International Journal of Agricultural and Applied Sciences, June 2021, 2(1):130-133
https://www.agetds.com/ijaas
ISSN: 2582-8053
https://doi.org/10.52804/ijaas2021.2115

**Short Communication**

OPEN ACCESS

# Assessment of In-vitro culture through Nodal explants of *Dendrocalamus hamiltonii*

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(**Received:** 11/11/2020; **Revised:** 10/04/2021; **Accepted:** 15/06/2021)

**ABSTRACT**
The present experimental study was aimed to overcome the traditional methods of propagation that limit the number of propagules by in-vitro regeneration through nodal explants of *Dendrocalamus hamiltonii* with a comparative study of growth regulators during the shooting and rooting process. *Dendrocalamus hamiltonii* is distributed from the Himalayas (Nepal) to the northern part of Burma. Collection of explants was done from different selected sites of CPTs. There was the use of HgCl2 and Ca (OCl)2 as sterilizing agents in different concentrations and its effect was visualized during the sprouting stage. Culm explants were cultured in a bottle containing White media (Wm) supplemented with BA and Kinetin for sprouting and IAA, IBA, NAA for rooting. There is also the use of IAA+IBA+NAA in combined form as a supplementary solution 0.1% HgCl2 treatment for 20-minute results into77.80% aseptic buds and 72% bud -break. Among the used growth-hormones, BA with concentration 0.25mg/l and 0.50mg/l respectively were appropriate for shoot-multiplication rate, 4.01±0.3 and 4.3±0.4 were ideal observation incorporation with BA (1.00mg/l) and BA (1.50mg/l) respectively. Maximum sprouting rate14.77±3.37with application of BA (2.00mg/l) and maximum shoot length4.3±0.4 is observed at BA (1.50mg/l). The applications of rooting hormone IAA+IBA+NAA in the concentration of 1.0 mg/l results in 72.5±0.3(rooting) and 11.1±0.3 (av. No. of the root).
**Keywords:** $CPT_{s,}$ $HgCl_{2,}$ sterilizing agents, BA, Kinetin, sprouting rate

## INTRODUCTION

*Dendrocalamus hamiltonii* Arn Ex Munro is a large tufted thin-walled bamboo with erect culms and large branches. It belongs to the grass family Poaceae; and subfamily Bambusoideae. The distinguishing features of this species are brown pubescent culm with bent top in mature culms, root verticils that are seen in almost all the nodes of the culm, largely broad ovate branch buds, and zig-zag internodes in some culms (Alam 1982). It grows very fastly and rapidly. The culms reached a height of 24-27m. with a diameter of 15-18cm. This species is distributed scarcely in Northeast Himalayas, Sikkim, Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, and Tripura. It is versatile and used as a very widely renewable resource. More than 3500 traditional and 1200 commercial applications have been identified and applied to our daily life. The species can be multiplied from seedlings by separating tillers (Adarsh Kumar et al., 1985). This species can also be propagated easily by rooting culm cuttings using growth regulators (Nath et al. 1986). For rooting, culm cutting from the basal nodes of the one-year-old culm is ideal (Sharma and Kaushal, 1985). Like other bamboos, it is also propagated vegetatively through stem and rhizome cuttings. Conventional methods of propagation are based on seeds and vegetative methods. As the availability of seeds is limited for a certain specific period and the viable period is very short, to overcome this problem; propagation through offset, rhizome cutting, culm cutting, and layering is the alternate option. All these conventional methods do not fulfill the demand of the market. In this condition, micropropagation ensures a continuous supply of bamboo planting materials in a very short time. The supply and demand scenario in bamboo-producing countries indicates that demand is much greater than production (Ramanuja Rao 1994). It is only in Japan that demand for bamboo has declined (Masatoshi, 1994). Successful multiplication of shoots derived from nodal explants from the adult plants of B. bamboos, *B. vulgaris*, and *D. strictus* (Nadgir, Phadke, Gupta, 1984).

