KFRI Research Report 308

Final Technical Report

POST- HARVEST PROTECTION OF BAMBOO FROM INSECT BORERS BY A TECHNIQUE ENHANCING STARCH HYDROLYSIS

Submitted to:
National Mission on Bamboo Applications
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ABSTRACT

The reduction of storage starch in harvested bamboo culms during post-harvest period was investigated in two common species of bambos, namely, *Bambusa bambos* and *Dendrocalamus strictus*. The extent of starch storage in culm tissues varied between the different height levels of the culms and between seasons. Also, there was no definite pattern of seasonal variation in starch content and the extent of starch stored. On an average, approximately 60% reduction in starch content occurred in a fortnight following harvesting. Starch depletion trend was identical in samples stored at room temperature (30º C) and at lower temperatures (20º C) but was more pronounced in the former. Maximum starch depletion occurred during May- August. The starch depletion was attributable to the activity of β-amylase enzyme which showed high activity during the first week after harvesting. The enzyme activity rose from a low value recorded on day-2 and attained a peak, usually on day-6, and then declined gradually. However, in samples where the enzyme activity was high right from the day-2 (or probably, right from the time of harvesting), the activity reduced gradually without a subsequent rise. The declining trend in enzyme activity evident during the second week was more gradual as compared to its initial increase which was more rapid. It was also found that lower temperature was not as favourable as room temperature for amylolytic depletion of storage starch. In general, the enzyme activity was high from July to December as compared to the rest of the period. The sugars released due to the amylolytic hydrolysis of starch were probably utilised for the respiratory activity in the culm tissues since there was an obvious increase in respiratory activity in harvested culms during the first week of post-harvest storage. Application of end coats of cashewnut shell oil or a wall paint called black japan on cut en ends and other exposed parts of the culm segments decreased the rate of moisture loss from the culms. However, the effect of the treatment on starch depletion was not very encouraging. Thus it is concluded that end coating may

serve only as a barrier to the entry of borer beetles into the culms during the post-harvest period. Steeping the culms with amylase solution during harvesting did not result in appreciable increase in subsequent starch depletion as probably the solution taken up by the transpiration stream was not distributed laterally. The stomatal conductance and transpiration rates were high during summer months in both the species. The *Fv/Fm* values obtained from chlorophyll fluorescence measurements which are indicative of photosynthetic efficiency showed a reduction as the dry period progressed.

INTRODUCTION

In many parts of the world, bamboo has been an integral part of rural lifestyle for centuries. It has served as a popular and handy material for a number of applications such as household articles, tools, agricultural implements and inexpensive housing structures. The importance of bamboo as a raw material for pulp and paper industry in countries like India needs no mention. Bamboo-craft industry has been a means of employment and livelihood for a significant proportion of rural population in the country.

In recent years, there has been an increased awareness of the value of bamboo all over the world particularly in the context of growing timber shortage. The innovative processing technologies developed over the years have led to utilisation of bamboo for manufacture of diverse end products such as boards, parquet, panelling, furniture and handicrafts. Thus the potential of bamboo as an acceptable substitute for wood is increasingly becoming established. It is obvious that in the years to come, bamboo is going to be a vital raw material for a number of forest-based industries producing diverse products of human consumption.

The merits of bamboo as compared to timber obtained from trees mainly include comparatively low input and shorter gestation period required for its production besides faster growth and maturation of culms. The lignocellulosic material of bamboo is almost comparable to timber in mechanical properties and processing characteristics and is usable for similar purposes for which wood is used. It can be machined, glued and finished just like wood without much difficulty. As there are hundreds of species of bamboos with a wide range of qualities available, it is possible to exercise a choice of optimum quality to suit the intended use.

However, the woody material of bamboo has some inherent limitations. The major problem with bamboo for most purposes is related to its natural durability; the material is perishable and highly susceptible to insect borers. The powder-post beetles (*Dinoderus* spp.) and their larvae, which feed on the culm tissues, are capable of causing enormous damage to the harvested culms and articles made from them. The incidence of borer damage is of widespread occurrence. The beetles infest the harvested culms during the

post-harvest period and lay eggs in minute tunnels made by them. The emerging larvae and the adults feed actively by nibbling the culm tissue and making tunnels, thus producing a powdery mass of frass.

It has been found that, in a stack of bamboos subject to borer damage, culms that are heavily damaged are those with abundant starch; the culms that evade the attack are those with little or no starch. There are also other evidences indicating that storage starch is the main attractant for the borer infestation in bamboo. Thus some possible methods suggested to protect bamboos from borers include harvesting during 'low starch periods' or subjecting the culms to some treatments like 'clump curing' which involves cutting the culms at their base and leaving them vertically leaning against other culms of the clump for a few days. Among other non chemical treatments, submersion of harvested culms in water for a month or more is another widely followed 'curing' method. Alternatively, in some countries burying the harvested culms in muddy water or sand are also followed.

It was found in a previous study that in felled culms of *Bambusa bambos* and *Dendrocalamus strictus* stored under shade, there was a gradual reduction in starch as days passed which was attributable to the activity of amylase enzyme in the culm tissues. It was found that starch content could drop from over 15 per cent in freshly felled culms to as low as 2 to 3 per cent in a fortnight if the culms remained green. It was also found that if the culms remained uninfected by borers during this period, they remained without damage subsequently. These observations prompted us to explore the possibility of manipulating the process and developing a technique of bamboo protection based on the natural process of starch hydrolysis.

Objectives of the study

The aim of the study was to see if this natural phenomenon could be manipulated into a technique to achieve total depletion of starch in felled bamboo. Hence this investigation was undertaken to examine the factors conducive to enhanced amylase activity in culm tissues during post-harvest period. Also it was thought useful to examine if some artificial treatments to promote starch degradation in culm tissues would be helpful to speed up the

process and achieve the desired result in a shorter time. The specific objectives of the study were:

a. To determine the rate of amylolytic activity in harvested culms of *Bambusa bambos* and *Dendrocalamus strictus* during different seasons
b. To assess the influence of climatic factors on starch degradation in felled culms and to attempt to promote the process by artificial means
c. To determine the sites of active metabolic sinks in the culms after harvesting
d. To examine the efficacy of application of exogenous amylase and different end-coat formulations in promoting starch depletion in culms, and
e. To optimize the technique for utilization and future popularization

REVIEW OF STATUS OF RESEARCH

Although there are various views regarding the susceptibility of bamboo to borer infestation, starch content is more commonly regarded as a predisposing factor for the borer incidence and several studies have correlated the borer attack with the occurrence of starch in bamboo (Plank and Hageman, 1951; Joseph, 1958; Sen Sarma, 1977; Nair *et al*., 1983; Dhamodaran *et al*., 1986; Liese, 1980; Mathew and Nair, 1994). Many of these studies have indicated a positive relationship between storage starch and borer damage. The damage caused is said to be proportional to starch stored in the culms and starch is detected by the beetles almost immediately after the culms are felled (Hidalgo, 2003). We have observed that the inner portion of the culm wall which is rich in starch is selectively chosen for feeding by the borer beetles and their larvae, as compared to the outer, more fibrous part (Bhat *et al*., 2005). It is also found that flowered clumps with depleted starch content usually evade the borer damage (Liese, 1998; Bhat and Varma, 2006). Thus it is well established that the borer damage to bamboo culms is closely related to the level of starch storage in culms.

