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In-vitro culture of *Dendrocalamus strictus* with respect to apical shoot and its traditional importance

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Abstract
Mass propagation of bamboo became needful that to bamboo considered as poor man timber for rural and traditional people. Vegetative propagation by rhizome cutting, layering, pre-rooted and pre-rhizome, branch cutting, nodal branch chips, offset and culm division are also common in various bamboo. But such methods have various limitations like large scale multiplication, cost of production, microbial infection, difficult in transportation, etc. So, in this paper special attention is given to mass propagation of *Dendrocalamus strictus* using apical shoot as explants in vitro process.

Keywords: In-vitro culture *Dendrocalamus strictus* Lakhimpur district, Assam

Introduction
There are 45 species of bamboo recorded from Assam. Bamboo is considered as the life line of traditional people for their livelihood. It is also named as "poor man timber" of traditional people. From the beginning of human civilization, bamboo has been of great importance to mankind. The multiple uses of bamboo meet the basic needs of villagers, farmers, and rural people. Hence it is inseparable part of the culture of rural people owing to its various uses like food, fodder, fuel, fencing, house construction, etc. Pulp of *Dendrocalamus strictus* and *Bambusa arundinaceae* contain about 85% cellulose that is used in paper industry in India, which consumes a huge proportion of the total annual bamboo production (Vatsala, 2003) [1]. So, further research is clearly required on propagation techniques to increase the multiplication rates of bamboo. For mass propagation of bamboo, in-vitro culture is one of the best available technique (Gielis and Oprins, 2002). Micropropagation ensures the supply of quality planting material on regular basis (Paranjothy *et al.*, 1990; Arya *et al.*, 2006) [4]. In this paper special attention is given to mass scale production of Bamboo (*Dendrocalamus strictus*).
Our study is specially restricted to Micropropagation of bamboo (*Dendrocalamus strictus*). Assam is predominantly inhabited by traditional people and in different culture bamboo plays a significant role. As this species is comparatively infrequent in the study area, its importance is significant to the socio-cultural impact of the traditional people. Though bamboo is not considered under RET plant, but special attention is outmost necessary in order to maintain livelihood of the local people.
Dendrocalamus strictus, commonly called as "Lathi bamboo" flower very infrequently with an interval of 60 to 120 years so there is scarcity of the seeds (Ramayana and Yakandawala, 1998) [5]. Besides seed culture apical shoot culture is one of the significant culture technique to obtain disease free stock. Moreover, seeds also suffer much damage due to rodents attack and there is rapid loss of Viability due to poor storage. So special care has to be taken for storage of the apical shoot. Vegetative propagation by rhizome cutting, layering, pre-rooted and pre-rhizome branch cutting, nodal branch chips, offset and culm division are also common in various bamboo. But such methods have various limitations like large scale multiplication, cost of production, microbial infection, difficult in transportation, etc. So, in this paper special attention is given to mass propagation of *Dendrocalamus strictus* using apical shoots as explant, collected from Lakhimpur district of Assam.

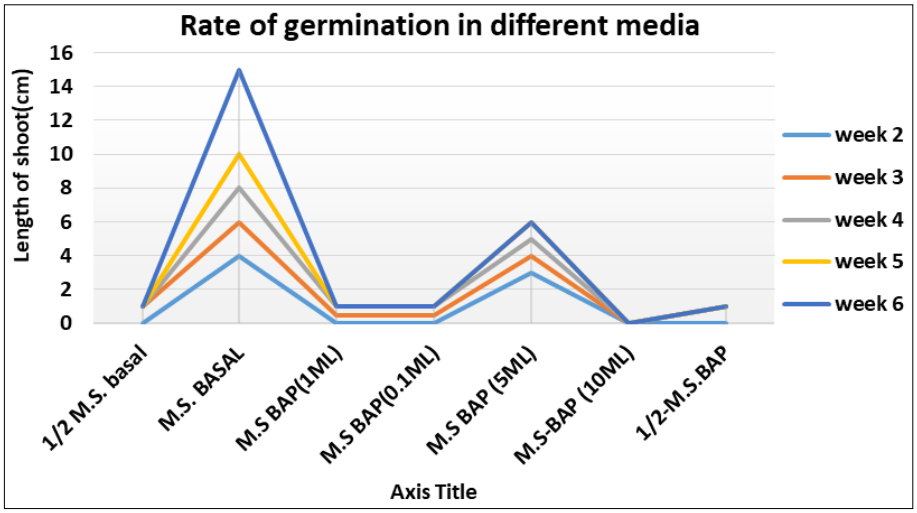
Materials and Method
Apical shoots of *Dendrocalamus strictus* were collected from Lakhimpur district of Assam. They were dehusked and sterilized in normal tap water with a drop of teepol for 6mins and washed with distilled water for 10 minutes to remove foreign debris. Apical shoots were further surface sterilized in mercuric chloride solution (8%, v/v) for 30secs and rinsed with distilled water for 5 times. Inside laminar flow, treated explants were again rinsed with water mixed with little alcohol for 30s and then decant off. Now the explants were inoculated in 100×10mm test tubes containing M.S Culture supplemented with 2% sucrose (w/v) and 7% agar (w/v). After inoculation the test tubes were placed in racks and incubated at 28º C in dark till germination. Keeping one culture media as control (M.S media), three other experiments were conducted using ½ M.S Media, M.S-BAP and ½M.S-BAP with various concentrations like 1ml, 0.1ml, 5ml, 10ml. 15 test tubes were observed for each experiment and results were noted every 2 weeks. The experiments were repeated 3 times to confirm the results. After germination the test tubes were transferred to continuous light for further growth.
Culture conditions: The P^H of the medium was adjusted with 1N NaOH and 1N HCl to 5.7 + 0. Prior to addition of 0.8% Agar, all culture media were autoclaved at 121 degree Celsius for 20mins. Cultures were maintained in a growth chamber at 25+3 degree Celsius.

