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Cleofé E. Calderón (1929-2007)

PROFESSIONAL HISTORY

Cleofé E. Calderón was born on 26 October 1929 in Buenos Aires, Argentina. Although we lack details of her childhood and schooling, we do know that she did work toward a Ph.D. with Prof. Ing. Lorenzo Parodi, the renowned agrostologist (specialist in grasses), at the University of Buenos Aires (Dept. of Agronomy). In 1961 or 1962, an opportunity to visit the United States of America, including Washington, D. C., became available to Cleo. While there, Cleo, as she was known to her colleagues, visited the U.S. National Herbarium in the National Museum of Natural History of the Smithsonian Institution and met Dr. Thomas R. Soderstrom, then the curator of grasses in the Department of Botany. This fortuitous meeting marked the start of a long and productive collaboration between Dr. Calderón and Dr. Soderstrom and led to a number of discoveries and papers that were extremely significant for bamboo systematics and evolution. This work ultimately also provided the foundation for our modern understanding of grass evolution through the extensive collections made by both Calderón and Soderstrom.

Tom Soderstrom, a dynamic young curator who was just discovering the fascinating world of bamboo, invited Cleo to conduct research at the grass herbarium. At that time, Tom's interest was focused on herbaceous bamboos, especially those in the tribe Olyreae. Dr. Floyd McClure was working on woody bamboos in the Grass Lab at the same time, so their research was complementary. And Agnes Chase, the Honorary Curator of Grasses, was still actively curating the grass collection until shortly before her death in September of 1963, so Cleo would have met her as well. The opportunity to study grasses with these colleagues and at one of the premier grass herbaria in the world was irresistible and Cleo stayed on, eventually spending most of the rest of her life in Washington, D. C. When the U.S. National Herbarium moved across the Mall to the west wing of the Natural History Museum building (Morton & Stern, 1966), Cleo moved into the newly established Laboratory of Agrostology in Room W408.

Initially Cleo and Tom concentrated on the olyroid bambusoid grasses (tribe Olyreae), but quickly their interest extended to encompass all the forest understory grasses then considered to be herbaceous bamboos. A number of tribes of broad-leaved, primarily tropical forest-dwelling grasses, including Anomochloeae, Streptochaeteae, Phareae, Puelieae, Guaduelleae, Streptogyneae, and the olyroid grasses (tribes Buergersiochloeae, Parianeae, and Olyreae), all fell into this category. Little was known about them compared to other herbaceous grasses or even the woody bamboos. Several of these tribes were restricted to the New World (Western hemisphere) or had some representatives there, so it was a logical choice for Tom and Cleo to concentrate on the Neotropics. In addition to her native Spanish, Cleo also spoke English and Portuguese fluently, which greatly facilitated her field work.

With support from the Smithsonian Research Foundation and the Smithsonian's Office of Systematics and Office of Ecology, the Office of Scientific Affairs of the Organization of American States, and the National Geographic Society, Cleo was able to undertake field work in Central and South America to collect and study bamboos as well as to spend time in Europe and India consulting with influential botanists such as Dr. C. R. Metcalfe, Dr. C. E. Hubbard, Prof. Dr. H. Weber, Prof. Dr. W. Troll, Dr. H. Jacques-Félix, and Dr. V. Puri, among others. These funding sources also supported Cleo when she was based at the National Herbarium. In 1966, she also had the opportunity to assist Dr. Richard W. Pohl (Iowa State University) in teaching a course on tropical agrostology in Costa Rica for the Organization for Tropical Studies.

Cleo and Tom began publishing their results on the Olyreae and the other herbaceous bambusoids in earnest in the 1970's (see Calderón bibliography below) but they also continued to conduct extensive field work, especially in Brazil (Table 1). Cleo devoted much time to collecting in Eastern Brazil because of its rich bamboo flora and because it was the most likely place to relocate *Anomochloa* (see *Collections* below), but she also was determined to learn more about the bamboos of Brazil's Amazonian region. Her last extended collecting trip was to the states of Amazonas and Rondônia, where she made a number of important bamboo collections representing many undescribed species.

Both Cleo and Tom had very strong but in many ways complementary personalities. Although their collaboration was productive and resulted in a number of papers and discoveries that were of fundamental importance to bamboo and grass systematics, it was often a stormy relationship. After Cleo's return from her extended 1979 trip to Brazil, Tom abruptly ended their collaboration and Cleo moved to a different office within the U.S. National Herbarium. Cleo continued to work on her collections and maintained her association with the herbarium, but in order to support herself she turned to catering for a period of time. Cleo had professional training as a chef and was

Table 1.
A summary of field trips by C. E. Calderón

Dates	**Itinerary**
Apr, Jun, Jul 1966	Costa Rica (April with R.W. Pohl; June and July with T. R. Soderstrom)
Dec 1967-Feb 1968	December: Brasilia, Santa Catarina, São Paulo, Rio de Janeiro, Brazil; January: Rio de Janeiro, Bahia, Brazil; February: Pará, Brazil
Mar 1968	Panama, Costa Rica (with T. R. Soderstrom)
Oct-Nov 1971	Panama
late Feb-May 1972	Late February and early March: Rio de Janeiro, Brazil (with T. R. Soderstrom); mid-March through May: Bahia, Brazil
Nov-Dec 1974	Pará and Amapá, Brazil
Feb-May 1976	February: Acre, Amazonas, Brazil; April through May: Bahia, Brazil
Apr 1977	Bahia, Brazil
late Jan-Feb 1978	Bahia, Brazil
Oct 1978	Indonesia (with T. R. Soderstrom)
Feb 1979	Bahia, Brazil
Jun-Sep 1979	June and July: Amazonas and Rondônia, Brazil; Aug-Sep: Amazonas, Brazil (late August and early September with K. Kubitzki)
Aug 1981	Colombia (with L.G. Clark, in part)
late Aug-Sep 1982	Ecuador (with L.G. Clark)

an outstanding cook. Friday afternoon tea at the herbarium was always especially well attended when it was Cleo's turn to help with the refreshments.

In 1981, Arq. Oscar Hidalgo, a Colombian architect who is well known for his development of bamboo as a building material (Hidalgo-Lopez, 2003), decided to organize a Latin American bamboo symposium in Manizales, Colombia. He originally invited Tom Soderstrom to lead a workshop on bamboo systematics as part of the symposium, but just a few weeks before the event, Tom became seriously ill and had to withdraw. Oscar turned to Cleo and she agreed to fill in for Tom, even though she had little time to prepare. Lynn Clark and Dr. Pohl traveled to Manizales to assist Cleo with the workshop. This event also marked a turning point in the study of American bamboos, as Ximena Londoño was one of the participants. The symposium was so successful that another Latin American bamboo symposium was organized the following year in Guayaquil, Ecuador, by Ing. Jorge Moran. For this event, Cleo was invited directly to conduct a workshop on bamboo systematics and identification with Lynn's assistance. Dr. Pohl and Ximena Londoño also attended, and it was during this workshop that Cleo strongly encouraged Ximena to continue her studies of bamboo and to visit Cleo at the U.S. National Herbarium. After the symposium in Manizales, Colombia, and prior to the symposium in Guayaquil, Ecuador, Cleo and Lynn were able to conduct field work in these two countries; the last collections made by Cleo in her botanical career were during these two trips.

Upon her return to Washington, D. C., Cleo came less and less frequently to the herbarium and by 1985 had left botany altogether. She obtained a job at a bibliographic service and continued to live in the District. She maintained a few contacts with friends and colleagues at the herbarium but after attending the memorial service for Tom Soderstrom in September 1987, she essentially severed all ties to her botanical life. Cleo retired and returned to Argentina in 2005 to be closer to her sister and the rest of her family. Cleo died in Buenos Aires on 19 March 2007.

BOTANICAL RESEARCH CONTRIBUTIONS

Collections. Approximately 1,000 collections by Cleo, the majority of them bamboos, are deposited at the U.S. National Herbarium (US). Duplicates are widely distributed but at least one set is deposited in a major herbarium in the country of origin. At least 40 additional collections in which Cleo participated but was not the principal collector are also deposited at US. These include collections made primarily with T. R. Soderstrom, K. Kubitzki, R. W. Pohl, and L. G. Clark (Table 1).

Cleo Calderón's collections, although not numerous, are of great significance to grass systematics due to both their quality and the large number of novelties represented among them. Cleo's rediscovery of *Anomochloa* ranks as her greatest collecting achievement, but a number of her collections represented the first time species and even genera (e.g., *Maclurolyra, Alvimia*) were collected, or collected in flower, and over 30 have become type specimens, particularly for bamboos. Many of these new species or genera were described after Cleo left botany, and we expect that some additional taxa may yet be described from her collections, especially those from Brazil.

One noteworthy non-bamboo collection, however, was Cleo's very complete gathering of vegetative and mature flowering material that became the type specimen for *Arundoclaytonia*,

an unusual grass of white sand vegetation collected during her last trip to Brazil (Davidse and Ellis, 1987). As Gerrit Davidse (Missouri Botanical Garden) recalled "I well remember the afternoon in 1985 during a visit to the National Herbarium when she brought me the unmounted collection of what we would later name *Arundoclaytonia dissimilis.* The moment I opened the newspaper and saw those distinctive spikelets, I realized that it was a new genus of the Steyermarkochloeae, a new tribe that Roger [Ellis] and I had just recently described and published in 1984. When she encountered the plant in the field along the Transamazon Highway in 1979, she had recognized that this was something very unusual and something probably undescribed. Because of this she took extra care to collect beautiful specimens and fully documented its various growth stages from sterile, vegetative plants, burned flowering plants that exposed the culm morphology, and, of course, mature flowering plants."

Cleo's collections are remarkable for their consistently high quality. When Cleo came upon an interesting bamboo or other grass, she would take whatever time was necessary to ensure that a thorough collection was made. She would take detailed field notes, including measurements, and photographs before pressing the specimens. Cleo usually carried two camera backs, one loaded with black and white film and the other with color film, so that she could simply switch lenses as needed to document the habit and other important features of the plant in both formats. Cleo and her assistants would routinely press several sets of duplicates for each collection number in the field, rather than putting plant parts in plastic bags for pressing that night. Cleo would not be hurried, often to the consternation of those accompanying her, but for this reason, her collections retain enormous scientific value and represent perhaps her most important scientific legacy.

Cleo collected all bamboos, both herbaceous and woody, that she encountered but she and Tom were particularly interested in relocating the mysterious tropical forest grass *Anomochloa*, whose name means "anomalous grass". Plants of *Anomochloa* were cultivated at various European conservatories through the 1850's, which came from propagules sent to France from Brazil in the 1840's, but all of the living plants eventually died out, so that the only available material consisted of a few herbarium sheets (Soderstrom, 1984). Both Tom and Cleo undertook several expeditions to Brazil in an effort to relocate *Anomochloa*, but it was Cleo, along with her Brazilian assistant Talmon dos Santos, who finally found it in 1976 in Bahia. A detailed account of the history of Cleo's rediscovery of this plant is provided in Judziewicz and Soderstrom (1989: 2-5). Cleo could never explain exactly why she and Sr. dos Santos stopped at that particular location, but she always insisted that her poor eyesight (we affectionately referred to her as "Mrs. Magoo") actually helped her in finding herbaceous bamboos because she had to walk so carefully and slowly in the forest.

For anyone interested in the evolutionary history of grasses, it would be difficult to overstate the importance of the rediscovery of *Anomochloa*. The material collected by Cleo and Sr. dos Santos as well as specimens and live plants collected subsequently by Sr. dos Santos accompanying variously T. R. Soderstrom, Gustavo Martinelli, Victoria Hollowell, and Emmet Judziewicz among others, formed the basis for the detailed morphological and anatomical study of *Anomochloa* by Judziewicz and Soderstrom (1989). This work confirmed that *Anomochloa* was indeed a grass and provided insights into the peculiar nature of its flowering structures. Clark et al. (1995) and the Grass Phylogeny Working Group (GPWG, 2001) later established through analysis of DNA sequence data that *Anomochloa* and *Streptochaeta*, another grass traditionally classified as an herbaceous bambusoid, actually comprised the earliest-diverging living lineage of grasses.

Herbaceous bamboos. In addition to the description of new species and genera of herbaceous bamboos (e.g., *Arberella, Maclurolyra*), two areas of special interest for Tom and Cleo were pollination biology and inflorescence architecture.

Although there were a number of reports in

the pollination biology literature that insects were found in association with the inflorescences of grasses, this had always been assumed to be a casual relationship since grasses are well known as a wind-pollinated group. A few intriguing reports relating to tropical rain forest grasses, however, suggested otherwise, and Tom and Cleo set out to examine the possibility that some of the herbaceous bamboos were insect-pollinated. This required patient observation of a variety of species along with collection of both insect visitors and voucher specimens for the herbaceous bamboos. Cleo carried out the bulk of this field work in Brazil and Panama, while Tom carried out observations in Colombia and Venezuela. Although the results were not conclusive (Soderstrom and Calderón, 1971), they nonetheless strongly suggested that insect pollination occurs in some herbaceous bamboos of tropical rainforests, primarily due to the exclusive association of some species of midges with the inflorescences of *Pariana* species. This work represents the first and to date only set of rigorous observations on the pollination biology of herbaceous bamboos.

McClure's detailed work on the morphology of woody bamboos (e.g., McClure, 1934, 1966) provided an excellent model for similar studies in the herbaceous bamboos. Cleo and Tom used the description of the new olyroid genus *Maclurolyra* (Calderón and Soderstrom, 1973) as an opportunity to conduct a thorough morphological and anatomical investigation and to apply the principles of Troll (e.g., 1964) to an analysis of inflorescence structure in herbaceous bamboos. The epidermal and cross-sectional leaf anatomy of Maclurolyra was described and illustrated in great detail, as were its gynoecium and endosperm. This work included the first report of plastids in the outer bundle sheaths in bamboos and provided a means to refine the concept of bambusoid leaf anatomy. Cleo and Tom also reported a chromosome count for *Maclurolyra*, as was a detailed description and illustration of the seedling. Beautiful line drawings of the new (and only) species *M. tecta* were also included. And a rigorous, comparative analysis of the inflorescence of *M. tecta* and two other olyroid species, *Olyra latifolia* and *Rehia nervata* (as *Bulbulus nervatus* in the publication) was provided. Troll's ideas regarding innovation, inhibition and supplementing (enrichment) zones were applied and the various orders of successive branching were identified. Although Cleo and Tom regarded their conclusions about inflorescence structure in the herbaceous olyroid bamboos as tentative, this was, to our knowledge, the first application of this type of analysis to bamboos and it paved the way for subsequent analyses of bamboo inflorescences (e.g., *Alvimia* in Soderstrom and Londoño, 1988). The *Maclurolyra* paper was an exemplary monograph that served as a model for several later papers, including Judziewicz and Soderstrom (1989), which have had enormous impact on our understanding of grass evolution.

General Bambusoideae. Cleo and Tom continued to focus on herbaceous bamboos, although they did describe several new species of the Neotropical woody bamboo *Chusquea* (Soderstrom and Calderón, 1978a, b). Their reviews of bamboo systematics and ecology (Soderstrom and Calderón, 1979a, c) highlighted the importance of bamboos in many different habitats worldwide and their economic value but also emphasized the need for continuing systematic and ecological study of bamboos. They also discussed the accelerating destruction of tropical forests and its impact on bamboo diversity, a common theme in many of their papers. Their final publication on the bamboos of the Americas (Calderón and Soderstrom, 1980) was their most synthetic work. They presented an updated and well reasoned recircumscription of the subfamily Bambusoideae and included keys to the tribes and genera. Although their concept of the subfamily Bambusoideae was much broader than is currently accepted, they nonetheless called attention to the diversity of bamboos and how much still needed to be learned about bamboos. In effect, this work provided a roadmap for bamboo systematics and their classification system was not revised until DNA sequence data provided the foundation for a truly phylogenetically based system of grass classification a generation later (Clark *et al.*, 1995; GPWG, 2001).

TEACHING, MENTORING AND COLLEGIALITY

Although Cleo never taught formal classes in botany, she nonetheless had a profound impact on bamboo systematics through her participation in workshops (e.g., the bamboo systematics workshops given at the two Latin American bamboo symposia in the early 1980's) and her individual training and mentoring of those students and botanists with whom she interacted during her career. Although Tom Soderstrom was responsible for recruiting Lynn Clark as a summer volunteer at the herbarium (during the summers of 1973 through 1977), Cleo provided a great deal of training during this time. Cleo also taught Lynn how to collect bamboos during their joint field work in Colombia and Ecuador. Cleo was the inspiration for Ximena Londoño to come to the U.S. National Herbarium starting in 1984 to learn English and to study bamboo. As mentioned above, Cleo assisted Richard Pohl in teaching the OTS course on Biology of Tropical Grasses in July and August 1966; among the students were Scott Mori, Melinda Denton, James Payne Smith, Jr., and Gerrit Davidse.

When Tarciso S. Filgueiras visited the U.S. National Herbarium in Washington, D. C. en route to Corvallis, Oregon in 1974, he was met by Cleo who kindly showed him the grass collections and strongly suggested that he should study grasses during his graduate work, not legumes as he had originally planned. "Será melhor para seu país," she said to him. This simple but effective advice was decisive in Tarciso's decision to study the grasses when he started his Master's degree program at Oregon State University.

The late "Brazilian" botanists Tatiana Sendulsky and Alasdair G. Burman visited the U.S. National Herbarium many times during the years that Cleo and Tom were collaborating. In all of those visits, Cleo played a key role, providing housing, full access to grass specimens and literature and generally facilitating the work of these two visiting colleagues, who always referred to Cleo as a dear, close friend.

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TAXA NAMED BY CLEOFÉ E. CALDERÓN

Arberella Soderstr. & C. Calderón

Arberella costaricensis (A. Hitchc.) Soderstr. & C. Calderón

Arberella dressleri Sodestr. & C. Calderón

Arberella flaccida (Doell) Soderstr. & C. Calderón

Chusquea angustifolia (Soderstr. & C. Calderón) L. G. Clark

Chusquea circinata Soderstr. & C. Calderón

Chusquea coronalis Soderstr. & C. Calderón

Chusquea longiligulata (Soderstr. & C. Calderón) L. G. Clark

Chusquea scabra Soderstr. & C. Calderón

Chusquea vulcanalis (Soderstr. & C. Calderón) L. G. Clark

Eremitis parviflora (Trin.) C. Calderón & Soderstr.

