Growth of Bamboo Shoots Measured using X-ray Diffraction

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The purpose of the present study is to investigate the changes in the internode length and cellulose crystallinity of moso bamboo (Phyllostachys pubescens Mazel) cell walls during the growth process of bamboo, including a few days growth young bamboo shoots. Specimens were prepared from bamboo culms that either continually grew throughout the growth period or stopped elongation growth in the course of growing. Cellulose crystallinility showed clear differences between above two types of bamboo culm regardless of growth period, while no significant changes were observed in internode length in each growth periods of bamboo culms. Crystallinity of continually growing bamboo culms increased from lower to higher internodes with the progress of growth. On the other hand, crystallinity of culms that stopped elongation growth remained relatively lower values compared to the former type even though the growth period was same. These microscopic features of bamboo cell walls may help to predict whether bamboo culms will stop their elongation growth.

Key words: Bamboo shoot, Crystallinity, Maturation, Stopped elongation growth, Self-thinning

1. INTRODUCTION

Bamboo is monocotyledonous plants in the family Gramineae found in tropical, subtropical, and temperate regions around the world [1]. The main stem of bamboo culm is lignified during growth process without secondary growth in thickness [2]. Despite the current availability of many artificial materials, various daily necessities are still made of bamboo in various fields in recent years, including laminated bamboo timber for architecture member, filling materials for bamboo fiber composites, and base materials for bioethanol [3–5].

Bamboo is among the fastest growing plants because the internodal growth occurs at the growth zone in all internode, and thus, the elongation growth of bamboo culm is quite rapid during its growth season. Nomura [2] reported that moso bamboo (Phyllostachys pubescens) and madake bamboo (Phyllostachys bambusoides) can grow more than 1 m/day. In addition, many bamboo shoots grow in bamboo forest every year. Because of these growth properties, bamboo is considered one of the valuable biomass resources, even though the rates of the bamboo forest in the total forest are relatively very low.

Some studies investigated microscopic changes in bamboo cell walls during growth process using X-ray techniques to measure changes in cellulose crystallinity, crystal size, and microfibrils angle [6–8]. In particular, Toba et al. [8] traced the changes in the crystallinity throughout the eleven growth stages, including short bamboo shoots.

However, these previous studies have focused on bamboo culms which continually grew and never stop their elongation growth during the growth process, although more than half of bamboo shoots stop their elongation in the growth process [1]. In the case of moso bamboo, as many as 40–80% of them in bamboo forest are thinned and die off during the growth season. Katanoda [9] suggested that the oral setae of bamboo shoots showed different colors between the continually growing one and dying. This variation in appearance is useful for harvesting and forestry management. However, few studies have investigated the cell walls properties of dying bamboo culms in microscopic region.

The purpose of this study is to investigate the self-thinning tendency of bamboo shoots. To this end, we compared the cellulose crystalline features of bamboo culms that either continually grew or stopped elongation growth in various growth periods.

2. EXPERIMENTAL

2.1 Specimen preparation

The studied specimens were prepared from culms of moso bamboo (Phyllostachys pubescens Mazel) obtained from the bamboo forest in Matsue (35.26°N, 133.50°E), Japan. On April 23, 2012, we selected dozens of freshly bamboo shoots which emerged a few centimeters off the ground, and sequentially harvested them at intervals of 2, 9, 15, 18, 23, and 43 days after selection. After cutting the bamboo culm from the rhizome, all specimens were immediately covered with plastic wrap to prevent drying. To avoid decaying and dimensional changes due to drying, specimens were temporarily stored in 50% (v/v) aqueous ethanol solution.

All internodes of obtained bamboo culms were numbered from the base to the top except the bamboo base, as shown in the left of Fig. 1 which presented the process flow of the specimen preparation. Flat-sawn specimens with dimensions 1 × 10 × 10 mm (thickness × length × width) were taken from the exterior of each internode using a saw, chisel, and cutter knife under the wet conditions to prevent shrinkage.
The cellulose crystallinity of bamboo cell walls was calculated using the Segal method [10]. The dried using a vacuum freeze dryer (VD-400, Taiyo Kagaku Co., Ltd., Japan) to maintain cell walls structure.

2.2 Measurement of internode length
The internode lengths of bamboo were measured using image processing software (QuickGrain Standard, Inotech Co., Japan). All bamboo culms were cut in half in longitudinal direction to obtain the digital image of the vertical section.

2.3 X-ray diffraction measurements
X-ray diffraction (XRD) measurements were performed to assess the cellulose crystalline properties of bamboo cell walls using an X-ray diffractometer (XD-Dw, Shimadzu Co., Japan). All measurements were performed by the reflection technique using characteristic Cu Kα X-ray passing through a nickel filter with a power of 30 kV and 30 mA. Both the air-scattering prevention slit and the divergence slit were 1°. The width of the detection slit was 0.1 mm, and scanning speed and integration time in the measurements of crystallinity were 2.0°/min and 2.0 s, respectively. Scanning ranges of 2θ angle was 5.0–40.0°. Flat-sawn specimens obtained from each internode were vertically fixed on the specimen holder of the XRD device to irradiate the tangential section.

