J. Bamboo and Rattan, Vol.6, Nos. 1&2, pp. 51-60 (2007)

Amylolytic breakdown of storage starch in felled bamboo culms during post-harvest period

K. V. Bhat†, K. Sunil Kumar, P. M. Sumesh, K. S. Renjithkumar and Jose Kallarackal
Kerala Forest Research Institute, Peechi 680 653, Kerala, India

Abstract: The gradual reduction in starch content noticed in culms of *Bambusa bambos* and *Dendrocalamus strictus* during post-harvest period was examined in detail by analyzing the activity of amylase and the respiratory enzyme succinate dehydrogenase within the tissues of harvested culms. The cut ends of the harvested bamboo segments which dried up quickly under ambient conditions and the nodal portions showed only little reduction in starch content. However, there was appreciable reduction in starch content in remaining portions of harvested culms which continued for over a week, after harvesting. Activity of β-amylase which was, in general, moderate on day-2 increased to maximum in a week, then gradually declined towards the end of the following week. The reduction in storage starch is thus attributed to the activity of β-amylase found in the living tissues of the culms. It is suggested that the sugars released from the amylolytic activity are utilized for continued respiration occurring in living tissues of the culm. Evidently, to minimize borer damage, it is advantageous to store harvested bamboo culms in shade for a certain length of time, than utilizing them fresh.

Key words: Bamboo, starch hydrolysis, β-amylase, succinate dehydrogenase, amylolytic activity, post-harvest period.

INTRODUCTION

Starch is a common constituent of bamboo culms and rhizomes although it does not contribute to the structural framework of tissues. Being a reserve carbohydrate produced from excess photosynthates, it is stored in living tissues during periods of high photosynthetic output and is utilised during lean periods and during flowering (Liese, 1998). Several studies have found that the amount and distribution of starch in bamboo culms is uneven within a culm and is also variable during different parts of the year (Abd. Latif *et al*., 1994; Liese, 1998; Kumar and Mohinder Pal, 2003; Bhat and Varma, 2006).

The presence of starch in bamboo culms has great significance with respect to their utilization for structural applications, handicrafts and other utility items. The durability

†To whom correspondence should be addressed; E-mail: kvbhat@kfri.org

of bamboo in service is dependant on the extent of starch stored in it; generally culms having abundant starch are found to be highly susceptible to borer damage, at the same time, those with low or no starch are less attractive to borer beetles. It has been found that bamboo culms that have exhausted all their reserve starch during seed setting after flowering are not attacked by borer beetles (Bhat and Varma, 2006). Thus a number of studies have attempted to identify 'low starch periods' for bamboo harvesting, with a view to develop a method to overcome the borer damage to harvested bamboo (Beeson 1941; Sulthoni, 1987). However, all the studies so far conducted have assumed that starch content present in the culm at the time of harvesting continues to remain constant during subsequent post-harvest period; there has been no attempt so far to examine if there is any change in its amount during the post-harvest period. The present study examines the aspect in detail and reports the reduction of starch in culms of *B. bambos* and *D. strictus* subsequent to harvesting and relates the same to the activity of β-amylase and succinate dehydrogenase occurring in the culm tissues.

MATERIALS AND METHODS

Culm samples for the study were collected from Attappady, Kerala, India. One mature culm each of *B. bambos* and *D. strictus* was collected at monthly intervals for six months.

