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Longitudinal and radial distribution of free glucose and starch in moso bamboo *(Phyllostachys pubescens* Mazel)

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Abstract: The longitudinal and radial distribution of the free glucose and starch was determined in moso bamboo (*Phyllostachys pubescens*) culms by the 'Alkaline extraction-Glucoamylase hydrolysis' method. The free glucose content was generally lower in the upper parts of the culm, the starch content was highest in the mid height (6 m) section, and it decreased almost linearly in the lower and the higher sections. Regarding the radial distribution, the free glucose and starch contents were generally higher in the inner part of the culm. This characteristic localization of free glucose is likely to be associated with the distribution of the particular nutrient storage cells, *i.e.*, parenchyma cells. On the other hand, it is suggested that the starch content of moso bamboo does not depend on the proportion of parenchyma cells alone but also on the abundance of starch grains in the cells.

Key words: Phyllostachys pubescens, free glucose, starch.

INTRODUCTION

In Japan, bamboo has been used as a building material for centuries; however, the extent of the production of bamboo culms has fallen recently in the country (Ministry of Agriculture and Fisheries, 2002) due to a decrease in its use resulting from changes in lifestyles and in architectural designs. This decrease in the use of bamboo has played an important role in the drastic increase of uncontrolled bamboo forests, resulting in local environmental problems, since bamboo grows faster than other woody plants (Nakashima, 2002; Nishikawa *et al.*, 2005). The effective utilization of bamboo has become an important factor in the overall effective utilization of sustainable bio-resources as well as in the maintenance of the forestry environment.

One serious problem with the use of bamboo as a building material is its low durability. Bamboo has little decay resistance due to its high content of sugar and starch, which are excellent nutrients for fungi or insects (Morita, 1985; Yoshimoto and Morita, 1985; Hirano *et al.*, 2003). Ninomiya and Kotani (2002) showed that damage by *Dinoderus*

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minutus Fab., which is a serious pest affecting bamboo, appeared only on the inner side of the culm wall. Morita (1985) indicated that the inner side of the culm wall, where there is abundant parenchyma in which nutrients are stored contained more sugars than the outer side. In bamboo, proportion of parenchyma is greater in the inner regions of the culm, and fibro-vascular bundles are abundant in the outer parts of the culm wall (Liese and Mende, 1969; Grosser and Liese 1974; Shimaji *et al.*, 1976). However, there has been no detailed study on the distribution of sugars and starch in the bamboo culm. In the present study, therefore, the longitudinal and radial distribution of the free glucose and starch in moso bamboo (*Phyllostachys pubescens* Mazel) culms was determined by a method newly designed by Okahisa *et al.* (2005).

MATERIALS AND METHODS

Culms of moso bamboo (*P. pubescens*) of over 3-years of age were harvested from the Ashu Forest Research Station of the Field Science Education and Research Centre, Kyoto University, on October 20, 2005. Three culms with an average height of 16.37 m were used for the study. The culms were cut into internode segments and oven-dried at 65°C for 48 h after their surfaces were cleaned with acetone.

Sample preparation for chemical analysis

Test samples with 2-4 cm thickness were cut from the internode segments taken from 0, 2, 4, 6, 8, 10, 12 and 14 m height levels of the culm from the bottom to measure the longitudinal variation in free glucose and starch contents. For the radial distributions, the samples were split into 5 portions from the inner side to the outer side (No. 1- No. 5) from all the above height levels. The average radial wall thickness of the culms was 15.07, 10.96, 8.39, 7.45, 6.36 and 5.51 mm respectively at these height levels. The specimens were ground using a grinder mill (Vita-Mix Absolute Mill, Osaka Chemical, Osaka, Japan) into a powder fine enough to pass through an 80 mesh sieve.

