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# Standardization of *in vitro* organogenesis technique in bamboo (*Dendrocalamus stocksii*)

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**Abstract**
Sterilized explants were inoculated on MS media containing different concentrations and combinations of cytokinins (BAP, BAP+TDZ) 90% bud break was obtained in the MS medium supplemented with 1.0 mg/l BAP and 0.5 mg/l TDZ. The MS medium with 1.5 mg/l BAP and 1.0 mg/l TDZ had the highest average of 10 shoots/explant and a shoot length of 3 cm. The highest shoot multiplication was obtained with a clump of 9 shoots on MS medium containing 1.0 mg/l BAP and showed 39 shoots/culture with the highest average shoot length of 6.00 cm. A clump of 4–5 shoots were subculture for root induction on MS medium containing different concentrations of IBA and NAA. The combination of MS Medium with IBA 0.5 mg/l + NAA 1.5 mg/l resulted in 67.21% rooting and 7.80 roots/explant. The medium with 35 gm/l sucrose shoots had an average length of 6.83 cm, a 4.5 cm length of roots, and 8 roots. So, it's considered the optimum concentration of sucrose. Rooted plants were transferred to a mixture of soil: cocopeat: FYM The maximum number of plants that survived was 56.77% in a 1: 1: 1 ratio of mixture.

**Keywords:** *Dendrocalamus stocksii*, nodal explant, MS medium, BAP, TDZ, IBA, NAA

## 1. Introduction
Bamboo is one of the most widely used plant commodities in human history, giving food, shelter, and even medicine to the majority of the world's people. Poaceae is a family of 75 genera and 1250 species found all over the world. In India, there are 125 species in 23 genera scattered throughout 8.96 million hectares in 25 states and union territories, accounting for 12.8 percent of the country's total forest area (Filgueiras and Goncalves 2004) [7]. Bamboo is a cultural aspect of Southeast Asia, and there is an indigenous bamboo flora in every nation in the region. Because of its many applications, titles like "bamboo culture," "green gold," "poor man's timber," "bamboo friend of the people," and "the cradle to coffin timber" have been coined (Tewari, 1992) [16].

Bamboos are plants with a huge economic impact and a wide range of applications. They're used for scaffolding, ladders, mats, baskets, fences, containers, tool handles, pipes, toys, musical instruments, cooking posts, and furniture, among other things. They are also utilized in the production of food, feed, and biofuel (Lessard and Chouinard, 1980). Bamboo's cover 10.03 million hectares of the 75 million hectares of natural and cultivated forest, accounting for 12.8 percent of the total forest area. Arundinaria, *Bambusa*, *Chimonobambusa*, *Dendrocalamus*, *Dinochloa*, *Gigantochloa*, *Indocalamus*, *Ochalandra*, *Oxytenanthera*, *Phyllostachys*, *Pleioblastus*, *Pseudoxytenanthera*, *Schizostachyum*, *Semiarundinaria*, *Sinobambusa*, are among the bamboo genera found in India (Tewari, 1992) [16].

Bamboos are propagated both vegetatively (mainly from rhizomes) and sexually (through seeds). Bamboos are grown artificially using a variety of techniques, including seeding and growing seedlings. However, because of their long and unpredictable blooming habits, short seed dormancy periods, low seed viability, inborn microbial infection, poor seed set during off season flowering, and seeds eaten by rats and wild animals, using seeds as propagation material becomes difficult and unreliable. Bamboo propagation methods that have been used in the past have a number of issues that make large-scale replication difficult. As a result, there is a need to create a method for propagating bamboos on a big scale. For the creation of new bamboo plantations, efficient *in vitro* propagation might be a dependable and effective strategy.

Traditional procedures are typically insufficient for large-scale propagation (>50,000 plants per year), and tissue culture is the only dependable option.

Due to the challenges with traditional propagation methods, *in vitro* methods of propagation (micropropagation) give an option to fulfil market demand, providing the fast multiplication of disease-free and true to type selected clones. In this study, an effective and consistent micropropagation protocol for mass production and generating identical clones for the bamboo species *Dendrocalamus stocksii* was established.