From each culm 1.5 m long segments from base, mid-height and top portions were brought to the laboratory for starting the analysis from the subsequent day (day-2). Samples were stored in two experimental conditions, namely in room temperature (30 °C and R.H. 71%) and at lower temperature (20 °C) of an air conditioned room. Starch estimation was done on alternate days from smaller sections (1 cm length) obtained from these segments. While cutting such samples each time, a 1 cm long portion from the exposed cut ends that was subject to desiccation was trimmed off and sample meant for analysis was oven-dried. Amylase activity was also estimated on alternate days from green intermediate portion of the culm segments, obtained in the same way. The moisture content (MC) percentage of the samples was estimated on alternate days by oven-drying the samples, to study its influence on enzyme activity. For microscopic examination of starch in tissues, 30 µm thick longitudinal sections cut on a sliding microtome were stained with I_2KI (Johansen, 1940). Whenever fresh material was required for histochemical tests, samples were collected locally from KFRI campus at Peechi. For visual assessment of the extent of starch in culm specimens, both in the field and laboratory, I_2KI reagent was applied to the culm wall of vertically split culm segments.

Estimation of starch

Starch was estimated by Humphreys and Kelly's (1961) method. Oven-dried samples of bamboo were ground into meal and the material passing through a 200-mesh screen was used for estimation. To 0.4 g of meal 4.7 ml of 7.2M perchloric acid was added

and the reaction allowed to continue for 10 min with occasional stirring. The contents were then transferred to a 50 ml volumetric flask and brought to volume with distilled water. The solution was centrifuged at 4000 rpm and 10 ml of aliquot was made alkaline with 2N sodium hydroxide using phenolphthalein indicator. To the solution 2N acetic acid was added until the indicator colour discharged and then a further 2.5 ml was added. Then 5 ml of 10 per cent (w/v) potassium iodide and 5 ml of 0.01N potassium iodate were added. The colour was allowed to develop for 15 min and the solution was brought to volume. The absorbance was measured on a spectrophotometer at 650 nm with a blank prepared without starch as zero. The starch content was estimated with the help of a reference curve set up using potato starch. The percentage of starch was expressed with respect to gram dry weight of the material. For estimating starch content in individual culms, values determined for the three height levels were averaged and to reveal the overall trend of change in each species these mean values were pooled for six culms each per species.

Determination of amylase activity

Activity of β-amylase was estimated according to the procedure described by Sadasivam and Manikkam (1992). Extraction of amylase enzyme was done by wet grinding fresh tissue of bamboo in a mortar using 5 ml of 66 mM phosphate buffer (pH 7) and 5 ml of 0.5M NaCl. The extract was centrifuged at 10,000 rpm for 15 min and the supernatant was used as enzyme extract. To 1 ml of starch solution, 1 ml of enzyme extract of known dilution was added which was subsequently incubated at 27 °C for 15 min. The reaction was stopped by addition of 2 ml of dinitro-salicylic acid reagent. The solution was heated on a boiling water bath for 5 min. After cooling in running tap water, the solution was made up to 10 ml and absorbance was read at 560 nm. In control tubes, the reaction was terminated at zero time. The standard curve was plotted by using 0-1000 µg maltose. The amylase activity expressed in units as mg of maltose produced from 1 g of oven-dry tissue during 5 min incubation with 1 per cent starch.

Histochemical localization of succinate dehydrogenase

Succinate dehydrogenase (SDH) enzyme was localized in microtome sections of fresh tissue as described by Berlyn and Miksche (1976). Sections were incubated for 30-45 min at 37 °C in a freshly prepared mixture of equal volumes of 0.2M sodium succinate and phosphate buffer (pH 7) to which 1 ml of 0.1 per cent nitro-blue tetrazolium was added. Sites of SDH activity showed blue diformazan deposits within cells.

RESULTS

Starch was found stored in ground parenchyma tissue of bamboo culms. In sections stained with I_2KI, starch was found accumulated within the cells as granules (Fig. 1). In unmagnified view of specimens stained with the reagent, starch-containing tissue