## MATERIALS AND METHODS

The nodal explants (2.0-2.5cm) had been collected from the selected healthy CPTs of *Dendrocalamus hamiltonii* of natural bamboo clumps from Pauhaddi village of Benipur under Darbhanga district of Bihar state. Survey and sampling of CPTs were done preciously during the transact walk of identified geography. The collection

of explants was done very carefully and minutely from selected CPTs. Collected nodal segments were cut into single nodal explants. the laboratory work was carried out at C.M. Science College, LNMU Darbhanga; Bihar, India during the rainy season of 2012.It is situated between longitude 85 degree 45minute-86 degree 25 minute East and latitude 25 degrees 53 minute-26 degree 27 minute North and is bounded by Madhubani district on the south by Samastipur district, on the east by Saharsa district, and on the west by Sitamarhi and Muzaffarpur district. These explants were pre-treated with a 0.5% dilute solution of Bavistin, a fungicide for 15 minutes. Thereafter, pre-treated segments were washed with antiseptic (Savlon) solution and then under running tap water 2-3 times, consequently for 2-min. each. Now the basal sheaths covering the axillary buds were to be removed and shaped with the help of scalpels. Thereafter, segments were surface sterilized with 0.1% HgCl2 solution for 5 min. The experimental contaminants were easily removed by washing in running water for 30-90 minutes and disinfected with surface sterilizing agents such as fungicides, alcohols, HgCl2, and NaOCl. The success of sterilization depends upon the concentration, duration, and antimicrobial agents (Oyebanji et. al., 2009). Mercuric chloride is often used to overcome microbial contamination, however, it is considered as one of the most toxic elements for the ecosystem, capable of causing a major alteration in the tissue of both animals and humans (Lund et. al., 1993). Others have used Sodium hypochlorite and chlorine water for surface sterilization (Rao et al.; 1985, Yeh and Chang, 1986 a, b). White's medium containing solidifying agents' agar with or without growth regulators IAA, IBA, NAA, Kinetin, and IAA+IBA+NAA in different concentrations, were prepared. Before this, sterilization of lab agents, culture-bottle, and other using apparatus was done very carefully. First, the collections of explants were done in September and were cultured in prepared media Whites media supplemented with 100mg/l, myoinositol, sucrose (3%), and 0.75%agar were used for culturing the explants. Different concentrations of growth regulators like BA, IAA, NAA, IBA, and Kinetin were used in the medium. The pH of the medium was adjusted to 5.7. The media was sterilized in an autoclave at 1210• C, 15lb pressure for 15min. Sterilized nodal segments were cultured in the semisolids White's agar medium in a culture bottle in an inoculation chamber under controlled conditions. Cultured buds sprouted within 3-5 weeks; sprouted buds were sub-cultured and transferred in other bottles containing the same media. After some time, sprouted portion developed and growth took place up to elongated shoots. Elongated shoots were used as explants either in a single form or in cluster form to produce multiple shoots and root inductions. When roots were well developed, plantlets were removed from culture media and tap water and thus transferred to hykopot containing cocopeat and vermicompost into 3:1ratio.

**Hardening, Acclimatization, and Field Transfer**

After 3 weeks, healthy rooted plantlets were transferred to seedling trays containing transplanting media (soil+ riverbed sand+ FYM/vermicompost) in a maintained mist chamber for 2-3 weeks. After that under optimum condition; prepared plantlets were to be transferred to the trial field for better survival and vigorous growth.

## RESULTS AND DISCUSSION

Statistically analyzed mean data disclosed that all characters under observation have a significant role during the experiment. After 2-3 weeks; cultured plants were sprouted, but some explants failed to green for a long time, didn't sprout, and dried up. Seasonal effect on axillary bud breaking was observed by Saxena and Bhojwani in 1993 on *D. longispathus*. Different methods of sterilization were applied for different durations such as 10, 15, and 20min.with both HgCl2, and Ca (OCl)2. Different results were observed with the application of different sterilizing agents for different durations. Effect of different surface sterilization treatments on explants of *D. hamiltonii* depicted in table 1, no. of shoots/clumps and Shooting-length (cm) in table 2 and average root number with rooting in table 3. According to the data record appropriate agent is HgCl2 for 20 minutes of treatment whereas the percentage of Aseptic buds and the percentage of bud break were 77.80% and 72% respectively. The application of Ca (OCl)2 for 15 minutes gave 36.57% of Aseptic buds and 46% of Bud-break. Maximum multiplication was recorded at BA (2.0mg/l) and maximum shoot length at BA (1.5mg/l) and rooting data record was predicted that the ideal condition for rooting is at concentration IAA+IBA+NAA(1.00mg/l). The application of 2mg/l concentration of Benzyl aminopurine; it results in 14.77±3.37 and (no. of shoots/clumps) and 3.00±0.4(length of shoots in c.m.). Similarly, the applications of growth regulators of IAA+IBA+NAA in the concentration of 1mg/l results in 72.5±0.3 (rooting) and 11.1±0.3 (Av. No. of the root). A comparative study of kinetin and Benzyl aminopurine- effective use on shooting results that BA is most effective than Kn. Adopting appropriate sterilization techniques gave good results.

