

Original Research Article

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## Effect of Different Concentrations of BA with Constant NAA on Shoot Proliferation of BAMBOO (*Dendrocalamus asper*)

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### ABSTRACT

The healthy, disease free, young and juvenile shoot explants were selected for the experiment. The explants were collected from the fields of Ishved biotech Pvt. Ltd., washed several time with tap water for 30 min in order to wash off the external dust/contaminants and then by using liquid biological detergent Bavistin (2% w/v). Again 70% ethanol is used for 1 minute, after that process 0.1% mercury chloride ( $HgCl_2$ ) for 5 min. and rinsed the explants three to four time to remove the traces of  $HgCl_2$  and ethanol the by sterile water. BA concentrations 0.0, 0.5, 1.0, 1.5, 2.0 mg/L. in combination with NAA 0.1mg/L were prepared by dissolving them in 100% sterile water as stock. Then, MS media (34.4gm/l), sucrose was dissolved in distilled water. The pH of the medium was adjusted between 5.6-5.8 by using either 0.1 N Hcl or 0.1 N NaOH with the help of a digital pH meter. The volume (1000 ml) was finally adjusted and required amount of agar added into the media. Agar in the medium was completely melted by gentle heating up to 90°C and 40 ml of medium was poured into pre sterilized culture bottles. The media was autoclaved at 121°C at 15 lbs/square inch pressure for 20 minutes and then allowed to cool to room temperature and stored in culture rooms until further use. After sterilization explants were trimmed to 1.5-2 cm and inoculated on MS media supplemented with different combination of BA with constant NAA inoculation and room temperature was maintained at  $25\pm 2^\circ C$  with 16 hour photoperiod. Biometric observation No. of Shoots, shoot length, No. of roots, root length were observed periodically.

#### Keywords

Bamboo,  
Micropropagation,  
Shoot, Growth  
hormones, Rooting

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### Introduction

Bamboos are perennial, evergreen woody monocots that belong to the family Poaceae, subfamily Bambusoideae and tribe Bambuseae. The plant is distributed at latitudes from 46°N to 47°S and at altitudes up to 4,000 amsl in the subtropical and temperate zones of all continents except Europe. Asian countries such

as Nepal, Vietnam, Laos, Thailand, China, India, Bhutan, Bangladesh and Myanmar account for about 1000 species, covering an area of over 180,000 km<sup>2</sup> (Yadav *et al.*, 2008). Among these, India and China together contribute more than half of the total bamboo resources of the world (Anonymous, 2018). Bamboo is considered to have originated in China (Anonymous, 2014).

There are about 90 genera and about 1200 species of bamboo found in the world. It is estimated that bamboo plants constitutes about 13% of the total forest area of India. About 50% of bamboo produced in North Eastern region and West Bengal of India (Diab and Mohammed, 2008).

The plant is an important structural component of many types of vegetation and plays a major role in ecosystem dynamics (Keelaey and Bond, 1999). It has gained importance in social forestry programs due to its short rotation cycle, fast growth and ease of progressive harvesting on suitable basis.

As per records India alone constitutes a market of 26,000 corers of rupees from bamboo segment. Major portion of this contribution is from the north eastern states of the country whereas the portion given by South India is also notable one. About 2.5 billion people in the world depend economically on bamboo In India some 8.6 million people depend for their livelihoods on bamboo and the industries it supplies. Indian bamboo is currently estimated to create value equal to USD 4.4 billion – approximately 130 times the USD 34 million recorded in 2003 and international trade in bamboo amounts to about USD 60 billion (Singh *et al.*, 2012).

In India, bamboo currently generates 432 million workdays annually, employing nearly 10 million people. With 8.96 million hectares of India's forest cover containing bamboo, there is potential to create approximately 129 million jobs or, even by more conservative estimates, at least 50 million.

India's current demand for bamboo is estimated at 27 million tons per year; only 50% of that demand can be met domestically because of lack of facilities for value addition and transportation. The world market for bamboo has been estimated at over USD 10 billion in 2001 and is expected to grow to

USD 20 billion by 2015. Although India has 30% of the world's bamboo resources, it constitutes only 4% of the global market (Jamatia, 2014).

It is the fastest growing canopy, releasing 35% more oxygen than any other tree. In bamboo, breeding is seriously handicapped because of its long vegetative phase, monocarpic flowering behavior and poor seed set.