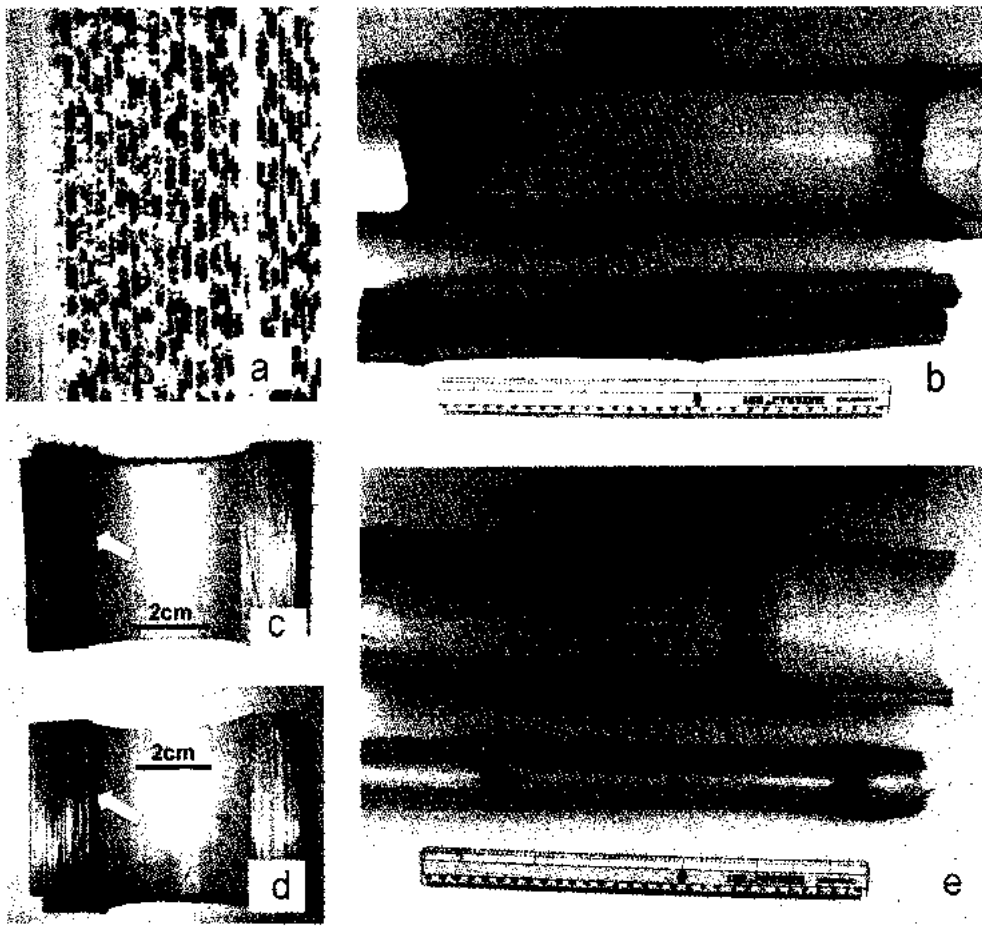

Figure 1. a: LS of *D. strictus* internode showing starch grains in ground parenchyma cells. Scale bar represents 170 µm; b: Longitudinally split culm segments of freshly felled *B. bambos* (above) and *D. strictus* (below) stained with I_2KI to show abundance of starch; c: A portion of internode of *B. bambos* culm showing deep staining of the culm wall (at arrow) in comparison to the unstained wall (on right); d: Reduced I_2KI stainability of the culm wall (at arrow) of *B. bambos* three days after harvesting in comparison to fresh material; e: Split culm segments of *B. bambos* (above) and *D. strictus* (below) indicating depletion of starch from internodal portions while the nodal diaphragms and cut ends stained intensely with I_2KI.

appeared as dark blue striations separated by fibrous strands. Thus from I_2KI staining it was possible to make a visual assessment of the abundance of starch in the culm segments.

Among the samples studied from three height levels of *B. bambos* and *D. strictus* culms, starch content was highest in the mid-height level of the culms; the basal portion of the culm was low in starch content. Thus along the culm length there was considerable variation in starch content. The distribution appeared more or less even

throughout the internode. However, the nodal portions were generally richer in starch as evident from visual difference in staining intensity (Fig. 1b). Therefore, for estimating average starch content of a culm, values obtained for three height levels were pooled.