Maclurolyra C. Calderón & Soderstr.

Maclurolyra tecta C. Calderón & Soderstr.

Otatea (McClure & E. W. Smith) C. Calderón & Soderstr.

Otatea acuminata (Munro) C. Calderón & Soderstr.

Otatea aztecorum (McClure & E. W. Smith) C. Calderón & Soderstr. = Otatea acuminata subsp. aztecorum

Raddiella esenbeckii (Steudel) C. Calderón & Soderstr.

Raddiella molliculma (Swallen) C. Calderón & Soderstr.

Streptogyneae C. Calderón & Soderstr.

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Trends in fiber characteristics of Nigerian grown bamboo and its effect on its impact and tensile strengths

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ABSTRACT

Variations in impact bending and tensile strengths of *Bambusa vulgaris* (Schrad) with increasing culm height were studied considering its fiber characteristics at the nodes and internodes along the length of the culm. At the nodes, statistical analysis revealed a weak but direct correlation between fiber length and tensile strength while fiber diameter had a strong but inverse relationship with it. Impact bending showed a direct but weak relationship with fiber length while it had an inverse and weak correlation with fiber diameter. For the internodes, a strong but inverse relationship was established between fiber length and tensile strength while fiber diameter showed a direct but weak relationship. Impact bending and fiber length had a strong but inverse correlation while fiber length and impact bending revealed a direct and significant correlation.

INTRODUCTION

Bamboo has long been in existence and its use in housing has been traced back to the pre-ceramic period 9500 years ago while relics from bamboo mats and baskets were dated at 3,300 to 2800 B.C (Liese, 1999). It remains the single most important organic building material in Asia accounting for over 70% of rural housing in Bangladesh for example (INBAR 2007).

Going from one climate to another bamboo varies in size and height e.g. dwarf bamboo, which is about 30cm in height to giant timber bamboos that may reach heights above 30m (Gib, 2005). Owing to the wideness of its distribution, it has a very wide variety of uses. Even though it is best known for its use as a construction material, it is also grown for shade, animal fodder and food. As a result of this diversity of species, it is safe to say that there is a bamboo to suit any need (Ahmad, 2000).

Bamboo grows fast and matures quickly. It is hard, strong, flexible and more importantly renewable. It is regarded as the fastest growing woody plant in the world (Janssen, 2002) as some species are reported to have a growth rate of about 1m per day. Bamboo species are seen as a good alternative to wood owing to their good qualities in physical and mechanical attributes (Li, 2004).

Bamboo has been recognized as an acceptable raw material for the manufacture of paper. Utilization research has shown that bamboo is not only well adapted to the production of most usual types of paper but is also suitable for the manufacture of special papers, that may not be readily produced from traditional materials (Perdue, 2005; Jiang, 2004). Dhamodaran *et al.* 2003) reported that fiber dimensions show the suitability of a fibrous raw material for producing pulp and that relative fiber length influences tearing resistance.

Liese (2004) observed that fibers amount to about 40% of total culm mass and about 60-70% of its weight just as fiber content and fiber length affects specific gravity and strength properties hence, the need to ascertain the extent of the influence of fibers on the strength of *Bambusa vulgaris* (Schrad).

MATERIALS AND METHODS

The culms of *Bambusa vulgaris* (Schrad) used in this work were collected from the Olodo bamboo forest in Egbeda Local government area of Ibadan, Nigeria and the physical conditions of the bamboos to be felled were inspected to

make sure that only mature and healthy bamboos were felled. *Bambusa vulgaris* (Schrad) is the predominant bamboo species found in Nigeria being one of about five known species (Oyebode and Ogedengbe, 2001).

The total length of the useful part of the bamboo culms was recorded after which they were divided into three equal parts in line with the recommendations of ISO standard DIS 22157 (2000). The portions were labeled Base, Middle and Top. From these, samples were taken with some containing nodes at mid-span within the test region while others were taken from the internodes only. Labeling of experimental samples was done immediately after conversion to facilitate easy identification and also to avoid mixing up of samples.

The experimental investigations performed were fiber length, fiber diameter, tensile strength and impact strength with particular reference to the presence or absence of nodes.

Fiber Characteristics: The *Bambusa vulgaris* (Schrad) culm portions collected from the field were cut into pieces of 2 x 1 cm dimensions and boiled in water for 24 hours to soften it. Portions of the *Bambusa vulgaris* (Schrad) culm that had been softened were sliced into small pieces and macerated using Schluz's fluid which was prepared by mixing equal volume of 10% chromic acid and 10% nitric acid. The maceration was carried out in a beaker kept in the sun for 2 days. The macerates were washed in five changes of water and then preserved in 50% ethanol. From these, fiber length (FL) and fiber diameter (FD) were determined in millimeters using the ocular/stage micrometer.

Tensile Strength TS: This was done using the Monsato Tensometer in the Mechanical Engineering Laboratory of the University of Ibadan. The samples were prepared in accordance with the recommendations of ISO DIS 22157 (2000). The total length of each specimen was 120mm while that of the gauge portion was 50mm. Samples were taken from both nodal and internodal areas with the nodes being at mid-span.

The equation used in calculating the tensile stress as stated in the standard is:

$$\mu_{ult} = F_{ult}/A$$

where μ_{ult} = ultimate tensile stress rounded up to the nearest whole N/mm^2

F_{ult} = maximum load in Newton at which the piece fails

A = mean cross sectional area of the gauge portion in mm^2

Sampling intensity was 12 specimens per test.

Impact Bending Strength IB: This was performed using the Hatt-Turner impact testing machine at the Department of Forestry, University of Ibadan. This test was performed according to British Standards BS 373 (1957) which recommends dimensions of 2cm by 2cm by 30cm (i.e. breadth by thickness by length) for wood. However, a modification in which the thickness of the samples was made to be equal to the wall thickness of the portion of bamboo from which they were taken as against 2cm recommended in the standard was made.

Samples were taken from the internodes as well as from those with the nodes being present at mid-span of the sample after which they were conditioned to 12% moisture content. The samples were clamped to the test area of the machine and the metal hammer which was suspended at an initial height of 50cm was released to create the impact. Increments in height of impact were made at an interval of 2.54cm and the height at which failure occurred was recorded.

Energy required to cause failure was then calculated from the following general equation for the determination of potential energy:

$$E = m*g*h$$

where E = energy absorbed at failure in Joules,

m = mass of hammer in kg

g = acceleration due to gravity in m/s^2 and

h = height at which failure occurs

Culm wall thickness was measured using the micrometer screw gauge.

Statistical Analysis: In order to find the correlation between fiber length, fiber diameter, tensile strength and impact strength of *Bambusa vulgaris* culms, statistical analysis was done using the Pearson's Product Moment Correlation Coefficient to make multiple comparisons between different parameters as well as to establish the kind of relationship existing between them.

Table 1: Mean Values of Experimental Data Obtained from Specimens from the Node

	Fiber Length (mm)	Fiber Diameter (mm)	Tensile Strength (N/mm²)	Impact Energy (J)	Culm Wall Thickness (mm)
Base	1.8	0.023	19.3	10.7	17.1
Middle	1.7	0.028	15.7	9.8	13.2
Top	1.4	0.031	11.1	6.3	8.9

Table 2: Mean Values of Experimental Data Obtained from Specimens from the Internode

	Fiber Length (mm)	Fiber Diameter (mm)	Tensile Strength (N/mm²)	Impact Energy (J)	Culm Wall Thickness (mm)
Base	2.8	0.033	26.4	9.5	14.8
Middle	3.4	0.018	23.1	7.6	6.7
Top	3.7	0.013	20.1	5.6	5.3

RESULTS AND DISCUSSION

Tables 1 and 2 show the mean values obtained for the fiber characteristics, culm wall thickness and strength properties determined.

Variations in Fiber Length (FL), Fiber Diameter (FD), Tensile Strength (TS) and Impact Bending (IB) with increasing Culm Height

Figures 1-4 show variations in the FL, FD, TS and IB of *B. vulgaris*. FL, TS and IB at the nodes reduce with increasing culm height, being highest at the base and lowest at the top while FD behaves conversely; increasing with increasing culm height. However, FD, TS and IB at the internodes reduce with increasing culm height, being highest at the base and lowest at the top while FL behaves conversely; increasing with increasing culm height.

When compared with the experimental values obtained from samples taken from the node, TS and IB of samples from the internode show the same trend while FL and FD behaves conversely as FL was highest at the top where FD was found to be lowest.

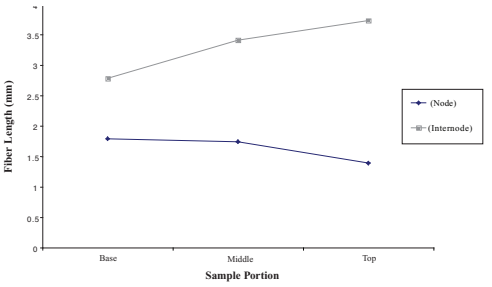

Figure 1: Fiber Length of *Bambusa vulgaris* (Schrad) with increasing culm height

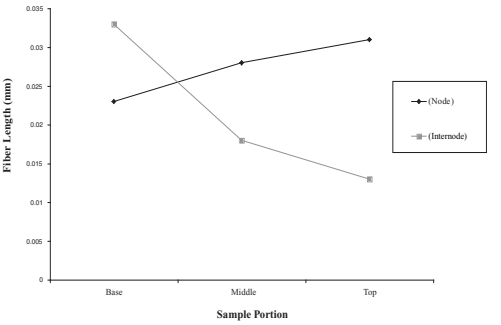

Figure 2: Fiber diameter of *Bambusa vulgaris* (Schrad) with increasing culm height

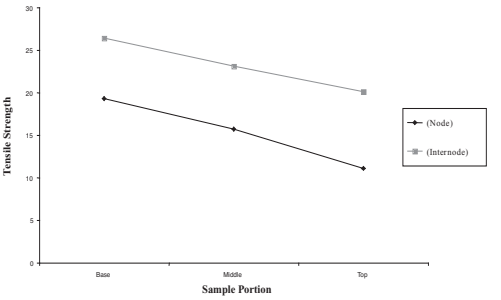

Figure 3: Tensile strength of *Bambusa vulgaris* (Schrad) with increasing culm height

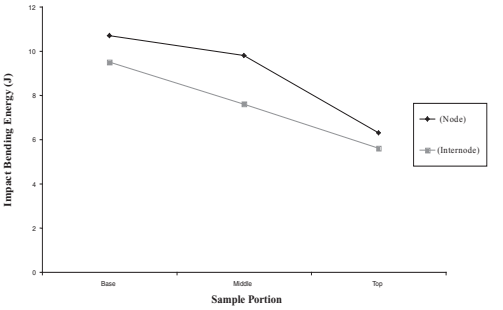

Figure 4: Impact Bending Energy of *Bambusa vulgaris* (Schrad) with increasing culm height

Correlation between Fiber Length (FL), Fiber Diameter (FD), Tensile Strength (TS) and Impact Bending (IB)

Statistical analysis was done to ascertain the effect of FL and FD on the TS of the *B. vulgaris* and this yielded a Pearson's correlation coefficient r^2 (at $P < 0.05$) of 0.158 and -0.642 for FL and FD respectively at the node. This implies that FL has a direct but weak relationship with nodal TS (i.e. as FL increases, TS increases) while FD has an inverse but significant correlation (as FD increases, TS decreases).

However, a difference was observed at the internodes as r^2 values were -0.574 and 0.479 for FL and FD respectively. This implies a significant but inverse relationship between FL and internodal TS (i.e. as FL increases, TS decreases) while FD has an insignificant but direct relationship with it (i.e. as FD increases, TS increases).

Also, statistical analysis performed to ascertain the effect of FL and FD on the IB of *B. vulgaris* yielded a Pearson's correlation coefficient r^2 (at $P < 0.05$) of 0.444 and -0.486 for FL and FD respectively for the node. This implies that FL has a direct but weak relationship with nodal IB (i.e. as FL increases, IB increases) while FD has an indirect and weak correlation (i.e. as FD increases, TS decreases).

At the internodes however, Pearson's correlation coefficient r^2 (at $P < 0.05$) were -0.625 and 0.738 for FL and FD respectively. This implies that FL has an indirect but strong correlation with internodal IB (i.e. as FL increases, IB decreases) while FD has a direct and strong correlation with internodal IB (i.e. as FD increases, IB increases).

CONCLUSIONS

The study shows that FL influences nodal strength positively both in TS and IB while FD behaves conversely. At the internodes however, TS and IB is negatively influenced by FL while FD has a positive influence on TS but a negative one on IB. Moreover, the presence of nodes serves as reinforcement against impact loads by improving the shock resistance of the culm while the absence of nodes is an added advantage when tensile stresses are to be applied because bamboo is weak in tension at the nodes.

Liese (2004) reported that FL is known to vary between different bamboo species and this has been reported to be in the range of 1.5mm to 3.5mm. More so, Li (2004) gave an average value of 2.3mm for FL while Horn and Setterholm (1990) gave and average value of 2.7mm and 15μm for FL and FD respectively. Liese (1992) similarly confirmed that bamboo nodes have shorter fibers than the internodes just as Oyebode and Ogedengbe, (2001) reported similar trends for impact bending test performed on bamboo. These all fall within the range established by this research.

However, Liese (1992) noted that FL is positively and strongly correlated with FD. This is not the case for *B. vulgaris* as FL was found to have a weak and indirect correlation with FD at the node while it was found to be strong but indirect at the internode. Such differences are not strange in different bamboo species. Dhamodaran *et al.* (2003) also reported variations for 12 Indian bamboo species in which FL was found to be higher than at the lower portion of the culms. They pointed out however, that the pattern of variation differs from species to species.

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Direct regeneration of shoots from immature inflorescences in *Dendrocalamus asper* (edible bamboo) leading to mass propagation

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ABSTRACT

Flowering in bamboos is a catastrophic event that leads to a number of post-flowering consequences and is hazardous for plants, which are monocarpic in nature. Direct shoot regeneration from immature inflorescence explants will help in overcoming the potential loss caused by unpredictable flowering in bamboos. Plant regeneration from cultured immature inflorescences of *Dendrocalamus asper* was obtained by direct shoot regeneration on Murashige & Skoog's 1962 (MS) medium supplemented with 7mg/l BAP. Best-regenerated shoots were obtained when 0.5-1.0 cm sized immature inflorescences were used. Regenerated shoots were grown on MS medium supplemented with 3mg/l BAP for further multiplication and development. Twelve to fifteen fold shoot multiplication rate was observed on MS medium. *In vitro* rooting was observed in 90-95% shoots on MS medium supplemented with 10mg/l IBA. After hardening and acclimatization plantlets were transferred to field and showed a 80-90% survival rate.

Keywords: – Shoot-regeneration, immature inflorescence culture, *Dendrocalamus asper*, micropropagation, bamboo.

Abbreviations: ABA- Abscisic acid, BAP- 6-Benzylaminopurine, 2,4-D- 2,4-Dichloro phenoxyacetic acid, GA_3 -Gibberellic acid, IAA-Indole-3-Acetic acid, IBA-Indole-3-butyric acid, NAA -1-Naphthalene acetic acid, FYM-farmyard manure, RH-relative humidity, CRD- Complete randomized design, SPSS-Statistical package for social science.

INTRODUCTION

Bamboo is a very important plant affecting the livelihood of millions of people around the world. However, research efforts on bamboos are very small compared with that on agricultural crops. *Dendrocalamus asper* is a sympodial tropical bamboo growing in large, dense clumps of a single genotype, and it is known for its edible tender shoots, which are also used for building material. It is commonly called "sweet bamboo" and is one of the best tropical bamboos in Asia in terms of its shoot quality. It grows up to 20-30m tall and individual stems are 8-20 cm in diameter. The species is heavily exploited as a food source and currently demand outstrips supply. The flowering of bamboo is poorly understood. Bamboo is monocarpic, i.e. a plant flowers only once and then dies. The flowering of bamboo needs to be controlled to avoid the losses in agroforestry resulting from the death of monocarpic bamboos.

Dendrocalamus asper flowers gregariously after 60-100 years, although some sporadic flowering has been reported (Anantachote 1998, Wang 1995, Satsangi *et al.* 2001). Nevertheless, diffuse sporadic flowering suffers from poor seed set and low viability. Flowering in vegetatively propagated plants, like those from micro propagation remains in synchrony with that of the mother plant (John *et al.* 1995). Consequently, vegetative propagation does not normally overcome the problem of monocarpy. However, it has been suggested / reported (Gielis *et al.* 1999) that shoot regeneration from pseudospikelets cultures from the inflorescence can produce plants that will be start of a new vegetative

generation. Regeneration from inflorescence tissue also offers the opportunity to overcome the problems associated with mass gregarious clump mortality after a flowering event.

Totipotent inflorescence cultures have been achieved from many monocots and dicots (Vasil 1982, Eapen and George 1997), *Dendrocalamus giganteus* (Ramanayake and Yakandwala 1998), wheat, rye and triticale (Eapen and Rao 1985), *Zingiber officinale* (Babu *et al.* 1992), *Amaranthus* (Arya *et al.* 1993), *Curcuma alismatifolia* (Wannakrairaj 1997), *Curcuma longa* (Salvi *et al.* 2000). The present paper describes for the first time the direct shoot regeneration from immature inflorescence in *Dendrocalamus asper*.

