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Tissue Culture of *Bambusa nutans* Wall ex Munro and *Dendrocalamus asper* (Schult. & Schult.f.) Backer ex Heyne: Optimizing Protocol for Commercial Exploitation

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ABSTRACT

Bamboo is a fast growing, highly versatile bioresource plant that significantly contributes to both industrial applications and ecological balance. The present study focuses on optimizing tissue culture protocols for commercial exploitation of

two economically important bamboo species *Bambusa nutans* Wall ex Munro and *Dendrocalamus asper* (Schult. & Schult.f.) Backer ex Heyne. We employed nodal segments as explants, which were surface sterilized and grown in Murashige and Skoog (MS) medium enriched with different plant growth regulators (alone or in various combinations). Seasonal variations in bud initiation and proliferations were monitored. The experiments were conducted in triplicate using a completely randomized design with 10 replicates each and significance levels were determined through Analysis of variance (ANOVA), with mean comparison performed using Duncan's Multiple Range Test (DMRT) at p≤0.05. The rate of bud initiation was observed during mid spring to summer. The maximum number of shoots (4.80 ± 0.28 in *B. nutans* and 5.04±0.05 in *D. asper*) were achieved in MS medium containing 5.0 mg/l BAP, however, the shoot length was significantly high in 2.5mg/l of BAP. Liquid media promoted better shoot growth and quality. NAA and additives had positive influence on shoot multiplication in both the species. For root formation in both species, NAA (5.0 mg/l) in half strength of basal medium was the most effective among all the auxins.

Keywords: *B. nutans, D. asper*, Bud proliferation, BAP, NAA, Hardening.

INTRODUCTION

Bamboo is a versatile plant that has been woven into the fabric of human culture for centuries. With over 1821 species spread across the globe, bamboo is renowned for its remarkable strength, durability and adaptability (Canavan *et al*., 2017; Hyde *et al*., 2024; Choudhary *et al*., 2025a). From its uses in construction, furniture, crafts, paper, textiles and fashion, cosmetics, medicines, bioenergy, bioplastics, biocomposite to its role in soil conservation, carbon sequestration and food protection, bamboo has proven to be an invaluable resource for communities world-wide (Choudhary *et al*., 2022). In recent years, bamboo has gained increasing attention for its potential to address some of the world's most pressing environmental and economic challenges (Rathour *et al*., 2022; Li *et al*., 2025). Considering the high potential of this plant, India Govt. has reclassified bamboo from a tree plant to grass through India Forest (Amendment) Bill, 2017, aims to promote cultivation, ease to promotes bamboo industries regulation, foster sustainable development and also to boost livelihood. Bamboo is a valuable component in agroforestry systems due to its fast growth rate, multipurpose uses and ecological benefits (Solomon *et al*., 2021; Devi *et al*., 2021; Gairola *et al*., 2025; Naik *et al*., 2025). Bamboo is integrated into agricultural landscapes to provide timber and raw material for various purposes, enhance soil health, prevent soil erosion and flood, generate income and employment for rural communities and climate change mitigation (Maina *et al*., 2023; Pan *et al*., 2023; Dutta *et al*., 2025; Choudhary *et al*., 2025b). Bamboo cultivation forms a vital component of reforestation and sustainable rural livelihood strategies under ***Jal Jeevan* Haryali Mission of Bihar State Govt. India**. Bamboo plantations on degraded lands, riverbanks, and community wastelands have improved the state's green cover and it has also strengthened the ecological resilience (FSI, 2023). According to recent Forest Survey of India (FSI) assessments, the state's green cover has shown a marked increase from approximately **5.4% in the early 2010 to 12–14% in 2023**, with bamboo plantations accounting for a substantial proportion of this improvement. Among the different commercially viable bamboo species, *Bambusa nutans* **Wall. ex Munro and** *Dendrocalamus asper* **(Schult. & Schult. f.) Backer ex K. Heyne** are highly valuable for their superior culm quality, rapid growth and