Starch is stored as minute granules in the subterranean axes and in the culm tissues as energy resource for production of new shoots as well as for mobilization of wound responses (Liese, 1998). It is found that there is a wide variation in the amount of storage starch between different species of bamboos. Even within the same culm, the distribution of starch is found to be variable. Starch content is generally higher in the upper parts of the culm than in the lower portions (Abd. Latif, 1995; Liese, 1998; Kumar and Mohinder

Pal, 2003; Bhat and Varma, 2006). Culm age is also reported to influence the amount of storage starch. Usually young, growing culms lack starch during the first year of their growth but as age increases there is accumulation of starch in the tissues (Alwin and Murphy, 1988; Weiner and Liese, 1996). However, there also reports to show the absence of any definite relationship between culm age and accumulation of starch (Bhat, 2001).

The amount of starch in culm tissues is also found to be season-dependant. It is found that the percentage of storage starch decreases during certain periods of the year as a consequence of the seasonal, developmental processes taking place in the clump. The variability of starch content in bamboo in relation to season and culm age is available from studies by Abd. Latif *et al.*(1994) and Liese (1998). Thus as a method to overcome the borer problem, harvesting bamboos during such 'low starch periods' has been suggested (Beeson, 1941; Sulthoni, 1987).

The reduction in starch content in felled culms during the post-harvest period is a new observation not reported so far. However, there are reports of mechanical injury triggering enzymatic reactions, which continue to occur up to a certain period in standing culms (Liese, 1998). 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 rate of amylolytic activity observed in bamboo is found to be spectacular. This gives an indication of the possibility of devising some very simple eco-friendly method of protecting stored bamboo and bamboo products.

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 one full year starting from July 2006 to June 2007. 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 per cent) 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 for analysis each time, a 1 cm 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 5ml 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 plotted using potato starch. The percentage of starch was expressed with respect to gram dry weight of the material. For estimating starch content in individual culm, 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 12 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 minutes 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 the addition of 2 ml of dinitro-salicylic acid reagent. The solution was heated on a boiling water bath for 5 min. After cooling under 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 was 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 37ºC 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% nitro-blue tetrazolium was added. Sites of SDH activity showed blue diformazan deposits within cells.

Determination of moisture content

The loss of moisture from culms subsequent to harvesting was assessed by determination of residual moisture content. Moisture content percentage of the culm segments was determined on alternate days following harvesting by oven-drying the samples. After recording the fresh (green) weight of the samples, the latter were dried for 24 hours in a hot-air oven maintained at 105º C and the oven-dry weight was again recorded. The

difference between the green and oven-dry weights was expressed as percentage of oven-dry weight of the samples. Moisture content of samples stored at room temperature as well as that of samples at lower temperature was similarly determined.

Experiments on end-coat application

In order to study the effect of end coating on the moisture loss and amylase activity in harvested culms, experiments were conducted with two types of end coat substances – cashewnut shell oil and a locally made emulsion paint called black japan. Moisture content in the treated samples was determined and compared with untreated (control) samples. Starch percentage and amylase activity were determined on day-6 after harvesting when amylolytic activity is known to attain peak level as found from previous months' observations. The experiment was repeated for three successive months.

Experiments on infusion of exogenous amylase

Infusion of exogenous amylase into the culms was conducted in the field. After cutting the culm at its base and leaving it vertically with intact branches and leaves, the cut end was kept immersed in a 5 per cent solution of amylase for four hours. At the end of this period, the culm was subdivided as usual and brought to the laboratory for determination of starch degradation from the following day. The extent of starch degradation was determined on alternate days during the post-harvest period for a fortnight and was compared with control. This experiment was also repeated for three months.

Physiological studies

Selection of plots

Experimental plot for the study was selected from the KFRI bamboosetum. The culms of the selected species of bamboo were tagged and readings of 5 replications in each species mentioned above were taken at 1 hr interval from 10hrs to 17hrs for the following:

1) Photosynthetic efficiency using the Plant Efficiency Analyzer
2) Stomatal conductance using the Steady State Porometer

Photosynthetic efficiency

The chlorophyll fluorescence technique has been efficiently used to know the photosynthetic performance of the plant species. Measurement of Chlorophyll fluorescence is a non-invasive and rapid method to study the photosynthetic performance of a plant (Bilger *et al*., 1995). Plant Efficiency Analyzer (PEA, Hansatech, Kings Lynn, U K) is a portable non-destructive instrument which utilizes continuous excitation fluorescence measurement principle. The studies on chlorophyll fluorescence parameters provided information on the quantity of light energy absorbed, trapped, utilized for electron transport and re-emitted from the photosynthetic system. It helps in determining the quantum use efficiency in the photosynthetic system II (PSII). The ratio of maximum variable fluorescence (F_V/F_M, where $F_{V=} F_{M-} F_0$) is linearly correlated with the quantum yield of net photosynthesis (Krause and Somersalo, 1989).

Principle: Chlorophyll fluorescence will be in ground state (F_0) when all the electron acceptors are fully oxidized. However, when actinic light is given, the electron acceptors are reduced and fluorescence level increases. The maximum chlorophyll fluorescence (F_M) will be reached when the chlorophyll molecules become unable to accept and transfer any more electrons. Chlorophyll fluorescence then decreases slowly to a steady state (F_S) as photochemistry and CO_2 assimilation increases (Krause and Weis, 1991).

Measurement procedure: Before measurement, leaves were darkened with leaf clips at least for 20 minutes in order to make the photochemical and non-photochemical processes of the seedlings to relax. Later, the plant was illuminated with an extremely dim light (<1µmol m^{-2} s^{-1}) for measuring F_o, the dark adapted yield of chlorophyll fluorescence (F_m). Subsequently, illumination with a brief pulse of extremely bright light (>2000 m^{-2} s^{-1}) helped to saturate the electron transport through chlorophyll fluorescence. From these two values, the quantum use efficiency of PS II (Fv/Fm) was calculated using the Biolyser program (Rodriguez, 2002). The quantum use efficiency of a healthy plant generally ranged from 0.80 to 0.83. The values lower than this are recorded in plants under stress regime due to damage occurring to a proportion of PS II reaction centers (Johnson *et al*., 1993).

