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RAPID *IN-VITRO* REGENERATION FROM 40- YEAR- OLD CLUMP OF*BAMBUSA NUTANS* WALL. EX MUNRO

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Bambusa nutans Wall. ex Munro is a very common cultivated evergreen bamboo species. It is a multipurpose plant with ecological and economical importance. For regeneration of shoots, nodal segments were taken from 40 year old clump, and surface sterilized using 0.1% (w/v) $HgCl_2$ solution for 15-20 min. and placed aseptically on media supplemented PGRs. 7.5 µM 6-benzylaminopurine (BAP) and 1.25 µM (NAA) exhibited maximum bud break within a period of three weeks. Proliferating shoots were excised along with small piece of mother tissue and kept on medium supplemented with 5.0 µM BAP for multiplication for 4 - 5 subculture cycles. Multiplied shoots were grouped into bunches of 2 -5 shoots each. Bunch of three shoots exhibited faster shoot multiplication when cultured on multiplication media. During multiplication phase, BAP alone proved to be superior to kinetin as well combination of BAP with NAA. Shoots multiplied at faster rate on media supplemented with 5.0 µM BAP and adjuvated with 50 mM glutamine. Glycine, adjuvated at equimolar concentration as that of glutamine to media, was found to be deleterious. Microshoots exhibited higher rooting percentages on media supplemented with IBA than other auxins (IAA or NAA). Effective IBA concentration was found to be 10 µM on which 72.22 % of propagules rooted. Hardening was effectively carried out on half strength MS media without growth regulators, vitamins and sucrose. Success was achieved in acclimatizing 73.33 % rooted propagules on mixture carrying potting mix of sand: FYM: Soil in 1:1:1 in polytunnels. Acclimatized plantlets were successfully transplanted to pots.

India is the second richest country in bamboo genetic resources after China. These two countries together have more than half the total bamboo resources globally. Sharma (1987) reported 136 species of bamboos occurring in India. Fifty-eight species of bamboo belonging to 10 genera are distributed in the northeastern states alone. The forest area, over which bamboos occur in India, on a conservative estimate, is 9.57 million hectares, which constitutes about 12.8 % of the total area under forests (Bahadur and Verma 1980). Out of the 22 genera in India, 19 are indigenous and three exotic. The annual production of bamboo in India is about 4.6 million tonnes; about 1.9 million tonnes is used by the pulp industries. The annual yield of bamboo per hectare varies between 0.2 and 0.4 tonnes with an average of 0.33 tonnes per hectare, depending upon the intensity of stocking and biotic interferences. The economic impact of the agroforestry-based

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bamboo system may influence general economic development considerably.

Bambusa nutans is one of the most economically important species; it is distributed between an altitude of 600–1500 m from Uttaranchal to Arunachal Pradesh. It is a sympodial bamboo with clump flowering and 15 - 20 m tall culms. The species is exploited for its use in paper pulp industry and for its usage as poles. The indiscriminate exploitation of natural resources of *Bambusa nutans* has

