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Effects of Carbohydrates on *in vitro* axillary shoot initiation and multiplication of *Bambusa pallida* Munro.

Beena D.B [1,2*], Rathore T.S[2,3] and Rao P.S[4]

[1]PG Department of Biotechnology, CMR Institute of Management Studies, Kalyana Nagar, Bangalore – 560 043, India
[2]Institute of Wood Science and Technology, 18th Cross, Malleswaram, Bangalore-560 003, India
[3] Director, AFRI, P.O Krishi Upaz Mandi, New Pali Road, Jodhpur – 342005, India.
[4]Director, Life Science and R&D, Life Science and Engineering Division, Dayananda Sagar College of Engineering, Bangalore, India.

Abstract
The purpose of the current study was to optimize the carbohydrate sources and sucrose concentrations for mass clonal propagation of *B. pallida*. Sucrose in MS liquid medium consisting additives (ascorbic acid, 50mg/l + citric acid, 25 mg/l + cysteine, 25 mg/l) was found to be the best carbohydrate source for shoot induction and shoot multiplication. NAA 0.25mg/l in combination with TDZ 0.25mg/l in the medium exhibited high frequency shoot induction and NAA 0.25mg/l with BAP 1.0mg/l helped for further multiplication of quality shoots. IBA pulse treated shoots were rooted in the MS half strength agar gelled medium fortified with sucrose (2%) and glucose (1%). Rooted plantlets were well established in the green house with more than 95% survivability within four weeks period.

Keywords: *Bambusa pallida,* NAA, TDZ, NMBA, Carbohydrates, Axillary shoot

INTRODUCTION

Bambusa pallida belongs to the family poaceae, is one of the most commercially important species listed by National Mission on Bamboo Appliances (NMBA). It is well distributed in North Eastern India and Burma. In India, it is found in Arunachal Pradesh (37%), Nagaland (13%), Mizoram, Tripura, Assam, West Bengal, Meghalaya, Sikkim and Myanmar [1]. It constitutes about 4% out of the 67% clump forming bamboos total growing stock. It has a huge demand in paper and pulp industries. Long flowering cycle and less viability of the seeds are the main constraints in propagation of the species. Vegetative propagation by using two year old, two noded culm-cuttings treated with Naphthalene acetic acid (NAA) and kinetin (filled in internodal cavity) is the only method available for its propagation [2]. Genetic improvement work had been initiated in 1980 at Arunachal Pradesh [3] and Candidate Plus Clumps (CPCs/superior genotypes) have been selected by the Arunachal Pradesh Forest Research Institute, Itanagar, form North East based on the morphological traits [4]. In addition, Rain Forest Research Institute, (RFRI), Jorhat, Assam also selected CPCs and established germplasm bank for improvement of this high value species. Since there is a large gap between the demand and supply, the need for application of biotechnological methods for mass propagation of such an industrially important species is required. The present study was undertaken with the objective to optimize the factors viz; carbohydrate sources and sucrose concentrations for mass clonal propagation of *B. pallida*.

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*Corresponding Author
Beena D.B
PG Department of Biotechnology, CMR Institute of Management Studies,
Kalyana Nagar, Bangalore – 560 043, India