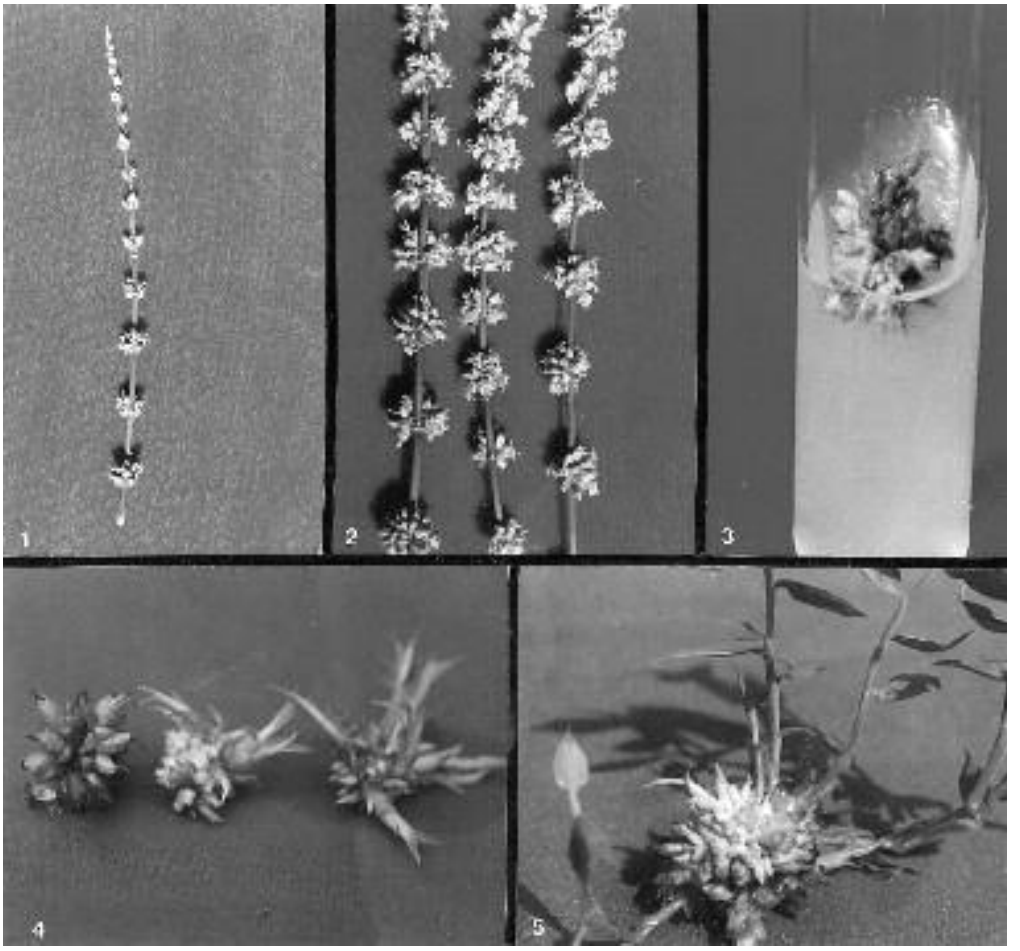
MATERIALS AND METHODS

Inflorescences at different developmental stages were collected from flowering branches of culms. These clumps were two-year-old tissue culture raised plants growing at experimental fields of Forest Research Institute, Dehradun, India. Explants (flowering buds) from immature inflorescences (Pre-anthesis buds of 0.5-2.5 cm) and of mature inflorescences (Post anthesis buds of 2.5-3.5 cm) were harvested from flowering clumps. They were cleaned with 70% alcohol soaked cotton and then sterilized with 0.1% mercuric chloride for 8-15 min. Finally they were washed three times in sterilized distilled water. Inflorescence tissues at different developmental stages were cultured (Table 1). Immature inflorescences (Figs.1&2) of six different bud sizes (0.1; 0.5; 1.0; 1.5; 2.0 and 2.5 cm) were cultured on MS medium supplemented with BAP. The explants were plated on MS medium supplemented with 1, 2.5, 3.0, 4.0, 5.0, 7.0 and 10 mg/l BAP. In all the experiments, data collected were computed using SPSS ver 8.0 software packages and analyzed by one-way ANOVA using CRD design of experiments. For each experiment five replicates were taken with six explants (in two flasks) per replicate and each experiment was repeated three times.

The basal medium used was solidified with 0.8% agar (Hi-media laboratories, Bombay, India) and supplemented with 2% (w/v) sucrose. The pH was adjusted to 5.8 prior to autoclaving. The shoots regenerated from these explants (immature-spikelets) were excised in clusters of 3-5 shoots called propagule. Three propagules per cultured flask were subcultured for shoot multiplication on MS medium supplemented with 1-10 mg/l BAP. Ninety shoots were cultured using CRD design per experiment on each media. Once the *in vitro* shoots were established the shoots were excised into groups of 3-4 shoots (propagule), which were subcultured and maintained on fresh MS medium at an interval of four weeks. The rate of multiplication was determined from the number of propagules at the end of each subculture divided by total number of propagules cultured.

Table 1: Effect of developmental age and size of explants for induction of shoots. MS medium was used. Data were collected after 4 weeks.

Explants	Size of explants (cm)	BAP (mg/l) of shoots	% Response	Average Number
Immature inflorescence tissue	0.5	7	36.67	2.0
	1.0	7	52.67	4.0
	2.0	7	56.67	5.5
Mature inflorescence tissue	2.5	7	40.00	2.5
	3.0	7	33.33	2.0
	3.5	7	15.00	1.0
Significance level 'F'			***	***
CD at 5%			9.72	0.904
S.E ±			4.36	0.405



Figures 1-5: Plant propagation through shoot regeneration from immature inflorescence.
Fig.1: Immature inflorescence buds suitable for culture response.
Fig.2: Mature floral buds at nodes of inflorescence.
Fig.3: In vitro culture of inflorescence segment on MS + 7 mg/l BAP.
Fig.4: Shoot regeneration from cultured inflorescence segment.
Fig.5: Regenerated shoot after four weeks from cultured inflorescence.

Rooting was induced in regenerated *in vitro* shoots on full strength MS medium supplemented with 10 mg/l IBA as described by Arya *et al.* (2002). Cultures were maintained at a temperature of 25 °C + 2°C under 16/8hrs (light/dark) photoperiod at a high light intensity (60-70 Em^{-2} s^{-2} of cool white light) provided by fluorescent lamps (Philips, India).

Rooted plantlets were transferred to polybags containing vermiculite and kept under green house at 30°C temperature and 90% RH through misting for hardening. These plants were fed with half strength MS salts without organics twice a week for 1 month. Subsequently these plants were transferred to polybags containing sand: soil: FYM in 1:1:1 ratio. After 1 month, plants were acclimatized in a shade house for the next 4 weeks before they were ready for field planting.

RESULTS AND DISCUSSIONS

Mercuric chloride proved to be the best sterilant. Surface sterilization with 0.1% $HgCl_2$ for 8 min yielded 60% results for inflorescence sterilization. An increase in treatment duration to 10 min resulted in necrosis in buds. Inflorescences if cleaned with ethyl alcohol

Table 2: Size and corresponding developmental stages of inflorescence

Explants	Size (cm)	Developmental stage
Immature inflorescence	0.5-1.0	Young initiating floral buds.
	1.0-1.5	Condensed growing floral buds.
	1.5-2.0	Floral buds with developing floral parts.
Mature inflorescence	2.0-2.5	Pre-anthesis buds
	2.5 or above	Post anthesis buds

(70%) swabbed cotton prior to surface sterilization with mercuric chloride increased the percentage of explants free of contamination by 10%. 50% of the explants cultured on MS medium supplemented with 7mg/l BAP regenerated and therefore this was considered as the optimal medium. On MS medium with 1-4 mg/l BAP only 1-2% of the explants responded and up to 20% on 5-6 mg/l BAP medium. At 8-10mg/l of BAP their generation frequency of shoots was also 50% but the shoots were condensed and small in size. Shoots regenerated from the explants after 10-14 days of culture depending on their size. At the optimal medium with 7mg/l BAP, 52% and 56% explants of 1 cm and 2 cm respectively responded by producing an average 4-6 shoots (Fig. 3). As the explants matures, the shoot regeneration response decreased from 40% to 15% (Table 1). In the present investigations inflorescences were cultured during 3-4 months of their development and their response as explants for the induction of direct shoot regeneration (Fig. 4 & 5) in culture were studied (Table 1&2).

IN VITRO SHOOT MULTIPLICATION

Regenerated shoots from inflorescences were excised from explants and were maintained separately on MS medium supplemented with BAP (1-5 mg/l). A 12 to 15 fold shoot multiplication rate was observed in inflorescence-derived shoots on MS medium supplemented with 3mg/l BAP (Table 3). Shoot multiplication rate decreased at reduced BAP levels (1-2mg/l) in MS medium. Also at increased BAP level (5-10 mg/l) condensation of shoots was observed resulting in decline in the multiplication rate. Thus, 3mg/l BAP supplemented MS medium proved the best for shoot multiplication (Fig. 6).

During initial cycles of shoot multiplication 3-6 folds multiplication was observed for the first to the fourth subculture. After subculture

Figures 6-8: Plant propagation through shoot regeneration from immature inflorescence.
Fig.6: In vitro shoot multiplication on MS + 3 mg/l BAP.
Fig.7: Tissue culture plantlet on MS + 2 mg/l NAA.
Fig.8: Hardened and acclimatized plants ready for field transfer.

Table 3: Effect of BAP concentration in MS medium on *in vitro* shoot multiplication and development. Propagules of three shoots were cultured and data were recorded after 4 weeks.

BAP mg/l	No. of shoots developed	Shoot length (cm)	Size of leaves (width in mm)	Multiplication Rate
0.0	–	4.5+1.0	9.0+2.1	0.0
1.0	14.1+1.0	3.3+0.7	6.2+1.0	5.6
2.5	33.4+3.2	2.3+0.2	4.2+0.6	12.2
3.0	34.0+3.8	2.2+0.4	3.0+0.1	12.6
4.0	30.3+3.6	1.2+0.5	2.3+0.4	11.3
5.0	30.2+2.5	1.2+0.2	2.0+0.1	10.2
7.5	16.3+2.5	1.0+0.1	2.0+0.5	6.2
10.0	17.0+4.0	0.8+0.2	1.6+0.2	7.0

cycle 5-6 multiplication rate increased to 10-16 folds and then became constant 12 to 15 fold. When MS medium was used without a supplement of BAP, the cultured *in vitro* shoots increased in length but without shoot multiplication. Uses of BAP for *in vitro* shoot multiplication in bamboos have been reported by many researchers (Arya *et al.* 2003, Arya and Sharma 1998, Arya and Arya 1997, Prutpongse and Gavinlertvatana 1992, Chambers *et al.* 1991, Saxena 1990). Also, for *in vitro* shoot multiplication clusters of three shoots (propagule) were subcultured together. The shoot multiplication rate declined sharply if propagules of less than 3 shoots were cultured. The multiplication rate also declined if propagules of more than 3 shoots were used for multiplication. Liquid as well as semisolid medium showed significantly similar shoot multiplication rate. However, on long subculture cycles in the liquid medium shoots become brittle and vitrified. Hence semisolid medium was used for multiplication cycles.

IN VITRO ROOTING

Attempts for *in vivo rooting* of *in vitro* raised shoots were only partially (15%) successful during rainy season, and the rest of the shoots turned pale in 5-7 days and eventually died. *In vitro* rooting was highly successful. For *in vitro* rooting shoot clusters of 3-5 shoots were transferred on MS medium supplemented with IBA or NAA. A success rate of 95% of *in vitro* rooting of *in vitro* shoots was obtained both in liquid and agar-gelled MS medium. However, in the liquid medium root initiation occurred 4 days earlier. Shoots rooted readily within 8-12 days on MS medium. Maximum *in vitro* rooting (80-95%) was achieved on MS medium supplemented with 10mg/l IBA or 3mg/l NAA. The root induction effect of IBA and NAA in the present case is similar to earlier reports on *Morus indica* (Chand *et al.* 1995), *Bambusa tulda* (Saxena 1990), *Bambusa bambos* (Arya and Sharma 1998). A propagule of 3 shoots of 1-2 cm in length developed 10-15 roots in 30 days.

HARDENING AND ACCLIMATIZATION

Four-week-old plantlets with well-developed root systems (Fig. 7) were hardened in Soilrite for 20 days. Plants were fed with half strength macro- and micronutrients of MS medium thrice a week in the mist chamber under RH 85-90% and at 30° ± 2°C. Acclimatization of these plants was carried out in an open agronet shade house in polybags containing a mixture of sand: FYM: soil in a ratio of 1:1:1 for 2 months. Rhizome formation occurred within two months and the plants eventually were established in the soil (Fig. 8).

Thus, present study shows that inflorescence culture is amenable to high frequency shoot regeneration and opens up the possibility of using this for further research in bamboos. The studies have shown that inflorescence explants could differentiate into plants under *in vitro* conditions. Since pseudospikelets are modified

vegetative buds, it is quite likely that immature floral buds have the capacity to revert to vegetative buds under appropriate *in vitro* conditions (Gielis *et al.* 1997; George and Sherrington, 1984; McClure, 1966). Determination of the appropriate stage of explant from the clump after onset of flowering may be an ineffective marker because the size of inflorescence and its developmental stages differed even between branches of the same clump. Inflorescence size is the best parameter for explant selection. Regeneration of plants from inflorescence culture has been previously reported in several plants such as wheat, rye, triticale (Eapen and Rao, 1985), ginger (Babu et al, 1992), *Amaranthus* (Arya *et al.* 1993) and *C. alismatifolia* (Wannakrairoj, 1997). In *D. asper* inflorescence segments (0.5-2.0 cm) with intact floral buds were cultured on MS medium supplemented with 7mg/l BAP. At the end of 4 weeks shoot primordia emerged from the surface of the explants (Fig. 4). Later with the passage of time number of shoot primordia differentiated from complete surface of the cultured inflorescence and developed into clusters of well-developed shoots in four to five weeks of culture (Fig. 5). Explants cultured on 7mg/l BAP gave optimal results as on this medium maximum number of shoot buds and shoots per explant were scored. The inflorescence is composed of many meristems which develop buds larger than 2.0 cm had mature stages of floral primordia, which did not regenerate into *in vitro* shoots, when cultured on MS medium supplemented with 7mg/l of BAP.

Thus in the present case it was found that subculture of the proliferating explants on a medium with BAP enhanced the formation of additional meristematic centers, which continued to grow and developed into buds. Regeneration occurred, as long as the tissue is young and actively growing. However, it is also reported in some species that regeneration of shoots occurred even when the flowers were still enclosed by the bracts. The inflorescence is composed of many meristems, which develop sequentially to form the florets. These meristems can develop into vegetative buds in several species if they are isolated at the appropriate developmental stage and cultured *in vitro* on suitable medium. The shoot buds often form clusters that can be used for subsequent mass propagation. Ziv and Kipnis (2000) reported in geophytes that at appropriate developmental stage under optimal growth hormones, reversion of meristems is possible, which later developed sequentially to form florets. The inflorescence can be reversed from flowering to vegetative states (Gielis and Goetghebeur, 1997), and buds proximal to the inflorescence can develop into innovation shoots. Size of explants at different developmental age of the spikelets plays an important role. Tanimoto and Harada (1979) in their studies on *Torenia* reported that 10-12 mm inflorescence segments produced vegetative buds.

Arya *et al.* (1993) developed *in vitro* plantlets from inflorescence cultures of *Amaranthus* cultured on MS medium supplemented with BA. Similarly, Salvi *et al.* (2000) supported similar findings that BAP at higher concentrations formed direct shoots from inflorescence tissues in case of *Cucurma* species. They also found cytokinins have the ability to stimulate flowering but tend to be more supportive of vegetative growth.

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Large scale plant production of edible bamboo *Dendrocalamus asper* through somatic embryogenesis

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ABSTRACT

Dendrocalamus asper, an edible bamboo, has a high international trade potential. Somatic embryos and embryogenic callus are induced in *D. asper* and are used for large-scale propagation. In bamboos somatic embryogenesis has great importance as it resets the internal calendar of plants produced which can be relied upon to live full life span. Embryogenic callus was developed from explants excised from nodal tissues and basal part of leaves that were harvested from *in vitro* shoots. Somatic embryos were induced on Murashige and Skoog's (1962) medium supplemented with 30µM 2,4-D and 2% (w/v) sucrose in dark conditions. On MS medium supplemented with 9µM 2,4-D + 2.85µM IAA + 0.88µM BAP callus proliferated to 3-5 folds every 4 weeks of subculture with efficiency of 21.7-32.8 globular somatic embryos per embryogenic callus. 6% (w/v) sucrose or 5µM ABA enriched medium enhances maturation of globular somatic embryos to scutellar and coleoptillar somatic embryos within 8 weeks. Somatic embryos developed into plantlets within 30 days on MS medium supplemented with 4.4µM BAP and 2.8µM GA_3 with a conversion rate of 70%. Thirty percent of embryos formed only shoots on germination medium. These somatic embryo shoots were multiplied on MS + 13.2µM BAP for 4-5 times of subculture. Later these shoots rooted on 16µM NAA supplemented MS medium with 98% root induction. The plantlets recovered from germination of somatic embryos and micropropagation of somatic embryo derived shoots were successfully hardened, acclimatized and transplanted to the field. The multiplication of *D. asper* through somatic embryogenesis is now routine procedure without the need for fresh explants. The system of somatic embryogenesis was efficiently utilized for direct plant formation and through *in vitro* shoot development; more than 10,000 plants have been produced during last year.

Keywords: Somatic embryogenesis, bamboo, *Dendrocalamus asper*, plant regeneration, micropropagation.

Abbreviations: ABA- Abscisic acid, BAP- 6-Benzylaminopurine, 2,4-D- 2,4-Dichloro phenoxyacetic acid, GA_3- Gibberellic acid, IAA- Indole-3-acetic acid, IBA- Indole-3-butyric acid, Kn- Kinetin, NAA- α-Naphthaleneacetic acid, FYM-farmyard manure, RH-relative humidity, CRD- complete randomized design, SPSS- statistical package for social science.