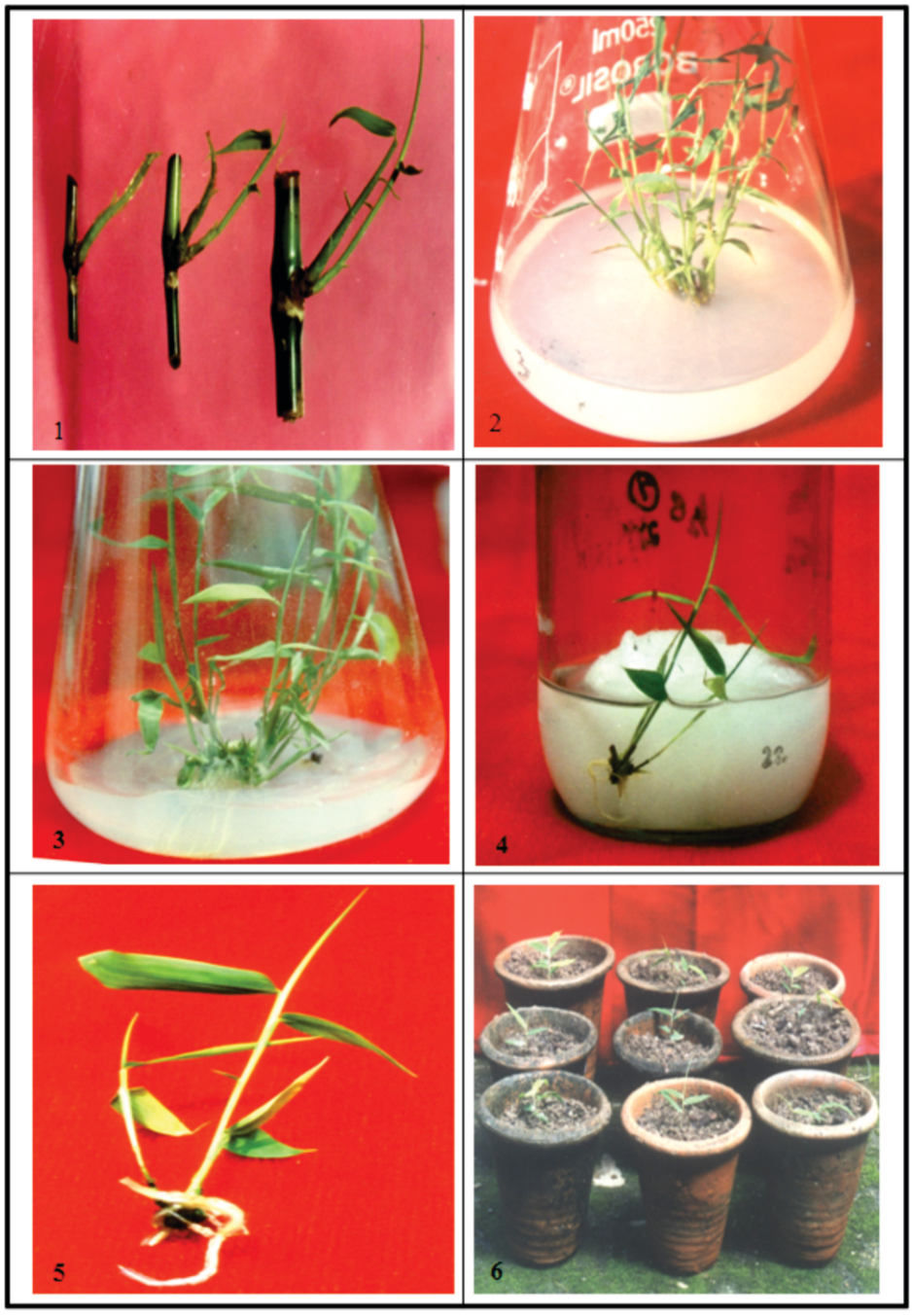

Figure1-6 *B. nutans* :1. Nodal explant .2. Established culture 3. *In-vitro* rooting .4. In-vitro hardening5. *In-vitro* hardened plantlet 6. Plantlets of the *B. nutans* growing in the pot.

reached threshold limit of depletion. Conventional method of propagation through seeds is limited due to unpredictable flowering and short seed viability period. Vegetative propagation by cuttings, offset and rhizome are also inadequate to cope up the demand of planting stock due to large propagule size and low multiplication rate. To meet the growing demand and conserve its resources, both conventional and non-conventional methods should be exploited. Hence, development of non-conventional method of micropropagation is required.

Regeneration of bamboos through application of tissue culture technique using juvenile and mature explants has been successfully achieved in *D. asper* (Arya *et al.* 1999), 54 bamboo species (Prutpongse and Gavinlertvatana 1992), *B. multiplex* and *P. aurea* (Huang *et al*. 1988), *P. viridis* (Hassan and Debergh 1987), *D. strictus* (Chaturvedi *et al*. 1993, Preetha *et al*. 1993, Nadgir *et al*. 1984), *B. tulda* (Saxena 1990) and callogenesis and organogenesis in *Bambusa vulgaris* (Rout and Das 1997). Till date micropropagation of *B. nutan*s using seed as source of explant has been reported (Yashoda *et al*. 1997) and regeneration from leaf and internode (Kalia *et al.* 2004a).

In the present communication successful establishment, multiplication and rooting of *Bambusa nutans* using nodal segment from the mature clump of about 40-yr-old, has been described.

MATERIALS AND METHODS

Explant and surface sterilization

Shoots were collected from current years growth from mature and healthy clumps of *B.nutans* established at bamboosetum of the campus. Shoots were cut down to single node segments measuring 2 to 3cm long (Fig. 1). Leaf sheath was removed from the explants and they were thoroughly cleaned with cotton scrub dipped in 70 % ethanol. These nodal segments were surface sterilized with 0.1% (w/v) $HgCl_2$ solution for 15-20 min. Later these explants were given 2 - 3 washings in sterile distilled water to remove the traces of sterillants. Finally, these aseptic nodal segments were inoculated in culture tubes containing 10 – 15 ml medium.

Bud break

In order to optimize mineral nutrient requirements for bud break, four basal media viz. MS (Murashige and Skoog 1962), B_5 (Gamborg *et al*. 1968), SH (Schenk and Hildebrandt 1972) and NN (Nitsch and Nitsch 1968) supplemented with either 2.5 or 5.0 µM BAP.