Tel: +91-9844760457; Fax: +91-8025424433
Email: beena2631@yahoo.com

MATERIALS AND METHODS

Material collection and surface sterilization

CPCs offset cuttings of *B. pallida* were collected from germplasm bank of State Forest research Institute, Itanagar and Rain Forest Research Institute, Jorhat. Collected cuttings were established at Institute of Wood Science and Technology (IWST) nursery (Fig. 1) and germplasm bank of bamboo, Gottipura, Bangalore as a source of material. The newly grown healthy branches were procured and explants of size 2.5 to 3.5cm length and 3-4mm in diameter with dormant buds were excised. Explants were then processed with 0.01% (v/v) liquid detergent (Tween 80, Himedia, India) for 5 minutes and 0.2% (w/v) Bavistin (systemic fungicide) for 5 minutes. In between each treatment, 5-6 thorough washes were given with distilled water and it was followed by surface sterilization in the Laminar Air Flow. For surface sterilization, explants were first treated with 70% ethanol (v/v) for 30 sec and washed with sterile distilled water for 5-6 times before immersing in 0.075 to 0.1% (w/v) Mercuric chloride (Himedia, India) for 5 minutes. Finally, 5-6 thorough washes were given with sterile distilled water and explants were used for shoot induction experiments. For shoot multiplication, 2-3 shoot clumps were taken from the cultures which were already maintained for two passages (each passage for 15 days) in the Murashige and Skoog (MS) liquid medium supplemented with additives, NAA (0.25mg/l) and 1.0mg/l of 6-Benzylaminopurine (BAP).

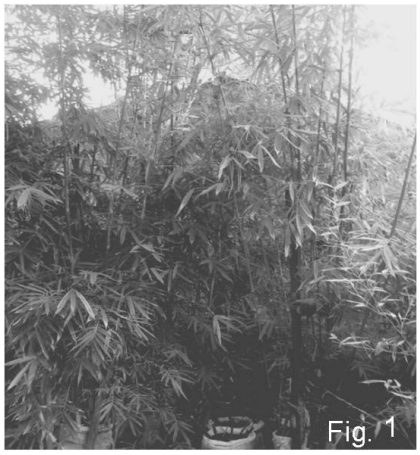

Fig 1. *B. pallida* plants established at IWST nursery as a source of material

Effect of various carbohydrates and sucrose concentrations

In order to optimize the carbohydrate source and its concentrations for *in vitro* clonal propagation of *B. pallida*, various carbohydrates (glucose 3%, fructose 3%, sucrose 3% and glucose 1.5% + sucrose 1.5%) and sucrose concentrations (2.0-6.0%) were tested for high frequency shoot induction and shoot multiplication. For shoot multiplication, maltose (3%) was also tested in addition to the above mentioned carbohydrates. Medium was supplemented with growth regulators NAA 0.25mg/l + Thiodiozuron (TDZ) 0.25mg/l for shoot induction and NAA 0.25mg/l + BAP 1.0 mg/l for shoot multiplication (unpublished data). The *in vitro* multiplied shoots (2-3 shoot clumps) were pulse treated with IBA 500ppm for 30 minutes and then transferred to hormone free MS half strength medium consisting sucrose (2%) and glucose (1%). The rooted plantlets were transferred to poly bags (600cc) containing sand, soil and compost in the ratio 4:2:4 (v/v) and kept inside the poly tunnel in the mist chamber with 90% relative humidity for three weeks. Plantlets were then kept outside the poly tunnel for one week before keeping in the open nursery.

Culture media and Culture conditions

MS liquid medium [5] supplemented with growth regulators, additives (ascorbic acid, 50mg/l + citric acid, 25mg/l + cysteine, 25mg/l) was used for shoot induction and shoot multiplication. Agar (Himedia India) 0.6% was used as a solidifying agent in rooting medium. The pH of the medium was adjusted to 6.2 for the medium consisting additives and 5.8 for the rooting medium before autoclaving at 15 pounds per square inch (15psi) for 20 minutes. The cultures were maintained at 25±2^{0}C temperature with 2500 lux light intensity for 12h photoperiod and 55 ± 5% relative humidity.

Experimental design and data analysis

For shoot initiation, minimum of 12 tubes were taken per treatment, whereas for shoot multiplication, four clumps with 2-3 shoots/clump were taken in each bottle and each treatment consisted four replicates. The experiment was repeated thrice and data was recorded after 15 days considering the parameters like percentage of bud break shoot number and shoot length for shoot induction and shoot number and length for multiplication. Experiments were set up in completely randomized design. Analysis of variance (one way or single factor) treatment means and standard errors were determined, followed by the least significant difference (LSD) test at *P*=0.05 level to compare means.

