Plant Tissue Cult. & Biotech. **15**(2): 103-111, 2005 (December) PTC&B

Micro-cloning in Commercially Important Six Bamboo Species for Mass Propagation and at a Large Scale Cultivation

S. A. M. Nurul Islam and Md. Mahbubur Rahman

Bangladesh Forest Research Institute, Sholoshahar, P.O. Amin Jute Mills, Chittagong-4000, Bangladesh. E-mail: nislam@abnetbd.com

Key wards : Nodal bud, Cloning, Mini-clump, Proliferation, Cultivation

Abstract

In vitro cloning of some commercially important bamboos (*viz*. *Bambusa balcooa*, *B. nutans*, *B. salarkhanii*, *B. vulgaris*, *B. vulgaris* var. *striata* and *Thyrsostachys oliveri)* were tried by using nodal buds. Excised shoots were used as explants in transfer cultures and BAP (1 - 5 mg/l) was found to be effective growth regulator in liquid media for inducing multiple shoots (3 - 30). Half strength MS was supplemented with NAA (1 - 3 mg/l) and IBA (1 - 5 mg/l) for root induction. Two to three weeks old 3 - 5 cm long shoots in cultures were suitable for inducing rooting. Frequent changes of rooting media had improved production of rooted plantlets. Rooted plantlets were cultured in non-sterile water in the growth room for one week and after the plantlets were transferred to the propagation bed. The plantlets in propagation beds produced mini-clumps with 3 - 10 shoots within five - eight weeks. Rooted shoots of the mini-clumps were separated into two - five parts. The younger parts of mini-clumps were transplanted in the propagation bed for further proliferation of shoots; older parts were transferred in the soil-filled bags for field planting. These bagged plantlets were vigorous in growth and found suitable for large scale cultivation.

Introduction

Bamboo is a fast-growing plant. It grows naturally in the forest and is cultivated in the village homestead. Village farmers have limited knowledge of bamboo cultivation based mostly on their own personal experiences and not on systematic scientific studies. The situation calls for immediate attention to more scientific cultivation and mass reproduction to meet increasing demands for planting materials (Banik et al. 1993; Banik 1997). There are 26 species of bamboo under seven genera including the natural and cultivated exotics in Bangladesh (Alam 2001).

All bamboo species under study were measured from bambusetum, BFRI but *B. balcooa* from Sarkarhat of Chittagong; internodes length, diameter and wall thickness are shown in average of 40 internodes from base and parentheses

indicated maximum value of internode number from basal internode. The bamboos cultivated in villages are well adapted, thick walled and have a multipurpose uses (Tables 1 and 2).

The cultivated bamboos are conventionally propagated by rhizome/offset planting but recently branch cuttings are widely used for some bamboo species. For improvement and large scale cultivation of village bamboos *in vitro* cloning and mass propagation techniques are urgently needed. The six important village bamboos *viz*. *Bambusa balcooa* Roxb.(Borak), *B. nutans* Wall. ex Munro (Makla), *B. salarkhanii* M.K.Alam (Karjaba), *B. vulgaris* Schard, ex Wendl. (Baijja), *B. vulgaris* var. *striata* (Sharna) and *Thyrsostachys oliveri* Gamble (Rangoon) have been selected for cloning and mass propagation by means of tissue culture in addition to the standard practice of using nodal buds and subsequently produced proliferating multiple shoots of mini-clumps.