INTRODUCTION

Dendrocalamus asper is a densely tufted, sympodial tropical bamboo known for its edible tender shoots, also used for building materials. It is commonly called sweet bamboo, one of the best in terms of its shoot quality among tropical Asiatic bamboos. It grows up to 20-30m heights and 8-20cm in diameter. The high utility of this species has led to its severe exploitation and currently demand outstrips supply. Conventional propagation methods are beset with many problems such as seed sterility, non-availability of seeds, unpredictable flowering nature and bulkiness of rhizome. Moreover, *D. asper* has intermast period of 100 years. When plants produced through vegetative propagation and micropropagation carry the floral stimulus from their mother plants they cannot be relied to live full life span. So far, since 1993 authors have observed flowering twice under two different sets of experimental

conditions in micropropagated plants (Satsangi *et al.* 2001). Whereas sexual reproduction or somatic embryogenesis is reported to reset the internal calendar at zero, thus the plants enable to live their life span (Rao *et al.* 1987; John *et al.*1995; Joshi *et al.* 1997).

Somatic embryogenesis offers an attractive alternative over conventional methods for mass propagation of bamboo. It has been reported that embryogenic calli maintained their regeneration competence for a long time and gave rise to genetically uniform plantlets (Vasil 1982) and plantlets produced could be relied upon to last for their full vegetative life span (John *et al.* 1995). Many workers have emphasized somatic embryogenesis as a preferred method for rapid *in vitro* multiplication of plants. It provides an ideal experimental process for investigating differentiation as well as for understanding the mechanisms of expression of totipotency in plant cells (Arya *et al.* 2000). It is expected that somatic embryogenesis could be used for *Agrobacterium* mediated transformation and regeneration of transgenic plants and that protoplasts isolated from embryogenic callus may provide the necessary vehicle for application of genetic engineering to bamboos. The advantage of this system is compact organized embryos, which can be screened visibly, multiplied and germinated easily. Somatic embryogenesis in bamboo has been reported for the first time in *Bambusa arundinacea* (Mehta *et al.* 1982). To date, in most of the bamboo species, somatic embryogenesis has been achieved from reproductive tissues, seeds and seedlings whereas few reports are available where vegetative explants were used for embryogenesis. Earlier reports described somatic embryogenesis from nodal segments and leaves/leaves sheath derived from *Bambusa vulgaris*, *Dendrocalamus giganteus, Dendrocalamus strictus* (Rout and Das 1994), *Dendrocalamus membranaceus* (Vongvijitra 1988) from nodal segments and leaves or basal part of leaves of 'Golden Goddess' (Jullien and Tran Thanh Van 1994) and *Dendrocalamus hamiltonii* (Godbole *et al.* 2002).

On the other hand, there is no report on reliable systems for somatic embryogenesis in *D. asper*. The present communication describes an efficient and reproducible protocol for large-scale propagation of *Dendrocalamus asper* through direct regeneration of plants from somatic embryos and plants obtained as a result of axillary branching initiated from shoot differentiation of somatic embryos.

MATERIALS AND METHODS

Initiation of callus

Somatic embryogenic callus was developed using *in vitro* shoots. *In vitro* shoots were developed through axillary bud culture. For this, nodal segments measuring 2-4cm in length and 0.4-0.8cm in diameter were selected from secondary branches of 5-7 year old bamboos. After removal of leaves and surface cleaning of explants with 70% alcohol, explants were sterilized with 0.1% $HgCl_2$ for 15 min and washed thoroughly with sterilized distilled water. Explants were cultured on MS basal medium (Murashige and Skoog's 1962) supplemented with 22µ BAP. Shoots proliferated were excised and multiplied on MS medium supplemented with 13.2µM BAP. These *in vitro* raised shoots and leaves were used for initiation of embryogenic callus. Nodal segments and leaves sheaths were dissected from *in vitro* shoots and were inoculated on MS media supplemented with 2, 4-D (10-50µM). Media were solidified with 0.8% agar (Himedia laboratories, India) and supplemented with 2% (w/v) sucrose. The pH of the medium was adjusted to 5.8 prior to autoclaving. Effect of age of *in vitro* shoots (subculture duration) for induction of callus was assessed for 15-60 days old cultures. Explants harvested from these sources were inoculated on MS medium supplemented with 30µM 2,4-D (induction medium). Explants were inoculated in horizontal position to remain in contact with media. The cultures were incubated in dark at 25°C ± 2°C. A regular 4 week subculture on fresh medium was carried out. After 12 weeks on induction medium calli produced from explants were transferred to multiplication medium. Cultures were scored after 30 days at the end of experiments in terms of induction percentage and fresh weight of callus was also recorded.

PRODUCTION OF SOMATIC EMBRYOS

12 weeks old compact embryogenic calli with proembryos were transferred on multiplication medium. The effect of phytohormones i.e. 2,4-D, IAA, BAP and Kn in various combinations was studied for optimization of multiplication of embryogenic callus. The effect of subculture duration was assessed for multiplication of embryos from 2-8 weeks. The basal media used were MS and B5 with 2% (w/v) sucrose and 0.7% agar. The embryogenic calli were subcultured subsequently every 4 weeks. Embryogenic cultures were scored for mean number of embryos per embryogenic culture (fresh weight of callus) under stereozoom microscope. Embryogenic frequency was also recorded. During each subculture dead and decaying callus, and non embryogenic callus was discarded, callus with immature embryo were transferred on fresh medium for further multiplication. Approximately 50mg of embryogenic callus was inoculated in all the related experiments and after 4 weeks duration the final weight of the callus was recorded. Globular embryos in callus needs maturation for further development of embryos to scutellar and coleoptillar stages. Calli with scutellar somatic embryos were transferred on MS medium supplemented with different concentrations of 2-10 % (w/v) sucrose or 0.5-20µM ABA for maturation of embryos. Callus was incubated on maturation medium for 8 weeks with subculture duration of 4 weeks, in dark. Efficiency of embryos calculated was the product of maturation frequency and mean number of embryos (different stages) seen per embryogenic culture under stereozoom microscope. Callus with coleoptillar stage embryos were transferred to germination medium and were incubated in light for 8 weeks. The effect of different combinations of phytohormones was studied for germination of somatic embryos. Germination was carried out at 25°C ± 2°C under 16/8hrs (light/dark) photoperiod at a high light intensity (60-70µEm^{-2} s^{-2} of cool white light) provided by fluorescent lamps (Philips, India).

PLANT REGENERATION AND TRANSPLANTATION

Somatic embryos that produced only shoots on germination medium were separated and these *in vitro* shoots were multiplied on MS medium supplemented with 13.2µM BAP as an alternative method for large-scale plant production. The roots were induced in these *in vitro* shoots on MS medium supplemented with 16µM NAA as described by Arya *et al.* (2002). Germinated embryos and rooted plantlets were transferred to polybags containing vermiculite and kept under mist house conditions of 30°C and 90% RH for hardening. The half strength solution of MS medium devoid of organics was added to the polybags twice a week for one month. These plants were transferred to polybags containing sand: soil: FYM in 1:1:1 ratio at FRI, Dehradun, India. After one month, plants were acclimatized in shade house for another 4 weeks before they were ready for field plantation. Hardened and acclimatized plantlets were transferred to the field using simple silvicultural practices of 6m x 6m spacing. So far 10,000 plants have been produced.

STATISTICAL ANALYSIS

All data collected and analyzed by one-way ANOVA using CRD design of experiments using SPSS ver 8.0 software packages. For each experiment 25 explants and five replicates were taken and each experiment was repeated three times. However, shoot multiplication is going on for the last year through routine subculturing every 4-weeks interval. Cultures were scored after 4 weeks duration of its inoculation. Four parameter were used to assess embryo response as reported by Lazzeri *et al.* (1987).

Embryogenesis frequency = Number of embryogenic calli / Total explants inoculated.

Embryogenic efficiency = Embryogenesis frequency x Mean embryo number.

Mean embryo number = Mean number of somatic embryos per embryogenic calli.

Multiplication rate = Final fresh weight of callus / Initial weight of callus inoculated.

RESULTS AND DISCUSSION

Initiation and Multiplication of Embryogenic Callus

In the present study it was found that the age of *in vitro* shoots was critical for the induction of callus. Only new shoots (30 day old) which developed by subculturing of shoot propagule consisting of 3-5 shoots were competent to respond. In the present investigation only the nodal segments and leaves sheath base of new shoots responded for embryogenic callus. In 4 weeks of subculture callus developed from the cultured nodal segments and also from the leaves sheath base which was carefully excised from the new *in vitro* shoots. Callus initiated from 15 day onwards in culture. Maximum induction response of 100% was recorded from 30-day-old nodal segments and only 87% from 30-day-old basal part of leaves (Table 1) as leaf sheaths were prone to dry may be due to cultural conditions (temperature shock, rupturing of cells during isolation). Explants, nodal segments and leaf sheaths isolated from *in vitro* shoots more than 30 days showed reduced frequency of embryogenic callus formation perhaps the explants matures to be responsive. Thus, determination of the appropriate stage based on subculture duration is a pre-requisite for selecting responsive explants.

Callus was induced from 77.7% leaves in dark. The callus size varied from 2-4mm in diameter (5 weeks) to 8mm-1cm in diameter in 12 weeks. 91.6% nodal segments induced callus in dark. Of various 2,4-D concentrations tried (10-50µM), 30µM 2,4-D concentration was found to be the best for callus induction (Table 2). After 4 weeks of culture 19mg callus was produced from nodes. Callus was induced from nodal region of the explants and from basal portion of leaves sheath only (Fig. 1A). On increased concentration of 2,4-D (50µM) cultures stopped proliferating after 2-3 weeks and callus turned brown on prolonged culture.

Different media, i.e. MS and B5 were tested for callus induction and results showed that MS medium was suitable for callus initiation and development of embryos (data not shown); therefore, all the cultures were initiated and maintained on MS medium. After 4 weeks of culture generally three types of callus were produced as 1) Yellow friable, non-embryogenic

Table 1: Effect of age of *in vitro* shoots for harvesting nodal segments and leaf sheaths base on induction of embryogenic callus. Observations recorded after 4 weeks of culture in the dark.

Age of shoot cultures (days)	Callus formation (%)		Fresh callus weight (mg)	
	Leaves	Nodal segments	Leaves	Nodal segments
15	72.2[a]	100[a]	54[a]	21[a]
30	87.7[a]	100[a]	36[b]	25[a]
45	19.4[b]	38.8[b]	12[c]	4[b]
60	16.6[b]	33.3[b]	10[c]	5[b]
S.E ±	20.0	20.4	4	4

Mean values followed by same letters are not significantly different according to DMRT at p=0.05%

Table 2: Effect of concentration of 2,4-D on induction of embryogenic callus using nodal Segments and leaf sheaths base. Data collected after 4 weeks of culture.

2,4-D (µM)	Callus formation (%)		Fresh callus weight (mg)	
	Leaves	Nodal segments	Leaves	Nodal segments
10	63.8[b]	77.7[a]	36[a]	14
30	77.7[a]	91.6[a]	33[a]	19
50	43.4[c]	58.3[b]	9[b]	15
S.E ±	6.9	15.1	6	2

Mean values followed by same letters are not significantly different according to DMRT at p=0.05%

Table 3: Effect of subculture duration on multiplication of embryogenic callus on MS + 9 µM 2,4-D + 0.88 µM BAP + 2.85 µM IAA. 50mg was inoculated initially.

Subculture interval (weeks)	Embryogenic response (%)	Fresh weight of callus (mg)	Callus multiplication rate (folds)	Dry weight of callus (mg)	Mean number of embryos (50mg)	Embryogenic efficiency	% Dry weight of callus
2	72.2[b]	140[b]	2.8[c]	21[b]	17.9[c]	12.9	15.0
4	100[a]	250[b]	5.0[b]	48[a]	32.8[a]	32.8	19.2
6	66.6[c]	300[a]	6.0[a]	40[a]	26.8[b]	17.7	13.3
8	50.0[c]	315[a]	6.3[a]	39[a]	33.8[a]	16.9	12.3
S.E ±	1.97	21	0.4	72	0.5		

Mean values followed by same letters are not significantly different according to DMRT at p=0.05%

callus; 2) White compact nodular embryogenic callus; 3) Translucent mucilaginous, partly embryogenic callus. These three different forms of callus were intermixed with each other and during each subculture the friable callus was removed as it grows faster than embryogenic callus. Mainly 2,4-D is used for induction of embryogenesis in bamboos and occurrence of 3 types of callus was observed by many workers (Rao *et al.* 1985; Rout and

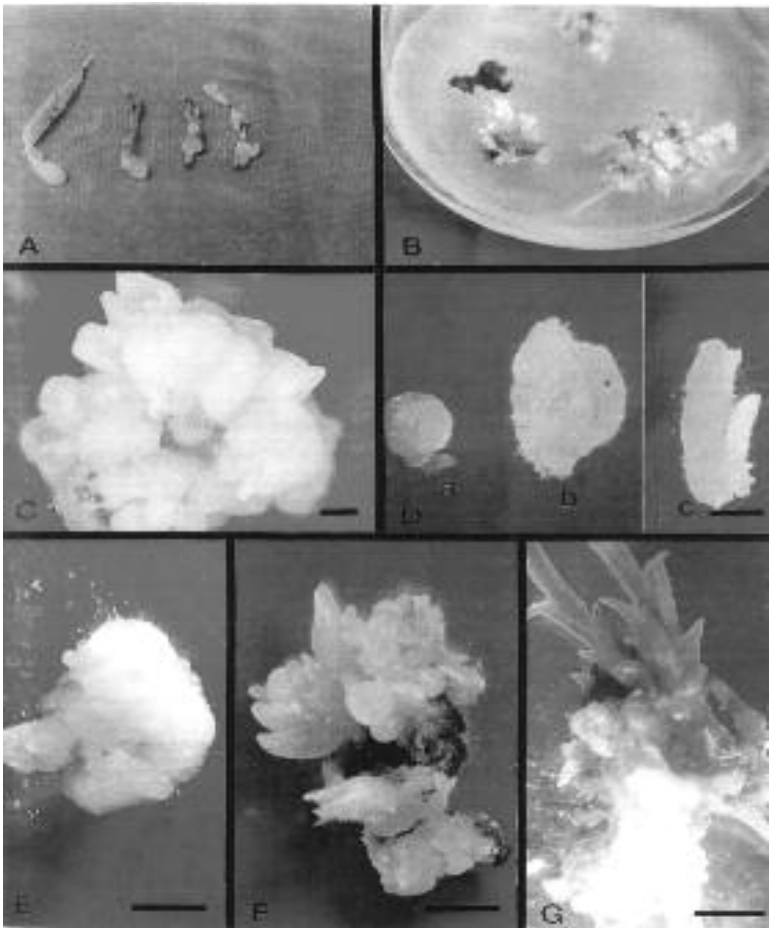

Figure 1

Table 4: Effect of different phytohormone combinations on multiplication of embryogenic callus on MS medium. Observations recorded after 4 weeks. 50mg was inoculated initially.

MS+ phytohormones (µM)				Embryogenic response (%)	Fresh weight of callus (mg)	Callus multiplication rate	Mean number of embryos per embryogenic callus	Embryogenic Efficiency
2,4-D	BAP	Kn	IAA					
10	–	–	–	88.8[a]	72[b]	1.4[b]	10.0	8.8
30	–	–	–	83.3[a]	151[a]	3.0[a]	15.4	12.8
50	–	–	–	72.2[a]	51[c]	1.0[b]	3.6	2.6
9	0.88	–	–	73.3[a]	97[b]	1.9[b]	9.8	6.9
9	–	0.88	–	50.0[b]	57[b]	1.1[b]	6.2	3.1
9	0.22	–	1.14	77.7[a]	75[b]	1.5[b]	11.5	8.8
9	0.88	–	2.85	100[a]	175[a]	3.5[a]	21.7	21.7
13.5	9.3	–	–	77.7[a]	83[b]	1.6[b]	8.4	6.4
13.5	–	9.3	–	68.3[a]	62[b]	1.2[b]	11.0	7.5
S.E ±	16.7	21	0.4					

Mean values followed by same letters are not significantly different according to DMRT at p=0.05%

Das 1994; Woods *et al.* 1992; Yeh and Chang 1987; Saxena and Bhojwani 1993; Vongvijitra 1988; Chan and Lan 1995). 12 weeks old compact nodular embryogenic callus with proembryos was selected and transferred to multiplication medium (MS + 9µM 2,4-D +0.88µM BAP + 2.85µM IAA). A subculture interval of 4 weeks was needed for the maintenance of embryogenic callus. Callus growth curve studies revealed that during a 6-week subculture period callus growth as measured by fresh weight showed a slight lag phase (during first week) followed by exponential growth. The percent dry weight increased steadily between 2 and 4 weeks and declined thereafter. This correlates well with the formation of non-embryogenic callus, which accumulates more rapidly towards the end of subculture interval (Table 3). Cultures left without subculturing beyond 4 weeks produce non-embryogenic callus. Therefore the entire callus was subcultured at 4 week intervals in which embryogenic callus also showed secondary embryogenesis. Secondary embryos were formed directly on the primary scutellar stage somatic embryos resulting in chains of globular somatic embryos on scutellar surface as observed in many instances under stereo zoom microscope in 10-11 weeks of culture (Fig. 1E).

SOMATIC EMBRYOGENESIS

Variation in developmental potential of somatic embryos was observed among the calli multiplied on different 2,4-D concentrations and other combinations (Table 4). Prolonged subculturing on 2,4-D alone (10-50µM) on induction medium did not improve the production of somatic embryos. In the present case callus produced maximum embryogenic response with highest efficiency when transferred from higher 2,4-D concentration (induction medium) to lower 2,4-D concentration on multiplication medium. Moreover continuous multiplication of callus on reduced concentration of 2,4-D (9µM) was preferred. Efficiency of embryogenesis was improved when multiplication medium containing 2,4-D was supplemented with BAP and IAA. Woods *et al.* (1992) also reported the important role of BAP in promoting the production of embryogenic callus in Mexican weeping bamboo.

