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Rapid Multiplication of *Dendrocalamus hamiltonii* in *vitro* Regeneration Techniques from Nodal Explants

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***Abstract*:** The present study reported an advanced, effective and reproducible *in vitro* propagation protocol for *Dendrocalamus hamiltonii* using nodal explants. Secondary branches of the mother plant of *D. hamiltonii* were used to collect nodal explants of length 12-15 mm. Explant sterilization was carried out with 70% ethyl alcohol for 30s followed by mercuric chloride (0.1%) for 5 min and then inoculated on MS basal media for establishment. For multiplication, established shoots were implanted on MS media supplemented with 4.0 mg/l BAP, which resulted in a greater number of shoots multiplication after 21 days of incubation. Clumps of excised propagules transplanted on ½ MS media supplemented with 3 mg/l IBA + 3 mg/l IAA and 2 % sucrose induced rooting after 25 days and profuse rooting of shoots after 40 days of incubation. A two-step acclimatization process was done, in which soil: sand: vermicompost (1:1:1) media showed 80 per cent survival in *in vitro* raised shoots of *D. hamiltonii*. Field-transplanted plants exhibit prolific growth and development. This study was carried out to standardise multiplication of *Dendrocalamus hamiltonii through In vitro* regeneration.

***Keywords*:** Acclimatization, Bamboo*, In vitro* regeneration, Multiplication, Shoot establishment

Dendrocalamus hamiltonii Nees et Arn. Ex Munro, family *Poaceae*, commonly called Maggar, is a versatile bamboo with numerous prominent uses, and a source of nutritive green fodder for the cattle. *D. hamiltonii* is an economically important species of bamboo that is distributed in the northwest Himalayas, Sikkim, Arunachal Pradesh, Assam, Manipur, Meghalaya, Mizoram, Nagaland, Tripura of India, Bhutan, and Bangladesh. The blooming cycle of this species has been reported to last 30-40 years (Arya et al., 2012). Many bamboo species are commercialized based on its productive potential and utility (Divya et al., 2023, Twinkle et al., 2023). *D. hamiltonii* is also one of the species under domestication and is utilized for construction, fencing, baskets, containers, and shoots eaten fresh or pickled. Tremendous socio-economic pressures besides jhum cultivation in the natural niche have often compelled the utilization of raw bamboo resources to a critical level. *In vitro* regeneration technique offers an effective policy for the expeditious propagation and mass propagation of edible bamboo species, considering their sustainable development and utilization (Devi and Sharma 2009). It can propagate through seed, culm, or rhizome cuttings using conventional propagation methods. Even though method of mass propagation has been emergence through the use of single node culm cuttings in Maggar bamboo, but due to limitations like time consumption, labor intensive conventional technique of macro propagation and less availability of explants creates hurdles in mass propagation. Another problem with bamboo propagation through seeds is the unfamiliar age of the mother plant, which shows mass flowering and the death of flowered clumps. Bamboo seeds are available only for specific period of time and their viability is very short. These conventional methods do not satisfy the demand for bamboo in the market; as a result, micropropagation secured a continuous supply of bamboo materials to the market in a very short period of time. Pest and disease also play significant roles in the success or failure of authentication of nurseries and plantations of bamboo stands (Singh et al., 2013). Micropropagation techniques can solve many of the problems associated with conventional methods and provide proficiency in developing large progenies from elite genotypes (Mustafa et al., 2021). Trial and error experiments are carried out to identify specific conditions for individual species, genotypes and the donor plant development stages when designing protocols for *in vitro* plant propagation in most of the cases. The purpose of designing a bamboo tissue culture protocol is to accomplish large-scale production of plants for operation, to conceive disease-free and genetically uniform planting material, and to furnish material for breeding programs and germplasm conservation (Sandhu et al., 2018). Hence, present study was undertaken to standardize protocol for tissue culture in *D. hamiltonii.*

MATERIAL AND METHODS

Explants source: Healthy segment (2.0- 2.5 cm length) of *D. hamiltonii,* were collected from Bambusetum established in the premises of Bamboo Resource Center, CoF, NAU, Navsari. Explants were collected from the secondary branch of the new culm of *D. hamiltonii* during December to March 2022. Bamboo internodes are highly loaded with bacteria, fungi, and endophytes; therefore, internodes with young

buds covered with culm leaves were selected as explants for micropropagation.

