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Effect of growth regulators on the *in vitro* multiplication of *Dendrocalamus Hamiltonii*

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Abstract:
Bamboos are versatile multipurpose forest product, which are important economically and are often referred to as 'GREEN GOLD'. *Dendrocalamus hamiltonii* is one of the economically important species of Bamboo in India. Government of India is running National Bamboo Mission to encourage the production of Bamboos in India. The present work was undertaken to study the effect of Auxins and Cytokinins on the *in vitro* multiplication of nodal cuttings with axillary buds *Dendrocalamus hamiltonii* a bamboo species growing in North east region of India and north western Himalayas. The growth medium used was MS (1962) basal medium supplemented with BAP, Kn and NAA at varying concentrations. The multiplication rate of shoots increased with increasing the concentration of NAA and Kn. However the optimum results were obtained on MS medium supplemented with a combination of 0.5 mg/l NAA, 0.5 mg/l Kn and 1 mg/l BAP. Effect of TDZ concentration was also observed, and the results revealed that 0.25 mg/l TDZ, 0.25 mg/l PGH with 1 mg/l BAP were found to be most suitable for in vitro multiplication of *Dendrocalamus hamiltonii.*
Key Words: *Dendrocalamus hamiltonii*, M S Medium, multiplication, TDZ.

I. Introduction:

Bamboo occupies an important place in the diverse phases of life and culture of the people. Bamboos are short rotation, arbores cent, perennial, giant grasses that constitute a major non-timber forest produce (NTFP) of India. Bamboo has an advantage of being a renewable resource and can be repeatedly harvested as sustainable source of raw material. Bamboo is increasingly identified as potential species for poverty reduction programs in India.

Bamboos are among economically most important plants worldwide and important forest resource in many countries of Asia. The *Dendrocalamus hamiltonii* is one such important species which is distributed in the North West Himalaya, Sikkim, Arunachal Pradesh, Assam, Manipur,Meghalaya, Mizoram, Nagaland, Tripura of India, Bhutan and Bangladesh. Its flowering cycle is of 30-45 years (Tiwari, 2002). It is used for used in construction, fencing, baskets, and containers, shoots eaten fresh or pickled. Tremendous socio-economic pressures besides jhum cultivation in the natural habitats have often compelled over-exploitation of raw bamboo resources to a critical level. Tissue culture techniques offer an effective strategy for rapid propagation and mass multiplication of edible bamboo species keeping in view of their sustainable development and utilization (Devi and Sharma, 2009). At present Government of India is running National Bamboo Mission to support and encourage the Bamboo cultivation in India. An integrated approach of biotechnology has played a key role in the fast development and improvement programme of forestry species, including bamboos. Tissue culture based propagation through axillary shoot proliferation and somatic embryogenesis have potential for mass production and improvement (Thorpe *et al.*, 1992).

II. Material and Methods:

The explants used in the present study were auxiliary buds as these preformed buds are the potential source for the large scale clonal propagation of the plants. These were excised from authentic plants of *Dendrocalamus hamiltonii* washed first under tap water with Labklin detergent and then with double distilled water. Nodal segments in separate wash bottles were then given fungicide treatment (1% Bavistin, Copper Blue -50, Care, Colt or mixture of any two) for 10-15 minutes after that again washing was done with double distilled water. These explants were disinfected by 0.1% & 0.15% $HgCl_2$ solution for 10 to 15 minutes and then thoroughly washed with autoclaved double distilled water under laminar airflow. These explants were then inoculated on sterilized culture medium in suitable culture vials.

The culture medium used was MS basal supplemented with different concentrations and combinations of auxins and cytokinins. The pH of the medium was adjusted to 5.8 with 0.1 N NaOH / HCl. The media were sterilized by autoclaving at 15 lbs pressure and 121°C for 15 minutes. After inoculation the cultures were maintained under controlled conditions (16/8 h photoperiod, 24 ± 2°C temperature, and light intensity of 500lx and relative humidity of 80%).

(Figure 1) Bud Induction : (a) In 0.5(mg/l)TDZ+ 1.0(mg/l) BAP

(b) In 1.0(mg/l)BAP+ 0.5(mg/l)NAA+ 0.5(mg/l)Kn

(Figure 2) Shoot proliferation: (a) In 0.5(mg/l)TDZ + 1.0(mg/l) BAP

(b) In 1.0(mg/l)BAP+0.5(mg/l)NAA+ 0.5(mg/l)Kn

III. Results:

Micro-propagation protocols through axillary shoot proliferation from field grown mature nodal shoot explants have been developed for many bamboo species. Bamboos are a group of tall, arborescent, perennial grasses that grow very fast and are evergreen. It flower at long intervals and in many instances (when gregarious flowering) the plants die after flowering. It can be propagated by tissue culture under the influence of various growth regulators. The results included in this paper depict the influence of auxins, cytokinins and their combinations on the multiplication of axillary buds. The nodal segments with axillary buds were taken as explants from *D. hamiltonii* grown in Bambusetum, FRI, Dehradun and inoculated on MS medium supplemented with various concentrations of BAP, NAA and Kinetin. The effect of TDZ and PGH was also analysed.