Moreover, it is near impossible that two desirable plants will flower simultaneously; therefore, conventional breeding also seems to be difficult (Das and Pal, 2005). Thus for meeting the raw material demand, the best possible way to manage the bamboo forest is through scientific management like plant tissue culture (Sood *et al.*, 2016).

## **Materials and Methods**

### **Experimental site**

The experiment was conducted at Plant Tissue Culture Laboratory "Ishved Biotech" Pvt. Ltd. Sindhkhedraja, Buldhana, Maharashtra.

### **Lab conditions**

The cultures were incubated in a culture room at  $25 \pm 2^\circ\text{C}$  under 16 hrs photoperiod provided by cool white fluorescent tubes.

### **Treatments of hormones**

Different concentrations of BA (0.0, 0.5, 1.0, 1.5, 2.0 mg/L) in combination with constant NAA (0.1 mg/L).

### **Source of explants**

Healthy, young and juvenile plants were obtained from the fields of Ishved biotech Pvt.Ltd. Sindhkhedraja, Buldhana.

### **Collection of plant material**

Young and juvenile plant of 3-4 years old was selected as explants of Bamboo. Nodal shoot segments of 5-6cm in length were selected as explants for shoot proliferation of Bamboo (*Dendrocalamus asper*).

### **Chemicals for surface sterilization**

Chemicals used in the experiment were 2% Bavistin (A biological detergent), 70% ethyl alcohol and 0.1% Mercuric chloride. The chemicals were used in surface sterilization in specific time period with explants both ends exposed to the chemicals.

### **Preparation of stock solutions**

At the time of media preparation, it is practically not possible to weigh each of the ingredients needed in the medium. Hence, for the sake of convenience, concentrated stock solutions of basal MS medium containing different ingredients were prepared in double distilled water and stored in jam bottles in refrigerator at 5°C temperature.

### **Preparation of culture media**

After addition of various kinds of adjuvant (after bringing stock solutions to room temperature) to the MS basal medium, the pH of medium was adjusted to 5.8 using 0.1N HCl. The final volume adjusted and 8 gm/l agar-agar was added to the medium and then hormones in respective treatments were added to the media and then dispensed in suitable container i.e. culture bottles. Autoclaving was done using horizontal steam sterilizer at 121°C and 1.5 lbs/cm<sup>2</sup> pressure for 20 min. After sterilization, the medium was allowed to cool down then medium were poured in culture bottles and allowed to solidify at room temperature and stored in dust proof room for at least 3 days before use to check for any contamination.

### **Aseptic techniques**

The standard sterilization techniques were followed as per standard tissue culture guidelines for inoculation and sub culturing of explants in culture bottles.

Inoculations of explants were carried out under aseptic conditions in laminar air flow bench. During the course of transfer of explants, all surgical instruments were dipped in alcohol, incinerated in glassbeed sterilizer and cooled before use.

### **Explants selection and surface sterilization**

The disease free young and healthy plants were selected for carry out the experiment. The nodal explants Bamboo were used as explants. Some of the branches were pruned periodically to obtain new sprouts. Nodal shoot segments (4-5cm in length) with dormant auxiliary buds were collected from the Ishved biotech Pvt. Ltd Sindhkhedraja, Buldhana.

Dipped in a 2% (w/v) liquid biological detergent (Bavistin) for 5 min and washed four or five times with distilled water. Subsequently, the explants were surface sterilized with 0.1% (w/v) mercuric chloride for 5–6min. and thoroughly washed 3 to 4 times with sterile distilled water, with both exposed ends of the explants.

### **Inoculation on micro propagation media**

Sterilized nodal explants were trimmed and inoculated on micro propagation media and incubate in culture room at 25°C temperature with 16 hours photoperiod.

### **Results and Discussion**

The results obtained in the present investigation of Bamboo (*Dendrocalamus asper*) on shoot proliferation and rooting in

bamboo by using nodal explants are presented by the following headings.

**Days required for shoot initiation**

Present investigation showed that the number of days required for shoot initiation was influenced due to different levels of BA and constant NAA. The minimum number of days for initiation of shoots i.e. (15-21days).