Stomatal conductance

Steady State Porometer: A porometer is an instrument used for rapid, precise measurement or water loss and diffusive resistance in leaves. We have used a Null balance Porometer for our experiment, generally referred to as Steady State Porometer (LI-1600, LI-COR, Nebhaslea, USA). The humidity in the cuvette is set constant by the controlled flow of dry air to balance the rate of transpiration, so a steady state condition (null-balance) is achieved. The null adjust meter indicates if more or less dry air is required to reach a steady state condition.

Field measurements were done on days which were not rainy or fully overcast. Only mature and well-exposed leaves were used for measurement of transpiration rate and stomatal conductance. Leaves of the selected species of bamboo were tagged and porometer readings of 5 replications in each species mentioned above were taken at 1 hr interval from 10 hrs to 17 hrs, enclosing the sample leaf in the cuvette. However, the measurements had to be completely abandoned during March, April and May as the experimental plants shed their leaves, and from June to September, due to heavy rainfall. The year round weather parameters like rainfall, and temperature were recorded near the experimental plot.

RESULTS AND DISCUSSION

STARCH AND ITS DEPLETION

The storage sites for starch in the culm tissues of bamboos are mainly the ground parenchyma cells. Starch was found in the form of minute granules within these cells (Fig. 1a). In split surfaces of the culm stained with iodine reagent (I_2KI), the starch-containing strands of parenchyma appeared as fine longitudinal striations of dark blue colour (Fig. 1b). The size and abundance of starch grains within the cells was variable. Similarly, there was considerable variation in starch content between different height levels of a culm and between the outer and inner portions of the culm wall. These observations conform to our earlier observations on distribution of starch in *B. bambos* and *D. strictus* and their relative susceptibility to borer damage (Bhat and Varma, 2006; Bhat *et al*., 2005). Studies have shown that the inner part of the culm wall with abundant starch content is generally subject to intensive damage as compared to the outer part since the borers and their larvae feed selectively on the starch-rich outer part (Plank and Hageman, 1951; Bhat *et al*., 2005). Usually the nodal regions including the diaphragms showed denser accumulation of starch as compared to internodal portions (Fig.1b). Hence

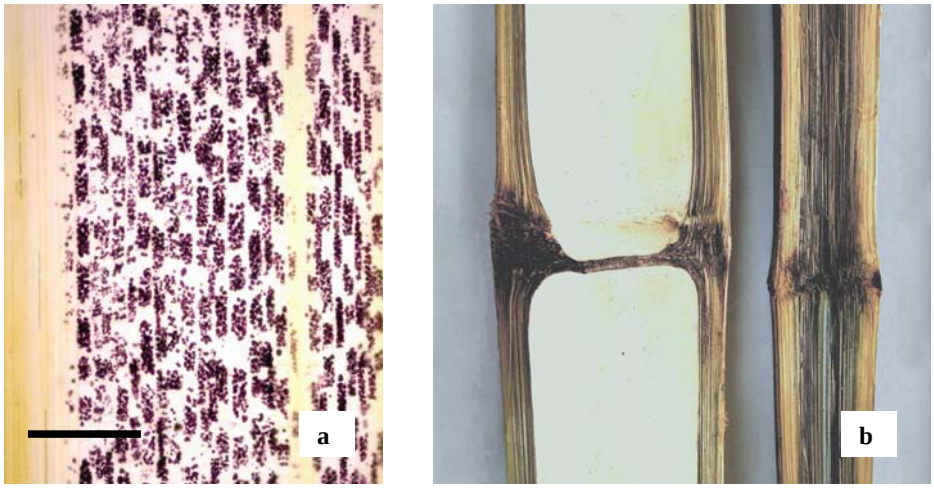

Fig. 1a. Longitudinal section of *Dendrocalamus strictus* showing distribution of starch grains in parenchyma tissue; scale bar represents 200µm. **1b.** Longitudinally split culms of *B. bambos* (left) and *D. strictus* (right) showing dark coloration in response to IKI staining; scale bar in Fig. 1a represents 4 cm.

for comparison of starch on successive days and between the months, culm average values were obtained by pooling together values obtained for basal, mid-height and top height levels of each culm.

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. At the end of two weeks from harvesting, there was conspicuous reduction in starch content (Fig.

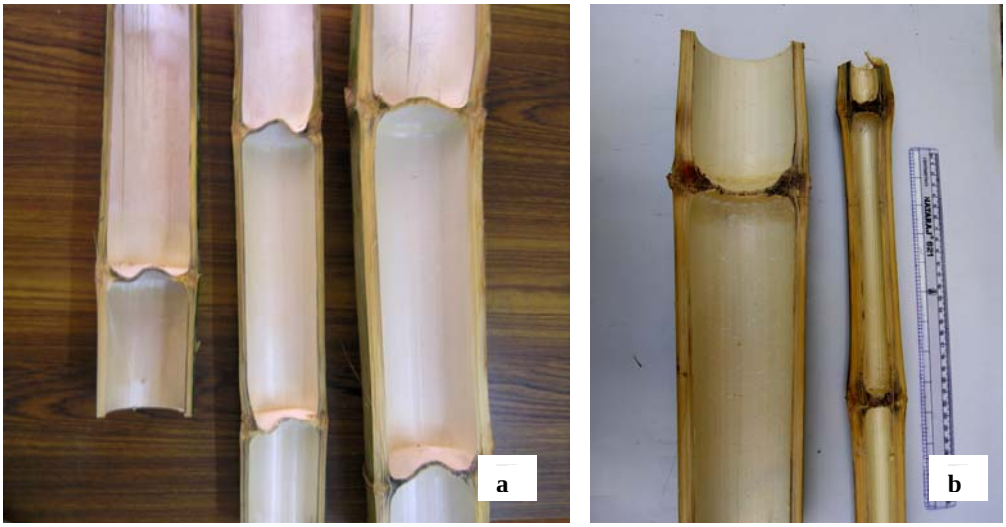

Fig. 2a. Culms of *B. bambos* after two weeks of post-harvest storage under shade; split longitudinally and stained with I_2KI reagent showing decreased staining intensity. **2b.** Split culm of *B. bambos* (left) and *D. strictus* (right) showing residual starch at nodes and cut ends.