Addition of IAA and BAP was found to be preferential for callus multiplication and formation of somatic embryos. It was observed that the cultures, which were established on 30µM 2,4-D, became highly embryogenic when transferred on MS medium supplemented with reduced level of 9µM 2,4-D and 0.88µM BAP. Moreover, efficiency of formation of embryos

Table 5: Effect of ABA on maturation of somatic embryos on MS medium. Observation recorded after 4 weeks of culture. Initially callus of 50mg fresh weight was used in each experiment (per replicate 5 pieces of 50mg callus)

ABA (µM)	Responding calli (%)	Efficiency of total no. of embryos	Efficiency of formation of scutellar/coleoptillar embryos	Stages of embryos in callus
0.5	70.0[c]	39.7	6.2	G
1.0	91.6[b]	28.6	9.5	G/S
2.0	100[a]	31.5	19.6	G/ S/C
5.0	100[a]	36.6	25.0	S/C
10	73.5[b]	7.9	6.9	Fused G/ S/ C
20	75.0[b]	5.9	3.0	Fused G/ S/ C
S.E ±	8.6			

G; Globular, S; Scutellar, C; Coleoptillar
Mean values followed by same letters are not significantly different according to DMRT at p=0.05%

Table 6: Effect of sucrose concentration on maturation of somatic embryos. Observation recorded after 4 weeks of culture. Initially callus of 50mg fresh weight was used in each experiment (per replicate 5 pieces of 50mg callus)

Sucrose (%)	Embryogenic calli (%)	Efficiency of total no. of embryos	Efficiency of formation of scutellar/coleoptillar embryos	Stages of embryos in callus
2	83.3[a]	20.4	2.9	G/S
4	75.0[a]	13.0	3.8	G/S
6	75.0[a]	17.1	13.6	S/C
8	50.0[b]	3.4	2.6	Fused G/ S/ C
10	33.3[b]	6.3	4.2	Fused G/ S/ C
S.E ±	7.9			

G; Globular, S; Scutellar, C; Coleoptillar
Mean values followed by same letters are not significantly different according to DMRT at p=0.05%

increased if IAA (2.85µM) was added to this multiplication medium. On this medium largest number of well-developed globular embryos with 21.7 efficiency (embryos per culture) and 3.5 fold callus proliferation rate was observed (Table 3). Therefore after 12 weeks of culture on induction medium callus with proembryos were transferred to multiplication medium (MS + 9µM 2,4-D + 2.85µM IAA + 0.88µM BAP) which resulted in differentiation of pro embryos to globular stage of embryos (Fig. 1B). An inductive effect of IAA on the production of embryogenic callus was reported by Nabors *et al.* (1983) on different cereal tissue cultures and a combination of IAA and cytokinin produced a synergistic increase in embryogenic callus production of wheat. In *B. glaucescens* callus remains friable on 2,4-D rich medium though supplementing it with IAA and BAP favored embryogenesis (Jullien and Tran Thanh Van 1994).

These well-developed globular somatic embryos were formed after 2-3 subcultures on multiplication medium in dark (Fig. 1C). All cultures were proliferated on this multiplication medium and regular subculturing was carried out every 4 weeks to multiply the embryos and to maintain the embryogenic potential of callus. On this medium callus retains its potential for more than 24 months with same efficiency. 2% (w/v) sucrose was found to be the best for multiplication of somatic embryos.

At the end of multiplication cycle callus with embryos of different developmental stages were selected. The globular embryos were transferred to fresh multiplication medium, whereas scutellar somatic embryos were transferred to abscisic acid (ABA) or high concentrations of sucrose for the maturation or development of somatic embryos. Exposure of tissue to abscisic acid (ABA) or high concentrations of sucrose was essential for the maturation or development of somatic embryos. Maturation is final event of embryogenesis and is characterized by the attainment of mature embryo morphology. Exposure of tissue to abscisic acid (ABA) may be required for further maturation or development of somatic embryos after scutellar stage as ABA has been found to be suitable for maturation of somatic embryos, in many species it prevented precocious germination (Von Arnold and Hakman 1986). Low concentration of ABA is known to influence maturation by accumulation of storage carbohydrates, lipids, proteins (Phillips and Collins 1981). Chan and Lan (1995) in *B. beechyana* Munro var beechyana reported use of ABA (0.1-2 mg/l) and other osmoticums (PEG, polyamine, mannitol) for further development of embryos. In *D. asper* maximum maturation of somatic embryos, scutellar and coleoptillar stages was achieved within 8 weeks of incubation on medium supplemented with 5µM ABA or on 6% sucrose in dark (Tables 5 and 6). On this medium the scutellar somatic embryos developed coleoptillar notch, which emerge from its center (Fig. 1F). These coleoptillar somatic embryos later developed shoot and roots when transferred to germination medium. Prolonged culturing beyond 8 weeks on high sucrose concentration (6%) retarded the callus growth and multiplication rates.

Microscopic observation revealed the presence of proembryo on induction medium after 12 weeks, which further proliferated to globular somatic embryos on multiplication medium after 2-3 times of subcultures. In established embryogenic cultures different developmental stages of somatic embryos were observed under stereo zoom microscope. Three types of embryos were formed, i.e. white round glossy smooth bodies (globular), cup shaped (scutellar) and tubular (coleoptillar) arising form center of scutellar somatic embryos (Fig. 1D). Globular and scutellar developmental stages were observed on multiplication medium whereas scutellar and coleoptillar stages were prominent on maturation medium. In established cultures sometimes, fused somatic embryos were also seen along with secondary embryos in fast growing embryogenic calli.

PLANT REGENERATION

Coleoptillar stage somatic embryos present in callus developed shoot axis and roots when transferred to germination medium. Maximum germination response (70%) was observed on MS medium (germination medium) with 4.4µM BAP + 2.8µM GA_3 under 16/8hrs (light/dark) photoperiod and 25°C ± 2°C (Table 7). Somatic embryos also germinated on 0.8µM BA + 3.4µM IAA, but the duration was longer (one week) more when compared to

Table 7: Effect of different phytohormones combination on germination of coleoptillar somatic embryos. Data collected after 4 weeks of culture in the light conditions.

Phytohormones (µM)					Germination (%)
2,4-D	**BAP**	**IAA**	**GA_3**	**NAA**	
–	4.4	–	2.8	–	70.5[a]
–	0.8	1.75	–	–	70[a]
2.26	3.11	1.75		–	41.6[a]
–	8.8	–	–	10.75	16.6[b]
2.26	13.2	–	–	–	57.6[a]
S.E ±	16.2				

Mean values followed by same letters are not significantly different according to DMRT at p=0.05%

germination medium. Complete plantlets with well-developed shoots of 1-3cm in size and 1-2 taproots were obtained (Fig. 1G & 2H). On germination medium 30% of embryos produced only shoots. Shoots devoid of roots on germination medium were harvested and were multiplied on MS medium supplemented with 13.2μM BAP. Initially these shoots showed a gradual increase in multiplication with every decrease of subculture cycle till 5th-6th cycle. Later these shoots multiplied with 17-20 folds constant shoot multiplication rate (Fig. 2I), when subcultured every 4 weeks. Other attempts made to germinate coleoptillar stage embryos gave reduced response (16% to 40%) when subcultured on different phytohormone supplemented media (Table 7). Rao *et al.* (1985); Saxena and Dhawan (1999) also reported that some embryos produced only shoots on germination medium. In monocots although the root meristem is generally established in the embryoids but root induction takes place only by forcing its growth by physicochemical stimuli. Significantly high sucrose concentration provides that impetus to the growing embryoids leading to their germination into plantlets (Godbole *et al.* 2002).

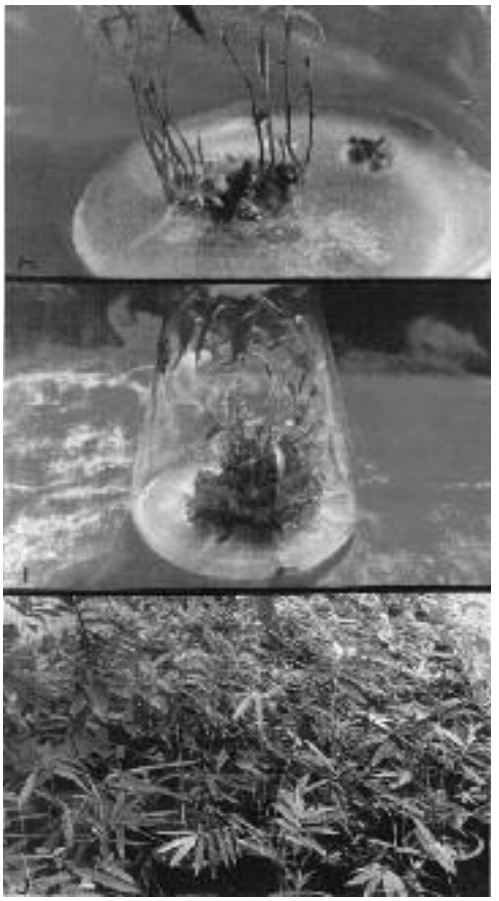

Figure 2

In vitro shoots developed from somatic embryos (30%) produced rooting, when cluster of shoots (3-5) were cultured on MS medium supplemented with 16μM NAA. On this medium 98-100% rooting was achieved. The root initiation was observed after 2 weeks from the *in vitro* shoots on rooting medium. Usually 8-10 roots developed from each shoot propagules. Plantlets recovered from germination of embryos and from rooting of *in vitro* shoots were transplanted in polybags containing vermiculite and were kept in the mist chamber under 80% RH and 30° ± 2°C for hardening. The plants were irrigated with half strength macro and micronutrients of MS salts. After 20-25 days the hardened plants were transferred to polybags containing a mixture of soil: sand: FYM and were kept under high-density agro net shade house for acclimatization. Plants were ready for field transfer within two months after hardening and acclimatization (Fig. 2J). A survival rate of 100% was observed in field conditions. Plants produced through germination of somatic embryos as well as from shoots derived from somatic embryos through axillary branching were phenotypically similar and growing well in field conditions. So far, 10000 plants have been produced.

In conclusion, as an attempt to produce large scale planting stock, which is becoming scarce and depleted, this paper describes for the first time the somatic embryogenesis and mature field grown plants of edible bamboo *Dendrocalamus asper*. Response of embryogenesis was assessed for the first time in terms of callus multiplication rates, efficiency of production of embryos and different developmental stages observed under microscope were also reported in this system. Production of efficient protocol, which is feasible for commercial production is an asset of this report as the somatic embryogenesis offers an attractive fast alternative

non-conventional method for mass propagation either through direct regeneration or through enhanced axillary shoot proliferation from somatic embryos. Another important advantage of recovering plants through somatic embryogenesis is that somatic embryos like their zygotic counterparts also arise from single cells (Haccius, 1978). Owing to their origin from single cells, plants obtained from somatic embryos are non-chimeric and show genetic uniformity (Vasil, 1982). This will reduce pressure on conventional production of this important edible bamboo, which is very slow due to non-availability of seeds. Also the embryogenic calli so developed have a great potential for number of other experiments using protoplasts and genetic transformation as reported by Arya *et al.* (2000).

In bamboo sexual reproduction resets the internal calendar-controlling flowering. It is reported that a rejuvenation by embryogenesis results in the resetting of the internal calendar at zero (Rao *et al.* 1987; John *et al.* 1995; Joshi *et al.* 1997). A similar resetting of internal calendar by somatic embryogenesis would be of much practical use in *D. asper* where now methods have been standardized for somatic embryogenesis from mature plants. The plants can be rapidly propagated in large scale without losing the vegetative growth phase. These plants can be relied upon to last for their full vegetative life span, which is very important for commercial purposes, when compared with the plants regenerated through *in vitro* axillary bud culture technique which had exhibited a carry over of age factor as studied earlier during micropropagation of *D. asper* (Satsangi *et al.* 2001).

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Above ground biomass, production and carbon sequestration in farmer managed village bamboo grove in Assam, northeast India

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Village bamboo forms an important component in the traditional landscape of North East India. Bamboos growing in the rural landscape can be the ideal choice for creating sinks for CO_2. For biomass estimation of village bamboos of Barak Valley, North East India, allometric relationships were developed by harvest method describing leaf, branch and culm biomass with DBH as an independent variable. The culm density of the stand was 3380 culms ha^{-1} in 2003 and increased to 11030 culms ha^{-1} in 2006. Total above ground standing biomass of the grove was 42.98 Mg ha^{-1} in 2003 that increased to 152.15 Mg ha^{-1} in 2006 with a mean stand biomass of 99.28 Mg ha^{-1}. Mean productivity of the stand was 42.5 Mg ha^{-1}. Carbon storage in the above ground biomass ranged from 21.69 - 76.55 Mg ha^{-1}. Current and one year old culm ages represented 58-73% (15.86-35.63 Mg ha^{-1}) of the total carbon storage in the grove. The rate of above ground carbon sequestration was 18.93-23.55 Mg ha^{-1} with the mean of 21.36 Mg ha^{-1}. Carbon storage estimated in the bamboo stand of the present study offer insight into the opportunity of village bamboos in the rural landscape for carbon storage through carbon sequestration.

INTRODUCTION

In India clumps of bamboo form an important component in the village landscape of most Northeastern states, Kerala and Karnataka. Rural landscape of Barak Valley in Southern Assam is rich in bamboo resources and *Bambusa cacharensis* R. Majumder (*betua*), *B. vulgaris* Schrad. ex Wendl (*jai borua*) and *B. balcooa* Roxb. (*sil borua*) form important village bamboos prioritized by the rural people (Nath *et al.* 2006). Village bamboo grove forms an important land use system managed by the farmer to fulfill the economic necessitates. Farmer management system involves vegetative propagation through offset method, application of leaf litters and farmyard manure to meet the nutrient demand of the culms in the clump. Harvesting of culms from a newly developed clump begins only after it exceeds five years of age. Clear-felling harvesting strategy is practiced in bamboo grove and the felling cycle of 5-6 year is maintained for the re-growth of the bamboo stand.

Studies on bamboo biomass are scarce (Veblen *et al.* 1980; Tripathi and Singh 1994; Chandrashekara 1996; Shanmughavel and Francis 1996; Isagi *et al.* 1997; Singh and Singh 1999; Embaye *et al.* 2005; Das and Chaturvedi 2006) and most of these studies are carried out in natural stands. A key issue in the profitability of bamboo plantations is the productivity that can be expected. There is little published data on the productivity of bamboo plantations (Hunter and Junqi 2002).

Global climate change has inspired an increasing interest of scientific and political communities in the study of global carbon

storage and of the carbon balance (Landsberg *et al.* 1995; INBAR 2006). Isagi *et al.* (1997) studied the carbon balance in *Phyllostachys pubescens*. Bamboo-based land-use systems in rural landscape sequester CO_2 through the carbon stored in their biomass. By promoting such land-use systems that have higher C contents significant increases in C storage can be achieved by altering lower-biomass land-use systems to bamboo-based systems. Present paper examines the above ground stand biomass, production and carbon sequestration in the farmer managed bamboo grove in village Dargakona of Barak Valley, northeast India.

MATERIALS AND METHODS

The species

B. cacharensis, B. balcooa and *B. vulgaris* are widely distributed and frequently cultivated bamboo species in the traditional homegardens and bamboo groves of Barak Valley (Nath *et al.* 2006, 2008). *B. cacharensis* is endemic to Assam and distributed abundantly in the Barak Valley of northeast India. Adaptability to different agro climatic condition, rapid extension growth, desirable growth architecture and multiple uses made the species highest prioritized village bamboo of Barak Valley (Nath *et al.* 2004). *B. balcooa* is indigenous to the northeastern India and cultivated in villages of different states in India and is grown up to an altitude of 600 m (Seethalakshmi and Kumar 1998). *B. vulgaris* is cultivated extensively in many parts of the world, in India mainly in northeast and also in many other parts of the country and is grown up to an altitude of 1200 m (Seethalakshmi and Kumar 1998). Both the species are listed among the14 Indian priority bamboo species (NMBA 2004) and 38 priority bamboo species for international action (Williams and Rao 1994).

Study area

The study was conducted in a farmer managed bamboo grove in Dargakona village, Cachar district of Barak Valley of northeast India and is situated between longitude 92°45' East and latitude 24°41' North. The bamboo grove was raised in 1985-1986 through offset plantation of *B. cacharensis*, *B. vulgaris* and *B. balcooa* after clearing secondary forest patches. Felling cycle of 5-6 year are maintained and in each felling 80-90% of total culms/ clump in *B. vulgaris* and *B. balcooa* and 40-50% of total culms /clump in *B. cacharensis* are felled. The most recent harvest was carried out in June 2002 and the present study commenced from May 2003.

The climate of the study site is sub-tropical warm and humid with average rainfall 2660 mm. most of which is received during the southwest monsoon season (May to September). The mean maximum temperature ranges from 25.1°C (January) to 32.6°C (August). The mean minimum temperature ranges from 11°C (January) to 25°C (August).

Stand characteristics

The bamboo grove is composed of *B. cacharensis*, *B. vulgaris* and *B. balcooa*. The clump density of the stand was 380 clumps ha^{-1} and 66% was represented by *B. cacharensis*, followed by *B. vulgaris* (18%) and *B. balcooa* (16%). The mean clump CBH was 10.95 m, 11.82 m and 12.45 m for *B. cacharensis*, *B. vulgaris* and *B. balcooa* respectively. The mean culm height and culm DBH for *B. cacharensis*, *B. vulgaris* and *B. balcooa* was 11.35 m and 5.1 cm, 14.52 m and 7.35 cm and 14.25 m and 7.39 cm respectively. Basal area of the stand was 8.32 m^2 ha^{-1}, 19.02 m^2 ha^{-1}, 30.80 m^2 ha^{-1} and 36.16 m^2 ha^{-1} for 2003, 2004, 2005 and 2006 respectively. Culm density of the stand is described in Table 1.

Biomass determination

Biomass was determined by harvesting randomly selected culms of different sizes for current, one, two and three year old culms for the three species and developing regression equations relating dry weight of different culm components to culm diameter at breast height (Nath 2006). Culm, branch and leaf biomass was determined from their respective dry weight to fresh weight ratio. Total biomass of the different culm ages for the three species were multiplied with their respective stand density to obtain the total stand biomass and then computed on hectare basis.