**No. shoots per explants**

Numbers of shoot were produced in media supplemented with BA and combination with NAA. Data presented in Table 1 and Fig. 1 would reveal the data for result of the No. of shoots produced per explants for different concentrations of BA. The shoot number was

influenced due to different levels of BA in combination with constant NAA. The highest No. of shoots 32 shoots per explant was produced by BA @ 2.0mg/L followed by 0.0, 0.5, 1.0 1.5mg/L and NAA @ 0.1 mg/L shoots per explants.

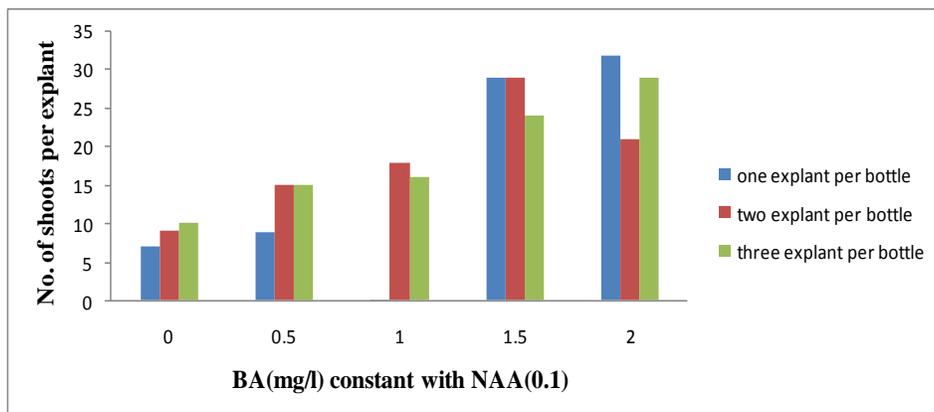
**Length of shoot**

Data on results of length of explants (cm) of various treatments shoots as influenced by various treatments of BA and constant NAA levels in 21 DAI are given in Table 1 and Fig. 2 Indicated that maximum height of explants (cm) of Bamboo was 7.0 cm showed for BA @ 1.0 mg/L and followed by 0.0, 0.5, 1.5, 2.0 mg/L and NAA @ 0.1 mg/L respectively.

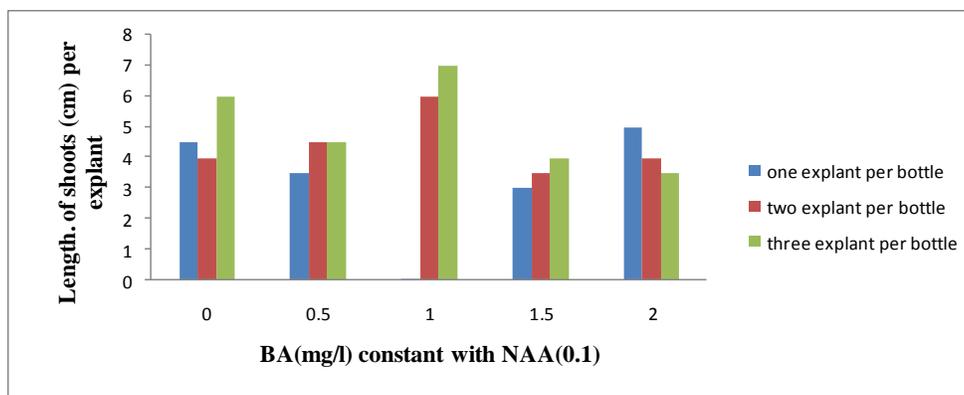
**Table.1** Effects of different concentration of BA with constant NAA (0.1 mg/L) on growth Bamboo

Sr. no	Explants / bottle	Growth Parameter	Media composition / treatments				
			BA(0.0)	BA(0.5)	BA(1.0)	BA(1.5)	BA(2.0)
01	One culture/ bottle (5 bottles)	No of shoots	7	9	10	29	32
		Shoot length (cm)	4.5	3.5	4.5	3	5
		No of root	Single rooting	3	4	Single rooting	No rooting
		Root length (cm)	4	1.3	6	1.8	Nil
02	Two culture/ bottle (10 bottles)	No of shoots	9	15	18	29	21
		Shoot length (cm)	4	4.5	6	3.5	4
		No of roots	4	6	3	No rooting	Single rooting
		Root length (cm)	6	8	3.5	Nil	1.2
03	Three culture/ bottle (10bottles)	No of shoots	10	15	16	24	29
		Shoot length (cm)	6	4.5	7	4	3.5
		No of roots	13	7	2	10	3
		Root length (cm)	15	6.2	5.5	8	6

