickness was determined from the formula:

$$\text{Cell wall thickness} = \frac{A - B}{4}$$

where :

A = the total width of two common cell walls of three adjoining fibers and the intervening lumen

B = the width of the cell lumen

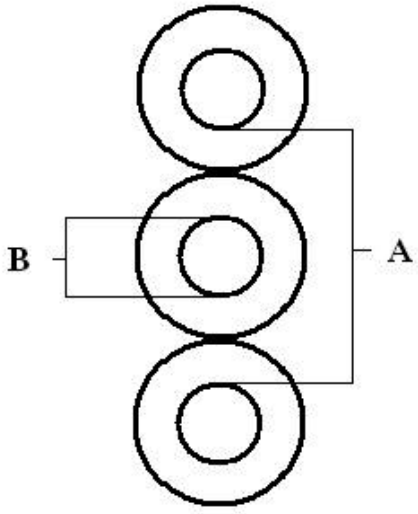


**Figure 2** Total width of two common cell walls of three adjoining fibers and the intervening lumen (A) and the cell lumen (B).

## RESULTS AND DISCUSSION

### Microfibril angle (MFA)

Clone 1 showed the smallest mean MFA of 18.0°, followed by clones 4, 2, 5, 3, and the seedling with 19.8°, 20.7°, 21.6°, 22.8° and 24.1°, respectively (Table 1). The variation among the five clones and the seedling was statistically significant at the 0.05 level, namely clone 1 was different from the seedling and clone 3 but not different from clone 4, clone 2, and clone 5 (Table 1). The MFA across the radial gradient of every sample slightly decreased with fluctuations from the pith to the bark but the MFAs near the bark of clone 4 and clone 3 were a little larger than those which were near the pith (Figure 3). This result was in good agreement with the data of Stuart and Evans (1995) who reported that the radial variation of the MFA in *E. nitens* decreased from the pith to the bark .

Figure 5 shows the brightness distribution curves obtained along the (002) arc in X-ray fiber diagrams near the pith, in the middle between the pith and the bark, and near the bark in clone 1 and Figure 6 shows the same in the seedling. The results mentioned above revealed that the MFA near the pith was larger than near the bark and the MFAs in the clones were smaller than in the seedling.

### Cell wall thickness

The seedling had the thickest mean cell wall of 3.0 μm but the variation in the cell wall thickness among the five clones and the seedling was not significantly different (Table 1). The radial variation in the cell wall thickness of all samples steadily increased from the pith to the bark (Figure4).

### Correlation of MFA with cell wall thickness

The investigation of the microfibril angle and the cell wall thickness mentioned above revealed that the MFA near the pith was larger than that near the bark. On the other hand, the cell wall thickness near the pith was thinner than near the bark. This implied that a thin cell wall and large MFA were found in the region near the pith whereas near the bark, we could find thick cell walls and small MFAs. These results corresponded to Hiller (1964) who found that the fibril angles in slash pine and loblolly pine were large in the thin cell walls. Tomolang *et al.*(1967) determined fiber strength and stiffness of holocellulose fibers in 17 tropical hardwoods. They found these parameters to be highly related to the cell-wall area and the fibrillar angle, and that the fiber strength per unit area of the cell wall was strongly influenced by the fibrillar angle.