Free glucose and starch analysis

Free glucose and starch were analyzed by the Alkaline extraction-Glucoamylase hydrolysis method (Okahisa *et al.*, 2005). Five hundred mg of dry bamboo powder and 10 ml of 0.5 N sodium hydroxide were mixed in the centrifuging tube, and the mixture was sonicated for 30 min (5510J-DTH, Branson, Kanagawa, Japan). After neutralization by 0.5 N acetic acid, the mixture was centrifuged for 10 min at 3000 rpm and the supernatant was recovered. The free glucose content (Gl) in the supernatant was analyzed with a glucose oxidase reagent kit (Glucose C II -Test Wako, Wako Pure Chemical Industries, Osaka, Japan), using a UVNIS spectrometer (U-2001, Hitachi, Tokyo, Japan) at 505 nm. 0.5 ml of 0.1M sodium acetate buffer (pH 4.8) containing glucoamylase from *Rhizopus* sp. (38.5 U/mg, Toyobo, Osaka, Japan) and α-amylase from *Bacillus* sp. (1870 U/mg solid, Nacalai Tesque, Kyoto, Japan) were added to 0.5 ml of the supernatant, and the solution was shaken for 2 h at 40 °C

(Thomastat T-N22S, Thomas Kagaku, Tokyo, Japan). The concentrations of glucoamylase and α–amylase were 40 mg per 10 ml (Okahisa *et al*., 2005). To remove the inhibition of the alkaline extracts, 20-30 mg of activated charcoal was added to the solution, and the solution was kept for 10-15 min, and then centrifuged for 10 min at 3000 rpm. The glucose content of the supernatant (G2) was analyzed with the glucose oxidase reagent kit as described above. The amount of starch in the bamboo was calculated using the following equation: (G2 - Gl) x 0.9 (Pucher *et al*., 1948).

Parenchyma cells in cross section

Cleanly cut transverse surface of the internode sample located at heights of 0, 6, and 12 m were digitized directly by a scanner with a resolution of 530 dpi (PM-A890, Seiko Epson, Nagano, Japan), at which the image allows to see the distribution of parenchyma cells. Three different sectors in each image were then selected for image analysis. In each sector, the culm was divided into 5 portions from the pith cavity to the epidermis. The proportion of parenchyma ratio was measured from each portion using the image analyzing software Scion Image Beta 4.02 Win (Scion Corporation).

Observation of starch grains in parenchyma cells

The iodine/potassium iodine solution (IKI) was prepared by dissolving 0.17 g of iodine crystals and 0.31 g of potassium iodine in 50 ml water. The 20 µm thick radial sections of the internode samples from 0, 6 and 12 m positions were cut using conventional sliding microtome. Starch grains in parenchyma cells were observed with an optical microscope (BX51 TF, Olympus, Tokyo, Japan) after staining with IKI solution.

Statistical analysis

The average free glucose and starch contents of bamboo in each portion and the percent area of parenchyma cells in the 0, 6 and 12 m sections were statistically analyzed by Tukey's test (Vargas, 1999).

RESULTS

Longitudinal distribution of free glucose and starch in the culm

The glucose and starch contents of bamboo samples obtained from the 0, 2, 4, 6, 8, 10, 12 and 14 m sections are shown in Figures 1A and B, respectively. The average values of free glucose are presented in Figure IA. Free glucose decreased from the bottom to the upper height levels of the culms. Compared to the specimens obtained from the 0 m section, the glucose content was significantly lower in the specimens from the upper sections with the exception of the specimen from the 2 m section ($P<0.05$ for 4 m and 6 m, $P<0.01$ for 8, 10, 12 and 14).