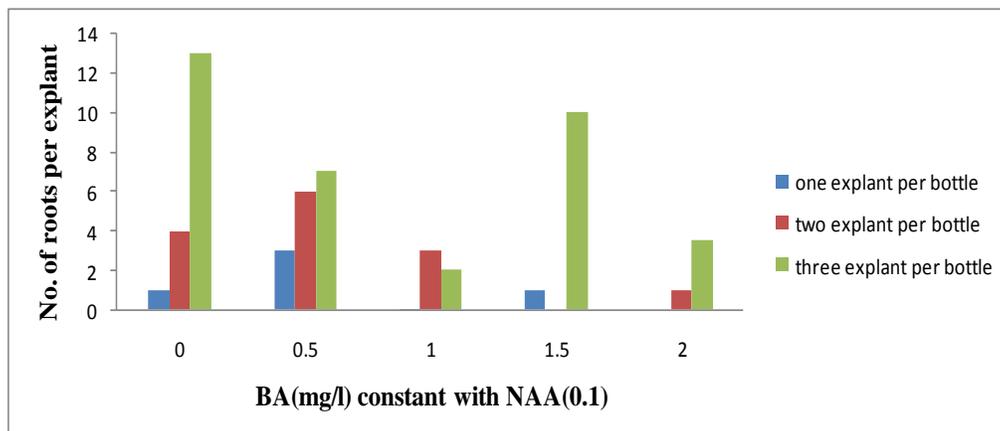
**Fig.1** Effect of different concentrations of BA with constant NAA (0.1 mg/L) on No. of Shoots per explant of Bamboo



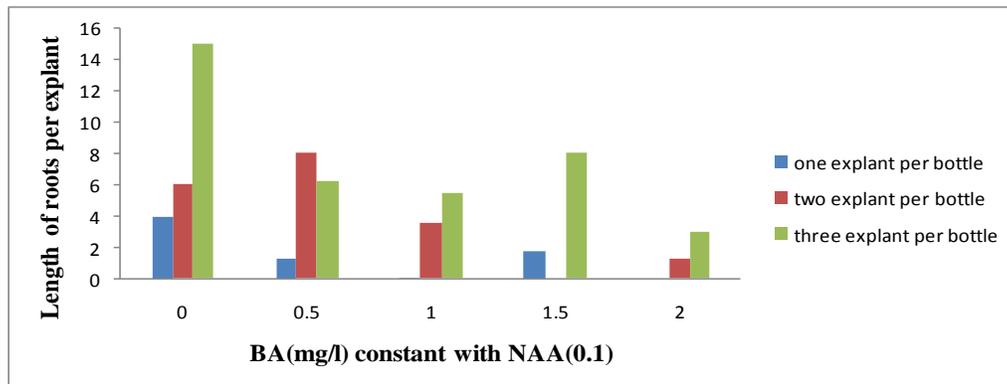
**Fig.2** Effect of different concentrations of BA with constant NAA (0.1 mg/L) on Length of Shoots (cm) per explant of Bamboo



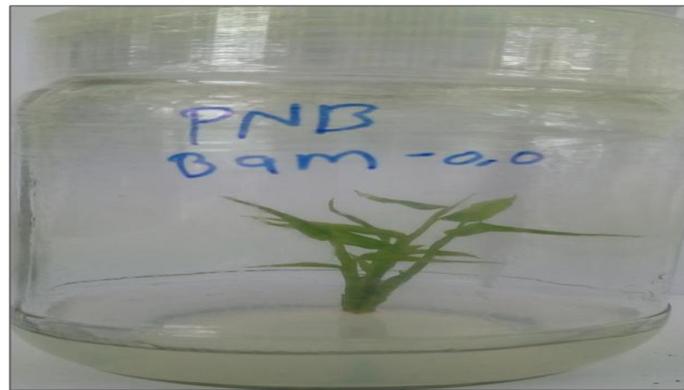
**Fig.3** Effect of different concentrations of BA with constant NAA (0.1 mg/L) on No. of Roots per explant of Bamboo