Nodal explants were cultured on MS media supplemented with cytokinins (BAP, Kinetin, BPA, Zeatin) in order to assess their effectivity to induce bud break. The cytokinin concentration used in the experiment was 2.5 - 15 µM. Effect of BAP-NAA interaction to achieve bud break was also investigated. BAP was tried in concentration range of 2.5 – 5 µM and NAA was used in range of 1.25 -2.5 µM.

Shoot multiplication

Effect of propagule size was studied for enhancing multiplication potential, for the purpose propagules with varying number of shoots (one to five shoots) were cultured on MS medium supplemented with 5.0 µM BAP.

Effect of cytokinins viz. BAP and Kinetin was investigated in the concentration range of 2.5-15 µM for shoot multiplication experiments. The effect of cytokinin-auxin interaction on shoot multiplication was investigated by supplementing media with 2.5 – 5 µM BAP and 1.25 - 2.5 µM NAA. To enhance the rate of multiplication glutamine and glycine (50, 100,

150, 200 mM) were adjuvated to the medium supplemented with 5 µM BAP.

Rooting

Rooting of shoots was carried out on MS media (half strength of major salts) supplemented with auxins (IBA or NAA or IAA) alone in the concentration range of 2.5 –15 µM. Bunch of three shoots each were cultured for rooting.

In all the experiments MS media (Murashige and Skoog 1962) was used unless and otherwise stated. All media were supplemented with 3 % sucrose and gelled with 0.7 % agar (Qualigenes, India). pH of all media was adjusted to 5.8 prior to autoclaving at 15 lbs pressure at 121^{0}C for 15-20 min. All cultures were incubated under 3000 lux with fluorescent light with photoperiod 16 hrs light and 8hrs dark and temperature 25 ± 2^{0}C.

Data recording

Observations were recorded for percentage of cultures exhibiting bud break and average number of shoots proliferated. Data was recorded on number of shoots multiplied and their length in shoot multiplication experiments. Shoot multiplication rate in the shoot multiplication experiments was calculated by subtracting initial number of shoots from the total number of shoots at the end of subculture cycle of three weeks. In rooting experiments, data were recorded on percentage of rooted shoots, average number of roots and root length.

For each treatment, twenty-four replicates were used and each experiment was repeated thrice. Data were recorded after twenty-one days of culture. Data subjected to GLM analysis using statistical analysis software package version SPSS 10.0 accordingly analysed data presented in Table 1 to Table 7 for describing the result of the study.

RESULTS

Bud break

Nodal explants were surface sterilized as per method described in materials and method section. The explants so sterilized were inoculated on MS medium supplemented with

Table 1. Efficacy of media in inducing bud break on nodal explants.

Media	BAP (µM)	Bud break (%)	Average no. of proliferated shoots
MS	**2.5**	62.49±4.17	3.36±0.13
	5.0	71.82 ±2.75	3.89±0.09
SH	**2.5**	55.54 ±4.80	2.94±0.28
	5.0	65.07 ±6.11	3.55±0.07
NN	**2.5**	49.99 ±4.16	2.74±0.11
	5.0	58.32 ±4.17	3.29±0.13
B_5	**2.5**	44.47 ±2.41	2.65±0.14
	5.0	48.61 ±6.36	3.08±0.08
Factor	**Variable**	**CD at 0.05**	**F Calculated**
Media	**Bud break**	**10.63**	**12.71**
	Shoot No.	**0.564**	**6.35**

Table 2. Effect of different concentration of cytokinins on bud break on nodal explants of *Bambusa nutans*.

Cytokinin	Concentration (µM)	Bud break (%)	Average number of proliferated shoots
BAP	**2.5**	58.33±4.17	3.08±0.28
	5.0	69.24±6.30	3.84±0.22
	7.5	79.16±7.22	3.86±0.12
	10.0	74.99±8.33	3.50±0.18
	12.5	70.82±7.21	3.32±0.27
	15.0	66.66±11.02	2.91±0.16
Kinetin	**2.5**	44.44±6.36	2.43±0.10
	5.0	47.22±2.41	2.68±0.04
	7.5	52.77±8.67	2.76±0.21
	10.0	62.49±4.17	3.29±0.19
	12.5	69.44±6.36	3.61±0.13
	15.0	66.66±4.16	3.41±0.22
BPA	**2.5**	40.27±2.40	1.91±0.19
	5.0	48.61±6.36	2.30±0.05
	7.5	52.77±8.67	2.38±0.22
	10.0	55.55±6.36	2.71±0.17
	12.5	61.10±10.42	2.91±0.07
	15.0	54.16±4.16	2.67±0.19
Zeatin	**2.5**	36.10±4.81	1.89±0.21
	5.0	41.66±4.68	2.22±0.09
	7.5	48.60±6.36	2.46±0.06
	10.0	51.39±9.62	2.69±0.17
	12.5	58.33±7.22	2.80±0.17
	15.0	50.00±4.16	2.47±0.20
Factor	**Variable**	**CD at 0.05**	**F calculated**
Treatment	**Bud break**	**34.56**	**8.92**
	Shoot number	**0.92**	**29.60**
Cytokinin	**Bud break**	**9.13**	**18.30**
	Shoot number	**0.26**	**26.01**