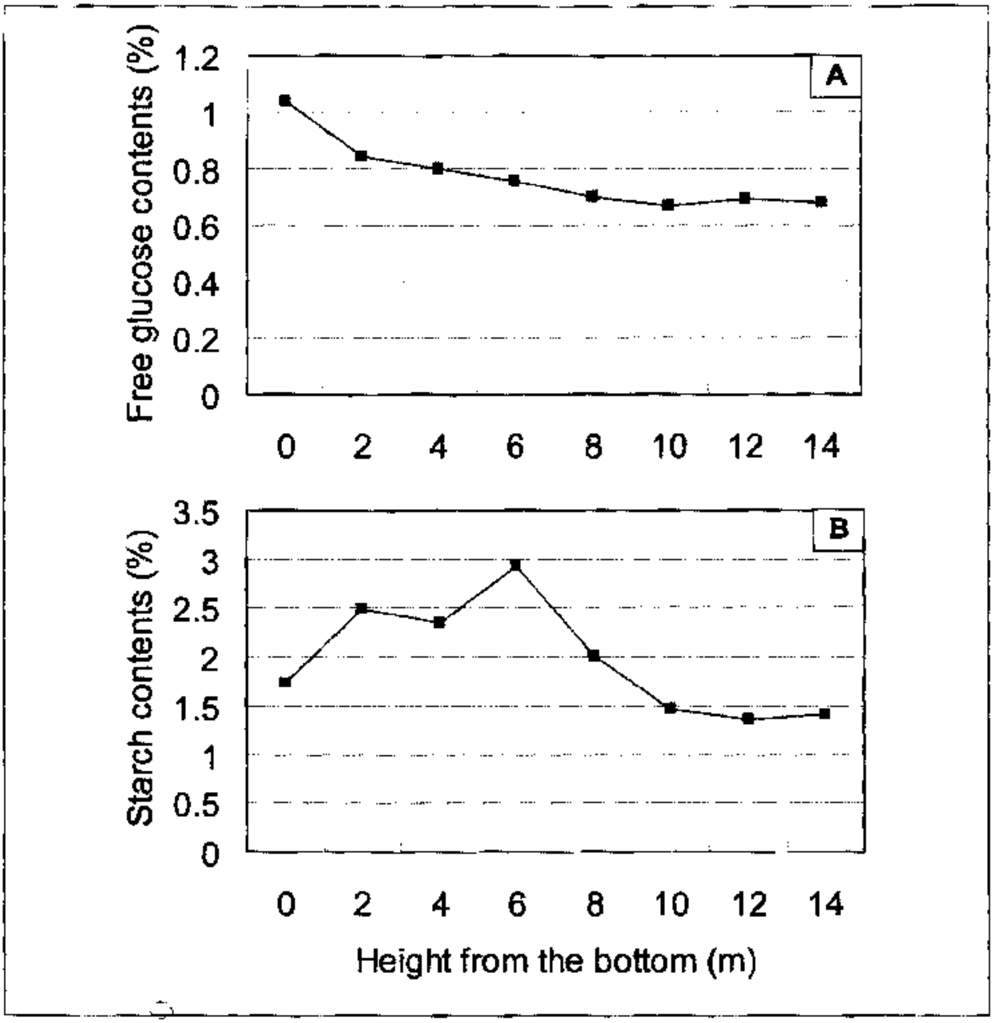

Figure 1. The free glucose and starch contents at different height levels of the culm of moso bamboo.

The starch content of the specimens showed a different tendency from that of free glucose content (Fig. 1B). The highest average starch content was obtained in the internode from 6 m level (2.92%), and the content decreased almost linearly towards the lower and higher levels, and remained steady in internodes above 10 m. In comparison with the specimens obtained from the 6 m section, the starch content was significantly lower in the specimens from the 10, 12, and 14 m sections ($P<0.05$).