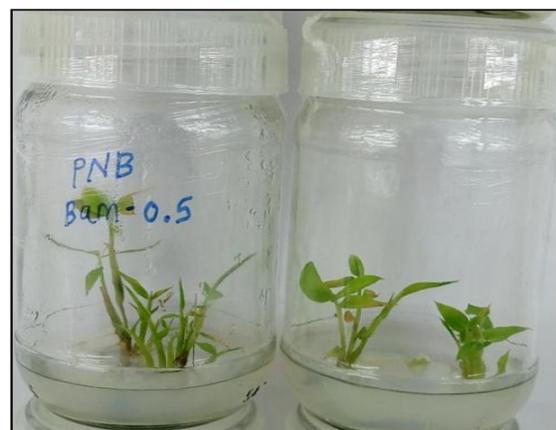
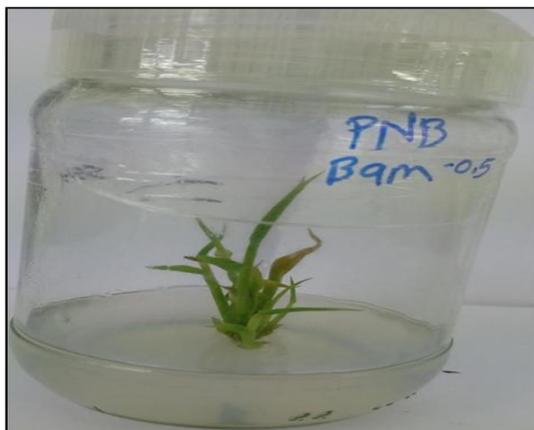
**Fig.4** Effect of different concentrations of BA with constant NAA (0.1 mg/L) on Length of Roots (cm) per explant of Bamboo



**Plate.1** Shoot initiation from nodal explants of Bamboo



**Plate.2** Shoot proliferation in Bamboo



**Plate.3** Shoot length of Bamboo



**Plate.4** Rooting in Bamboo



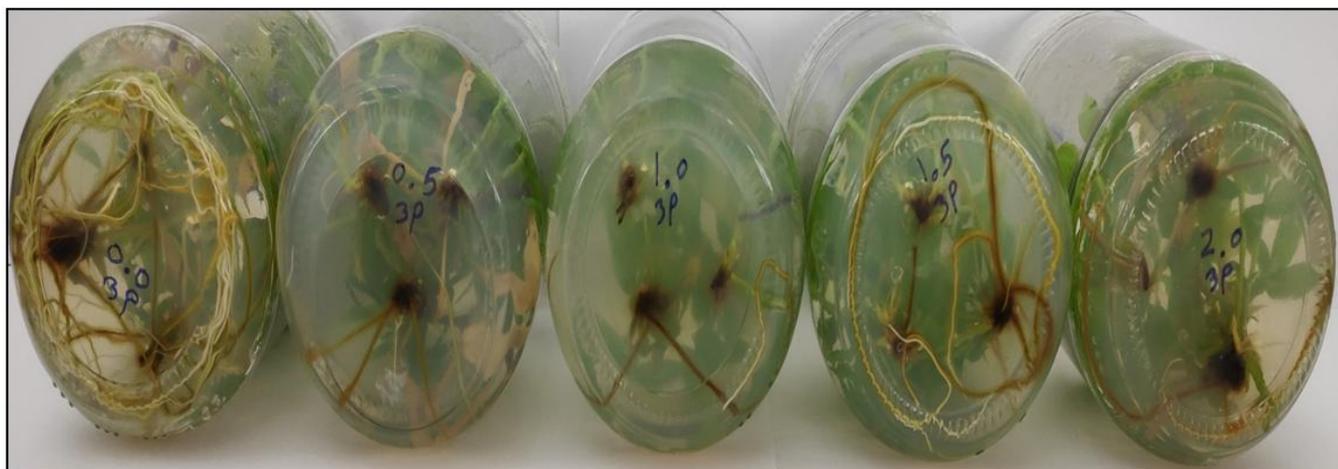
**Plate.5** Root length of Bamboo



**Plate.6** Effect of different concentrations of BA with constant NAA (0.1 mg/L) on shoot proliferation of Bamboo



**Plate.7** Effect of different concentrations of BA with constant NAA (0.1 mg/L) on Rooting in Bamboo



Present investigation showed that the number of days required for shoot initiation was influenced due to different levels of BA and constant NAA. The minimum number of days for initiation of shoots i.e. (15-21days).