## 2. Materials and Methods

The species of bamboo used in this study was *Dendrocalamus Stocksii*, in order to multiply them using micropropagation techniques. Explants were collected from the lateral branches of young culm thicket grown in the campus of Biodiversity Park of Forestry, Dr. BSKKV, Dapoli. The nodes containing pre-existing axillary bud explants and apical explants were used to initiate and establish the in vitro culture. Explants were washed and sterilized with DDW, $HgCl_2$, Tween-20, Savlon, 70% Ethanol, Sodium Hypochlorite, $HgCl_2$, Carbendazim (Bavistin).

The sterilized nodal segments were then trimmed at both ends and cultured individually in MS medium (Murashige and Skoog, 1962) [8] supplemented with various concentrations and combinations of Cytokinins (BAP, TDZ and Kn). The pH of the medium was adjusted to 5.8 using a pH meter before autoclaving at 15 lbs pressure and 121°C for 15 minutes. There were two types of explants taken for the research such as apical and axillary buds. The side branches with axillary buds and apical buds with sheaths were obtained from the natural clump's current year culms. Single nodal segments and apical segments of these immature shoots were cut into various sizes with the help of secateur.

Nodal type of explant were selected for the further procedure. Different size of explants in length and width were taken and inoculated on MS medium with BAP 5mg/l. The explants were surface sterilized and inoculated on MS basal medium supplemented with various amounts of hormones/regulators, both alone and in combination. The cultures were then moved to the culture chamber. The cultures were incubated in the growth chamber for 30 days while the media content was maintained by introducing new media to the same cultures every week for a month. Each treatment has three repetitions, with each replicate containing four explants. At the conclusion of three weeks, the data was recorded. After inoculation, the cultures were transported to a culture room, where *in vitro* investigations were carried out under strict photoperiod and temperature control. The culture chamber was equipped with a photoperiod control system (temperature controller) that kept the temperature at 25 + 2 ºC. Fluorescent tubes and bulbs were used to provide a light intensity of 100 E $M^{-2}$ $sec^{-1}$ during a 16-hour light cycle and an 8-hour dark cycle, respectively.

After initiation, the shoots were sub cultured on MS basal medium supplemented with various quantities and combinations of growth regulators for shoot development. Multiplication and proliferation. Shoot proliferation and multiplication were observed and recorded. The studies were carried out to see what effect combining BAP with various concentrations had. For 2-3 months, subculturing was done every 1-2 weeks at intervals of 1-2 weeks. The dried leaves, cells/material, shoots, and sheath were removed from each subculture, allowing the underlying buds to proliferate. Each treatment has three repetitions, with each replicate containing four explants. The data was collected at the conclusion of the eight weeks.

*In vitro* regenerated elongated bamboo shoots were inoculated on various rooting media. For root induction, clusters of 2-3 shoots were seeded on a variety of rooting medium supplemented with varied concentrations and combinations of auxins such as IAA and NAA. (Table 4). Each treatment had three replicates, each of which contained four explants, and the data was collected at the end of the month.

*In vitro* regenerated elongated bamboo shoots were inoculated on various rooting media supplemented with varied concentrations of sucrose for shoot development and rooting. For hardening, individually separated *in vitro* rooted plantlets were rinsed under running tap water to eliminate agar-agar particles from the roots. These rooted plantlets were transplanted into pots with a variety of potting soil compositions.

The experiment used a Completely Randomized Design (CRD) as suggested by Panse and Sukhatme (1995) [12] and the data was analysed using OPSTAT (Online Software).

## 3. Result and Discussion

Explant selection is the first and most important step in the *in vitro* propagation of bamboo to obtain desired results. The process of organogenesis is depended upon the explant type and explant size. From this study nodal/axillary explant with 3.5 cm length and 1.5 cm width found to be optimum for shoot initiation. The nodal segments are considered as much efficient explants for *in vitro* culture because of reserved food materials and due to the presence of highly active meristematic tissue in nodal segments, it develops into new plantlets (Oprins *et al.* 2004) [11]. Similarly, nodal segments were found best for *in vitro* regeneration of bamboo and used as explant to initiate the *in vitro* cultures by Arya and Sharma (1998) [5], Nayak *et al.* (2010) [10], Anand *et al.* (2013) [2] and Brar (2014) [2].