Radial distribution of free glucose and starch in the culm

Figures 2A and B show the free glucose and starch contents of the 5 portions from the inner side to the outer side of the culm wall. The free glucose content of the specimens was generally lower in the outer side, in portions No. 4 and 5, and the tendency was most obvious in the 0 m section (Fig. 2A). The highest average free glucose content was obtained for the second inner portion (No. 2) at the 0 m section, and the lowest content was in the No. 5 portion at the 10 m section. On an average, the total free glucose content of the samples in Nos. 1, 2, 3, 4, and 5 were 0.54, 0.70, 0.62, 0.42 and 0.30 per cent, respectively. Compared to the specimens in No. 5, the outermost side, the glucose content was significantly higher in the specimens in Nos. 1, 2, and 3 (Tukey's test, $P<0.01$). As shown in Figure 2B, the starch content decreased from the inner side to the outer side in the lower parts of the culms, *i.e.,* at the 0 and 2 m internodes. In the samples from 4, 6, 8 and 10 m the second inner portion (No. 2) tended to have the highest starch content. The highest and the lowest starch contents were 9.18 per cent in No. 1 from the 0 m internode and 0.72 per cent in No. 5 from the 10 m internode, respectively. The average total starch contents of the 5 portions were 4.93 per cent for No. 1, 4.21 per cent for No. 2, 2.49 per cent for No. 3, 1.38 per cent for No. 4, and 0.94 per cent for No. 5. The total starch content in No. 1, the innermost

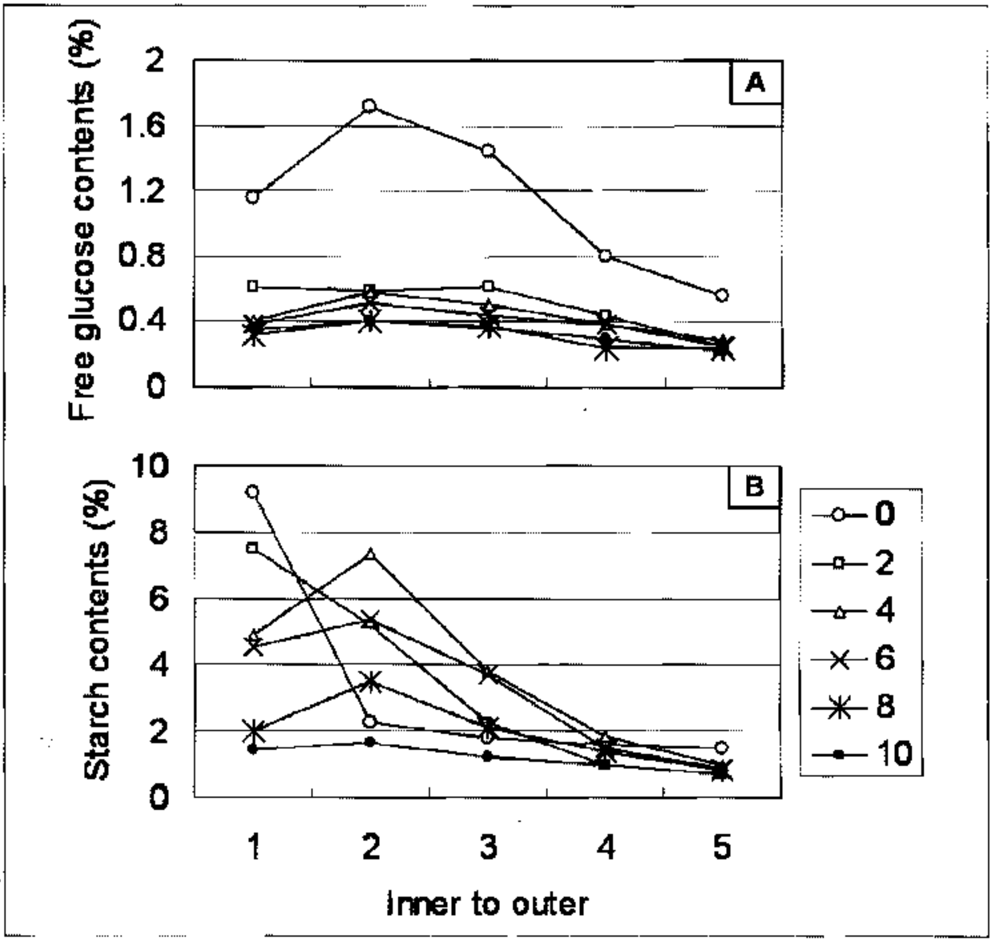

Figure 2. Variation in free glucose (A) and starch (B) contents between five radial positions of the culm wall of moso bamboo.

side, was significantly highest among the portions (*P<0.05* for No. 2, *P<0.01* for Nos. 3, 4, 5), and the content in No. 2 was significantly higher than those in Nos. 3, 4, and 5 (*P<0.01*). Significantly higher starch content was obtained in No. 3 than in Nos. 4 and 5 (*P<0.01*).