2a). However, in the nodal portions and the cut ends of the culm segments, decrease in starch was relatively less (Fig. 2b) as evident from I_2KI stainability.

The starch percentage in culms estimated on alternate days following harvesting for two successive weeks ascertained that there was appreciable reduction in starch content of culms during the post-harvest period in both the species. Mean of 12 month values

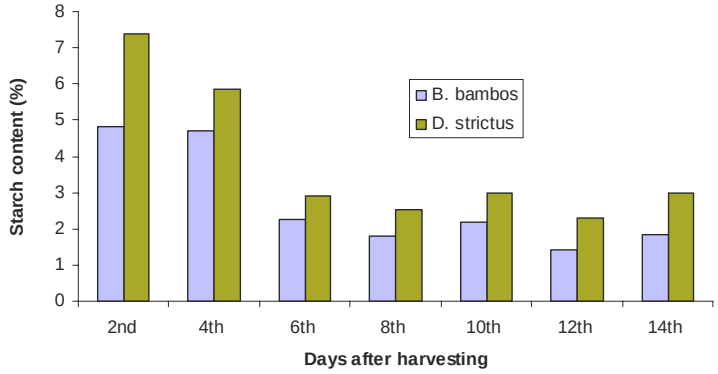

Fig. 3. Reduction in starch content in *B. bambos* and *D. strictus* during post-harvest period.

obtained for day-2 up to day-14 are shown in Fig. 3. It is evident that starch content decreased from an average initial value of nearly 5% (on day-2) to less than 2% towards the end of two weeks in *B. bambos* and from 7.5% to nearly 3% in *D. strictus*. The

fluctuation in average values between day-6 to day-14 is attributable to the wide variation in starch distribution within a culm and also probably the difference between the culms. However, the overall tendency was a drastic reduction in starch percentage during the first week (towards day-6) and then a gradual reduction to reach more or less a stable level subsequently.

Influence of temperature

Comparison was made between the two temperature conditions of post-harvest storage tested using paired *t-test*. There was no statistically significant difference between treatment and control. However, from Fig. 4 it is seen that the trend of decrease in starch content was almost identical at room temperature (30º C) and lower temperature (20º C). However, there was a tendency of lower rate of starch depletion in samples stored at lower temperature. While in *B. bambos* there was a decrease of 60 to 70% starch as compared to

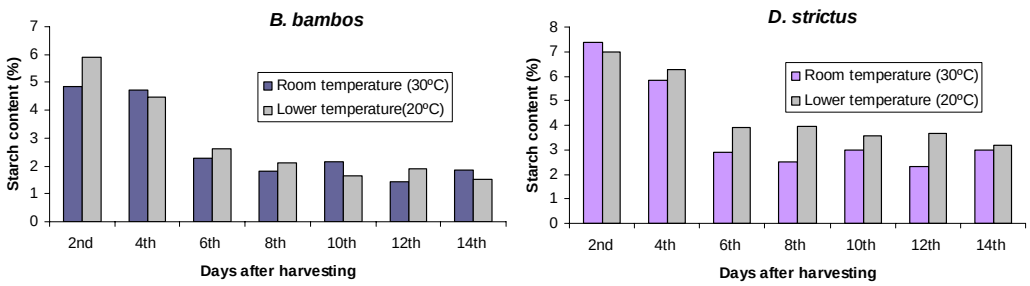

Fig. 4. Starch depletion at two different temperature conditions in *B. bambos* and *D. strictus*

the original, *D. strictus* samples stored at 20º C showed only 50% reduction. It is possible that room temperature is more suitable than lower temperature for achieving enhanced rate of starch depletion although the difference between the two temperature conditions was not much pronounced.

Seasonal variation in starch content

The average starch content of the culms as estimated on day-2 fluctuated between different months without a definite pattern (Fig. 5). Starch was low during December to May except for the high values obtained for January and February in *D. strictus.* The period from June to November were months with relatively high starch content. These

observations deviate from those recorded in the earlier study (Bhat and Varma, 2006), in which starch content was relatively low during September to December and moderate during December to May. Several studies on seasonal variation of starch in bamboo have shown contradictory observations. For example, Sulthoni (1987) reported low starch in

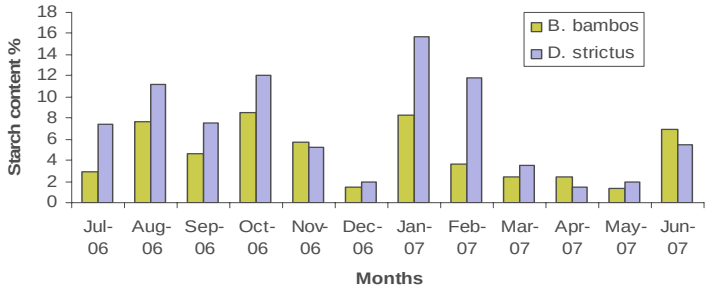

Fig.5. Monthly variation in starch content in *B. bambos* and *D. strictus*

April-May in bamboos of Java, Indonesia. Abd. Latiff (1995) found highest starch content in July-August and lowest in January in Malaysian species *Gigantochloa scortechini*. Okahisa *et al*. (2006) found lowest starch content in August which increased almost linearly up to February and March. Chang *et al*. (2004) reported that in *Dendrocalamus latiflorus* starch content was higher from February to September than rest of the months while in *Phyllostachys makinoi* January to July were the months when starch was high. Seki and Aoyama (1995) found that Starch is low in summer and high throughout the rest of the year in *Sasa senanensis.* Thus it is clear that there is no definite pattern of seasonal variation in starch content and the extent of starch stored is dependent on a number of factors related to growth, age and clump health. Chang *et al*. (2004), based on their study, conclude that the extent of starch found is dependent on physiological factors.

Difference in starch depletion between months

As the initial and final starch content varied between culms, for a meaningful comparison

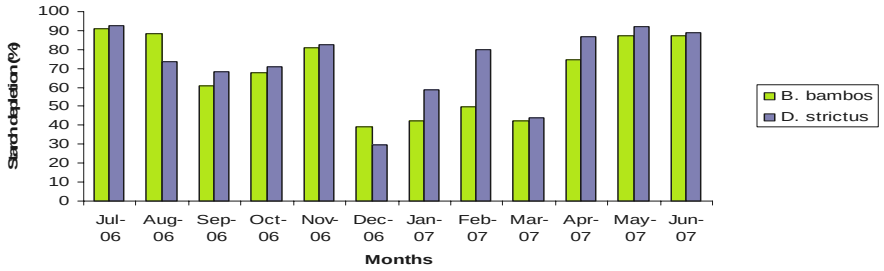

Fig. 6. Differnce in starch depletion between different months in *B. bambos* and *D. strictus*

between months, the extent of starch depletion that occurred in two weeks of post-harvest Period was analysed. From December to March the extent of decrease in starch content was low. Maximum starch depletion occurred during May- August. Thus, it is evident that a seasonality in utilization of storage carbohydrates exists in bamboo. It was found that the starch percentage present in the culm and that depleted during post-harvest period were not significantly correlated.