varying levels of plant growth regulators. Maximum number i.e. 100% uncontaminated established from the explants which were collected during months of March – April (Fig 1).In experiment to select suitable basal media, four media (MS, SH, NN and B_5) were supplemented with equimolar concentration of BAP (0, 2.5 and 5.0 µM). Explants cultured on MS medium exhibited higher bud break (Fig. 2) percentages compared to other media tried (Table 1). On MS media supplemented with BAP (2.5 and 5 µM) multiple shoots were induced on 59.71% and 67.85% of explants and the average number of induced shoots was 3.02 ± 0.17 and 3.61± 0.26 respectively. Average number of shoots induced on MS medium supplemented with 2.5µM BAP was nearly twice as induced on SH with equimolar concentration of BAP. On all media except B_5, normal healthy, green shoots were induced. Shoots induced from explants cultured on B_5 medium were pale green, stunted, and exhibited necrosis, and failed to multiply on multiplication medium. Due to better performance of explants cultured on MS medium, it was selected for further experiments.

Effect of different cytokinins (BAP, Kinetin, BPA, Zeatin) at their equimolar concentrations was assessed on the percentage bud break and average number of shoots induced (Table 2). Buds sprouted on 33.33% explants cultured on basal medium and only one to two shoots emerged from these explants. Shoots formed on explants cultured on the basal medium remained green for 15-18 days, thereafter these shoots failed to grow further. BAP was superior to other cytokinins tried at equimolar concentrations, followed by Kinetin, Zeatin and BPA. There was gradual

Table 3. Effect of BAP-NAA interaction on bud break on nodal explants of *Bambusa nutans*.

BAP(µM)	NAA(µM)	Bud break (%)	Average number of proliferated shoots
5.0	**0.00**	65.27±8.67	3.63±0.26
7.5		77.77±6.36	3.82±0.28
10.0		73.60±4.80	3.41±0.06
5.0	**1.25**	74.99±7.21	3.89±0.27
7.5		86.11±2.41	4.43±0.21
10.0		79.16±4.16	4.28±0.13
5.0	**2.50**	72.21±6.36	3.73±0.25
7.5		80.55±2.41	3.95±0.19
10.0		74.79±4.47	3.78±0.14
Factor	**Variable**	**CD at 0.05**	**F calculated**
Treatment	**Bud break**	**19.11**	**3.76**
	Shoot No.	**0.78**	**6.36**
BAP x NAA	**Bud break**	**8.06**	**3.24**
	Shoot No.	**0.32**	**11.36**

increase in percentage bud break and average number of shoots induced per explant with increase in concentration of BAP up to 7.5 µM, which thereafter, exhibited a decline. On the contrary, number of buds induced increased till 10 µM BAP but these buds were condensed and clustered and only few buds elongated into shoots. However, there was no parity between the optimal concentration of each of the cytokinin for percentage bud break and average number of shoots induced. The optimal concentration of BAP was 7.5µM on which the explants exhibited 77.78% bud break with 3.86 shoots per explant. Kinetin, BPA and Zeatin were effective at 12.5µM concentration in the medium at which 70.83%, 62.49% and 59.72% explants exhibited bud break and induced an average of 3.61, 2.91 and 2.80 shoots respectively. Bud break percentage (77.78%) and average number of shoots proliferated per explant (3.86) was significantly higher on optimal concentration of BAP (7.5µM) than other cytokinins. On the contrary, average length of induced shoots was more on Kinetin supplemented media than the media supplemented with corresponding concentration of other cytokinins. Addition of small amounts of Kinetin to the best BAP concentration failed to enhance bud break on cultured explants. Average period for bud break varied between 7-12 days on explants cultured on cytokinin-enriched medium.

BAP supplemented media was enriched with auxin (NAA) and their interaction was found superior with respect to all cultural parameters studied (bud break percentage, number and length of induced shoots) as compared to cytokinins alone. Responsiveness of explants was enhanced on all the concentrations of BAP when NAA (1.25µM) was supplemented to the medium in comparison to the medium supplemented with BAP alone (Table 3). On the medium supplemented with 7.5µM BAP and 1.25µM NAA, explants exhibited highest percentage of bud break (86.11%) with average of 4.46 shoots per explant. Decline in percentage of explants exhibiting bud break and number of shoots proliferated per explant was observed when NAA concentration in the medium was increased from 1.25µM. Increase in NAA concentration (>1.25µM) enhanced the frequency of hyperhydricity of induced shoots which exhibited glassy green appearance. Frequency of hyperhydricity was more on combination of NAA with higher BAP content (12.5µM and 15µM). Vitrified shoots failed to convert into normal shoots when transferred to basal media or media with lower concentration of BAP.