Proportion of parenchyma

Table 1 shows the percentage of parenchyma cells in the cross section in five portions from the inner side to the outer side (No. 1 to No. 5) at 0, 6 and 12 m sections. The percentage of parenchyma cells was generally higher in the inner side of the culm wall and in the lower height levels of the culm. The highest percentage was obtained in the portion No. 2 at the 0 m section (83.6%), and the lowest value was in portion No. 5 at the 12 m section (15.6%). Between the culms, specimens 1 and 2 had significantly higher proportion of parenchyma at 0, 6, and 12 m levels. For specimen No. 5, the per cent area at the 0 m section was significantly higher than that at the 2 m section (*P<0.05*). Figure 3 shows the relationship between the free glucose content and the per cent area of parenchyma cells obtained from 0 and 6 m height levels of the culms. There was a positive correlation between the free sugar content and parenchyma percentage.

Starch grains in parenchyma cells

Figures 4-6 show radial sections of the inner part (Fig. 4), middle part (Fig. 5), and the outer part (Fig. 6) of the culm wall at 0 m (a), 6 m (b), and 12 m (c) sections stained with IKI solution. Figures 4 (a), (b), and (c) represent the inner portions, namely No. 1, Nos. 1-2, and Nos. 1-2, respectively. The middle portions, No. 3, Nos. 3-4, and Nos. 2-

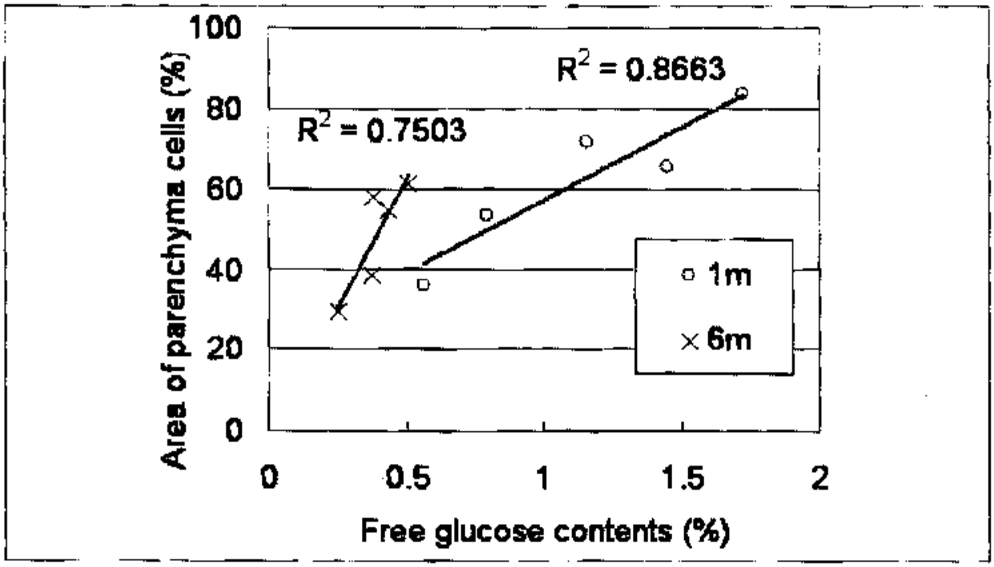

Figure 3. Relationship between free glucose and proportion of parenchyma at different height levels of moso bamboo culms.