Materials and Methods

Buds with 1.0 - 1.5 cm nodal segments were used to initiate and establish *in vitro* culture. Nodal buds were collected from branches of one - two years old culms of six different bamboos (*viz. Bambusa balcooa*, *B. nutans*, *B. salarkhanii*, *B. vulgaris*, *B. vulgaris* var. *striata* and *Thyrsostachys oliveri*). Clumps of donor bamboos were about 10 - 40 years old. Explants of nodal buds were collected in late February or in March. Nodal buds were green and generally buds of upper nodes were initiated to sprout. Collected buds were cleaned, sized and washed under running water. $HgCl_2$ (0.1%) and a few drops Tween 20 (Polyxyethylene sorbitan Monolaurate) were used to surface sterilized explants. The sterilized nodal buds were cultured in semi-solid gel of MS and supplemented with BAP (1.0 mg/l). Selected buds sprouted within three - four weeks. The sprouted buds were cultured in liquid MS supplemented with BAP (1.0 mg/l) for two - three weeks. By this time sprouted buds elongated and developed into a number of multiple shoots. The latter shoots were used as explants either as a single or a cluster of two - three shoots for production of additional multiple shoots and root induction. Macro- and micronutrients of MS medium and vitamins and other organics of B5 medium were used in the transfer cultures. In the second phase also BAP (1 - 5 mg/l) was added to semi-solid gel or liquid media for induction of multiple shoots. For induction of roots in the excised shoots, NAA (1 - 3 mg/l) and IBA (1 - 3 mg/l) were used.

Prior to final transfer into soil, plantlets which were already in soil with well developed roots were cultured for one - two weeks in water. Within a period of three - four months the plantlets transferred to the propagation bed produced mini-clumps of shoots in abundance. Roots grew on the shoot bases of mini-clumps. The rooted shoots of mini-clumps proliferated further when individual

mini-clumps were separated and planted in the specially prepared planting beds. This procedure was found most suitable for large scale shoots multiplication or for their storage in bags until field planting. Bagged plantlets were planted in June, 2004 on different locations at the bambusetum in BFRI farmer's field at Harbang, Jahangirnagar University campus at Savar near Dhaka city and Bangladesh Agriculture Research Institute (BARI) research station at Chandraghona, Chittagong. Data on the field performance i.e., the growth of six to nine months old bamboo species and their survival rate were collected in April, 2005.

Results and Discussion

The sterilized nodal buds (Fig. 1) were cultured in agar-gelled MS medium either alone or supplemented with BAP (1 mg/l). In both cases, buds sprouted but BAP enhanced sprouting of buds (Fig. 2). The nodal buds of all six species sprouted within one to three weeks but those, which did not sprout, remained green for a long period. For inducing multiple shoots, the sprouted buds were transferred in liquid MS supplemented with BAP (1 mg/l) with constant shaking (100 rpm) under the light in an orbital incubator. The elongated shoots were separated from nodes by a sharp knife and transferred in the same fresh medium. Initially sprouted nodal buds produced thick shoots but after subsequent three - four transfer culture in the same medium, the shoots multiplied constantly by forced axillary's branching and became thin shoot clusters. These shoots were suitable for multiplication of shoots and induction of roots.

Breaking of nodal buds and sprouting of shoots depend on the condition of explants, season of the year and culture conditions. Ramanayake et al. (1995) studied *in vitro* bud breaking of two bamboos (*Dendrocalamus giganteus* and *Bambusa vulgaris*) from April, 1994 to April, 1995 and found seasonal effect on bud-breaking. Similar observations were also made by Saxena and Dhawan (1994) on *D. longispathus*.

The excised shoots (either single or two - three together) of shoots cluster, established from nodal buds of the six bamboo species were used as explants. These were cultured in liquid and semisolid gelled (7 g/l agar) MS supplemented with vitamins of B5 medium and BAP (l – 5 mg/l). New shoots emerged from the cultured shoot(s) on the third day and continued to grow further for 10 to 12 days (Fig. 3). In media with a lower concentration of BAP, (1 mg/l) shoots elongated more than in those fortified with a higher concentration of BAP (5 mg/l). All explants did not produce equal number of new shoots within the same period of time in the same species. The variation of new shoot emergence may be due to size, age or other conditions of explants (Table 3). Saxena and Dhawan (1994) also observed that shoots induced from nodes of *B. vulgaris*

multiplied at a slower rate than those from *D. longispathus*. Healthy shoots (Fig. 4) from profusely growing multiple shoots were found to be more suitable for root induction provided they were cultured beforehand in the semi-solid gel medium with a low BAP content. When profuse shoots (> 20) were grown in cultures, they started browning on the third week; in the liquid medium they

Table 1. Yield and use related characters of six bamboo species.