**Table 1.** Effect of different surface sterilization treatments on Explants of *D. hamiltonii* collected from Pauhaddi Darbhanga

| Treatments | Aseptic Buds (%) | Bud break (%) |
|---|---|---|
| HgCl2 (10minutes) | 41.42 | 69.7 |
| HgCl2 (15minutes) | 67 | 54.3 |
| HgCl2 (20minutes) | 77.80 | 72 |
| Ca(OCl)2 (10minutes) | 17.31 | 29.63 |
| Ca(OCl)2 (15minutes) | 36.57 | 46 |
| Ca(OCl)2 (20minutes) | 18.62 | 22 |

Micropropagation techniques produced the improved quality of clones with the treatment of different growth hormones in different concentrations like BA, IAA, IBA, NAA, and Kinetin. According to the data record BA (2.0mg/l) gave maximum multiplication rate whereas maximum shoot length was observed at 1.5 mg/l. According to the data record, ideal results of rooting were observed at NAA (5.0mg/l) and maximum rooting was observed at IAA+IBA+NAA (1.0mg/l). In an earlier study, only 30% rooting was reported in 4-weeks in *D. hamiltonii* shoots cultured on IBA or NAA supplemented media (Sood et al. 2002a and 2002b).

**Table 2.** Shooting data of *D. hamiltonii* collected from Pauhaddi Darbhanga

| Growth regulators(mg/l) | No. of shoots/clumps | Shooting-length (cm) |
|---|---|---|
| Kn 0.25 | 1.6±1.1 | 1.1±0.05 |
| Kn 0.50 | 1.3±0.47 | 1.1±0.32 |
| Kn 1.00 | 2.06±1.50 | 2.0±1.1 |
| Kn 1.50 | 2.25±1.3 | 3.02±1.21 |
| Kn 2.0 | 3.65±2.07 | 1.1±0.05 |
| BA 0.25 | 4.01±2.2 | 3.24±0.5 |
| BA 0.50 | 4.46±2.02 | 3.22±1.3 |
| BA 1.0 | 7.00±1.4 | 4.01±0.3 |
| BA 1.5 | 10.3±2.44 | 4.3±0.4 |
| BA 2.0 | 14.77±3.37 | 3.00±0.4 |

**Table 3.** Rooting data of D. hamiltonii collected from Pauhaddi Darbhanga

| Auxins concentrations (mg/l) | Rooting | Av. root no. |
|---|---|---|
| NAA 1.00 | 26.2±0.4 | 3.3±0.1 |
| NAA2.00 | 46.6±0.7 | 2.6±0.2 |
| NAA3.00 | 59.8±0.3 | 1.7±0.6 |
| NAA5.00 | 63.6±0.3 | 8.2±0.3 |
| IBA1.00 | 19.7±0.5 | 4.0±0.3 |
| IBA 2.00 | 47.9±0.4 | 5.7±0.4 |
| IBA 3.00 | 32.6±0.2 | 4.5±0.5 |
| IBA 5.00 | 45.1±0.7 | 4.0±0.7 |
| IBA 7.00 | 66.9±0.6 | 5.1±0.5 |
| IAA+IBA+NAA (S1.0) | 72.5±0.3 | 11.9±0.3 |

## CONCLUSION

With experimental study, we depicted that through micropropagation technique control over the traditional methods of propagation were done very easily and a comparative study of effects of growth regulators were summarized by collected data. High multiplication rate, better rooting proficiency and easy establishment in the soil were observed

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**Citation:** Jha, A. and Das, S. 2021. Assessment of In-vitro culture through Nodal explants of *Dendrocalamus hamiltonii*. *International Journal of Agricultural and Applied Sciences*, **2**(1):130-133. https://doi.org/10.52804/ijaas2021.2115