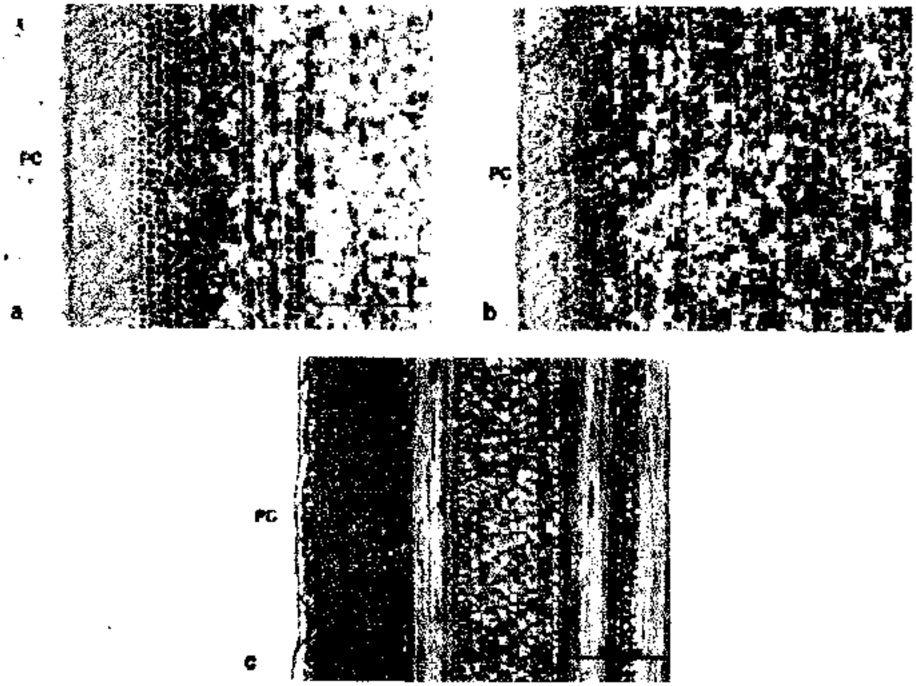

Figure 4. Photomicrographs of IKI-stained radial sections of the inner portions at 0 m (a), 6 m (b), and 12 m (c) height positions of the moso bamboo culms.