In harvested culm segments stored under shade there was a gradual reduction in starch content as judged from the staining intensity of the specimens with I_2KI reagent. For instance, samples from freshly harvested culms showed intense staining (Fig. 1c) whereas samples extracted from these segments after a gap of three days had relatively feeble stainability (Fig. 1d) which continued to decline further as days passed. At the end of two weeks after harvesting, there was conspicuous reduction in starch content. However, in the nodal portions and the cut ends of the culm segments, decrease in starch was relatively less (Fig. 1e) as evident from I_2KI stainability.

The initial starch content varied spatially and temporally. The highest initial starch content observed was 16.36 and 20.23 per cent in mid-height level of *B. bambos* and *D. strictus* respectively. November and December were the months when starch was found to be specifically low. Similarly, the peak amylase activity noted in *B. bambos* was to the tune of 11.3 units in *B. bambos* (basal segments) and 13.6 units in *D. strictus* (mid-height level segments), in both instances, on fourth day after harvesting. The enzyme activity reduced to zero on 14th day or, more commonly, on 12th or 10th day after harvesting. Exceptionally, high amylase activity was noticed on day-2 itself in a few culms, in which case the activity declined to zero much before this period.

Data on moisture content showed that there was a difference in the rate of moisture loss from culms subsequent to harvesting among different months. Moisture loss during the two weeks of post-harvest storage was generally less (20-30%) when culms were cut during September and October (when average atmospheric humidity was nearly 83%) whereas moisture loss was over 50 per cent of the original during November to February (when humidity was around 65%). Invariably the rate of loss of moisture was higher from material stored under ambient conditions than from that at lower temperature (20 °C). It was noted that amylase enzyme was active even when the culm moisture content was as low as 16 per cent. On the other hand, decline in enzyme activity occurred even when moisture content of the culm was well over 40 per cent suggesting that culm moisture content was not strictly a limiting factor for enzyme activity under ambient conditions.

The average values for starch content and amylase activity estimated on alternate days in culms of *B. bambos* and *D. strictus* subsequent to harvesting are shown in Figures 2 and 3 respectively. In order to show the general trend of change during the fortnight following harvesting, mean values for each culm have been pooled for six culms each per species. There was an overall decreasing trend in starch content from an average of 6.5 per cent to as low as 2 per cent in *B. bambos* and from over 8 per

cent to about 2 per cent in *D. strictus* in a fortnight. The average reduction in starch content noticed was thus substantial amounting to 65 to 70 per cent of the original. The decrease was more prominent during the first week after harvesting.

The activity of β-amylase also showed more or less identical trend in both the species. The moderate level of enzyme activity noticed on day-2 rose to maximum on fourth or sixth day and then gradually declined in the succeeding week (Figs. 2,3). The period of high amylase activity during the first week after harvesting almost coincided with decrease in starch content. The declining trend in enzyme activity evident during the second week was more gradual as compared to its initial increase which was more rapid. Samples stored at ambient temperature and at 20 °C did not show much difference in the basic trend and length of the period of enzyme activity.

In freshly harvested mature culms, respiratory activity as assessed from histochemical localization of succinate dehydrogenase (SDH) was generally more intense in the cortical parenchymatous tissue (Fig. 4). Moderate activity of the enzyme was also observed in a few layers of ground parenchyma next to the cortex and the lining layers of the culm cavity (Fig. 9). However, in major portion of the inner culm tissue SDH activity was limited to a low level in bundle parenchyma surrounding xylem vessels. After four to six days after harvesting, there was a decline in SDH activity in cortical layers and outer ground parenchyma. On the other hand, bundle parenchyma cells surrounding xylem vessels showed enhanced SDH activity (Fig. 10, at arrows). Ground parenchyma cells occasionally showed feeble activity at this stage.