The hormone BAP was found effective in inducing the sprouting of the buds at a concentration of 1.0 mg/l. BAP (1.0mg/l) in combination NAA (0.5mg/l) showed 60% sprouting of buds and the average shoot length was found 2.11±0.75 mm only, at the same time BAP (1.0mg/l) in combination NAA (2.5 mg/l) induced sprouting in 30% buds but the shoot length measured was 3.65±0.35 mm only. The percentage sprouting decreased with increasing the concentration of the NAA and the optimum results were obtained at 1.0mg/l BAP and 0.5mg/l NAA. However, when the nodal cuttings axillary buds were treated with various combinations of BAP, Kinetin and NAA, the results improved with maximum sprouting on 1.0mg/l BAP, 0.5mg/l NAA and 0.5mg/l Kn in 70% cultures .The optimum length of the *in vitro* raised shoots (4.85±0.44 mm) was also achieved at this combination (Table 1, Figure 2a and b).

The Combination of BAP with TDZ and PGH showed maximum sprouting of the buds at 1.0mg/l BAP: 0.5mg/l TDZ : 0.5mg/ l PGH i.e. 90%. With increasing concentration of TDZ the sprouting of buds reduced to 80%. It was seen that increasing concentration of TDZ has negative effect on sprouting of buds and shoot length.

Table 1- Effect of different growth regulators on the multiplication of axillary buds of *Dendrocalamus hamiltonii*

Growth Medium	% of Buds sprouted	Shoot length (mm±SD)
MS Basal + BAP(mg/l)		
1.0	50	4.05± 0.95
2.0	60	4.55±0.27
3.0	60	4.81± 0.25
MS+ BAP+NAA(mg/l)		
1.0+0.5	60	2.11±0.75
1.0+1.0	50	2.22±0.08
1.0+1.5	40	2.50± 0.09
1.0+2.0	40	2.82± 0.13
1.0+2.5	30	3.65±0.35
MS+BAP+Kn+NAA(mg/l)		
1.0+0.5+0.5	70	**4.85±0.44**
2.0+0.5+0.5	60	3.41±0.16
3.0+0.5+0.5	60	2.47 ±0.95
MS+BAP+TDZ+PGH(mg/l)		
1.0+0.25+0.25	90	**6.85± 0.95**
1.0.+0.50+0.25	80	5.56±0.24
1.0+1.0+0.25	60	4.47 ±0.87

IV. Discussion:

The present *in vitro* studies on *D. hamiltonii* were carried out to identify the factors which are responsible for *in vitro* micropropagation of this edible bamboo species which is over exploited. The axillary buds when subjected to *in vitro* studies did not produce any morphogenetic response on MS basal medium without any growth regulator thus signifying the importance of growth inducers which vary from species to species and explant to explant. Bonga and Von aderkas, (1992) studied the effect of different plant growth regulator either alone or in combinations at various concentrations for successful shoot initiation in different bamboo species and reported that the Plant growth regulators particularly auxins (IAA, IBA and NAA), cytokinins (BA/BAP, Kn and 2-iP and Zeatin) and cytokinin like substances (TDZ) have specific roles in cell elongation, cell division and differentiation. During the present investigation it was established that the cytokinins (BAP and Kn) were effective in inducing the sprouting of the axillary buds and their subsequent elongation into a shoot. Similar observations have been reported in *Bambusa bambos* (Arya and Sharma, 1998), in *Arundinaria callosa* Munro (Devi and Sharma, 2009), and *Dendrocalamus strictus* (Chaudhary et al., 2004). Gielis *et al*., 2002 reported that Axillary shoot proliferation is preferred for mass scale production of bamboo and is highly efficient ensuring clonal fidelity and true-to-type propagation. Researches carried out on micropropagation of bamboo showed variation in species in response to levels of BAP for

shoot multiplication as in the case of *Dendrocalamus longispathus* (Saxena and Bhojwani, 1993), *Dendrocalamus giganteus* (Ramanayake and Yakandawala, 1997) and *Bambusa bambos* (Arya and Sharma, 1998).. Similarly the combined effect of NAA and BAP on multiplication of shoots has also been earlier reported in *Inula recemosa* (Kaloo and Shah.1997), *Piper longum* (Sonia and Das, 2002). Thus suggesting a viable system of shoot/ plant production which can be utilized for the micropropagation of the plant in question.

V. Conclusion:

The effect of cytokinins (BAP and Kn) and TDZ and PGH on the micro-propagation of *Dendrocalamus hamiltonii was* studied and it was concluded that these hormones were more effective, when used in combination with auxins, in increasing the rate of shoot multiplication. *In vitro* bud-break was enhanced up to 90% by supplementation of BAP in combination with TDZ and PGH.

In conclusion, the present study presents effects of different hormones on rapid micropropagation of *Dendrocalamus hamiltonii*, an edible bamboo from nodal explants of adult plant. *In vitro* bud-break was enhanced by supplementation of BAP in combination with NAA, Kn, TDZ and PGH.

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