VOLUME 6

Horticulture

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Preface

Chair, Yves Crouzet, French
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Research on bamboo has made enormous progress these last 20 years, stimulated by the increasing demand of poles for industry, construction, handicraft... and also by the new methods of investigation. But we all agree that there is still so much to discover that we can consider ourselves to be just at the beginning.

We are not going to point out to you the value of this meeting, your presence shows you don't need to be convinced. We will speak briefly about the World Bamboo Organization's mission which is: "To promote and support the use of bamboo as a sustainable and alternative natural resource through the development of partnerships and alliances, and the creation of mechanisms for global communication, information exchange and technology transfer."

The papers that will be presented here on the biology, cytology, physiology, embryology and so on, are of substantial scientific interest. Science has contributed to the tremendous progress we know. Presently however, we should not forget the disastrous consequences of some scientific discoveries. Of course it's not the discoveries that are to blame, but the way they have been used.

If we do not know how to propagate, grow and cultivate bamboo, the threat diminishing bamboo resources will prevail.

Let us continue going forward, there is still a long way to go, but let us take care to keep in mind the fragile balance of our planet. François Rabelais, a French writer warned us 500 years ago when he wrote: "Science without conscience is nothing but ruin of the soul". Let us never forget.

Micropropagation Protocol for *Melocanna baccifera* using Nodal Explants from Mature Clump

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Abstract

A complete protocol for micropropagation of *Melocanna baccifera* using nodal segments from mature clumps is described. Single nodal segments collected from the 18 years old clumps were pretreated with a mixture of fungicide bavistin and bactericide streptomycin, followed by surface disinfection with 0.1% $HgCl_2$ solution for 10-12 min., and inoculated on liquid Murashige and Skoog (MS) medium supplemented with cytokinin. Highest bud break with multiple shoots was obtained on liquid MS medium supplemented with 20µM 6-benzylaminopurine (BAP). *In vitro* differentiated shoots were further multiplied on liquid MS medium supplemented with 15µM BAP and 3µM 6-furfurylaminopurine (Kn) at a rate of 2.99 folds, every 4 weeks. Nearly sixty five percent of *in vitro* shoots were rooted on half-strength MS medium supplemented with 25µM indole-3-butyric acid (IBA). Later on *in vitro* raised plantlets were hardened and acclimatized.

Keywords: *Melocanna baccifera*; bamboo; micropropagation; tissue culture.

Introduction

Melocanna baccifera (Roxb.) Kurz commonly known as 'muli' is an important bamboo from South East Asia, distributed chiefly in the Northeast India, Bangladesh and Myanmar region. The culms are thin walled with long internodes and are ideal for splitting, and widely used in house building, to make woven wares (baskets, mats, handicrafts, wall plates, screens, hats) and domestic utensils, and are an important source of superior paperpulp (Alam 1995). The young shoots are edible and during the rainy season constitute one of the important foods of tribal peoples in association with which it grows. The remarkable large fruits of *M. baccifera* are fleshy and edible; they are used as famine food and relished by wild and domestic animals. The leaves may be used in brewing liquor. Tabasheer, a medicine, as a cooling tonic and as an aphrodisiac, is the residue of the watery sap and found in the internodes of the culms (Singh 1986). Its net like extensive rhizome system protected the forest soil from erosion (Banik 1989).

This bamboo is accredited as one of the priority bamboo species for international action (Williams and Rao 1994). This bamboo is flowering since 2004 throughout in its natural range. Maximum forest cover is now denuded due to its gregarious flowering habit. Myanmar reports that the share of the bamboo species *Melocanna*

baccifera declined from 51.3 percent in 1990 to 36.2 percent in 2000 due to overexploitation (Lobovikov *et al*. 2007).

Considering the erratic flowering behaviour of bamboo and seed viability for a very short period, it is difficult to use seeds for raising large plantations continuously. Although propagation is possible by variety of vegetative methods, each method has its own limitations. Supply of planting material remains critical in establishing bamboo plantations and grove, from which culms may be taken to support bamboo based industries. Use of bamboos as industrial raw material besides their traditional uses, is however increasing day-by-day creating a wide gap between demands and supply which is escalating at alarming pace. In such conditions, *in vitro* methods remain an alternative solution. These techniques can be applied either for a practical purpose viz. mass propagation by micropropagation and/or for some fundamental studies like understanding flowering phenomenon, genetic studies etc.

Probably there is no report on *in vitro* regeneration in the genus *Melocanna*. The aim of the present study was to set up a protocol for the establishment, regeneration, multiplication and rooting *in vitro*, of *Melocanna baccifera* using nodal explants from mature clump.

Materials and methods

Explant source: Single nodal segments were collected during March to May from 18 years old clumps growing at the experimental plot of Forest Research Institute, Dehradun, India. After removal of the leaf sheath, individual nodes, each with the bud, were washed with dilute solution of 5-10 drops Tween-20 (HiMedia, India) per 100 ml of distilled water for 10 min. followed by running tap water for 15 min. Pre-disinfection treatments was given to reduce the contamination, where nodal segments were treated with a mixture of aqueous solution of fungicide bavistin (BASF, India) and bactericide streptomycin (HiMedia, India) at a concentration of 0.1% each for 20 min. For surface disinfection, nodal segments were treated with aqueous solution of 0.1% $HgCl_2$ (HiMedia, India) for 10-12 min. To remove the traces of disinfectant, nodal segments were finally washed with sterilized distilled water in laminar air flow for 3-4 times. The surface disinfected axillary buds were inoculated on liquid MS (Murashige and Skoog 1962) medium supplemented with cytokinin.

Effect of growth regulators on bud break: Different concentrations of 6-benzylaminopurine (BAP; 0-40µM) or 6-furfurylaminopurine (Kn; 0-40µM) alone were used in liquid MS medium for bud break. The pH of the medium was adjusted to 5.8 by using 1 N NaOH prior to autoclaving. The liquid medium (10ml) was dispensed into 25 x 150mm test tubes (Borosil, India). The culture tubes with media were autoclaved at 105kPa and 121^0C for 20 min.

Shoot multiplication: Proliferated *in-vitro* shoots were separated into clumps (four shoots) and used for further shoot multiplication. Various concentrations of BAP (0-25µM) or Kn (0-25µM) alone or in combinations were used in liquid MS medium. Subculturing was carried out every 4 weeks on fresh shoot multiplication medium. The number of shoots cultured and the number of shoots derived at the end of subculture gave the multiplication rate.

Effect of auxin on root induction: *In-vitro* raised shoot clumps (three or four shoots) derived from shoot multiplication medium were used for root induction. Half strength liquid MS medium with various concentrations of indole-3-butyric acid (IBA; 5-30µM) or α-napthalene acetic acid (NAA; 5-30µM) alone were used.

Culture conditions: All cultures were incubated at 27 ± 2ºC temperature and illumination of 16hrs photoperiod with light intensity of 2400 lux, obtained by white cool fluorescent tubes of 40 watts (Philips, India).

Hardening and acclimatization: Rooted plantlets were taken out from the flasks, washed to remove adhered medium and then transferred to autoclaved 250 ml screw cap glass bottle containing 1/3 volume of soilrite. These plantlets were nurtured with half strength MS medium (without organics) twice a week for two weeks and were kept in tissue culture incubation room. After two weeks these bottles were shifted to mist chamber having relative humidity of 70-80% with a temperature of 30 ± 2^0C. The caps of bottles were removed and plantlets were allowed to remain in the bottle for one week before they were transferred to polybags containing a mixture of sand, farmyard manure and soil in a ratio of 1:1:1. In the mist chamber, the plants were kept for four weeks and were irrigated with half strength MS medium. Later, these polybags were shifted to high-density double deck agronet open shade house for acclimatization.

Statistical analysis: A completely randomized design was followed for all the experiments. Each experiment was repeated thrice and data represents the mean of three experiments. Each treatment consisted of minimum twelve replicates. Data was subjected to one way Analysis of Variance (ANOVA) using Microsoft Excel ver. 2007 © Microsoft Technologies, USA. Degree of variation was shown by Standard Error (SE), Critical Difference (CD) at 5%. The CD values computed, were used for comparing differences in means of various treatments.

Results

Effect of growth regulators on bud break: Axillary bud break was observed in nodal segments within 15-20 days, when cultured on MS medium supplemented with cytokinin. The morphogenic response of explant towards axillary bud proliferation was markedly influenced by the concentration of growth regulator in the medium. Nodal segments cultured on liquid MS medium without plant growth regulator, yielded only 10.33% bud break response. Amongst cytokinin tried, BAP proved its superiority in inducing multiple buds (Fig. 1). Maximum bud break response (82.80%) was obtained on liquid MS medium supplemented with 20µM BAP (Fig. 2-A). However, among kinetin treatments, maximum responding explants (55.40%) were recorded at 25µM concentration.

Shoot multiplication: Response of *in vitro* shoot multiplication varied with cytokinin type and its concentration used in the medium. 15µM BAP concentration gave an average of 10.40 shoots with a multiplication rate of 2.60 folds in a period of four weeks, while 7.09 shoots developed from a propagule (four shoots) with a multiplication rate of 1.77 folds on MS medium supplemented with 20µM Kn . However, a synergistic effect was observed when lower concentration of Kn was added to the medium with BAP (Table-1). Thus, optimal *in*

vitro shoot multiplication was found to be 2.99 folds on MS supplemented with 15µM BAP and 3µM Kn (Fig. 2-B).

Effect of auxin on root induction: Shoots of 2-3cm length were used for various *in vitro* rooting experiments. *In vitro* rooting was obtained when *in vitro* grown shoots were transferred on half strength MS medium supplemented with auxins. However, spontaneous rooting was observed in few cultures if they remained on multiplication medium for more than 2 months duration. A thin one root per propagule was obtained and during hardening the plantlets did not survived. Amongst auxins tried for *in vitro* rooting, IBA was found to be better for *in vitro* rooting response as compared to NAA (Table-2). On medium supplemented with 25µM IBA, 64.66% rooting was observed (Fig. 2-C). Any increase or decrease in IBA levels in MS medium reduced the rooting percentage.

Hardening and acclimatization: Healthy plantlets with good roots and shoot system developed within 5-6 weeks when *in vitro* raised shoots were transferred on rooting medium. During hardening and acclimatization the shoots elongated, leaves turned greener and expanded. Over 65% of tissue culture plants were hardened and acclimatized (Fig. 2-D).

Discussion

For tissue culture of bamboo the use of starting material (seeds or adult plants) and the choice of the propagation method are crucial (Gielis 1999). The disadvantages of using seed are insufficient or no knowledge of genetic background, restricted availability of seeds for most species and rapid loss of germination capacity. In addition there is a huge variability in responsiveness in tissue culture (Saxena and Dhawan 1994). In the present investigation, nodal segments containing pre-existing axillary bud from mature clumps were used to initiate the *in vitro* cultures. The suitability of nodal segments is further reported in the micropropagation of bamboos (Nadgir *et al.* 1984; Prutpongse and Gavinlertvatana 1992; Saxena and Bhojwani 1993; Hirimburegama and Gamage 1995; Ramanayake and Yakandawala 1997; Arya and Sharma 1998; Bag *et al.* 2000; Das and Pal 2005; Sanjaya *et al.* 2005; Arya *et al.* 2006; Jimenez *et al.* 2006; Arya *et al.* 2008).

Presence of cytokinin in the medium leads to the promotion of bud differentiation and development. Hirimburegama and Gamage (1995) found cytokinin to be essential for bud break. In present study, axillary bud proliferation was more in number on BAP supplemented medium as compared to medium supplemented with Kn. Highest bud break of 82.80% was obtained on MS medium supplemented with 20µM BAP. The efficiency of BAP for shoot culture initiation is also reported in *Bambusa ventricosa* (Huang and Huang 1995); *B. bambos* (Arya and Sharma 1998)*; D. strictus* (Mishra *et al.* 2001); *D. asper* (Arya *et al.* 2002); *D. hamiltonii* (Sood *et al.* 2002); *D. giganteus* (Arya *et al.* 2006); *Guadua angustifolia* (Jimenez *et al.* 2006); *Drepanostachyum falcatum* (Arya *et al.* 2008).

In the present study a synergistic effect was evident when combination of BAP and Kn was tried for *in vitro* shoot multiplication. MS medium supplemented with 15µM BAP + 3µM Kn gave the optimal multiplication rate of 2.99 fold, which was significantly higher as compared to the multiplication obtained on MS medium supplemented with BAP or Kn alone. Similar positive effects of BAP and Kn interactions for *in vitro* shoot

multiplication were reported in *Dendrocalamus longispathus* (Saxena and Bhojwani 1993); *D. giganteus* (Ramanayake and Yakandawala 1997); *D. strictus* (Ravikumar *et al.* 1998); *Bambusa balcooa* (Das and Pal 2005; Arya *et al.* 2008).

Occasionally spontaneous rooting in the absence of exogenous auxin was observed, which was not significant and the plantlets thus obtained were not able to survive during hardening. Spontaneous rooting have already been reported by Shirgurkar *et al.* (1996) who obtained 50% spontaneous rooting on multiplication medium in *Dendrocalamus strictus*. Jimenez *et al.* (2006) and Ramanayake *et al.* (2008) have described spontaneous root development in shoots raised from adult culms of *Guadua angustifolia* and *Bambusa atra*, respectively. Our observation on root induction in shoots of *Melocanna baccifera* reveals that IBA alone was more effective. Efficacy of IBA on root induction is well reported in *Dendrocalamus strictus* (Nadgir *et al.,* 1984), *B. vulgaris* (Hirimburegama and Gamage 1995); *D. membranaceous* and *B. nutans* (Yasodha *et al.* 1997); *D. giganteus* (Ramanayake and Yakandwala 1997); *D. strictus* (Ravikumar *et al.* 1998); *Thamnocalamus spathiflorus* (Bag *et al.* 2000); *D. asper* (Arya *et al.* 2002); *B. balcooa* (Das and Pal 2005); *Pseudoxytenanthera stocksii* (Sanjaya *et al.* 2005); *Drepanostachyum falcatum* (Arya *et al.* 2008).

Conclusion

This is a first report for micropropagation of *Melocanna baccifera* using nodal segments from mature clumps. A multiplication rate of 2.99 folds was obtained with a good rooting percentage. The protocol can be used as a non conventional method for rapid propagation of this bamboo.

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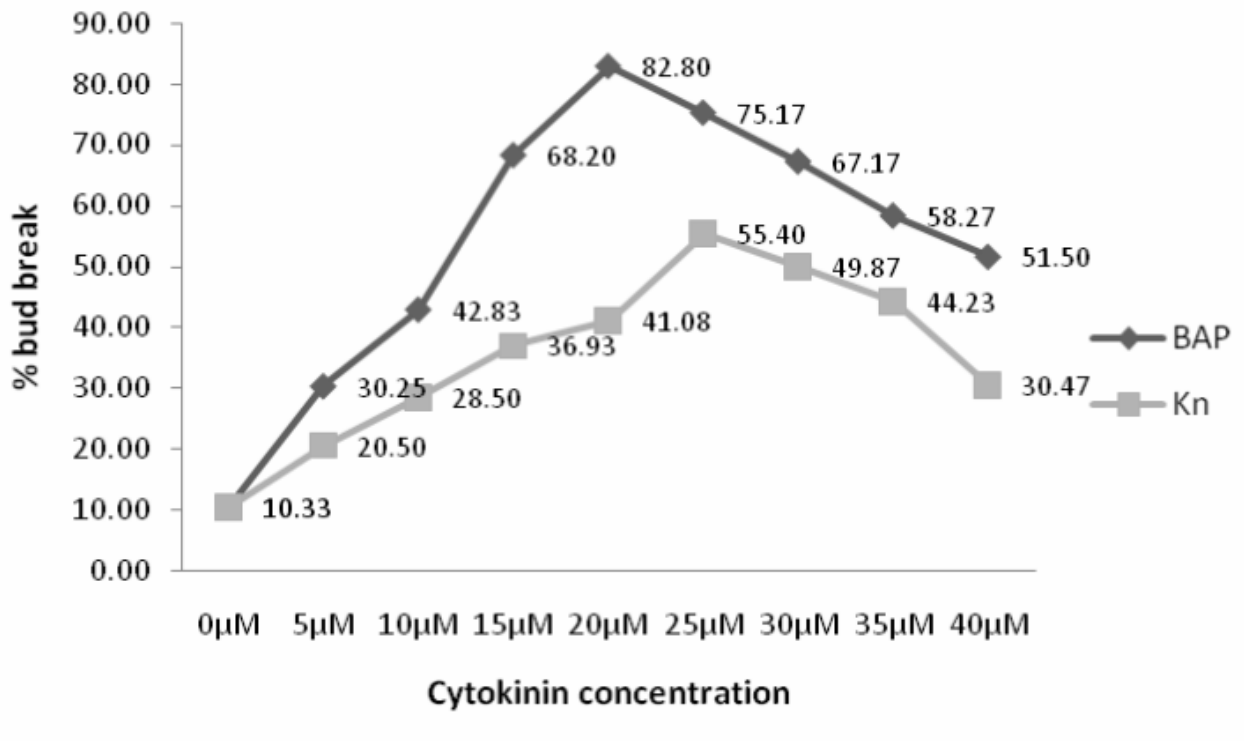

Figure 1: Effect of cytokinin concentrations on bud break from nodal segments of *Melocanna baccifera*, after 15 days in culture.

Figure 2: Micropropagation of *Melocanna baccifera* using nodal segments from mature clumps. (A) nodal segment showing bud break after 15 days of culture on MS medium + 20µM BAP; (B) *in vitro* multiplication on MS medium +15µM BAP .+ 3µM Kn after 3 weeks of culture; (C) *in vitro* rooting on half strength MS medium with 25µM IBA; (D) Hardened tissue culture raised plantlets in polybags.

Table 1: Effect of cytokinin in MS medium on shoot multiplication of *Melocanna baccifera*, after 4 weeks in culture. Propagules of four shoots were cultured.

Treatment	Mean shoot number	Mean shoot length (cm)	Multiplication rate
Control	03.47 ± 0.27	1.43 ± 0.11	0.87 ± 0.07
BAP			
5µM	06.51 ± 0.19	1.82 ± 0.15	1.63 ± 0.05
10µM	09.05 ± 0.25	2.35 ± 0.17	2.26 ± 0.06
15µM	10.40 ± 0.30	2.45 ± 0.12	2.60 ± 0.08
20µM	09.92 ± 0.17	2.06 ± 0.22	2.48 ± 0.04
25µM	08.82 ± 0.20	1.96 ± 0.13	2.20 ± 0.05
30µM	08.24 ± 0.19	1.88 ± 0.14	2.06 ± 0.05
Kn			
5µM	04.86 ± 0.19	1.71 ± 0.09	1.22 ± 0.05
10µM	05.42 ± 0.15	1.78 ± 0.07	1.35 ± 0.04
15µM	06.10 ± 0.20	2.17 ± 0.13	1.52 ± 0.05
20µM	07.09 ± 0.15	2.02 ± 0.08	1.77 ± 0.04
25µM	06.61 ± 0.29	1.85 ± 0.07	1.65 ± 0.07
30µM	05.91 ± 0.26	1.67 ± 0.09	1.48 ± 0.06
BAP + Kn			
10µM +1µM	09.27 ± 0.12	2.20 ± 0.23	2.32 ± 0.03
10µM +3µM	09.50 ± 0.20	2.18 ± 0.16	2.38 ± 0.05
10µM +5µM	10.03 ± 0.26	2.32 ± 0.17	2.51 ± 0.07
15µM +1µM	10.75 ± 0.27	2.46 ± 0.17	2.69 ± 0.07
15µM +3µM	11.94 ± 0.35	2.50 ± 0.20	2.99 ± 0.09
15µM +5µM	11.23 ± 0.24	2.22 ± 0.22	2.81 ± 0.06
20µM +1µM	10.19 ± 0.23	1.97 ± 0.11	2.55 ± 0.06
20µM +3µM	09.95 ± 0.19	1.87 ± 0.20	2.49 ± 0.05
20µM +5µM	09.35 ± 0.68	1.89 ± 0.22	2.34 ± 0.17
CD at 5%	**0.22**	**0.12**	**0.06**

Control: liquid MS basal medium

Table 2: Effect of auxin (IBA) in MS medium on rooting of *in vitro* shoots of *Melocanna baccifera*, after 4 weeks in culture. Propagules of four shoots were cultured.

Treatment	Response %	Mean root number	Mean root length (cm)
IBA			
5µM	20.70 ± 3.31	1.57 ± 0.19	2.07 ± 0.28
10µM	35.10 ± 4.20	2.08 ± 0.18	2.39 ± 0.22
15µM	52.77 ± 3.82	2.39 ± 0.19	2.70 ± 0.23
20µM	59.14 ± 2.91	3.69 ± 0.28	3.32 ± 0.34
25µM	64.66 ± 3.24	5.08 ± 0.21	4.07 ± 0.25
30µM	58.83 ± 2.64	4.64 ± 0.35	3.52 ± 0.20
NAA			
5µM	37.36 ± 3.53	2.25 ± 0.16	1.81 ± 0.07
10µM	53.84 ± 4.44	3.94 ± 0.19	2.37 ± 0.09
15µM	48.48 ± 2.69	3.48 ± 0.17	2.12 ± 0.12
20µM	38.42 ± 3.85	3.05 ± 0.25	2.08 ± 0.10
25µM	34.03 ± 3.43	2.70 ± 0.17	1.94 ± 0.15
30µM	27.62 ± 2.14	2.48 ± 0.15	1.70 ± 0.07
CD at 5%	**4.78**	**0.33**	**0.35**

Bamboo Propagation: Practical Experiences of Some Private Nursery Operators in Laguna, Philippines

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Abstract

Keeping in mind that the demand for bamboo propagules is increasing very fast, the author took a close look at the nursery practices of five bamboo propagators operating in the province of Laguna, the province where the foremost research organizations of the country in agriculture and forestry are located. His major findings: (1) most propagators are unaware of research findings on the use of improved methods; consequently they continue to use traditional methods that gives high survival rates but limited quantities, (2) propagators use a light weight mixture consisting of garden soil, rice hull and coconut coir as propagation medium, (3) a few propagators use growth hormones to induce root formation, and (4) they sometimes conduct research that once in a while produces surprising results. The author urges research institutions to assist bamboo nursery operators by giving them training and information materials that are easy to understand and follow.

Introduction

There is some kind of revolution going on in the Philippines right now and it involves bamboo. More and more people, NGOs, local government units (LGU), private land owners, resort managers, environmentalists and many others are increasingly getting interested in planting bamboo for various reasons (e.g. pole production, landscape beautification, erosion control, carbon sequestration, food production, bambusetum development and tourism enhancement). Consequently, the demand for planting materials has increased tremendously, prompting many enterprising individuals to go into the business of bamboo propagation.

At present, the local demand for bamboo propagules is greatest for ornamental species but that for timber or construction bamboo is expected to catch up soon because many LGUs, private landowners and companies engaged in bamboo processing are expected to put up their own plantations in the very near future.

Although much work by scientists has been accomplished in almost every country where bamboo is being planted, their research results often do not trickle down to bamboo propagators, and even if they do, many local propagators have found that some methods that work satisfactorily under experimental conditions actually do not turn out to be as effective in practice.

Because they have years of practical nursery experience, bamboo nursery operators are valuable sources of information. As the saying goes "experience is the greatest teacher". For sure, there is much that we can learn about bamboo propagation from experienced nursery operators. For one thing, they are the most reliable judges of whether technologies developed by experimentation do really work in practice. And bear in mind, some of them also do carry out some improvisations that sometimes bring about positive results.

The province of Laguna is located at the southern portion of Metro Manila. The province is famous for its gardens that supply the landscaping plants needed by Metro Manila and nearby provinces. The country's foremost educational and research institutions in agriculture and forestry are located in Los Baños, including the University of the Philippines at Los Baños, the Ecosystems Research and Development Bureau, the Philippine Council for Agriculture, Forestry and Resources Research and Development (PCARRD) and the Forest Products Research and Development Institute.

This paper presents a summary of what the author has learned from the experiences of co-nursery operators in Laguna, Philippines and his own 12 years of experience in propagating different species of bamboo.

Nurseries Included in the Survey

Name and address	Nursery size	Bamboos raised	APC**
Sardo's garden* Lalakay, Los Baños, Laguna	1,500 sq. m	ornamental	1,500
Tony's Garden*	1,500 sq. m	ornamental	1,500
Lito's Garden* Maahas, Los Baños, Laguna	2,000 sq. m	ornamental	2,000
Laguna Botanic Nursery* Timugan, Los Baños, Laguna	1,000 sq m	ornamental	1,500
CDC Bamboo Nursery Sto. Tomas, Calauan, Laguna	3,000 sq m	ornamental timber	2,000 6,000

*Also raises non-bamboo garden plants

**APC – annual production capacity

Note that of those included in the survey, only CDC Bamboo Nursery (or CDC for short) which is owned and operated by the author and two partners, is engaged in the production of both ornamental and timber bamboos. CDC has been in existence for less than a year.

The quantities produced by the nurseries are dependent largely on demand, especially for timber bamboos. Large quantities are not raised unless there are firm orders from customers.

Species Being Propagated

With the exception of CDC, nurseries in Laguna are involved only in the production of propagules of a few species, all ornamental. We can't blame them from specializing because ornamental bamboos are not only in high demand. They also command prices many times higher than other species. The ornamental species being propagated in large numbers include: (1) *Thyrsostachys siamensis*, (2) *Bambusa dolichomerithalla*, (3) *Bambusa multiplex* forma *variegata*, (4) *Schizostachyum brachyladum*, (5) *Bambusa vulgaris wamin* and (6) *Phyllostachys aurea*. There is much interest in producing propagules of black bamboos (*Bambusa lako* and *Gigantochloa atroviolacea*) but mother plants of these species are still quite scarce and very expensive.

Insofar as timber or construction bamboos are concerned, the emphasis has been in the raising of planting stocks of three species: *Bambusa blumeana* (Kawayan tinik), *Dendrocalamus asper* (giant bamboo) and *Bambusa merriliana* (bayog). However, small quantities of the following species are also being propagated: *B. philippinensis* and *Schizostachyum lumampao.*

Propagation Methods

By Seeds

Bamboo seeds are seldom available so like other propagators in the country and other parts of the world, bamboo propagators in Laguna rarely use seeds to produce new plants. Recently, however, one operator bought seeds of two species of black bamboo, Java black bamboo (*Gigantochloa atroviolacea*) and Timor black bamboo (*Bambusa lako*), from eBay. He placed an order for 100 seeds for each species and sowed them immediately when they arrived. More than 50% of the Java black bamboo germinated within two weeks after sowing. No germination was obtained from Timor black bamboo; the reason could be that the seeds have lost their viability after many months of storage at the sellers' outfit.

In the Philippines, there is one species that produces flowers almost every year---*Schizostachyum brachycladum*. There has been no report, however, that seeds have been produced from clumps of the said species. Amazingly, no clump of the species has been reported to die out after flowering.

Clump Division and Offset Planting

Almost every bamboo nursery operators in Laguna uses these two traditional methods of propagation (Banik, 1995; PCARRD, 2006) to produce new planting stocks. Clump division is the most widely employed method for species that have small diameters and do attain heights greater than 2 to 3 meters such as *Bambusa multiplex*, *B. muliplex* forma *variegata* and *B. multiplex* var *riviereorum*. Clump division is carried out by using machetes or saws. The divisions are commonly planted in large plastic bags filled with a medium consisting of a mixture of decomposing rice hull, garden soil and coconut coir.

Although rice hull is nutrient poor and does not decompose easily, it is very popular among plant propagators in the Philippines because of its light weight, which makes handling and transport of potted plants easy and less costly. Another advantage is that they increase the porosity of the medium allowing air circulation to proceed

easily. Because rice hull is nutrient poor, most plant propagators apply fertilizers to make their plants more vigorous and nice looking. For bamboo, the most common fertilizer is urea.

Clump division is definitely not a satisfactory technique for large scale production over short periods because it takes time for clumps to grow to a size that will make them ready for another round of division. Laguna bamboo propagators like the technique, however, because it is simple, easy to implement, cheap, and most of all, it gives 100% survival most of the time.

The use of offsets is also a favorite method for propagators of ornamental bamboo in Laguna because it offers the same advantages as the clump division method. Propagators use it for species that are bigger than those propagated by clump division and which command very high prices in the market. Thai monastery bamboo (*Thysostachys siamensis*) which sells like hot cakes in the country is propagated by offsets. Each offset consists of a rhizome and a portion of the culm cut just above the 4^{th} or 5^{th} node from the base. The offsets are planted in plastic bags filled with the medium described above and kept under partial shade until they produce new leaves. Watering is done every day and urea fertilizer is applied once a month or once every two months.

Other ornamental plants propagated by offsets in Laguna are *Bambusa dolichomerithalla*, *Schizostachyum brachyladum*, pink bamboo (probably a *Bambusa*), Australian bamboo (also probably a *Bambusa*) and *Phyllostachys aurea*, a running species.

By Culm Cuttings

Among those included in the survey, only CDC propagates bamboo by culm cuttings. The main reason for this could be that the other nursery operators have no technical background on bamboo, do not know what publications are available and have not been in contact with government extension workers. Because of their poor technical background, they find it quite difficult to understand many of the existing references. the fact that most of the publications available There has been very little attempt on the part of research agencies to carry out public education and information activities that would spread the technologies developed by research to reach their intended beneficiaries.

Propagation by culm cuttings is definitely an improvement over the traditional methods, i.e. clump division and offset. The principal advantage of using culm cuttings over clump division and the offset method is that more propagules can be obtained from a single culm within a matter of months.

The procedure employed by CDC in propagating culm cuttings evolved from carefully studying techniques described in various references (Anonymous (a), 1997; Anonymous (b), 1997; Banik, 1995; Lantican, 2008; PCARRD, 2006; Virtucio and Roxas, 2003). It consists of the following steps:

1. Selection of the culm to be cut
2. Cutting of the selected culm near the base
3. Trimming of the branches to 2 or 3 internode lengths
4. Cutting of the culm (with a saw) into segments consisting of 1, 2 or 3 internodes with the cut positioned 2 to 3 cm below the basal node
5. Planting of the cuttings in black polyethylene bags or in propagation plots

6. Watering of the cuttings daily at least 3 times a day.
7. Transferring the rooted cuttings

Based on the experience of the CDC, the best culms to use for culm cuttings are those that are mature and with well developed branches and lateral buds. According to Virtucio and Roxas (2003), a culm is perceived to be mature when all its culm sheaths have already fallen off. This rule of thumb would of course not work in the case of species with persistent culm sheaths such as *Thrysostachys siamensis*.

Between cuttings with branches and those only with buds, CDC's observation is that cuttings with branches give a higher percentage of rooting than those with nothing but buds. Cuttings that only have buds may root but rooting is very much longer compared to those with branches.

In terms of the number of internodes in a cutting, CDC prefers the use of single node cuttings. The cuttings are planted vertically or in a slanting position and then their cavities are filled with water. Filling the cavities with water prevents the fast drying of the cuttings, increasing their chances to produce roots.

When cuttings are made up of two or more internodes, the cuttings are buried in the soil in a horizontal position. Before they are buried, a hole is usually made in each internode and water is poured into the cavity.

The planting medium used by the propagators varies from one individual to another but the most widely used is a mixture of garden soil, rice hull and coconut coir. Several propagators who use garden soil only as a medium claim that they get similar results as those who use mixtures but they said using rice hull and coconut coir would greatly reduce the weight of their rooted cuttings.

CDC prefers the use of polyethylene bags over propagation plots because once rooted, those planted in the bags can be sold and transported right away but those planted in propagation beds have to be dug, planted in a container and conditioned for a week or two before they can be sold.

Keeping the cuttings moist everyday before rooting takes place is of key importance in growing cuttings. CDC uses a misting system constructed using PVC pipes and nozzles.

As many propagators have observed, there is a great variability in the rooting ability among different species of bamboo when propagated by culm cuttings. There are those that root easily (within a couple of weeks in the case of *Bambusa vulgaris*, *B. philippinensis* and several varieties of *B. multiplex*) and there are those that are very difficult to root such as *Dendrocalamus latiflorus* and *Thyrsostachys siamensis*. Portions of the culm that give high percentages of rooting also vary substantially from one species to another (see Table 2).

Incidentally, to educate some people who repeatedly call rooted bamboo cuttings (culm and branch) as "seedlings", which is absolutely inappropriate because seedlings are produced from seeds, the author has proposed that the word "cutlings" be used instead.

By Branch Cuttings

Branch cuttings have the same advantage as culm cuttings over the traditional methods of propagation (PCARRD, 2006). Many propagules can be obtained from a single culm.

In Laguna, only CDC uses this method for propagating some species of bamboo. The CDC findings:

1. Some species respond very well to the method but some do not.
2. The method works very well with branches that have "swollen" bases.
3. Branches that have adventitious roots below the branch bases (e.g. *Bambusa blumeana* and *Schizostachyum brachycladum*) are rather easy to propagate.
4. Many species of bamboo form adventitious roots below some of their branches when the culm is cut some distance from the ground.
5. Branch cuttings root faster when the branches have 2 to 3 internodes.
6. The application of a growth hormone (IBA + NAA) induces roots to form earlier in some species, even when the branch has no swollen base. In fact, species like *Bambusa multiplex* forma *variegata* would root easily even if the branch does not include its base if treated with a growth hormone and planted in an enclosed plastic bag.

Marcotting

Marcotting is the term used by some bamboo growers to refer to a method of bamboo propagation in which branch bases are covered with a propagating medium (usually coconut coir) and held in place using transparent plastic sheets tied around the culm with strings or fine wire, PCARRD (2006), Virtucio and Roxas (2003).

The method was tried in the author's farm on *Dendrocalamus latiflorus* by the instructors of a training course but it did not work well. The author tried it on several other species but it also did not work well. Although the author hasn't tried it, he believes wounding the culm just below the branch base and applying a growth hormone may lead to better rooting.

Summary and Conclusions

Table 2 summarizes the findings obtained by the author in the survey that he conducted. The table shows the ranges in the percentage of rooting for different bamboos and vegetative propagation methods.

There is a great need to establish more bamboo nurseries in the Philippines, not only to fill the requirements of those involved in landscaping but more so to produce propagules in large quantities for plantation establishment, watershed protection, carbon sequestration, erosion control, riverbank stabilization and the reduction of the incidence of landslides. There is clearly a need to upgrade the knowledge of small-scale propagule producers through training and information materials. Concerned agencies of the government are definitely not doing enough to make sure that their research findings find their way to their intended beneficiaries.

One of the research areas worth looking into concerns the observation made by CDC that the rooting ability of cuttings of some species seems to be affected by the season of the year. For example, *Bambusa philippinensis*

cuttings root more easily during summer than in the rainy months. Another area worth looking into is the development of a culm maturity index that is based on easily observed characters so that propagators can be guided accordingly in choosing the culms that they will use for propagation.

In closing, the author is convinced that researchers can learn a lot from private bamboo propagators' experiences. Learning what their practices are could lead them to research problems that are worth investigating.

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Table 1. Findings of the CDC on the propagation of different species of bamboo by cuttings.