Multiplication

Proliferated shoots were excised from the mother explant with small part of it attached to it and then cultured on hormone free medium for elongation until appropriate size of shoots (2.5-3.0 cm) were available. This treatment helped to overcome the deleterious effect of higher plant growth regulator treatments and uniform crop of shoots were available for multiplication. The shoots were then multiplied on 5µM BAP for 6 weeks until a sufficient shoot number was there for multiplication experiments (Fig 2).

For shoot multiplication, a cluster of 2-5 shoots was used as propagule and cultured on medium supplemented with 5µM BAP. Cultured propagules of three shoots each were found suitable as they exhibited enhanced multiplication rate (3.66) compared to isolated single shoots, which failed to multiply at faster rate. The shoot multiplication potential of cluster of two, four and five shoots was lower than the cultured propagule of three shoots each and was 2.78, 3.56 and 2.05 respectively. Therefore, in subsequent experiments cluster

of three shoots each were used for shoot multiplication.

In order to enhance shoot multiplication, propagules of three shoots each, were cultured on media supplemented with either BAP or Kinetin. On the media without cytokinins shoots failed to multiply but exhibited copious elongation of shoot (Table 4). BAP proved to be superior to kinetin in respect to shoot multiplication potential. The optimal concentration of BAP was 5.0µM at which highest multiplication rate (4.07) was recorded. On BAP concentration beyond 5µM there was decline in multiplication rate. The optimal concentration of Kinetin was 10µM at which multiplication rate of 3.34 was achieved. Although, kinetin was less effective in multiplying shoots at faster rate, but the length of multiplied shoots, was more than that on the equimolar BAP concentrations. Shoot length decreased with increasing cytokinin concentration in the medium. Shoot hyperhydricity was observed on some shoots

Table 4. Multiplication rate of cultured *Bambusa nutans* propagules of three shoots each as affected by different cytokinins.

Cytokinin	Concentration(µM)	Average number of multiplied shoots	Shoot length (cm.)	Multiplication rate
BAP	**2.5**	11.73±0.58	3.29	3.91
	5.0	12.22±0.77	3.15	4.07
	7.5	11.770.62±	3.13	3.92
	10.0	10.70±0.41	2.82	3.56
	12.5	9.89±0.50	2.45	3.29
	15.0	8.18±0.47	2.27	2.72
Kinetin	**2.5**	7.35±0.42	3.87	2.44
	5.0	8.49±0.53	3.71	2.81
	7.5	8.96±0.48	3.63	2.98
	10.0	10.14±0.39	3.38	3.34
	12.5	8.47±0.37	3.06	2.85
	15.0	7.57±0.35	2.82	2.52
Factor		**Variable**	**C D at 0.05**	**F calc**
Treatment		**Shoots**	**0.86**	**27.92**
		Shootslt.	**0.26**	**17.89**
Cytokinin		**Shoots**	**2.03**	**34.09**
		Shootslt.	**0.46**	**53.48**

multiplied on media supplemented with 12.5 and 15µM BAP. Leaf morphology of shoots also changed with increase in BAP concentration in the medium; leaves were normal at lower concentration (2.5-10µM BAP), which turned rudimentary at higher concentrations of BAP (12.5-15µM).

Addition of NAA (0, 1.25 or 2.5 µM) to the BAP (2.5-5 µM) supplemented medium was found deleterious for shoot multiplication (Table 5). Decline in the multiplication potential was observed when NAA concentration in the medium was increased from 0 to 2.5 µM. Shoots multiplied on medium supplemented with NAA exhibited browning of sheaths and gave vitrified appearance. BAP alone at 5 µM was found to be effective in establishing cultures with faster rates of shoot multiplication (3.94) with shoots of average length 3.11 cm. Therefore, the cultures were subsequently multiplied on media supplemented with 5 µM BAP.

Effect of adjuvants (amino acids glutamine and glycine) revealed that the addition of glutamine improved the multiplication rate whereas glycine was found to be deleterious. Increment in shoot multiplication rate was observed when glutamine concentration in the medium was enhanced from 0 to 100 mM (Table 6). On media adjuvated with 100 mM shoot multiplication rate was 4.48 which declined with further increase in its concentration. On media adjuvated with glycine multiplication rate declined with increase in concentration.

Rooting

In vitro multiplied shoots (3-4 cm) were harvested and cultured on MS basal medium for one week before their culture on rooting

Table 5. Interactive effect of BAP-NAA on *Bambusa nutans* shoot multiplication.