**Table 1:** Selection of explant type

| Tr. No. | Tr. code | Types of explants | Per cent of survivability | Sprouting % | Time required for sprouting (days) |
|---|---|---|---|---|---|
| 1 | E-1 | Axillary/Nodal explant | 99.58 | 86.18 | 14 |
| 2 | E-2 | Apical explant | 0.00 | 0.00 | Explants turn brown and dead in 7 days |

Note: E- Explant type

In the current study significant effect of cytokines combination of BAP and TDZ (MS + BAP 1.0 mg/l + TDZ 0.5 mg/l), gave the best result for bud sprouting which was 90%, the average number of shoots was 10 with an average shoot length of 3.00 cm in 18 days. Superiority of BAP with TDZ for shoot induction may be attributed to the ability of plant tissues to metabolize BAP and TDZ more readily than other synthetic growth regulators or to the ability of BAP to induce production of natural hormones such as zeatin within the tissue (Zaerr and Mapes 1982) [17]. Similar to Raju and Roy (2016) [13], who used BAP and TDZ to induce shoot induction and get the greatest results, The most effective medium was discovered to be one that had 2.0 mg/l BAP and 1.0 mg/l TDZ added to it. 90% of the explants in this concentration responded to shoot induction after 18 – 25 days of inoculation, resulting in an average of 3.14±0.16 shoots per explant.

**Table 2:** Effect of plant growth regulators on shoot initiation

| Tr. No. | Tr. code | Composition of media | Frequency % |
|---|---|---|---|
| 1 | SIM-1 | MS Media (**control**) | 54.90 (47.79) |
| 2 | SIM-2 | MS+BAP 2.0 mg/l | 63.00 (52.51) |
| 3 | SIM-3 | MS+BAP 2.5 mg/l | 65.90 (54.24) |
| 4 | SIM-4 | MS+BAP 3.0 mg/l | 86.00 (68.00) |
| 5 | SIM-5 | MS+BAP 3.5 mg/l | 71.84 (57.92) |
| 6 | SIM-6 | MS+BAP 4.0 mg/l | 77.18 (61.44) |
| 7 | SIM-7 | MS+ TDZ 2.5 mg/l | 65.17 (53.81) |
| 8 | SIM-8 | MS+ TDZ 3.0 mg/l | 64.96 (53.68) |
| 9 | SIM-9 | MS+ TDZ 3.5 mg/l | 77.48 (61.65) |
| 10 | SIM-10 | MS+ BAP 1.0 mg/l + TDZ 0.5 mg/l | 100.0 (90.00) |
| 11 | SIM-11 | MS+ BAP 1.0 mg/l + TDZ 1.0 mg/l | 90.07 (71.62) |
| 12 | SIM-12 | MS+ BAP 1.5 mg/l + TDZ 0.5 mg/l | 87.66 (69.42) |
| 13 | SIM-13 | MS+ BAP 1.5 mg/l + TDZ 1.0 mg/l | 99.00 (85.35) |
| 14 | SIM-14 | MS+ BAP 2.0 mg/l + TDZ 0.5 mg/l | 90.44 (71.97) |
| 15 | SIM-15 | MS+ BAP 2.0 mg/l + TDZ 1.0 mg/l | 98.34 (83.94) |
| SE (m)± | | | 0.58 (1.07) |
| CD (at 1%) | | | 1.69 (3.12) |

Note: SIM- Shoot Initiation Media

In the present study, different concentrations of BAP were used out of all the treatment the combination of MS + Additives + BAP (1.0 mg/l) was found best with a shoot multiplication rate of 1: 4.76, the average number of shoots produced was 28.12 and the average length of the shoot was 4.1 cm. According to this research result, the alone use of BAP is optimum for shoot multiplication. Somashekar *et al.* (2008) [14] found that a lower concentration of BAP (1.0 mg/l) was ideal for shoot multiplication, which is in line with the results indicated above. Choudhary *et al.* (2022) [6] also reported similar outcomes but semi-solid media were used. In the case of *D. stocksii*, the largest number of shoots (24.87±0.62) and the average length of the shoots (5.23±0.11) were seen in semisolid media supplemented with BAP 2.5 mg/l, without combination with any other auxin or cytokinin.