Species	Age	# of nodes	Part	Direction
Bambusa bambos	1 - 2	2 - 3	B - M	U, S
B. blumeana	1	1 - 2	B - M	U, S, H
B. dolichomerithalla	1	1	Branchy	U, S
B. merrilliana	1	1 - 2	Branchy	S, H
B. multiplex	1	1	Branchy	U, S
B. multiplex f. variegatum	1	1	Branchy	U
B. oldhamii	1 - 2	1 - 2	Branchy	U, S
B. philippinensis	1 - 2	1 - 2	Branchy	U, S
B. vulgaris	0.5 - 1	1 - 3	Branchy	S, H
B. vulgaris vittata	1 - 2	1 - 3	Branchy	U, S, H
B. vulgaris wamin	1 - 2	1 - 3	Branchy	U, S
Dendrocalamus asper	1 - 2	1 - 3	B - M	H
Gigantochloa levis	1	1 - 3	B - M	H
Schizostachyum brachycladum	1 - 2	1	Branchy	U, S
Schizostachyum lima	1 - 2	2 - 3	Branchy	H

Legend:
B - M: base to middle
U - upright
S - slanting
H - horizontal

Table 2

Ranges in percentage rooting for different species and vegetative methods (based on the experiences of bamboo nursery operators in Laguna, Philippines)

	Scientific Name	Stem cuttings	Branch cuttings	Clump division	Offset	Marcot
1	*Bambusa atra*			>90	>90	
2	*B. bambos*	50-70	50-70			
3	*B. blumeana*	50-70	50-70			
4	*B. dolichomerithalla*	50-70		>90	>90	<5
5	*B. merrilliana*	40-50	50-60			
6	*B. multiplex*	25-40		70-80	70-80	
7	*B. multiplex f. variegata*	60-70	60-70	>90	>90	
8	*B. multiplex var. riviereorum*	<5		>85	>85	<5
9	*B. oldhamii*	50-70				
10	*B. philippinensis*	70-85				
11	*B. sp. (pink bamboo)*	80-100				
12	*B. sp. (Australian bamboo)*	50-70				
13	*B. vulgaris*	70-85	70-85			
14	*B. vulgaris var. vittata*	70-85	70-85			
15	*B. vulgaris var. wamin*	50-70	50-70			
16	*Dendrocalamus asper*	50-70	50-70			
17	*D. latiflorus*	<10				<10
18	*D. strictus*	50-70	50-70			
19	*Gigantochloa atter*	50-70				
20	*Gigantochloa levis*	40-60	50-70			
21	*Guadua angustifolia*		50-60			
22	*Meloccana baccifera*	<10			>90	
23	*Phyllostachys aurea*				>80	
24	*Sasa fortunei*	<10	<10	70-80	70-90	
25	*Schizostachyum brachycladum*	50-60			>90	<5
26	*S. lima*	50-60		>70	>80	
27	*S. lumampao*	50-60			75-90	
28	*Thyrsostachys siamensis*	<5			>90	

Cyanogenic Glucosides in Juvenile Edible Shoots of some Indian bamboos

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Abstract

The cyanogenic glucosides, secondary metabolites of plants, are glucosides of the α-hydroxynitrile which plants use to protect themselves against predators. There are approximately 25 cyanogenic glucosides with the major cyanogenic glucosides *viz.* amygdalin, dhurrin, linamarin, lotaustralin, prunasin and taxiphyllin being found in the edible parts of plants. Bamboo, a tall grass with about 1250 species in 75 genera, is one of the most primitive grasses surviving today. Apart from the diverse use that the various parts of the plant are put to, the tender juvenile shoot is consumed. While shoots of some species can be eaten raw and without any pretreatments, there are a large number of species whose shoots possess an acrid taste due to the presence of cyanogenic glucosides and have to be pre-boiled in water for varying periods of time to make them fit for consumption. The cyanogenic glucosides present in bamboo shoots are taxiphyllin, a *p*- hydroxylated mandelonitrile tiglochinin, which is rapidly hydrolysed to glucose and hydroxybenzaldehyde cyanohydrins. Hydroxybenzaldehyde cyanohydrin then decomposes to hydroxybenzaldehyde and hydrogen cyanide. Cyanogenic glucosides in fresh shoots of four species of edible bamboos (*Bambusa balcooa, B. bambos, B. tulda* and *Dendrocalamus giganteus*) at three different stages of growth were studied. The cyanogenic glucoside content showed variation in quantity in all species and also as the shoot advanced in age. There was also difference in the quantity of the glucoside in the tip, middle and basal portion of the same shoot. The findings of this study may be valuable for use in food composition databases and for prevention of diseases caused by cyanide toxicology due to consumption of bamboo shoots.

Keywords: Bamboo shoot, Cyanogenic glucosides, Picrate method, Taxiphyllin.

Introduction

Known more for their uses in the industry, bamboos have an additional usage in utilization of its young shoots as food. Juvenile shoots are not only delicious but have high nutritive value. Most bamboo species produce edible shoots, fibre and timber but less than 100 species are commonly grown or utilized for their edible shoots (Midmore 1998; Collins and Keilar 2005). The species employed for commercial production of edible bamboo shoots belong to a few genera like *Bambusa, Dendrocalamus* and *Phyllostachys* (Kleinhenz et al. 2000) which are consumed in different forms as fresh, fermented, pickled, dried or canned in East and South East Asia,

including the major countries of Japan, China, Korea and Indonesia. In India, the consumption of tender shoots is confined mainly to the North-Eastern states of the country where it is a part of the traditional cuisine, both in fresh and fermented form. Bamboo shoots are nutritionally very rich in proteins, carbohydrates, dietary fibre, and amino acids and low in fats and cholesterol; the juvenile younger shoots being much richer in nutrients than the fermented and canned form of shoots (Nirmala et al. 2008). The freshly emerging juvenile shoots are further more nutritionally superior than the older emerged shoots (Nirmala et al. 2007). While shoots of some species can be eaten raw and without any pretreatments, there are a large number of species whose shoots possess an acrid taste due to the presence of cyanogenic glucosides and have to be pre-boiled in water for varying periods of time to make them fit for consumption.

In the present study, the total cyanogenic glucoside content in fresh shoots of some selected bamboo species, *viz. Bambusa balcooa, B. bambos, B. tulda* and *Dendrocalamus giganteus*, at three stages of growth were analyzed. These bamboos were selected on the basis of their nutritive value and also because of the fact that they are popularly consumed and have commercial value.

Materials and methods

Samples.

Juvenile shoots of four edible bamboos (*Bambusa balcooa, B. bambos, B. tulda* and *Dendrocalamus giganteus)* at three different stages of growth, *viz.* soon after emergence above ground, two weeks old and three weeks old, were obtained from the bamboos growing in the P.N. Mehra Botanical Garden of Panjab University, Chandigarh, India and from those growing in the wild. The culm sheaths were removed and the shoots were cut longitudinally in half (Figure 1). The half shoot section was cut transversely at the tip, middle and basal portion and a small shoot section from each was sliced and ground in a pestle and mortar and used for estimation. Because of rapid breakdown of the bamboo cyanogenic glucoside to HCN, the ground material was processed immediately.

Picrate Method.

25 mg of the shoot ground in pestle and mortar, was weighed out accurately and put in a flat-bottomed plastic vial immediately after grinding (Egan et al. 1998, Bradbury et al. 1999). 0.5 ml of 0.1 M phosphate buffer at pH 6 was added to each and a picrate paper attached to plastic strip was put and the vial immediately closed with a screw capped lid.

After about 16-24 hours at 30^0C, the picrate paper was then carefully removed and immersed in 5 ml of distilled water for about 30 min. The absorbance was measured at 510 nm and the total cyanide content (ppm or mg/kg) was determined by the equation

Total cyanogen content (mg/kg) = 396×absorbance×100/z

Where z = weight (mg) of ground shoot (Bradbury et al. 1999)

Results and discussion

Variations could be seen in the amount of cyanogenic glucoside from species to species as well as the shoot ages. In one week old emerging bamboo shoots, a trend could be seen in all the species as one goes from the apical, the middle portion and towards the basal portion of the same shoot. The cyanogenic glucoside content is highest in one week old shoots in the tip portion and gradually decreases towards the base. As indicated in Table 1, the highest amount is present in *Dendrocalamus giganteus* (1164 mg/kg) while lowest in *Bambusa bambos* (223 mg/kg).

As the shoot ages, the cyanide content in the shoot is seen to increase more towards the middle portion of the shoot while it decreases in both the basal and tip portion of the shoot (Table 2). It reaches a maximum in the middle portion of the shoot of *Dendrocalamus giganteus* (1012 mg/kg). This same trend of increase in the middle portion can further be seen in the 3 weeks old shoot of all selected species (Table 3), with highest in the middle portion of the shoot of *D. giganteus* (1132 mg/kg).

The cyanide content is minimum in the emerging shoots of *B. bambos* (tip=859; middle=371; base=223 mg/kg) as also indicated by Haque and Bradbury (2002) (Table 1). Taxiphyllin content is highest in the shoots of *D. giganteus* at all 3 selected stages of growth. The values are quite high with minimum of 139 mg/kg in basal portion of *B. bambos* and maximum in the tip portion of *D. giganteus* (1164 mg/100 kg). Generally, plants which contain more than 20 mg/ 100 g of fresh plant material are considered potentially dangerous (Kingsbury 1964). European Food Society Authority (EFSA 2004) have stated that a level of up to 10 mg/kg HCN is not associated with acute toxicity. Thus, the cyanide content in the bamboo shoots in the raw state without any pretreatments or processing could prove potentially toxic for the consumer. However, the total HCN content in the shoots decreases substantially following harvesting and the bamboo shoots sold commercially as food can be processed adequately by boiling before consumption (FSANZ 2004). Ferreira et al. (1995) reported that the acridity caused by HCN can be removed progressively upto 70 % from bamboo shoots by boiling them in water for 20 minutes at 98^0C while boiling them at higher temperatures and longer intervals removes progressively up to 96% of the acridity. Thus, the glucoside content in the bamboo shoots would be reduced to lesser than the permissible limits as determined by health standards and organizations by proper processing of the shoots before consumption.

The cyanogenic glucosides are glucosides of the α-hydroxynitriles and belong to the secondary metabolites of plants. They are amino acid derived plant constituents. The biosynthetic precursors of the cyanogenic glucosides are different L-amino acids, which are hydroxylated, then the N-hydroxylamino acids are converted to aldoximes and these are converted into nitriles and hydroxylated to α-hydroxynitriles and then glycosylated to cyanogenic glucosides (Vetter 2000; FSANZ 2004). All known cyanogenic glucosides are β-linked, mostly with D-glucose. Many edible plants contain cyanogenic glucosides, whose concentrations can vary widely as a result of genetic and environmental factors, location, season, and soil types (Ermans et al. 1980; JEFCA 1993). Table 4 summarises some of the main food sources of cyanogenic glucosides and their estimated potential yield of hydrogen cyanide released on hydrolysis. There are approximately 25 known cyanogenic glucosides, and a number of these can be found in the edible part of some important food plants. These include amygdalin (almonds), dhrrin (sorghum), lotaustralin (cassava), linamarin (cassava, lima beans), prunasin (stone fruit) and

taxiphyllin (bamboo shoots) (JEFCA 1993; Padmaja 1995). The cyanogenic glucosides in bamboo shoots is in the form of taxiphyllin, a *p*- hydroxylated mandelonitrile tiglochinin, which is rapidly hydrolysed to glucose and hydroxybenzaldehyde cyanohydrins (FSANZ 2004). Hydroxybenzaldehyde cyanohydrin then decomposes to hydroxybenzaldehyde and hydrogen cyanide. This is a means by which plants protect themselves against predators (Jones 1998; Moller and Seigler 1999). However, taxiphyllin is unusual in being comparatively thermolabile (Davies 1991). Of the 4 species studied, *B. bambos* has the least amount of cyanogenic glucosides and the young shoots are delicious.

The present study is a preliminary study and may help to throw more light on the way of consumption of bamboo shoots and help in developing protective steps towards the potential toxic effects caused by the cyanogenic glucosides present in them. The findings of this study may further be valuable for use in food composition databases and for prevention of diseases caused by cyanide toxicology due to consumption of bamboo shoots.

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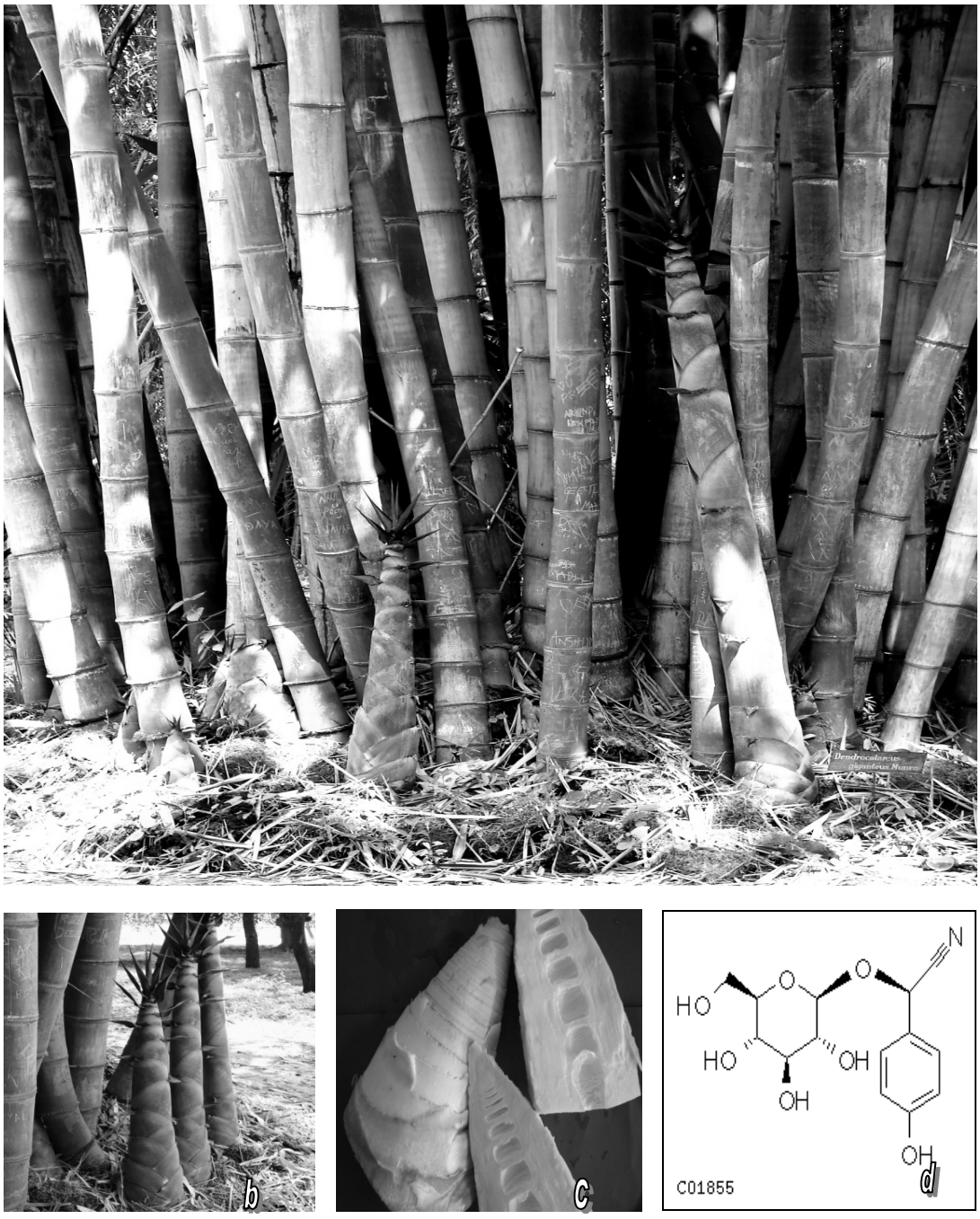

Fig.1. a. Plant morphology of *Dendrocalamus giganteus*; b. Emerging shoots; c. Sliced shoots; d. Taxiphyllin.

Table 1. Total cyanide content in mg/kg of one week old fresh bamboo shoots.

Portion of Shoot / Species	Tip (mg/kg)	Middle (mg/kg)	Base (mg/kg)
Bambusa balcooa	1006	811	304
B. bambos	859	371	223
B. tulda	746	455	331
Dendrocalamus giganteus	1164	974	857

Table 2. Total cyanide content in mg/kg of two weeks old fresh bamboo shoots.

Portion of Shoot / Species	Tip (mg/kg)	Middle (mg/kg)	Base (mg/kg)
B. balcooa	659	890	293
B. bambos	504	678	171
B. tulda	564	629	302
D. giganteus	943	1012	380

Table 3. Total cyanide content in mg/kg of three weeks old fresh bamboo shoots.

Portion of Shoot / Species	Tip (mg/kg)	Middle (mg/kg)	Base (mg/kg)
B. balcooa	445	944	188
B. bambos	422	997	139
B. tulda	391	1061	280
D. giganteus	911	1132	281

Table 4. Some of the main food sources of cyanogenic glucosides and their estimated potential yield of hydrogen cyanide released on hydrolysis

Food source	Cyanogenic glucoside	Hydrogen cyanide yield (mg/100 g fresh weight)
Almond bitter seed	Amygdalin	290
Apricot kernel	Amygdalin	60
Bamboo stem (unripe)	Taxiphyllin	300
Bamboo sprout tops (unripe)	Taxiphyllin	800
Cassava tuber bark (less toxic clones)	Linamarin and Lotaustralin	69
Cassava inner bark (very toxic clones)	Linamarin and Lotaustralin	7
Cassava tuber bark (very toxic clones)	Linamarin and Lotaustralin	84
Cassava inner tuber (very toxic clones)	Linamarin and Lotaustralin	33
Flax seedling tops	Linamarin, Linustatin, and Neolinustatin	91
Black Lima bean, Puerto Rico (mature seed)	Linamarin	400
Peach kernel	Prunasin	160
Sorghum shoot tips	Dhurrin	240
Wild cherry leaves	Amygdalin	90-360

(adapted from: Frehner et al.. 1990, Plant Physiology, 94, 28-34)

In-vitro Organogenesis and Simultaneous Formation of Shoots and Roots from Callus in *Dendrocalamus asper*

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Abstract

Dendrocalamus asper, popularly known as sweet bamboo, is an edible bamboo having high international trade potential. Direct axillary shoot and simultaneous formation of shoot and root from callus regenerated from mature nodal explants was, obtained on MS medium supplemented with different concentrations of BAP and, 2, 4 -D in combination with BAP and with different auxins IAA, IBA and NAA. Highest frequency of axillary shoots regenerated in medium containing 7.0 mgl^{-1} BAP. Effect of 2,4- D in combination with BAP, IAA,IBA and NAA on organogenesis was studied. When the medium was supplemented with 1mgl^{-1} each of 2,4-D+BAP, 2.4-D+IAA+NAA and 2,4- D+IAA+NAA+IBA, explants developed callus which subsequently produced shoots and roots simultaneously in the same medium after 6 weeks. These further developed into plantlets and were transferred successfully to living soil. To the best of our knowledge, this is the first report of simultaneous development of shoot and root from callus in *D. asper* and is a novel technique for propagation of bamboos wherein culture initiation and root induction is the main constraint.

Keywords: Shoot-regeneration, callus, organogenesis, *Dendrocalamus asper*, micropagation, bamboo.

Abbreviations: BAP-6-benzylaminopurine, 2,4-D- 2,4-dichlorophenoxyacetic acid,IAA-indole-3-acetic acid, IBA-indole-3-butyric acid, MS-Murashige and Skoog medium, NAA -1-naphthalene acetic acid

Introduction

Dendrocalamus asper is a densely tufted sympodial bamboo and plays a major role in both shoot and timber production. In addition to being used as raw material for pulp in the paper industry, it is one of the most favored edible bamboo as its shoots are tender and sweet. Though native to South East Asia, it has been introduced in many countries due to its high economic value, especially the shoots. Due to its high shoot quality, it has tremendous demand in the international market and the species is being over exploited leading to dwindling of the natural resources. *In-vitro* propagation techniques can provide alternative means for rapid propagation of this economically important bamboo species.

For bamboo propagation, different techniques are available such as seeds, clump division, rhizomes and culm cutting (Banik 1994, 1995). But these methods suffer from serious drawbacks for large or mass scale

propagation. Micropropagation offers a rapid means of producing plant stock and overcome the draw backs of conventional propagation (Gielis 1999). *In-vitro* culture has been successful for various bamboo species using different explants *viz* seeds, seedling, embryo, stem, and leaves (Rao et al. 1990; Zamora 1994). Regeneration of *D. asper in vitro* has been achieved through induction of multiple shoots and somatic embryogenesis by using different explants seeds, and *i*n vitro shoots (Arya and Arya 1997, 2002; Arya et al. 1999, 2008a, 2008b). Organogenesis has been reported in *Bambusa multiplex, Bambusa nutans Dendrocalamus giganteus, Phyllostachys nigra* (Huang et al. 1989; Ramanayake and Wanniarachchi 2003; Kalia et al. 2004; and Ogita 2005).However, till date, there has been no report of shoots and roots being formed directly from the callus. The present paper describes for the first time regeneration of shoot and root from the callus of *D. asper* by using nodal explants.

Materials and methods

Initiation of aseptic culture

Single nodal segments (3-4 cm in length) with unsprouted bud were collected from secondary and tertiary branches of bamboo plants grown in Botanical Garden Panjab University, Chandigarh, India.. The outer prophyllus covering the axillary bud were removed and the explants swabbed with 70% alcohol, washed with fresh soapy water of surgical hand wash - chlorohexidine gluconate solution, for 10 min. Explants were treated with solution containing bavistin (0.5% w/v), streptocyclin (0.1% w/v) and rifampicin (0.1% w/v) for 10 min, followed by treatment with streptocyclin and rifampicin(0.5% w/v) and ciprofloxacin (0.25% w/v) for 2 min. The explants were then surface sterilized with 70% alcohol for 1 min followed by immersion in 0.1% mercuric chloride for 5 min. After sterilization, explants were rinsed 5 times with autoclaved distilled water. Explants were inoculated vertically to jars containing half strength Murashige and Skoog (1962) basal medium (MS) supplemented with sucrose (3% w/v), and agar (0.8% w/v) for bud sprouting and screening for any contamination.

Shoot multiplication

Sprouted buds with 3 shoots (Figure 1A) were transferred to MS medium supplemented with 3% sucrose 3.5 and 7 mgl^{-1} BAP for shoot proliferation. The elongated single shoots of about 3-4 cm in length were severed and small segments (5-6 mm) were transferred to MS medium supplemented with 1-4 mgl^{-1} 2, 4-D, BAP, NAA, IBA and IAA in different combinations to induce callus and organogenesis (Figure 1 E,F). pH was adjusted to 5.8, agar added (0. 8% w/v) and the medium autoclaved at 121^{o}C and 1.5 kg/cm^3 for (20 min). Cultures were kept in growth chamber at (35^{o}C ± 2^{o}C, 16 hr light: 70 ± 5 µmol m^{-2} s^{-1} and 8 h. dark).

Rooting

A cluster of 4-8 shoots were separated and transferred to root induction medium. The shoot cluster were placed on liquid MS medium supplemented with 1-3 mgl^{-1} NAA and in combination with 1mgl^{-1}IAA. Percentage of rooted propagules, numbers, and length or roots was noted after 4 weeks in culture. Plantlet derived from callus and rooted shoot, were transferred to a mixture of soil, fine sand and peat moss (1:1:1). Humidity and light

conditions were controlled. After an acclimatization period, a cluster of 3-4 the plants were re-potted into polythene bags.

Statistical analysis

All the experiments were laid out in completely randomized design with five replications of a set of five, 25 explants were used. Induction percentage is expressed as the average percentage of explants that developed into callus, shoots or roots divided by the total number of explants used. Counts of shoots per explants and multiplication rate was presented as the mean ± standard error. The data was subjected to statistical analysis to analyse 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

Micropropagation by development of axillary buds.

The axillary buds sprouted within 10-14 days and grew to 3-4 cm in length within 3 weeks. Number of axillary buds increased after transferring the sprouted nodal explants to solid MS medium supplemented with different concentrations of BAP. The shoot proliferation response varied depending on the concentration of BAP (Table 1 and Figure 1B,C). A maximum of 91.66 shoots were obtained within 12 weeks on medium containing 7.0 mgl^{-1} BAP. Number of shoots and multiplication rate is higher than that advocated by Arya and Arya (1997, 2002) wherein they obtained 65 shoots using 3mgl^{-1} BAP. Subsequently, a stock of actively proliferating shoots were maintained in medium containing 7 mgl^{-1} BAP using clumps of 3-5 shoots.

Rooting of shoots

The effect of different concentrations of NAA singly or in combination with IAA on number, length and root formation *in vitro* was studied (Table-2). Roots emerged from shoots within 2 – 3 weeks on MS medium supplemented with NAA or in combination with IAA. When NAA is used singly, roots are induced only in 60% of shoots. The medium with NAA 3 mgl^{-1} + IAA 1 mgl^{-1} was most effective as roots developed in 80% shoots (Table 2, Figure 1D). Also, root number and root length was maximum in this combination (Table 2).

According to Shirgaurka et al. (1996), bamboo species vary widely in their ability to develop roots, during vegetative propagation by classical or *in vitro* methods. Axillary shoots of *D. asper* and *Bambusa bambos* rooted well in the presence of NAA and IBA in the rooting medium (Arya and Arya 1997; Arya and Sharma 1998; Arya et al. 2002). *B. tulda* and *D. giganteus* required coumarin in addition to auxin in the medium to induce roots (Saxena 1990; Ramanayake and Yakandawala 1997). Ramanyake et al. (2006) working on *B. vulgaris*, achieved a high degree of shoot proliferation with BAP in the medium . The shoots were subsequently transferred to the rooting medium supplemented with 3- 10 mgl^{-1} IBA Roots were formed but the frequency was very low compared to rooting in shoots pretreated with TDZ for 2-3 subcultures. These findings show the variability of requirements for roots induction in some bamboo species. The auxin IAA were used in

combination with IBA and coumarin for rooting of *in vitro* grown shoots (Saxena and Bhojwani 1991). Sanjay et al. (2004) found that treatment of culm cuttings with IAA increased the roots numbers.

Micropropagation via organogenesis.

Table 3 indicates the effect of 2, 4-D in combination with BAP or with different auxins (IAA, IBA, NAA) on organogenesis . When 1-5 mgl^{-1} of 2,4-D was used singly, callus was formed but there was no further growth. Of all the growth regulator combinations used, a combination of $1mgl^{-1}$ 2,4-D+BAP, 2,4-D+IAA+NAA and 2,4-D+IAA+NAA +IBA each resulted in plantlets, where in all the others, only non regenerable callus was formed. In a medium supplemented with both 1 mgl^{-1} of 2, 4-D and BAP, 75% of explants developed callus at the cut ends within 4 – 6 weeks. Callus did not proliferate further in the same medium and 5 – 10 shoots with more then 8 – 10 roots were regenerated (Figure 1F,G,H). When 2,4-D +IAA+NAA ($1mgl^{-1}$) was used in the medium, though there was prolific production of callus, only 25% developed into shoots and roots. These further developed into plantlets and were transferred successfully to soil (Figure 1 I). A high rate of transplantation and plant survival of 95% was obtained. The results indicate that 2,4-D controls the development pathway as in all three instances where plantlets are formed, it is present in the medium. Plantlets raised from axillary shoots and organogenesis were transplanted successfully to soil with 80-90% survival rate (Figure 1J,K).

In earlier work done in bamboos, only shoots have been formed from callus. In *D. hamilltonii*, Sood et al. (1994) reported development of friable and compact-nodular callus in a combination of 2,4-D and BAP. The later formed shoot buds as well as embryoids. It is known that the identity of induced tissues in *in vitro* system is driven by the ratio of auxins and cytokinins. The different quantitative requirements for auxin and cytokinin in order to induce various tissues in culture is probably part due to different endogenous concentrations of these hormones within explants. Transfer of tissue explants to medium with higher levels of auxin induce development of root regenerative tissues, whereas transfer of explants to medium with higher levels of cytokinin induce new shoot regenerative tissues and inductive media containing both auxin and cytokinin induce proliferation of callus (Gordon et al. 2008). Many studies have confirmed that auxins like 2,4-D activate auxin response factors and auxin signal pathways and regulate genes related to growth and development (Tao et al. 2002; Yazawa et al. 2004, Che et al. 2006; Piscke et al. 2006). In *D.asper*, organogenesis via callus takes place when 2,4-D is used in combination with BAP, IAA, NAA and IBA. There is simultaneous development of shoots and roots from callus in low concentrations of growth hormones. Che et al. (2008) have reported that callus follow three basic developmental programs – somatic embryo development, shoot organ differentiation and mixed development pathway with both somatic embryogenesis and shoot organogenesis. Probably, a fourth developmental program exists wherein both shoots and roots develop simultaneously from the callus as has been seen in our present work in *D. asper*. This strengthens the suggestion that there exists a diversity of developmental programs in cultured explants. Gordon et al. (2008) have characterized early patterning during *de novo* development of *Arabidopsis* shoot meristem using fluorescent reporters of known gene and protein activities required for shoot meristem development and maintenance. Some progenitor cells have been identified that initiate development of new shoot meristems. Such progenitor cells could also be present for development of new shoot meristems. In the present work, both shoots and roots are formed from the callus in a medium supplemented with 2,4-D in combination with BAP, IAA, NAA and IBA. The combination of $1mgl^{-1}$ 2,4-D and BAP is most suitable for inducing both shoots and roots and further plantlet development. This is a novel

technique and would be of immense help in large scale propagation of *D.asper* and if applicable, to other bamboo species also, wherein culture initiation and root induction is the main constraint.

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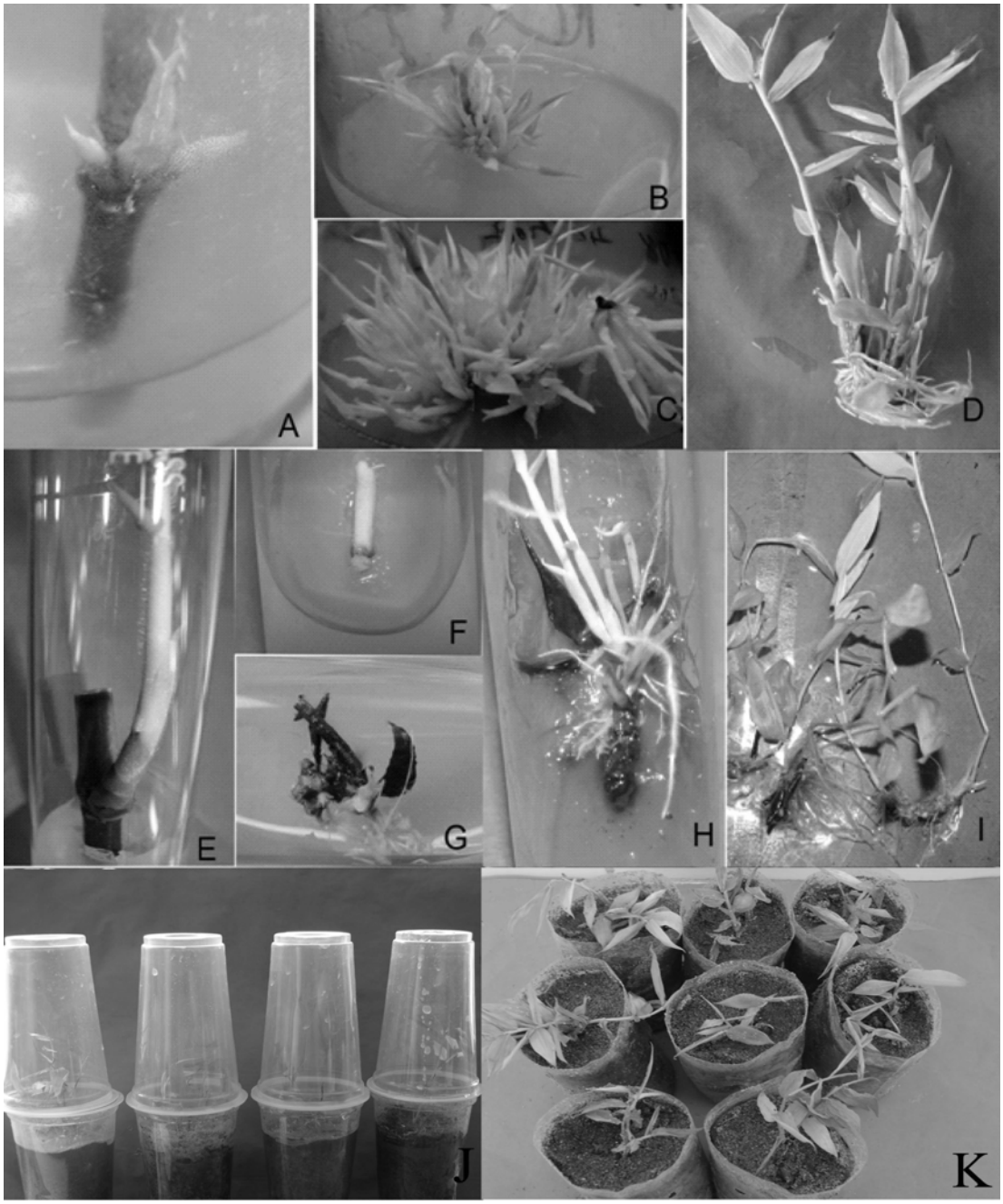

Fig. 1. A,E Bud sprouting in MS 1/2 medium containing 3% sucrose. B,C Direct regeneration of axillary shoots from nodal explants in a cluster of 3 shoots. D Rooted plant obtained through axillary shoots. F Callus induction at the cut end of nodal explants. G Shoot and root regeneration directly from the callus. H Indirect shoots developing to plantlet form. I 8-12 weeks old recovery plantlet. J Plantlet hardening. K Plants after 3-4 months

Table 1 – Effect of BAP concentration in MS medium on shoots growth after 12 weeks.

BAP Conc. mg/l	Shoot Numbers	Multiplication rate
0	Nil	Nil
3	65.00 ± 5.00^{a}	21.66 ± 0.28^{b}
5	75.66 ± 4.04^{a}	25.22 ± 0.34^{b}
7	91.66 ± 7.63^{a}	30.55 ± 0.39^{b}

Value suffixed in the rows with same letters are not significantly different with each other at < 0.05 probability level.

Table 2 – Effect of auxin on root formation of *in vitro* shoots.

NAA(mg/l)	IAA (mg/l)	Rooting (%)	No. of Roots	Root Length(cm)
0	0	0	0	0
1	0	0	0	0
2	0	20	2-3	1- 4
3	0	60	2-4	3 – 5
1	1	20	1-3	2 – 3
2	1	50	3-5	2 -4
3	1	80	8-9	4-7

Table 3 – Effect of auxins and cytokinin on regeneration.

MS +PGR (Plant growth regulators)					Phase I %	Phase II %	Results
2,4-D mg/l	BAP mg/l	IAA mg/l	NAA mg/l	IBA mg/l			
1	1	0	0	0	75% callus	75% shoots + roots	Plantlets
2	1	0	0	0	80% callus	23% roots	Roots
3	1	0	0	0	80% callus	60% callus	Callus
4	1	0	0	0	100% callus	40% callus	Callus
1	0	1	1	0	100% callus	25% shoots + Roots	Plantlets
2	0	2	2	0	100% callus	20% roots	Roots + callus
3	0	3	3	0	100% callus	100% dead brown callus	dead brown callus
4	0	4	4	0	100% callus	100% dead brown callus	dead brown callus
1	0	1	1	1	100% callus	40% shoots + roots	Plantlets
2	0	2	2	2	100% callus	100% callus	20% roots
3	0	3	3	3	100% callus	100% callus	callus
4	0	4	4	4	100% callus	100% callus	callus
5	0	5	5	5	100% callus	100% callus	callus

Micropropagation and Evaluation of Growth Performance of the Selected Industrially Important Bamboo Species in Southern India

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Abstract

This paper deals with micropropagation and field evaluation of six industrially important bamboo species viz; *Bambusa balcooa, B. bambos, Dendrocalamus asper, D strictus, D. stocksii* and *Guadua angustifolia*. Maximum multiple shoot induction was observed from the nodal shoot segments in MS liquid medium as compared to agar gelled medium with additives (ascorbic acid 50 mg/l + citric acid 25 mg/l + cysteine 25 mg/l) + NAA (0.1 -0.25 mg/l) + BAP 1.0 – 2.5 mg/l. Number of shoots per explant varied (3-8 shoots/explant) with the species. Further multiplication of shoots was the best in fresh MS liquid medium with additives + NAA (0.1-0.25 mg/l) + BAP (1.0 – 2.5 mg/l). In case of *D. asper* and *D. stricuts,* MS liquid medium with additives + NAA (0.25mg/l) + TDZ (0.25mg/l) proved the best for shoot multiplication. Optimum requirement of cytokinin varied with the species. Shoot multiplication rate varied from 2.5 – 5.0 fold in 4 weeks period. In most of the species, MS/4 basal salts medium with IBA/NAA (1.0 – 2.0 mg/l) proved the best for rooting. Hardening was found essential for 4-5 weeks.

Multilocational field trials of micropropagated plants of five species viz; *B. balcooa, B. bambos, D. asper, D. strictus* and *D. stocksii* were established in Karnataka and Andhra Pradesh during July-August, 2005. Field trials of *D. asper* and *G. angustifolia* were established in Karnataka, Andhra Pradesh and Kerala during 2005-2006. Initial survival rate of five bamboo species varied from 81-100%. Maximum survival rate was in *D. strictus* and minimum in *D. asper.* At the age of 40 months, overall growth in terms of culm height and diameter were maximum in *B. balcooa* and minimum in *D. asper.* In another trial, the initial survival rate varied from 89 to 100 per cent for *D. asper* and 81 to 100 per cent for *G. angustifolia.* At the age of 40 months, survival rate was minimum in both the species under semi arid condition as compared to high rainfall tropical humid condition. Maximum culm height and diameter were found in *G. angustifolia* in high rainfall area in Karnataka. Details of micropropagation protocols and results of the field performance are discussed in this paper.

Keywords: Micropropagation, industrially important bamboo species, multilocational trials, growth performance, Southern India.