BAP(µM)	NAA(µM)	Average number of multiplied shoots	Shoot length(cm.)	Multiplication rate
2.5	**0.00**	11.37±0.62	3.30	3.79
5.0		11.83±0.65	3.11	3.94
7.5		11.19±0.38	3.02	3.73
2.5	**1.25**	10.41±0.57	3.18	3.47
5.0		11.13±063	3.01	3.71
7.5		10.19±0.40	2.85	3.39
2.5	**2.50**	9.14±0.96	3.08	3.04
5.0		9.37±0.25	2.76	3.12
7.5		8.55±0.48	2.65	2.85
Factor		**Variable**	**C D at 0.05**	**F calc**
Treatment		**Shoots**	**2.14**	**11.19**
		Shoot lt	**0.47**	**7.71**
BAP X NAA		**Shoots**	**0.76**	**35.17**
		Shoot lt	**0.74**	**6.03**

Table 6. Effect of adjuvants supplemented in media (MS + 5.0 µM BAP) on multiplication of *Bambusa nutans* shoots.

Adjuvants	Concentration (mM)	Average number of multiplied shoots	Shoots of length (cm.)	Multiplication rate
Control	**0.00**	12.19±0.75	3.32	4.06
Glutamine	**50**	12.74±0.36	3.13	4.25
	100	13.45±0.31	3.02	4.48
	150	12.59±0.35	2.86	4.19
	200	11.78±0.56	2.75	3.92
Glycine	**50**	11.98±0.51	3.04	3.99
	100	10.13±0.27	2.80	3.38
	150	9.11±0.54	2.76	3.04
	200	7.89±0.31	2.63	2.61
Factor		**Variable**	**CD at 0.05**	**F calc.**
Treatment		**Shoots**	**1.71**	**48.02**
		Shoot lt.	**0.53**	**7.02**

medium. It was found that IBA was most effective for rooting than IAA or NAA (Table 7, Fig. 3-4). For rooting 10µM concentration of IBA was most effective on which 72.22% of cultures developed 5.13 roots of average length 4.01 cm. Although percentage of rooted shoots was highest on medium supplemented with 10 µM IBA, the average number of roots as well as root length was highest on media supplemented with 10 µM and 2.5 µM NAA respectively. NAA and IAA were found to be effective at 7.5 µM and 10 µM concentration respectively on which 54.99% and 47.21% cultures developed roots. On the rooting medium supplemented with IAA, the cultured shoots exhibited rapid chlorosis.

Hardening and acclimatization

Rooted shoots were transferred to liquid medium (MS half strength) without plant growth regulators and vitamins for hardening. After hardening period of three weeks, the hardened plantlets were transferred to polybags with different potting mixtures. Plantlets transferred to 1:1:1 mixture of sand: FYM: soil, 73.5% of the plantlets successfully acclimatized as against 55% plantlets in mixture of sand: soilrite (Fig. 5 and 6)

Present procedure ensures faster multiplication of cultures and ensures regular supply of planting material for plantation as well as afforestation programmes.

DISCUSSION

Propagation of bamboos through single node culture provides an efficient means of propagation. The plantlets arising via this

Table 7. Effect of auxins on induction of roots on shoots of *Bambusa nutans.*

Auxin	Concentration (µM)	Rooting(%)	Root Number	Root Length (cm)
IBA	**2.50**	37.49±7.21	2.16±0.15	4.53±0.43
	5.00	47.22±2.40	2.71±0.21	4.65±0.48
	7.50	58.33±4.17	3.43±0.25	4.10±0.22
	10.0	72.22±4.81	5.13±0.35	4.01±0.38
	12.5	65.27±6.36	4.06±0.23	3.78±0.11
	15.0	55.55±2.41	3.51±0.33	3.52±0.29
IAA	**2.50**	20.83±4.17	2.11±0.15	4.71±0.33
	5.00	29.16±4.16	2.90±0.62	4.60±0.27
	7.50	30.55±8.67	3.35±0.56	4.33±0.23
	10.0	47.21±6.36	3.08±0.42	4.26±0.33
	12.5	41.66±7.21	2.66±0.28	4.10±0.15
	15.0	38.33±2.40	2.26±0.25	3.86±0.12
NAA	**2.50**	31.945±2.40	2.96±0.15	5.04±0.39
	5.00	48.60±8.67	3.86±0.59	4.39±0.39
	7.50	54.99±5.46	5.45±0.32	4.03±0.25
	10.0	52.77±8.67	4.10±0.20	3.38±0.13
	12.5	38.88±6.36	3.42±0.28	3.24±0.13
	15.0	27.77±4.80	3.03±0.15	2.86±0.11

Factor	**Variable**	**CD at 0.05**	**F calc**
Treatment	**Rooting (%)**	**26.96**	**17.23**
	Rooting No.	**1.37**	**11.04**
	Rooting length	**1.62**	**21.62**
Auxin	**Rooting (%)**	**9.73**	**15.59**
	Rooting No.	**0.49**	**3.17**
	Rooting length	**0.72**	**7.67**

method are generally free from chromosomal aberrations and are true to type copy of the parental stock. In past two decades cloning via tissue culture has received a great impetus. Successful propagation of bamboos through single node culture has been documented in *Bambusa arundinacea* (Mehta *et al*. 1982), and *Dendrocalamus strictus* (Preetha *et al.* 1992, Chaturvedi *et al.* 1993, Ramanayake and Yakandawala 1997, Ravikumar *et al.* 1998).