Amongst, the different cytokinins, BAP was found superior for shoot multiplication. The shoot multiplication frequency increased with increase in concentration of cytokinin up to an optimal concentration.

A clump size of 6 shoots with an average multiplication rate of 1: 5.26 is the ideal number of shoots to be inoculated for excellent growth and to increase shoot multiplication.

**Table 3:** Effect of plant growth regulators on shoot multiplication

| Tr. No. | Tr. code | Cytokinin (mg/l) | Multiplication rate | No. of Shoots | The average length of the shoot (cm) |
|---|---|---|---|---|---|
| | | BAP | | | |
| 1 | SM-1 | 0 | 1: 2 | 10 | 5.51 |
| 2 | SM-2 | 0.25 | 1: 3 | 11 | 5.08 |
| 3 | SM-3 | 0.50 | 1: 3.16 | 13 | 4.86 |
| 4 | SM-4 | 0.75 | 1: 4.16 | 19.44 | 4.2 |
| 5 | SM-5 | 1.00 | 1: 4.76 | 28.12 | 4.1 |
| 6 | SM-6 | 1.25 | 1: 4.66 | 23.10 | 3.12 |
| 7 | SM-7 | 1.50 | 1: 4.06 | 25.21 | 3.08 |
| 8 | SM-8 | 1.75 | 1: 4.3 | 20.92 | 2.71 |
| 9 | SM-9 | 2.0 | 1: 3.0 | 24.06 | 2.51 |
| SE (m)± | | | 0.46 | 0.60 | 0.33 |
| CD (at 1%) | | | 1.94 | 1.81 | 1.37 |

Note: SM- Shoot Multiplication Media

The most important stage is to establish the roots, particularly for shoots coming from old woody plants like some types of bamboo. For the mass generation of clonal planting material, high-frequency root induction is essential. Initial shoot culture media, shoot quality, shoot length, nutrients medium, auxin concentrations and auxin levels are significant determinants of rooting frequency, root number, length and future shoot growth following rooted.

It is generally accepted that auxins have a certain role in the rooting initiation. Auxins control growth and development in plants

Different auxin concentrations in medium, such as IBA alone and in conjunction with NAA, were examined in the current study. The outcomes showed that treatments had a statistically significant impact on the rate of root initiation. (MS + IBA 1.0 mg/l + NAA 1.0 mg/l) demonstrated the greatest rate of root sprouting at 69.58 % and the most roots started per explant at 8.9 out of the thirteen different IBA and NAA concentrations used in MS medium. Whereas Raju and Roy (2016) [13] achieved rooting within 15-22 days when 2.5 mg/l IBA was added in conjunction with 2.5 mg/l NAA with 86.67% rooting efficiency. The combined use of IBA and NAA for rooting was also reported by Islam and Rahman (2005). In contrary Arya & Arya (2009) [3] obtained 80–85% rooting in 3–4 weeks of culturing on MS + 3.0 mg/l NAA and on MS + 10 mg/l IBA.

According to this study, clumps of shoots are preferable than single shoots for root induction in *D. stocksii*. Similarly, as reported by Arya and Sharma (1998) [5] in *B. bamboosa*, it was shown that clusters of shoots (4-5) worked better than

individual shoots to promote roots. In addition, Arya *et al.* (2012) [4] studied *Dendrocalamus hamiltonii*, as did Sood *et al.* (1992) [15], Bag *et al.* (2000), Agnihotri *et al.* (2009) [1] and Nadha *et al.* (2013) [9] in other bamboo species.