Introduction

India is rich in genetic resource of bamboo. There are 125 indigenous and 10 exotic species in the country, which constitute 12.5% of forest area. National Mission on Bamboo Application (NMBA), National Bamboo Mission (NBM) and Department of Biotechnology (DBT) Govt. of India are promoting cultivation and improvement of 15 industrially important bamboo species in the country (Swarup and Gambhir, 2008). India is second after China in bamboo resource and having 125 species in 23 genera spread over an 8.96 million hectare area in 21 states and union territories, which is 12.8% of total forest area (Anonymous, 2003). Genetic diversity between and within bamboo species provide scope for the selection of the species and best genotypes for commercial cultivation. Apart from indigenous bamboo species, exotic species like; *D. asper*, *G. angustifolia, Phyllostachys bambusoides* and *P. pubescens* are fast growing and industrially important species have high potential for commercial cultivation in India. Demand of bamboo is estimated to 26.6 million tones and supply is only 13.47 million tones per year (Anonymous, 2003). New uses of bamboo, particularly as a substitute of wood, housing sector and value added product will increase further demand of bamboo (Pandey, 2008). Currently, India is importing timber approximately rupees 10,000 crore annually, which can be partly prevented by use of bamboo as a substitute of commercial timber. The Institute of Wood Science and Technology, Bangalore has pursued research work on bamboo on propagation, improvement and cultivation for the past one decade. With the support of NMBA, NBM and DBT Government of India, several projects are ongoing on development/refinement of protocols for macro and micropropagation, production of quality planting material, establishment of germplasm bank, field evaluation and development of agroforestry models of industrially important bamboo species.

Most of the industrially important bamboo species have long flowering cycle (30 to 120 year) which is a limiting factor for planting programme, in addition, sporadic flowering is uncertain, and short viability of seeds further restrict the availability of seeds as and when required for planting programmes. Vegetative propagation through offset cutting, rhizome splitting, culm and branch cuttings are successful in bamboo species (Banik, 1994; Seethalakshmi *et al*, 2008). Most of the classical techniques of vegetative propagation are low cost infrastructures based but are useful for small scale production of clonal planting material (Nautiyal *et al.,* 2008). For mass scale propagation (>50,000 plants/year) classical techniques are largely insufficient and inefficient and tissue culture is the only reliable method (Geilis *et al.,* 2002).

Most of the studies on micropropagation of bamboo species are based on seedling explants either through axillary shoot proliferation or somatic embryogenesis (Rao *et al.,* 1985; Saxena, 1990; Chambers *et al.,* 1991; Sood *et al.,* 1992; Woods *et al.,* 1995; Chang *et al.,* 1995; Arya *et al.,* 1999). Success rate of micropropagation of bamboo species from mature clumps have been reported with limited success rate (Hassan and Debeg 1987; Huang *et al.,* 1989; Prutpongse and Gavinlertvatana 1992; Chatuvedi *et al.,* 1993; Ravikumar *et al*., 1998; Lin and Chang, 1998; Bag *et al.,* 2000; Sanjay *et al.,* 2005; Victor *et al.,* 2006).

The present paper deals with micropropagation and evaluation of growth performance of *Bambusa balcooa, B. bambos, Dendrocalamus asper, D strictus* and *D. stocksii* in Karnataka and Andhra Pradesh and two exotics viz; *D. asper* and *G. angustifolia* in semi arid and high rainfall area in Karnataka and Kerala.

Materials and Methods

Nodal shoot segments of 2.0 -2.5 cm in length were used as a explant. Explants were surface sterilized with 70% ethanol for 30-45 seconds, followed by 0.075-0.1% Mercuric chloride for 4-5 minutes depending on the explants. Explants were washed 6-7 times with sterile distilled water. Culture tubes of 25X150 mm were used for the shoot initiation. Each culture tube consisted single explant.

Shoot initiation

In case of Dendrocalamus asper, D. stocksii and G. angustifolia, explants were used from field grown mature culms, where as, in case of B. bambos and D. strictus explants were collected from seedlings. MS medium (Murashige and Skoog, 1962) liquid and agar gelled with additives ascorbic acid (50 mg/l), citric acid (25 mg/l) and cysteine (25 mg/l) were tested with auxins (IBA and NAA 0.1-0.25 mg/l) and cytokinins (BAP 1.0-5.0 mg/l and TDZ 0.1-1.0 mg/l) to standardize the best combination and concentration of auxin and cytokinin. Cultures were incubated at 28 + 2oC temperature and 2500 lux intensity of light for 12 hours photo period.

Shoot multiplication

In vitro differentiated shoots were subcultured on fresh MS liquid medium with additives, NAA (0.1-0.25 mg/l), Kn (1.0-5.0 mg/l), BAP (1.0-5.0 mg/l) and TDZ (0.1-0.5 mg/l) either alone or in combination for shoot multiplication. Subculturing for further shoot multiplication was carried out within 2 weeks on fresh MS liquid medium. For comparison MS liquid and agar gelled media were tested for shoot multiplication. Shoot multiplication cultures were incubated at 28 + 2oC temperature and 2500 lux intensity of light for 12 hour photo period.

Rooting

Shoot clumps (2-3 shoots/clump) were used for in vitro rooting. MS/2 and MS/4 basal salt media with various auxins viz., IAA, IBA, NAA and NOA (0.5-2.5 mg/l) were tested for high frequency rooting. Initial 2-3 days cultures were kept in dark conditions, followed by under light condition at 28 + 2oC temperature and 2500 lux intensity of light for 12 h photo period for early rooting.

Hardening

In vitro regenerated plantlets of B. bambos, B. nutans, D. strictus D. stocksii and G. angustifolia were transplanted in containers (polybags of 600 cc) consisted of sand, soil and compost in the ratio of 4:2:4. In vitro raised plants were kept in polyglobules for 3 weeks. Inside polyglobule humidity was about 90% and temperature was 30+3oc. Plants were kept in shade for 2 weeks before keeping in open nursery.

Field evaluation

Micropropagated plants of B. balcooa were outsourced from Growmore Biotech, Hosur, where as the rest of the species were raised at IWST, Bangalore. Field trials of industrially important five species viz., Bambusa

balcooa, B. bambos, Dendrocalamus asper, D. strictus and D. stocksii were established at three locations (Nallal, near Bangalore, Yelawala near Mysore and Dullapally near Hyderabad) during July-September, 2005. In another trial, micropropagated plants of D. asper and G. angustifolia were used for the field trials at Aluva and Palakkad (Kerala); Thithimathi (Coorg, Karnataka) (falling under high rainfall tropical humid zone); Nallal, near Bangalore, Yelawala near Mysore and Dullapally near Hyderabad (falling under semiarid conditions) during 2005-2006. The experiments were laid in Randomized Block Design at an spacing of 5x5 m. Pits of 1 cum were made by employing mechanical jaw cum bulldozers (JCB). Periodical watering, weeding and soil working were carried out as and when required. Growth parameters such as, survival rate, culm height, culm number and culm diameter was recorded at periodical intervals. Data pertaining to survival per cent was subjected to arc-sine transformation and two way analysis of variance (ANOVA) (Jayaraman, 2001) was carried out for all the growth parameters and critical difference ($p<0.01$) value was estimated to know the significant difference.

Results and Discussion

Shoot initiation

Optimum requirement of PGR's varied with the species. MS liquid medium with additives + NAA 0.1 mg/l + BAP 2.5 mg/l favored multiple shoot induction in B. bambos and D. strictus. Where as, medium consisted NAA 0.25 mg/l + BAP 2.5 mg/l favoured rapid multiple shoot induction in D. stocksii (Figure 1) and NAA 0.25 mg/l + BAP 5.0 mg/l in G. angustifolia. In case of D. asper, medium with NAA 0.25 mg/l + TDZ 0.25 mg/l proved the best for multiple shoot induction and growth.

Shoot multiplication

Shoot clumps (3-4 shoots/clump) were essential for further shoot multiplication. MS liquid medium proved better than agar gelled medium for shoot multiplication and growth. Shoot multiplication rate improved after 4-5 passage of subculturing on shoot multiplication medium. Optimum requirement of PGR's also varied with species. MS liquid medium with additives + NAA 0.1 mg/l + BAP 1.0 mg/l favoured shoot multiplication and growth in B. bambos. Where as, medium consisted NAA 0.25 mg/l + BAP 2.5 mg/l proved suitable for shoot multiplication and growth in D. stocksii. G. angustifolia shoots multiplied the best in MS liquid medium with NAA 0.25 mg/l + BAP 5.0 mg/l. Medium with NAA 0.25 mg/l with TDZ (0.25 mg/l) favoured shoot multiplication in D. asper and D. strictus (Figure 2). Subculturing was found essential within 2 weeks medium to maintain growth and vigour. Shoot multiplication rate varied from 2.5-5 fold depending on the species. Minimum shoot multiplication was in G. angustifolia and maximum in D. asper.

Rooting

Shoot clump of 2-3 shoots were found to be ideal for rooting. Requirement of type of auxins and its concentration varied with the species. MS/4 basal salts agar gelled medium with NAA 1.0 mg/l favoured high rate (>95%) in B. bambos (Figure 3), D. stocksii and G. angustifolia (Figure 5). In case of D. asper, IBA 2.0

mg/l proved the best (100%) (Figure 4). Where as in D. strictus combined use of IBA (2.0 mg/l) + Kn / BAP (0.5 mg/l) favoured 60% rooting.

Hardening

Plantlets with 5-7 cm of shoot length with 2-3 roots proved ideal for hardening. Keeping plants in polyglobules in mist chamber for 3-4 weeks, favoured high rate of survival (>90%) in all the species. New growth of shoots and roots initiated in the 3rd week of hardening. Plantlets of 3-4 tillers with shoot length of 25-30 cm attained within 4-5 months period after hardening (Figure 6-8).

Fig 1 to 8. Micropropagation of the selected bamboo species; 1) Multiple shoot induction in *D. stocksii,* 2) Shoot multiplication of *D. strictus* in liquid medium, 3) Rooted shoots of *D. asper*, 4) Rooted shoots of *B. bambos,* 5) Rooted shoots of *G. angustifolia,* 6) Hardening of micropropagated plants of *D. asper*, 7) Hardened plants of *D. asper* and 8) Hardened plants of *D. stocksii.*

Field performance

In the first trial, the initial survival of micropropagated plants of five bamboo species in three different locations varied from 81 to 100 per cent (Figure 9). Maximum survival rate was in *D. strictus* (Bangalore) and minimum in *D. asper* (Mysore and Hyderabad). The results after 40 months showed statistically significant difference ($p<0.01$) for all the growth parameters (survival rate, culm height, culm number and culm diameter) between species and location. However, the interaction between location and species was not significant for all the growth parameters (Table 1).

Highest survival rate was found in *D. strictus* in all the three locations (100% in Bangalore, 96% Mysore and 90% in Hyderabad) and minimum in *B. balcooa* at Bangalore (85%). In case of Hyderabad, least survival rate was recorded in *D. stocksii* (54%). The results did not reveal any significant difference between the sites. *B. balcooa* culm height was significantly superior from other species in all the three location (4.34 m in Bangalore, 3.92 m in Mysore and 3.38 m in Hyderabad) (Figure 11) and was minimum in *D. asper* (1.83 m in Bangalore, 1.75 m in Mysore and 1.42 m in Hyderabad). However, culm number showed inverse pattern with maximum culm numbers in *D. asper* (15.00 in Bangalore, 11.75 in Mysore and 10.87 in Hyderabad), which was significantly superior from other bamboo species and minimum culm number was in case of *B. balcooa* (6.77 in Bangalore, 4.63 in Mysore and 4.10 in Hyderabad) in all the three locations. Culm diameter also showed similar trend as culm height (Table 1)

In another field experiment, the initial survival rate of two non indigenous species varied 89 to 100 per cent for *D. asper* and 81 to 100 per cent for *G. angustifolia* (Figure 10). The performance after 40 months of planting showed significant difference ($p<0.01$) for all the growth parameters at species level, locations wise and also the interaction between latter two levels (Table 2). The results on survival rate of *D. asper* was maximum in Kodagu location (100%) followed by Alwaye (83.5%). The per cent survival in latter two tropical humid locations was significantly different from other four semi arid areas. Least survival was observed in Hyderabad (54%). The growth parameter such as height, culm number and culm diameter was highest in Kodagu (6.83 m, 45 and 32.1 mm respectively) (Figure 12) and was least in Hyderabad (1.42 m, 7.9 and 8.5 m respectively). The values of all the growth parameters for Kodagu were significantly better from semiarid conditions such as Bangalore (Figure 13 and 14), Mysore (Karnataka), and Hyderabad (Andhra Pradesh).

The results on another exotic Columbian bamboo (*G. angustifolia*) showed similar field performance as like in *D. asper*. Where, Kodagu location showed significant higher values for survival rate (100%), culm height (13.89 m), culm number (27.3) and culm diameter (56.1 mm) compare to other locations. Minimum values for survival rate (10%) was recorded in Mysore and for rest of the growth parameters such as culm height (0.43 m), culm number (3.3) and culm diameter (7.9 mm) were low in Hyderabad trial, respectively.

The result after 40 months on overall field performance of five different bamboo species in first experiment indicated that the performance of *B. balcooa* under rain fed semiarid condition was superior. Though, the survival rate and culm number was found to be minimum, with respect to culm height and culm diameter, the result showed best performance. And also, the results showed similar kind of performance among the species in all the three locations. Another significant observation was that, the performance of local species such as *B. bamboos, D. strictus* and *D. stocksii* showed equal field performance to *B. balcooa*. But from the point of

industrial requirement criteria's such as, culm straightness, culm diameter, easy harvesting and culm wall thickness, *B. balcooa* is desirable and considered to be promising species under rain fed semiarid conditions.

Similarly, the important finding of the second experiment is that the performance of two exotic bamboo species was found to be better in Kodagu and Alwaye. The best performance in these two locations could be due to favourable locality factors and which falls under tropical humid conditions compare to other four locations, which fall under semiarid type of zones. Hence, the result showed significant interaction between location and species. The performance from the present findings clearly indicated that the two exotic bamboo species adopted well and performed better under tropical humid with high rainfall and soil factors than semiarid conditions.

Figure 9. Initial survival rate of micropropagated plants of the selected five bamboo species in three locations in southern India under semiarid condition.

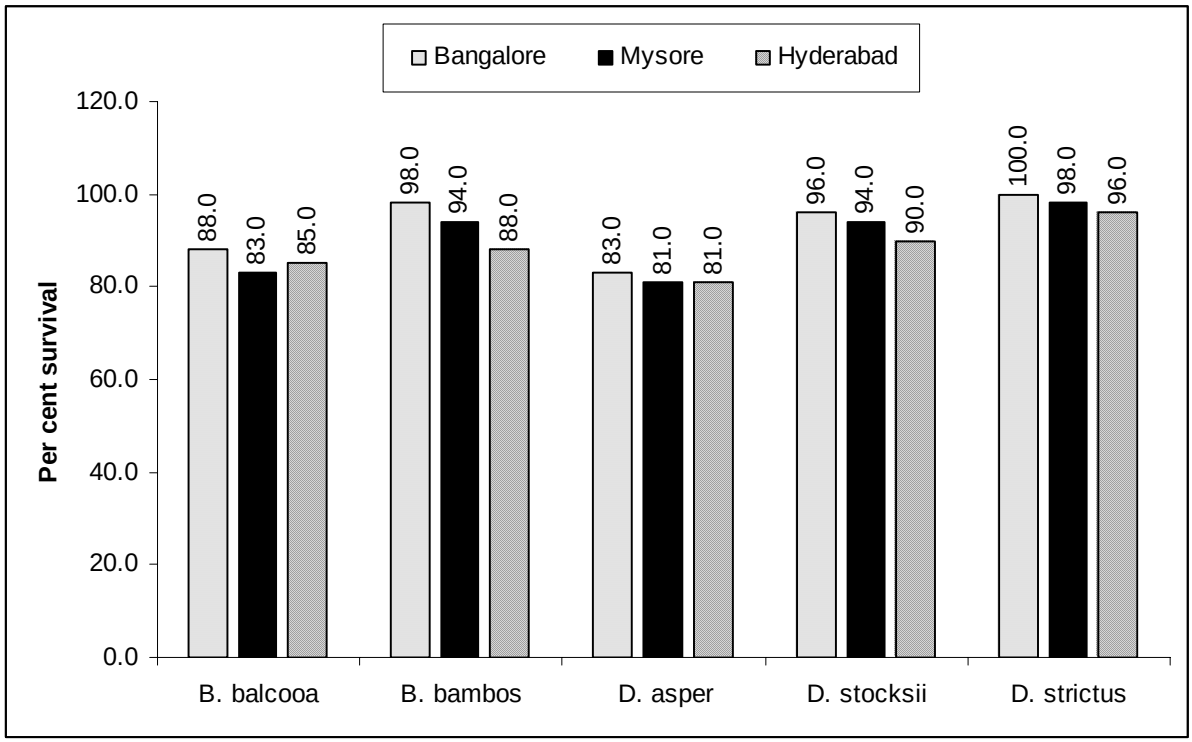

Table 1. Growth performance of micropropagated plants of the selected five bamboo species in three locations in southern India under semiarid condition after 40 months of planting.

Source	Species	Survival %	Height (m)	Culm No.	DBH (mm)
Bangalore	***B. balcooa***	85.00 (69.0)	4.34[a]	6.77[c]	22.2[a]
	B. bambos	98.00 (85.1)	3.70[b]	9.25[bc]	14.1[b]
	D. asper	98.00 (85.1)	1.83[c]	15.00[a]	8.1[c]
	D. stocksii	96.00 (83.1)	3.56[b]	9.38[bc]	12.6[bc]
	D. strictus	100.00 (90.0)	3.98[ab]	9.75[b]	16.0[b]
	F - test	**NS**	**<0.01**	**<0.01**	**<0.01**
Mysore	***B. balcooa***	58.00 (50.3)[b]	3.92[a]	4.63[c]	18.1[a]
	B. bambos	81.00 (68.6)[ab]	3.17[b]	7.00[b]	12.4[b]
	D. asper	59.00 (51.9)[b]	1.75[c]	11.75[a]	10.2[b]
	D. stocksii	76.00 (65.2)[ab]	3.20[b]	7.13[b]	13.1[b]
	D. strictus	96.00 (83.1)[a]	3.39[b]	8.38[b]	14.6[ab]
	F - test	**<0.01**	**<0.01**	**<0.01**	**<0.01**
Hyderabad	***B. balcooa***	78.00 (62.1)	3.38[a]	4.10[c]	16.3[a]
	B. bambos	72.00 (58.2)	2.85[b]	7.20[b]	10.8[b]
	D. asper	74.00 (59.6)	1.42[c]	10.87[a]	7.6[b]
	D. stocksii	54.00 (47.4)	2.69[b]	6.82[b]	10.2[b]
	D. strictus	90.00 (74.5)	2.97[ab]	8.16[b]	12.4[ab]
	F - test	**NS**	**<0.01**	**<0.01**	**<0.01**
Location X Species		**NS**	**NS**	**NS**	**NS**
SE		**10.32**	**0.15**	**0.98**	**1.81**

* Values in parentheses are arc-sine transformed. Values with the same superscripts in a column do not vary significantly and means compared column-wise; NS = not significant and SE = standard error.

Figure 10. Initial survival rate of micropropagated plants of the selected two exotic bamboo species in six locations in southern India.

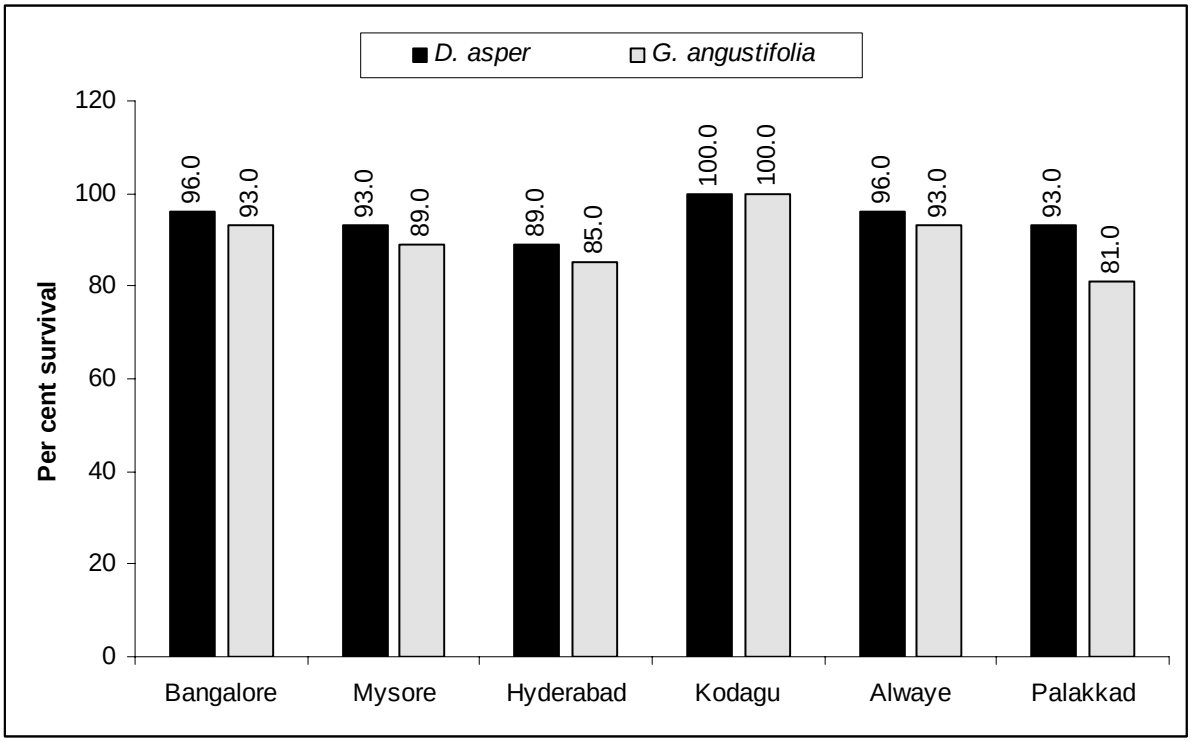

Table 2. Growth performance of micropropagated plants of the selected two exotic bamboo species in six locations in southern India after 40 months of planting.

Source	Species	Survival %		Height (m)	Culm No.	DBH (mm)
	Bangalore	91.00	$(72.7)^{bc}$	2.12^{d}	16.3^{d}	9.0^{d}
	Mysore	60.00	$(50.6)^{de}$	2.23^{d}	15.3^{d}	11.6^{c}
	Hyderabad	54.00	$(47.1)^{e}$	1.42^{e}	7.9^{e}	8.5^{d}
D. asper	**Kodagu**	100.00	$(90.0)^{a}$	6.83^{a}	45.0^{a}	32.1^{a}
	Alwaye	96.00	$(83.5)^{ab}$	5.75^{b}	18.9^{c}	27.5^{b}
	Palakkad	80.00	$(63.7)^{cd}$	3.20^{c}	31.8^{b}	12.6^{c}
	F - test	**<0.01**		**<0.01**	**<0.01**	**<0.01**
	Bangalore	55.00	$(48.1)^{c}$	2.79^{c}	11.4^{b}	14.8^{c}
	Mysore	10.00	$(18.8)^{d}$	1.25^{e}	8.0^{b}	9.0^{d}
	Hyderabad	41.00	$(39.6)^{c}$	0.43^{f}	3.3^{d}	7.9^{d}
G. angustifolia	**Kodagu**	100.00	$(90.0)^{a}$	13.89^{a}	27.3^{a}	56.1^{a}
	Alwaye	90.00	$(75.2)^{b}$	7.70^{b}	11.3^{b}	40.4^{b}
	Palakkad	15.00	$(22.3)^{d}$	2.10^{d}	11.7^{b}	15.9^{c}
	F - test	**<0.01**		**<0.01**	**<0.01**	**<0.01**
Location X Species		**<0.01**		**<0.01**	**<0.01**	**<0.01**
SE		**10.32**		**0.15**	**0.98**	**1.81**

* Values in parentheses are arc-sine transformed. Values with the same superscripts in a column do not vary significantly and means compared column-wise; NS = not significant and SE = standard error.

Fig 11 to 14. Field trials of micropropagated plants of selected bamboo species; 11) Over view of micropropagated plants of five bamboo species trial at the age of 40 months at Bangalore, Karnataka, 12) Over view of *G. angustifolia* (left) and *D. asper* (right) trial at the age of 40 months in Kodagu, Karnataka, 13) Over view of *G. angustifolia* at the age of 40 months in Bangalore, Karnataka and 14) Over view of *D. asper* trial at the age of 40 months in Bangalore, Karnataka

Conclusion

Optimum requirement of auxin and cytokinin vary with the species for shoot initiation, multiplication and rooting. Multiplication rate also varied with species. Large scale production of clonal planting material of *D. asper*, *D. stocksii* and *G. angustifolia* can be carried out from the explants collected from field grown mature clump. Where as, in case of *D. strictus* shoot necrosis and comparatively low rate of rooting (about 60%) from the shoots of mature clump. Comparatively, *B. bambos*, *D. asper* and *G. angustifolia* are easy to root than other species tested.

Field evaluation of five micropropagated bamboo species viz., *B. balcooa, B. bambos, D. asper, D. stocksii, and D. strictus* revealed that, the *B. balcooa* is most suitable species, followed by *D. strictus* based on the survival rate and growth performance in all the three locations. Based on the 40 months performance, it is concluded that *D. asper* is not suitable for semiarid conditions. The micropropagated plants of *B. bambos, B. balcooa, D. stocksii* and *D. strictus* can be used for establishment of plantations in the localities similar to the study sites. The results on field performance of two exotic species such as *D. asper* and *G. angustifolia* indicated the best performance under high rain fall areas than semiarid conditions. The results clearly indicate that, these two exotic species can be used for large scale plantation only in high rainfall tropical humid climatic zones with good deep loamy soils.

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Identifying new *Fargesia* Introductions and Predicting their Cold Tolerance using AFLP markers

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Abstract

New collections of bamboos from Sichuan and Yunnan have enriched the sortment of ornamental clumping bamboos in horticultural trade in Western Europe and North America. Various of these bamboos have been under study by collectors for almost two decades and some species have proven a success in the market. However, a lot of uncertainty remains about precise identity of these clones and their placement in genera and species. In order to resolve at least some of these issues, AFLP™ was used. The main result is that bamboos group according to geography, with a clearly delineated *Fargesia* group from Sichuan, and a group comprising *Fargesia/Borinda/Yushania* from Yunnan. As a corollary AFLP™ markers can be used to predict winterhardiness and growth performance.

New *Fargesia* bamboos in the horticultural trade

Up to the late eighties only a few types of bamboo could be found in gardens or the horticultural trade. One century earlier, over 100 different bamboos were found in European collections, following the first introduction of *Phyllostachys nigra* (Black Bamboo) in 1827. The turbulent twentieth century however, led to the destruction of many collections. In botanic gardens more types survived of course, but in Western Europe one could find only about four types of bamboo which were more or less widely distributed in gardens. These were two clumping types, namely *Fargesia murieliae* (Umbrella Bamboo) and *Fargesia nitida* (Fountain Bamboo), and two types with running rhizomes (although they behave more or less reasonably in our climate conditions), namely *Phyllostachys aurea* (Golden Bamboo) and *Pseudosasa japonica* (Arrow Bamboo).

The large scale monocarpic flowering of *Fargesia murieliae* led to the death of millions of plants in the eighties and nineties. All these plants originated from one original mother plant, collected by Ernest Wilson in the early twentieth century. Its history is a fascinating one, and during the past eighty years, the species has been subject of various name changes. During the twentieth century, *Fargesia murieliae* became one of the most popular garden bamboos, but all plants died during the flowering (Gielis et al., 1999). This flowering started in the seventies in Denmark, and peaked in the mid nineties. Also in another popular garden bamboo, *Fargesia nitida* (fountain bamboo) flowering has started in the early nineties of last century. Contrary to *Fargesia murieliae*,

Fountain Bamboo is not descending from a single plant, but is a collection of types regenerated from seeds collected by Berezowski in 1894.

Large scale flowering has prompted collectors to search for bamboos which could replace Umbrella and Fountain bamboos, mainly in SW China, where the original *Fargesia*'s were selected. These expeditions by various collectors as well as introductions from botanical gardens in China has led to a variety of new introductions in Europe and the US such as *F. denudata, F. dracocephala* (Figure 1)*, F. rufa* and *F.* 'Jiuzhaighou'. Many of these collections have been under observation for over 10 years in Europe now, and some recent articles by Jos Van der Palen (see Publications at www.kimmei.com) give extensive descriptions of the best of these new introductions.

Many of these are still under observation with collectors, but a few such as Jiuzhaighou, *F. scabrida, F. robusta* (Figure 1) and *F. rufa* are already very successful in trade. *Fargesia rufa* has been introduced at large scale since 2000, with a Silver Medal in Boskoop, and in Germany *Fargesia* Jiuzaighou and *Fargesia scabrida* have been named Bamboo of the Year by the bamboo society EBS-Germany, in 2006 and 2008 respectively.

Identifying and placing the new Fargesia where they belong

Since time immemorial, bamboo has been a real challenge to taxonomist, and has been very cumbersome in many cases. The Southwest Chinese bamboos, growing in colder regions of Gansu and Sichuan are very hard and comprise the 'core' *Fargesia*'s. *F. murieliae* and *F. nitida* were collected there, and also the more recent *F. rufa* and *robusta*, among others were collected there. The problems arises when one goes more south, towards Yunnan; here one finds bamboos which are less frost resistant, and which have a taller stature and more open growth. This has caused confusion and, although no clearcut distinction could be made, many bamboos were transferred provisionally to *Borinda* (Stapleton, 2009). Even more south, one will find subtropical species, some of which tend to have long but very tender shoots, which rather climb than grow upright. In the whole southwest of China, one will find a large diversity of clumping bamboos.

In the 1980's Chao and Renvoize (1989) grouped all these bamboos into one large clade, the "Sinarundinaria" group. This however, was not satisfactory because it was clear that many of the species and genera were too different from each other to belong to just one genus (Stapleton, 2009). In botany, one has to rely on flowering for classification, but for most of these species, flowering has never been observed. For example in the genus *Fargesia*, only in one third of the species flowers have been described. So taxonomists have had to rely on other characteristics such as the rhizomes, the culms and internodes and the growth habit.

Table 1: Differerence between *Fargesia* and *Borinda* (according to Stapleton), and the current description in the Flora of China (right column)

Characteristic	Borinda	Fargesia	Fargesia (incl. Borinda) in Flora of China
Plant	**Shrub-like to subarboreous, usually densely clumping**	**Shrub-like, densely clumping**	**Small (ca 1 m) to subarborescent (15 m) bamboos.**
Rhizomes	**Pachymorph, necks similar in length,up to 30cm**	**Pachymorph, necks to 30 cm**	**Pachymorph, short relative to culm height 10-30-(50) cm with short neck**
Growth Habit	**In single dense to loose clump (unicaespitose)**	**Unicaespitose**	**Unicaespitose**
Culms	**To 7 m tall and 3,5 m in diameter, erect or curving at base, apically nodding to pendulous**	**To 6 m tall and 2,5 cm in diameter, basally erect, apically nodding to pendulous**	**Basically erect, apically nodding to pendulous; New shoots May-Sept.**
Internodes	**To 50 cm, terete, usually finely ridged, without fine purple spots, usually blue-grey with light persistent wax, becoming glossy**	**To 30 cm, terete, usually smooth and not finely ridged, glabrous, finely purply spotted, rarely with light to dense wax at first, becoming glossy**	**Terete, smooth or finely ridged**
Nodes	**Scarcely to moderately raised**	**Scarcely to moderately raised**	**With level or weakly prominent supranodal ridge, usually narrower than sheath scar**
Branches	**5-7(-14) per mid-culm node at first, above promontory, subequal, initially erect, becoming deflexed, lateral branch axes lacking subtending sheaths**	**5-10 per mid-culm node at first, 7-20 later, often above promontory, subequal, initially erect, becoming deflexed, lateral branch axes lacking subtending sheaths.**	**Branches initially 7-15 per node in mid-culm, above promontory, initially erect becoming deflexed, subequal.**
Buds	**At mid-culm lanceolate, with 2 often very tall, single keeled bracts, open at front, closed at culm base, 3-9 initials visible within**	**At mid-culm ovate to lanceolate, with 2 tall single keeled bracts, dorsally fused in lower culms open at front (closed at culm base), several initials visible**	**Ovoid to lanceolate, branch sheathing reduced**
Culm sheaths	**Usually long-triangular, papery and deciduous (rarely oblong, thickened and persistent)**	**Oblong, shorter than internode, deciduous,**	**Linear, rounded or triangular**
Blades	**Long, reflexed, deciduous**	**Usually reflexed**	**Usually reflexed**
Leaf sheaths	**Usually persistent**	**Persistent**	
Blade	**Usually matt, thin, venation distinctly tesselate, either persistent or deciduous in winter**	**Small to medium sized, usu. glossy and thickened, normally not deciduous, transverse veins prominent**	**Small to medium sized; transverse veins prominent, blades glossy and thickened, or matt and delicate.**

Inflorescence	**(Synflorescence) ebracteate, semelauctant, branching paniculate, erect, never unilateral, ususally long exserted from narrow subtending sheath, often fasciculate in dense panicles**	**(Synflorescence) ebracteate or branches subtended by a series of small persistent delicate sheaths, semelauctant, compressed, branching racemose, unilateral**	**Racemose to paniculate, compressed or open, ebracteate or branches subtended by a series of persistent small delicate sheaths, unilateral or not**
Spikelets	**Several flowered**	**Few flowered**	**Several flowered**

This has led Dr. Chris Stapleton, then at Kew Botanic Gardens, to establish a new genus, *Borinda*. His initial studies were done in Bhutan and Nepal (Stapleton, 1994 a and b), which directly border the South-West of China, but back in England he expanded *Borinda* to include some of the *Fargesia* (Stapleton, 1998). He considers the difference between *Fargesia* and *Borinda* mainly in terms of the open growth of the (known) inflorescences (synflorescences). In colder regions, the true *Fargesia*'s are smaller and the inflorescences are very compact. More to the south similar bamboos grow taller, more open, and the inflorescence is also more open, like a panicle (see Table 1).

In the most recent *Flora of China*, which is a contemporary snapshot of the state of the art in taxonomy of this group, all the *Borinda*'s are still included under *Fargesia*, and molecular work of Chinese taxonomists has not found real evidence for the existence of a separate genus *Borinda* (Guo and Li, 2003). A very recent article (Stapleton et al., 2009) discusses this situation, in particular the state of knowledge in this group of bamboo with molecular markers at the time of publication of the Flora of China (Li et al., 2006).

The new introductions, often made by non-taxonomists, should be carefully named in order not to confuse matters further. Especially since flowering of *F. nitida* and *F. murieliae* clones has also led to the introduction of numerous seedlings under a variety of trade names. Within the group of new seedlings of *Fargesia murieliae* and *F. nitida*, a lot of mixing has taken place in trade, which adds to the confusion about the correct names. In the past we have used ALFP for various purposes, e.g. assessment of genetic stability (Gielis et al., 1997, 2001, 2004). In this study AFLP was used to unequivocally identify bamboos with the aim of specific grouping of genera and species, and additionally the hope of, either confirming the validity of a separate genus *Borinda*, or providing evidence against a seperate genus for pachymorph bamboo with open inflorescences in Yunnan.

AFLP molecular markers for identification

Both for identification and positioning of new introductions, and for the precise identification of the progeny of *F. nitida* and *F. murieliae,* AFLP™ was undertaken. AFLP™ (Amplified Fragment Length Polymorphism in full) is a method of molecular markers, which is probably the most sensitive method to identify plants correctly. In *Phyllostachys* for example, we has been used to distinguish among species and even cultivars (unpublished results). It uses DNA of the plants under study, and compares the precise DNA sequences, allowing to group the plants more closely when their DNA matches most. When the DNA diverts too much between two genotypes, they will be grouped in different groups.

For this analysis we collected material at the garden of Jos Van der Palen (www.kimmei.com) in Valkenswaard, The Netherlands, with main emphasis on the true, very hardy Fargesia's, but also less hardy bamboos from Yunnan were included. These bamboos included species which Chris Stapleton would place under *Borinda* (and these names were also used in Figure 2), and we also included *Yushania* and *Bashania* from the same region, for comparison. Also even less hardy bamboo, from India, Nepal and Bhutan were included, of the genera *Himalayacalamus, Drepanostachyum, Ampelocalamus* and *Thamnocalamus.* As a very distant relative, which is custom in DNA analysis,Thamnocalamus of South Africa was included as well. The *Fargesia murielieae* seedlings Bimbo, Kranich and Mae were included as well.