Period between March-April provided suitable environment for culture establishment. Similar

effect of season on culture establishment has been reported in variety of tree species and bamboos (Chaturvedi *et al.* 1993, Thakar and Bhargava 1998). Amongst the trial of different media for bud break MS proved to be superior over other salt formulations tried (SH, B_5, NN). This was also observed in the reports on axillary bud proliferation using nodal segments from mature trees of *Prosopis cineraria* (Shekhawat *et al*. 1993) and *Prosopis alba* (Tabone *et al.* 1986).

Role of propagule size during multiplication phase was investigated and propagule size of three shoots each was found to multiply at the faster rates. A faster rate of shoot multiplication taking propagules of three shoots each was observed in *Bambusa tulda* (Saxena 1990) and. *Dendrocalamus asper* (Arya and Arya 1997). Of the cytokinins tested BAP proved its superiority during culture establishment as well as shoot multiplication phase. BAP has proven to be an effective cytokinin in the micropropagation of bamboos in several studies (Arya *et al.* 1999, Saxena 1990, Ramanayake *et al.* 2001, Kalia *et al.* 2004a). Failure of shoots to multiply on medium without cytokinins as in the present report is observed in case of *Dendrocalamus asper* (Arya and Arya 1997) and *Bambusa bambos* (Arya and Sharma 1998). In present investigation, addition of auxin (NAA) to the BAP supplemented medium for shoot multiplication was deleterious. Our findings are in close agreement with observations of Saxena (1990) in *Bambusa tulda* where auxin-cytokinin interaction was found to be deleterious for bud break as well as shoot multiplication. Some of the cultures in the present study particularly at the higher concentrations of BAP developed vitrified shoots. The role of cytokinins in medium on explant/shoot hyperhydricity has been mentioned by Debergh (1983), Bornman and Vogelman (1984) and Pasqualetto *et al*. (1986). Lishem *et al*. (1988) suggested that hyperhydricity is the buds reaction to an excess cytokinin. In the present study shoot multiplication rate of 3.5 after three weeks of culture was observed.

Significance of auxins in root development has been investigated by several workers in their studies. In present study, IBA was found more effective than IAA or NAA. Similar efficacy of IBA on root induction is reported in *Acacia auriculiformis*, *Prosopis cineraria* (Nandwani and Ramawat 1993), *B. nutans* (Kalia *et al.* 2004a) and *D. sissoo* ((Kalia *et al.* 2004b).

Present study documented higher rate of shoot acclimatization (72%) in the potted plantlets of *Bambusa nutans*. Such high success rates of acclimatization have been reported in *Dendrocalamus asper* (Arya *et al*. 1999). In addition, in the present procedure there is no visible evidence of variants (albino shoots) as observed in other studies. Therefore, present study provides an effective methodology of multiplying *Bambusa nutans* at faster rates. Efforts are on to develop a cost effective and highly reliable methodology to ensure sufficient planting stock for industry and agro-forestry.

REFERENCES

Arya ID & Arya S 1997 *In vitro* culture and establishment of exotic bamboo *Dendrocalamus asper*. *Indian Journal of Experimental Biology*, 1252-1255

Arya S & Sharma S 1998 Micropropagation technology of *Bambusa bambos* through shoot proliferation. *Indian For.* **124** 725-731.

Arya S, Sharma S, Kaur R & Arya I D 1999 Micropropagation of *Dendrocalamus asper* by shoot proliferation using seeds. *Plant Cell Rep*. **16** 877-882.

Bahadur K N & Verma J 1980 Country Report India. 19-46 In: *Proceedings of the Workshop on Bamboo Research in Asia, Singapore* eds. Lessard G & Chorinard A IDRC, Ottawa, Canada.

Bornman C H & Vogelmann T C 1984 Effect of rigidity of gel medium on benzyladenine induced adventitious bud formation and hyperhydricity in *Picea abies*. *Physiol. Plant*. **61** 505-512.

Chaturvedi H C, Sharma M & Sharma A K 1993 *In vitro* regeneration of *Dendrocalamus strictus* Nees through nodal segments taken from field grown culms. *Plant Science*. **91** 97-101.

Debergh PC 1983 Effects of agar brand and concentration on tissue culture medium, *Physiol Plant* **59** 270-276.

Gamborg O L, Miller R A & Ojima K 1968 Nutrient requirements of suspension cultures of soyabean root cells. *Exp. Cell Res*. **50**:151-158.