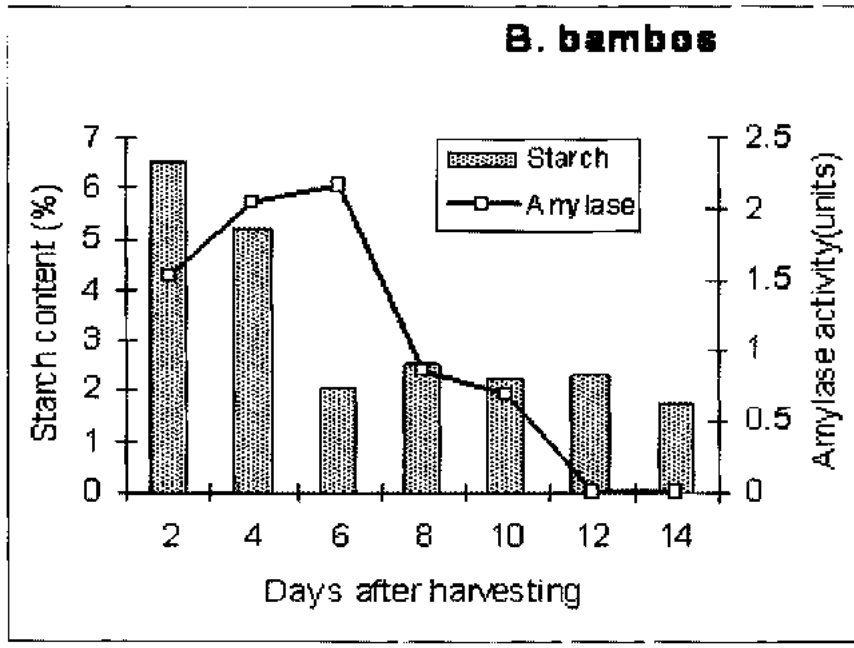

Figure 2. Levels of starch and amylase activity during post-harvest period in *B. bambos*.

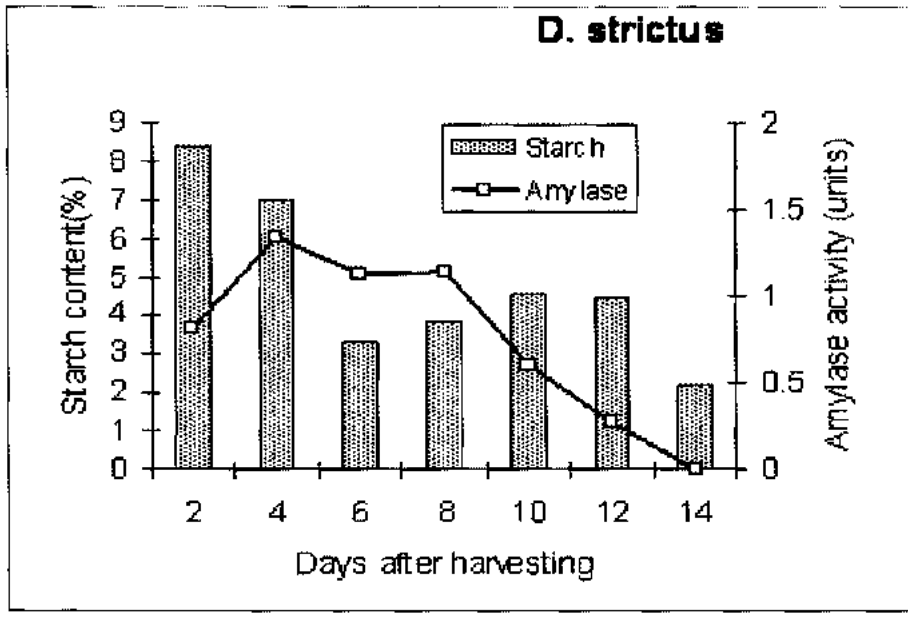

Figure 3. Levels of starch and amylase activity during post-harvest period in *D. strictus*.