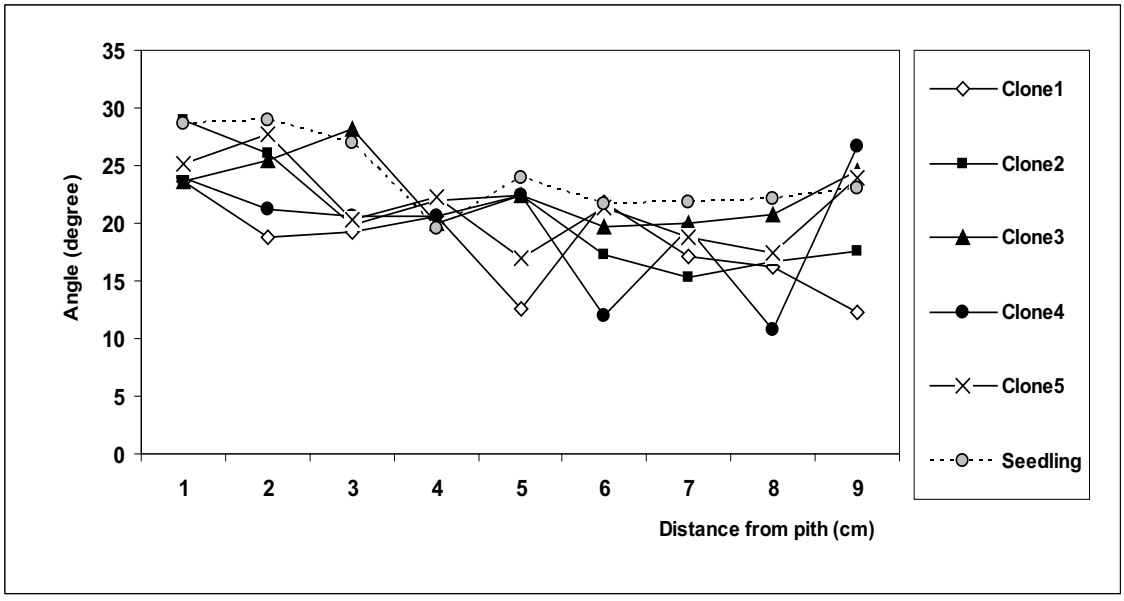


**Figure 3** Radial variation of microfibril angle of five clones and one seedling.

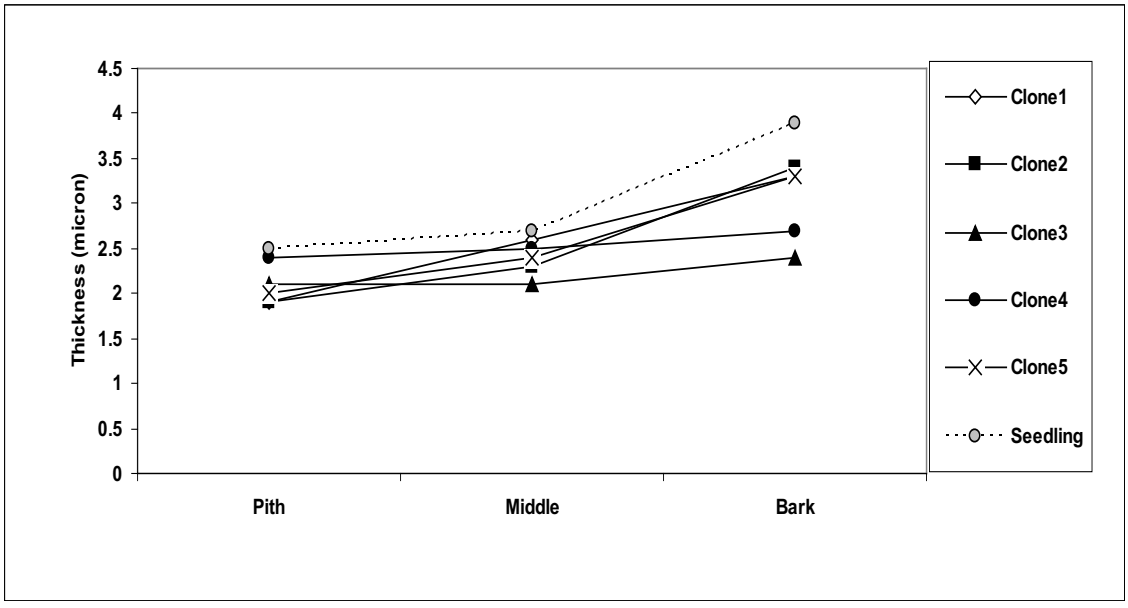


**Figure 4** Radial variation of cell wall thickness of five clones and one seedling.