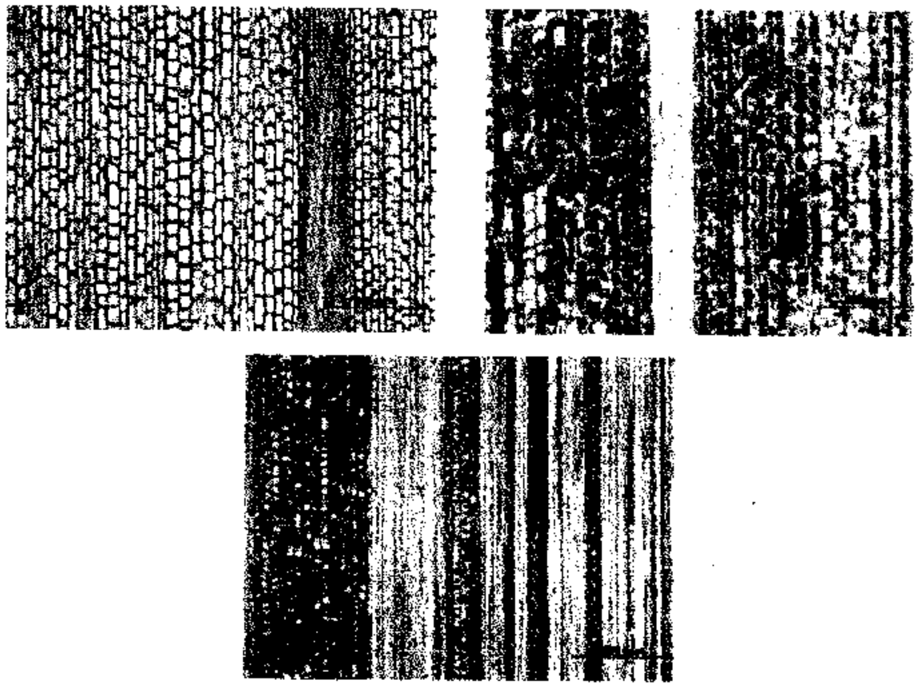

Figure 5. Photomicrographs of IKI-stained radial sections of the middle portions at 0 m (a), 6 m (b), and 12 m (c) height levels of the moso bamboo culms.

Table 1. Proportion of parenchyma in different portions of *P. pubescens* culms

Position within culm wall*	Proportion of height levels		
	0 m	6 m	12 m
No. 1	71.5 ± 13.6	58.0 ± 10.1	67.0 ± 8.2
No. 2	83.6 ± 13.1	61.3 ± 7.4	52.6 ± 17.5
No. 3	65.6 ± 13.0	54.9 ± 10.6	44.7 ± 10.0
No. 4	53.5 ± 13.0	38.5 ± 6.6	32.4 ± 10.3
No. 5	36.3 ± 9.8	29.6 ± 6.8	15.6 ± 4.9

* Positions 1to 5 refer to 5 radial positions within culm wall from inner to outer part.

4, are shown in Figures 5 (a), (b), and (c), respectively. Figures 6 (a), (b), and (c) show the outer portions; No. 5, Nos. 4-5, and Nos. 4-5, respectively. The average radial thicknesses of the culms were 15.07, 7.45, and 4.21 mm for 0, 6 and 12 m, respectively. Generally, the parenchyma cells of the inner parts contained greater accumulation of starch grains. Most starch grains were found within 1 mm of the pith cavity (PC) at the 0 m section, and they spread to the outer side at the 6 and 12 m sections (Fig. 4). Very small amount of starch grains was observed at the mid-wall portion of 0 m section, whereas there were a lot of grains in parenchyma cells at other height levels, particularly at the 6 m section (Fig. 5). However, these grains decreased in the outer part even at the 6 m section, and were absent at the 0 m and 12 m sections (Fig. 6).

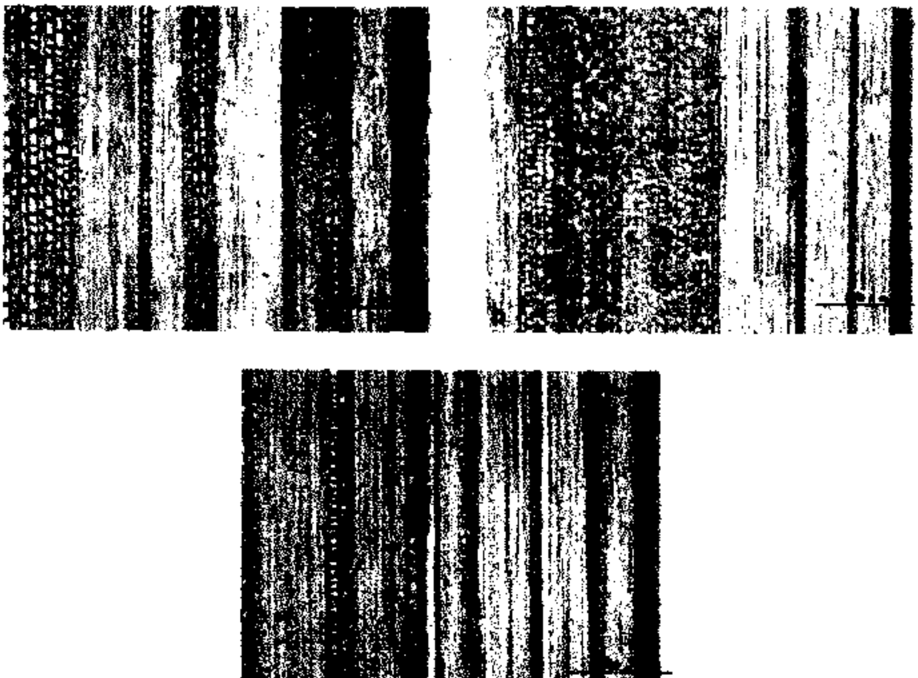

Figure 6. Photomicrographs of IKI-stained radial sections of the outer portions at 0 m (a), 6 m (b), and 12 m (c) height levels of moso bamboo culms.