diverse applications in construction, paper and handicrafts, making them economically and culturally significant (Lobovikov *et al*., 2019; Devi *et al.,* 2021; Widjaja *et al*., 2023). ***D. asper***, is commonly known as sweet bamboo, dragon bamboo, giant bamboo, rough bamboo, and clumping bamboo. (Lobovikov *et al*., 2020). The tender shoots of this plant are edible (vegetables, pickles, ingredients grains/ bamboo rice) and valued for their high protein and fiber content along with bioactive compounds known for reducing cholesterol levels (Nirmala *et al*., 2018; Choudhury *et al.,* 2022). Its leaves also serve as nutritious fodder for cattle during winter, making it an economically and ecologically valuable species for agroforestry and rural livelihoods (Singh *et al*., 2012; Sharma *et al*., 2023). Another bamboo, *B. nutans* is a fast-growing clumping tropical bamboo species characterized by erect culms with drooping tips, reaching heights up to 15–25 m and diameters of 8–12 cm. The species is valued for its strong, flexible culms and is widely used in construction, handicrafts, scaffolding and as raw materials for the paper and pulp industries. It also plays an ecological role in soil conservation and climate resilience (Rao *et al*., 2021; Singh *et al.,* 2023).

Propagation of bamboos through seeds is often unreliable due to infrequent and unpredictable flowering cycles, poor seed viability and rapid loss of germinability, necessitate alternative propagation strategies (Negi *et al*., 2022; Pothula *et al*., 2023; Chauhan *et al*., 2024). Vegetative propagation through culm or rhizome cuttings, although effective technique, however, it is limited by seasonal dependency, low multiplication rates and susceptibility to pests and pathogens (Singh *et al*., 2020; Trivedi *et al*., 2024). Tissue culture offers a rapid and season-independent alternative method for large-scale production of elite bamboo planting stock. Earlier tissue culture studies on these species faced challenges such as low multiplication rates, difficulties in rooting, poor survival during hardening and economic viability issues (Arya *et al*., 2001; Negi and Saxena,2011; Mudoi *et al* 2014; Maiya *et al* 2021; Mustafa *et al*.,2021; Zani *et al*.,2024). Therefore, optimizing protocols for these species is essential for their commercial utilization.

The present investigation is aimed to develop a rapid micropropagation protocol for producing, disease-free, genetically uniform, and high-quality planting materials of *B. nutans* and *D. asper*. The main objective of these studies was (i) To evaluate season for explant collection, development of aseptic cultures, multiplication, rooting and (ii) optimization of protocol for commercial-scale production.

MATERIALS AND METHODS

Sources of Explants Collection

Nodal segments lacking visible bud primordia were collected in the morning from healthy and juvenile culm of both the species of Bamboos*, B. nutans and D. asper* maintained at the Plant Tissue Culture (PTC) Laboratory, *Tej Narayan Banaili* (TNB) College, *Tilka Manjhi* Bhagalpur University (TMBU), Bhagalpur in Bihar, (India). The super quality germplasms of mother plants were brought from IWST, Bangalore, Karnataka. Prior to 15 days of explants collection, the mother plants were pruned before 15 days from explants collection and treated with systemic fungicide through foliar spray application. The experimental site is located at 25.24° N latitude and 86.98° E longitude on the south of Bihar on the bank of the river Ganges, and the region has a humid subtropical climate with hot summers (18.7 -36.0°C) and cool winters (5.2 –23.4°C).

Seasonal Variation

To access the impact of seasons on the establishment of aseptic cultures, nodal explants were collected throughout the year in different seasons winter (October–December), spring (January–March), summer (April–June), and monsoon (July–September), then the explants were inoculated in initiation MS media with suitable cytokinin at different concentrations. Thereafter the percentage of bud initiation and contamination levels were monitored.

Surface Sterilization of Explants

At first healthy juvenile shoots which contained nodal segments (with dormant auxiliary buds) were wiped by cotton swap dipped in ethanol (70%) for removing dust and surface microbes. Then shoots were thoroughly washed by tap water followed by removal of sheath carefully. After that healthy nodal segment of 1.5-2.5 cm in length (with single dormant auxiliary bud) were taken washed in running tap water for 10 minutes and subsequently rinsed with double sterilized distilled water. Further, six sterilization procedures (P1-P6) were used to determine the most effective treatments for establishment of aseptic cultures (Table: 1).

Table: 1 Steps for sterilizing surface of nodal explant of nodal explants of B. *nutans* and D. *asper*.