**Table 4:** Effect of plant growth regulators on root initiation

| Tr. No. | Tr. Code | Treatments | Auxin | | Avg. No of roots/ propagule | % Rooting response | Day of root induction |
|---|---|---|---|---|---|---|---|
| | | | IBA | NAA | | | |
| 1 | RIM-1 | Full MS (control) | 0 | 0 | 0 | 0 (0.00) | 0 |
| 2 | RIM-2 | MS + IBA | 0.5 mg/l | 0.00 | 1.16 | 10.01 (18.38) | 20 |
| 3 | RIM-3 | MS + IBA | 1.0 mg/l | 0.00 | 3.00 | 38.05 (38.06) | 17 |
| 4 | RIM-4 | MS + IBA | 1.5 mg/l | 0.00 | 4.00 | 45.00 (42.10) | 18 |
| 5 | RIM-5 | MS+ IBA + NAA | 0.5 mg/l | 0.5 mg/l | 5.00 | 54.00 (47.27) | 14 |
| 6 | RIM-6 | MS+ IBA + NAA | 1.0 mg/l | 0.5 mg/l | 6.00 | 60.00 (50.75) | 15 |
| 7 | RIM-7 | MS+ IBA + NAA | 1.5 mg/l | 0.5 mg/l | 6.80 | 68.00 (55.53) | 13 |
| 8 | RIM-8 | MS+ IBA + NAA | 0.5 mg/l | 1.0 mg/l | 7.20 | 72.11 (58.11) | 14 |
| **9** | RIM-9 | MS+ IBA + NAA | 1.0 mg/l | 1.0 mg/l | 8.90 | 87.66 (69.58) | 12 |
| 10 | RIM-10 | MS+ IBA + NAA | 1.5 mg/l | 1.0 mg/l | 8.00 | 84.13 (66.52) | 12 |
| 11 | RIM-11 | MS+ IBA + NAA | 0.5 mg/l | 1.5 mg/l | 7.80 | 85.00 (67.21) | 11 |
| 12 | RIM-12 | MS+ IBA + NAA | 1.0 mg/l | 1.5 mg/l | 7.60 | 84.00 (66.42) | 12 |
| 13 | RIM-13 | MS+ IBA + NAA | 1.5 mg/l | 1.5 mg/l | 7.50 | 82.00 (64.89) | 13 |
| SE (m)± | | | | | 0.54 | 1.50 (1.05) | |
| CD (at 1%) | | | | | 1.59 | 4.40 (3.09) | |

Note: RIM- Root Initiation Media

In this experiment different concentrations of sucrose in MS media were used which affected the rooting and hardness of shoots. MS media containing 1mg/l BAP and 35 gm/l sucrose was found to be best for the good length of shoots, roots and no. of roots. As explant grown in this media had 6.83 cm average length of shoots, 4.5 cm length of roots and 8 roots.

**Table 5:** Effect of Sucrose concentration on rooting and hardness of shoots

| Sr. No. | Treatment Code | Sucrose | Length of shoots | No. of roots | Length of roots |
|---|---|---|---|---|---|
| 1 | SC-1 | MS + Sucrose 15 gm/l | 3.5 | 2 | 5.3 |
| 2 | SC-2 | MS + Sucrose 20 gm/l | 4.33 | 4 | 5.9 |
| 3 | SC-3 | MS + Sucrose 25 gm/l | 5 | 4.3 | 6.2 |
| 4 | SC-4 | MS + Sucrose 30 gm/l | 5.63 | 5.4 | 5.5 |
| 5 | SC-5 | MS + Sucrose 35 gm/l | 6.83 | 8 | 4.5 |
| 6 | SC-6 | MS + Sucrose 40 gm/l | 6.43 | 6.7 | 5 |
| SE(m)± | | | 0.39 | 0.57 | 0.53 |
| CD (at 1%) | | | 1.23 | 1.79 | 1.63 |

Note: SC- Sucrose Concentration

In the present investigation, *in vitro* regenerated cultures were transferred to the composition of soil, cocopeat and FYM. Different concentration of these three potting mixtures were tested and among all soil: cocopeat: FYM in 1: 1: 1 ratio showed the greatest percentage of plantlets survival that was 56.77% under controlled conditions. Somewhat the same line of result was obtained by Mishra et al., (2011) using the potting mixture containing soil: sand: farmyard manure (1:1:1).