To obtain good and reproducible AFLP™ patterns, the method of material collection and storage is crucial. Suboptimal treatment or storage of the plant material will lead to DNA degradation and unreliable AFLP™ patterns. The easiest method for use in field situations, and this method is actually yielding very good DNA, is as following: Fresh, young leaves are collected and directly stored in plastic bags containing silica gel (for example Silica gel blue in bags, 1 kg, Fluka, catnr: 93505). To avoid contact between the leaves and the silica, silica in small paper bags is to be used (contact with silica results in low DNA quality!!!). To protect the DNA from degradation, the silica is renewed every day to dry the material as quickly as possible. Silica can be reused after drying it (dry silica is blue, humidity changes the color to pink).

The dried leaf material can be transported at room temperature, but for long term storage, -80º C is preferred. Alternatively, freshly harvested material is immediately frozen in liquid nitrogen and stored on dry ice for transportation to -80º C conditions.

DNA-extraction and AFLP™ analysis

DNA extraction was carried out with DNeasy Plant Mini Kit (Qiagen) according to the manufacturers instructions. The DNA is eluted in a volume of 100 µl.

Aliquots from the DNA preparations were used for AFLP™. AFLP™ analysis was performed essentially as described by Vos *et al.* (1995). Primary template DNA was prepared in a restriction-ligation reaction. Genomic DNA (250 ng) was digested with 2,5 U of *Eco*RI (Invitrogen) and 2,5 U of *Mse*I (Invitrogen) for 2 h at 37 °C in a final volume of 25 µl containing 10 mM MgOAc, 10 mM Tris-HCl buffer (pH 7,5) and 50 mM KOAc. An *Eco*RI- and a *Mse*I-adaptor, designed to avoid the reconstruction of the restriction sites, were ligated to the restriction fragments by adding 24 µl of a mix containing 5 pmol *Eco*RI-adaptor (Invitrogen), 50 pmol *Mse*I-adaptor (Invitrogen), 10 mM ATP, 10 mM Tris-HCl, 10 mM MgOAc, 50 mM KOAc and 1 µl T4 DNA-ligase (1U) (Invitrogen) and keeping for 2 h at 37 °C. The resulting primary template was diluted to 100 µl with 10 mM Tris-HCl (pH 8,0) and 0,1 mM EDTA. AFLP fingerprints were generated using a two-step PCR amplification. The first step, a preamplification, was performed with primers complementary to the *Eco*RI- and *Mse*I-adaptors with one additional selective 3' nucleotide. The PCR reactions were performed in a 50 µl volume of 5 µl primary template, 25 ng of each primer (Invitrogen), 1 U *Taq* DNA Polymerase (Applied Biosystems), 0,2 mM of each dNTP, 10 mM Tris-HCl (pH 8,3), 50 mM KCl and 1,5 mM $MgCl_2$. The PCR amplifications were carried out in a Gene Amp PCR system 9600 (Perkin Elmer) using 20 cycles, each cycle consisting of 30 s

at 94 °C, 60 s at 56 °C and 60 s at 72 °C. The preamplification products were diluted tenfold in 10 mM Tris-HCl (pH 8,0) and 0,1 mM EDTA and used as template in the fluorescent selective amplification.

The selective PCR amplification was performed using an *Eco*RI-primer tagged with a fluorescent dye and an *Mse*I-primer. The primer combinations *Eco*RI-GCT/*Mse*I-ACTA, and *Eco*RI-GCT/*Mse*I-ACTT were used. The primers contained the same sequences as those used in the preamplification but with three or four selective nucleotides at the 3' end instead of one. The reactions were carried out in a total volume of 20 µl consisting of 3 µl diluted preamplification product (1/10 of their initial concentration), 1 µl *Mse*I primer (Invitrogen) at 5 µM, 1 µl *Eco*RI primer (MWG Biotech) at 1 µM, 1 U *Taq* DNA polymerase (Applied Biosystems), 2 µl 10 x PCR Buffer (Applied Biosystems) and 0.2 µl of dNTP's (20 mM each)(Amersham Biosciences). A GeneAmp PCR system 9600 (Perkin Elmer) was used, according to the following parameters: 1 cycle of 2 min at 94 °C, 30s at 65 °C, 2 min at 72 °C, followed by 8 cycles in which the annealing temperature decreases 1 °C per cycle and the denaturation was carried out at 94°C for 1 s, followed by 23 cycles of 1 s at 94 °C, 30s at 56 °C and 2 min at 72 °C. The sample were kept at –20°C until further analysis.

The AFLP-reaction mixtures were loaded on a 3130 Genetic Analyzer (Applied Biosystems) for fragment separation according to the manufactures instructions. GeneScan-500 Rox labelled size standard (Applied Biosystems) was loaded in each lane in order to allow the automatic sizing of the DNA fragments using GeneMapper 3.7 (Applied Biosystems). The results were submitted for manual size correction. The database presents all the markers in the sample along with the marker name (e.g. GCT-ACTA-350 for the marker of 350 bp obtained after selective PCR with primers *Eco*RI-GCT + *Mse*I-ACTA), the calculated size, the annotated size and the peak height (signal intensity). These data are used to generate in MS Excel a 1/0 matrix representing the presence or absence of each marker (50-450 bp) in each plant. All AFLP data are grouped andavailable in an AFLP database with marker positions, size and height of the peak.

AFLP molecular markers reveal cold tolerance

The results are straightforward and very interesting, as can be seen from Figure 2 and Table 2, with three to four distinct groups. Table 2 lists the hardiness as it is assumed in Western European horticulture (see for example the species list of Jos Van der Palen at www.kimmei.com). The AFLP™ groups coincide precisely with the division into hardiness groups. The "Sichuan" group (Figure 2,blue arrow) is the core *Fargesia* group of very hardy bamboos, of the type *rufa, scabrida, denudata, robusta, dracocephala*, *nitida* and Jiuzaighou. Some of the names, like Wolong and Xian, indeed refer to the places where these bamboos have been collected, which is in very cold Panda region of Sichuan.

The second group is the "Yunnan" group (Figure 2, red arrow) which groups the less hardy Fargesia, like *F. utilis* and *F. angustissima*, and all of the *Borinda*. Also *Yushania* types and *Bashania* group with these *Borinda*'s indicating that geographical speciation has an important role in bamboo taxonomy, apparently even more important than the differences in growth habit, or inflorescence.

This is also clear in the subtropical group "Himalaya" (green and yellow), where no clear distinction can be made between the various genera. Also here, it is geography, rather than anything else, which determines the group. *Thamnocalamus crassinodus* and *T. aristatus*, which clearly form a separate group.

Table 2: Groups according to winter hardiness in Europe

Group	Region	Hardiness	Genera
"Sichuan"	Sichuan, Gansu, Shaanxi	-20 to -25°C	*Fargesia*
"Yunnan"	Yunnan (Sichuan)	-8 to -20°C	*Fargesia, Borinda, Yushania, Bashania*
"Himalaya"	India, Bhutan, Nepal, Tibet	0 to -8°C	*Himalayacalamus, Drepanostachyum, Ampelocalamus, Thamnocalamus*

Fargesia resolves to be a nice clade, with distribution in cold areas (mainly Sichuan). The problematic second group consists of species of *Fargesia, Borinda, Yushania* and *Bashania fangiana.* Many of the Yunnan *Fargesia species* were transferred to *Borinda* by Stapleton, although in the Flora of China (2006) *Borinda* is included in *Fargesia.* So far, molecular markers have not been able to resolve these issues. However, when he published the genus, Stapleton (1994a and b) pointed out that *Borinda* inflorescences are similar to those of *Yushania*. The species were not transferred to *Yushania* however, because *Yushania* has usually been interpreted as a genus of spreading bamboos with long, running rhizomes while species placed in *Borinda* are all clump-forming with short rhizomes (Stapleton, 2009).

In our study it becomes clear that biogeographical considerations seem to be more important than morphological differences. Characteristics considered special to *Borinda*, such as ridged culms might have other functions, e.g. adding extra strenght to taller culms. With this grouping of *Fargesia/Borinda, Yushania* and *Bashania fangiana* into one large "Yunnan" group, it might be considered to recognize *Fargesia* in Sichuan as a separate genus, and one large clade from Yunnan, perhaps as *Yunarundinaria* in analogy to *Sinarundinaria* of Chao & Renvoize (1989), although this will obviously lead to new questions and difficulties, for example in the vicinity of separated biogeographical zones. This will be discussed in more depth in a forthcoming paper.

The professionalisation of bamboo cultivation and trade in horticulture

The production and trade of bamboo in horticulture is increasing rapidly, and sales are rising year after year, despite problems related to flowering and dying of bamboo in the past. In present times, bamboo has become an interesting tradename. The market for ornamental bamboos in Europe has increased by a factor of ten compared to the early nineties, and an estimated 5 million bamboos are produced and sold today. In the US the market is slowly emerging, in part because the market structure for ornamentals differs considerably from Europe.

A shift is also taking place however in species in trade, in production and distribution channels. More and more growers produce bamboo in larger numbers. In addition a shift towards clumping bamboos is observed. At Oprins Plant NV, the main production of *Fargesia* is through tissue culture, and the annual production is over half a million per year for this group alone. In addition, marketing and distribution has been completely changed for bamboo, compared to one decade ago. Then plants were offered sporadically at high prices, with limited availability. Today, millions of bamboo plants find their way to the market through different distribution channels. Also marketing bamboo has been revised completely: From plants with confusing latin names, today bamboos are marketed and sold under trade names like Asian Wonder, Great Wall, Green Panda and Green Screen, and under brand names like BambooSelect (www.bambooselect.com) with clear information on planting and control.

As bamboo production and trade is becoming more and more professionalised, also the quality needs to be carefully monitored, not only throughout the production and sales chain (Figure 3), but there is a clear need to identify bamboos unequivocally, to avoid any of the confusion which has plagued the sector in the past. We have applied AFLP™ since 1997 to identify genotypes, to assess genetic stability of our propagatin methods and to aid in understanding taxonomy (Gielis et al., 1997, 2001, 2004).

With molecular markers like AFLP we can resolve some important problems. It is not only possible to clearly identify bamboos and place them, but we can even predict their winterhardiness, and to some extent, how they will grow, and their overall appearance. Our results can also aid in resolving taxonomic questions and difficulties in this fascinating group of bamboos, or proposing new solutions. In any case, from a horticultural point of view we see no need to change names and genera from *Fargesia* to *Borinda* as the latter genus has no support from molecular studies, neither studies focusing on phylogeny (Guo and Li, 2003), nor from this study.

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Figure 1: Hedge of *F. robusta* Pingwu (left) and new shoots of *F. dracocephala*

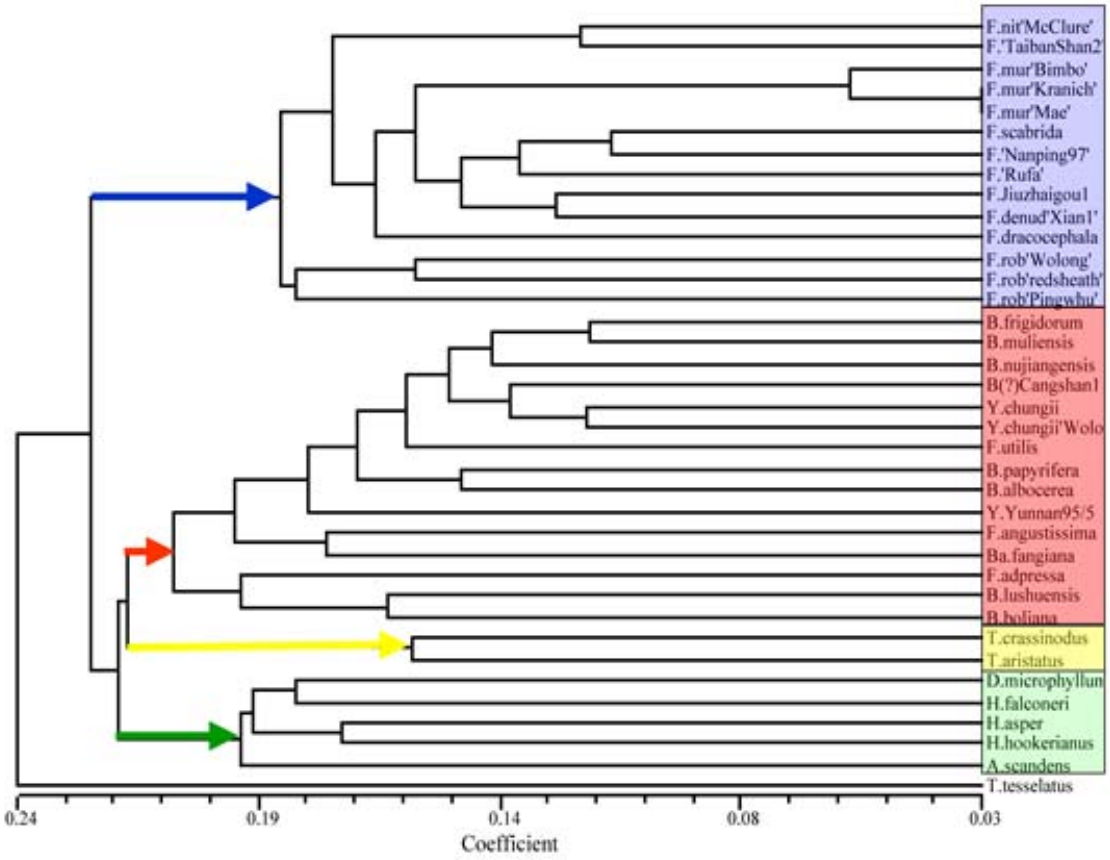

Figure 2: Dendrogram based on AFLP data

Figure 3: Large scale *Fargesia* production at Oprins Plant NV, Belgium

Allelopathic Effect of *Parthenium hysterophorus* L. on Germination and Growth of Seedlings of *Bambusa bambos* (L) Voss and *Dendrocalamus strictus* Nees

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Abstract

The allelopathic effect of *Parthenium hysterophorus* on *Bambusa bambos* (L) Voss and *Dendrocalamus strictus* Nees was investigated with water soluble extracts. The experiment was carried out under laboratory condition, using the aqueous extracts of *Parthenium hysterophorus* to determine its effect on seed germination, shoot and root length of 12 day-old seedlings over a different range of extract concentrations. The aqueous extract at 6-10% concentrations significantly inhibited seed germination and seedling growth of both the bamboo species, and the degree of inhibition increased with the incremental extract concentration. The inhibitory effect was much pronounced in root and lateral root development as compared to germination and shoot development of the test species. Number of roots increased with increasing concentrations of the extract. However the length of root was greatly reduced at high concentration. Formation of lateral roots and root hairs was inhibited in the extract treated seedlings. In 2% aqueous extract (100%) relative germination ratio (RGR) was observed in *Dendrocalamus strictus* while (61.1%) RGR occurred in 10% aqueous extract. Reduction in Vigour Index (VI) was noted in all the cases in comparison to control.

Keywords: Allelopathy, aqueous extracts, *Bambusa bambos*, *Dendrocalamus strictus*, Germination, *Parthenium hysterophorus*, Seedling length.

Introduction

Bamboos are one of the most versatile groups of plants with peculiar flowering behavior such that most of them flower at the end of a long vegetative phase which is followed by death of the culms. After flowering, germination and seedling establishment are the most vulnerable period in the life cycle of bamboos. Seedling mortality is often high with seedlings being unable to tolerate environmental fluctuations (biotic, abiotic) easily endured by mature plants. Bamboo is enduring versatitle and highly renewable resource with multifarious impact on environment and ecology. As bamboo can tolerate diverse soil and moisture regime, it has the ability to stitch and repair degraded areas, conserve soil and moisture and act as drought proof material. Its foliage shelter topsoil from the onslaught of tropical downpours while its leaf litters (up to 10cm in a year) also cushions the soil from the impact of rain and eases the soil's absorption and retention of moisture. Bamboo

preserves many exposed areas, providing micro-climates for forest regeneration and watershed protection (the plants vast underground rhizome network may cover upto 100m around one bamboo clump). It is known to be hardy, light and flexible and sought for its nutritional and environmental value, all these features impart bamboos a unique quality of resilience required for sustainable growth and development of any resource. Bamboo should be promoted as an important component of agro-forestry system and as an agri-horticultural crop as it is amenable to annual harvesting particularly edible shoots.

Parthenium hysterophorus L (Family:Asteracea; common names: Bitter weed, Parthenium weed, Ragweed, white top, etc; vernacular names: Kanike ghans, Bethughans or Padke phul) is an annual, erect and profusely branched herb. *Parthenium hysterophorus* appears to be potentially harmful weed that affect the growth and development of many crop plants. Present study reports the potential of *Parthenium hysterophorus,* the effect of its aqueous extracts on seed germination and seedling growth of two bamboo species, *Bambusa bambos* and *Dendrocalamus strictus* considered as important timber yielding plants in India. The study can be further assessed for understanding the interactions between plants like bamboos that possess high growth potential and weeds like Parthenium which can cause severe damage to the existing flora of an area due to their allelopathic effects.

Material and Methods

Plant Materials

The *Parthenium hysterophorus* L. plants growing naturally in the Delhi University campus were uprooted and collected during the month of August, 2007, sun dried, crushed and stored at room temperature (30±2°C).

Preparation of aqueous extract of the weed

Aqueous extracts were prepared by soaking 10g of crushed plant material in 100ml of sterilized distilled water at room temperature for 24 h followed by filtration through Whatman filter paper No.1 and the final volume was made up to 100ml, making a 10% aqueous extract. The extract was considered as stock solution and a series of solution with different strengths (2, 4, 6 and 8%) were prepared by further dilution of the stock solution. Twenty uniform and surface sterilized seeds (0.045% $HgCl_2$ for 5 seconds) of two commonly used varieties of bamboos, *Dendrocalamus strictus* and *Bambusa bambos* were germinated in sterilized 9.0 cm Petri dishes lined with blotting paper and moistened with five milliliter of different concentrations of aqueous extracts. Each treatment had three replicates (total number of test seeds: 20x3=60). One treatment was run as control with sterile distilled water only. The Petri dishes were maintained under laboratory conditions at the temperature 25±2°C.

Determination of seed germination and seedling growth

The emergence of plumule and radicle was considered as the criteria for seed germination. The results were determined by counting the number of germinated seeds which was recorded daily beginning from the first day of germination up to ten days and expressed as per cent seed germination. Number of lateral roots was recorded

and the length of primary root and main shoot were a measured. Ratio of germination and elongation were calculated as suggested by Rho and Kil (1986).

$$\text{Relative germination ratio (RGR)} = \frac{\text{Germination ratio of tested plants}}{\text{Germination ratio of control}} \text{ x } 100$$

$$\text{Relative elongation ratio (RER) of shoot} = \frac{\text{Mean length of shoot of tested plant}}{\text{Mean length of control}} \text{ x } 100$$

$$\text{Relative elongation ratio (RER) of root} = \frac{\text{Mean length of root of tested plant}}{\text{Mean length of control}} \text{ x } 100$$

The response index (RI) was calculated according to Williamson and Richardson (1988):

Germination of treatment (T) was higher than control (C)

RI= 1-(C/T)

When germination of treatment (T) was lower than control (C)

RI= (T/C)-1

If RI>1= Treatment stimulates germination

If RI=0 Effect of treatment is nil

If RI<1= Treatment inhibits germination

Statistical Analysis: Significance of the difference in root and shoot length of seedlings under different treatments were tested and compared using Analysis of Variance (ANOVA). All statistical analysis was done using Statistical Package for Social Sciences (SPSS version 11.5, 2002).

Results

Germination

In control, seeds germinated within 72h. Whereas in seeds treated with equal amount of different concentrations of the aqueous extract of *Parthenium hysterophorus,* the rate of germintion varies from three days to six days. In 2% concentration, seeds germinated after three days while in 10% concentration it took place after five days of treatment. As compared to the control, the aqueous extract of *Parthenium hysterophorus* exhibited significant ($P<0.05$) inhibition on seed germination. With the increase in concentration of aqueous extract, the inhibitory effect increased progressively. In both the test species maximum inhibitory effect was found in 10%

concentration. Maximum (100%) relative germination ratio (RGR) was found in *Dendrocalamus strictus* at 2% concentration while minimum (61.1%) RGR in *D. strictus* was observed at 10% concentration (Fig. 1A). *Bambusa bambos* was less sensitive to the application of crude aqueous leaf extracts of *Parthenium hysterophorus* as compared to *D. strictus*.

Seedling growth

Analysis of variance showed significant difference ($P< 0.05$) between treatments in influencing seedlings shoot and root length of the test species. As compared to control, aqueous extracts had deleterious effect on shoot and root length. In control, bamboo seedling showed fully developed leaf with well expanded leaf lamina after two weeks (Fig. 2 A&B). At 2-4% concentration of treatment there was no significant ($P< 0.05$) difference in the length of shoots as compared to the control. However, the shoot length was significantly different from that of control at 6% concentrations of *P.hysterophorus* leaf extract. At 8-10% concentration in both the test species the length of and the formation of leaves got highly suppressed (Table 1). The inhibitory effect of treatments got progressively pronounced with the increase in concentration of the extract. Among the germinated seedlings, the maximum (98.25%) relative elongation ratio of shoot was observed in *D.strictus* followed by *B.bambos* (94.52%) in control treatment while the minimum (46.73%) was in *B.bambos* followed by *D.strictus* (48.16) at 10% concentration of the aqueous extract (Fig. 1B).

ANOVA showed significant difference ($P< 0.05$) between treatments in root length of the test species. In control, bamboo seedling showed formation of primary root from the radicle. The primary roots elongated and formed several lateral roots with numerous root hairs. At 2-4% concentration, there was no significant difference in the length of roots as compared to the control but the primary roots formed less number of lateral roots and root hairs in the treatment. 6% aqueous extract of *P. hysterophorus* leaves cause significant difference in the root length in comparison to control. At 8-10% concentration, in both the test species the length of roots was highly reduced. In 85% of the tested seeds at 6-10% concentrations, there was rudimentary roots formation (three to four small root-like short, dark and thick protrusions appeared at the base of each shoot) and lateral roots and root hairs were completely absent. Maximum (97.24%) and minimum (3.10%) relative elongation ratio (RER) of root were observed in *B.bambos* at control and 10% concentration of the extract treatment, respectively (Fig. 1C).

The vigour index (VI) was also greatly affected by *P.hysterophorus* extract in both the species of bamboo. According to Williamson and Richardson (1988), the values of Response Index (RI) indicates stimulatory (if $RI>1$), inhibitory (if $RI<1$) and no effect (if $RI=0$) on seed germination. In the present study, from 6-10% concentration of the extracts, RI values of *P.hysterophorus* was ($RI<1$) inhibitory in both the varieties, indicating that *P.hysterophorus* extracts exhibited allelopathic effect on seed germination of both the test species.

Discussions

This study demonstrated that aqueous extracts of *Parthenium hysterophorus* exhibited allelopathic activity on bamboo seed germination as well as seedling growth. The degree of inhibition was significantly dependent on

the concentration of the extract being tested. These results indicate that the allelochemicals present in the aqueous extract of *Parthenium hysterophorus* adversely affect seed germination, growth and development of shoot, root, root hairs and lateral roots. The effect was more pronounced at higher concentrations, where the effect was so severe that, the 10% concentration completely inhibited the growth of roots in both the test species. Overall growth of seedlings was also reduced in almost all the treatments as compared to the control as also reported by Donger and Singh (2007). A number of studies have suggested that the degree of germination, shoot and root growth inhibition increased with increasing extract concentration of allelopathic species (Han et al. 2008; Kumar and Gautam 2008; Rai and Triparthi 1984; Rizvi and Rizvi 1987).

In the present study, there was significant inhibition in seed germination at high concentrations of aqueous leaf extracts. Among the treatments, 8% and 10% aqueous extracts had the strongest inhibitory effect on germination. However, complete suppression of seed germination was not observed in both the test species even at the maximum (10%) concentration. It is in contrast with the report of Tefera (2002) who found that 10% aqueous extracts of *Parthenium hysterophorus* resulted in complete failure of seed germination in *Eragostis tef*. The complete inhibition of seed germination could occur only when some allelochemicals present in the leaf extract prevented growth of embryo, or caused the death of embryo (Rajendiram 2005). Maharjan et al. (2007) also reported that 10% aqueous extracts of *Parthenium hysterophorus* completely inhibited seed germination of *Oryza sativa, Triticum aestivum, Ageratina adenophora, Artemisia dubia, Raphanus sativus, Brassica campestris* and *Brassica oleracea*.

From the analysis of variance it was found that the shoot length of both the test species (*B.bambos* and *D.strictus*) at 2-4% concentration was not significantly different from that of the control. Reduction in shoot length was significantly different at 6% and above concentrations. At higher concentration (10%) there was formation of more than one shoot from the embryonal end of the caryopsis of *D.strictus*. However there was complete failure of leaf formation in both the test species and the shoot length was significantly different from the control. Similar effects of leaf extract of *Parthenium hysterophorus* was reported by Maharjan et al. (2007) on some cultivated and wild herbaceous species, Tefera (2002) on *Eragostis tef* and Rajan (1973) on wheat. Singh et al. (2005) also reported a strong positive correlation between extract concentration of residues of *Parthenium hysterophorus* and reduction in seedling length of *Brassica* species.

In the present investigation, roots appeared more sensitive to the allelopathic extracts than shoots. Lateral root development was significantly decreased with increasing concentrations of the extract. This finding is congruent with the results of Amoo et al. (2008) who found that the root length is a more sensitive indicator of phytotoxic activity. The stronger inhibitory effects on roots might have been caused by the fact that roots were in direct contact with the extract and subsequently with inhibitory chemicals (Bhowmik and Doll 1984; Quasem 1995). The reduction in root length may indicate that cell division was affected as allelopathic chemicals have been found to inhibit gibberellin and indole acetic acid function (Tomaszewski and Thimann 1966). Such an outcome might be expected, because it is likely that roots are the first to absorb the allelochemical compounds from the environment (Turk and Tawaha 2002).

Present results showed that the aqueous extract of *Parthenium hysterophorus* inhibited seed germination and seedling growth of *Bambusa bambos* and *Dendrocalamus strictus.* Reduction in shoot length, formation of more

than single shoot and reduction in root formation as observed in the present study corresponds to the function of growth regulator (cytokinin) which is known to induce multiple shoots and reduction in root formation. However, further studies are required to identify and check the levels of its concentration in different parts of this weed. It can be concluded that there are compounds in the leaves of *Parthenium hysterophorus* which may cause allelopathic effects and mimic the effects of growth regulator on the germination and seedling growth of both the test species.

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Table 1. Effect of the aqueous extracts of Parthenium hysterophorus on length of shoot, length of root, number of roots and lateral roots of *Bambusa bambos* and *Dendrocalamus strictus.*

Bambusa bambos

Treatment	Shoot Length	Root Length	No. of roots	No. of lateral roots
0%	4.41±0.16	3.63±0.19	1.1±0.10	11.8±0.72
2%	4.17±0.15	3.57±0.05	1.0±0.14	9.5±0.10
4%	3.95±0.22	3.14±0.31	1.8±0.13	8.2±1.52
6%	3.0±0.09*	0.53±0.12*	2.6±0.13	0.09±0.10*
8%	2.91±0.17*	0.39±0.03*	2.8±0.19	0.03±0.61*
10%	2.51±0.11*	0.35±0.29*	3.2±0.41	0

Dendrocalamus strictus

Treatment	Shoot Length	Root Length	No. of roots	No. of lateral roots
0%	5.07±0.19	3.92±0.40	1.0±0.22	13.5±1.92
2%	4.69±0.21	3.85±0.16	1.30±0.15	11.7±0.30
4%	5.02±0.22	3.53±0.27	1.5±0.52	5.5±0.71
6%	3.99±0.12*	0.99±0.19*	1.8±0.13*	3.9±0.83*
8%	3.45±0.23*	0.79±0.16*	2±0.21*	1.6±2.9*
10%	3.09±1.68*	0.36±0.03*	2.2±0.20*	0.9±0.31*

Values are mean±SE of ten replicates. *indicates significant difference

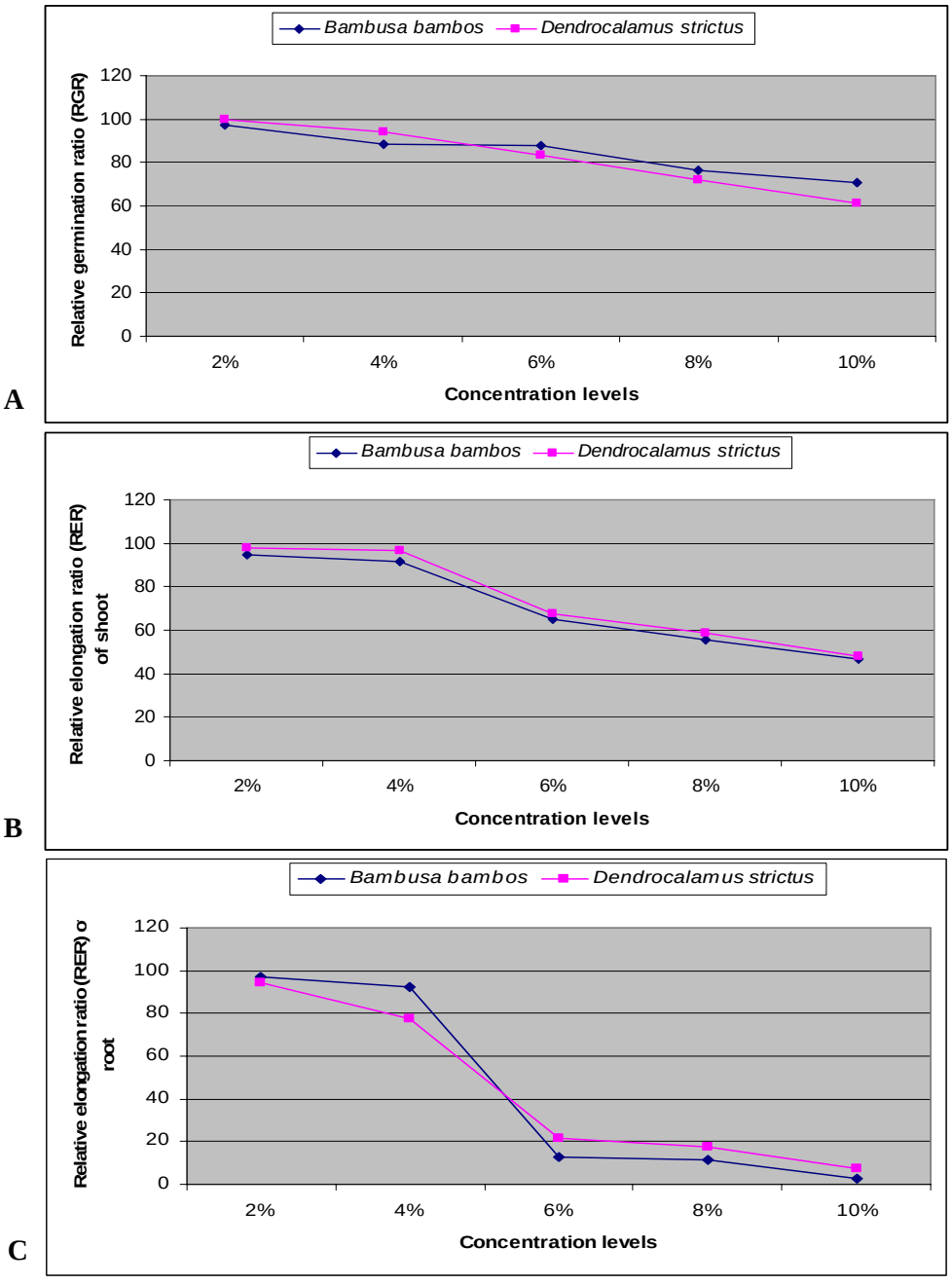

Figure 1. Line graph showing (A) the relative germination ratio (RGR), (B) the relative elongation ratio (RER) of shoot and (C) the relative elongation ratio (RER) of root on bioassay studies of *B.bambos* and *D.strictus* caryopses treated by different concentrations of water soluble extracts of *Parthenium hyterophorus*.

Figure 2. Effect of *Parthenium hysterophorus* plant aqueous extracts on seedling growth of *Bambusa bambos* (A) and *Dendrocalamus strictus* (B)

Exploring the Nutraceutical potential and Food Safety Aspect of Bamboo shoot of Some Indian Species

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Abstract

Young bamboo shoots are extensively eaten as pickle, vegetable or as a fermented product in most of the eastern countries like China, Japan, Thailand etc. The nutraceutical potential of bamboo shoots remains unexplored despite these being rich in dietary fiber and protein content and low in fat percentage. Similarly, cyanogenic toxicity in bamboo shoot - an important aspect related to food safety has not been given sufficient attention. The present study is designed to investigate the functional properties of bamboo shoots harvested from 4 edible species namely *Dendrocalamus strictus, Bambusa tulda, B. vulgaris and B. balcoa* in order to explore their nutracentical potential. Bamboo shoot samples are analyzed for protein, vitamin C, total phenol content and antioxidant activity by DPPH assay and TAA. The protein, Vitamin C & total phenolic content of the shoots ranged between 18.74-25.84g/100g, 5.0-6.6 mg/100g and 153.91-222.81 GAE/g dry powder, respectively. It was found that amongst all the species *B. balcoa* contained highest content of total phenolics (222.81 GAE/g dry powder), crude protein (25.84%) and vitamin C (6.6%). It also possesses highest antioxidant activity by DPPH assay (39.85% free radical scavenging power) and TAA (3.15 mg GAE). Raw fresh shoots of *D. strictus and B. vulgaris* were found to contain 386 and 200 ppm of cynogenic glycoside respectively which can be reduced to permissible limit by suitable processing methods. The results obtained indicate a strong correlation between the nutrient components and antioxidant power in bamboo shoot and its potential as a nutraceutical for prevention of metabolic disorders. Since studies on the antioxidant properties and toxicity related issues of bamboo shoots are scanty, R&D work on preparation of bamboo shoot based nutraceutical formulations as well as recipes after eliminating the toxicity would open new avenues in the area of nutraceuticals.

Introduction

Bamboos a group of giant arbores cent grasses belong to the family Poaceae and sub- family Bambuseae. More than 1250 species belonging to 75 genera have been reported to be distributed worldwide out of which 125 species are growing in India spreading over an area of 9.57 million hectare. India has rich bamboo resources after China. The North Eastern states are endowed with more than 50% of the Indian bamboo genetic resources (Sharma et al 1992)

Application of bamboo in the structural and building materials along with its use in cottage industry is globally recognized. Recently, R&D work on bamboo as a modern engineering material for structural and agricultural applications has been taken up at IIT Delhi. Similarly the edibility of tender shoots has generated a lot of business potential in countries like China, Japan, and Thailand etc. The tender shoots of a few species are consumed either as vegetables or in curries or as pickles. Extensive practice of fermenting shoots is carried out in the North Eastern states of India and other countries since ancient times (Singh et al, 2003). Surprisingly in India despite of several edible species being grown in certain states, potential of bamboo shoot as food has not been explored in areas other than North Eastern Region.

Bamboo shoots contain several nutritional components like protein, carbohydrates, fat, vitamins, minerals, enzymes, coenzymes, reducing and non-reducing sugars, lactic acid and citric acid (fermented products) etc. Bamboo shoot being rich in fiber and protein and low in fat (Yamaguchi M, 1983) may be considered a nutraceutical product. It also contains lignans and phenolic compounds which may contribute to its antimicrobial activity and antioxidant property. Fermented bamboo shoots are an excellent source of phytosterols which are the precursors of pharmaceutically important steroidal products such as sex hormones and oral contraceptives. (Srivastava and Sarangthem, 1994). Recently compounds of nutritional importance like α-tocopherol (0.26 mg/100 g), γ-tocopherol (0.42 mg/100 g), β-carotene (1.9 μg/100g) and lutein (35.6 μg/100g) have been reported in raw bamboo shoot (Kim et al, 2007).

Along with above excellent food and nutritional characterteristics, raw bamboo shoots have been found to contain cyanogenic toxicity. Taxiphyllin (4-hydroxy-(R)-mandelonitrile-b-D-glucopyranoside), a cynogenic glycoside (Conn 1969) has been found to be the potential toxic component. The acute lethal dose of HCN for humans is 0.5-3.5 mg/kg body weight (Jones 1998). But FAO/WHO Codex Alimantarius has defined a safe limit for human consumption which is 10mg HCN equivalent per kg dry weight (FAO 1991). However, when the dose is relatively small, humans are able to detoxify and excrete in the urine. Chronic cyanide poisoning sets in due to lack of nutrients like riboflavin, protein, vitamin $B_{12,}$ sodium & methionine. Chronic sub-lethal dietary cyanide has reportedly caused serious reproductive effects (Food standards Australia 2005).

In view of the above, present study aims at exploring the nutraceutical potential of bamboo shoot of 4 bamboo species namely *Dendrocalamus strictus, Bambusa tulda, B. vulgaris and B. balcoa*. Cyanogenic toxicity in bamboo shoot – an important but overlooked aspect is also studied for food safety. Cyanide and its removal by optimum processing method have been attempted thus reflecting light on the processing methods by which toxicity in bamboo shoots can be eliminated.