Procedure	Outside laminar air flow	Inside laminar air flow
P1	Treatments of Tween20+Dettol for 10 minutes followed by 1% Bavistin treatment for 10 minutes	Treatment of Ethanol (70%) for 30 seconds followed by 1%NaOCl treatment for 5 minutes
P2	Treatments of Tween20+Dettol for 10 minutes followed by 1% Bavistin treatment for 10 minutes	Treatment of Ethanol (70%) for 30 seconds followed by 1%NaOCl+Tween20 treatment for 5 minutes
P3	Treatments of Tween20 + Dettol + NaOCl (0.25%) for 10 minutes followed by 1% Bavistin treatment for 10 minutes	Treatment of Ethanol (70%) for 30 second followed by 1%NaOCl+Tween20 treatment for 5 minute
P4	Treatments of Tween20+Dettol+$HgCl_2$ (0.025%) for 10 minutes followed by 1% Bavistin treatment for 10 minutes	Treatment of Ethanol (70%) for 30 seconds followed by 0.1%$HgCl_2$ + Tween20 treatments for 5 minutes
P5	Treatments of Tween20+Dettol for 10minutes followed by 1% Bavistin treatment for 10 minutes	Treatment of Ethanol (70%) for 30 seconds followed by 0.1% $HgCl_2$ + Tween 20 treatments for 5 minutes
P6	Treatments of Tween20+Dettol for 10minutes followed by 1% Bavistin treatment for 10 minutes	Treatment of Ethanol (70%) for 30 seconds followed by 0.1% $HgCl_2$ treatment for 5 minutes

Procedure (P1)

- **Outside Laminar Air flow** -The explants were immersed in a Tween 20 and Dettol solution (1-2 drops in sterilized distilled water) for 10 minutes, leveraging the surfactant and antiseptic properties to reduce microbial load and surface debris, followed by 3-4 rinses with autoclaved distilled water to remove foam and impurities. This was followed by immersion in 1% Bavistin (carbendazim) for 10 minute to inhibit fungal growth and then after again 3-4 times rinses with distilled water (autoclaved).

- **Inside Laminar Air Flow-** Explant samples were surface sterilized with 70% ethanol for 30 seconds, followed by 3-4 rinses with sterile distilled water, and then treated with 1% NaOCl for 5 minutes to ensure through disinfection.

Procedure (P2)

- **Outside laminar air flow-** Method were followed similar to sterilization produce P1.
- **Inside laminar air flow-** After ethanol treatment (70**%**) for 30 seconds explants were exposed to 1% NaOCl along with Tween 20 (1–2 drops) for 5 minutes

Procedure (P3)

- **Outside laminar air flow-** Explants were treated with a solution of Tween 20, Dettol and 0.25% NaOCl solution for 10 minutes, combining surfactant and chlorine-based disinfection, followed by a 10 minutes treatment with Bavistin (1%).
- **Inside laminar air flow -** Procedures similar to those in P2 were conducted.

Procedure (P4)

- **Outside laminar air flow**-Explants were initially immersed in a solution containing Tween 20, Dettol, and 0.025% $HgCl_2$ for 10 minutes, followed by a 10 minutes treatment with Bavistin (1%).
- **Inside the laminar air flow**–Explant samples were surface sterilized under aseptic conditions using two-step process 30 seconds in 70% ethanol and then 5 minutes in 0.1% $Hgcl_2$ solution with Tween 20 (1-2 drops).

Procedure (P5)

- **Outside laminar air flow** -In this procedure, nodal explants were first subjected to an external wash with Tween 20 (1–2 drops) and Dettol for 10 minute to facilitate removal of adhering dust, debris, and superficial microbial load. This was followed by immersion in 1% Bavistin solution for 10 minutes.
- **Inside the laminar air flow**- Explants were briefly treated with 70% ethanol for 30 seconds to remove surface residues and then sterilized with 0.1% $HgCl_2$ solution with 1-2 drops of Tween 20 for 5 minutes.

Procedure (P6)

- **Outside the laminar air flow-**This treatment was the same as those in P5, involving a 10 minute wash in solution of Tween 20 (1–2 drops) and Dettol, followed by a treatment of 10 minutes Bavistin solution (1%).
- **Inside laminar air flow** -The nodal segments were dipped in 70% ethanol for 30 seconds to disrupt microbial lipid membranes, enhancing steriliant penetration followed by a 5 minutes, immersion in $HgCl_2$ solution for final disinfection.

After each surface sterilization step, both outside and inside the laminar airflow, explants were thoroughly rinsed with sterile distilled water to remove residual sterilant. The efficacy of different surface sterilization procedures in establishing aseptic cultures was evaluated, with observations on contamination rates and bud initiation survival.