AMYLASE ACTIVITY

The activity of β-amylase enzyme showed wide variation on successive days of analysis in both *B. bambos* and *D. strictus;* however, the general trends observed were almost similar

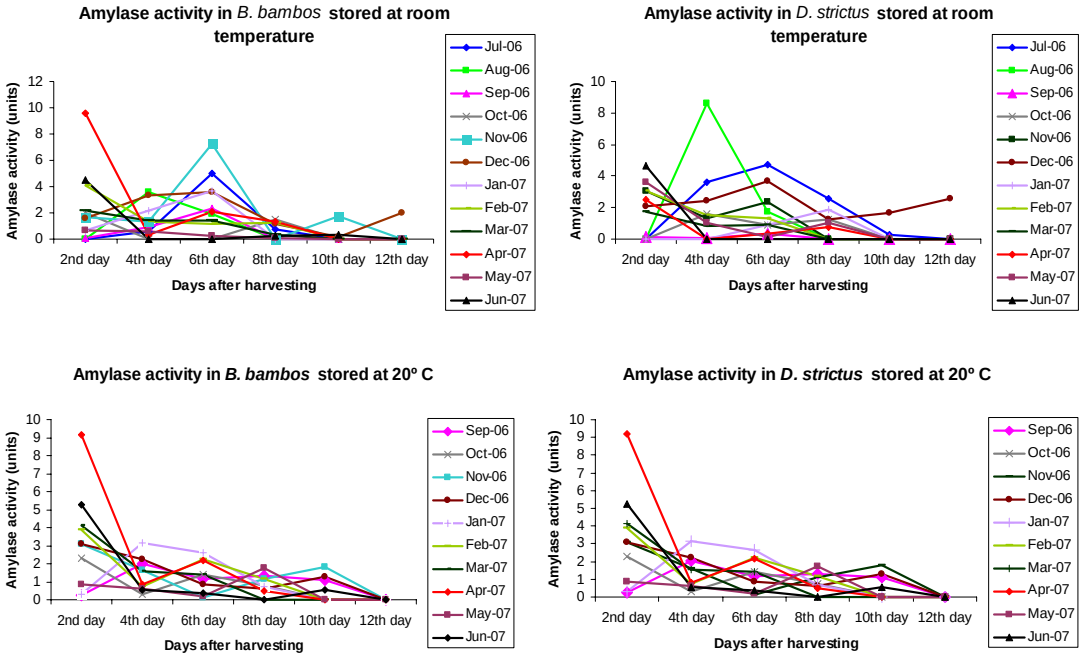

Fig. 7. Trend of amylase activity during post-harvest storage at two different temperature conditions in different months in *B. bambos* and *D. strictus*

in both the species. More commonly, the enzyme activity rose from a low value recorded on day-2 and attained a peak, usually on day-6, and then declined gradually. In contrast to this, in samples where the enzyme activity was high right from the day-2 (or probably, right from the time of harvesting), the activity reduced gradually without a subsequent rise. The declining trend in enzyme activity evident during the second week was more gradual as compared to its initial increase which was more rapid.

Influence of temperature

Samples stored at room temperature and at 20º C did not show much difference in the basic trend and length of the period of enzyme activity. However, the level of enzyme activity did not reach a peak on day-6 or day-4 in samples stored at 20º C unlike in samples kept at room temperature (Fig. 7). Comparison of residual starch on different days in samples stored at two temperature conditions (Fig. 4) also indicates that lower temperature is not as favourable as room temperature for amylolytic depletion of storage starch. The possible reason is probably the higher temperature requirement for the enzyme system for maximum activation.

Monthly variation in amylase activity

No distinct pattern of variation was evident in the extent of amylase activity between different months of the year. However, in general the enzyme activity was low from

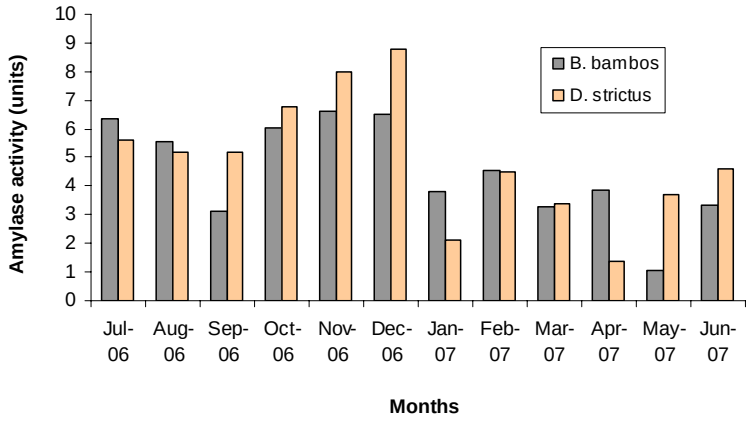

Fig. 8. Variation in amylase activity between months in *B. bambos* and *D. strictus*

January to June as compared to the rest of the period. Despite the low values recorded for the month of September, the extent of amylase activity was relatively higher between July and December. Although the trend of starch depletion between months (Fig. 8) does not match fully with that of high amylase activity, it is evident that both of them almost coincide with the late summer/monsoon period during which fresh growth of leafy branches and subsequently new shoots emerge. It is generally believed that such developmental processes occurring in the clumps are at the expense of reserve

carbohydrates stored in the tissues (Liese, 1998). Therefore it is logical to believe that the period from June to November is ideal for achieving maximum depletion of starch from harvested culms through simple storage under shade.