Nutrition and Nutraceuticals

The term “Nutraceutical” was coined from “nutrition” and “pharmaceutical” in 1989 (DeFelice 1995). In 1992 De Felice defined Nutraceuticals as any substance that may be considered as food or a part of food and provide medical and health benefits including prevention and treatment of disease. Nutraceuticals may range from isolated nutrients, dietary supplements, herbal products and processed products such as cereals, soups and beverages (DeFelice 1992). Based on the chemical characteristics, nutraceuticals are broadly put up into seven different classes (Wildman 2006) as shown in Figure 1. In order to have a close look at the nutritive value of

bamboo shoot of different species, a brief compilation of reported data has been done (Table 1). It indicates the need of systematic scientific investigation of bamboo shoot of selected species for exploring its nutraceutical potential.

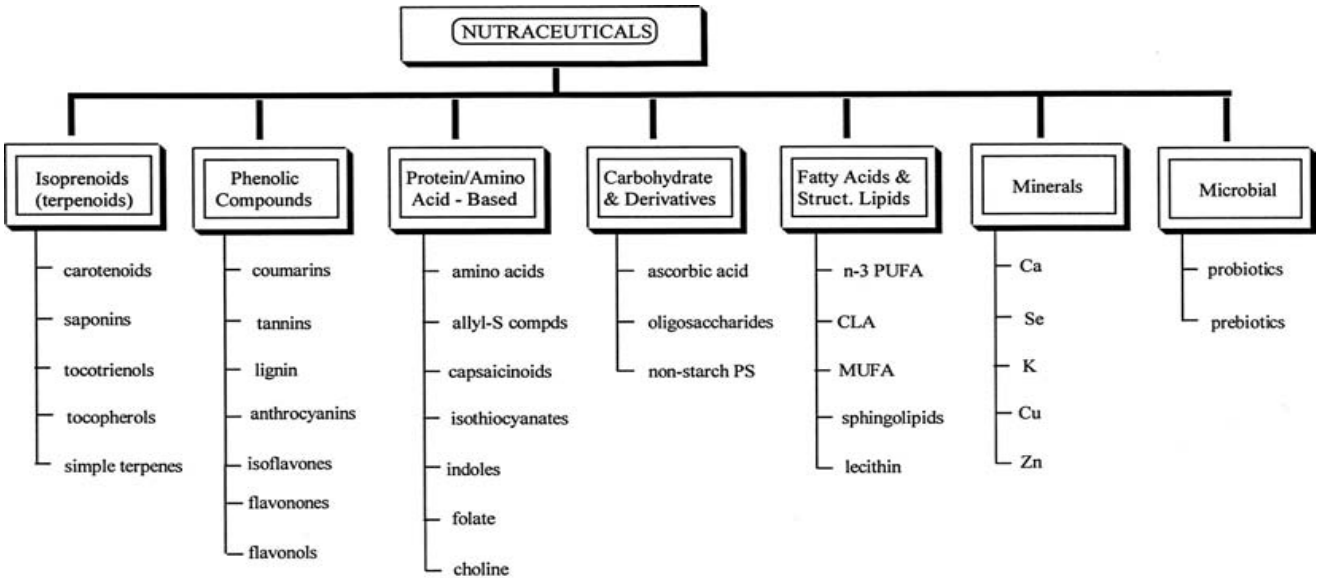

Figure 1: Classification of nutraceuticals

Table 1: Nutritive value of Bamboo Shoot – General Scenario

S.No	Species	Calories (Kcal)	Moisture (%)	Crude Protein (%)	Crude Fat (%)	Carbohydrate (%)	Ash (%)	Crude fiber (%)	References
1	*Bambusa arundinacea*	—	54.0	5.33	—	10.0	—	—	Bhargava et al, 1996
2	Species not mentioned	294	11.6	29.6	0.4	43	8.5	6.9	Rajyalakshmi & Geervani, 1994
3	Species not mentioned	27	91	2.6	0.3	5.2	—	—	Yamaguchi M, 1983
4	*Bambusa bamboos*	--	89.83	3.57	3.55	5.42	1.38	3.535	Nirmala et al, 2007
5	*Bambusa balcoa*	15.5-15.6	84-86.3	3.3-3.87	0.6-1.0	5.2-5.23	3.1	26.4	Bhatt et al, 2003
6	*Dendrocalamus. strictus*	—	—	19.2	—	2.6	0.90	0.98	Kumbhare and Bhargava, 2007

Materials and methods

Collection of raw material (bamboo shoot) and sample preparation:

Bamboo shoots of four species of viz. *Dendrocalamus strictus, Bambusa tulda, B. vulgaris and B. balcoa* were procured from Bamboo Forest TERI Gram, Mandi Village, near Delhi (India) during rainy season. The samples were washed with water and the sheaths were removed in a concentric manner from the base to the top. Nodes and internodes were separated. Internodes were oven dried at 60 ± 2°C for 8-10 hours and finely powdered for further analysis. For toxicity determination the shoots were washed, peeled and cut into rings as above. 200mg of fresh shoots were taken for cyanide analysis. The samples were analyzed in triplicates.

Analysis of bamboo shoot for nutrients

Preparation of methanolic extract

The dried and finely ground shoot samples (0.4g) were extracted with 20 ml methanol in a shaking incubator at 45°C for 2 h. The mixture was centrifuged at 5000 rpm for 10 min and subsequently decanted. The residue was re-extracted for 2 h and supernatants were mixed together. The mixture was concentrated using a rota evaporator and stored at 0°C in freezer until analyzed for total phenolics and antioxidants.

Determination of Total Phenolics (TP)

Amount of TP was assessed using Folin–Ciocalteu reagent procedure as described by Li et al, 2008. 0.5 ml of Folin–Ciocalteu reagent and 7.9 mL deionized water were added to a test tube containing 0.1 mL of methanolic extract of bamboo shoot. The mixture was kept at room temperature for 10 min, and then 1.5 mL of 20 g/100 mL sodium carbonate was added. The mixture was heated on a water bath at 40°C for 20 min and then cooled in an ice bath before absorbance at 755 nm was measured. The results are expressed as gallic acid equivalents (GAE) per g of dry matter.

Determination of Vitamin C

Extract 0.5g of the sample in 4% Oxalic Acid and make up to 100ml and centrifuge. Pipette out 5ml of the supernatant and add 10ml of 4% Oxalic acid and titrate against the dye containing 2,6-dichlorophenol indophenol till a light pink colour appears and persists for a second. Similarly titrate the standard solution containing ascorbic acid of 100µg/ml concentration (Harris and Ray 1935)

Protein estimation

Crude protein was estimated using elemental analyzer (Elementar analysensysteme GmbH, Germany) model Vario EL III, in which 10 mg of dry powder was sealed in a tin boat and introduced into the instrument. The nitrogen value was obtained as percent total nitrogen content and it is factored with 6.25 to get the crude protein content.

Antioxidant analysis

Free radical scavenging activity using 1,1-diphenyl-2-picryl hydrazyl (DPPH)

DPPH, a commercial oxidizing radical is reduced by antioxidants. The disappearance of the DPPH radical absorption at a characteristic wavelength is monitored by decrease in optical density (Singh, Murthy and Jayaprakasha, 2002). To 0.1mL methanolic extract of bamboo shoot, four ml of 60µM methanolic solution of DPPH was added. The tubes were shaken vigorously and allowed to react for 30 min at room temperature in the dark. The control prepared without any sample was used for base line correction. Changes in absorbance of samples were measured at 515 nm. Free radical scavenging activity was expressed as inhibition percentage and was calculated using the following formula.

$$\% \text{ Free radical scavenging activity} = \frac{(\text{Control OD} - \text{Sample OD}) \times 100}{\text{Control OD}}$$

Total Antioxidant Activity (TAA)

The assay was based on the reduction of Mo (VI)-Mo (V) by the extracts and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH (Prieto et al, 1999). 0.1 ml of the methanolic extract of bamboo shoot was combined with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were incubated at 95°C for 90 minutes, after which the mixture was cooled to

room temperature; the absorbance of the solution was measured at 695 nm against blank. The total antioxidant activity is expressed as Gallic acid equivalent.

Determination of Total Cyanide content

Total Cyanide content was determined by the acid hydrolysis method (Rezaul Haque & Howard Bradbury 2002). Fresh bamboo shoot samples (200mg, triplicate) were taken in a stopperd test tubes followed by 20ml of Phosphoric acid (0.1M). The tubes were centrifuged and then 10 ml of the supernatant solution was mixed with 10 ml of H_2SO_4 (4M). The mixture was boiled for 10 minutes and then ice cooled. Further 10 ml of NaOH (3.6M) was added and make up to 25ml in a volumetric flask. Absorbance solution was prepared by using 3 ml of the solution from the flask with little NaOH (0.04M) and 0.5ml acetate buffer. After adding 1ml of Chloramine T, the solution was allowed to stand exactly for 2 minutes. Then 5ml of Pyridine-Barbituric acid reagent was added dilute to 25ml with NaOH (0.04M) and let stand exactly 8 minutes. Measurements were taken at 578nm using UV-Visible spectrometer (PerkinElmer Inc., USA).

Results and discussion

Data of bamboo shoot samples analyzed for protein, vitamin C, total phenol content and the antioxidant activity for different species of bamboo shoots namely *B. balcoa , B. vulgaris, D. strictus and B. tulda* are presented in Table 2. cyanoglucoside content in these samples is also given in the last column.

Table 2: Analysis of bamboo shoot for 4 different species

Bamboo shoot species	Total phenolics (GAE/g dry powder)	DPPH (% free radical activity)	TAA (mg/ml) gallic acid equivalent	Protein (g/100g dry matter)	Vitamin C (mg/100g)	Cyanoglucosides (ppm)
D. strictus	153.91	13.97	1.12	21.51	5.8	386
B. tulda	162.66	15.94	0.88	18.74	5.0	77*
B. vulgaris	191.41	28.21	1.31	20.60	5.0	200
B. balcoa	222.81	39.85	3.15	25.84	6.6	67*

*Bamboo shoot stored at -18°C for 2 months

Nutraceutical Potential

Proteins

The protein content ranged from 18.74 – 25.84 % on dry weight basis, highest being in *B. balcoa* and lowest being in *B. tulda*. Yamaguchi M (1983) had reported protein value of 2.6 g in shoots but investigations made by Kumbhare and Bhargava (2007) gave higher values for crude protein content which ranged from 9.6 to 17.2% on fresh weight basis and 19.2 –25.8% on dry weight basis. Ferriera et al (1992) reported much higher values in the apical and basal portion of *D. giganteus* which were 46.1 and 40.4% respectively. The wide variation in the protein content of bamboo shoots reported in literature may be attributed to differences in species, growing site, climatic factors and method of analysis.

Vitamin C

Ascorbic acid values ranged from 5.0-6.6 % in different species when determined by the titremetric method. *B. tulda* and *B. vulgaris* were found to contain the same amount of ascorbic acid. The results were in accordance with the earlier investigations made by Yamaguchi M (1983) who reported a value of 4%. Vitamin C content reported by Bhargava et al (1996) in a few species was as high as 23%. Bhatt et al (2003) has also reported vitamin C content for a number of bamboo species ranging from 3.0 to 12.9, highest being in *D. hamiltonii* and lowest being in *D. sikkimensis.*

Total phenolics

The most commonly observed and predominant phenolic acids have been reported to be ferulic acid and p-coumaric acid. p-coumaic acid content increases in good accordance with the increase of lignin content while ferulic acid is inversely proportional to the lignin content. It has been observed that the chemical composition varies from top to bottom in immature moso bamboo (Fujii et al 1991). Total phenolics in the present study were present in the range of 153.91-222.81 GAE/g dry powder in different species of bamboo shoots. *B. balcoa* was found to contain the highest content of phenols (222.81GAE/g dry powder). Values obtained were higher than previously reported values in different species of shoots. Total phenols in three species of bamboo shoots viz. *Dendrocalamus latiflorus, Phyllostachys nigra, Bambusa oldhamii* were found to be 31.7, 115, 114 mg per 100 g shoots (Huang et al, 2002). The total phenolic content in *Kaeng kae* and *Kaeng naw mai bai* (northeastern Thai foods) containing 6.9 % and 21.6 % Bamboo shoot as the main ingredient was found to be 111.69±1.45 and 60.79±6.57 mg. gallic acid equiv./100g food for *Kaeng kae* and *Kaeng naw mai bai* respectively (Tangkanakul et al 2006)

Antioxidant activity

The methanol extracts of bamboo shoot were evaluated for their antioxidant effect by DPPH scavenging activity and total antioxidant activity (TAA).

DPPH is a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction in DPPH radical was determined by the decrease in its absorbance at 517 nm induced by antioxidants (Blois 1958). Hence, DPPH radical is usually used as a substrate to evaluate the antioxidative action of antioxidants. Methanol extracts of *B. balcoa* exhibited higher ability (39.85 %) in scavenging DPPH

free radical than that of *D. strictus* which has a free radical scavenging potential at 13.97%. Earlier reports suggest that methanol extracts of culm of moso bamboo (*Phyllostachys pubescens*) showed a higher free radical scavenging activity (41.41%) as compared to madake bamboo (*P. bambusoides*) (29.44%) when determined by DPPH scavenging assay (Jun et al, 2004). Bamboo shoot exhibited a value of 17.8 mmol Trolox Equivalent (TE) kg−1 fresh weight and hence seemed to show moderate antioxidant capacity as compared to the other light colored vegetables (32.3-0.7 mmol TE/kg) when determined by hydrophilic assays such as the oxygen radical absorbance capacity (ORAC) (Cho et al, 2007). *Kaeng kae and Kaeng naw mai bai* (northeastern Thai foods) containing 6.9 % and 21.6 % Bamboo shoot as the main ingredient were found to possess some antioxidant activities. *Kaeng kae* exhibited moderate antioxidant capacity at a level of 54.77±0.29 mg.vit.C equiv./100 g food where as *Kaeng naw mai bai* possessed a lower value at 30.25±1.23 mg.vit.C equiv./100 g food (Tangkanakul et al 2006).

Total antioxidant capacity of bamboo shoots is expressed as number of equivalents of gallic acid. The assay is based on the reduction of Mo (IV) to Mo (V) by the extract and subsequent formation of green phosphate/Mo (V) complex at acid pH. The result of the study shows that total antioxidant activity varied from 0.88 to 3.15 (mg/ml) gallic acid equivalent was in the order of *B. balcoa* > *B. vulgaris* > *D. strictus* > *B. tulda*. Because of scarcity of literature the value were matched with some Nigerian vegetables which possessed similar TAA by this assay in the range of 0.13-1.60 gallic acid equivalent (Salawu et al 2006)

Food Safety aspect

Raw bamboo shoots of *B. vulgaris* and *D. strictus* contained higher cynogenic glucoside content of 200 and 386 ppm. The values were found to be low as compared to the WHO Report (1993) where immature bamboo shoot tip contained as high as 8000 mg of HCN/kg (WHO 1993). However, some Asian species like *D. giganteus Munro* and *D. hamiltonii* Nees et Arnott have been reported to contain only 90-100 mg HCN/100g fresh weight (Schwarzmaier 1977). However, the tip usually contains higher HCN content (1600 ppm) as compared to (110 ppm) in the base (Rezaul Haque and Howard Bradbury 2002). The toxic content varies with different parts, geographical and climatic conditions, soil conditions and also with method choosen for analysis.

The toxic content in the present investigation was found to decrease when two different species namely *b. balcoa and B. tulda* were stored at -18°C for 2 months. The toxic content reduced by 82.7 % upon storage. The significant reduction in the toxic content may be due to degradation of the toxic compound taxiphyllin into HCN. Cyanide content has been reported to reduce with prolonged fermentation by lowering the pH through microbial activity (Bhardwaj et al 2007). Other methods of removing HCN include processing (boiling, canning, soaking, and fermentation) before consumption. Boiling bamboo shoot in an open vessel for 3-4 hours can reduce the toxicity by 97% (Ferreira et al 1995). If not processed by proper means, food borne botulism has been found to be associated with the consumption of home canned bamboo shoots (Swaddiwudhipong and Wongwatcharapaiboon 2000)

Conclusions and future prospects

In the present study the chemical composition in relation to protein, vitamin C and total phenol content and the antioxidant activity of different species of bamboo shoots was evaluated. It was found that amongst all the species *B. balcoa* contained the highest content of total phenolics, crude protein and vitamin C. It also possesses highest antioxidant activity. *D. strictus and B. vulgaris* were found to contain 386 and 200 ppm of cynogenic glycoside respectively which can be eliminated by boiling bamboo shoot in open vessel or pressure cooker for a specified time.

Based on the data obtained from this study it can be concluded that the selected species of bamboo shoots possesses an exceptionally high level of protein almost comparable to certain legumes, moderate amount of vitamin C required as an antioxidant in the body and total phenols. The shoots also exhibited good antioxidative capacity which may limit free radical damage occurring in the human body. Overall, this reveals that consumption of bamboo shoots may supply essential nutrients and substantial antioxidant which may provide health promoting and disease preventing effect. Cynogenic toxicity present in bamboo shoot can be minimized by suitable processing method.

Since studies on combining the antioxidant capacity and nutritional composition of shoots are very scarce. Therefore further research is warranted on this important aspect along with variations with changes in parameters like season, climate etc. Cynogenic toxicity in young shoots has generally been ignored, indicating the need of developing methods by which the toxicity can be eliminated without affecting the nutritive value of bamboo shoot. Thus bamboo shoots would exhibit a great potential to prevent metabolic diseases and with R&D inputs suitable nutraceutical formulation can be prepared through rural entrepreneurship.

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Production of Quality Planting Material of Sympodial Bamboos for Raising Plantations in India

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Abstract

Production of quality planting material of bamboos is one of the most important aspects for raising successful industrial and commercial plantations. The main problem faced in the establishment of large scale plantations is the non availability of quality planting material in sufficient quantities. Although large number of plantlets are being produced through micro-propagation: tissue culture or somatic embryogenesis but this requires highly trained staff and sophisticated expensive infra-structure. Macro-propagation methods using conventional methods appear to be of limited value because seed availability is rare due to very long seeding cycles. The seeds are short lived hence cannot be stored for longer time. Rhizome and off-set plantings, culm and branch cuttings are useful only for propagation on a very small scale. However, macroproliferation technology developed for mass propagation of bamboos, offers the possible solution to the ever existing problem regarding production of field planting stocks on mass scale for raising large plantations. Macroproliferation technology shall be able to play major role in production of field planting stocks of sympodial commercially important bamboos identified by National Bamboo Mission (India) in order to increase the coverage of area under bamboo in potential forest and non forest lands with suitable species to enhance yields. This technology is being used by forest managers, scientists, farmers and bamboo growers for multiplying quality planting material for raising plantations. Further, it has been found highly advantageous in enhancing the multiplication rate of tissue culture plants which results in reduction of the cost of production of tissue culture saplings remarkably. The paper highlights the usefulness of macroproliferation technology for further mass multiplication of saplings developed earlier by conventional methods of propagation and also through tissue culture.

Keywords: Sympodial, Bamboos, Macroproliferation, Technology, Massive planting stocks, Plantations

Introduction

Bamboo is one of the most important and valuable plants occurring wild as well as grown in India and entire south-east Asia. It is linked with the rural life and culture for its multifarious uses ever since the beginning of civilization. In India, its versatility has led to the coinage of such sobriquets as “bamboo culture”, “green gold”, “poor mans timber” and “cradle to coffin timber”. There are over 75 genera and 1,250 species of bamboos

found in tropical, subtropical and temperate regions of the world (Sharma, 1980). India has the world's second richest bamboo genetic resource after China with about 130 species occurring over an area of 8.96 million hectares which is about 12.8 per cent of the total forest area of the country and represents 20 per cent of the India's total production of forest produce (Shanmughavel, 1997). Bamboos are widely grown in homesteads on unproductive lands and also occur as an understorey in natural forests. Looking to the diverse and varied uses of bamboos in rural areas, bamboo farming has great potential in rural development. The constant use of bamboo for various products and its economic value as raw material for paper industry and cottage industries is continuing to result in gradual disappearance of its natural source in forests and rural areas. Emphasis has now been given to introduce bamboos in agroforestry systems, on the harsh and eroded lands, hill slopes and ensure productivity and quick returns (Kishwan *et al.,* 2005).

Bamboos are natural choice to encourage sustainable integrated farming systems like agroforestry, and an excellent resource for development, income and employment generation opportunities (Melkania, 2008). Recently, the social and industrial demand of bamboo has increased at a much faster rate than its supply. In order to enhance the production of bamboo to meet the increasing demand, the expansion of bamboo areas in and out side the forest has become the necessity. The agroforestry and farm forestry are possible approaches for sustained supply of bamboo for commercial purposes. Bamboos grow much faster than trees and begin to yield from three to four years of planting. Plantation establishment needs very little capital investment with routine plant cultivation skills of farmers and foresters. Bamboos are highly suitable for agroforestry system in India. These are suitable for intercropping, soil conservation, wind break and yield value added products such as 'bamboo timber', forages, edible shoots, fiber and craft. Bamboo based agroforestry models can provide higher economic returns to the farmers, improve the soil fertility, bridge the gap of targeted national forest cover (33 per cent) and provide raw material to industry as well as for domestic use of the rural community. By the use of various intercrops, produces are obtained even during the initial stages of bamboo plantings and the income would be much higher than any other system. Bamboo can be harvested every year from third or fourth year onwards and hence regular income starts much earlier than from any other woody component. Agroforestry practice will also benefit the bamboo plants due to sharing of the inputs of irrigation, manure and fertilizers, weeding etc. applied to agricultural crops, hence bamboo growth and yield here would be better than in unmanaged plantations. Hence the total returns are likely to be much higher than other wood based agroforestry systems (Ahlawat *et al.,* 2008). Economically bamboo cultivation ensures internal rate of return (IRR) of more than 25%, almost equal to the *Eucalyptus* but returns are recurrent on annual basis after 5-6 years upto 30 years or more without recurring investments on plantations (Ashutosh *et al*., 1996).

More than 4,000 traditional uses of bamboos have been estimated (Hsiung, 1991). Bamboo is a very important raw material for several small and large scale industries besides it is also used as construction material. Several thousands of rural people are engaged in the traditional bamboo based crafts of making mats, baskets etc. to earn their livelihood. Intensive bamboo propagation is necessary not only to increase biomass and conservation of rare and threatened species but also to cultivate the economically important species for financial gains and to supply bamboo to meet the market demands (Rao, 1992). Increasing demand and over exploitation is continuously depleting the bamboo production in most of the Asian countries. A time has come to take this matter seriously and devise ways, means and measures for management of bamboo areas properly to bring back to rejuvenation and enhance productivity. As a highly renewable and versatile resource, bamboo receives more

attention from many sectors of modern civilization. In the post industrial world the outstanding productivity of this plant and versatility of the material will ensure the global importance of bamboo (Hanke, 1990).

Troup (1921) described the seed germination and seedling growth pattern followed by the 'seedling division' as one of the methods of propagation besides other methods viz. off-set planting, culm cuttings and branch cuttings etc. for propagation of *Dendrocalamus strictus*: "The 'seedling division' consisted in dividing up the mass of rhizome and transplanting the culms in small clump of two or three with rhizomes attached; transplanting is best carried out immediately before the growing season commences". Banik (1987) reported the growth pattern of *Bambusa tulda* from seed to seedling and observed that its seedling attains 4 -5 culms stage at the age of nine months. Seedlings at this stage are ready for multiplication and may be separated into three units in such a way that each piece has roots, old and young rhizome with buds and shoots. Thus every year the seedlings get multiplied three times of the initial stock. Out of this, two-thirds of the seedlings may be planted in the field, the rest can again be multiplied after nine months (April-May) and the process can be repeated every year. However, Banik (1987) further continued, "detailed scientific study is essential on such a macroproliferation of bamboo seedlings to develop a new dependable technique for bamboo propagation at least for a few years". Adarsh Kumar (1991, 1992, 1993) studied the bamboo propagation and developed a new low cost universal macroproliferation technology for mass propagation of sympodial bamboos, for desired number of years without dependence on seed production in nature, from second year onwards.

Rapid Increase in Bamboo Demand

The estimated bamboo resources of the world are about 20 million hectares and the estimated current market of bamboo is US $ 10 billion that is expected to increase to US $ 20 billion by 2015. In India, it encompasses about 8.96 million hectares of forest area which is equivalent to 12.8% of the total forest cover consisting of 130 MT with estimated annual harvest being 13.47 MT. The current market of bamboo/ bamboo products in India is estimated to be Rs. 4,500 crores which is expected to increase to Rs. 20,000 crores by 2015 with major contribution from wood substitute, processed bamboo shoots, industrial products (activated charcoal etc.) and structural applications segments (Gupta, 2008). The employment potential of bamboo is very high and the major work force constitutes of the rural poor, especially women and 432 million work days per annum are provided by the bamboo sector in India (Adkoli, 1994).

Rapid increase in the demand of bamboos in the industrial sector coupled with increase in domestic demand due to rising population have caused depletion of the natural bamboo resources which calls for concerted efforts for the awareness to raise bamboo plantations in land hitherto barren, degraded or in association with agriculture crops. With the trend of decrease in production and rise in human population, the gap between supply and demand is going to be larger. Srinivasan (Anon., 1994) stressed that in India the demand for bamboo planting stocks are 90-120 million per annum, which is expected to increase to up to 300 million seedlings per annum. Large scale cultivation is the only way to prevent further depletion of bamboo resource, and to ensure a regular and sustained supply of raw material for growing industrial uses (John, *et al.,* 1995). This situation elucidates the need for increase in bamboo production. Due attention on raising bamboo plantation under various programmes has not been paid so far. Now farmers and villagers need to be involved in bamboo cultivation /

production. Apart from protecting natural vegetation of bamboos, the activity has to be brought to the non forest lands (Kamesh Salam, 2002).

National Bamboo Mission (India)

The requirement of "bamboo wood" for multiple uses by the industries and the common man will definitely increase in far greater dimensions. In India, the total demand of various bamboo consuming sectors is estimated at 26.9 million tonnes. The estimated supply is only 13.47 million tonnes i.e. only half of the total demand. The pulp and paper industry, construction, cottage industry and handloom, food, fuel, fodder and medicine annually consume about 13.4 million tonnes of bamboo amounting to Rs. 2042 crores. Demand of bamboo for industrial use is met from state owned forests, while for non industrial purpose it comes from private as well as state owned resources. Keeping abreast of versatility of bamboo uses and its potential to build up the rural economy, Government of India launched massive programme viz. National Bamboo Mission for over all development of bamboo sector in the country and also to improve the Indian representation in global bamboo market. Bamboo has also been recommended for plantations for a greener, pollution free environment along with economic prosperity.

Based on India's rich culture, bamboo utilization has triggered several programmes in the country for economic and industrial development through the use of bamboo. Large targets for plantations across the country have been fixed. The National Bamboo Mission (India) envisages covering over 1.76 lakh hectare area through bamboo. This will need over 70 million field plantable saplings to raise bamboo plantations. The emphasis of the National Bamboo Mission is on an area based regionally differentiated strategy, for both forest and non-forest areas. A number of activities are proposed to be taken up for increasing production of bamboo through area specific species/varieties with high yield, plantation development and dissemination of technologies through a seamless blend of traditional wisdom and scientific knowledge, along with the convergence and synergy amongst stakeholders. Besides ensuring proper post-harvest storage and treatment facilities, marketing and export National Bamboo Mission is committed to assure appropriate returns to growers/producers. Also, bamboo development is viewed as an instrument of poverty alleviation and employment generation for skilled and unskilled persons, especially unemployed youth particularly in the rural sector through eco-rehabilitation purposes.

Selection and Propagation

The selection of clump is carried out to obtain significant quantum of genetic gain as quickly and inexpensively as possible keeping in view the end use and habitat suitability. Banik (1995) described selection criteria and species selection for specific end use listing various bamboo species fitting in different criteria. Individual selection of phenotypically superior clumps from a large population is the common practice. Bamboos are reported to be highly cross pollinated, this gives enormous opportunities for selection of superior seedlings having desired combination of characters (Venkatesh, 1984). Natural seedling population of bamboos with genotypic diversity may afford an opportunity for selecting superior clones and individual plants (McClure 1966). Thapliyal *et al.,* (1991) stated that propagation through seed is desirable to maintain genetic diversity in

bamboo plantations. It also helps to develop *ex-situ* conservation strategies through seed which is the easiest and cheapest method of propagation. Gurumurti *et al.,* (1995) are of the opinion that identification of patterns of variation, collection of suitable material followed by mass multiplication would help in strategically enhancing the genetic quality of bamboos. Banik (1997) described seedling selection methods for *Bambusa tulda* and *B. polymorpha*. Ombir Singh (2008) has listed several traits regarding selection of candidate plus clumps.

The abundant seed production after gregarious flowering and scanty seed production through sporadic flowering in some bamboo species is utilized for production of base population of seedlings. The base population is further multiplied using macroproliferation technology. After selection of natural variations existing in bamboos, followed by clonal propagation, is utilized for production of quality planting stock. Only the best performers are selected based on certain desired morphological characters. The germplasms of selected performers are established in a centralized germplasm bank cum vegetative multiplication garden. The base population saplings are produced through clonal propagation using conventional methods of propagation viz. off-sets, culm and branch cuttings and through tissue culture. These are further multiplied using macroproliferation technology repeatedly (Prasad and Pattanaik, 2002). The production of clonal base population (saplings) needs to be carried out at the centralized nursery also having tissue culture laboratory. After selection, the seedling stocks can be raised through macroproliferation technology for establishment of pilot plantations. These pilot plantations after evaluation are screened for growth performance, culm production and other desired characters for identifying 'elite clumps'. Then the elite clumps have to be further multiplied to produce field planting stocks through macroproliferation technology for raising commercial and industrial plantations. A part of the base population (saplings) so developed is transferred to the farmer's nurseries for mass multiplication through macroproliferation technology for production of field planting stocks for raising plantations.

Constraints for Mass Propagation of Bamboos

Prof. Liese (1985) was of opinion that vegetative propagations by cuttings from culm, branch or rhizome is commonly practiced. So far several methods are applied, but for practical purposes especially for establishing larger plantations, the degree of failure is still rather high. Prof. Liese (1991) again stated that in spite of intensive efforts made at various institutions, universally applicable method for vegetative propagation of bamboo is not yet available. Bamboo seems to be a difficult species to multiply, no body seems to understand the bamboo just enough to propagate it in massive numbers (Anon., 1990). None of the conventional methods of propagation is universal and effective for all the species of bamboos. Each carries its own inherent risks (Anon., 1994). Sharma (1990) stated that Pathak (1899) was perhaps the first to attempt the propagation of the common 'male bamboo' (*Dendrocalamus strictus*) by cuttings. Since then several papers have appeared dealing with the vegetative propagation of bamboos. None of these earlier attempts have standardized the technique of bamboo propagation by vegetative methods. The vegetative methods of bamboo propagation viz. off-set planting, rooting of culm and branch cuttings are of limited value for the large scale propagation of clump forming sympodial bamboos. The propagules, thus produced are bulky, heavy, difficult to handle and transport. Thus to generate field planting stocks of bamboos on mass scale for raising industrial and commercial plantations is definitely an uphill task. Rao and Rao (1990) stated that vegetative propagation by offsets and culm cuttings has proved to be of limited value as the daughter clumps are bound to flower at the same time as the parent clump. Nawa Bahar

and Singh (2008) mentioned that bamboos can be multiplied vegetatively with ease but large plantations are difficult to raise because lack of regular and plentiful supply of seed / propagation material is a serious constraint for mass propagation of bamboos. Similarly, Manoj Chandran (2008) has also cautioned that propagation from rhizome, offset and culm cuttings, of unknown age, is also unreliable as the saplings developed and planted in the field for raising bamboo plantations, may flower, dry and die before their economic use starts or much before utilization of full potential of bamboo culm production of the plantation. Sastri (2008) is of opinion that supply of planting material for large scale commercial plantations of the desired species could be a limiting factor due to lack of seed and/or other propagules. Srinivasan (Anon. 1994) mentioned that the key to the success will be cost effective and simple plant propagation technique.

Bamboo Propagation

Bamboo is a unique plant, which has not easily lent itself to modern methods of macro-propagation and genetic improvement owing to its long vegetative phase and monocarpic behaviour. Conventional breeding is difficult because of the near impossibility of getting two desirable parents to flower simultaneously (Rao and Rao, 1990). Thus for meeting the raw material demand scientific management of bamboo forests is *sine qua non*. Regeneration of bamboos takes place sexually as well as asexually. However, both the methods of propagation are beset with many problems that restrict their large scale use. In view of the constant increase in demand, the scarcity of planting material and the problem associated with the conventional methods of vegetative propagation, development of an effective method of vegetative propagation of different sympodial bamboos is highly required. Bamboo propagation can be carried out by the under mentioned some of the important methods:

(i) Seed sowing **(ii) Rhizome / Off-set planting**

(iii) Culm cutting **(iv) Branch cutting**

(v) Macroproliferation technology **(vi) Tissue culture**

Depending on the availability of seeds, technical feasibility of propagation by vegetative propagation techniques and suitability of micropropagation protocols for large scale multiplications, the bamboo species can be propagated either by clonal methods or through tissue culture or both.

(i) Propagation by seed: It is the easiest method of propagation requiring low technical skills. Bamboo seeds are sown directly in the soil already dug up to a depth of 15-20cm or deeper in case of poor soils either in lines 5-6 m apart or in pits of 30-45 cm^3 dug at the spacing of 5m x 5m or 6m x 6m apart and cleared of weeds. As direct sowing in lines is often liable to fail due to relatively (i) uneven germination (ii) slow growth of resultant plants and (iii) exposure to animal damage etc. The usual procedure is to raise plants, by seed sowing in a nursery bed and then transplanting entire plants. But the seed of commercially important bamboo species is not available every year because of very long interval of time of the flowering cycles ranging from 30 to 60 or more years. Secondly, the bamboo seeds when available are short lived hence cannot be stored for longer durations. The availability of seeds is a major constraint and it would be difficult to multiply bamboos on a large scale

through seed alone. Therefore, in order to sustain large supply of planting material year after year the focus should be towards producing planting material by vegetative methods and tissue culture.

(ii) Propagation by rhizome / off-set planting: This is the traditional and common method of vegetative propagation. The off-set consists of one year old culm of about 1m length and a part of rhizome with roots attached excavated from the ground. Off-sets are planted in the field on the advent of monsoon season. This traditional method is applicable only in cultivating few clumps. The various limitations with this method are:

1. Limited availability of rhizome/offsets
2. The method is very expensive as it is labour and time intensive
3. Off-sets and rhizomes are bulky and very heavy as such it is difficult to handle and transport
4. The survival success is low

(iii) Propagation by Culm cutting: Bamboos can be propagated by rooting culm cuttings (Pathak, 1899; Dabral, 1950; Mc Clure, 1966; Adarsh Kumar *et al*., 1988; Seethalakshmi and Surendran, 1990; Reddy, 2006). This method is well studied. One to two years old culm is cut into 1, 2 or 3 noded segments placed in the nursery bed horizontally and covered with soil during April – May. In some species solution of growth promoting substances like IBA, NAA, boric acid, coumarin etc. are filled singly in the internodes before planting in the nursery for improvement in rooting percentage. This method has a limited use because of the limited availability of the planting material. This method is also labour intensive, expensive and propagules are difficult to transport.

(iv) Propagation by Branch cutting: Propagation of bamboo through branch cuttings appears to be a promising method. Branches with 3 nodes from 1-2 year old culms are planted in sand after treatment with 100 ppm IAA. Normally branch cuttings develop roots after only 3-6 months and rhizomes after 12-15 months. Propagules bearing roots, rhizomes and shoots are essential for the successful establishment and development of bamboo plantations. Bakshi and Rakesh (2008) reported IBA 100 ppm was found to induce rooting 75%, sprouting 80% and survival 80% of branch cuttings of *Bambusa vulgaris*.

(v) Propagation through Macroproliferation technology for Mass Propagation of Bamboos: A major breakthrough (Anon. 1992) was achieved for mass production of field plantable saplings of economically important sympodial bamboos viz. *Bambusa bambos, B. tulda, Dendrocalamus hamiltonii* and *D. strictus* vegetatively through macroproliferation. This technology (Adarsh Kumar, 1991, 1992 & 1993) was developed for production of field plantable saplings in large numbers for any desired number of years depending upon the targets and the facilities available, solves the ever existing problem pertaining to non-availability of planting stocks for raising large plantations. According to this technology:-

In July, bamboo seeds are sown in germination boxes or in the nursery beds. After one month, in August young seedlings of 3-5 leaf stage are pricked out: *Bambusa bamboos* - 7,000 nos.; *Bambusa tulda*– 5,000 nos.*; Dendrocalamus hamiltonll* – 4,000 nos.*; Dendrocalamus strictus* - 6,000 nos. are planted in polybags. From August to March bamboo seedlings are maintained by regular watering, weeding and soil working. The tillers

ranging from 3-10 in number developed in each polybag of *B.tulda*, *D. hamiltonii* and *D.strictus.* Whereas in *B. bambos* their number ranged between 6 to 14. The averages being *B. bamboos* = 7.0; *B. tulda* = 5.0; *D. hamiltonii* = 4.0 and *D. strictus* = 6.0.