RESULTS

Among all the carbohydrates (glucose, fructose, sucrose and maltose) tested alone or in combination, sucrose at 3% produced highest (98.52%) bud break with 4.85shoots/explant (Fig. 2). Even though the combination of glucose and sucrose exhibited 97.69% bud break compared to glucose alone, shoot number/explant was less (Table. 1). Medium consisting 3% sucrose proved to be the best with 6.52 shoots/clump and 4.46cm shoot length for shoot multiplication (Fig. 3b). Fructose and maltose were found to be inhibitory for shoot multiplication (Table. 2)

Table 1.Effect of various carbohydrate sources on shoot initiation from nodal shoot segments of *B. pallida* in MS liquid medium supplemented with additives + NAA (0.25mg/l) and TDZ (0.25mg/l).

Tr. No	Carbohydrates	% of responnse	No. of shoots/ explant	Shoot length (cm)
1	Glucose 3%	95.57 ^{c}	3.81 ^{b}	2.58 ^{c}
2	Fructose 3%	60.78 ^{d}	2.73 ^{d}	2.07 ^{d}
3	Sucrose 3%	98.52 ^{a}	4.85 ^{a}	3.23 ^{a}
4	Glucose 1.5% + Sucrose 1.5%	97.69 ^{b}	3.51 ^{c}	2.92 ^{b}
	SE (0.05)	0.23	0.08	0.11
	CD (0.05)	0.42	0.15	0.20

Treatments followed by different letters are significantly different from each other, SE: Standard error of the mean, CD: Critical difference at ∞ =0.05

Table 2.Effect of different carbohydrates on shoot multiplication from shoot clumps of *B. pallida.* in MS liquid medium supplemented with additives + NAA (0.25mg/l) + BAP (1.0mg/l).

Tr. No.	Carbohydrate (%)	No. of shoots/Clump	Shoot length (cm)
1	Glucose 3%	4.48^c	4.31^a
2	Sucrose 3%	**6.52^a**	**4.46^a**
3	Maltose 3%	3.57^d	3.57^b
4	Fructose 3%	3.23^e	2.93^c
5	Glucose 1.5% + sucrose 1.5%	**5.35^b**	4.29^a

SE (0.05)	**0.14**	**0.20**
CD (0.05)	**0.25**	**0.36**

Treatments followed by different letters are significantly different from each other, SE: Standard error of the mean, CD: Critical difference at ∞ =0.05

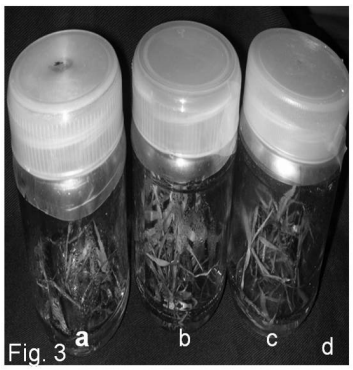

Fig 2. *B. pallida* shoots initiated in MS liquid medium consisting 3% sucrose + additives+ NAA 0.25mg/l + TDZ 0.25mg/l.
Fig 3. Shoot multiplication of *B. pallida* in MS liquid medium Consisting additives + NAA 0.25mg/l + BAP 1.0mg/l and sucrose concentrations a) 2% , b) 3% and c) 6%.
Fig 4. Hardened plants of *B. pallida* after three months

Further optimization determined no significant difference in the bud break with respect to the sucrose concentrations (3-6%), but shoot number was varied. Highest (5.73) shoots/explant with 3.71cm shoot length was obtained in 6% sucrose. Though, 3% sucrose showed comparatively less (5.01) numbers of shoots/explants, however, the shoots were healthy and multiplied further (Table. 3). For shoot multiplication, shoot number was directly proportional to the sucrose concentrations. But 3% sucrose was found to be ideal for multiplication of healthy shoots, which exhibited 6.41 shoots/clump and 4.48cm shoot length (Table. 4).

Table 3.Effect of various sucrose concentrations on shoot initiation from nodal shoot segments of *B. pallida* in MS liquid medium supplemented with additives + NAA (0.25mg/l) and TDZ (0.25mg/l).