RESPIRATORY ACTIVITY IN HARVESTED CULMS

Respiratory activity in harvested culms was assessed by histochemical localisation of the respiratory enzyme succinate dehydrogenase (SDH). Thus, only visual comparison was made to assess the overall extent of SDH activity on the basis of the bluish, diformazan deposits formed in cells as a result of enzyme reaction. In freshly harvested young culms

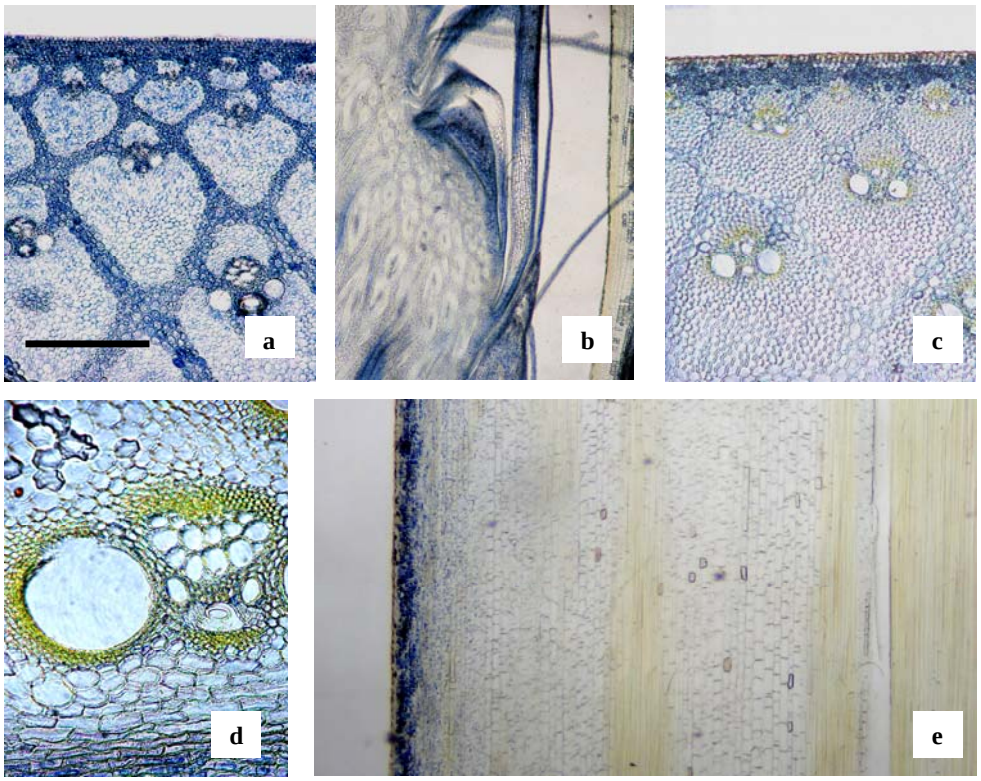

Fig. 9. Succinate dehydrogenase activity. **a.** T. S. of young culm of *B. bambos* with high SDH activity in ground parenchyma tissue. **b.** Nodal bud showing high activity. **c.** T. S. of mature culm of *B. bambos* with enzyme activity confined to cortical and peripheral ground parenchyma cells. **d.** T. S. of mature culm showing SDH activity in inner lining layers and no activity in other parenchyma cells. **e.** L. S. of a mature culm of *D. strictus* with SDH localised only in outer cortical zone and no activity in parenchyma surrounding vessels. Scale bar represents 270, 310, 270, 100 and 250µm for Figs a, b, c, d and e respectively.

undergoing maturation, high SDH activity was evident in cortical parenchyma, ground tissue, lining layers of culm cavity and parenchyma associated with vascular bundles (Fig. 9a). The nodal buds showed high respiratory activity (Fig. 9b). However, in freshly harvested mature culms, SDH activity was mostly restricted to the cortical layers (Fig. 9c).

Feeble activity was also noticed in outer layers of ground parenchyma. Similarly, moderate enzyme activity was also evident in inner lining layers of the culm cavity (Fig. 9d). However, no appreciable SDH activity was evident in parenchyma cells contiguous to the vessels (Figs. 9d, 9e).

During the post-harvest period there was a difference in the extent of SDH activity. Three to four days after harvesting, not only in cortical parenchyma cells but the sub-cortical layers of ground parenchyma cells showed prominent increase in SDH activity. At this

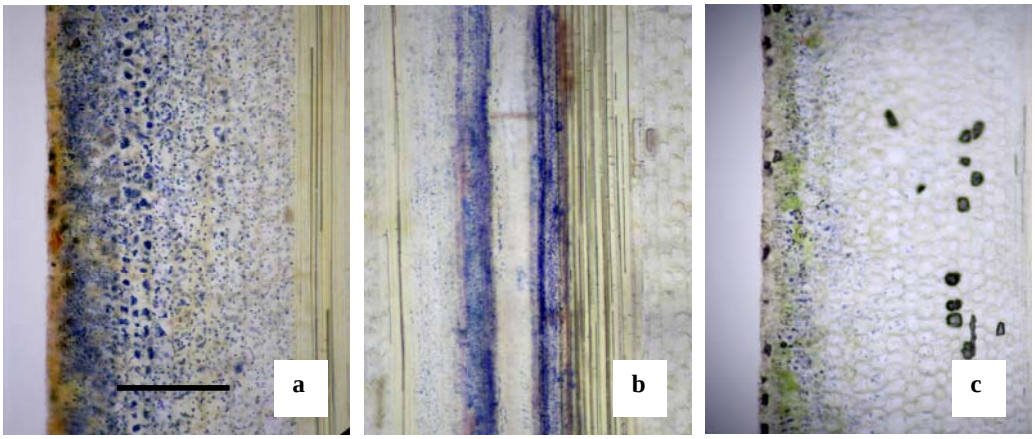

Fig. 10a. L. S. of *B. bambos* showing SDH activity in cortical parenchyma on 4th day after harvesting. **b.** High activity of SDH in parenchyma cells contiguous to metaxylem vesels. **c**. Declined activity of SDH in *D. strictus* during the second week. Scale bar represents 220, 190 and 220µm for Figs. a, b and c respectively.

stage, the bundle parenchyma cells contiguous to the metaxylem vessels also displayed enhanced SDH activity (Fig. 10b). However, towards the end of the first week after harvesting there was a decline in activity of the enzyme and after 7 days there was only feeble activity even in the cortical cells (Fig. 10c).

Overall, there was an obvious increase in respiratory activity in harvested culms during the first week of post-harvest storage implying utilisation of sugars released from hydrolysis of starch. The enhanced respiration is probably due to the disturbance caused in the normal metabolism of the culm resulting from the harvesting. Whether the enhanced respiration in cells contiguous to vessels is related to a wound healing response of the culm or due to penetration of air through open vessels remains to be studied.

EXPERIMENTAL STUDIES

End coat application

Two end coat substances were tested for application on cut ends of the harvested bamboo. One was the common cashewnut shell oil, known for its insect repellent properties. The second one was a locally manufactured wall paint popularly known as 'Black Japan' or 'Japan Black'. The experiment on end coat application was repeated for four successive months. Analysis of starch, amylase and moisture loss was done on day-6 after harvesting when amylase activity was commonly found to be high.