Culture Conditions

Media were autoclaved at 121°C for 15 psi for 20 minutes for sterilization. Cultures were then maintained at 25 ± 2 °C, 60 ± 5%, RH and a16 hours photoperiods under cool white fluorescent light.

Inoculation of Explants and Shoot Initiation

Each explant was aseptically transferred into the autoclaved culture tubes with 10 ml of modified liquid and semi-solid (MS) Murashige and Skoog basal medium containing various cytokinins, BAP (6-benzylaminopurine), Kn (kinetin) or TDZ (thidiazuron) along with myo-inositol 100 mg/l, (w/v) sucrose 3%. Additionally, 1–2 pinches of streptomycin were added to minimize microbial contamination. The pH of the medium was adjusted to 6.0–6.2. Dormant buds were initiated sprouting within 1–2 weeks in culture tubes containing initiation medium. The sprouted buds were sub-cultured 2-4 times in culture bottles with liquid media at 7-8 days intervals to enhance proliferation. Upon achieving sufficient proliferation, shoot clumps were excised from the mother explant and subsequently sub-cultured in MS medium with appropriate cytokinins for further multiplication. Effects of PGR's and physical state of media on shoot length and shoot number were also monitored after 15 days of explants inoculation.

Shoot Multiplication

Once sufficient shoot multiplication was attained, shoot clumps were excised from the mother plant and transferred into MS liquid media containing varying concentrations of BAP (1.0, 2.5, or 5.0 mg/l), either alone or in combination with NAA (0.1 mg/l). Additives including ascorbic acid (50 mg/l), citric acid (25 mg/l) and cysteine (25 mg/l) were incorporated in order to minimize phenolic oxidation. A hormone-free (HF) MS medium served as the control. Proliferated cultures were sub cultured in fresh liquid medium at the interval of 6–8 days, maintaining 2-3 clumps with 5-6 shoots. After 3rd and 4th cycle of subculturing shoot length and shoot number were monitored to evaluate the influence of various concentration of BAP and interactive effect of BAP with NAA. The effect of additives on the rate of multiplication was also calculated.

Induction and Elongation of *in vitro* Rooting

During the 4th subculture cycle and beyond, 5–7 elongated shoots were sub-cultured in half strength of MS medium (solid) containing different auxins such as NAA, indole-3-butyric acid (IBA), or indole-3-acetic acid (IAA) along with 2% (w/v) sucrose. Influence of different auxins of varying concentrations (1.0,2.5,5.0 mg/l) were tested for their efficacies in relation to number of roots per shoot and also the average length of roots.

Acclimatization

Primary Hardening:

Following 20-25 days of rooting in culture room, plantlets were taken out of the culture vessels in Greenhouse, thoroughly washed to remove medium residues and dipped in Bavistin solution (1%) to control fungal growth. Treated plantlets were transferred to root trainers containing various transplanting media and then they were placed under a closed tunnel of the greenhouse for primary hardening. After 10–15 days, plantlets were shifted to an open tunnel for further acclimatization in controlled environmental conditions with 80 to 85% of RH and 26 ± 2°C temperature.

Secondary Hardening:

For secondary hardening, acclimatized plantlets were transplanted into mother beds with different potting mixtures and maintained in a net house with partial shade before field transfer.

Experimental Design with Statistical Interpretation

The study was designed as a completely randomized experiment with three replications, each consisting of 10 explants and was repeated three times. During initiation, the impact of seasonal variations, physical state of the media (Semi solid vs liquid) and PGR's were studied whereas during multiplication, the effectiveness of PGR's was monitored. Data were analyzed using Analysis of variance (ANOVA) to determine significance levels and Duncan's Multiple Range Test (DMRT) was used to compare differences between means. ANOVA results showed significant differences among treatments.