In the first week of April, the saplings are carefully removed from the polybags. Each proliferated tiller alongwith some rhizome and roots is separated by cutting the rhizome. These act as propagules. On an average *Bambusa bambos*- 49,000; *Bambusa tulda– 25*,000*; Dendrocalamus hamiltonii– 16*,000*; Dendrocalamus strictus -3*6,000 propagules are produced. Each propagule is planted in polybag in April. From April to June, these propagules are maintained by regular watering, weeding and soil working to grow into saplings by the first week of July.

In the first week of July, field plantable bamboo saplings are available in massive members. *Bambusa bambos*- 49,000 saplings; *Bambusa tulda– 25*,000 saplings*; Dendrocalamus hamiltonii – 16*,000 saplings*; Dendrocalamus strictus -3*6,000 saplings are available. Out of these, 1000 or in multiples, as per the requirements, are retained in the nursery for future propagation work. The field planting stock of remaining saplings in sufficient numbers i.e. *Bambusa bambos* – 48,000 saplings; *Bambusa tulda– 24,*000 saplings*; Dendrocalamus hamiltonii – 15*,000 saplings*; Dendrocalamus strictus* -35,000 saplings are available for raising plantations.

In August, the propagules (*Bambusa bambos* - 7,000; *Bambusa tulda* – 5,000*; Dendrocalamus hamiltonii* – 4,000*; Dendrocalamus strictus* - 6,000) are prepared by separating the tillers of 1000 saplings of different species retained in the nursery by cutting the rhizome and planted in polybags. These are maintained by regular watering, weeding and soil working till April. Again the tillers ranging from 3-10 in number developed in each polybag of *B.tulda*, *D. hamiltonii* and *D.strictus w*hereas in *B. bambos* these range between 6 to 14. The averages being *B. bambos* = 7.0; *B. tulda* = 5.0; *D. hamiltonii* = 4.0 and *D. strictus* = 6.0 as was found earlier. In the first week of April, as in the previous year, each proliferated tiller along with some rhizome and roots is separated by cutting the rhizome. These act as propagules. On an average *Bambusa bambos*- 49,000; *Bambusa tulda– 25*,000*; Dendrocalamus hamiltonii– 16*,000 and *Dendrocalamus strictus* -36,000 propagules are produced and planted in polybags for further growth and development into field plantable saplings by July.

Thus 49, 25, 36 and 16 field plantable saplings can be developed from each of the propagule in one year in case of *Bambusa bambos*; *B. tulda*, *Dendrocalamus strictus* and *D. hamiltonii* respectively as under:

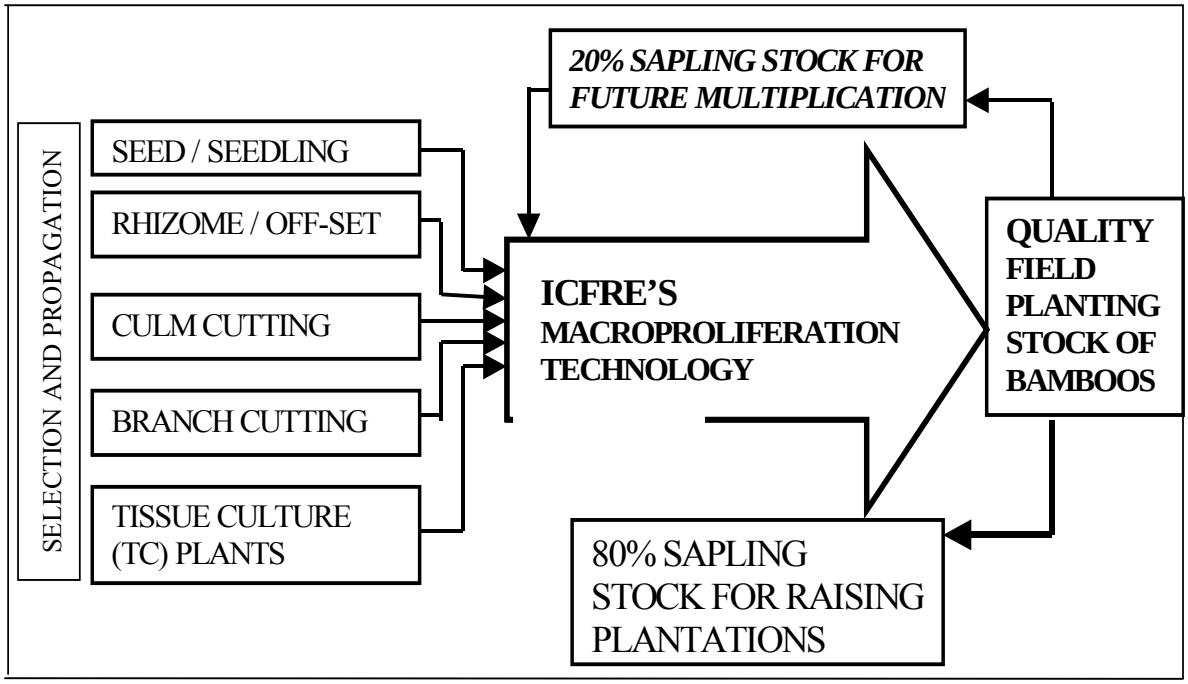
Productivity of Macroproliferation Technology

Species	No. of tillers developed in one seedling / sapling		
	Range	Mean	Multiplication index per year
B. bambos	6-14	7	49
B. tulda	3-8	5	25
D. hamiltonii	3-7	4	16
D. strictus	3-10	6	36

From second year onwards, the whole methodology as mentioned above is repeated and field planting stocks of bamboo are produced year after year for any numbers of years. The dependence on bamboo seed production in nature is totally eliminated from second year onwards for production of field planting stocks.

The macroproliferation technology of vegetative propagation is universally applicable and can be used for mass production of field plantable saplings of sympodial bamboos. Dransfield and Widjaja, (1995) stated that vegetative propagation system called 'macroproliferation of seedlings' has been successfully developed in India for large scale propagule production. This method ensures a continuous supply of propagules. Kishwan *et al*. (2005) considered macroproliferation technique as the current technique of mass multiplication of bamboos. This technology shall also be able to play major role in production of huge quantities of field planting stocks of commercially important sympodial bamboos identified by National Bamboo Mission (India) in order to increase the coverage of bamboo in potential forest and non-forest areas with suitable species to enhance yields.

Multiple Approaches to Macroproliferation Technology: The saplings available from selected superior / elite mother clumps, through (i)seedlings raised by seed sowing (ii) conventional methods of macro-propagation of bamboos *viz.* off-sets, culm and branch cuttings etc. and (iii) micro-propagated tissue culture plants may be successfully used for multi-way approachable this technology for production/enhancement of bamboo planting stocks during the prolonged vegetative phase of sympodial bamboo clumps for raising industrial and commercial plantations as shown in the plan -1.



Plan-1. Multiple approaches to macroproliferation technology for massive production of quality planting stock of bamboos from macro and micro (tissue culture) propagated saplings.

This technology is approachable through:

(i) Seeds of Bambusa bambos, B. tulda, Dendrocalamus hamiltonii and D. strictus (Adarsh Kumar, 1991, 1992, 1993, 1995).

(ii) Off-sets of Bambusa balcooa and B. vulgaris (Koshi and Gopakumar, 2005).

(iii) Culm and Branch Cuttings: Rain Forest Research Institute, Jorhat, India, has developed a protocol to induce juvenility and generate saplings from the mature culms, followed by mass multiplication through macroproliferation technology (Katwal, 2002); Dubey et al. (2008) developed the quality planting stock by using macroproliferation technology on the saplings developed from the two noded culm cuttings of Bambusa vulgaris var. vittata, B. balcooa, B. bambos, B. nutans, B. tulda, and Dendrocalamus hamiltonii with survival rate of 90-100 per cent. Through this technique 60-180 nos. of bamboo saplings (propagules) could be produced in a year (April to March) depending upon the species selected from a single bamboo node. Dubey et al. (2008) further stated that the best part of the technique is that it can produce bamboo planting stock round the year without involving many technicalities. Koshi and Gopakumar (2005) studied the rooting of branch cuttings of Bambusa vulgaris and B. balcooa which were further propagated by macroproliferation technology to enhance the production of planting stock.

(iv) Tissue culture plants: Preetha et al. (1993); Arya and Arya, (1999) used macroproliferation technology for enhancement of the multiplication rate of tissue culture (TC) bamboo plants several times for production of field plantable saplings quickly and economically in large quantities.

Earlier Adarsh Kumar and Mohinder Pal (1993) found that conventional methods are still useful for raising planting stocks on very small scale, but for large scale propagation of bamboos, macro-proliferation method has many benefits and can be directly used in the forest nurseries. Recently, Banik (2008) has also stated that macroproliferation method has subsequently also been used to multiply plants generated by culm cuttings, branch cuttings and micro-propagated tissue cultured (TC) plants.

Farmer's Friendly Macroproliferation Technology: Macroproliferation technology very well meets the requirements of farmers, NGO's and other agencies involved in the production of bamboo field planting stocks for raising multipurpose massive plantations as this technology is simple, easy and involves the use of locally available materials involving the routine plant cultivation skills of farmers and foresters. It neither requires highly trained staff nor sophisticated high value infra structure for mass production of bamboo planting stocks for raising plantations.

(vi) Propagation by Tissue culture: Details of the study on *in-vitro* seed germination are provided in recent publications by Saxena and Dhawan (1991), Rao *et al.* (1985; 1990), Preetha *et al*. (1993), Dhawan (1993) Rout and Das (1994), Ravikumar *et al.* (1998) and Arya *et al.* (2002) where a large number of plantlets are produced either through micropropagation or somatic embryogenesis. However, this is an expensive technique and requires highly trained staff and sophisticated expensive infrastructure.

Enhancement of Multiplication Rate of Tissue Culture Bamboo Plants: The macroproliferation technology of mass propagation of bamboos has also been found highly advantageous by the tissue culture scientists to multiply *Dendrocalamus asper* plants which were earlier developed through tissue culture (Arya and Arya, 1999). They reported that this technology enhanced the multiplication rate of tissue culture (TC) bamboo plants and ensured a very high rate (95 per cent and above) of establishment and survival in the field in a short interval of time. These plants were multiplied twice a year for two years as per this technology. Preetha *et al.* (1993) used the technology to enhance the quantity of *Dendrocalamus stictus* and *Bambusa bambos* rooted plants which were developed through tissue culture and hardened in the shade house for 20-30 days. Thus the field planting stock was increased 4-5 times. Shanmughavel *et al*., (1997) suggested that in order to increase the tissue culture plants before transfer to the field, macroproliferation should be practiced. This technology shall also effect reduction in the cost of production of field plantable tissue culture saplings remarkably.

Economics of Planting Stock Production: Nautiyal *et al*. (2008) found that unlimited planting stock may be produced at the lowest cost i.e. @ Indian Rs. 2.50 (=US$ 0.05) per sapling through macroproliferation technology. They have worked out the comparative economics of bamboo planting stock production by different methods of vegetative propagation as under:-

Sl No.	Method	Cost per plant(Rs.)	Remarks
1.	Off-set planting	50.00	Labour intensive, heavy planting stock
2.	Rhizome planting	30.00	Labour intensive, heavy planting stock
3.	Whole culm cutting	15.00	Labour intensive
4.	Layering	05.00	Labour intensive
5.	Culm cutting	08.00	Limited planting stock may be produced
6.	Branch cutting	03.00	Unlimited planting stock may be produced
7.	Macroproliferation Technology	02.50	Unlimited planting stock may be produced

Conclusion

Regeneration of bamboos takes place sexually as well as vegetaively. However, both the methods of propagation are beset with many problems that restrict their large scale use. Macroproliferation technology is both, cost and time effective and without doubt is the best method for bamboo planting stock production, when more than million plants annually are required to be produced. The bamboo saplings remain in field plantable size which is light in weight, easy to handle and transport. Further the farmer's friendly macroproliferation technology is simple, easy, involves the use of locally available materials. It neither requires highly trained staff nor sophisticated high valued infra-structure for mass production of bamboo planting stocks for raising plantations. Unlimited planting stocks may be available at the lowest cost i.e. @ Indian Rs. 2.50 (=US$ 0.05) per sapling through macroproliferation technology. Ever since this technology was developed in 1991, great interest is being continuously shown by the bamboo scientists, researchers and growers to further explore the full potential of macroproliferation technology. Once some saplings are produced through offsets, culm or branch cuttings of selected superior clumps or by any other means viz. tissue culture etc., they can be used for further propagation by macroproliferation technology for production of quality field planting stocks for raising bamboo plantations. In short, it can be said that every action counts, every person counts, bamboo plantations could emerge as "Green Gold Mines world over" in near future.

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Clonal Propagation of *Bambusa vulgaris* Schrad ex wendl by Leafy Branch Cuttings

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Abstract

***Bambusa vulgaris* Schrad ex wendl** is a widely cultivated bamboo species in rural Bangladesh for its versatile uses. The species does not set seed after sparse flowering which makes seedling progenies unavailable and rendered vegetative propagation as the only viable alternative. But information on convenient vegetative propagation methods of the species is still lacking. We therefore carried out a low-cost propagation trial to explore the clonal propagation techniques for the species with two types of small branch cuttings - nodal leafy cuttings and tip cuttings. The cuttings were treated with 0%, 0.1%, 0.4% and 0.8% IBA solutions and kept in non-mist propagator for letting them root with the objective of assessing the rooting ability. The cuttings were rooted in four weeks and were allowed to grow in the polybags for ten months under nursery condition to assess their steckling capacity. The study revealed that both types of branch cuttings are able to develop roots, shoots, to survive and to form rhizome in the nursery condition. Rooting ability of the cuttings was significantly enhanced by the application of rooting hormone - IBA. The highest rooting percentage in nodal leafy cuttings and the tip cuttings (56.67 and 51.0, respectively) were observed in 0.8% IBA treatment followed by 0.4% IBA and the lowest (34.3 and 30.0 respectively) was in control. The highest number of root developed per cutting (9.77 and 8.33 in nodal leafy cuttings and the tip cuttings, respectively) was also obtained from the cuttings treated with 0.8% IBA solution followed 0.4% IBA treatment and the lowest (3.1 and 2.1 respectively) was in the cuttings without treatment. However, the length of the longest root varied significantly neither with the cutting types nor the concentrations of IBA solution. Survival percentage of the stecklings in nursery condition was significantly enhanced by IBA.

Keywords: *Bambusa vulgaris*, leafy branch cuttings, non-mist propagator, rooting ability, steckling capacity.

Introduction

Bamboos of Poraceae Family and Bambosidae Sub-family are the fastest growing woody species. These multipurpose plants (Shanmughavel et al. 1997) play vital roles in every-day life of millions of people the South-East Asia by meeting their basic needs in the forms of food, fodder, fuel, clothing, medicine, shelter and

raw materials for industries including paper and pulp, furniture, construction etc. Their versatile use (Banik et al.1997) is due to their excellent splitting ability (Banik 2002), tensile and compressive strength, amenability of being harvested within five years after planting (Negi 1996) etc. With the swelling population, the demands for bamboos in housing, agricultural activities and paper industries are in rise. However, the area and quantity of bamboo stocks in the country are declining alarmingly due to destruction of tropical forests, indiscriminate harvesting associated with increasing demand, lack of proper knowledge and suitable technique for cultivation (Banik 1995). It is, therefore, an urgent need to develop and maintain the bamboo resource bases through massive plantation program with genetically improved planting materials.

Among 75 genera and 1250 species in the world (Sharma 1980), 33 are grown in Bangladesh - of which 7 occur naturally in forests and rest are cultivated in homestead throughout the country (Banik 1980). *Bambusa vulgaris* Schrad ex wendl is the most prevalent and preferred bamboo species in rural homesteads in Bangladesh. However, flowering is sparse in the species (Koshy and Pushpangadan 1997) and the flowering that is seen is not good enough to set viable seeds due to the cumulative effect of a number of physical and physiological factors (Banik 1979; Koshy and Jee 2001). This indicates the inadequacy of seedling progenies for the species (McClure 1966). Consequently, the knowledge on alternative means of obtaining propagation materials became inevitable wherein vegetative propagation methods for the species fits perfectly (Banik 2000; Koshy and Pushpangadan 1997).

Rhizome cutting, offset planting, culm or stem cutting, branch cutting are the various approaches of vegetative propagation for bamboos with known issues for some of them like the low rate of multiplication, need for skilled workforce, or high capital investments etc. Among these approaches, rhizome cutting is popular in the villages but the lower rate of survival and bulkiness (4-30Kg) of rhizomes makes it an expensive option in terms of obtaining and transporting propagules let alone the limited availability of rhizomes due to the risk of detrimental effect of removing rhizomes on the regeneration potential of the source clump. Pre-rooted and pre-rhizomed branch cuttings (Banik 1989) are the other good choices, however, pre-rooted branch cuttings is limited only to the wet seasons and very few branches attached with the mother culms can develop root at their base which makes it inapplicable for large-scale propagule production. The recently developed branch-cutting technique can overcome many of these problems (Seethalakshmi et al. 1983) as it is inexpensive, produces a bulk of propagation materials with high survival potential in short time and reduces the labor and transportation cost (Banik 2000**)**. A branch with the base or one node from the main branch and 3–4 nodes from secondary branches is cut for propagation (Banik 2000**)**. In our previous studies (Hossain et al. 2005; Hossain et al. 2006), we have shown that the cuttings start to develop active buds within 7–10 days and produce profuse roots in the propagation beds within 4–8 weeks, depending on the season. However, the numbers of suitable branches (larger than 5mm diameter with base or node) available from a clump to produce root in the propagation bed is inadequate and the branch cutting method limited only to rainy season during June-July. Our approach to overcome the issue is to use small, leafy branch cutting for vegetative propagation to produce cuttings in high quantity from a single clump, inexpensively round the year. In this research we are reporting the rooting ability of small leafy branch cuttings (around 20 cm in length and 3mm in diameter) in non-mist propagator with or without rooting hormone IBA and their steckling capacity in the nursery condition so that large number of propagules can be produced from a single clump of bamboo.

Materials and Methods

Study area and climatic conditions

The study was conducted over a period of one and half year from June 2005 to November 2006 in the nursery of Institute of Forestry and Environmental Sciences, University of Chittagong, Bangladesh. It lies at 22°27′27″ N latitude and 91°48′30″ E longitude (Figure 1) and enjoys typical tropical monsoon with hot humid summer and cool dry winter having a mean monthly temperature between 21.8 and 29.2°C, the maximum and minimum being 26 and 15°C, respectively. Relative humidity is lowest (64%) in February and the highest (95%) in June to September and mean annual rainfall is about 300 cm, which occurs mostly between June and September (Gafur et al. 1979). Mean monthly day length varies between 10 h 35 min in December and 13 h 20 min in June (Hossain et al. 2005).

Methodology

Clump selection

Vigorous five-years old clumps were selected based on 1) maturity in terms of their ability to produce sufficient number of branches, 2) growth potential in terms of the number of culms per clump, height, diameter and length of internodes and 3) disease- and pest-freeness.

Preparation of cuttings

Cuttings were collected from the selected branches of the pre-selected clumps by excising the small secondary or tertiary branches. Two types of small branch cuttings were made - nodal leafy cuttings and tip cuttings. The nodal leafy cuttings were the small branches with one node from the primary or secondary branch (approximately 2 cm from both side of node as the base of leafy branch) along with 3-4 nodes and fleshy leaves with tip (Figure 2 and table 2). Tip cuttings were the healthy leading shoot apex along with 2-3 nodes of the secondary or tertiary branch without swollen base. Average length of tip cutting was 18.94 to 20.97 cm and diameter was 2.24 to 3.05 mm (Figure 2 and table 2).

Treatment of the cuttings

A total of 480 cuttings, taking 240 of each type, were used for the rooting trial in the study. Cuttings were immersed briefly in a solution of fungicide, Diathane M45 (Rohm & Co. Ltd., France; 2 g. per litre of water) to avoid fungal infection. Then they were rinsed and kept under shade for 10 minutes in open air. Finally the cuttings of each type were treated with 0%, 0.1%, 0.4%, and 0.8% (w/v) Indole 3-Butyric Acid (IBA) solution to assess the effect of applied rooting hormone on rooting ability of cuttings by dipping the base of cuttings briefly into the solution.

Rooting trials

The cuttings were planted in the perforated plastic trays filled with coarse sand mixed with gravel. Each tray contained 10 cuttings which made 60 replicate cuttings for each of the treatment (six trays; 10 cuttings in each

tray). Trays containing the cuttings were placed in the non- mist propagator (Kamaluddin 1996) for rooting (Figure 3).

Propagator environment

About 85-90% humidity was maintained within the propagator. Every day the propagator was opened briefly in the early morning and the late afternoon for gaseous exchange to avoid excessive heat accumulation. Again, the propagator was kept under bamboo made shed to avoid excessive heat on the propagator. Further shading was achieved by putting jute mat over the roof of the shed. Thus the photosynthetic photon flux inside the propagator was reduced to about 12% of full sun . During the experiment mean maximum temperature were 32ºC and the mean minimum temperature 25ºC.

Four weeks after setting the experiment, the rooted cuttings were subjected to weaning before transferring to polybags, particularly towards the end of rooting period during root lignifications. For weaning the shed was kept open at night for three days and then day and night for another three days.

Transferring of rooted cuttings

The rooted and weaned cuttings were then transferred to the polybag (25cm x 15cm) filled with soil and decomposed cow dung at a ratio of 3:1 and were placed in the nursery bed. They were allowed to grow for ten months for assessing the steckling capacity of the cutlings developed under various treatments. Proper care and maintenance were done from the time of setting the experiment up to the final assessment.

Record keeping

During transferring the rooted cuttings from the rooting medium to the growth medium (polybags filled with soil and decomposed cowdung) the rooting percentage, root number, root length, cutting length and cutting diameter of each cutting were recorded. Survival percentage of the cutlings was assessed by counting the number of rooted cuttings survived and thrived ten months after transferring them into the poly bags in the nursery condition.

Data Analysis

All data were analyzed with Microsoft Excel and SPSS ver.13.0 (SPSS Incorporation, Chicago, USA) Possible treatment variations were explored by analysis of variance (ANOVA) and Duncan multiple range test (DMRT). Rooting percentage values were adjusted accordingly by using arc sign root square before putting the data into analysis since the percentage of cuttings rooted were distributed between the range of 30 to 60 and proportions were based on equal denominator.

$$Y = \mathrm{Sin}^{-1} (x)^{1/2}$$

Where, Y= Arc sign transformed value

X = Proportion of number of cuttings rooted to the number of cuttings substituted (100-1/4 n) where ‘n’ is the number of units upon which the percentage data is based i.e., the denominator used in compiling the percentages.

Results and Discussion

Rooting Ability of Cuttings

Rooting percentages

Rooting percentage of *B. vulgaris* branch cuttings varied from 36.7 to 56.7 in nodal leafy cuttings and 30.0 to 51.7 in tip cuttings. Rooting percentages of both types of cuttings were significantly varied among the treatments. Exogenous rooting hormone IBA and its various concentrations affected the rooting percentage of cutting types (nodal leafy cuttings and tip cuttings) remarkably. In the nodal leafy cuttings, the highest rooting percentage (56.7) was observed in the cuttings treated with 0.8% IBA solution followed by the cuttings treated with 0.4% IBA (48.3) and the lowest (36.7) was in control (Figure 4). The highest rooting percentages (56.7) in tip cuttings was also obtained from the cuttings treated with 0.8% IBA solution followed by 0.4% IBA (42.3) and the lowest rooting percentage (30.0) was in cuttings without any treatment (Figure 4). However, the rooting percentage in nodal leafy cuttings was significantly higher than the tip cuttings among the treatments.

In the present study, rooting percentages of branch cuttings of *B. vulgaris* varied significantly among the treatments with the varying concentrations of IBA solution. This finding is validated by several reports in the influence of IBA on the rooting percentage of bamboo branch cuttings. For instance, Somashekar et al. (2004) reported the highest rooting percentage (85, in leafy branch cuttings with tip and 80 in nodal cuttings) in the cuttings treated with 2500 ppm IBA. Hossain et al. (2005) observed that rooting ability of cuttings and growth performance of cutlings were affected significantly by IBA treatment. They recorded the highest rooting percentage (84) in 0.2% IBA treated cuttings compared to untreated cuttings (73.3). In a separate experiment Hossain et al. (2006) reported that the highest percentage of rooting (63.33) was observed in the *B. vulgaris* var. *striata* branch cuttings treated with 0.4% IBA followed by 0.2% IBA (60) and the lowest (30) was in the cuttings without treatment. Sharma (1980) reported that hormone treatment accelerated the success of rooting in branch cuttings and it was 80% in *B. vulgaris*. Moreover, Surendran and Seethalakashi (1985) reported significant enhancement in rooting and sprouting responses of bamboos by the application of growth regulators - IBA and NAA. Sing et al. (2002) found that application of IAA, IBA, NAA, NOA either alone or in combinations have influence on rooting percentage from culm and culm branch cutting as they augment the endogenous level of auxins promoting early and high rate of root induction as compared to the control. In cuttings with low endogenous auxin contents, applied auxin causes significant increases in both percentages rooting and number of roots (Bowen et al. 1975).

Number of roots per cutting

Number of roots per cutting varied from 3.1 to 9.8 in nodal leafy cuttings and 2.1 to 8.3 in tip cuttings. In nodal leafy cutting, the highest number of roots (9.8) was observed in 0.8% IBA treated cuttings followed by the cuttings treated with 0.4% IBA (7.0) and the lowest number of roots (3.1) was in cuttings without treatment (Figure 5 and 6). Similarly, in tip cuttings, the highest number of roots for (8.3) was obtained in cuttings treated with 0.8% IBA solution followed by 0.1% IBA (7.3) and the lowest (2.1) was in untreated cuttings (Figure 5 and

6). The number of root developed in the nodal cuttings was significantly higher than the tip cuttings in all concentrations of IBA treatment.

In both types of cuttings, the number of roots were significantly increased by IBA treatment which strengthen previous reports, for instance, Hossain et al. (2005) observed significant increase in the number of roots in IBA treated cuttings (6.8 to 7.9 in 0.2% IBA treated cuttings) compared to in untreated cuttings (4.1 to 6.6). In a separate work Hossain et al. (2006) reported that the number of roots in the branch cutting of *B. vulgaris* var. *striata* was the maximum for 0.4% IBA treatment followed by 0.2% IBA treatment and the lowest was in the control cuttings. Similar result was reported by Castillo (1990) as they observed the maximum number of roots in base cuttings treated with 1000ppm IBA. Kamaluddin et al. (1996) reported a significant increase in root number as well as rooting percentage with the application of IBA in vascular plant, *Artocarpus heterophyllus* and recorded the highest number of roots (9.4) in cuttings treated with IBA.

Applied auxin is known to intensify root-forming process in cuttings. For instance, polysaccharide hydrolysis is activated under the influence of applied IBA, and as a result, the contents of physiologically active sugar increases providing materials and energy for meristematic tissues and later for root primordia and roots in cuttings of vascular plants (Ermakove and Zhuravieva 1976). Hassig (1983) examined the function of endogenous root forming components of vascular plants and demonstrated that auxin is required for the development of callus in which root premordia are initiated.

Root length of cuttings

Average lengths of longest roots in nodal leaf cuttings were within the range between 10.1 cm to 14.0 cm and in tip cuttings between 9.9 cm and 12.1 cm without any significant difference in the average lengths of the longest roots among the treatments or between the cutting types. However, in nodal leafy cuttings, the highest root lengths (14.0 cm) was obtained from the cuttings treated with 0.4% IBA solution followed by the 0.1% IBA (13.9 cm) and the lowest (10.1cm) was found in untreated cuttings (Table 1 and figure 6). Interestingly, the highest root lengths in tip cuttings (12.1 cm) was observed in the cuttings treated with 0.1% IBA solution followed by 0.4% IBA (12.0 cm) and the lowest (8.2 cm) was found in cuttings treated with 0.8 % IBA solution (Table 1 and figure 6) – which is totally reverse compared to nodal leafy cuttings. Further experiment is needed to explain the reason behind this total contrast between nodal leafy cutting and tip cuttings. Hossain et al. (2006) reported earlier that in *B. vulgaris* var. *striata* branch cuttings, average length of the longest roots was maximum for 0.2% IBA treatment followed by 0.4% IBA treatment and the lowest was in the control.

Cutting Morphology

Cutting lengths and cutting diameters

Cutting lengths varied from 25.7 cm to 28.3 cm in nodal leafy cuttings and 18.9 cm to 20.9 cm in tip cuttings (Table 2 and figure 2). Cutting diameter varied from 4.6 mm to 5.4 mm in nodal leafy cuttings and 2.2 mm to 3.1 mm in tip cuttings (Table 2 and figure 2). There was no significance difference among the cutting lengths or cutting diameters between the types of cuttings due to the treatment. However, the length and diameter of the cuttings in each cutting types were kept indifferent to avoid the non-treatment variation in the experiments.

Steckling Capacity

The survival percentage of rooted cuttings of *B. vulgaris* in the nursery was observed significantly higher in the cuttings treated with IBA than control. In nodal leafy cuttings and tip cuttings, survival percentages were 73.3 to 93.3 and 66.7 to 90 respectively (Figure 7). The highest survival percentage in nodal leafy cutting (93.3) was observed in cuttings with 0.8% IBA treatment followed by 0.4% IBA (86.7) treated cuttings and the lowest (73.3) was in untreated cuttings. In tip cuttings, the highest survival percentage (90) was for 0.8% IBA treatment followed by 0.4% IBA treatment (83.3) and the lowest (66) was in control (Figure 7). The cutlings' growth patterns are also shown in figures 8 and 9 - three and ten months after transferring them to polybags.

The survival percentage of cutlings (rooted cuttings) was higher in IBA treated ones in both types of branch cuttings. The result of the present study is in line with the report by Hossain et al. (2005) who observed the highest survival percentage (95.2) was in the cuttings rooted with IBA solution and the lowest (90) in the controlled cuttings. Pattanaik et al. (2004) reported 100% field survivals of *B. balcooa* cutting treated with 200 ppm IBA two years after field planting. However Hossain et al. (2006) reported that the survival percentage did not vary between the cutting types and the concentrations of rooting hormones in the rooted branch cuttings of *B. vulgaris* var. *striata*. The higher survival potential in the cuttings rooted with IBA solution over the control cuttings is due to the higher number of roots produced in the cuttings treated with IBA as evident in figures 5 and 6. However, there was no report found that discussed the inner mechanism behind such augmentation in the survival percentage of the rooted cuttings of bamboo which can be an interesting new field of investigation.

Conclusion

Rooting ability of two types of branch cuttings (nodal leafy cuttings and tip cuttings) of *B. vulgaris* was investigated under four concentrations of IBA solution with respect to a control. The highest rooting percentage and the number of roots developed per cutting were observed in the cuttings treated with 0.8% IBA solution in both the types of cuttings and the lowest was in the cuttings without treatment. In steckling performance IBA treated cuttings showed better survival capacity in the nursery condition than control. The result of the present study could help in opening a new avenue for the propagation of this variety of multipurpose bamboo since both types of branch cuttings showed potential in developing roots, survive and rhizome formation. However the rooting percentage of leafy branch cuttings of *B. vulgaris* in this study was 56 and we believe there is further scope of increasing the rooting ability for the species. Furthermore, the performance of planting stock developed through these methods was not assessed in field due to the limitations in time for the study. This might be one of the important aspects for future study.

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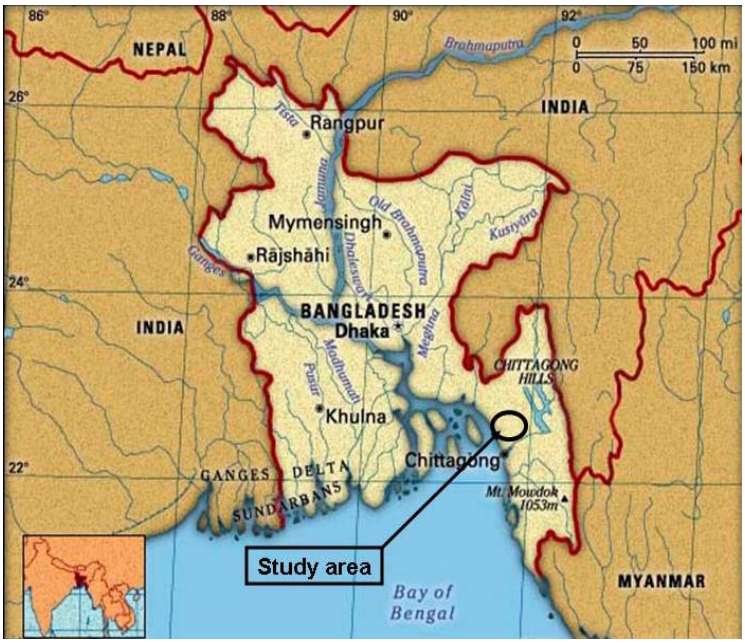

Figure 1: Study area (Adopted from Encyclopaedia Britannica inc. 1999)

Figure 2: Nodal leafy cuttings with base (left) and tip cuttings (right) of *B. vulgaris* are ready for rooting trial.

Figure 3: Non-mist propagator (left) and the cuttings rooted in perforated plastic trays filled with coarse sand mixed with gravel in the propagator (right).

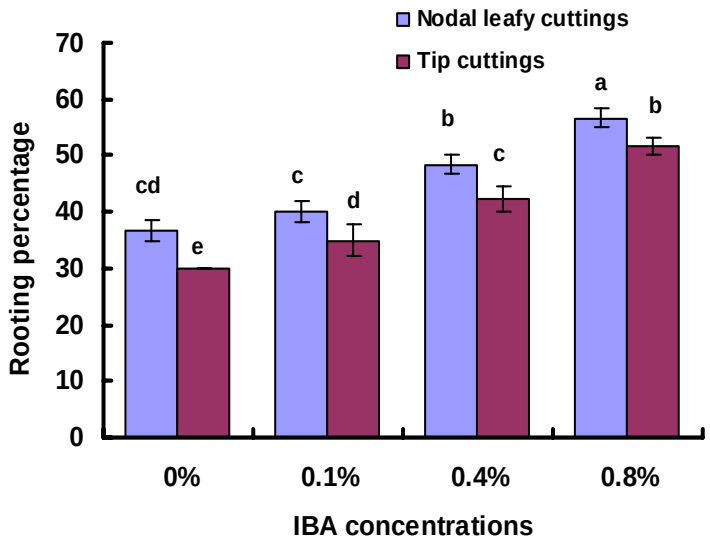

Figure 4: Rooting percentage of nodal leafy cuttings and tip cuttings of *B. vulgaris* under various treatments. Same letters indicate no significant difference at $p<0.05$ (ANOVA and DMRT). Bar indicates the standard error of means.

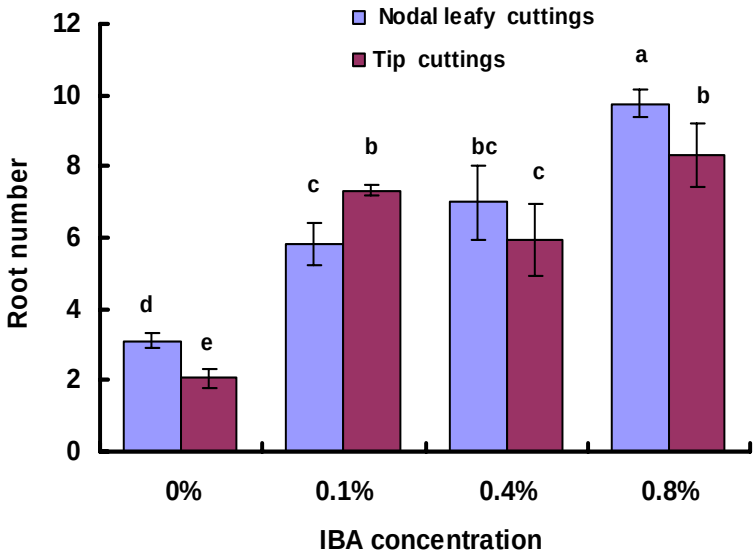

Figure 5: Number of root developed in nodal leafy cuttings and tip cuttings of *B. vulgaris* under various treatments. Same letters indicate no significant difference at $p<0.05$ (ANOVA and DMRT). Bar indicates the standard error of means.