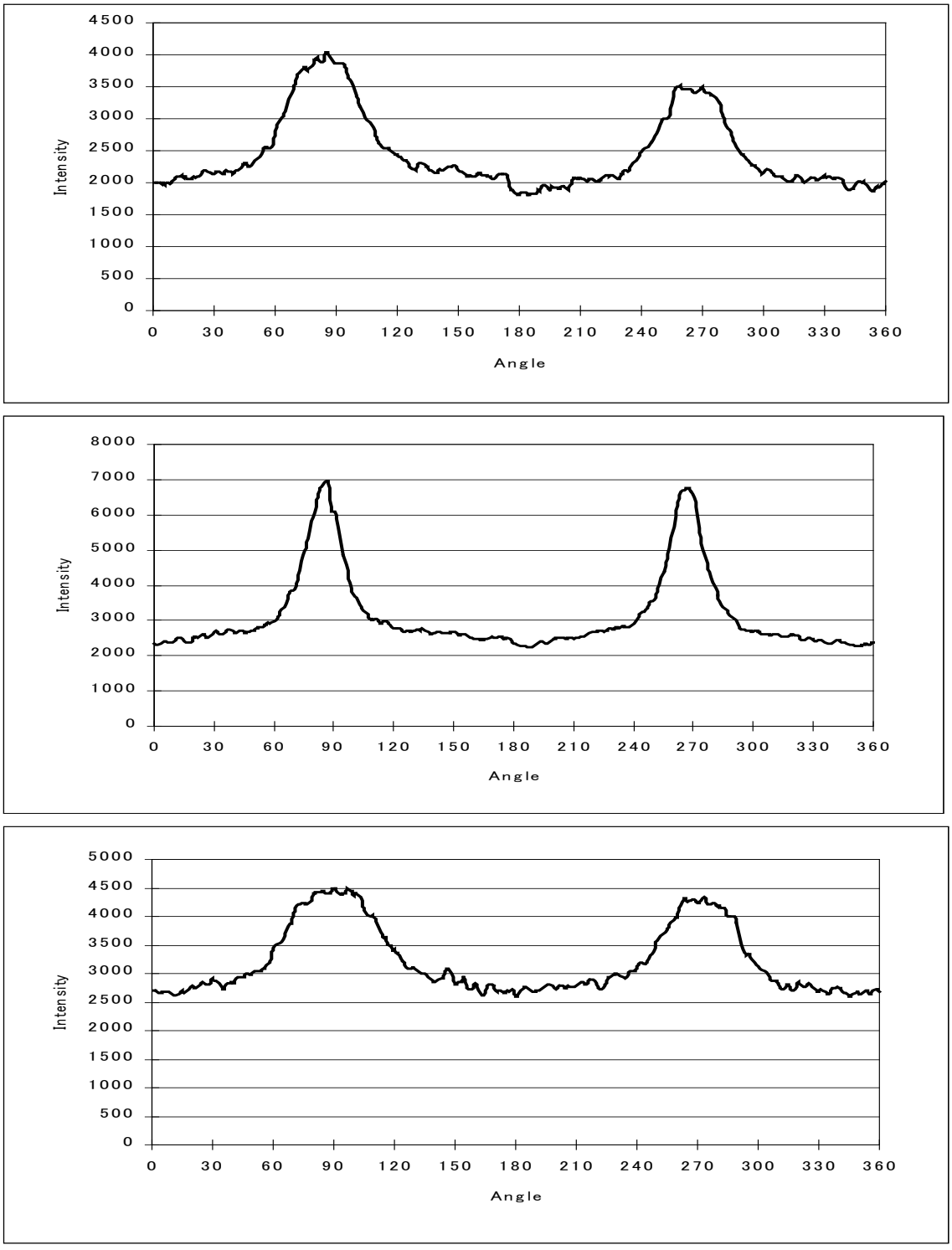


**Figure 5** Brightness distribution curves obtained along the (002) arc in X-ray fiber diagrams near pith (a), in the middle (b) and near bark (c) in clone 1.