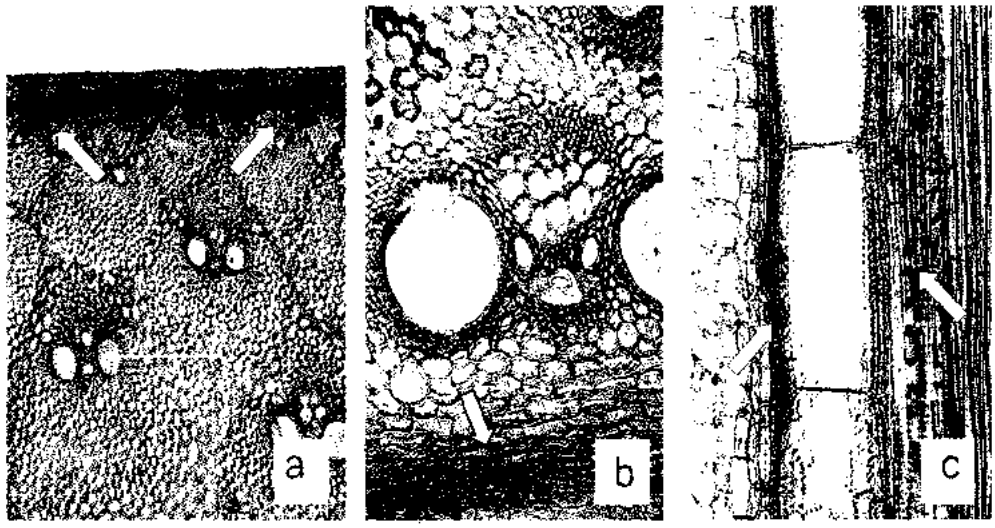

Figure 4. a: Cross sectional view of culm wall of *B. bambos* (freshly felled) showing intense SDH activity in the cortex and outer ground tissue (at arrows); b: SDH activity in the lining layers of the culm cavity (at arrow) of the same material; c: SDH activity in bundle parenchyma surrounding xylem vessels in *D. strictus* four days after harvesting. Scale bar represents 185 µm , 100 µm and 120 µm for Figs. 4a,b,c respectively.

DISCUSSION

It is generally agreed that starch stored in bamboo culms is a major attractant to borer beetles that feed and thrive on harvested culms and finished articles. A number of

studies have indicated a direct relationship between the extent of borer damage and the abundance of starch in harvested culms (Beeson, 1941; Plank and Hageman, 1951; Joseph, 1958; Liese, 1980; Nair *et al.*, 1983; Dhamodaran *et al.*, 1986; Bhat *et al.*, 2005). A threshold level of 5 per cent of starch has also been reported by Beeson (1941) above which bamboo is more susceptible to borer damage.

The extent of starch storage is generally variable within the culm and also with respect to culm age (Alwin and Murphy, 1988; Abd. Latiff *et al.*, 1994; Weiner and Liese, 1996; Kumar and Mohinder Pal, 2003; Bhat and Varma, 2006). It was observed in the present study that starch accumulation was generally greater in the nodal than in internodal portions as found earlier (Bhat and Varma, 2006). Such high degree of variability was the main reason for the disparity of values on starch content obtained among samples. Therefore, mean values were used for a reasonable estimate of starch content of the culms.