Figure 6: Rooting ability of nodal leafy cuttings (left) and tip cuttings (right) of B. vulgaris under various treatments.

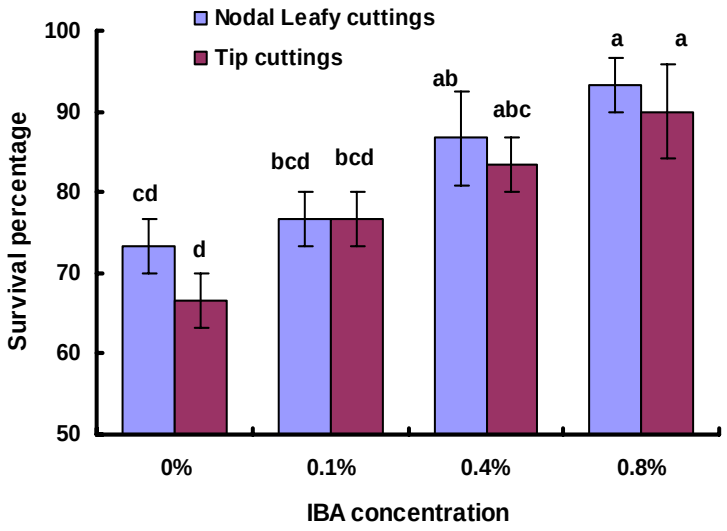

Figure 7: Survival percentage of nodal leafy cuttings and tip cuttings of *B. vulgaris* ten months after transforming the rooted cuttings in to the polybags. Same letters indicate no significant difference at $p<0.05$ (ANOVA and DMRT). Bar indicates the standard error of means.

Figure 8: Steckling performance of leafy cuttings (left) and the tip cuttings (right) of *B. vulgaris* three months after transferring the rooted cuttings in the polybags.

Figure 9: Cutlings of ten months old developed from nodal leafy branch cuttings (left) and tip cuttings (right) are ready for out planting.

Table 1: Average length (cm) of root developed in the cuttings under various concentration of IBA solution. NS: Not significant at *P<0.05* (ANOVA and DMRT).

Cutting types	IBA concentrations				
	0%	0.1%	0.4%	0.8%	*p*
Nodal leafy cuttings	10.1±0.41 [a]	13.9±0.95 [a]	14.0±0.74 [a]	12.8±2.03 [a]	NS
Tip cutting	10.2±0.70 [a]	12.1±2.09 [a]	12.0±1.68 [a]	9.9±0.44 [a]	NS

Note: Same superscript letters indicate no significant difference at $p<0.05$ (ANOVA and DMRT). ± indicates the standard error of means.

Table 2: Length and diameter of nodal leafy cuttings and tip cuttings of *B. vulgaris* under various treatments. NS: Not significant at *P*<0.05 (ANOVA and DMRT).

	Cutting types	IBA concentrations				*p*
		0%	0.1%	0.4%	0.8%	
Length (cm)	Nodal leafy cuttings	28.3±0.15 [a]	26.5±0.55 [a]	26.9±0.76 [a]	25.7±0.75 [a]	NS
	Tip cutting	20.3±0.58 [a]	19.8±0.87 [a]	20.9±0.87 [a]	18.9 ±0.13 [a]	NS
Diameter (mm)	Nodal leafy cuttings	4.6±0.30 [a]	5.4±0.54 [a]	5.3±0.85 [a]	4.7±0.39 [a]	NS
	Tip cutting	2.4±0.23 [a]	2.8±0.23 [a]	3.1±0.10 [a]	3.0±0.16 [a]	NS

Note: Same superscript letters indicate no significant difference at $p<0.05$ (ANOVA and DMRT). ± indicates the standard error of means.

Micropropagation of Economically Important Bamboo *Dendrocalamus hamiltonii* through Axillary bud and Seed culture

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Abstract

An efficient and reproducible protocol for the large scale propagation of *Dendrocalamus hamiltonii* is described. Nodal segments from mature clump and seeds were used as explants for establishment of cultures. To establish aseptic cultures nodal segments were surface sterilized with 0.1% $HgCl_2$ for 10-15 min. while the seeds were disinfected with sodium hypochlorite (4%) for 20 min. For axillary bud break and shoot induction from seeds, sterilized explants were inoculated on MS medium supplemented with cytokinins. Axillary shoots (3-4 shoots) from nodal explants proliferated within 10 days of culture on MS medium supplemented with 1.0mg/l BAP. Multiple shoots were formed within 3-5 weeks of seed culture. 7-8 shoots were obtained when seeds were inoculated on MS medium supplemented with 7.0mg/l BAP. The initiated shoots were excised from mother explants and further multiplied on MS medium supplemented with defined plant growth regulators. Best shoot multiplication was observed on MS medium supplemented with BAP (1.0-10mg/l). A regular subculture in every 3-4 weeks increased the rate of multiplication. To initiate *in- vitro* rooting, pulse treatment was given in 2-step procedure. Excised propagules of 3-5 shoots were inoculated on MS medium supplemented with high concentration of auxin (IBA) for 7 days, later on these *in- vitro* shoots were transferred to half strength MS medium without auxin for 10-15 days to obtain well rooted plants. Plantlets were hardened, acclimatized and established in soil, where they exhibited normal growth.

Keywords: *Dendrocalamus hamiltonii;* bamboo; micropropagation; nodal segment; seed.

Introduction

Bamboos are versatile multipurpose forest product, which are important economically and are often referred to as 'GREEN GOLD'. The bamboos occupy a special place in the lives of rural poor and rural industries, especially in Asia.The most important use of bamboo is as a raw material in pulp, paper and rayon industries. Apart from industrial use, bamboos are utilized in the making of mat boards, roofing, furniture, agriculture implements, and baskets for construction and for numerous traditional uses (Anonymous 1978; Rao et al. 1990).The multifarious uses of bamboos, have increased their demand much beyond the availability. Bamboo is threatened because of its monocarpic habit and increased market demand. It has traditionally been propagated through seed or through vegetative means, but these methods besets with many problems. Seeds of most

bamboo species have short viability, and lasts only for a few months (Nadgir et al. 1984). The conventional vegetative propagation through cuttings and rhizome is undependable due to the bulky size of the propagules in the required number (Rao et al. 1990). The potential of micropropagation has raised high hopes and a lot of research has been focused on the development of protocols for rapid and large scale propagation (Rao et al. 1985; Nadgauda et al. 1990, Godbole et al. 2002; Sood et al. 2002). The *Dendrocalamus hamiltonii* is one such economically important species which is distributed in the North- West Himalaya, Sikkim, Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Tripura of India, Bhutan and Bangladesh. Flowering cycle is reported to be 30-40 years. It is popular for its strong culms that are used for construction purposes, the tender shoots are used for preparation of ' hiyup' a sour pickle by the tribals of Arunachal Pradesh in India. Its leaves also serve as a fodder for animals.The present study was undertaken to establish a protocol for efficient *in-vitro* propagation of *D. hamiltonii* from nodal segments of mature clump and by high frequency shoot proliferation from the seeds since seed propagated plants are expected to last a full life span.

Materials and Methods.

Axillary bud proliferation from nodal segments

The efficiency of shoot multiplication from axillary bud proliferation was tested by culturing the nodal segment from the lateral branches of a 10 year old vegetatively propagated plant of *D. hamiltonii* growing at the Bamusetum, FRI, Dehradun. Nodal segments (about 2.5-3.0 cm in length) were disinfected with 0.1%$HgCl_2$ for 10-15 min., rinsed with distilled water for three to four times, both the ends were trimmed & segments were cultured on both liquid and semisolid MS medium supplemented with varying concentrations of cytokinins. The medium was adjusted to pH 5.8 prior to autoclaving at 121^{o}C for 15 min. Cultures were maintained at 25^{o}C ± 2 ^{o}C under a 16h photoperiod with a photon flux density of 2500 lux from white fluorescent tubes.

Seed germination

Mature seeds of *Dendrocalamus hamiltonii* were obtained from The Sheel Biotech Company, New Delhi, India. After dehusking carefully, the healthy seeds were selected and surface sterilized with sodium hypochlorite (4%) for 20 min. followed by three to four rinses with sterile distilled water to remove the traces of sterilant. Disinfected seeds were then cultured aseptically on semi- solid MS medium containing 3% sucrose supplemented with varying concentrations of cytokinin (1.0- 10mg/l BAP/ Kn). The medium was gelled with agar (0.7%), adjusted to pH 5.8 prior to autoclaving at 121^{o}C for 15 min. Cultures were maintained at 25^{o}C ± 2 ^{o}C under a 16h photoperiod with a photon flux density of 2500 lux from white fluorescent tubes.

In vitro shoot multiplication

Well developed *in vitro* shoots from both seeds and nodal segments were excised and transferred to MS medium (Murashige & Skoog's 1962) supplemented with different concentrations of BAP & Kn alone or in combination for achieving maximum shoot multiplication. Subcultures were performed at intervals of 3 weeks, by separation of shoots in propagules of varying number of shoots to see the effect of size of propagule on *in vitro* shoot multiplication and transferred to fresh medium. Effect of pH ranging from 4.0 to 7.0 in medium was studied for

shoot multiplication. Different concentration of sucrose (0-5%) was studied to see the effect on shoot multiplication. Different strength of MS medium i.e. full, ¾, ½ & ¼ were tried for optimization of multiplication medium.

In vitro Rooting of Shoots

After 3 weeks of incubation, propagules consisting of 3-5 shoots were transferred to rooting medium (MS medium supplemented with 1.0- 10.0mg/l NAA or 5.0-30.0mg/l IBA). To initiate *in- vitro* rooting, pulse treatment was given in 2- step procedure. Excised propagules of 3-5 shoots were inoculated on MS medium supplemented with high concentration of auxin (IBA) for 7 days, later on these *in- vitro* shoots were transferred to half strength MS medium without auxin for 10-15 days to obtain well rooted plants. After 4 weeks in culture, data were collected on the percentage of rooted propagules and the number of roots per propagule.

Hardening and acclimatization of in vitro propagated plants

The rooted plants were removed from the flasks, washed thoroughly with water to remove all traces of medium attached to the roots and then transferred to glass bottles containing 1/3 volume of soilrite. Plants were fed with half strength macro and micro nutrients of MS medium thrice a week in the mist chamber under RH 85-90% with a temperature of 32^{o}C. Acclimatization of these plants was carried out in shade house in polybags containing a mixture of sand: farmyard manure (FYM): soil in ratio of 1:1:1 for two months. Hardened plants were transferred to bigger pots and transferred to net house.

Results and discussion

Tissue culture technique is being applied for *in-vitro* propagation of *D. hamiltonii* using nodal segments from mature clump and seed as explants.

Axillary bud proliferation from nodal segments

In case of nodal explant from mature clump, axillary shoots proliferated within 10 days of culture on MS medium supplemented with BAP. Best bud break with 3-4 shoots was obtained on liquid MS medium supplemented with 1.0mg/l BAP (Figure A, Table 1). BAP when used along with Kn in the MS medium did not improve the seed response as compared to BAP when used alone in the MS medium. After 3 weeks these buds were excised from mother explant and cultured on MS medium containing different concentrations of BAP (1.0-5.0 mg/l).

Seed germination

In the present study 25% of seeds germinated and formed shoots without root formation on semisolid MS medium containing BAP. The ability of the seeds to form multiple shoots was dependent on the concentration of BAP in the medium (Table 2). Maximum germination was recorded at a concentration of 7.0 mg/l BAP supplemented in the MS medium where 7-8 shoots were formed immediately after seed germination within 3 weeks of culture (Figure B). BAP induced direct shoot regeneration from aseptic seed culture has also been

reported in *Dendrocalamus asper* (Arya et al.1999). BAP when used along with Kn in the MS medium did not improve the seed response as compared to BAP when used alone in the MS medium. Multiple shoots developed from the seeds were excised and subcultured for further multiplication on MS medium containing 1.0-5.0 mg/l BAP.

In vitro shoot multiplication

The shoots proliferated from the seeds and axillary buds were established on MS medium and were used for *in vitro* shoot multiplication. Best multiplication of proliferated shoots was obtained on MS medium supplemented with 3.0mg/l BAP, when propagule of four shoots was subcultured every four weeks. Shoot multiplication rate of 7-8 folds was obtained from seed culture on MS medium supplemented with 3.0 mg /l BAP in a period of every four weeks (Figure C, Table 3). Subculture period showed its effect on multiplication rate of *in vitro* shoots. Best multiplication rate was obtained when the shoot culture were regularly subcultured on fresh medium every four weeks. Cultures when left without subcuturing for 5 weeks showed necrosis. The shoot multiplication capacity of the *in vitro* shoots was greatly influenced by the BAP concentration in the medium and size of the propagule used for shoot multiplication. Similar results have been reported in *Dendrocalamus asper* (Arya et al. 1999). The best shoot multiplication rate was obtained on MS medium supplemented with 3.0mg /l BAP when propagule of four shoots was subcultured (Table 4). At decreased level of BAP the shoot multiplication rate decreased with an increase in shoot length. Similar results on BAP supplemented medium have been reported in a number of bamboos (Arya and Arya1997; Arya and Sharma 1998; Chambers et al. 1991; Purtpongse Gavinlertvatana 1992).The shoot multiplication rate declined sharply if propagule of less than 3-4 shoots was cultured. On decreased level of BAP (1.0-2.0 mg/l) as well as high concentration of BAP (4.0-9-0 mg/l) the multiplication rate of *in vitro* shoots was reduced. The *in vitro* shoot multiplication cycle were carried out in liquid as well as in semisolid medium. The shoot multiplication was better in the liquid medium as compared to semisolid medium in respect to multiplication rate and shoot development. The shoots were relatively healthy on liquid medium. This may be because of better uptake of nutrients by the cultures. Similar results were earlier reported in *Bambusa tulda* (Saxena 1990), *Dendrocalamus giganteus* (Arya et al. 2006) and *D. strictus* (Nadgir et al. 1984). However, the shoot multiplication cycles were carried out in semisolid medium due to easy handling of cultures and to avoid vitrification of shoots. The hydrogen ion concentration of the medium effect growth of the tissue by altering pH of the cells, because higher 'H' ion concentration induced precipitation of phosphates, gelatinization of agar and destruction of vitamins and growth regulators. In the present study, slightly acidic medium favours increase in shoot multiplication rate whereas, alkaline medium reduced multiplication rate. Maximum multiplication of shoots was obtained on MS medium having 5.8 pH. Effect of different strengths of MS medium supplemented with 3.0 mg/l BAP was also tested for *in vitro* shoot multiplication. Full strength MS salts yielded best shoot multiplication and overall growth of shoots and leaves. At reduced strength pale yellow leaves and thin shoots developed. Sucrose has been widely used as carbon source for various plant tissues in cultures. Therefore, study was conducted to see the effect of different concentrations of sucrose ranging from 0.0-5.0 % on *in vitro* shoot multiplication rate. Addition of sucrose in the medium enhanced shoot multiplication. 3.0 % of sucrose in the MS medium was found to be the optimal requirement for shoot multiplication. At lower concentration of sucrose, reduced multiplication rate was obtained along with yellowing of leaves.

In vitro rootingof shoots

The multiple shoots in both types of cultures were found to initiate roots following a pulse treatment in a MS medium supplemented with 20mg/l IBA for 7 days. After incubation in the above medium these were transferred to half strength MS medium for 10-15 days to obtain well rooted plants (Figure D). Effective role of IBA in bamboos for rooting has also been reported in *Dendrocalamus giganteus* and *D. strictus* (Das and Rout 1991).

Hardening and acclimatization of in vitro propagated plants

In vitro propagated plants are generally susceptible to transplantation shock due to delicate root system, reduced amount of epicuticular wax and reduced stomata. This can result in excessive dehydration, poor control of gaseous exchange. Therefore, a gradual procedure of hardening and acclimatization of the plantlets is required. The rooted plants were removed from the flasks, washed thoroughly with water, transferred to soilrite for hardening (Figure E). Plants were fed with half strength macro and micro nutrients of MS medium thrice a week in the mist chamber under RH 85-90% and at 32^{o}C. Acclimatization of these plants was carried out in shade house in polybags containing a mixture of sand: farmyard manure (FYM): soil in ratio of 1:1:1 for two months and established in pots where they exhibited normal growth (Figure F).

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Table 1: Effect of BAP concentration in MS medium on *in vitro* axillary bud proliferation of *D.hamiltonii*

BAP	Response %	Mean shoot number	Mean Shoot Length (cm)
0.0 mg/l	20.12 ± 2.85	3.26 ± 0.01	2.58 ± 0.01
1.0 mg/l	58.17 ± 4.43	4.03 ± 0.03	2.85 ± 0.03
3.0 mg/l	51.00 ± 3.10	3.01 ± 0.04	2.47 ± 0.01
5.0 mg/l	44.47 ± 2.34	2.03 ± 0.02	2.17 ± 0.01
7.0 mg/l	38.20± 3.70	1.47 ± 0.01	2.20 ± 0.02
9.0 mg/l	39.50 ± 4.58	1.27 ± 0.01	2.11 ± 0.01

Mean of 30 replicates ± Standard Error

Table 2: Effect of BAP concentration in MS medium on *in vitro* shoot formation from seeds of *D. hamiltonii*

BAP	Response %	Mean shoot number	Mean Shoot Length (cm)
0.0 mg/l	12.83 ± 1.42	1.11 ± 0.06	1.82 ±0.01
1.0 mg/l	17.87 ± 1.17	1.33 ± 0.05	1.71 ±0.01
3.0 mg/l	19.33 ± 0.76	1.89 ± 0.05	1.75 ±0.02
5.0 mg/l	22.67 ± 1.54	3.05 ± 0.05	1.89 ±0.01
7.0 mg/l	26.77 ± 1.75	8.03 ± 0.03	2.17 ±0.02
9.0 mg/l	22.50 ± 2.08	6.52 ± 0.04	1.96 ±0.01

Mean of 30 replicates ± Standard Error

Table 3: Effect of BAP concentration on *in vitro* shoot multiplication from seed culture of *D. hamiltonii*, after four weeks in culture. Propagules of four shoots were cultured.

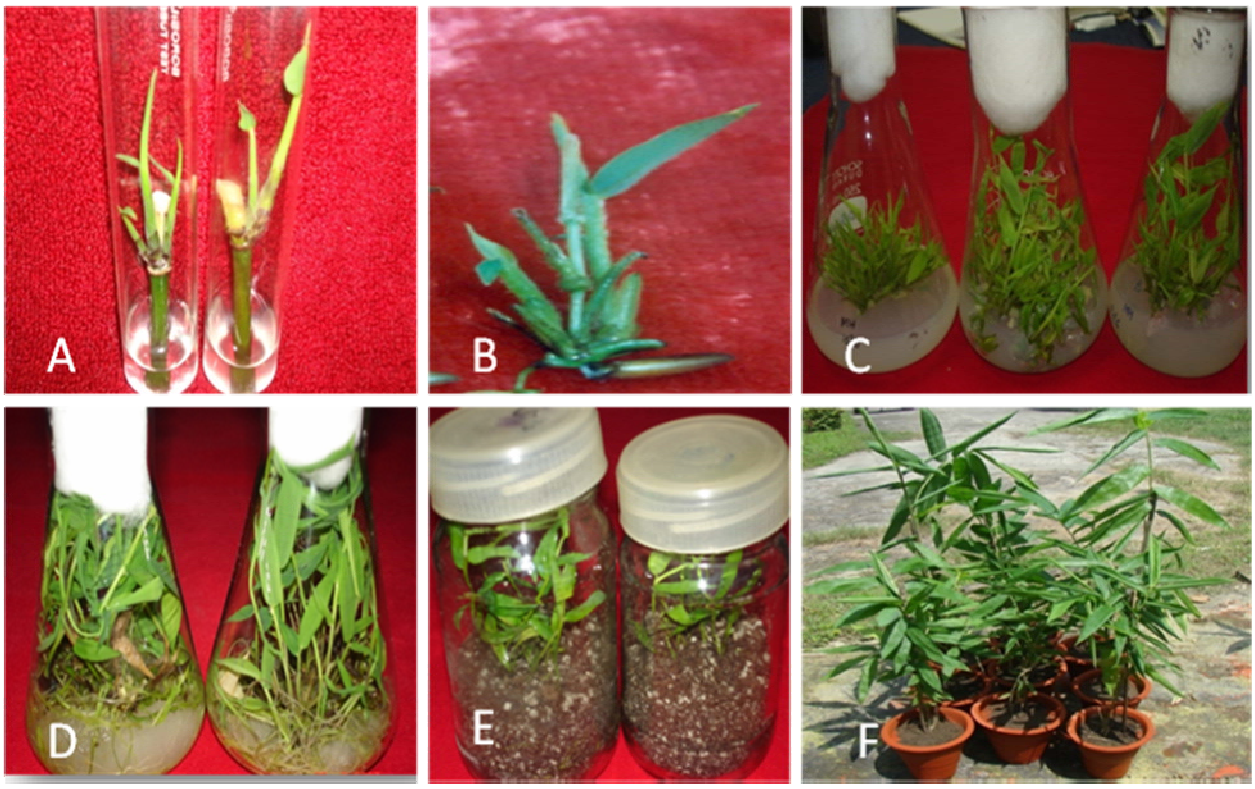
BAP	Mean shoot number	Mean Shoot Length(cm)	Multiplication fold
1.0 mg/l	19.10±0.06	2.04±0.01	4.78±0.01
2.0 mg/l	23.10±0.05	2.27±0.01	5.78±0.01
3.0 mg/l	29.10±0.06	2.32±0.02	7.28±0.01
4.0 mg/l	28.60±0.02	1.94±0.01	7.15±0.01
5.0 mg/l	27.90±0.04	1.88±0.02	6.98±0.01

Mean of 30 replicates ± Standard Error

Table 4: Effect of the size of *D. hamiltonii* propagule on *in vitro* shoot multiplication rate, after four weeks in culture on MS+3.0mg/l BAP.

No. of Shoots inoculated	Mean shoot number	Mean Shoot Length (cm)	Multiplication fold
1 shoot	1.00±0.00	1.72±0.02	1.00±0.00
2 shoots	4.03±0.02	1.75±0.03	2.02±0.01
3 shoots	15.83±0.03	1.90±0.01	5.28±0.01
4 shoots	29.27±0.12	2.20±0.02	7.32±0.03
5 shoots	26.73±0.09	2.18±0.01	5.35±0.02
6 shoots	24.80±0.12	2.13±0.01	4.13±0.02

Mean of 30 replicates ± Standard Error



Micropropagation of *Dendrocalamus hamiltonii* through axillary and seed culture
Fig.A Axillary bud proliferation from nodal segment on MS +1.0mg/l BAP.
Fig.B Multiple shoots formation from seed cultured on MS + 7.0mg/l BAP.
Fig.C *In vitro* shoots multiplication on MS + 3.0mg/l BAP.
Fig. D *In vitro* rooting
Fig. E *In vitro* hardening of plantlets
Fig. F *In vitro* raised plants in pots.

Propagation of Bamboos through Tissue Culture Technology and Field Plantation

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Abstract

An efficient and reproducible technology for the large scale propagation of bamboos is developed. High frequency direct shoot proliferation was achieved through axillary bud culture and through aseptic seed culture. So far tissue culture technology has been developed for nine economically important bamboo species for large scale multiplication. In all these species multiple shoots were induced in *in-vitro* culture of nodal shoot segment containing axillary bud through forced axillary branching in case of *Dendrocalamus asper, D.strictus, D. membranacaeus, D. giganteus, D. hamiltonii*, *Bambusa bambos, B. vulgaris, Drepanostachyum falcatum, Melocanna baccifera* and direct shoot proliferation obtained in aseptic seed cultures of *D. asper, D. hamiltonii* and *D. falcatum.* Best response towards axillary bud break was obtained on BAP supplemented medium. These axillary shoots were cultured and with the passage of subculture yielded 4-16 fold *in vitro* shoot multiplication. A propagule of three shoots was found to be the best for rapid shoot multiplication in 4 week subculture cycle. 80-98% *in vitro* rooting was obtained with IBA or NAA supplemented MS medium. Aseptic seeds when used as explant were cultured on MS medium supplemented with 1.0-10.0 mg/l BA. Multiple shoots were formed within 3-5 weeks of seed culture without root formation. The shoot forming capacity of seeds was critical and was influenced by the BA concentration in the medium. *In vitro* shoot cultures were established from the initial shoots that developed from the seed. These *in vitro* shoots were later rooted on auxin (NAA &IBA) supplemented medium. In both the cases a very high rate of transplantation (Lab to field) and plant survival was obtained. The success limits to the gradual procedure of hardening and acclimatization of plantlets. Plantation work undertaken at 5x5 field spacing gave good results and so far thousands of plants have been field planted. Both the procedures described is of high value as clonal propagation is promoted on one hand (through axillary bud culture) with genetic widening of planting stock is achieved on other hand through seed culture.

Introduction

Bamboos, the world's fastest growing and environment- friendly giant grass, has now gained international recognition and priority, leading to its recognition as an important non- woody timber resource and a versatile commodity of considerable economic importance. It perhaps has the singular distinction of being the only natural resource put so many and so varied uses (John and Nadgauda 1995). The multifarious uses of bamboos have increased their demand much beyond the availability. This trend of increasing demand and decreasing supply is expected to continue in future. With respect to vegetative propagation, cuttings, offsets and rhizomes

are bulky, difficult to handle and transport and survival of plantlets is also low (Hasan 1980). The production of seed is irregular with short viability, seed sterility, poor seed setting during off season flowering, and flowering once before culm death (McClure 1966; Janzen 1976; Nadgir et al. 1984). For mass scale propagation tissue culture is the only viable method. Indeed, the order of magnitude of the demand for bamboo planting materials indicates that micropropagation will inevitably be necessary for mass scale propagation (Subramanlam 1994; Gielis 1994).

Attempts have been made in the recent past to micropropagate bamboos, but plants have been produced in small numbers only in species mainly through somatic embryogenesis (Rao et al. 1985; Yeh and Chang 1986a, b, 1987), organogenesis (Huang et al. 1989) and axillary branching (Nadgir et al. 1984; saxena 1990). So far we have successfully developed tissue culture technology for large scale multiplication of nine economically important bamboo species, viz. *Dendrocalamus asper, D. membranaceus, D. strictus, D. giganteus, D. hamiltonii, Bambusa arundinacea, B. vulgaris, Melocanna baciferra* and *Drepanostachym falcatum* through axillary bud culture and aseptic seed culture. So far around 40, 000 plants have been produced and the protocol developed is presently used by Biotech companies producing tissue culture plants in million in India.

Material and methods

Axillary Bud Proliferation

Young and juvenile shoots of different species were collected from the mother plants. Nodal segments with single axillary buds were used as source material. After removal of the leaf sheath, individual nodes were washed with dilute solution of 5-10 drops Tween-20 (HiMedia, India) per 100 ml of distilled water for 10 min. followed by running tap water for 15 min. Pre-disinfection treatments was given to reduce the contamination, where nodal segments were treated with a mixture of aqueous solution of fungicide bavistin (BASF, India) and bactericide streptomycin (HiMedia, India) at a concentration of 0.1% each for 20 min. For surface sterilization, nodal segments were treated with aqueous solution of 0.1% $HgCl_2$ (HiMedia, India) for 8-10 min. After three washings in sterile distilled water the surface disinfected axillary buds were inoculated on liquid MS (Murashige and Skoog's 1962) medium supplemented with cytokinin. Different concentrations of 6-benzylaminopurine (BAP) or 6-furfurylaminopurine (Kn) alone or in combination were used in liquid MS medium for bud break. The pH of the medium was adjusted to 5.8 by using 1 N NaOH or 1 N HCl prior to autoclaving. The liquid medium (10ml) was dispensed into 25 x 150mm test tubes (Borosil, India). The culture tubes with media were autoclaved 121^0C for 20 min. Plants were field planted following simple silvicultural practiced keeping 5x5 m & 4x4 m spacing.

Seed germination

Mature seeds of *Drepanostachym falcatum, D. asper, D. hamiltonii* were collected. After dehusking carefully, the healthy seeds were selected and surface sterilized with sodium hypochlorite (4%) for 20 – 30 min. followed by three to four rinses with sterile distilled water to remove the traces of sterilant. Disinfected seeds were then cultured aseptically on semi- solid MS medium containing 3% sucrose supplemented with varying

concentrations of cytokinin (1.0- 10mg/l BAP/ Kn). The medium was gelled with agar (0.7%), adjusted to pH 5.8 prior to autoclaving at 121^{o}C for 20 min.

Shoot multiplication

Proliferated *in-vitro* shoots were separated into clumps (four shoots) and used for further shoot multiplication. Various concentrations of BAP (0-25µM) or Kn (0-25µM) alone or in combinations were used in liquid and semisolid MS medium. Subculturing was carried out every 4 weeks on fresh shoot multiplication medium. Established cultures were maintained on semi- solid medium. The number of shoots cultured and the number of shoots derived at the end of subculture gave the multiplication rate.

In vitro Rooting

In-vitro raised shoots (three or four shoots) were used for root induction. Half strength liquid MS medium with various concentrations of indole-3-butyric acid (IBA; 5-30µM) or α-napthalene acetic acid (NAA; 5-30µM) alone was used. The number of individual shoot propagules that responded for rooting were counted and expressed as percent rooting.

Culture conditions

All cultures were incubated at 25 ± 2ºC temperature and illumination of 16hrs photoperiod with light intensity of 2400 lux, obtained by white cool fluorescent tubes of 40 watts (Philips, India).

Hardening and acclimatization

Rooted plantlets were taken out from the flasks, washed to remove adhered medium and then transferred to autoclaved 250 ml screw cap glass bottle containing 1/3 volume of soilrite. These plantlets were nurtured with half strength MS medium (without organics) twice a week for two weeks and were kept in tissue culture incubation room. After two weeks these bottles were shifted to mist chamber having relative humidity of 70-80% with a temperature of 30 ± 2^{0}C. The caps of bottles were removed and plantlets were allowed to remain in the bottle for one week before they were transferred to polybags containing a mixture of sand, farmyard manure and soil in a ratio of 1:1:1. In the mist chamber, the plants were kept for four weeks and were irrigated with half strength MS medium. Later, these polybags were shifted to high-density double deck agronet open shade house for acclimatization.

Results

Axillary Bud Proliferation

Axillary bud break was obtained in nodal segments within 15-20 days, when cultured on MS medium supplemented with cytokinin. The morphogenic response of explant towards axillary bud proliferation was markedly influenced by the concentration of growth regulator in the medium. A cluster of 4-15 shoots normaly proliferated from the axilary bud depending on the culture conditions. Under the optimal media conditions the

axillary shoot proliferation reached upto 5-8 axillary shoots in *D. strictus*, *D. giganteus* (MS + 2.0 to 5.0 mg/l BAP), 8-10 shoots in *D. membranaceus* (MS + 1.0 to 5.0 mg/l BAP + 0.5 mg/l NAA), 10-15 shoots in *B. bambos* (MS + 5.0 mg/l BAP), 1-3 shoots in *D. asper* and *B. vulgaris* (MS + 5.0 mg/l BAP), 3-4 shoots in *D. hamiltonii* (MS + 1.0 mg/l BAP) and 10-12 shoots in *Drepanostachym falcatum* (MS + 5.0 mg/l BAP) (Figure 1A). Once the axillary bud break was achieved the axillary shoots production could be increased by regular subculturing on MS medium supplemented with defined concentration of BAP in the MS medium.

Seed germination

In the present study for multiple shoot formation through seeds in *D. falcatum*, maximum germination of 63% was recorded at a concentration of 3.0 mg/l BAP supplemented in the MS medium (Table 3, Figure 2A).In case of *D. hamiltonii* (Figure 2B) and *D. asper* maximum germination was recorded at a concentration of 7.0 mg/l BAP and 5.0 mg/l BAP supplemented in the MS medium respectively. The ability of the seeds to form multiple shoots was dependent on the concentration of BAP in the medium

Shoot multiplication

Response of *in vitro* shoot multiplication varied with cytokinin type and its concentration used in the medium. The proliferated shoots from the axillary buds were excised into groups of shoot clusters and subcultured on liquid (paper bridge) as well as on semisolid MS medium supplemented with 2.0 – 10.0 mg/l BAP for further shoot multiplication. During first to third subculture an average shoot multiplication rate of three fold was obtained in the liquid/semisolid MS medium supplemented with 2.0-5.0 mg/l BAP. Once the shoot cultures were established they were excised into groups of shoots (called as propagule), subcultured and multiplied on MS medium supplemented with 2.0-5.0 mg/l BAP. Regular (four week interval) subculturing of shoot propagules increased the multiplication rate. A propagule of three to four shoots gave best shoot multiplication rate as compared to smaller (1-2 shoots) or larger size (5-6 shoots) propagules in all the bamboos (Table 6). Repeated subculturing in liquid medium resulted in vitrification of shoots except in case of *D. strictus* and *B. vulgaris*(Figure 2D) . Hence semisolid medium was used in subsequent subcultures for other bamboo species. After 4-6 cycles of shoot multiplication the rate of shoot multiplication increased and later a consistent 4-6 folds average multiplication rate was obtained in case of *B. bambos(*Figure 1B*), B. vulgaris, D. strictus* and *D. giganteus* (Figure 2C). In case of *D. asper* 12-16 folds multiplication was obtained after every subcultured cycle (Table 1). In case of *D. membranaceus* the shoot multiplication rate obtained was 6- 15 fold. A multiplication rate of 9 – 11 folds was obtained in case of *D. falcatum* (Table 2) . In case of *D. hamiltonii* and *Melocanna baciferra* multiplication rate was 7-8 and 2-3 folds respectively.

Rooting of in vitro shoots

Shoots of 2-3cm length were used for various *in vitro* rooting experiments. *In vitro* rooting was obtained when *in vitro* grown shoots were transferred on half strength MS medium supplemented with auxins (IAA, NAA, and IBA). Rooting was obtained when shoot propagules were subcultured on MS medium supplemented with 1.0 - 5.0 mg/l NAA or 10-15 mg/l IBA depending on the bamboo species. Best rooting (80-85%) was obtained within 3-4 weeks of subculture on MS + 3.0 mg/l NAA and on MS + 10 mg/l IBA in case of, *B. bambos* (Table 5,

Figure 2E), *D. asper* (Figure 1D) and *D. membranaceus* (Table 4, Figure 2F). In case of *B. Vulgaris* (Figure 1C), MS + 4.0 mg/l NAA gave the best results for *in vitro* rooting. IBA was found to be better for *in vitro* rooting response as compared to NAA in case of *M. baciferra*. Generally 5-8 roots emerged from the basal end of the propagule. Single shoot when placed for rooting did not survive. Thus a propagule of minimum three shoots was required. Size of the shoot propagule was also found to be critical for rooting.

Hardening and acclimatization

Healthy plantlets with good roots and shoot system developed within 5-6 weeks on rooting medium. During hardening and acclimatization the shoots elongated, leaves turned greener and expanded (Figure 2G). After six months new shoots and rhizomes developed and the plants were ready for field transfer. Around 40,000 plants have been transferred in field so far (Figure 1E & F, 2H & I).

Discussion

For tissue culture of bamboo the use of starting material (seeds or adult plants) and the choice of the propagation method are crucial (Gielis 1999). In addition there is a huge variability in responsiveness in tissue culture (Saxena and Dhawan 1994). In the present investigation, nodal segments containing pre-existing axillary bud from mature clumps and seeds were used to initiate the *in vitro* cultures. The suitability of nodal segments is further reported in the micropropagation of bamboos (Nadgir *et al.* 1984; Prutpongse and Gavinlertvatana 1992; Saxena and Bhojwani 1993; Hirimburegama and Gamage 1995; Ramanayake and Yakandawala 1997; Arya and Sharma 1998; Bag *et al.* 2000; Das and Pal 2005; Sanjaya *et al.* 2005; Arya *et al.* 2006; Jimenez *et al.* 2006; Arya *et al.* 2008).

In present study, axillary bud proliferation was more in number on BAP supplemented medium as compared to medium supplemented with Kn. The efficiency of BAP for shoot culture initiation is also reported in *Bambusa ventricosa* (Huang and Huang 1995); *B. bambos* (Arya and Sharma 1998)*; D. strictus* (Mishra *et al.* 2001); *D. asper* (Arya *et al.* 2002); *D. hamiltonii* (Sood *et al.* 2002); *D. giganteus* (Arya *et al.* 2006); *Guadua angustifolia* (Jimenez *et al.* 2006); *Drepanostachyum falcatum* (Arya *et al.* 2008).