#### **No. of roots per explant**

Numbers of root were produced in media supplemented with BA and combination with NAA. Data presented in Table 1 and Fig. 3 would reveal the data for result of the No. of

concentrations of BA with constant NAA. The root number was influenced due to different levels of BA in combination with constant NAA. The highest No. of roots 13 roots per explant was produced by BA @ 0.0 mg/L followed by 0.5, 1.0, 1.5, 2.0 mg/L and NAA @ 0.1 mg/L roots per explants.

#### **Length of roots**

Data on results of length of explants (cm) of various treatments shoots as influenced by

various treatments of BA and constant NAA levels in 21 DAI are given in Table 1 and Fig. 4 Indicated that maximum length of explants (cm) of Bamboo was 15 cm showed for BA @ 0.0 mg/L and followed by 0.5, 1.0, 1.5, 2.0 mg/L and NAA @ 0.1 mg/L respectively.

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### References

Anonymous 2018, [www.wikipedia.com](http://www.wikipedia.com)  
Anonymous 2014, [www.bamboogrove.com](http://www.bamboogrove.com).  
Das M., Pal A. 2005. In vitro regeneration of *Bambusabalcooa Roxb*: Factors affecting changes of morphogenetic competence in the auxiliary buds. Plant cell, Tissue and organ culture, 81(1): 109-112.  
Devi W.S., Bengyella L., Sharma G.J.2012. In vitro seed germination and micropropagation of edible bamboo *Dendrocalamus giganteus* Munro using seeds. Biotechnology11 (2): 74-80.  
Diab E., Mohamed S. 2008. In vitro morphogenesis and plant regeneration of Bamboos. International journal of sustainable crop production, 3 (6): 72-79.  
Goodbole S., Sood A., Thakur R., Sharma M., Ahuja P.S. 2002. Somatic embryogenesis its conversion into plantlets in a multi-purpose Bamboo *Dendrocalamus Hamiltonian*. Current science, 83(7): 885-889.  
Iram siddique, Najat Abdullah W Ahab Bukhari, Kakhkashan Parveen and Iffat siddiqui. Influence of plant growth

regulators on in vitro shoot multiplication and plantlet formation *Cassia augustifolia* Vahl, Vol. 58, n. 5: pp. 686-691.  
Keely E., Bond W. 1999. Mast flowering and semelparity in bamboos: the bamboo fire cycle hypothesis the American Naturalist, 154(3):383-391.  
Mehta U, Rao IVR, Ram HYM (1982) Somatic embryogenesis in bamboo. In: Proc 5th Intl Cong Plant Tiss and Cell Cult 109–110.  
Murashige M., Skoog F. 1962. A revised media for rapid growth and bioassays with Tobacco tissue culture. Physiologia Planatrum, 15:473-474.  
Rao IU, Rao IVR, Narang V (1985) Somatic embryogenesis and regeneration of plants in the bamboo *Dendrocalamus strictus*. Plant Cell Rep 4:191–194.  
Jamatia S., 2016 Bamboo regulation in India: the need for reforms. Journal of bamboo and rattan 73 (2): 212-215.  
Singh S.R., Dalal S., Singh R., Dhawan A.K., Kalia, R.K. 2012. Seasonal influences on in vitro bud break in *Dendrocalamus hamiltonii*. Arn. Ex Munro nodal explants and effect of culture microenvironment on large scale shoot multiplication and plantlet regeneration. Indian Journal of Plant Physiology, 17: 9-21  
Sood A., Ahuja P.S., Sharma M., Sharma O.P., Godbole S. 2002. In vitro protocols and field performance of elites of an important bamboo *Dendrocalamus hamiltonii* Neeset Arn.ex Munro. Plant Cell, Tissue and Organ Culture, 71(1): 55-63.  
Yadav S., Patel, P., Shirin F., Mishra Y., Ansari S.A. 2008. In vitro clonal propagation of 1 year old clumps of *Bambus anutans*. Journal of Bamboo and Rattan, 7(3-4): 201-210.  
Yasodha R., Kamala S., Kalaiarasi K. 2010.

Anatomical and biochemical changes associated with in vitro rhizogenesis in *Dendrocalamus giganteus*. Journal of

Plant Biochemistry and Biotechnology, 19(2): 217-222.

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