Explants preparation, establishment and contamination control: *D. hamiltonii* explants were washed with tap water 2-3 times, consequently laboratory detergent Tween 20 (0.05%) added to distilled water and stir the solution with explants for 10 min and washed with sterile water 2-3 times, afterwards treated the explants with fungicide carbendazim (0.5%) and streptomycin (0.01%) for 30 min. The explants were washed 2-3 times with sterile distilled water at room temperature. Furthermore, plants were subjected to different contamination control treatments-C_1 -C_6 (Table 2) and observation on number of shoots, number of days taken for sprouting, length of longest shoot, per cent establishment and per cent contamination were recorded. Explants were washed 2-3 times with sterile distilled water to remove traces of mercuric chloride and inoculated on MS basal media (0.8%) gel at pH 5.8 and were incubated for 21 days at 24°C under 16 h light and 8 h dark cycles.

Shoot multiplication: Sprouted shoots were transferred onto MS medium containing cytokinines (BAP) at different concentrations (Table 3). The data were recorded for shoot multiplication. The excised shoots were incubated for 21 days at 24°C under 16 h light and 8 h dark cycles.

Rooting of shoots: Multiplied shoots were dissected into 3-4 shoots and inoculated on different rooting media. There were 33 combinations of rooting hormones (R_1 to R_{33}) were applied to *in vitro* regenerated shoots for root induction and root development (Table 1). Rooting treatments selected as per review in which generally three auxins (IAA, NAA and IBA) are widely utilized for inducing roots *in vitro*. Data were recorded after 40-45 days of incubation period at 24°C for 16 hours light and 8 hours dark cycles.

Acclimatization/ hardening: Rooted plants were transferred to seedling trays for 30-35 days. Trays were used, which contained different media for hardening (A_1 to A_4). The different types of media used in the study (Table 4). Trays were kept in a mist chamber for better survival of the tissue-cultured raised plants. *In vitro* regenerated plants were transferred to the field for better survival and vigorous growth.

Statistical analysis: Data was analyzed with the OPSTAT online.

RESULTS AND DISCUSSION

Establishment and contamination control: Significantly maximum number of shoots (2.21), length of longest shoot (5.35 cm), highest establishment (92.22 %), and lowest contamination (7.78 %) were in 70% ethyl alcohol for 30s followed by mercuric chloride (0.1%) for 5 min (Table 2). The 70% ethyl alcohol for 30s, followed by mercuric chloride (0.1%) for 3 min resulted in minimum number of days to sprout shoots (5.21 d). Culture establishment of *D. hamiltonii* in MS basal media without hormone is shown in Plate 1a.

Jha et al. (2013) reported similar results for the contamination control treatment of *D. hamiltonii* nodal explants. Mercuric chloride with 70 % ethanol is found satisfactory result in sterilized bamboo explants (Hu et al.,

Table 1. Rooting media treatments for *D. hamiltonii* multiplied shoots

Treatments code	Treatment details
R_1	MS + 1 mg/l IBA + 0.25 % Activated charcoal
R_2	MS + 0.1% IBA+ 2% Sucrose
R_3	MS + 1 mg/l IBA + 1 mg/l NAA + 2% Sucrose
R_4	MS + 1 mg/l IBA + 1 mg/l NAA
R_5	MS + 1.5 mg/l NAA + 3 mg/l IBA + 2% Sucrose
R_6	MS + 1 mg/l NAA + 0.3% Activated charcoal
R_7	MS + 20 mg/l IBA
R_8	MS + 0.5mg/l IBA
R_9	MS + 1 mg/l BAP + 3 mg/l NAA
R_{10}	MS + 1.8 mg/l NAA + 10 mg/l Coumarin
R_{11}	MS + 1.8 mg/l NAA
R_{12}	MS + 1 mg/l BAP + 1 mg/l NAA + 3% Activated charcoal
R_{13}	MS + 3 mg/l NAA+ 2% Sucrose
R_{14}	MS + 0.2 mg/l NAA + 0.2 mg/l IBA + 10 mg/l Coumarin
R_{15}	½ MS + 1.8 mg/l NAA + 2 mg/l IBA + 10 mg/l Coumarin + 2 % Sucrose
R_{16}	½ MS + 0.5 mg/l IBA + 0.5 mg/l NAA + 2 % Sucrose
R_{17}	½ MS + 1 mg/l IBA + 0.5 mg/l NAA + 2 % Sucrose
R_{18}	½ MS + 0.5 mg/l NAA
R_{19}	½ MS + 1 mg/l NAA
R_{20}	½ MS + 1 mg/l IBA
R_{21}	½ MS + 2 mg/l IBA
R_{22}	MS + 3 mg/l IBA
R_{23}	MS + 4 mg/l IBA
R_{24}	MS + 5 mg/l IBA
R_{25}	½ MS + 3 mg/l IBA
R_{26}	½ MS + 3 mg/l IBA + 3 mg/l IAA + 2% Sucrose
R_{27}	½ MS + 3 mg/l IBA + 10 mg/l Coumarin
R_{28}	½ MS + 0.2 mg/l IBA
R_{29}	½ MS + 1 mg/l IBA + 1 mg/l IAA
R_{30}	½ MS + 2 mg/l IBA + 1 mg/l IAA
R_{31}	½ MS + 5 mg/l IBA + 1 mg/l IAA
R_{32}	MS + 8µM BAP + 1µM NAA + 100 µM IBA
R_{33}	½ MS + 8µM BAP + 1µM NAA + 100 µM IBA