Table 1. Culm population structure and dynamics in bamboo grove (no. ha^{-1}) in Dargakona village, Barak Valley, northeast India

Study period	Culm age classes (year)					
	Current	One	Two	Three	Four	Total
2003(t_1)	1670	1240	370	100	0	3380
2004(t_2)	2720	1670	1240	370	100	6100
2005(t_3)	2850	2720	1670	1240	470	8950
2006(t_4)	2080	2850	2720	1670	1710	11030
Net change						
($t_2 - t_1$)	1050	430	870	270	100	2720
($t_3 - t_2$)	130	1050	430	870	370	2850
($t_4 - t_3$)	-770	130	1050	430	1240	2080
Rate of change						
(t_2/t_1)	1.63	1.35	3.35	3.70	0.00	1.80
(t_3/t_2)	1.05	1.63	1.35	3.35	4.70	1.47
(t_4/t_3)	0.73	1.05	1.63	1.35	3.64	1.23

Production estimation

Allometric equations used for quantification of stand biomass were used to calculate the site productivity (Nath 2006). During the study period current, one, two, three and four year old culms of each year were converted into one, two, three, four and five year old culm ages respectively in the next year. The new culms that recruited during each year (June and July) and after the completion of height growth were marked during November and treated as current year culms of that year. Conversion of different culm ages to its next higher age classes were characterized by accumulation of dry matter. The net biomass accumulation for the period 2003 (B_1) to 2004 (B_2), 2004 (B_3) to 2005 (ΔB_4) and 2005 (ΔB_5) to 2006 (ΔB_6) was calculated as $\Delta B_1 = (B_2\text{-}B_1)$, $\Delta B_2 = (B_4\text{-}B_3)$ and $\Delta B_3 = (B_6\text{-}B_5)$.

The equation for the production estimation is

$$\mathbf{B= (B_n - B_{n-1}) + H+L}$$

where, B_n is the stand biomass for nth year and B_{n-1} is the stand biomass of the previous year of the *n*th year, *H* is biomass increment in culm components with increase in culm ages to its higher age classes and *L* is the total litter production during the period.

Mean values *ΔB* over the study period yielded the net primary productivity of the site.

The amount of litter fall was calculated by summing leaf, sheath and branch litter. Litter fall was studied by randomly laying 30 (thirty) permanent quadrats of 50 cm X 50 cm and collecting the litter samples at monthly interval followed by sorting into leaf, sheath and branch component and oven drying at 70°C to calculate the oven dry weight.

Carbon content determination

Sub-samples of culm, branch and leaf from different culm age classes for the three species that were taken to the laboratory during the sampling periods were ground using wiley mill and analyzed for carbon content determination. A total of 50% of the ash free mass was calculated as the carbon (C) content (Allen 1989). The ash content was determined by igniting 1 g of powdered litter sample at 550°C for 6 hr in a muffle furnace (Allen 1989). The total C storage in the above ground standing biomass was determined by summing the C content values for leaf, branch and culm component.

RESULTS

Farmers' management system

Bamboos in bamboo grove are principally managed for commercial purpose i.e. for selling in paper industry. *B. cacharensis* is the most predominant species in bamboo grove followed by *B. vulgaris* and *B. balcooa*. Clear felling strategy of clump management is mainly practiced in bamboo grove and under this

Table 2. Estimated biomass (Mg ha^{-1}) and productivity (Mg ha^{-1} yr^{-1}) of the bamboo grove in Dargakona village, Barak Valley, northeast India

	Leaf	Branch	Culm	Total
2003(ΔB_1)				
Current	0.05	1.08	15.67	16.80
One	0.81	1.83	11.94	14.58
Two	0.57	1.04	9.33	10.94
Three	0.03	0.08	0.55	0.66
Total	1.46	4.03	37.38	42.98
2004(ΔB_2)				
Current	0.09	1.62	27.43	29.14
One	1.54	2.26	17.46	21.26
Two	0.87	1.97	17.40	20.24
Three	0.68	0.98	7.90	9.54
Four	0.03	0.08	0.55	0.66
Total	3.13	6.48	71.23	80.84
2005(ΔB_3)				
Current	0.12	1.95	30.91	32.98
One	2.07	4.18	31.25	37.50
Two	1.54	2.99	20.45	24.98
Three	0.61	1.85	12.99	15.45
Four	0.71	1.06	8.45	10.22
Total	5.05	12.03	104.05	121.13
2006(ΔB_4)				
Current	0.08	1.27	22.25	23.60
One	2.41	4.39	32.90	39.70
Two	2.63	4.42	33.91	40.96
Three	1.27	2.80	18.15	22.22
Four	1.32	2.91	21.44	25.67
Total	7.71	15.79	128.65	152.15
(ΔB_2 -ΔB_1)				
Current	0.09	1.62	27.43	29.14
One	1.49	1.18	1.79	4.46
Two	0.06	0.14	5.46	5.56
Three	0.11	-0.06	-1.43	-1.4
Four	0.00	0.00	0.00	0.00
Total	1.75	2.88	33.25	37.86
(ΔB_3 -ΔB_2)				
Current	0.12	1.95	30.91	32.98
One	2.18	2.56	3.48	3.84
Two	0.10	0.73	2.99	3.82
Three	-0.16	-0.12	-4.41	-4.69
Four	0.00	0.00	0.00	0.00
Total	2.24	5.12	32.97	35.95
(ΔB_4 -ΔB_3)				
Current	0.08	1.27	22.25	23.6
One	2.29	2.44	1.99	6.72
Two	0.56	0.24	2.66	3.46
Three	-0.37	-0.19	-2.30	-2.76
Four	0.00	0.00	0.00	0.00
Total	2.66	3.76	24.60	31.02

system few current and one year old culms are retained in the clump for re-growth of the clump. In all the three species, new culms emerging from clear-felled clumps were shorter and thinner. During the subsequent year culm height and size increases than of the previous year and after 5-6 year of felling cycle culm attains their height and size comparable with the culms under selectively felled clump. Bamboo growers prefer clear felling system during rainy season as the harvested culms are constructed into rafts and ferried through water to reduce transportation cost.

Culm density and age class structure

B. cacharensis represented 60-67% of the total growing stock, 16-20% of *B. vulgaris* and 15-20% of *B. balcooa*. The culm density of the stand was 3380 culms ha^{-1} during 2003 that increased to 11030 culms ha^{-1} during 2006 (Table 1). Culm population structure was represented by four culm ages during 2003 and by five culm ages for the rest three observation periods. During 2003, 50% of the total stand population was represented by current year culms that gradually declined and was 19% during 2006. The proportion of one year culm ranged from 26-37% of the total stand density. Contribution of two, three and four year old culms increased with increase in stand density. A stand population structure of 5:4:1; 4:3:2:1; 3:3:2:1:1 and 2:3:3:1:1 was recognized for current, one, two, three and four year old culms for the period 2003, 2004, 2005 and 2006. Net change of total culm was highest (2850 culms ha^{-1}) for the year 2004 to 2005. However, the rate of change was highest for the year 2003 to 2004. Rate of change was highest for three and four year old culms and lowest for current year culms.

Stand biomass and productivity

Total above ground standing biomass of the village bamboo stand was estimated to be 42.98 Mg ha^{-1} during 2003 that increased to 152.15 Mg ha^{-1} during 2006 with a mean stand biomass of 99.28 Mg ha^{-1} (Table 2). Of the total stand biomass 3-5% was contributed by leaf followed by branch (8-11%) and culm (84-89%). Current year old culm ages represented the highest proportion of the stand biomass that ranged from 16-39% of total stand biomass and together with one year culm ages constituted 59% of the total above ground biomass indicating maximum stand biomass is contributed by the younger culm ages (Figure 1). Productivity of the stand varied from 39.41 Mg ha^{-1} for the period 2005-2006 to 44.03 Mg ha^{-1} for 2004-2005 with the mean value of 42.5 Mg ha^{-1} yr^{-1} (Table 3). Allocation of stand productivity revealed 60-75% (23.6-32.98 Mg ha^{-1}) of the total production was contributed by the new culm recruitment followed by 14-21% (6.06-8.08 Mg ha^{-1}) through litter production and 7-20% (3.0-8.72 Mg ha^{-1}) of biomass accumulation through changes of culm ages to its higher age

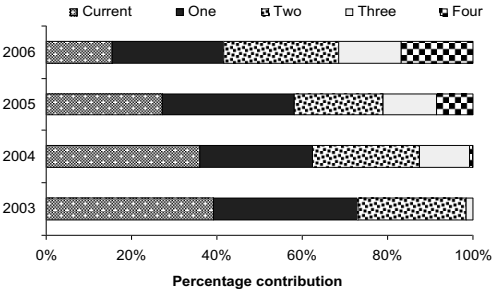

Figure 1. Contribution of different culm ages to the stand biomass of bamboo grove over the study period (2003-2006) in Dargakona village, Barak Valley, northeast India.

Table 3. Net production (Mg ha^{-1}yr^{-1}) of the bamboo grove in Dargakona village, Barak Valley, northeast India

Study Period	Biomass production from different component			Total production (Mg ha^{-1})
	New culm recruitment	Culm age changes	Litter production	
2003-2004	29.14	8.72	6.06	43.92
2004-2005	32.98	2.97	8.08	44.03
2005-2006	23.60	7.42	8.39	39.41

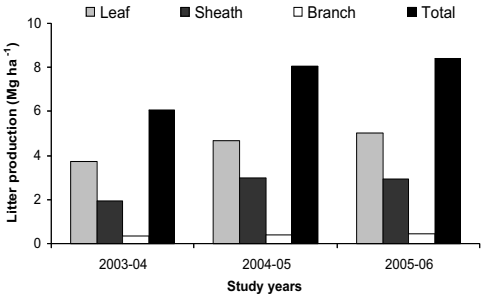

Figure 2. Litter production through different litter components in the bamboo grove in Dargakona village, Barak Valley, northeast India.

classes. Total litter production of the stand was 6.06-8.39 Mg ha^{-1} yr^{-1} with the mean value of 7.51 Mg ha^{-1}yr^{-1} (Figure 2). Leaf litter constituted 58-62% (3.73-5.03 Mg ha^{-1}) of the total litter production followed by sheath 32-37% (1.96-2.99 Mg ha^{-1}) and branch 5-6% (0.37-0.43 Mg ha^{-1}).

Carbon storage and carbon sequestration

The mean carbon content of the different culm component was in the order of culm (51.15%)> branch (47.51%)> leaf (42.11%). Statistically no significant difference in C content was observed in relation to culm ages and species.

Carbon storage in the above ground biomass ranged from 21.69 Mg ha^{-1} during 2003 to 76.55 Mg ha^{-1} during 2006. Allocation of C was more in culm components (85-89%) than in branch (8-10%) and leaf (3-4%). Both current and one year old culm constituted 58-73% (15.86-35.63 t ha^{-1}) of the total above ground carbon pool (Figure 3).

The rate of above ground carbon sequestration was 18.93-23.55 Mg ha^{-1} yr^{-1} with the mean of 21.36 Mg ha^{-1}yr^{-1}. Of the total annual carbon production, 82-89% was contributed by new culms and carbon production through culm age increment and 14-18% by annual total litter production (Table 4).

DISCUSSION

Stand biomass and productivity of the village bamboo stand

The above ground standing biomass of 42.98-152.15 Mg ha^{-1} of the present study is greater than/comparable with the corresponding reported values for *Fargesia spathacea* (Taylor and Zisheng 1987); *Dendrocalamus strictus* (Tripathi and Singh 1994; Singh and Singh 1999), *Gigantochloa atter* and *G. verticiliata*

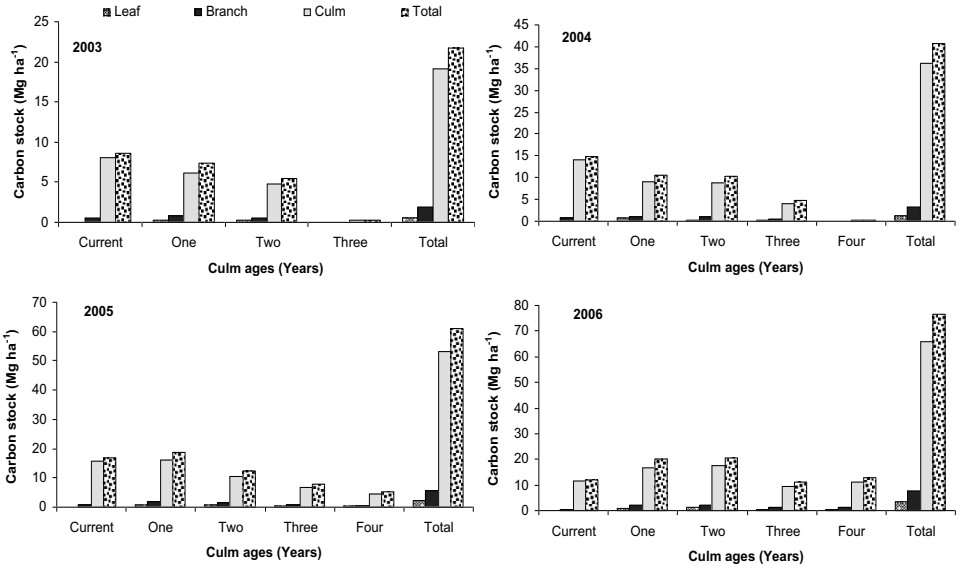

Figure 3. Estimated carbon pool of bamboo grove over study period (2003-2006) in Dargakona village, Barak Valley, northeast India.

Table 4. Carbon production/sequestration (Mg ha^{-1}yr^{-1}) of bamboo grove in Dargakona village, Barak Valley, northeast India

Observation period	C-sequestration through different culm component				C-sequestration through litter production				Total C-sequestration
	Leaf	Branch	Culm	Total	Leaf	Sheath	Branch	Total	
2003-2004	0.74	1.38	17.0	19.13	1.47	0.84	0.17	2.48	21.61
2004-2005	0.78	2.44	17.04	20.24	1.84	1.28	0.19	3.31	23.55
2005-2006	1.12	1.78	12.58	15.49	1.98	1.26	0.20	3.44	18.93

(Christanty *et al.* 1996); *Phyllostachys pubescens* (Isagi *et al.* 1997); *D. latiflorus* (Yiming *et al.* 1998); *Yushania alpina* (Embaye *et al.* 2005), whereas it is lower than *Chusquea culeou* (Veblen *et al.* 1980); *Bambusa bambos* (Shanmughavel and Francis 1996; Das and Chaturvedi 2006). Gradual increase in stand biomass over the study period is related with the increase in culm density of the stand. Higher number of culms increased the total leaf area implying greater capture of solar radiation and hence more photosynthetic production leading to increase in stand biomass over the study period. Current year culm contributed the highest proportion of the stand biomass that ranged from 16-39% of total stand biomass and together with one year culm constituted 59% of the total above ground biomass indicating maximum biomass contribution by the younger culm ages. A similar contribution across age structure can be calculated with data from the studies of Prasad (1987) in *D. strictus*. Above ground net production of 39.41-44.03 Mg ha^{-1} of the bamboo stand is higher than the values reported for bamboo forest and bamboo plantation (Veblen *et al.* 1980; Singh and Singh 1999) but lower than *B. bambos* (Shanmughavel *et al.* 2001). The net primary production depends on stand age (Shanmughavel *et al.* 2001). However, present study revealed other than stand age shoot productivity; dry matter increment with increase in culm age and culm age structure could also enhance greater biomass productivity. For example (i) compared to the shoot productivity of 8 t ha^{-1} in *Y. alpina* (Embaye *et al.* 2005) shoot productivity in the present study is much higher (23.6-32.98 Mg ha^{-1}yr^{-1}). (ii) Incorporation of dry matter increment with increase in culm age also yielded greater production of the stand; the feature that had been excluded by many workers in bamboo biomass/productivity estimation. (iii) Greater productivity of the bamboo stand can also be attributed to stand population structure with the preponderance of current and one year old culms as the stand age structure of *Y. alpina* was heavily skewed towards older culms that reduced the productivity in Masha forest (Embaye *et al.* 2005). This is also evident from the studies of Nath *et al.* (2006, 2007) where a culm population structure of five different ages preponderant towards younger culms could optimize yield by producing more new culms with superior height and diameter. Stand productivity (excluding litter production) declined from 37.86 Mg ha^{-1} (2003-2004) to 31.02 Mg ha^{-1} (2005-2006) and such trend can be related with the rate of change of total culms in the stand (Table 1).

C storage and C sequestration in the village bamboo stand

C content in the three bamboo species was higher in culm component followed by branch and leaf. Mean above ground carbon storage of 50.03 Mg ha^{-1} in the present study is comparable with the reported above ground carbon storage of 52.3 Mg ha^{-1} for *P. pubescens* (Isagi *et al.* 1997) but lower than total C storage of 83.3-103.8 Mg ha^{-1} for *B. bambos* (Das and Chaturvedi 2006). Above ground C sequestration of 18.93-23.55 Mg C ha^{-1} yr^{-1} of the present study is comparable with 20.5 Mg C ha^{-1} yr^{-1} in *B. bambos* (Das and Chaturvedi 2006). C storage of 2.48 - 3.44 Mg ha^{-1} through litter production reflects its importance as an important C sequester source, which in turn have positive impact on environmental services. The consequences of

an increased litter mass can increase the soil fertility, land productivity for greater production and prevention of land degradation.

Rapid phase of culm growth and subsequent greater resource acquisition resulted into higher shoot productivity and consequently greater biomass productivity of the village bamboo grove. Present findings reveal management of village bamboo grove as an important source of CO_2 sink. Short periodicity of culm growth, rapid culm elongation rate and brief clump development period makes village bamboo as a prospective resource to sink atmospheric CO_2. Village bamboos have socio-economic and ecological values and its management can provide benefits on a local, national and global level through livelihood, economic and environmental security for many million of the rural people. Farmer managed bamboo plantation in rural landscape, which although is cost effective in terms of carbon sequestered, can be considered under the Clean Development Mechanism (CDM) of Kyoto Protocol. Industrial utilization of bamboo fast degrades the bamboo by different mechanical and chemical processes for paper production and therefore limits the long term carbon storage potential of bamboo grove. However, farmers in homegarden practice selective felling and maintain a growing stock throughout the year (see Nath *et al.* 2006) that can provide long term carbon storage in homegardens compared to bamboo groves where a clear-felling strategy of bamboo harvesting is practiced. A shift in the utilization pattern of bamboos growing in the rural landscape especially in bamboo grove from paper industry to household, craft sector through value addition can enhance the carbon stock and carbon sequestration potential through longer lives of its materials/products.