As can be expected, the moisture loss from harvested culms in a week was lower from samples on which end-coat was applied as compared to the control segments stored without end-coating (Table 1). However, ANOVA showed insignificant difference between the treatment and the control and between the species. In general moisture loss was high during the month of February, obviously due to lower atmospheric humidity as compared to the rest of the months (March to May). It was also found that moisture content was not a constraint for amylase activity as it was found that the enzyme was found active even at a lower M.C. %. The end coat applied thus helped only in preventing the entry of borer beetles during the post-harvest storage and not in prolonging the period of enzyme activity.

Table 1. Moisture loss from harvested culms during the first week of post-harvest storage

Species	Treatments	Moisture loss in percentage of the original				
		Feb. 07	Mar. 07	Apr. 07	May 07	Average
B. bambos	C. shell oil	44.15	17.96	20.73	12.27	23.78
	B. japan paint	40.33	13.66	13.32	14.07	20.35
	Control	49.13	31.76	27.39	21.46	32.44
D. strictus	C. shell oil	52.20	36.24	28.62	41.09	39.54
	B. japan paint	39.04	18.82	11.36	30.75	24.99
	Control	47.79	48.58	26.52	40.71	40.90

On the other hand, the average residual starch at the end of one week of post-harvest storage was found to be higher for the two end coat treatments than in the control (Fig. 11) suggesting a lower rate of starch depletion in end coated culm segments as compared to

the control. It is not clear how the end coat chemicals interfere with the normal amylolytic metabolism occurring in harvested culms. Further studies are required to validate the

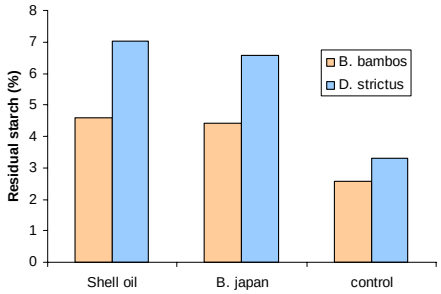

Fig. 11. Residual starch content (%) at the end of one week of post harvest storage of end coated culms

observation.

Application of exogenous amylase

Samples obtained from bamboo culms that were steeped with a dilute solution of amylase during extraction showed a slightly higher rate of starch depletion during the post-harvest storage. The experiment was repeated for three months (October, March and May, 2007) for reliable estimation of starch depletion. On an average, there was a 3.6 per cent increase in starch depletion in *B. bambos* and 3 per cent increase in *D. strictus* as compared to respective untreated samples maintained as controls. Evidently the difference obtained as a result of the treatment was not much pronounced and there was inconsistency of values obtained for different months. It is possible that the major part of amylase solution infused into the culm by steeping is taken up by transpiration stream through the conducting metaxylem vessels without appreciable lateral diffusion of the same to the ground parenchyma which are the main sites of starch hydrolysis. This could be the probable reason for the lack of appreciable difference between the treated and control samples.

PHYSIOLOGICAL STUDIES

Site and climatic details

The experimental site used for physiological studies was the bamboosetum in the campus of Kerala Forest Research Institute (Lat.10^{o}32', Long. 76^{o}20', Altitude 45 m). The climate

was very tropical with two clearly defined monsoons, South-west monsoon during June to September and North-east monsoon during October-November. The annual average rainfall for the location was 2650 mm (personal communication from data collected during 1990 to 2006 by Dr. Jose Kallarackal). During the study period, that is, during 2006 and 2007, the rainfall was normal in 2006 and unusually high during 2007. In 2007, a total rainfall of 4018 mm was obtained till the end of October. After the monsoons, there is a prolonged dry period starting from December to April when there will be not more than 200 mm of rainfall in a normal year. The soil gets progressively dry during these months

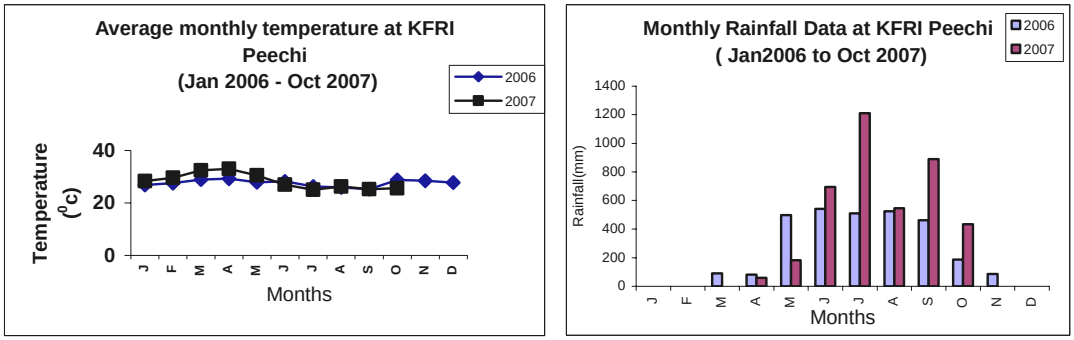

Fig. 12. Monthly climatic data at KFRI Campus, Peechi

and the vapour pressure deficit of the atmosphere also increases to a level of 4.5 kPa. During the months of November to February, there is a dry easterly wind passing through this location. The cumulative solar radiation during the dry period ranges between 25 and 30 Mega Joules m^{-2} day^{-1}.

The temperature in the locality usually ranged between 17 and 37^{o}C. The average monthly temperature and the monthly cumulative rainfall are shown in Fig.12. The maximum temperatures are shown during the month of March.

Stomatal conductance and transpiration

Measurements of stomatal conductance and transpiration (Figs. 13, 14) showed that conductance and transpiration values were relatively low during December when the soil had sufficient water, and not under water stress. The values for the above parameters increased as the dry period proceeded further. Leaf rolling was observed in both the species during periods of water stress. During the months of March and April, the plants

were devoid of leaves, so that no measurements could be taken. The conductance values were slightly more for *B. bambos* when compared to *D. strictus.*

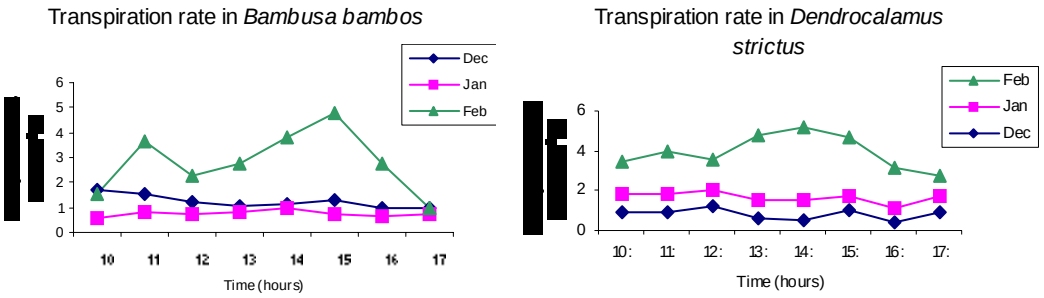

Fig. 13. Chamber transpiration rate (mmol m^{-2} s^{-1}) of *B. bambos* and *D. strictus*.