2011, Arshad et al., 2005). These entire chemicals successfully eliminated the surface contaminations; however, there is a hindrance in controlling the endophytic contamination. In such situation, antibiotics can be used during surface sterilization (Syandan and Md Nasim 2016).

Shoot multiplication: Significantly greater number of multiplied shoots was recorded in MS supplemented with 4.0 mg/l BAP in pooled over two years data (10.44), which was followed by treatment MS + 3.0mg/l BAP *i.e.,*7.66 shoots (Table 3). The treatment MS + 0.5 mg/l BAP resulted in poor shoot multiplication 3.80 (Table 3). Successful shoot multiplication of *D. hamiltonii* on MS media supplemented with 4 mg/L BAP is shown in Plate1b. As discussed by Jha and Das (2021) showed near to similar trend for number of shoot multiplication of *D. hamiltonii.* In contrast, Sayanika et al (2014) observed that the combined concentration of 2mg/L of kinetin and 3mg/L of BAP produced more shoots per explant in *B. tulda* and *M. baccifera.* Moreover, increase in the BAP concentration may escalate the multiplication but leaves of plants became tiny and more condense (Mudoi and Borkhadur 2009). This could be due to surplus of hormone which may leads to toxic for the plant, hence, smaller and abnormal leaves develop.

Root initiation and establishment: A total of 33 rooting treatment combinations ($R_1$to T_{33}) were applied for the rooting in *D. hamiltonii* (Table 1). Among them, only R_{26}*i.e.,* treatment combination of ½ MS + 3 mg/l IBA + 3 mg/l IAA + 2% sucrose responded to rooting and rest of treatments did not produce roots. T_{26} resulted in 86.68 per cent rooting with 12.84 roots per plant in the pooled data (Plate 1c). Length of longest root measured was 5.69 cm in the pooled data. Since all the treatments, except $T_{26,}$ showed zero result; hence, data is not provided in the table.

Murlidhran and Pandalai (2017) suggested that the best concentration of auxin for rooting is ½ MS + 3 mg/l IBA for *B. tulda.*Such combination also holds good for rooting of *D. giganteus* shoots (Ramanyake and Yakandawala 1997).Raju and Roy (2016) achieved optimum rooting efficiency in bamboo shoots within 15-22 days when 2.5 mg/l IBA was added in conjunction with 2.5 mg/l NAA. Effect of growth regulators on rooting also vary from species to species and also depend upon age of explants (Ramanayake et al., 2008).

Acclimatization / Hardening: The data on acclimatization of *D. hamiltonii* using *in vitro* regeneration techniques from nodal explants is given in Table 4. Result shows that the highest survival per centwas recorded in acclimatization

Table 3. Effect of shoot multiplication treatments on number of multiplied shoots of *D. hamiltonii*

Treatments	Pooled
T_1 - MS + 0.5 mg/l BAP	3.80
T_2 - MS + 1.0 mg/l BAP	4.67
T_3 - MS + 2.0 mg/l BAP	5.44
T_4 - MS + 3.0mg/l BAP	7.66
T_5 - MS + 4.0 mg/l BAP	10.44
CD (p=0.05)	0.46
CV %	5.95

Table 4. Effect of acclimatization treatments on survival per cent of *Dendrocalamus hamiltonii*