Species	Culms height (m)		Internode number		Internode length (cm)		Diameter (cm)		Wall thickness (cm)	
	Av.	Max.	Av.	Max.	Av.	Max.	Av.	Max.	Av.	Max.
Bambusa balcooa	11.3	13.3	60.6	70	22.94	34.5 (30)	6.02	10.00 (1)	1.23	3.30 (1)
B. nutans	19.0	20.0	49.3	57	49.97	69.0 (16)	4.60	8.32 (1)	0.615	2.27 (1)
B. salarkhanii	27.2	21.2	50.3	53	44.53	74.0 (12)	5.77	9.03 (2)	0.846	3.74 (1)
B. vulgaris	21.8	23.6	66.6	77	34.25	44 (15)	7.97	10.37 (15)	0.88	2.74 (1)
B. vulgaris var. *striata*	12.9	13.3	63.3	73	26.61	35.5 (22)	5.80	6.75 (1)	0.74	1.65 (1)
Thyrsostachyo oliveri	11.1	13.3	65.0	68	16.76	24.8 (29)	4.63	6.18 (1)	0.91	2.24 (1)

Figs. 1 - 10. 1. Nodal Buds of *B. vulgaris* var. *striata*. 2. Sprouted one nodal bud of *T. oliveri*. 3. Profuse shoots of *B. balcooa*. 4. Multiple shoots of *B. salarkhanii*. 5. Rooted plantlets of *B. salarkhanii*. 6. Mini-clumps of *B. nutans* with profuse shoots. 7. Multiple shoots with roots in mini-clumps of *B. nutans*. 8. Proliferated mini-clump shoots of *B. vulgaris*. 9. Polybag plantlets of *T. oliveri* and 10. Polybag plantlets of *B. balcooa*.

started browning earlier and in the semi-solid gel medium, browning took place later. MS at half strength supplemented with NAA/IBA (1 - 3 mg/l) induced roots (Fig. 5) in the excised shoots of all the six bamboo species. To induce root NAA was found to be more effective than IBA. At a low concen-tration of NAA or IBA (1 mg/l) in the medium, induced roots showed poor growth but the

Table 1. Yield and use related characters of six bamboo species.

Species	Culms height (m)		Internode number		Internode length (cm)		Diameter (cm)		Wall thickness (cm)	
	Av.	Max.	Av.	Max.	Av.	Max.	Av.	Max.	Av.	Max.
Bambusa balcooa	11.3	13.3	60.6	70	22.94	34.5 (30)	6.02	10.00 (1)	1.23	3.30 (1)
B. nutans	19.0	20.0	49.3	57	49.97	69.0 (16)	4.60	8.32 (1)	0.615	2.27 (1)
B. salarkhanii	27.2	21.2	50.3	53	44.53	74.0 (12)	5.77	9.03 (2)	0.846	3.74 (1)
B. vulgaris	21.8	23.6	66.6	77	34.25	44 (15)	7.97	10.37 (15)	0.88	2.74 (1)
B. vulgaris var. *striata*	12.9	13.3	63.3	73	26.61	35.5 (22)	5.30	6.75 (1)	0.74	1.65 (1)
Thyrsostachyo oliveri	11.1	13.3	65.0	68	16.76	24.8 (29)	4.63	6.18 (1)	0.91	2.24 (1)

attached shoots remained green for a long period. The roots were profuse in a medium with a higher concentration of NAA (3 mg/l) or IBA (5 mg/l) but soon thereafter they degenerated by browning, killing the regenerating shoots. Sometimes death occurred without browning even before the emergence of roots from the seedling.

Table 2. Uses of six bamboo species.