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A comparative study of nutrient components of freshly harvested, fermented and canned bamboo shoots of *Dendrocalamus giganteus* Munro

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ABSTRACT

Bamboo shoots are largely consumed in different forms as fresh vegetable, fermented and canned. They are nutritionally very rich and are a complete health food. *Dendrocalamus giganteus*, the tallest bamboo, is a commercially important edible bamboo as it produces large sized shoots. The present study was conducted to determine changes in nutrient components in freshly harvested, fermented and canned shoots of this species. The study has revealed that the freshly harvested shoots are richer in nutrient components as compared to canned and fermented shoots. There is an overall decrease of the nutrient components except the dietary fibre content during fermentation and canning. Fresh shoots have higher quantities of macronutrients such as amino acids, proteins, carbohydrates, fat and fibre than the fermented and canned shoots except vitamins (C and E) and mineral elements like calcium, iron, potassium and phosphorous. The moisture content was highest in the canned shoots. In view of the present findings, the freshly harvested shoots, being nutritionally richer than the fermented and canned shoots, are recommended for consumption as food.

INTRODUCTION

Bamboos, known for their multiple uses both in the traditional way for the rural people as well as the modern society, is gaining popularity worldwide in the utilization of its shoots as healthy and nutritious food (Xia 1989; Giri and Janmejoy 1992; Qiu 1992; Shi and Yang 1992; Sharma *et al.* 2004; Bhatt *et al.* 2005, Cheng; 2006, Nirmala *et al* 2007). The shoots are rich in mineral components, mainly proteins, carbohydrates, minerals, vitamins and fibre and low in fat and sugar. They can be consumed fresh, cooked, pickled, fermented and canned. In India, consumption of bamboo shoots is confined mainly to the Northeastern states. The most commercially marketed shoots are derived from a few selected species including *Dendrocalamus giganteus*. This bamboo is large and gigantic with culms attaining a height of about 24-30m. It is distributed naturally in India and Indonesia and is cultivated in several states of India. Its juvenile shoots are conical, large with an average weight of 1.2-1.8 kg with older shoots attaining a weight of up to 2.5 kg. The shoots of this species are consumed as fresh vegetable, or in fermented and canned form and are mostly used for fermentation and canning on a large scale in the north-eastern states of India. The present paper discusses the changes in nutrient components in the fermented and canned juvenile shoots of *D. giganteus* as compared to freshly harvested shoots.

MATERIAL AND METHODS

The fresh juvenile shoots of *D. giganteus* were harvested at a stage when their tips were just emerging from the ground. After harvesting, the shoots were peeled off their hard sheaths, washed under tap water and used for the estimation of different nutrient components. The fermented and canned shoots of this species were obtained from the Khwairamband local market of Imphal (Manipur state).

The various nutrients estimated (with method employed in parenthesis) include free amino acids (Lee and Takahashi, 1966), proteins (Bradford 1976), carbohydrates (Whistler 1971),

starch (Mecreddy et al 1958), fat (AOAC 1990), nutrient dietary fibre (NDF) and its components (Goering and Van Soest 1970), vitamin C (Reiss 1993), vitamin E (Baker *et al.* 1980), ash (Harbers 1994) and minerals viz. calcium (Ca), cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), magnesium (Mg), nickel (Ni), phosphorus (P), potassium (K), selenium (Se), sodium (Na) and Zinc (Zn) by using Atomic Absorption Spectrophotometer. Moisture content was determined by calculating the percentage loss of moisture after drying of the shoots. Data were subjected to statistical analysis to analyze Analysis of Variance (ANOVA) through General Linear Model (GLM). In order to differentiate the mean values, mean range test (LSD) was applied from SPSS version 10.5.

RESULTS AND DISCUSSION

Tables 1, 2 and 3 give the results of various estimations. A perusal of these tables indicate that there is significant depletion of all the nutrient components such as amino acids, proteins, carbohydrates, starch, fat vitamins (C and E) and mineral elements except in the dietary fibre content in the fermented and canned shoots of *D. giganteus* as compared to the freshly harvested shoots. The fermented and canned shoots possess amino acid content of 2.005 g/ 100g fresh weight and 1.980 g/100g fresh weight respectively which is less than the fresh shoots (3.863 g/ 100g fresh weight). Protein content also decreased in the fermented shoots (2.570 g/ 100g fresh weight) as compared to freshly harvested shoots (3.108 g/ 100g fresh weight). The canned shoots showed a lower amount of protein (1.930 g/ 100g fresh weight) than the fresh and fermented shoots (Table 1; Figure 1).

Table. 1. Comparision of macronutrients (g/100g fresh weight), vitamins C and E (mg/100g fresh weight), moisture and ash content in the freshly harvested, 10 days old emerged, fermented and canned (non-salted) shoots of *D. giganteus*. Values suffixed in the rows with same letters are not significantly different with each other at < 0.05 probability level.

Shoot nature / **Nutrients**	**Freshly harvested juvenile shoots**	**10 days old emerged shoots**	**Fermented shoots**	**Canned shoots (non-salted)**
Amino acids	3.863 ± 0.128^{a}	2.230 ± 0.009^{b}	2.005 ± 0.012^{c}	1.980 ± 0.024^{d}
Proteins	3.108 ± 0.173^{a}	2.600 ± 0.049^{b}	2.570 ± 0.030^{c}	1.930 ± 0.016^{d}
Carbohydrates	5.103 ± 0.044^{a}	5.020 ± 0.091^{b}	1.504 ± 0.048^{c}	1.450 ± 0.016^{d}
Starch	0.506 ± 0.061^{a}	0.460 ± 0.020^{b}	0.455 ± 0.030^{bc}	0.443 ± 0.019^{bd}
Fat	0.387 ± 0.029^{a}	0.320 ± 0.020^{bc}	0.315 ± 0.031^{c}	0.250 ± 0.043^{d}
Vitamin C	3.280 ± 0.021^{a}	2.150 ± 0.008^{b}	1.090 ± 0.014^{c}	1.800 ± 0.008^{d}
Vitamin E	0.690 ± 0.032^{a}	0.238 ± 0.013^{b}	0.210 ± 0.010^{c}	0.301 ± 0.014^{d}
Ash	0.890 ± 0.131^{a}	0.740 ± 0.041^{b}	0.780 ± 0.024^{c}	0.750 ± 0.014^{bd}
Moisture	90.70 ± 0.121^{a}	91.08 ± 0.200^{b}	88.83 ± 0.097^{c}	95.16 ± 0.121^{d}

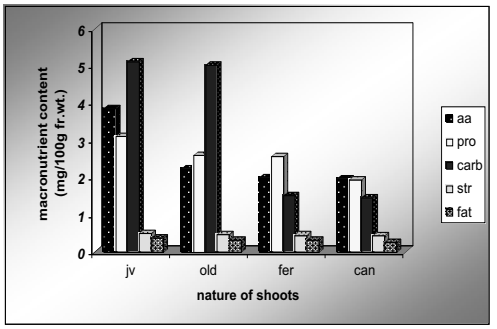


Fig. 1. Comparative content of macronutrient components in the freshly harvested juvenile shoots (jv), 10 days old emerged shoots (old), fermented (fer) and canned (can) shoots of *D. giganteus* (aa = amino acids, pro = proteins, carb = carbohydrates, str= starch).

There is a drastic reduction in the total carbohydrate content in fermented (1.504 g/ 100g fresh weight) and canned shoots (1.45 g/ 100g fresh weight) as compared to the freshly harvested shoots (5.103g/100g fresh weight). There was a decrease in the fat content of only 0.75 mg in the fermented (0.315g/100g fresh weight) and canned shoots (0.25 g/100g fresh weight) as compared to fresh shoots (0.3870g/100g fresh weight).

However, while there was a general reduction of various nutrients in the canned and fermented shoots as compared to the fresh shoots, the fibre content showed an increase in both the fermented (4.180 g/ 100g fresh weight) and canned shoots (3.04 g/ 100g fresh weight) as compared to the fresh shoots (Table 2; Figure 1). Whereas the fermented shoots have a lesser amount of Acid Detergent Fibre (ADF), the canned as well as the fresh shoots have nearly equal amount of ADF (Table 2). Lignin content in both the fresh and canned shoots was less than the fermented shoots (Fig. 2). The canned shoots (1.02 g/100 g fresh weights) have a comparatively higher content of hemicellulose than the fermented as well as the fresh shoots. The fermented shoots have higher amounts of cellulose (1.8 g/100g fresh weights) than the fresh shoots (1.589 g/100g fresh weight) while canned shoots have lower amount of cellulose (1.24 g/100 g fresh weight) than both the fresh and fermented shoot (Table 2).

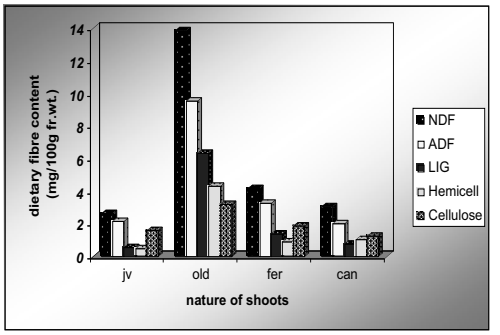


Fig. 2. Comparative content of dietary fibre in the freshly harvested (jv), 10 days old (old), fermented (fer) and canned (can) shoots of *D. giganteus.*

Table 2. Dietary fibre and its components (g/100g fresh weight) in the freshly harvested juvenile, 10 days old emerged, fermented and canned (non-salted) shoots of *D. giganteus*. Values suffixed in the rows with same letters are not significantly different with each other at < 0.05 probability level.

Shoot nature / Dietary fibre components	Freshly harvested juvenile shoots	10 days old emerged shoots	Fermented shoots	Canned shoots (non-salted)
NDF	2.645 ± 0.025[a]	3.840 ± 0.041[b]	4.180 ± 0.104[c]	3.040 ± 0.108[d]
ADF	2.150 ±0.009[a]	9.520 ± 0.021[b]	3.280 ± 0.076[c]	2.020 ± 0.095[d]
Lignin	0.560 ± 0.010[a]	6.320 ± 0.014[b]	1.398 ± 0.042[c]	0.780 ± 0.038[d]
Hemicellulose	0.495 ±0.016[a]	4.320 ± 0.020[b]	0.900 ± 0.028[c]	1.020 ± 0.013[d]
Cellulose	1.589 ±0.999[a]	3.200 ± 0.007[b]	1.882 ± 0.034[c]	1.240 ± 0.057[d]

Table 3. Summary of ANOVA for nutrient components and dietary fibre in freshly harvested, 10 days old emerged, fermented and canned (non-salted) of *D. giganteus*.

Nutrients	df	Mean square	F	p
Amino acid	3	2.440	11713.640	0.00
Protein	3	0.701	3821.86	0.00
Carbohydrate	3	12.882	154579.3	0.00
Starch	3	0.002675	13.375	0.02
Fat	3	0.009800	49.000	0.00
Vitamin C	3	2.503	807.548	0.00
Vitamin E	3	0.151	1506.750	0.00
Ash	3	0.01354	162.500	0.00
Moisture	3	21.289	1454.811	0.00
NDF	3	84.741	376627.0	0.00
ADF	3	38.071	147371.4	0.00
Lignin	3	22.371	335560.2	0.00
Hemicellulose	3	9.395	187890.8	0.00
Cellulose	3	2.224	22241.556	0.00
Residual	8			.

The vitamin C and E content was highest in the freshly harvested shoots (3.28 mg and 0.690 mg/100 g fresh weight) followed by canned (1.8 mg and 0.301 mg/100g fresh weight) and fermented shoots (1.09 mg and 0.210 mg/100g fresh weight (Table 1). The fermented shoots possess nearly the same amount of Cd, Co, Mn, Ni, P and Se content as the fresh shoots (Figure 4). However, Cd, Co, Cu, Mg, Mn and Na content in the canned shoots were lower than in the fermented shoots (Table 4). In comparison to the fermented shoots, canned shoots showed a higher content of Ca, Fe, K, P and Se (Table 4).

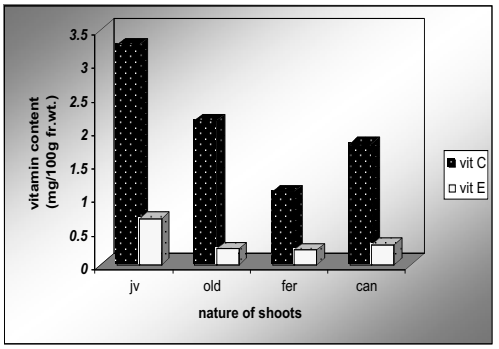
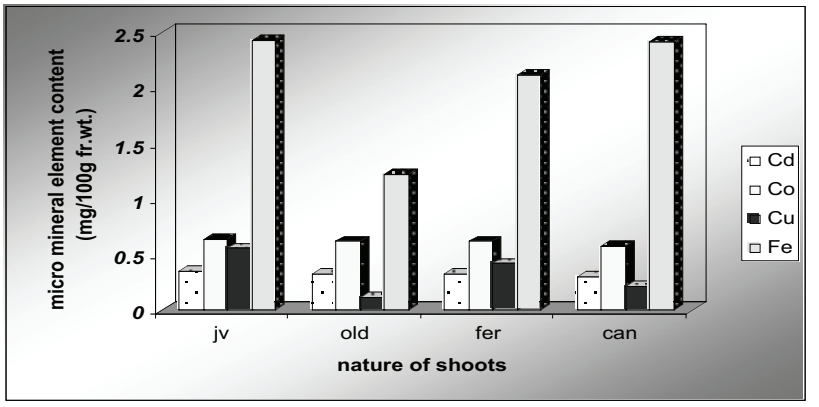


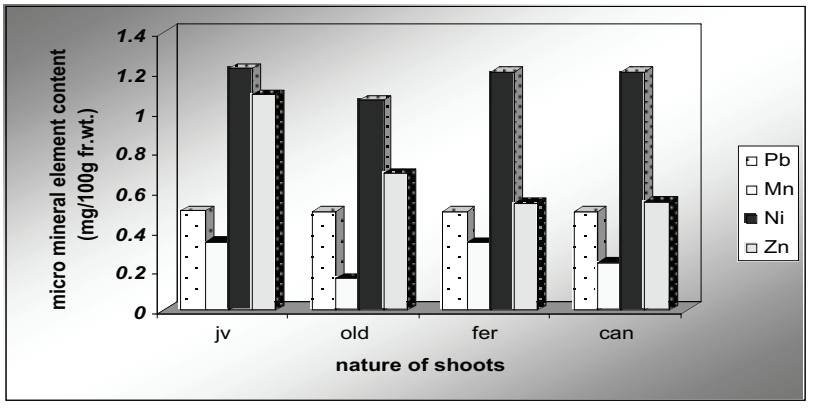
Fig. 3. Comparative content of vitamins C and E in the freshly harvested (jv), 10 days old (old), fermented (fer) and canned (can) shoots of *D. giganteus*

The moisture content is highest in the canned shoots (95.16g/100g fresh weight) followed by fresh (90.70g/100g fresh weight) and fermented shoots (88.83g/fresh weight). This is probably because the canned shoots are stored in preservative liquid inside the can. The canned and fermented shoots have lower amounts of ash content than the freshly emerging shoots (Table 1).

A comparative study of the freshly harvested, fermented and canned shoots of *D. giganteus* shows that there is an overall decrease in all the nutrients (except fibre) during fermentation and canning. The major decrease occurs in the carbohydrate content in both the fermented and canned shoots as compared to the fresh shoots. The loss in protein and Vitamin C content was also quite high while the reduction in starch and fat content was negligible. The fermented shoots have a comparatively higher content of macronutrients (amino acids, proteins, carbohydrates, fat and fibre) than the canned shoots except the vitamins (C and E), mineral elements like calcium, iron, potassium and phosphorus and moisture content. Giri and Janmejoy (2000) reported a total loss of vitamin C during fermentation of shoots. However, the present analysis has shown a decrease of only about



(a)



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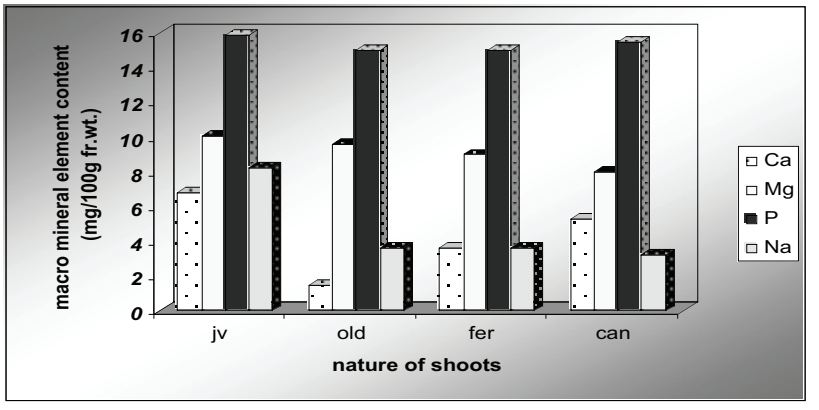

(c)

Fig. 4. Comparative content of **micro (a,b)** and **macro (c)** mineral elements in the freshly harvested juvenile shoots (jv), 10 days old emerged shoots (old), fermented (fer) and canned (can) shoots of *D. giganteus*.

Table 4. Comparison of mineral element content in the freshly harvested, 10 days old emerged, fermented and canned (non-salted) shoots of *D. giganteus* (mg/100 g fresh weight).