Observation and Discussion

Maximum germination was observed on M.S. Basal Media and signs of germination were also noticed more quickly as compared to other media (within 7 days). The optimum temperature for germination was 28-30 degree Celsius. In this medium rhizome induction was seen after 4 weeks in germinated shoots which transformed into plantlets. Rhizome induced plantlets show better survivability in field hence it increases the efficiency of micro-propagation. The rhizome helps in early establishment of plant in field and it also helps in early culm production. In M.S-Basal media the in-vitro germinated plantlet produced both shoot (culm) and root thus acting as a seed. As seen that rhizome was not induced in other media in this study, except M.S-Basal media, it would not be wrong if we say that M.S-Basal media contains some necessary elements that helps induction of rhizome (Fig-A).

On MS basal medium with 5ml (5×10^{-6}M) BAP, there was development of multiple shoots (Fig-C). This could be due to suppression of apical dominance. The Cytokinin 6-Benzylaminopurine is capable of inducing axillary shoot formation. The first hypothesis was reported that cytokinin could reduce IAA oxidase of axillary shoots thus it leads to the increase in axillary shoots elongation via the increase in endogenous auxin. The second hypothesis was reported cytokinin stimulated axillary shoots formation via the transportation of nutrients and vitamins Thus the cytokinin 6-Benzylaminopurine was found to develop multiple shoots in the seedlings of *Dendrocalamus strictus.* (Tran Trong Tuan *et.al.* 2012). Further propagation of multiple shoots in groups of 4-5 can also help in increasing the propagules of this species and help in micropropagation. However, rooting took place after a long time on BAP media.

The graph shows rate of shoot callusing in terms of length of shoots in increasing number of weeks on various combinations. In the experiments conducted high rate of germination was seen in M.S. Media and MS basal medium with 5ml (5×10^{-6}M) BAP. Whereas no germination was seen in MS basal medium with 10ml BAP. Dead shoot was observed in all the other concentration. However, growth of root was seen only in M.S basal media and a slight growth was observed in MS basal medium with 5ml (5×10^{-6}M) BAP.



Graph 1: showing rate of germination

Above all, it has also been observed that ½ MS Media lacks to fulfill the entire basic requirement for growth and development of *Dendrocalamus strictus.* While performing the experiment germination was observed in 11 out of 15 test tubes but after about 2 to 3 weeks' time period the shoots died. Thus, there was only dead shoot with roots. M.S.-BAP Media is however useful for the growth and development of *Dendrocalamus strictus*, especially in multiple shoot formation. After about two weeks of inoculation some explants starts to germinate which develop a small (about +0.5cm) whitish protruding from it. As weeks passes by it starts growing when transferred to 24 hours light condition. Finally, the shoot turns green slowly and leaves starts to develop. Meanwhile the fibrous root also started to develop slowly. It took more time for the roots to develop as compared to M.S. Basal media. Also, in 10 out of 15 germinating tubes multiple shooting was also observed which has given us a rough idea that 5×10^{-6} Molar MS-BAP Media also supports multiple shooting. However, the optimum composition of BAP suitable for such development is 5ml (5×10^{-6} M). While performing the experiment in various composition of BAP only 5ml (5×10^{-6} M) survived and 0.1ml (10^{-7} M) & 1ml (10^{-6} M) formed dead shoot after germination. But tubes with 10ml (10^{-5} M) BAP did not germinate. Earlier it was seen that MS-BAP media was useful for multiple shooting. When tested with ½ M.S-BAP it is seen that the time period taken for germination is more as compared to the M.S-BAP media. Moreover, there was no shooting within 3 weeks' time. Only small elongation was observed whose growth remained constant for a long time (21 days).

Result

It has been observed that MS Media is very useful for the growth and development of *Dendrocalamus strictus.* Initiation of root and shoot starts within a week when kept in dark at 28 degree Celsius. After about a week some explants starts to form callus which develop a small (about 0.5cm) whitish protruding from it. As weeks pass by it starts growing when transferred to 24 hours light condition. Finally, the shoot turns green slowly and leaves starts to develop. Meanwhile the fibrous root also develops and becomes denser. After about 60days, they can be shifted and plants in soil: sand: manure (1:1:1) and watered regularly.