Species	Uses
Bambusa balcooa	1. Structural and construction application. 2. Thatching, walling, roofing, handicrafts and novelty item. 3. Pulp, paper and rayon.
B. nutans	1. Structural and construction application. 2. Thatching, walling, roofing, handicrafts and novelty item. 3. Pulp, paper and rayon.
B. salarkhanii	1. Structural and construction application. 2. Thatching, walling, roofing, handicrafts and novelty item. 3. Pulp, paper and rayon.
B. vulgaris	1. Structural and construction application. 2. Thatching, walling, roofing, handicrafts and novelty item. 3. Pulp, paper and rayon. 4. Edible shoot.
B. vulgaris var. *striata*	1. Structural and construction application. 2. Thatching, walling, roofing, handicrafts and novelty item. 3. Pulp, paper and rayon.
Thyrsostachys oliveri	1. Structural and construction application. 2. Thatching, walling, roofing, handicrafts and novelty item. 3. Pulp, paper and rayon.

Table 3. *In vitro* shoot growth in the six bamboo species.

Species	BAP (1.0 mg/l)		BAP (5.0 mg/l)	
	Shoot number	Shoot height (cm)	Shoot number	Shoot height (cm)
Bambusa balcooa	3 - 24	1.0 - 6.0	5 - 54	0.5 - 3.5
B. nutans	3 - 21	1.0 - 7.0	4 - 28	1.0 - 5.0
B. salarkhanii	2 - 21	1.0 - 11.0	3 - 23	1.0 - 6.5
B. vulgaris	3 - 13	1.0 - 6.5	3 - 26	0.5 - 4.0
B. vulgaris var. *striata*	3 - 11	1.0 - 7.0	3 - 30	0.5 - 4.5
Thyrsostachys oliveri	3 - 14	1.0 - 9.0	3 - 21	0.5 - 4.0

Rooting improved when repeated subcultures were made using a medium with higher concentrations of NAA or IBA (> 3 mg/l) in the semi-solid gel medium followed by their transfer to a liquid medium culture with a low content of the same growth regulator.

Tablte 4. Multiple shoots growth in mini-clumps of the six bamboo species in propagation bed.

Species	1st transfer (TC Lab.)		2nd transfer (Mini-clump)		3rd transfer	(Mini-clump)
	Shoot number	Height (cm)	Shoot number	Height (cm)	Shoot number	Height (cm)
Bambusa balcooa	3 - 7	4 - 8	3 - 10	4.0 - 14.0	3 - 5	8.0 -82.0
.B. nutans	3 - 6	3 - 7	3 - 11	5.0 - 17.5	6 - 11	7.0 - 113.0
B. salarkhanii	3 - 11	4 - 15	3 - 12	4.5 - 16.0	3 - 5	13.0 -53.0
.B. vulgaris	2 - 6	5 - 12	3 - 7	5.0 - 7.5	3 - 4	56.0 -30.0
B. vulgaris var. *striata*	3 - 5	5 - 16	3 - 5	9.0 -14.0	2 - 3	20.0 - 33.0
Thyrsostachys oliveri	3 - 9	6 - 15	3 - 12	6.5 - 23.0	3 - 6	8.0 - 57.0

Table 5. Growth performances of six bamboo species in the field at different locations.

Species	BFRI campus*		Farmer field*		J. N. University*		BARI station*	
	Survival %	Growth vigor	Survival %	Growth vigor	Survival %	Growth vigor	Survival %	Growth vigor
Bambusa balcooa	100	+++	100	+	100	-	100	+++
B. nutans	100	+	100	+	100	-	100	+
B. salarkhanii	100	+++	100	+	100	-	100	++
B. vulgaris	100	++	100	+	100	-	100	++
B.vulgaris var. *striata*	100	++	-	-	100	-	100	++
Thyrsostachys oliveri	100	++	100	++	100	-	100	+

*Bambusetum of BFRI, Chittagong; *Faitong farmer, Mr. M.A. Ali; *Jahangirnagar University campus; *A research station of BARI located at Chandraghona, Chittagong.