Hassan A & Debergh P 1987 Embryogenesis and plantlet development in bamboo *Phyllostachys viridis*. *Plant Cell Tissue Org. Cult*. **10** 73-77

Huang L, Huang B & Chen W 1988 Tissue culture investigations of bamboos. IV. Organogenesis leading to adventitious shoot and plants in excised shoot apices. *Environ. Exp. Bot.* **29** 307-315.

Kalia Sanjay, Kalia Rajwant K & Sharma S K 2004a *In vitro* regeneration of an indigenous bamboo (*Bambusa nutans*) from internode and leaf explant. *JOBR* **3** 217-229

Kalia Sanjay, Kalia Rajwant K & Sharma S K 2004b Evaluation of clonal variability in shoot coppicing ability and *in vitro* responses of *Dalbergia sissoo* Roxb**.** *Silvae Genetica* **53(5-6)** 212-220

Lishem B, Shally D P & Izhar S 1988 Cytokinin as an inducer of vitrification in melon. *Ann. Bot*. **61** 255-260.

Murashige T & Skoog F 1962 A revised medium for rapid growth and bioassay with tobacco tissue culture. *Physiol. Plant*. **15** 473-497

Nadgir A L, Phadke C H, Gupta P K , Parasharami V A, Nair S & Mascarenhas A F 1984 Rapid multiplication of bamboo by tissue culture. *Silvae Genet*. **33** 219-223.

Nandwani D & Ramawat KG 1993 *In vitro* plantlet formation through juvenile and mature explants in *Prosopis cineraria. Indian J. Exp. Biol*. **31** 156-160.

Nitsch J P & Nitsch C 1969 Haploid plant from pollen grains. *Science*: **163** 85-87.

Pasqualetto P L, Zimmermann R H & Fordham I 1986 Gelling agents and growth regulator effects on shoot vitrification of Gala apple *in vitro*. *J. Am. Soc. Hort. Sci*. **111** 976-980.

Preetha N, Yashoda R, Sumathi R & Gurumurti K 1992 Continuous shoot proliferation of *Dendrocalamus strictus* in stationary liquid cultures. *J. Trop. For*. **8** 105-108.

Prutpongse P & Gavinlertvatana P 1992 *In vitro* micropropagation of 54 species from 15 genera of Bamboo. *Hort. Sci*. **27** 453-454

Ramanayake S M S D & Yakandawala K 1997 Micropropagation of the giant bamboo (*Dendrocalamus giganteus* Munro) from nodal explants of field grown culms. *Plant Sci.* **129** 213-223.

Ramanayake S M S D, Wanniasrachchi W A V R & Tennakoon T M A 2001 Axillary shoot proliferation and *in vitro* flowering in an adult giant bamboo *Dendrocalamus giganteus* Wall. Ex. Munro. *In Vitro Cell Dev. Biol.-Plant*. **37** 667-671.

Ravikumar R, Ananthakrishnan G, Kathiravan K & Ganpathy A 1998 *In vitro* shoot propagation of *Dendrocalamus strictus* Nees. *Plant Cell Tissue Org. Cult*. **52** 189-192.

Rout GR & Das P 1997 *In vitro* plant regeneration via callogenesis and organogenesis in *Bambusa vulgaris*. *Biol. Plant*. **39** 515-522.

Saxena S 1990 *In vitro* propagation of the bamboo (*Bambusa tulda* Roxb.) through shoot proliferation. *Plant Cell Rep*. **9** 431-434.

Schenk R U & Hildebrandt A C 1972 Medium and techniques for induction and growth of monocotyledonous and dicotyledonous plant cell cultures. *Can. J. Bot*. **50** 199-204.

Sharma YML 1987 Inventory and resources of bamboos 14-17 In: *Recent Research on Bamboos* eds Rao AN, Dhanarajan G & Sastry CB, CAF. China and IDRC, Canada.

Shekhawat NS, Rathore TS, Singh RP, Deora NS & Rao SR 1993 Factors affecting *in vitro* clonal propagation of *Prosopis cineraria. Plant Growth Reg*. **12** 273-280.

Tabone TJ, Felker P, Bingham RL, Reyes I & Loughrey S 1986 Techniques in the shoot multiplication of the leguminous tree *Prosopis alba* clone B_2V_{50} *Forest. Ecol. Manag*. **16** 191-200.

Thakar J & Bhargava S 1999 Seasonal variation in antioxidant enzymes and the sprouting response of *Gmelina arborea* Roxb. nodal sectors cultured *in vitro*. *Plant Cell Tissue Org. Cult.* **59** 181-187.

Yashoda R, Sumathi R, Malliga P & Gurumurthi K 1997 Genetic enhancement and mass production of quality propagules of *Bambusa nutans* and *Dendrocalamus membranaceous*. *Indian For.* **121** 303-306.