The shoot multiplication capacity of the *in vitro* shoots was greatly influenced by the BAP concentration in the medium and size of the propagule used for shoot multiplication. At decreased level of BAP the shoot multiplication rate decreased with an increase in shoot length. Similar results on BAP supplemented medium have been reported in a number of bamboos (Arya and Arya1997; Arya and Sharma 1998; Chambers et al. 1991; Purtpongse Gavinlertvatana 1992).The shoot multiplication was better in the liquid medium as compared to semisolid medium in respect to multiplication rate and shoot development. The shoots were relatively healthy on liquid medium. This may be because of better uptake of nutrients by the cultures. Similar results were earlier reported in *Bambusa tulda* (Saxena 1990), *Dendrocalamus giganteus* (Arya et al. 2006) and *D. strictus* (Nadgir et al. 1984). However, the shoot multiplication cycles were carried out in semisolid medium due to easy handling of cultures and to avoid vitrification of shoots.

The *in vitro* raised shoots failed to root on plant growth regulator free basal medium. Our observation on root induction in shoots of different species of bamboo reveals that IBA and NAA are effective. A very high rate (80-90%) of transplantation and plant survival was obtained in *D. asper, D. membranaceus, B. bambos* and *B. vulgaris*. The success was attributed to the gradual procedure adopted for hardening and acclimatization of the plants. Besides these values, transplantation rates of 70-80% have been reported only in *D. strictus* (Nadgir et al. 1984) and in *B. tulda* (Saxena 1990). Large scale micropropagation of bamboos has so far not been reported. A high rate of shoot multiplication averaging 5-20 folds with 80-90 percent rooting success and a high rate of plant survival are attractive findings of this study.

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Table 1. Effect of cytokinin (BAP) concentration in MS medium on the multiplication rate of D. asper.

BAP (mg/l)	Mean shoot number	Mean Shoot Length(cm)	Multiplication fold
1.0	14.1 ± 2.9	3.3 ± 0.07	5.6± 0.13
2.0	33.4 ± 3.2	2.3 ± 0.02	12.2±0.17
2.5	41.0 ± 5.1	2.2 ± 0.04	15.3±0.15
3.0	46.5 ± 5.1	1.8 ± 0.05	17.2±0.16
4.0	36.5 ± 3.6	1.2 ± 0.05	13.3±0.16
5.0	30.2 ± 6.5	1.2 ± 0.02	12.2±0.14
7.5	16.3 ± 2.5	1.0 ± 0.01	6.2±0.18
10.0	17.0 ± 4.0	0.8 ± 0.02	7.0±0.14

Mean of 30 replicates ± Standard Error

Table2. Effect of cytokinin (BAP) concentration in MS medium on the multiplication rate of *D. falcatum.*

BAP (mg/l)	Mean shoot number	Mean Shoot Length(cm)	Multiplication fold
1.0	13.92 ± 0.53	2.70 ± 0.01	3.48 ± 0.13
2.0	24.00 ± 0.52	2.32 ± 0.02	6.00 ± 0.13
3.0	43.50 ± 0.63	2.30 ± 0.02	10.87 ± 0.16
4.0	39.92 ± 0.38	2.00 ± 0.01	9.98 ± 0.09
5.0	30.83 ± 0.32	1.02 ± 0.02	7.71 ± 0.08

Mean of 30 replicates ± Standard Error

Table 3: Effect of cytokinin (BAP) in MS medium on shoots production through seeds of *Drepanostachym falcatum.*

BAP (mg/l)	Response %	Mean Shoot number	Mean shoot Length (cm)
0.0	33.33 ± 0.58	1.25 ± 0.13	1.03 ± 0.04
1.0	41.66 ± 0.58	1.40 ± 0.15	2.42 ± 0.05
3.0	62.50 ± 0.60	20.50 ± 0.69	2.68 ± 0.04
5.0	58.33± 0.60	12.83 ± 0.73	3.54 ± 0.10
7.0	50.00± 0.57	5.25 ± 0.35	3.12 ± 0.10
9.0	45.83± 0.58	4.50 ± 0.31	2.52 ± 0.10

Mean of 30 replicates ± Standard Error

Table 4. Effect of auxin (NAA) in MS medium on rooting of *in vitro* shoots of *D. membranaceus,* after 4 weeks in culture. Propagules of three shoots were cultured.

NAA (mg/l)	Mean root number	Response %
1.0	8.72 ± 1.9	73.33 ± 3.12
2.0	14.02 ± 1.2	98.26± 2.87
3.0	13.41 ± 2.1	97.08 ± 3.65
4.0	13.45 ± 2.6	91.66 ± 2.43
5.0	12.72 ± 1.5	73.33 ± 2.67

Mean of 30 replicates ± Standard Error

Table 5. Effect of auxin (NAA) in MS medium on *in vitro* rooting of *Bambusa bambos*, after 4 weeks in culture and the plant survival rate in field.

NAA (mg/l)	Rooting %	Plant survival rate (%)
1.0	15.32 ± 2.9	70.14 ± 2.12
2.0	30.33 ± 2.2	88.26± 2.87
3.0	58.66 ± 3.1	94.32 ± 2.65
4.0	85.32 ± 3.6	96.52 ± 1.43
5.0	81.28 ± 2.5	95.36 ± 1.67

Mean of 30 replicates ± Standard Error

Table 6. Effect of the size of the *D. asper* propagule on the development of the number of shoots and shoot multiplication rate, when cultured on MS + 3.0 mg/l BAP.

No. of Shoots inoculated	Mean shoot number	Multiplication fold
1.0	06.54 ± 0.95	06.3 ± 0.8
2.0	20.88 ± 1.09	10.4 ± 1.5
3.0	47.23 ± 1.56	14.7 ± 2.0
4.0	48.74 ± 2.55	12.3 ± 1.8
5.0	46.84 ± 2.33	09.5 ± 1.6

Mean of 30 replicates ± Standard Error

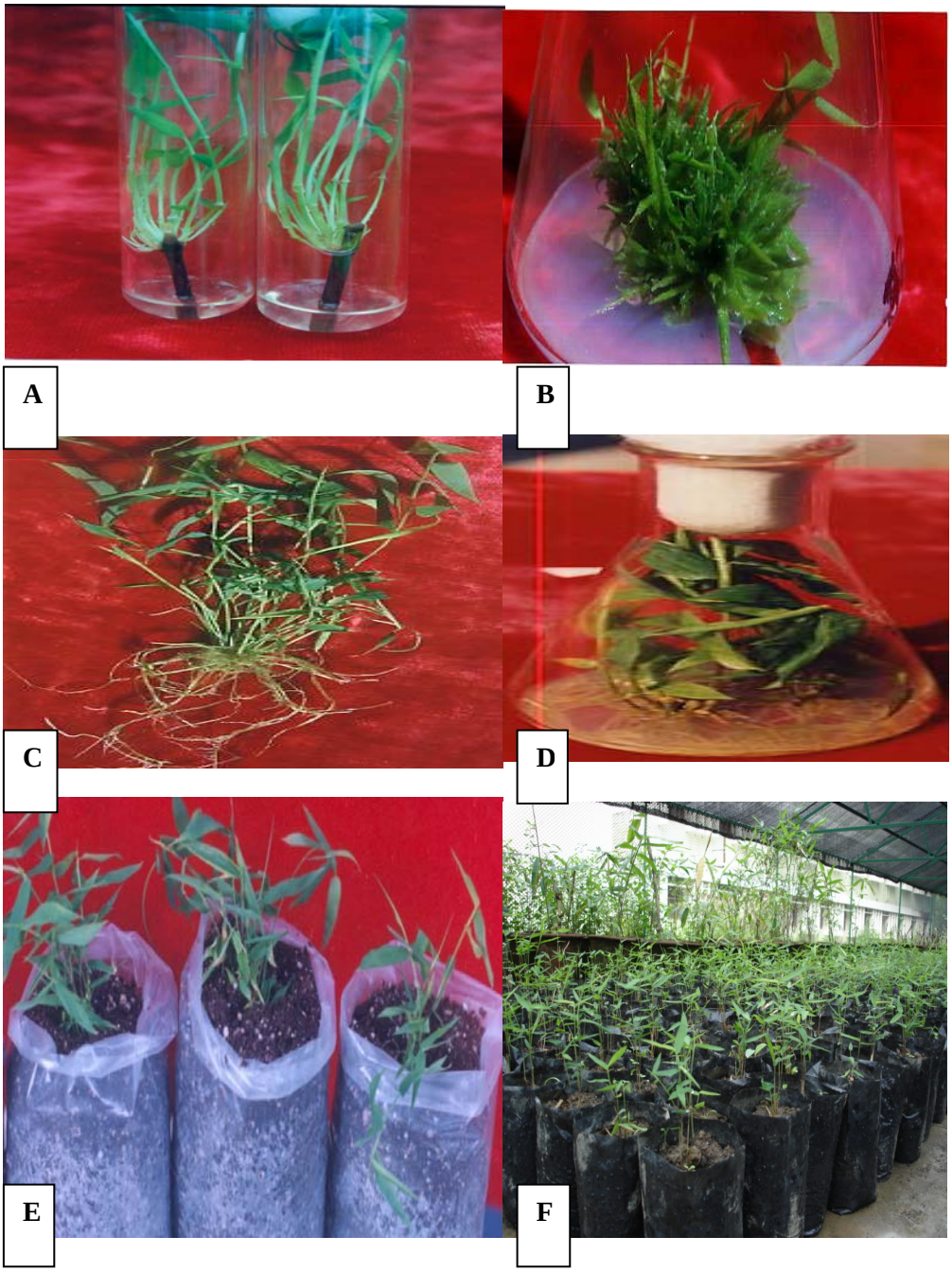

Figure 1:Various stages of plantlet regeneration through micropropagation A. Axillary bud proliferation from nodal segments of *Drepanostachym falcatum*. B. *In vitro* shoot multiplication in *Bambusa bambos*. C. *In vitro* developed roots in *Bambusa vulgaris* D. *In vitro* rooting in *Dendrocalamus asper*. E. *In vitro* raised plantlets of *Dendrocalamus giganteus* after hardening and acclimatization. F. *In vitro* raised plants ready for field transfer.

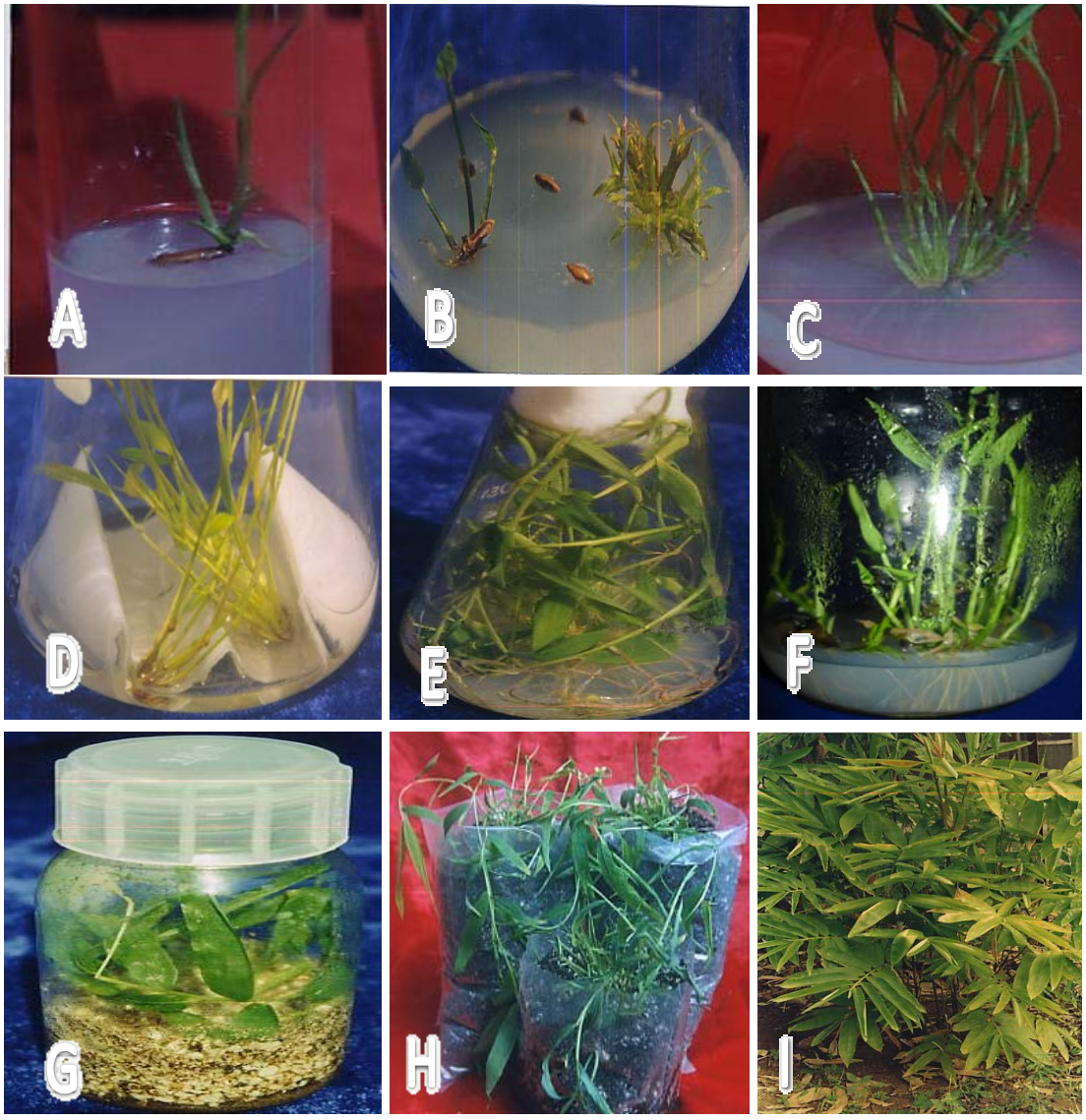

Figure 2:Various stages of plantlet regeneration through micropropagation A. Aseptic seed culture of *Drepanostachym falcatum*. B. *In vitro* shoot formation from seeds in *Dendrocalamus hamiltonii* . C. *In vitro* shoot multiplication of *Bambusa giganteus* in semisolid MS medium.D. *In vitro* shoot multiplication of *Dendrocalamus vulgaris in Liquid* MS medium. E. *In vitro* rooting in *Bambusa bambos*. F. *In vitro* root formation in *Dendrocalamus membranaceus.* G. *In vitro* hardening of plantlets in *Dendrocalamus asper*. H. Hardened and acclimatized plants of *Drepanostachym falcatum.* I. Tissue culture raised plants of *Dendrocalamus asper* in field.

Macro-Propagation of *Oxytenanthera abyssinica* (A. Rich Munro) from Culm Cuttings

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Abstract

Oxytenanthera abyssinica like most bamboos has very erratic and unpredictable seeding years. Vegetative propagation seems to be the best alternative for its regeneration. Therefore the objective of the present work was to investigate the effect of Indole -3 Butyric Acid (IBA), season of the year, number of nodes on culm cuttings and planting method (vertical or horizontal) on rooting and sprouting of cuttings. To fulfill this objective a completely randomized design experiment in a factorial setting was conducted (4 IBA concentrations x 2 seasons x 2 planting methods x 2 types of nodded cuttings (one node and two nodes cuttings) x 3 sources of cuttings from the explants).

Data were subjected to Analysis of variance (ANOVA). Results indicated significantly better rooting percentage in winter (42%) as compared to (30%) in summer. The two-nodded cuttings were significantly better than the one-nodded in both seasons. On the other hand, cuttings taken from the basal part of the culm were significantly better in rooting and sprouting than those taken from the middle, while those taken from the top failed to root. Horizontally planted cuttings were significantly better than the vertically planted ones. IBA at 4000 ppm gave significantly higher rooting of cuttings and sprouting than the 2000 ppm in both winter and summer seasons while the IBA1000 ppm produced significantly lower percentages for the number of roots and number of sprouts. However, cuttings failed to root without IBA application.

Based on the results of the present work and with due consideration to previous studies, it can be concluded that culm cuttings should be taken from the current year culms as they are better than old ones and that winter is the best season for propagating bamboo cuttings in the Sudan. It seems useful for ensured and successful propagation to use culm cuttings having at least two nodes treated with IBA of 2000 to 4000 ppm concentrations and planted horizontally.

Keywords: Macropropagation, *Oxytenanthera abyssinica*, culm cuttings

Introduction

Bamboo is a group of the family Poaceae, subfamily Bambusoideae, tribe Bambuseae. Some of its members are giant bamboos, forming by far the largest member of the grass family (David 2003). There are 91 genera and about 1000 species of bamboos. They are found in diverse climates within the tropical and subtropical regions of the world (Bystriakova *et al*. 2003).They are perennials, fast-growing woody grasses and are capable of producing utilizable annual production. They were not hitherto fully brought under sustainable management despite their vast biogeographical distribution. Consequently, alternative sustainable management and maintenance of the remaining bamboo forest resource of the Sudan has been strongly realized in conjunction with the present day deterioration of the natural forests of the country as a result of over exploitation and desertification. (El Houri. *et al.* 2001) A part from taxonomic studies, only very little and fragmented efforts were made to investigate the various aspects of bamboo growth, productivity, management and utility in the Sudan (Elamin 1990). *Oxytenanthera abyssinica* (A. Rich Murno) and *Arundinaria alpina* (K. Schuni) were the only two indigenous bamboo species endemic to Sudan. The former is considered more important as it has a wider distribution and therefore commonly used than the later.

Oxytenanthera abyssinica has a very long vegetative phase of growth before it flowers and this is followed by the death of the clump (Khan 1972; Kigomo 1989). However, it can be propagated from various vegetative parts using different methods viz. branch cuttings of *Dendrocalamus asper*, *Gigantocheoa aspera*, and G. *ligulat*a. (Saxena 1990; Shudong 1998). Successful propagation of bamboos from culms was reported for many species like *Bambusa valgaris, B. balcoa, B. nutani. Dendrocalamus hamittoni, D. hookeri* (Banik 1980; Das 1988), *Bambusa textitils var. fasca, B. multiplex, B. Gilla,* and *B. chungii* (Zhang 1997).

Difficulties encountered in the use of seeds as a means for regeneration in addition to their scarcity calls for research on alternative means. Vegetative propagation seems to be the best choice considering the encouraging results obtained elsewhere (Saxena 1990; Zhang 1997).

Therefore, the main objective of this investigation was to establish a protocol for a successful propagation of *Oxytenanthera abyssinica* from culm cuttings at normal nursery conditions. The specific objectives were to investigate the effect of season (Summer and Winter), type of cutting (one and two nodded cuttings), method of planting (horizontal-vertical planting), position on the culm from where cuttings were taken (top, middle and basal part of the culm) and different IBA concentrations on inducing rooting of cuttings.

Materials and Methods

Plant Material

Culm cuttings were prepared from new culms (less than one-year-old) from clumps of *Oxytenanthera abyssinica* at Abu Gaili Forest near Sinner (Blue Nile State at latitude 13° 36′ and longitude 23° 36′). The new culms were examined morphologically to ascertain the existence of buds, which may grow and form culms. Two types of cuttings were prepared viz. One-nodded cuttings, approximately 25 cm in length and 5 cm in diameter and two-nodded cuttings, approximately 40 cm in length and 5 cm in diameter.

The cuttings were defoliated and the axillary buds were left intact. Approximately equal proportions of the internodes were left on both sides of the single-nodded and two nodded cuttings. Cuttings were prepared from the top, middle and basal part of the culms.

Experimental Design and treatments

An experiment was performed utilizing a completely randomized design in a factorial setting. Treatments used included IBA concentrations at 4 levels (4000, 2000, 1000 and 0.00 ppm) x 2 methods of planting (horizontal and vertical) x 2 types of cuttings (one nodded and two nodded cuttings) x 3 positions on the culm from where cuttings were taken (top, middle and basal part) x 2 seasons (summer and winter). Treatments were replicated four times using ten plants for each treatment.

IBA preparation and mode of application

IBA was prepared to the required concentrations of 4000, 2000, 1000 and 0.00 ppm as a control. The dip method was used throughout and the basal ends of the prepared cuttings were dipped in the prepared IBA solutions, which were put in a plastic tray. The treated cuttings were left for 16-20 hrs in the solutions at laboratory temperature (24-25°C).

They were then planted in a soil medium consisting of a thin layer of silt covered with sand. This mixture was placed on concrete beds of 150x50x30 cm. The beds were covered with plastic sheets laid on a metal frame about 30-50 cm above the ground to create suitable conditions for rooting and to maintain high relative humidity. Planted cuttings were kept moist by daily watering and were given all necessary care and protection.

Growth Parameters and Data Analysis

Data on shoot length, root length, number of sprouted and rooted cuttings were recorded periodically for five months. Analysis of variance was carried out using Statistical Analysis Systems (SAS) and means were separated using Fisher protected L.S.D.

Results and Discussion

Effect of Season on the Performance of Cuttings:

Culm cuttings planted during winter showed significantly higher sprouting (49.2%) compared to those planted during the summer season (32.0%). On the other hand, winter planted cuttings produced significantly bigger percentage of rooted cutting (42%) than winter planted cutting with (30.0%). In addition, the number of roots produced by cuttings in summer (5) was significantly bigger than winter (2.4). As shown in Table (1) shoot and root growth varied significantly between the two seasons. Shoot length was significantly taller during winter (32.0) than summer, and root length was significantly longer in winter (18.1) than in summer (12.5).

Table (1): Effect of season on performance of *Oxytenanthera abyssinica* cuttings, after five months in the nursery.

Season	Sprouted cuttings %	Rooted cuttings %	Shoot length (cm)	Root length (cm)	Root number
Summer	32.0 [b]	30.0 [b]	22.0 [b]	12.5 [b]	2.4 [b]
Winter	49.2 [a]	42.0 [a]	32.0 [a]	18.1 [a]	4.8 [a]

Means followed by different letters are significantly different at P<0.05 using Fisher protected L.S.D.

Cuttings started to initiate roots in the second month after planting in winter whereas in summer the cuttings started to initiate rooting at the third month after planting. These results agreed with

Lin (1995), who recommended that cuttings taken from *Bambusa odonii* should be taken in winter season. Also, Sirikalyanon *et al*., (1997) concluded that the best season for planting cuttings is winter for *Dendrocalamus hamiltonii*.

Effect Cutting Position on the Performance of the Cuttings

Cuttings taken from the basal, middle and upper parts of the culm and planted during the summer season showed significant differences between them in sprouting percentage (Table 2).

Table (2): Effect of cutting position on the performance of *Oxytenanthera abyssinica* cuttings in summer and winter, after five months in the nursery

Position on the culm	Season	Sprouted cuttings %	Shoot length (cm)	Root length (cm)	Root number
Upper parts	Summer	17.0 [c]	14.9 [c]	0 [c]	0 [c]
	Winter	24.0[c]	17.6 [c]	0 [c]	0 [c]
Middle part	Summer	33.8[b]	22.9 [b]	12.0 [b]	3.4 [b]
	Winter	35.0[b]	24.5 [b]	15.7 [a]	4.8 [a]
Basal part	Summer	38.6[b]	28.6 [a]	13.6 [b]	4.2 [a]
	Winter	44.0[a]	30.4 [a]	16.8 [a]	5.4 [a]

Means in the same column followed by different letters, are significantly different at P<0.05 using Fisher protected L.S.D

The highest sprouting % (38.6%) was recorded by the cuttings taken from the basal part, but this was not significantly different from the sprouting percentage (33.8%) obtained from the middle part of the culm. However, cuttings taken from the upper part produced significantly lower sprouting percentage (17.0%). The same trend occurred in winter season where the percentage of sprouted cuttings taken from the upper part (24%) was significantly lower than those taken from middle and basal parts (35%) and 44% respectively.

Regarding the percentage of rooted cuttings, those taken from the upper part failed to initiate roots in both seasons, whereas those taken from the middle and basal parts initiated roots, giving (32.2%) and (35%) in summer and 36.8 and 41.8% in winter respectively (Fig.1).

The shoot length of the cuttings taken from the upper part and planted in summer were significantly shorter (14.9) cm than those taken from the middle and basal part that attained (22.9) and (28.6) cm respectively. However, the basal part cuttings were significantly taller than those taken from the middle of the culm. In winter the shoot length of cuttings taken from the upper part attained significantly lower length (17.6 cm) than those taken from middle and basal parts attaining lengths (24.5) and (30.4) cm respectively.

Cuttings taken from the upper part of the culm in both seasons failed to initiate roots. However, cuttings taken from basal and middle parts and planted during summer and winter showed no significant differences between them (Table 2.). These findings were in agreement with

Hormilson (1988) who investigated the influence of the position of the nodal buds in the culms on the bud break. The found that the position of the nodal buds on the culm affects bud break and those at the middle and basal part showed higher percentage of bud break and better growth compared with those at the upper part.

Effect of Type of Nodes

Single and double nodded cuttings planted in summer showed significant differences between then in the percentage of sprouted cuttings. Double nodded cuttings produced significantly higher percentage (54.0%) compared to (33.0%) for the single nodded. On the other hand the double nodded cuttings planted in winter showed significantly higher percentage (68.0%) than the single nodded cuttings(Table 3).

Table (3): Effect of type of cuttings in the performance of *Oxytenanthera abyssinica* cuttings in summer and winter after five months in the nursery

Type of node	Season	Sprouting %	Shoot length (cm)	Root length (cm)	Root number
Single node	Summer	33.0 [c]	29.3[a]	13.7 [b]	2.7 [b]
	Winter	38.0 [c]	31.9 [a]	15.2 [a]	2.9 [b]
Double node	Summer	54.0 [b]	30.1 [a]	15.2 [a]	4.2 [a]
	Winter	68.0 [a]	33.1 [a]	16.7 [a]	5.1 [a]

Means in the same column followed by different letters, are significantly different (P<0.05) using Fisher protected L. S. D

However, both types of cuttings showed similar shoot lengths in summer and winter.

The percentage of rooted cuttings was significantly greater in double nodded cuttings (33.4%) and (44.7%) in summer and winter seasons respectively compared with (25.9%) and (35.2%) for the single nodded cuttings in summer and winter respectively.

However, in both seasons the double nodded cuttings produced significantly bigger number of roots (4.2) and(5.1) than the single nodded cuttings (2.7) and (2.9) for summer and winter seasons respectively.

In summer, the double nodded cuttings produced significantly longer roots (15.2) compared to the single nodded (13.7) but in winter they showed no significant differences between them attaining lengths of (16.7) and (15.2) for the double and single nodded cuttings respectively. These results are in agreement with the results reported by Bohidar (1989) who recommended that the two nodded cuttings were usually more successful that one nodded cuttings. They are in contrast with the results obtained by Das (1988) who found a success rate of 80% from single-nodded culm cuttings in *Bambusa nutans* compared with 60% in the two- nodded cuttings of the same species. However, these results confirm the results obtained by Castillo (1990) who recommended the use of cuttings having at least two nodes for *Dendrocalamus merrillianus*.

Effect of IBA

Culm Cuttings treated with 4000 ppm IBA and planted during winter produced the highest percentage of sprouted cuttings (46.0%) while the untreated cuttings planted in winter produced the lowest percentage (22.0%) (Fig.2).

However, results showed no significant differences in sprouting between cutting which were untreated and planted in winter (25.0%), treated with IBA 1000 ppm and planted in summer and those treated with 1000 ppm and planted in winter (28.0%). In addition, there were no significant differences in sprouting between cuttings

treated with IBA 2000 ppm and 4000 ppm and planted in summer and those treated with 2000 ppm and planted in winter with sprouting percentage of (33.2), (38.8) and (33.9) respectively (Table 4.)

Table (4): Effect of IBA concentrations on the performance of *Oxytenanthera abyssinica* cuttings after five months in the nursery

IBA (ppm)	Season	Sprouting cuttings %	Shoot length (cm)	Root length (cm)	Root number
0.00	Summer	22.0 [d]	16.8 [c]	0.0 [c]	0.0 [f]
	Winter	25.0 [c]	27.0 [b]	0.0 [c]	0.0 [d]
1000	Summer	25.9 [c]	26.0 [b]	4.2 [b]	0.5 [c]
	Winter	28.0 [c]	27.0 [b]	6.0 [b]	1.5 [c]
2000	Summer	33.2 [b]	27.0 [b]	14.1 [a]	3.2 [b]
	Winter	33.9 [b]	30.0 [a]	15.7 [a]	4.0 [a]
4000	Summer	38.8 [b]	31.0 [a]	16.3 [a]	4.8 [a]
	Winter	46.0 [a]	33.0 [a]	17.7 [a]	5.9 [a]

Means in the same column followed by different letters, are significantly different ($P < 0.05$) using Fisher protected L. S. D

Cuttings treated with IBA at 4000 ppm initiated the highest rooting percentage (41.4%) and (46.2%) in summer and winter seasons, respectively. They were significantly different from those treated with IBA at 2000 ppm, which initiated rooting percentage of (28.1%) and (34.4%) in summer and winter seasons respectively. The untreated cuttings failed to initiate roots and those treated with IBA at concentration of 1000 ppm initiated roots with percentage of rooting of (10%) and (16.5%) in summers and winter season respectively.(Plate 1.)

Cuttings treated with IBA 4000 ppm and planted in summer and winter and those treated with IBA 2000 ppm and planted in winter showed no significant differences between them in shoot length attaining (31.0 cm), (33.0 cm) and (30.0 cm) respectively. The untreated cuttings which were planted in summer showed significantly shorter shoot length compared to the remainder of treatments attaining (16.8 cm). On the other hand the untreated cuttings planted in winter and the ones treated with IBA1000 ppm planted in summer and winter in addition to cuttings treated with IBA 2000 ppm and planted in summer, showed no significant differences between them in shoot length (Table 4).

Untreated controls failed to initiate roots in both seasons. However, the longest roots were produced when cuttings were treated with IBA 4000 and 2000 ppm. Their lengths ranged from (17.7) cm for the cuttings treated with IBA 4000 ppm planted in winter to (14.1) cm for the cuttings treated with IBA 2000 ppm planted in summer. However, cuttings treated with IBA 1000 ppm showed no significant differences between them in root length with (6.0) cm in winter and (4.2) cm in summer respectively.

The biggest number of roots were obtained from cuttings treated with IBA 4000 ppm planted in winter and summer (5.9) cm and (4.8) cm and IBA 2000 ppm planted in winter (4.0) cm. The fewest number of roots were produced when cuttings were treated with IBA 1000 ppm and planted in both seasons (1.5) and (0.5) cm for winter and summer respectively.

These results are in line with McClure (1986) who recommended the use of IBA for *Bambusa textis*. These findings are also in agreement with Rungnapar (1988) who reported that IBA and NAA as effective hormones increasing rootability *B. arundinacea*, *Dendrocalamus strictu*s and *D. scrpteria*. The results of this study conform to the findings of Abd Razak (1994) who showed that the use of IBA at 2000 ppm promoted the growth of the cuttings of *Gigantochloa Levis*.

These results also confirm the findings of Nagariaiah (1994), who reported an increased rooting percentage of the cuttings of *Bambusa vulgaris* by using growth regulating substances.

Effect of Method of Planting

The method of planting vertically or horizontally, produced significant effect on the percentage of sprouting, rooting cuttings, the shoot and root length. The cuttings planted horizontally gave successful propagules whereas the vertically planted ones especially the two nodded cuttings failed to sprout successfully in summer and winter seasons. So the recommended method of planting would be horizontally, and this agrees with Dong *et al*. (1999) and Pyare *et al*. (1998) who reported that, the best method of planting cuttings is to plant them horizontally.

Generally from the results of the present work and with due consideration of the previous studies discussed it can concluded that culm cuttings should be taken from the current year culms as they are better than old culms and that winter is the best season for propagating bamboo cuttings in the Sudan. It seems useful for ensured success of propagation to treat culm cuttings having at least two nodes with IBA 2000 to 4000 ppm concentrations and planted horizontally (Plate 2.)

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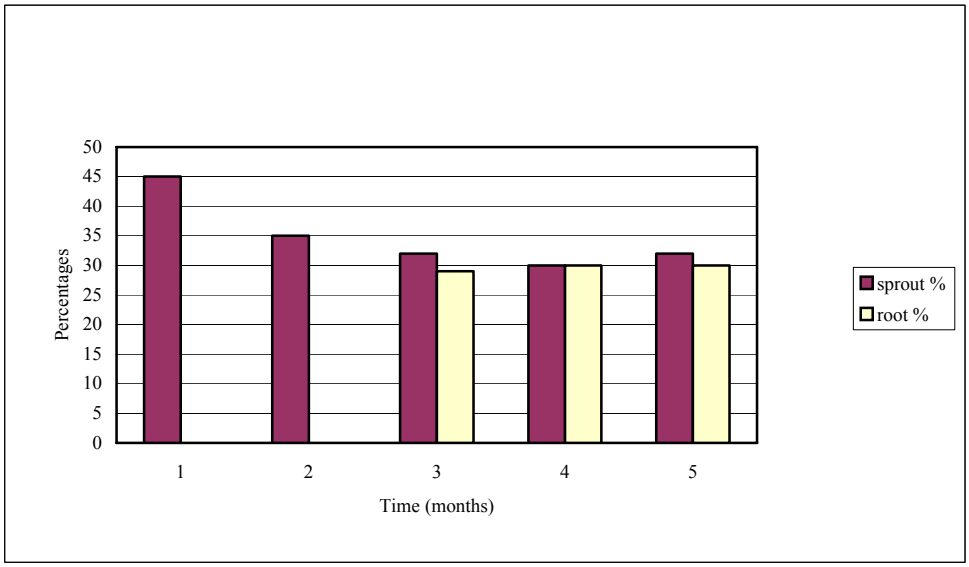

Figure 1. The percentages of sprouting and rooting of *Oxytenanthera abyssinica* culm cuttings during summer, after five month in the nursery

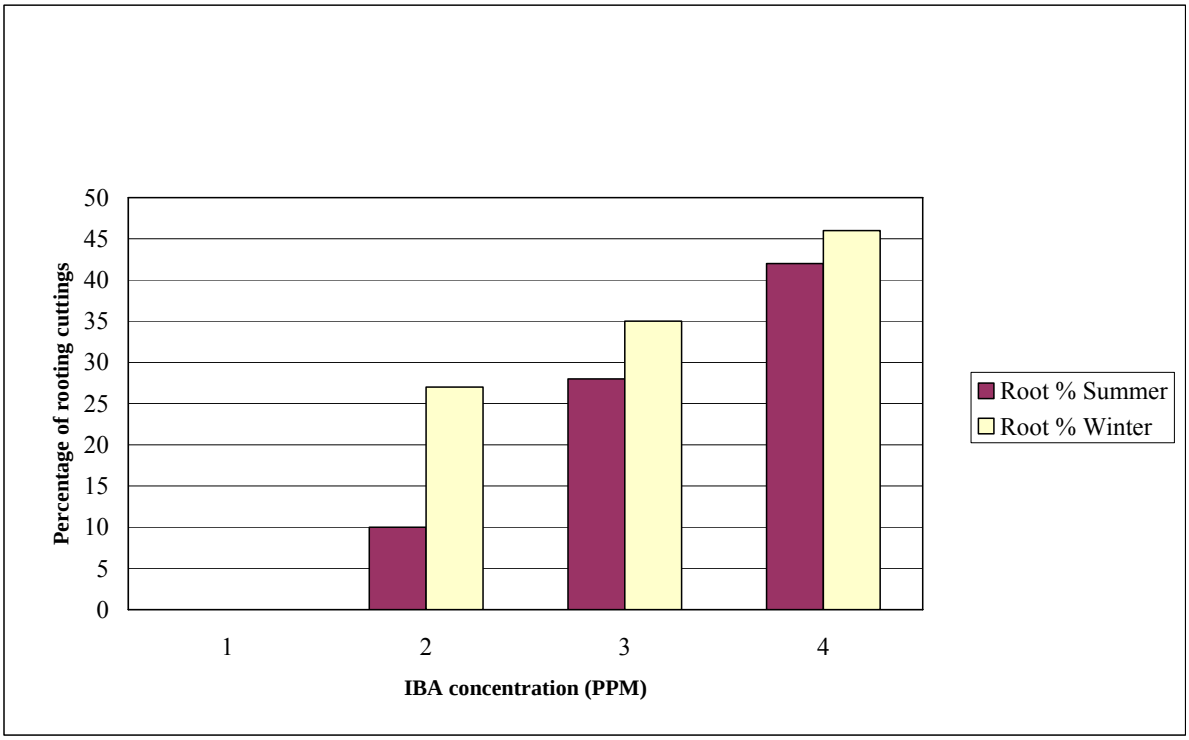

Figure 2. The effect of IBA concentration on *Oxytenanthera abyssinica* rooted cuttings percentage during summer and winter, after five month in the nursery

Plate 1. Successful rooting of *Oxythentherea abysssinica* culm cuttings treated with IBA 4000 ppm In Winter Season

Plate 2. Successful sprouting of *Oxythentherea abysssinica* culm cuttings treated with 4000 ppm IBA in winter