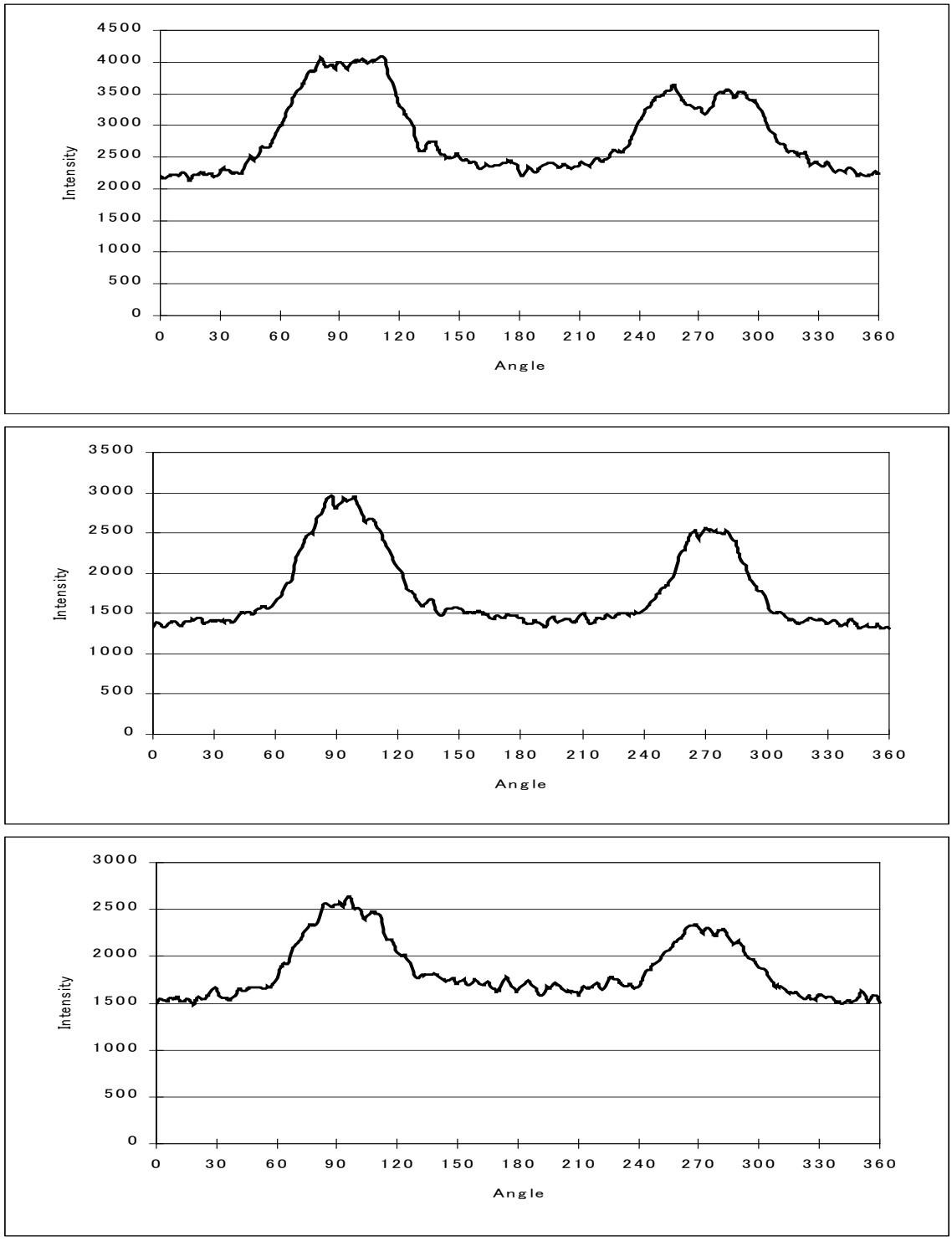


**Figure 6** Brightness distribution curves obtained along the (002) arc in X-ray fiber diagrams near pith (a), in the middle (b) and near bark (c) in the seedling.

**Table 1** Data and analysis of variance of microfibril angle (MFA) and cell wall thickness (CT) of five clones and one seedling.

| Clone | MFA (degrees) | | CT (µm) | |
|---|---|---|---|---|
| | Mean<br>Min - Max | S.D. | Mean<br>Min - Max | S.D. |
| 1 | $18.0^{c}$<br>12.3-23.6 | 3.88 | $2.6^{ns}$<br>1.9-3.3 | 0.70 |
| 2 | $20.7^{abc}$<br>15.3-29.0 | 4.59 | $2.5^{ns}$<br>1.9-3.4 | 0.78 |
| 3 | $22.8^{ab}$<br>19.7-28.2 | 2.96 | $2.2^{ns}$<br>2.1-2.4 | 0.17 |
| 4 | $19.8^{bc}$<br>10.8-26.6 | 5.20 | $2.5^{ns}$<br>2.4-2.7 | 0.16 |
| 5 | $21.6^{abc}$<br>17.0-27.8 | 3.64 | $2.6^{ns}$<br>2.0-3.3 | 0.67 |
| Seedling | $24.1^{a}$<br>19.5-29.0 | 3.38 | $3.0^{ns}$<br>2.5-3.9 | 0.76 |

**Notes:** S.D. = standard deviation.
ns = not significant.

Means with common letters are not significantly different at the 0.05 probability level as determined by Duncan's separation procedure.

**Discussion**

Based on this implication, it was considered that the wood properties near the bark were better than those near the pith. The physical and mechanical properties of clone 1 were the best, followed by clones 4, 2, 5, 3, and the seedling, respectively. Therefore, this might indicate that the wood properties of the clones were better than those of the seedling because the mean microfibril angle of the clones was smaller than that for the seedling. Andersson *et al*. (2000) reported that the microfibril angle was an important property of wood cells. A high MFA of the $S_2$ layer resulted in low stiffness and increased the longitudinal shrinkage of wood. Sahlberg *et al.* (1997) reported that the mechanical properties of wood fibers were highly dependent on the arrangement of the cellulose microfibrils within the different cell wall layers of the fiber. The smaller the angle between the orientation of the fibrils and the fiber axis, the stronger and stiffer the wood fibers were.

Although the age of the studied clones and seedling were different, all samples had the same diameter sizes and cell wall thicknesses with no significant difference.

## CONCLUSION

The results of radial variations in the microfibril angle and cell wall thickness were summarized as follows:

1. The microfibril angle across the radial gradient of every sample decreased slightly with some fluctuation from the pith to the bark, while the cell wall thickness steadily increased from the pith to the bark.

2. The mean microfibril angle in the clones was smaller than in the seedling which indicated that the wood properties of the clones might be better than those of the seedling.

3. A thinner cell wall and larger microfibril angle were found in all samples near the pith than near the bark.

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