Values of starch estimation in the culms following harvesting confirmed that the decrease in I_2KI stainability of culm tissue observed subsequent to harvesting was due to diminishing starch in the tissues. Since the periods of peak amylase activity and decrease in starch content of the culms almost coincided, it can be concluded that they are mutually related and starch is digested by amylolytic action of the enzyme. The activity of amylase probably serves to maintain an optimum level of sugars in the living tissues. Although studies on amylolytic hydrolysis of starch in bamboo culms are few, there are reports of reduction in scutellar starch and accumulation of amylase during development of somatic embryos in some bamboos (Godbole *et al.*, 2004). Since starch and free sugar contents within culms are highly fluctuating, obviously due to enzyme action, a few studies have attempted to examine seasonal and height-dependent variation of these carbohydrates in bamboos with respect to their biodegradability (Okahisa *et al.*, 2006). The present study also indicates that amylase enzyme was active right from the time of harvesting, or possibly even before, from the fact that in some samples peak activity was shown soon after harvesting which subsequently declined to zero. Storage of harvested culms at lower temperature (20 °C) to prevent faster drying of culms was not helpful to prolong the period of starch depletion since moisture content did not appear to be a constraint for cessation of amylase activity.

It is interesting to note that the average level of starch content at the end of two weeks of post-harvest storage was lower than the threshold level of 5 per cent suggested by Beeson (1941). As there is no possibility of appreciable photosynthetic input in harvested culms stored under shade since the branches and leaves are already removed, it is possible that the normal metabolic activities in the tissues are dependent on reserve carbohydrates stored. In this regard it is relevant to consider a traditional method of protection of harvested bamboo followed in some parts of eastern India such as West Bengal. In this practice, harvested bamboo culm is stored under shade for a few days

before it is put to use and this is found to be beneficial to minimize subsequent borer damage. In the light of observations from the present study, it can be concluded that the probable reason for effectiveness of the treatment is starch depletion during post-harvest storage. Other traditional practices such as 'clump curing' (which involves cutting the culm at base and leaving it vertically leaning against other culms for four weeks before it is used) followed in some parts of India (Mathur, 1958; 1961) are also probably treatments leading to starch depletion in culm tissues.

Results from succinate dehydrogenase localization indicate that in standing bamboo, normally the cortex and few outer layers of ground parenchyma are active sites of respiration besides the inner lining layers of culm cavity. The free sugars already available in cells or sugars released from starch hydrolysis perhaps act as substrate. However, for the continued respiratory activity in harvested culms, the sugars released by amylolytic activity are probably the chief source of substrate. Thus, the amylolytic breakdown of storage starch in culms can be linked to post-harvest respiration. Although the enhanced SDH activity in xylem parenchyma cells in harvested culms is an interesting observation, the behaviour cannot be readily explained from the present investigation.

CONCLUSIONS

The present study indicates that there is considerable reduction (65-70% of the original) in starch content of the culms during post-harvest period. Therefore from the point of view of protection of bamboo, it is advantageous to store harvested bamboo culms in shade for two weeks or more after harvesting, than utilizing them fresh. If borer entry into culms during this storage period is prevented by some means, probably damage can be minimized to a great extent.

ACKNOWLEDGEMENTS

The authors are indebted to NMBA, New Delhi for providing financial support to undertake this study. The encouragement and facilities provided for the study by Dr. R. Gnanaharan, Director, and Dr. J. K.Sharma, former Director are gratefully acknowledged.

REFERENCES

Abd. Latif, M., Khoo, K.C. and Nor Azah, M.A. 1994. Carbohydrates in commercial Malaysian bamboo. In: Bamboo in Asia and Pacific. Proceedings of the 4th International Bamboo Workshop, Chiangmai, Thailand. 27-30 Nov. 1991. IDRC. Ottawa. Canada and FORSPA, Bangkok, Thailand: 227-229.

Alwin, K.L. and Murphy, R. J. 1988. Variation in fibre and parenchyma wall thickness in culms of the bamboo *Sinobambusa tootsik*. *IAWA Bull.* (n.s.) 9: 353-361.

Beeson, C. F. C. 1941. The Ecology and Control of Forest Insects in India and Neighbouring Countries. Govt. of India: 767p.

Berlyn, G. P. and Miksche, J. P. 1976. Botanical Microtechnique and Cytochemistry. Iowa State Univ. Press. Iowa.