The crystallinity of the crystalline cellulose in bamboo cell walls was calculated using the Segal method [10]. The Segal method is widely used and simple method which does not require the separation of reflection peaks.

3. RESULTS AND DISCUSSION
3.1 Variability in internode length during the growth process of bamboo
Typical results of the internode length in each growth period of bamboo culm were shown in Fig. 2. For each growth period, some bamboo culms were obtained to measure internode lengths in each internode number. Growth periods were 2, 9, 18, 23, and 43 days, and the height were 19, 102, 263, 399, and 1540 cm. In young specimens, internode lengths were greatest in lower internodes (internode number < 10), while that of the older specimens were in high internodes. Thus, internode lengths of bamboo culm gradually increased from the lower internodes in their growing process. This tendency of bamboo growth coincides with previous study [2, 6, 8], however, these studies seemed to be based on the data of bamboo culms which continually grew and does not stop the elongation growth.

Some bamboo specimens in this study grew to more than 10 meters in height for about a month, while others seemed to stop the elongation growth during the growth season. The comparison of internode lengths between two types of bamboo culms in each growth period is shown in Figs. 3. Because the height of specimens had clear differences in the same growth period, it was possible to determine if the bamboo culm stopped elongation growth (Figs. 3c, d). Specimens shown by open circles in Fig. 3c must stop their growing. However, considering only from the internode length, it was difficult to determine whether bamboo culms had stopped elongation growth in the case of young specimens of less than 10 days old (Figs. 3a, b).

3.2 Variability in cellulose crystallinity during the growth process of bamboo
To investigate bamboo’s self-thinning tendencies, the cellulose crystallinity of bamboo cell walls was detected with XRD technique. Typical results of the crystallinity changing in each growth period of bamboo culm were shown
Fig. 3 Comparison of internode length of bamboo culms for each growth period. Growth periods in (a), (b), (c), and (d) were 2, 9, 18, and 23 days, respectively. Open circles represent culms that had stopped elongation growth and filled circles represent culms that continued growing.

Specimens for XRD measurement were prepared from the same one of internode length for each growth period. In young bamboo shoots, the crystallinity was greatest in lower internodes. This microscopic feature of crystalline cellulose in cell walls was already observed in two-day growth bamboo shoots. With the growth of bamboo culms, increasing in crystallinity was also observed in higher internodes. At the same time, gradual increasing in crystallinity progressed in all internodes. These tendencies in microscopic region suggested that increasing in crystallinity in bamboo cell walls progressed from the lower to higher internodes during growth process of bamboo culms.

Furthermore, for bamboo culms that stopped elongation growth during the growth season, cellulose crystallinity was measured at each internode and variation was compared in each growth periods as shown in Figs. 5. Growth periods of specimen were same as internode lengths (Figs. 3). Filled circles in Figs. 5 were results of specimens which seemed to maintain growing, because they tended to have higher crystallinity values especially in lower parts of bamboo culm in all growth periods. Therefore, we postulate that relatively low crystallinity in bamboo cell walls comparing to the same growth periods of other one contributed to halting elongation growth in our specimens. Moreover, this tendency was also observed in specimens with a short growth period of only two-day growth bamboo shoot (Fig. 5a), while no significant changes were observed in internode length over this time (Fig. 3a). Thus, self-thinning tendencies of bamboo culm may be related to the changes in cellulose crystallinity of its cell walls. In addition, the crystallinity of two-day growth bamboo culm that stopped elongation growth (open circles in Fig. 5a) showed almost constant values throughout whole bamboo culm, and was greater than the crystallinity of continually growing bamboo (filled circles in Fig. 5a) in higher internodes. This suggests that crystallinity of all bamboo culms kept increasing even if lower internodes did not sufficiently increase crystallinity during the growth period.

Furthermore, another interesting case of bamboo culms which stopped elongation growth was observed in 15-day growth specimen as shown in Fig. 6. Among the two bamboo culms with over 1.4 m in height, 86 cm higher one (open circles in Fig. 6) showed the tendencies of stopping elongation growth, that is, the cellulose crystallinity in the higher internodes became almost the same values as lower internodes even though the crystallinity was still as low as 20 %. In this case, it was impossible to make accurate
evaluation of the bamboo growth without detection of cellulose crystalline feature. In addition, these values of 15-day growth bamboo culm were slightly higher than in the two-day growth, thus the self-thinning tendencies of bamboo can occur regardless of growth period. Moreover, elongation growth of bamboo culm likely stops due to some factors not considered here, such as delayed growth and environmental changes. However, it is still difficult to conclude what plays leading role in the self-thinning tendencies of growing bamboo. Further investigation is required to understand whole picture of bamboo growth in microscopic level.

4. CONCLUSION

Internode length and cellulose crystallinity of bamboo culms including young bamboo shoots were measured and differences between culm types that either stopped or continued elongation growth were investigated. Within the same growth period, bamboo culms that stopped elongation growth had lower crystallinity in cell walls even if no significant change were observed in internode length and total height of bamboo culm. Finally, microscopic cellulose crystalline feature proves useful for determining if the bamboo culm had stopped elongation growth.

REFERENCES


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