The planted plants are raised with about 80% survivability. ½ MS Media lacks to fulfill all the basic requirement for growth and development of *Dendrocalamus strictus.*

Earlier it was seen that MS-BAP media helps in multiple shooting. When tested with ½ M.S-BAP it is seen that the time period taken for callus formation is more as compared to the M.S-BAP media. Moreover, there was no shooting within 3 weeks' time. Only small elongation was observed whose growth remained constant for a long time (21 days) (Fig-B). Thus, MS.-BAP Media is most useful for growth and development of *Dendrocalamus strictus* especially in multiple shoot formation. However, the optimum composition of BAP suitable for such development is 5ml (5×10^{-6} M) (Fig- D, E and F).

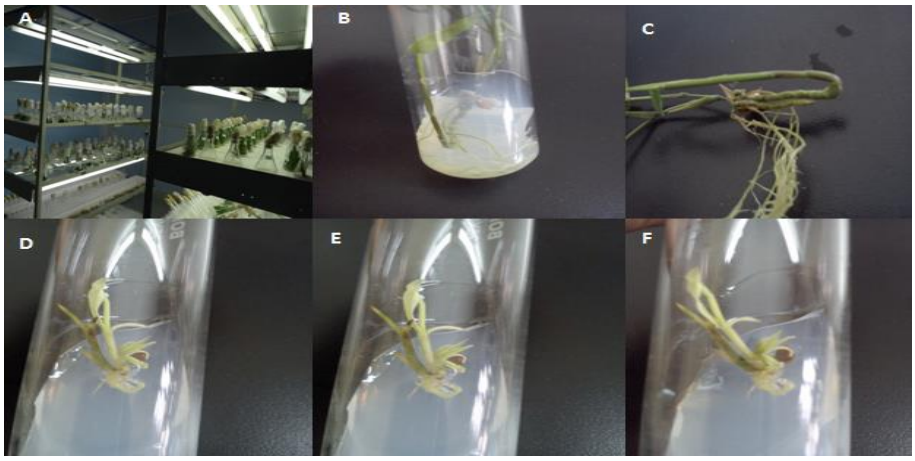


Fig 1

Fig-A Tissue Culture laboratory, Fig-B Rhizome induction, Fig-C Giving rise to new plantlet, Fig-D Multiple shoot {On MS basal medium with 5ml (5×10^{-6}M) BAP, Fig E & F. Multiple shoots on (5×10-6 M) BAP medium after 4thweek

References

1. Vatsala. Bamboos in India. National Institute of Science Communication and Information Resources, New Delhi, 2003, 111 pp.
2. Gielis J. "Micropropagation and in vitro flowering of temperate and tropical woody bamboos". (in the press), 1998.
3. Paranjothy, Paranjothy K, Saxena S, Banerjee M, Jaiswal VS, Bhojwani SS, *et al*. Clonal multiplication of woody plants. In: S. S. Bhojwani (Ed.), Plant Tissue Culture: Application and Limitations. Elsevier, Amsterdam, 1990.
4. Arya S, Rana PK, Sharma R, Arya ID. Tissue culture technology for rapid multiplication of *Dendrocalamus giganteus* Munro. Ind. For. 2006; 132:345-357.
5. Ramanayake SMSD, Yakandawala K. "Micropropagation of the giant bamboo (Dendrocalamus giganteus Munro) from nodal explants of field grown culms". Plant Science. 1997; 129:213-223.
6. Pandey BN, Singh NB. Micro propagation of *Dendrocalamus strictus* from mature nodal Explants; Journal of Applied and Natural Science. 2012; 4(1):5-9.
7. Chaturvedi HC, Sharma M, Sharma AK. In vitro regeneration of *Dendrocalamus strictus* Nees. through nodal segments taken from field-grown culms. Plant Sci. 1993; 92:97-101.
8. Chambers SM, Heuch JHR, Pirrie A. Micropropagation and *in vitro* flowering of the bamboo *Dendrocalamus hamiltonii* Munro. Plant Cell Tiss. Org. Cult. 1991; 27:45-48.
9. Arya S, Sharma S. Micropropagation technology of *Bambusa bambos* through shoot proliferation. Ind. For. 1998; 9:725-731.
10. Micropropagation of important bamboos: A review (Kalpataru Dutta Mudoi, Adrita Goswami, Debashish Bora and Mina Borthakur) African Journal of Biotechnology. 2013; 12(20):2770-2785.
11. Dekkers AJ, Rao AN. Tissue culture of four bamboo genera. In: A. N. Rao and A. M. Yusoff (Eds.), Proc. Seminar on tissue culture of forest species. FRI, Malaysia and IDRC, Singapore, 1989.
12. Saxena S. *In vitro* propagation of the bamboo (*Bambusa tulda*Roxb.) through shoot proliferation. Plant Cell Rep. 1990; 9:431-434.
13. Chaturvedi HC, Sharma M, Sharma AK. In vitro regeneration of *Dendrocalamus strictus* Nees. through nodal segments taken from field-grown culms. Plant Sci. 1993; 92:97-101.
14. Arya S, Rana PK, Sharma R, Arya ID. Tissue culture technology for rapid multiplication of *Dendrocalamus giganteus* Munro. Ind. For. 2006; 132:345-357.