Rooted shoots i.e., plantlets of the six different bamboo species were thoroughly washed under the running tap water. These were cultured in tap water for one-two weeks with or without macro- and microelements of MS. Thereafter, the plantlets were transferred to the propagation beds sheltered from direct sun and maintained there for a week. About 80% plantlets survived in the propagation beds.

Successful plantlets in the propagation beds produced profuse multiple shoots and grew luxuriantly. All the plantlets of six bamboo species showed juvenility like seedlings and grew into mini-clumps (Fig. 6) each with 3 - 23 shoots (Table 4). Most of the shoots produced underground rhizomatous bases with roots (Fig. 7). Young mini-clumps with two - five shoots each were divided into two - three parts and each was carefully planted for further proliferation in the propagation beds. At the older stage 10 - 15 weeks, mini-clumps stopped further divisions and neither they were suitable for further proliferation in the propagation beds nor they were usable for storage in bags (Figs. 8, 9, 10). It may be mentioned that Zamora and Gruezo (1999) found micro-cloned mini-clumps suitable as macro-propagation technology of bamboos and field planting.

During the nine-month period the field performances of all the six bamboo species were excellent including their good survival and growth rate (Table 5). Although no intensive care was taken, the propagules were in good condition throughout the period under observation and survived under general field conditions.

Banik (1983) initiated micro-propagation of bamboo species using nodal buds as explants. During the period that followed many other scientists worked on micro-cloning of bamboo species by using nodal buds (Ramanayake et al. 1996; Saxena and Dhawan 1994; Prutpongse and Vantana 1992). Here, we have shown that use of nodal buds is an alternative reproducible and dependable method for cloning of all the six bamboo species that we have tried. We have also shown that proliferation of mini-clumps of micropropagated plantlets is one more powerful and reliable method to attain the same objective, namely large scale multiplication of bamboo species available in the country.

References

Alam MK (2001) Bamboos of Bangladesh: A Field Identification Manual. Government of the People's Republic of Bangladesh, Bangladesh Forest Research Institute, Chittagong. pp. 35.

Banik RL (1983b) Micropropagation of bambusoid grass (*Phragmites communis* (Cav.) Trin and Stend.) and micropropagation of bamboo (*Bambusa glaucescens* Siebold); M.S. thesis, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.

Banik RL, Islam SAMN and **Hadiuzzaman S** (1993) *In vitro* regeneration of multiple shoots in three bamboo species. Plant Tissue Cult. **3**(2): 101-106.

Banik RL (1997) Domestication and Improvement of Bamboos. INBAR Working Paper No. 10. International Network for Bamboo and Rattan. International Development Research Centre, 17 Jor Bagh, New Delhi-110 003, India. pp. 53.

Prutpongese P and **Gavinlertvatana P** (1992) *In-vitro* micropropagation of 54 species from 15 genera of bomboo. Hortscience **27**: 453-454.

Ramanayake SMSD, Yakandawala K, Nilmini Deepika PKD and **Ikbal MCM** (1995) Studies on Micropropagation of *Dendrocalamus gigateus* and *Bambusa vulgaris* var. *striata*. Bamboo, People and The Environment. Vol.1, Propagation and Management. INBAR Technical Report No. pp. 8. 75-85.

Saxena S and **Dhawan V** (1994) Micropropagation research in South Asia. *In:* Constrains to production of bomboos and rattan. Report of a consultation held in Bangalore, India, 9-13 May 1994. INBAR Technical Report No. 5. International Network For Bamboo and Rattan, New Delhi, pp. 101-114.

Zamora Alfinetta B and **Gruezo Sunny S** (1999) Micropropagation of Bamboo through Tissue Culture Technology. Canopy International **25**(6). Ecosystem Research Development Bureau, College, Leguna 4031, Philippines.