Shoot nature / **Mineral elements**	**Freshly harvested** juvenile shoots	**10 days old emerged** shoots	**Fermented shoots**	**Canned shoots (non-salted)**
Cd	0.354 ± 0.002	0.330 ±.002	0.330 ±.001	0.300 ±.001
Ca	6.802 ± 0.001	1.472 ± 0.002	3.644 ± 0.001	5.302 ± 0.002
Co	0.636 ± 0.001	0.621 ± 0.002	0.620 ± 0.002	0.578 ± 0.001
Cu	0.560 ± 0.010	0.120 ± 0.001	0.420 ± 0.001	0.220 ± 0.001
Fe	2.433 ± 0.002	1.226 ± 0.001	2.122 ± 0.002	2.422 ± 0.001
Pb	0.502 ± 0.001	0.498 ± 0.003	0.500 ± 0.001	0.500 ± 0.001
Mg	10.090 ± 0.011	9.570 ± 0.010	9.000 ± 0.055	8.000 ± 0.02
Mn	0.342 ± 0.002	0.160 ± 0.002	0.340 ± 0.002	0.240 ± 0.002
Ni	1.220 ± 0.001	1.060 ± 0.015	1.200 ± 0.006	1.200 ± 0.015
K	2.88 ± 0.150	2.75 ± 0.011	2.70 ± 0.015	2.80 ± 0.015
P	15.90 ± 0.020	15.01 ± 0.006	15.00 ± 0.010	15.50 ± 0.010
Se	0.0005 ± 0.001	0.0004 ± 0.001	0.0004 ± 0.001	0.0005 ± 0.001
Na	8.220 ± 0.017	3.640 ± 0.010	3.620 ± 0.006	3.240 ± 0.06
Zn	1. 086 ± 0.001	0. 690 ± 0.002	0.540 ± 0.002	0.546 ± 0.002

1.09 mg/100g fresh weight of vitamin C after fermentation. Dietary fibre and its components have shown an increase in both the fermented and canned shoots as compared to the fresh shoots. The nutrition data given by, USDA (www.nutritiondata.com) shows an overall reduction in nutrients in the canned shoots whether (salted or non-salted) as compared to the fresh shoots.

The present analysis of nutrient components of fresh, fermented and canned shoots of *D. giganteus* indicates that fermentation and canning processes leads to the depletion of all the nutrient components present in the fresh shoots except only the fibre content. The fresh shoots are therefore nutritionally richer and better for health than the fermented and canned shoots.

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Flowering in *Chusquea riosaltensis* (Poaceae: Bambusoideae: Bambuseae: Chusqueinae)

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ABSTRACT

The synflorescences and spikelets of *Chusquea riosaltensis* L. G. Clark, previously known only from vegetative material, are described and illustrated, and the reproductive cycle of the species is discussed. Images of the habit, branching and synflorescences are also included. A new record of this species for the Serra Negra, municipality of Rio Preto, state of Minas Gerais, enlarges the known geographic distribution of the species. A re-description of the vegetative parts, based on material from both populations, is presented.

RESUMO

É apresentada a descrição e ilustração das sinflorescências e espiguetas de *Chusquea riosaltensis* L.G. Clark primeiramente descrita apenas em material vegetativo, além de comentários sobre seu ciclo reprodutivo. Um novo registro da espécie para Serra Negra, município de Rio Preto Minas Gerais, amplia sua distribuição geográfica. Uma nova descrição das partes vegetativas com base no material de ambas as serras é incluída.

Comprising approximately 140 described species, *Chusquea* Kunth is the most diverse genus of Bambusoideae (Poaceae) (Judziewicz *et al.* 1999). Restricted to the New World, the species of *Chusquea* are distributed from Mexico to Argentina and Chile, and present the greatest latitudinal (24°N to 47°S) and altitudinal (0 to 4,000 m) ranges among bamboo genera (Soderstrom *et al.* 1988; Clark 1989, 1992, 1995; Judziewicz *et al.* 1999). The species of *Chusquea* are generally associated with montane forests, although many species, especially of *Chusquea* subg. *Swallenochloa* (McClure) L. G. Clark, are characteristic of tropical high altitude grasslands (*páramos* in the Andes, *campos de altitude* in Brazil). Although *Chusquea* is considered to be an Andean-centered genus (Clark 1995), southeastern Brazil represents a major center of diversity for *Chusquea*, with 36 described species and at least a dozen that remain to be described. Of the Brazilian species of *Chusquea*, 12 described species are classified within *Chusquea* subg. *Swallenochloa*, 10 species

within *Chusquea* subg. *Rettbergia* (Raddi) L. G. Clark, and the remainder in *Chusquea* subg. *Chusquea* (Judziewicz *et al.* 1999; Clark *et al.* 2007).

A number of species within both *Chusquea* subg. *Rettbergia* and subg. *Swallenochloa* appear to have relatively short flowering cycles or they exhibit some form of continuous or sporadic flowering (Clark 1989, pers. obs.; Judziewicz *et al.* 1999), so flowering material in the form of herbarium specimens, often representing various stages, is typically available. Even in woody bamboos with longer flowering cycles, some herbarium material can usually be found to document flowering structures, at least for the purpose of species description, although the description may be incomplete if not all stages are represented in the collections (e.g., flowers present but fruits lacking). In some instances, especially for rare species, only one or a few specimens may exist and they may be only vegetative or only reproductive. Three species of *Chusquea* subg. *Swallenochloa* in Brazil (*C. caparaoensis* L. G. Clark, *C. erecta* L. G. Clark and *C. riosaltensis* L. G. Clark) were each known from a single locality (and for the first two, from a single collection) but were sufficiently distinct to be described as new species from only vegetative material (Clark 1992).

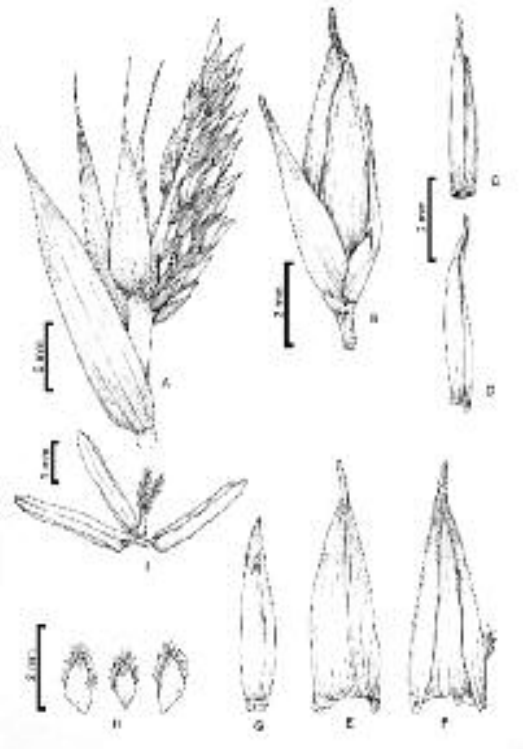

Figure 1. *Chusquea riosaltensis.* A. Synflorescence. B. Spikelet. C. Glume III. D. Glume IV. E. Lemma, abaxial view. F. Lemma, adaxial view. G. Palea. H. Lodicules. I. Androecium and gynoecium. (Based on Ferreira *et al*. 1148)

Field work in the Brazilian state of Minas Gerais by FM and PV for their graduate studies led to the discovery of a new locality for *Chusquea riosaltensis* and flowering material from both the original site and the new locality. We here describe and illustrate the synflorescences and spikelets of this species and document its occurrence in a new locality. We also emend the original description of its vegetative structures to include variation from the second population.

Chusquea riosaltensis L. G. Clark, Brittonia 44(4): 403. 1992. TYPE: BRAZIL. Minas Gerais: Mun. Lima Duarte, Serra do Ibitipoca, Parque Estadual (Florestal) do Ibitipoca, Paredão do Rio Salto, fork from trail to Ponte da Pedra, 1300 m, 2 Feb 1991 (veg), L. Clark & M. Morel 775 (holotype: SP!; isotypes: BHCB!, ISC!, MO, RB!, SJRP, US). Figs. 1-3.

Woody bamboo. *Rhizomes* pachymorph, necks 1-1.5 cm long. Culms 1.5-4 m tall, 0.3-1 (-1.5) cm diameter, erect; *nodes* solitary; *internodes* 4.5-9 cm long, slightly flattened above the branch complement, glabrous, green with glaucous wax below the culm leaves when young, becoming yellow to orange when mature or flowering, solid. *Culm leaves* 6.6-16.5 cm long, persistent, abaxially retrorsely scabrous, juncture of sheath and blade abaxially a more or less horizontal line; *sheaths* 3.6-8 cm long, 1.2-3.6 times as long as the blade, more or less triangular, the shoulders slightly rounded, margins smooth, ciliate near the apex; *girdle* 0.5-1 mm wide, pubescent; *inner ligule* 0.5 mm long, ciliolate; blades 2-5 cm long, triangular, erect, persistent, adaxially glabrous, the apex subulate. *Branching* intravaginal; *branch complement* with 8-12 branches per node; *central branch* 20-60 cm long, erect at

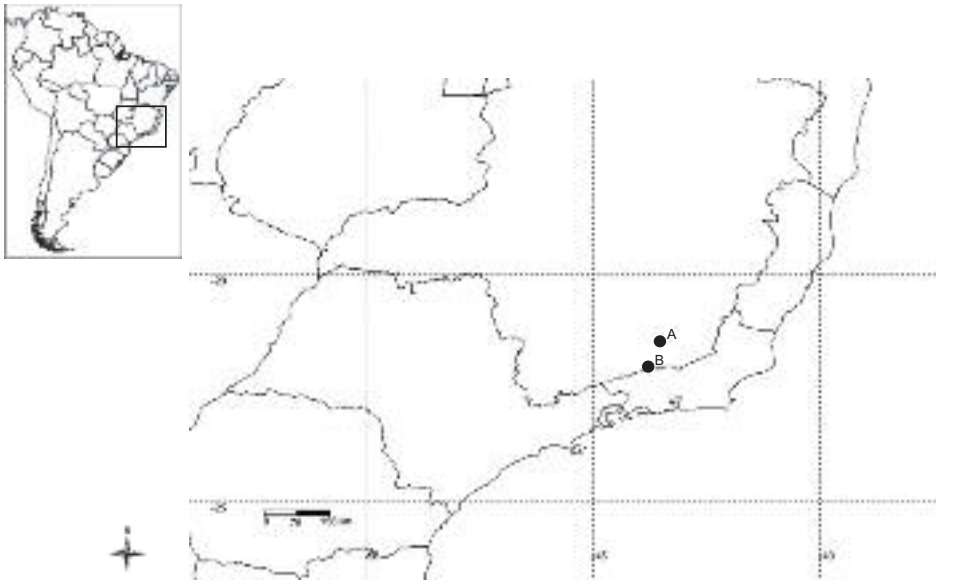

Figure 2. Distribution of *Chusquea riosaltensis*.A. Serra do Ibitipoca. B. Serra Negra.

the base, ascending but gently curving away from the main culm, rebranching; *subsidiary branches* 4-13 cm long, leafy, erect, not rebranching. *Foliage leaves* 8-10 (-13) per complement; *sheaths* persistent, pubescent between the nerves, margins smooth; *outer ligule* ca. 0.2 mm long, ciliolate; *inner ligule* 0.3-0.5 mm long, chartaceous, truncate; *pseudopetiole* ca. 0.5 mm long; *blades* 2.5-6 cm long, 0.2-0.6 cm wide, L:W = 10-13, lanceolate, glabrous, not tessellate, abaxially with a scabrous band along one of the margins, apex subulate, base rounded, the margins scabrous. Synflorescences 2-3 cm long, paniculate, contracted. *Spikelets* 6-7 mm long, 1-1.5 mm wide, laterally compressed. *Glumes* 4; *glumes I and II* 0.2-0.4 mm long, < 1/15 the spikelet length, scalelike; *glumes III and IV* scabrous, awned; *glume III* 3-4 mm long, 1.5-2 mm wide, ca. 2/3 the spikelet length, 1-nerved, awn 0.5-0.7 mm long; *glume IV* 3-4 mm long, 1-2 mm wide, ca. ¾ the spikelet length, 3-4-nerved, awn 0.6-0.8 mm long. *Fertile lemmas* ca. 5 mm long, 2-3 mm wide, abaxially scabrous with the margins ciliate toward the apex, 9-nerved, short-awned, awn ca. 0.6 mm long. *Palea* 4-5 mm long, 2.5-3 mm wide, 4-nerved, keeled only toward the apex, glabrous at the base becoming scabrous toward the apex. *Lodicules* 3, 1-2 mm long, acute, finely 2-3 nerved, the apices ciliolate; anterior pair obovate, posterior one oblanceolate. *Stamens* 3; anthers 3-4 mm long, stramineous when dry. *Gynoecium* with stigmas 2, hispid. *Fruit* not seen.

Additional Specimens Examined. BRAZIL. Minas Gerais: Parque Estadual Ibitipoca (PEIB), Lima Duarte, borda da mata ciliar de altitude, presente também no paredão do Rio do Salto, s.d., *Andrade 1082* (BHCB); região da Janela do Céu, 5 Feb 2004 (fl), Dias-Mello *et al.* 156 (RB); idem, 24 Nov 2004 (veg), Forzza *et al.* 3675 (RB); gruta dos Três Arcos, 7 Mar 2006 (veg), Ferreira *et al.* 989 (CESJ, R); gruta do Monjolinho, 19 Sep 2006 (veg), Ferreira *et al.* 1135 (R, RB); trilha entre o Monjolinho e Janela do Céu, 20 Sep 2006 (veg), Ferreira *et al.* 1144 (R, RB); aceiro norte do Parque, 20 Sep 2006 (fl), Ferreira *et al.* 1148 (CESJ, R, RB); idem, 24 Jan 2007 (fl), Ferreira *et al.* 1252 (CESJ, R, RB); Mun. Rio Preto, Serra Negra, região do Burro de Ouro, 21 Jan 2006 (fl), Souza *et al.* 138 (CESJ, R); idem, 26 Feb 2006 (veg), *Viana & Mota* 1936 (CESJ, R).

Distribution and Habitat. Clark (1992) indicated that the habitat for *C. riosaltensis*

was gallery and elfin forests above the Rio Salto and tributaries at elevations of 1200-1300 m in the Serra do Ibitipoca. The species is very common in the Parque Estadual do Ibitipoca, where it can be found along watercourses at the edges of forest and in *campo rupestre*. However, recent collections of this species document its occurrence in the Serra Negra (municipality of Rio Preto, state of Minas Gerais), an important mountain chain located about 30 km south of the Serra do Ibitipoca. In the Serra Negra, *C. riosaltensis* occurs in dense populations at the summit of the mountain from 1400 to 1650 m in elevation. The vegetation of the Serra Negra is somewhat similar to that of the Serra do Ibitipoca and both mountain chains have a quartzite rock substrate. In the Serra Negra, however, precipitation seems to be higher and the vegetation resembles high altitude grasslands (*campos de altitude*), whereas the vegetation of the Serra do Ibitipoca more closely approaches the *campo rupestre* of the Espinhaço Range (states of Minas Gerais and Bahia) (Joly, 1970).

Figure 3. *Chusquea riosaltensis* in habitat. A. Stand in Parque Estadual do Ibitipoca. B. Flowering branches. C. Intravaginal branching. D. Flowering culm. E. Synflorescence. (A by P.L.Viana; B-E by F. M. Ferreira).

These new records enlarge the distribution of this species such that it is no longer endemic to the Serra do Ibitipoca. Nevertheless, *C. riosaltensis* still presents a very restricted distribution, with only two documented populations, and it remains endemic to the state of Minas Gerais (Fig. 2).

Phenology. Clark (1992) observed many dead clumps and young individuals of this species with an estimated age of about three years in Parque Estadual do Ibitipoca in 1991, from which she inferred that the population had flowered gregariously three to four years previously. This would give an estimated previous flowering date of 1987 or 1988.

In the Park, flowering material was collected in February, 2004 in the area of Janela do Céu (Dias-Mello *et al.* 156); however, no information about the population or extent of flowering was included in the label. Other flowering collections within the Park were made in September, 2006 (Ferreira *et al.* 1148) and January, 2007 (Ferreira *et al.* 1252), in the *campo rupestre* at the extreme north end of the Park. In this population, only one clump was flowering while the rest remained vegetative. *Chusquea riosaltensis* was collected in January, 2006 (Souza *et al.* 138) in flower in the Serra Negra, but this was not a gregarious flowering event. Fruits were not found in any of the material examined.

Based on Clark (1992) and the recently collected specimens, *C. riosaltensis* may present a flowering cycle of close to 20 years, if the scattered flowering documented in the last few years marks the beginning of a more general flowering event in the species. Both populations will need to be monitored over the next few years to verify whether or not this is the case. Clark (1992) classified *C. riosaltensis* within *Chusquea* subg. *Swallenochoa* in sect.

Swallenochloa (McClure) L. G. Clark. Some species within this section have a tendency to flower frequently (Clark 1995); while *C. riosaltensis* does not appear to exhibit that pattern of flowering behavior, it may have some form of sporadic flowering.

Affinities. Within *Chusquea* sect. *Swallenochloa*, *C. riosaltensis* is most similar to *C. baculifera* Silveira, another member of section *Swallenochloa* from eastern Brazil, but they differ in the size of the culms, number of branches per complement and the length of the subsidiary branches, and indument of the culm leaves and sheaths of the foliage leaves (Clark 1992). Although the spikelets are about the same size in the two species, glumes III and IV and the fertile lemmas in *C. riosaltensi*s are short-awned while those of *C. baculifera* are subulate or awn-tipped, and glumes III and IV in *C. riosaltensis* extend for ⅔ to ¾ of the spikelet length while those of *C. baculifera* extend for ½ to ⅔ of the spikelet length. Based on Clark (1992) and data presented here, *C. riosaltensis* inhabits gallery forests and grasslands/camp rupestre at altitudes of 1,200 to 1,650 m whereas *C. baculifera* occurs in high altitude grasslands from 2,000 to 2,800 m in elevation. A revised key to the species of *Chusquea* subg. *Swallenochloa* in Brazil is in preparation (Clark and Blong, in prep.). *Chusquea riosaltensis* differs from all other species of the genus found in the Parque Estadual do Ibitipoca and also in the Serra Negra principally in its shrubby habit and intravaginal branching.

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