DISCUSSION

Yoshimoto and Morita (1985) found that the 5-year-old moso bamboo contained fructose, sucrose, and glucose as free sugars, and the seasonal fluctuation of these sugars was reported to show a similar tendency by Morita (1985) and Ninomiya *et al.* (1998). Based on these findings, we analyzed the glucose content as an indicator of the distribution of free sugars in moso bamboo.

In this study, the free glucose content was generally lower in the upper portion of the culms. On the other hand, previous studies showed that the glucose content in immature moso bamboo with a height of 5-6 m increased from the bottom to the top (Fujii *et al.*, 1993; Azuma *et al.*, 2000). These contradictory distribution patterns indicate the possibility that the distribution of sugars in moso bamboo varies with growth or age. With regard to starch, the contents in immature moso bamboo were reported to be higher in the upper height levels of culms. Fujii *et al.* (1993) reported that starch in 2- to 3-year-old ma bamboo *(Phyllostachys bambusoides)* was concentrated in the upper part of the culm and then decreased towards the culm base. In the present investigation, the starch content in moso bamboo was highest in the 6 m section, which decreased almost linearly towards either ends. The amount and distribution of starch in moso bamboo might change with age as free sugars do. Anatomical changes during the maturation of bamboo have been reported (Bhat, 2003; Liese and Schmitt, 2006). Bhat (2003) showed that the main change occurring during the maturation of *Bambusa bambos* and *Dendrocalamus strictus* was the thickening of cells and lignification. Bamboo was found to mature from the bottom to the top (Nomura and Yamada, 1991). Itoh (1990) investigated the lignification in the culms of *Phyllostachys heterocycla* and reported that the axial progress of lignification in the component cells of epidermal cells, fibres and parenchyma cells proceeded upward from the basal internode to the top. The present results suggest that the sugar and starch contents varied with the maturation of bamboo.

In the present study, free glucose and starch contents were found to be generally higher in the inner part of the culm. Morita (1985) measured the free sugar content of moso bamboo and reported that the specimens from the inner part contained approximately three times more free sugars than those from the outer part. Etoh (1996) reported that the starch in bamboo was concentrated in the inner part of the culm wall and decreased to the outer side. These results are consistent with the results of our study. In the present study, the majority of the free glucose and starch was distributed in the Nos. 1-3, No. 1, and No. 2 portions, respectively. Previous investigations on attack by the insect *D. minutus* showed that the damage caused by the pest increased with the higher starch content in the bamboo culms (Plank and Hageman, 1951) and the damage only appeared in the inner parts of the bamboo (Ninomiya and Kotani, 2002). Considering the free glucose and starch contents, the inner part (Nos. 1-2) of bamboo is recommended to be specifically treated or removed when bamboo is used as a building material.

This characteristic localization of free glucose and starch is likely to be associated with the distribution of the particular nutrient storage cells, *i.e.* parenchyma cells. It is well known that the proportion parenchyma is generally higher in the inner part of the culm wall. With increasing height level, the amount of parenchyma decreases with a corresponding increase in the proportion of fibres (Liese and Mende, 1969; Grosser and Liese, 1971). In the present study, there was a positive correlation between free glucose content and percentage of parenchyma. On the other hand, the starch content did not show a high correlation with proportion of parenchyma. Therefore, it is suggested that the starch content of moso bamboo depends not only on the ratio of parenchyma cells but also on the abundance of starch grains in the cells.

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