**Table 6:** Per cent survival after 30 days of transplantation in bamboo cultivar (*D. Stocksii*)

| Tr. No. | Potting code | Potting Mixture | Per cent of survival |
|---|---|---|---|
| 1 | PM-1 | Soil 50%: FYM 50% | 58.00 (49.58) |
| 2 | PM-2 | Soil 50%: Cocopeat 50% | 67.00 (54.92) |
| 3 | PM-3 | Soil 75%: Cocopeat 25 % | 60.00 (50.75) |
| 4 | PM-4 | Soil 75%: FYM 25% | 45.00 (42.11) |
| 5 | PM-5 | Soil 25%: Cocopeat 37.5%: FYM 37.5% | 50.00 (44.98) |
| 6 | PM-6 | Soil 50%: Cocopeat 25%: FYM 25% | 55.00 (47.85) |
| **7** | PM-7 | Soil 33.33%: Cocopeat 33.33%: FYM 33.33% | 70.00 (56.77) |
| SE(m)± | | | 1.15 (0.68) |
| CD (at 1%) | | | 3.53 (2.08) |

Note: PM- Potting Mixture

## 4. Conclusion

Studies on the selection of explants, the impact of plant growth regulators on shoot initiation and shoot multiplication, the effect of auxins on *in vitro* rooting, the effect of sucrose on shoots and roots, and the standardization of potting mixture for hardening were conducted in *D. stocksii* to improve *in vitro* regeneration protocols. According to the results of the experiment, it is crucial to properly sterilize the cultures before inoculation in order to entirely prevent contamination. Nodal explant was suitable for the *in vitro* organogenesis of *D. stocksii*. Explant with 3.5 cm length and 1.5 cm width showed the maximum sprouting. Cytokinins

significantly influence the initiation of shoots from nodal segments. After testing various BAP and TDZ concentrations, it was discovered that MS medium fortified with BAP 1.0 mg/l + TDZ 0.5 mg/l produced the best bud break results. Similarly, for shoot induction, MS medium fortified with BAP 1.5 mg/l + TDZ concentration of 1.0 mg/l produced the best results for shoot number and shoot length. The optimum media for shoot multiplication is MS media with BAP (1.0 mg/l). Prior to rooting, subculturing the induced shoot clumps for 2 months±15 days was discovered to be crucial. Among the different concentrations of IBA and NAA tested for *in vitro* root induction, MS media fortified with additives and IBA 1.0 mg/l and NAA 1.0 mg/l exhibited root sprouting and a maximum number of roots per explant. Different concentrations of sucrose were investigated, and among all the MS media with, 1 mg/l BAP and 35 mg/l sucrose concentration were determined to be ideal for healthy shoots, root number, and root length. Different pooting mixtures are utilized among all the treatments for hardening plantlets. The combination of Soil: Cocopeat: FYM with equal proportion had the highest per centage of plantlets that survived in greenhouse. The results of the study make it abundantly evident that the mass propagation of elite genotypes may be effectively accomplished through the application of the standardized *in vitro* regeneration approach of *D. stocksii* from nodal explants.


**Plate 1-7:** *In vitro* regeneration of *Dendrocalamus Stocksii* 1- Nodal explant sprouting. 2- Shoot initiation on MS medium supplemented with BAP 1.0 mg/l and TDZ 0.5 mg/l. 3- Multiplication of shoots in the same medium. 4- Rapid multiplication with elongated shoots in MS medium supplemented with 1.0 mg/l BAP. 5- *In vitro* root induction on MS medium supplemented with 1.0 mg/l IBA + 1.0 mg/l NAA. 6- Complete Plantlet 7- Acclimatization of the regenerated plantlets in poly bags containing soil, cocopeat and FYM (1:1:1).

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