Bhat, K.V. and Varma, R.V. 2006. Optimization of harvesting and post-harvest technology to economize bamboo resource utilization. KFRI Research Report No. 285. Kerala Forest Research Institute, Peechi, Kerala, India: 67p.

Bhat, K.V., Varma, R.V., Raju Paduvil, Pandalai, R.C. and Santhoshkumar, R. 2005. Distribution of starch in the culms of *Bambusa bambos* (L.)Voss and its influence on borer damage. *Journal of American Bamboo Society* 19: 1-4.

Dhamodaran, T.K., Mathew, G, Gnanaharan, R. and Nair, K.S.S.1986. Relationship between starch content and susceptibility to insect borer in the bamboo reed *Ochlandra travancorica*. *Entomon* 11: 215-218.

Godbole S., Sood A., Sharma M., Nagar, P.K. and Singh, Ahuja P. 2004. Starch deposition and amylase accumulation during somatic embryogenesis in bamboo (*Dendrocalamus hamiltonii*). *J. Plant Physiol.* 161: 245-248.

Humphreys, F. R. and Kelly, J. 1961. A method for determination of starch in wood. *Analytical Chemistry Acta* 24: 66-70.

Johansen, D. A. 1940. Plant Microtechnique. McGraw-Hill, New York.

Joseph, K.V. 1958. Preliminary studies on the seasonal variation in starch content of bamboos in Kerala State and its relation to beetle borer infestation. *Journal of the Bombay Natural History Society* 55: 221-227.

Kumar, R. and Mohinder Pal. 2003. Carbohydrates in relation to rooting of culm cuttings of *Dendrocalamus strictus* (Roxb.). *Indian Forester* 129:1355-1360.

Liese, W. 1980. Anatomy of Bamboo. In: G. Lessard and A. Chouinard (Eds.) Bamboo Research in Asia. Proceedings of the Workshop held in Singapore, 28-30 May, 1980. IDRC, Ottawa, Canada: 161-164.

Liese, W. 1998. The Anatomy of Bamboo Culms. INBAR, Beijing, China: 204p.

Mathur, R. N. 1958. Clump curing of bamboos and susceptibility to powder post beetle attack. *Indian Forest Bulletin* (New Ser.) Ent. No. 221: 19p.

Mathur, R. N. 1961. Effect of clump curing of bamboos and susceptibility to powder post beetle attack. *Indian Forest Bulletin*. (New Ser.) Ent. No. 233: 11p.

Nair, K.S.S., Mathew, G., Varma, R. V. and Gnanaharan, R.1983. Preliminary investigations on the biology and control of beetles damaging stored reed. KFRI Research Report No. 48. Kerala Forest Research Institute, Peechi, Kerala, India: 39p.

Okahisa, Y., Yoshimura, T. and Imamura, Y. 2006. Seasonal and height-dependent fluctuation of starch and free glucose contents in moso bamboo (*Phyllostachys pubescens*) and its relation to attack by termites and decay fungi. *Journal of Wood Science* 52: 445-451.

Plank, H. K. and Hageman, R. H. 1951. Starch and other carbohydrates in relation to the powder-post beetle infestation in freshly harvested bamboo. *J. Econ. Entomol.* 44: 73-75.

Sadasivam, S. and Manikkam, A. 1992. Biochemical Methods. Wiley Eastern Ltd., New Delhi: 246p.

Sulthoni, A. 1987. Traditional preservation of bamboo in Java, Indonesia. In: A.N. Rao, G. Dhanarajan and C. B. Sastry (Eds.). Recent Research on Bamboos. Proceedings of the International Bamboo Workshop, Hangzhou, China, 6-14 October, 1985. Chinese Academy of Forestry, Beijing, China and IDRC, Ottawa, Canada: 349- 357.

Weiner, G. and Liese, W. 1996. Wound reactions in bamboo culms and rhizomes. *Journal of Tropical Forest Science* 9: 379-397.