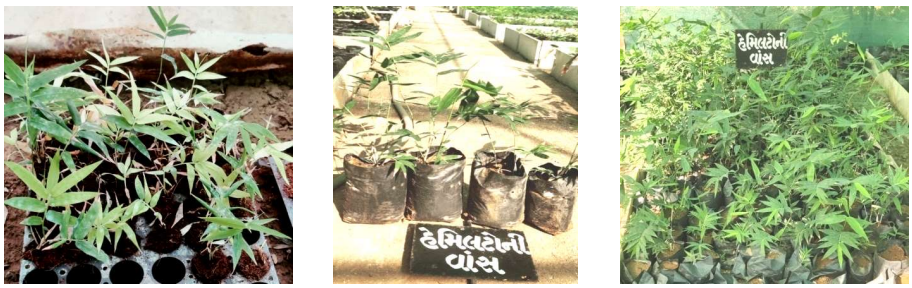
Treatments	Survival per cent pooled
A_1 - FYM + Soil + Sand (1:1:1)	63.33
A_2 - FYM+ Soil (1:1)	42.22
A_3 - FYM + Soil + Coco Coir (1:1:1)	55.00
A_4 - Soil + Sand + Vermicompost (1:1:1)	80.00
CD (p=0.05)	5.07
CV %	6.88

Table 2. Effect of contamination control treatments on shoot establishment parameters in *D. hamiltonii (*Pooled data for 2022 and 2023)

Treatments	No. of shoots	No. of days for sprouting	Length of longest shoot (cm)	Establishment (%)	Contamination (%)
C_1 - Mercuric Chloride (0.1%) for 3 Min	1.53	5.33	4.33	60.00	40.00
C_2 - Mercuric Chloride (0.1%) for 4 Min	1.64	5.34	4.06	66.11	33.89
C_3 - Mercuric Chloride (0.1%) for 5 Min	1.76	5.51	4.77	71.67	28.33
C_4 – Ethyl alcohol (70%) for 30 Sec + Mercuric Chloride (0.1%) for 3 Min	1.77	5.21	3.90	58.89	41.11
C_5 - Ethyl alcohol (70%) for 30 Sec + Mercuric Chloride (0.1%) for 4 Min	1.83	5.54	4.55	79.44	20.56
C_6 - Ethyl alcohol (70%) for 30 Sec + Mercuric Chloride (0.1%) for 5 Min	2.21	5.35	5.36	92.22	7.78
CD (p=0.05)	0.09	NS	0.24	3.31	3.31
CV %	4.02	3.87	4.54	3.89	9.71

(a) (b) (c)

Plate 1. a) Culture establishment in basal MS medium, b) Shoot multiplication in MS supplemented with 4mg/ l BAP, and c) Rooting of multiplied shoot in ½ MS media supplemented with 3 IBA+ 3 IAA with 2% sucrose



(a) (b) (c)

Plate 2. Acclimatized plantlets of *D. hamiltonii* through *in vitro* regeneration a) Primary hardening, b) Secondary hardening and c) Well-established plantlets

treatment A_4 [Soil + Sand + Vermicompost (1:1:1)] in individual years (78.89 % and 81.11 %, respectively) and it was 80 % in pooled data. In fact, A_1 treatment composed of FYM + Soil + Sand (1:1:1) also resulted in 64.44 and 62.22 per cent survival in the first and second year respectively with pooled value of 63.33 per cent survival (Table 4). In contrast, treatment- A_2 [FYM+ Soil (1:1)] resulted in lowest survival per cent of 42.22 % (in pooled data), 43.33 % in the first year and 41.11 % in second year. Acclimatized micro-propagated plantlet of D. hamiltonii is shown in Plate 2.

Jha and Das (2021) also suggested Soil: Sand: Vermicompost (1:1:1) as hardening media for obtaining optimum growth of *D. hamiltonii*. Arya et al. (2012) mentioned that plants, which are transferred into polybags containing Sand: Soil: FYM in a 1:1:1 ratio, resulted in elongated shoots as well as greener and expanded leaves.

CONCLUSION

Culture establishment of *D. hamiltonii* in MS basal media in the contamination control treatment containing 70% ethyl alcohol for 30 second + mercuric chloride @ 0.1% for 5 minutes performed well and produced a greater number of shoots, length of longest shoot with more culture establishment and less contamination. The least number of days taken for sprouting was recorded in C_4. Shoot multiplication was highest in the treatment containing MS media + 4.0 mg/l BAP (T_5). Roots were developed only in treatment combination of ½ MS media supplemented with 3mg/l IBA + 3mg/l IAA + 2% sucrose (R_{26}). Maximum *in vitro* plant survival was acclimatization treatment composed in soil: sand: vermicompost (1:1:1) (A_4). Study concludes that protocol developed in this study can be used for successful *in vitro* regeneration of *D. hamiltonii*.

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AUTHORS' CONTRIBUTION

Jayesh Pathak provided the study conception and design, Nidhi Patel helped in data collection, development of methodology, D.H. Prajapati and J.R. Chavda helped in statistical analysis and data interpretation, V. B. Patel supervised the experiment and helped in preparation of draft.

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