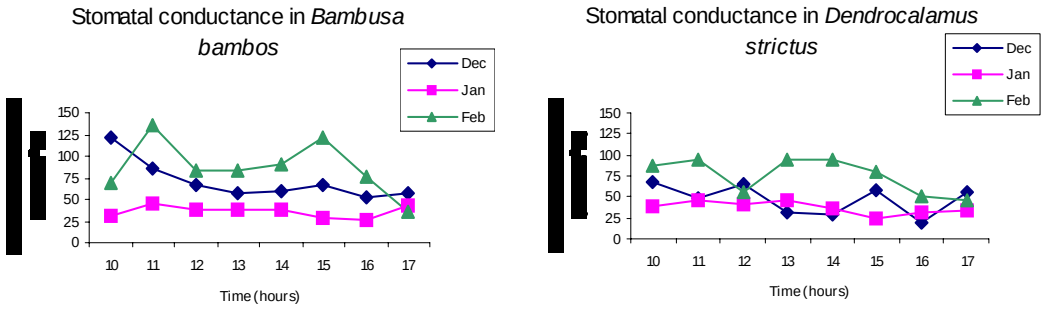

Fig. 14. Stomatal conductance (mmol m^{-2} s) of *B. bambos* and *D. strictus*

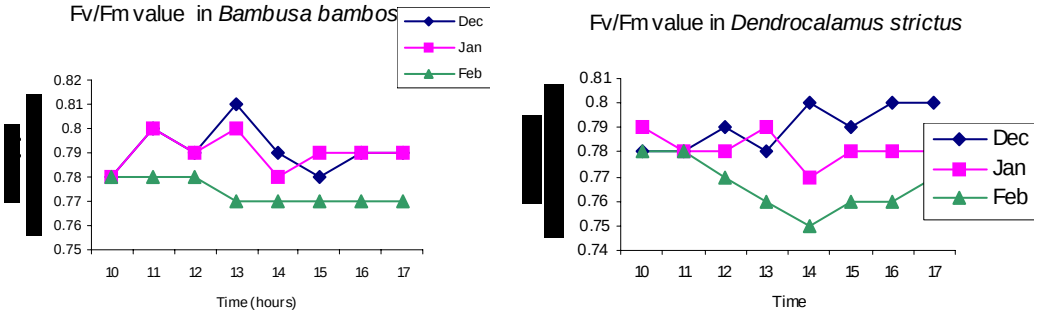

Fig. 15. Fv/Fm values of *B. bambos* and *D. strictus*

Chlorophyll Fluorescence measurements

The values of *Fv/Fm* obtained from chlorophyll fluorescence measurements (Fig. 15) did not show much diurnal variations in *D. strictus* as compared to *B. bambos*. However, the

monthly variations were remarkable. As the dry period progressed, there was a marked reduction in the *Fv/Fm* values, from 0.8 in December to 0.75 in February in *D. strictus* and 0.81 in December to 0.77 in February in *B. bambos*.

Measurements using the porometer throw some light on the stomatal behaviour of the two species of bamboo studied in this project. The conductance values which are in the range of 50 to 150 mmol m^{-2} s^{-1} are relatively low compared to several other tropical species. For example, in teak tree, the values sometimes cross 1000 mmol m^{-2} s^{-1} (Kallarackal and Somen, 2002). It does not probably mean that bamboos have good stomatal control on transpiration. As is evident from the present study, the stomatal conductance and transpiration values are more during the month of February compared to December. In February, the soil is getting drier compared to December; still the transpiration and stomatal conductance are more. It is also seen that the trend for stomatal closure during midday is also weakly developed in the two species studied here. All the above observations indicate that bamboos control the transpiration, probably not due to stomatal closure, but by leaf rolling, which is a well-developed mechanism for preventing water loss from the leaves. The complete shedding of leaves when the dry period progresses, is also another well developed mechanism for excessive water loss from the plants.

Bamboos in general are shallow rooted plants, the roots going not more than 60 cm deep (Divakara *et al*., 2001). However, they spread laterally to several meters. This means that they do not have access to water in the deep soil. In Kerala, during the dry season, the surface soil gets very dry and therefore leaf shedding must be occurring because of this. It could be reasonable concluded that the fast growing bamboo is a good water consumer during the monsoon period and during the dry season, they consume water very economically.

The differences in stomatal conductance and transpiration were not very apparent between the two species studied. However, in *B. bambos* the transpiration and conductance rates were relatively higher as compared to *D. strictus*.

The Fv/Fm values are a good indicator of the photosynthetic efficiency of photosynthesis, especially the PSII system. The optimum values during the non-stress period in most plants have been reported to be 0.83. The measurements in the present study show that the

photosynthetic capacity of the two species is drastically affected in the dry period. The values dropped from 0.83 to 0.75 in *D. strictus* and from 0.83 to 0.77 in *B. bambos* during the transition from December to February. As the photosynthetic output is low during December to February, it is likely that starch accumulation is also likely to be low during this period. However, diurnal variations were not much apparent. This is a good indication to show that drought affects the growth of bamboo.

CONCLUSIONS AND RECOMMENDATIONS

The important conclusions that emerged from the present study are:

- There is significant reduction in starch content from harvested bamboo culms during post-harvest period which is attributable to respiration in tissues. Therefore it is advantageous to store harvested culms under shade for 7-10 days than using them fresh.
- The activity of amylase enzyme is comparatively higher at room temperature (30º C). Therefore better rate of starch depletion can be achieved by storing the culms under warmer storage sheds.
- Although end-coating is not advantageous in enhancing the starch hydrolysis in harvested culms, it is vital for preventing borer entry. Since borer beetles enter the culms through cut ends and other exposed points it will be useful to coat all open wounds and ends of culms with some repellent substances.
- Steeping the culms with amylase solution at the time of harvesting does not help much in enhancing subsequent starch depletion.
- As the photosynthetic output is low during December to February (dry period), the starch accumulation is also likely to be low during this period.
- Since harvesting during the 'low starch periods' is not much practicable due to variability, it will be reasonable to schedule harvesting operation for the period when amylase activity and starch depletion are relatively higher (July-November).

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