Tr. No.	Sucrose concentration	% of response	No. of shoots/explant	Shoot length (cm)
1	2%	80.11[b]	1.82 [d]	2.11 [c]
2	3%	98.49 [a]	4.92 [c]	3.33 [b]
3	4%	98.55 [a]	5.01 [b]	3.50 [a]
4	5%	98.57[a]	5.22 [b]	3.55 [a]
5	6%	98.56 [a]	5.73 [a]	3.71 [a]
	SE (0.05)	0.13	0.13	0.18
	CD (0.05)	0.24	0.24	0.33

Treatments followed by different letters are significantly different from each other, SE: Standard error of the mean, CD: Critical difference at ∞ =0.05

Table 4. Effect of sucrose concentrations on shoot multiplication from the shoot clumps of *B. pallida* in MS liquid medium supplemented with additives + NAA (0.25mg/l) + BAP (1.0mg/l)

Tr. No.	Sucrose %	No. of shoots/Clump	Shoot length (cm)
2	2%	3.22[e]	4.39[c]
3	3%	6.41[d]	4.48[c]
4	4%	7.53[c]	4.51[c]
5	5%	7.98[b]	4.58[b]
6	6%	8.80[a]	5.01[a]
	SE (0.05)	0.17	0.25
	CD (0.05)	0.31	0.13

Treatments followed by different letters are significantly different from each other, SE: Standard error of the mean, CD: Critical difference at ∞ =0.05

Though, higher concentrations (4-6%) of sucrose produced more number of shoots at initiation and multiplication stages, shoots turned yellow after two weeks without further growth. IBA pulse treated shoots exhibited 67.4% root induction in MS half strength medium consisting sucrose and glucose. About 95% of the plantlets established well after four weeks of transfer to poly bags

(Fig. 4). No gross morphological variations were observed in the acclimatized plants.

DISCUSSION

The present study revealed that an appropriate type of carbohydrate and concentrations of sucrose plays an important role for high frequency bud break and shoot multiplication of *B. pallida*. Sucrose produced highest bud break as compared to other carbohydrates used. Similarly, Warieng and Philips [6] found that conjugation of sucrose with growth regulators were ideal to make sugar alcohols, which could be transported to the cellular system quickly and helps to maintain protein stability in the cell. Highest number of shoot buds per culture was also obtained in the medium consisting sucrose as compared to other carbohydrates. Frequency of bud break and shoot multiplication decreased in the presence of glucose whereas, fructose showed complete inhibition of shoots multiplication. Similar response was observed in *Zingiber officianalis* and *C.arundiaceum* [7, 8]. Hydrolysis of sucrose into glucose and fructose can bring about very small change in osmotic potential in the medium compared to monosaccharides which may cause more change in osmotic potential [9]. Though, increasing concentrations of sucrose produced more number of shoots in initiation and multiplication stage, shoots produced at 4-6% turned yellow and found not to be ideal for further multiplication. This may be possibly due to the enhancement of phenolics by the increasing sucrose concentrations [10]. Sucrose (3%) was proved to be ideal for shoot initiation and shoot multiplication like other bamboo species viz; *B. bambos*, *B. edulis and B. wamin* [11-13]. But in contrast, 2% sucrose favoured highest percentage of response and shoot development in *D. strictus* Nees and *Vaccinum vitis* [14, 15]. IBA pulse treated shoots were rooted in MS half strength agar gelled medium consisting sucrose and glucose. The above finding concord with the report of Yasodha *et al*. [16], where shoots treated with IBA and glucose for 3 days favoured highest rooting of *B. nutan in* MS basal medium.

CONCLUSION

The present work has demonstrated high-efficiency micropropagation of *B. pallida* for the first time by optimizing the type of carbohydrate and sucrose concentrations. It can be concluded that 3% sucrose is ideal for high frequency shoot induction and multiplication of this species. This would help to adopt the protocol in Industries for mass clonal propagation of the *B. pallida* species and to fill the gap between the present demand and supply.

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