RESULTS AND DISCUSSION

As evident from (Table -2) that there was marked influence of seasons on auxiliary bud initiation and also the levels of microbial contamination in both *B. nutans* and *D. asper*. While considering different seasons, it was derived that in case of *B.nutans* the explants collected during late spring and summer resulted comparatively high rate of survival (80 to 96.66%) followed by monsoons (73.3% to 83.3%) (Fig -1). However, spring season was congenial for initiation and per cent survival (83.33 to 93.33%) of *D. asper*. For both the species monsoons provided ideal conditions as the per cent survival was high (66.67 to 83.33%), however, the microbial contamination was significantly high ranging from 66.67 to 86.6% which spoiled the cultures. In winter, the survival per cent of initiating culture was significantly low (6.66 to 46.67%) in both the species. The maximum bud break percentage for (96.66%) *B. nutans* was observed in May followed by April (93.3%) and Feb-March (90.0%). In case of *D. asper*, the highest survival rate was recorded during February-March (90 to 93%). These findings indicated that the **late spring and pre-summer were favourable for establishment of aseptic cultures** for both the species. However, in case of *B. nutans* we found high survival of buds (96.66%) upto the month of May. This could be ascribed to the improved physiological condition of the explants and a manageable level of microbial contamination. During this period, the low relative humidity, moderate temperatures, lower microbial load along with increased meristematic activity, significantly improved the explant's survival and regeneration potential. In previous investigations, bamboo and other woody species, it was observed that the reduced humidity and favorable metabolic activity during of spring months there was enhanced high rate of survival and establishment of cultures (Arya *et al*., 1999; Singh *et al*., 2012; Negi and Saxena, 2011). However, the high temperature coupled with high RH provide ideal conditions for airborne and endogenous microbial contamination, which thereby, reduce the chances of the establishment of cultures. Monsoon-associated environmental conditions correlated with the reduced rate of the establishment of aseptic cultures, have been reported by various workers (Nadhae *et al*., 2013). The reduced morphogenic response in winter (October-December) has been attributed to plants natural dormancy during this season as well as the accumulation of phenolic compounds which triggered browning of tissue and necrosis (Luo *et al*., 2012). Phenolic oxidation is a well-known constraint in the tissue culture of woody and perennial plants (Choudhary *et al*., 2004; Banerjee *et al*., 2011). Climatological factors have pronounced influence on ecophytomorphophysiological state of the explants and also the

establishment of aseptic cultures (Kalptaru *et al*., 2014; Mudoi and Borthakur, 2012; Anand *et al*.,2013; Choudhary *et al*.,2020a).

Table 2: Season-wise bud initiation survival (%) and contamination levels (%).

Seasons	Month of explants collection	Survival Percentage (%) (Mean±S.E.)		Percent Contamination (Mean±S.E.)	
		B. nutans	***D. asper***	***B. nutans***	***D. asper***
Winter	Oct-2020	33.33 ± 12.01^{ij}	46.67 ± 6.67^{efghi}	6.66 ± 3.33^{ghijkl}	13.33 ± 3.33^{efghij}
	Nov-2020	20 ± 5.77^{ijk}	26.67 ± 8.82^{hijk}	10 ± 5.77^{efghijk}	26.67 ± 12.02^{defgh}
	Dec-2020	6.66 ± 3.33^{k}	10 ± 5.77^{kl}	26.66 ± 8.81^{defg}	30 ± 5.77^{def}
Spring	Jan-2021	40 ± 5.77^{i}	66.67 ± 12.02^{bcdef}	33.33 ± 6.66^{de}	36.67 ± 8.82^{de}
	Feb-2021	90 ± 10^{abcd}	90 ± 5.77^{ab}	30 ± 10^{def}	26.67 ± 6.67^{defg}
	Mar-2021	90 ± 5.77^{abc}	93.33 ± 3.33^{a}	16.66 ± 12.01^{defgh}	6.67 ± 3.33^{fghijkl}
Summer	Apr-2021	93.3 ± 3.33^{ab}	83.33 ± 8.82^{abc}	13.3 ± 6.66^{defghi}	10 ± 5.77^{fghijk}
	May-2021	96.66 ± 3.33^{a}	36.67 ± 8.82^{hij}	10 ± 5.77^{efghij}	13.33 ± 3.33^{efghi}
	Jun-2021	80 ± 5.77^{abcdef}	46.67 ± 6.67^{efgh}	36.66 ± 6.66^{d}	43.33 ± 8.82^{cd}
Monsoon	Jul-2021	76.66 ± 8.81^{abcdefg}	66.67 ± 6.67^{bcde}	73.3 ± 3.33^{abc}	80 ± 5.77^{ab}
	Aug-2021	73.3 ± 3.33^{bcdefg}	70 ± 5.77^{abcde}	86.66 ± 3.33^{a}	83.33 ± 8.82^{a}
	Sep-2021	83.3 ± 8.81^{abcde}	73.33 ± 3.33^{abcd}	80 ± 5.77^{ab}	66.67 ± 12.02^{abc}

Values are expressed in mean (n= 10). Means of with different superscript letters within the column differ significantly ($p\leq0.05$) according to Duncan's multiple range (DMR) test.

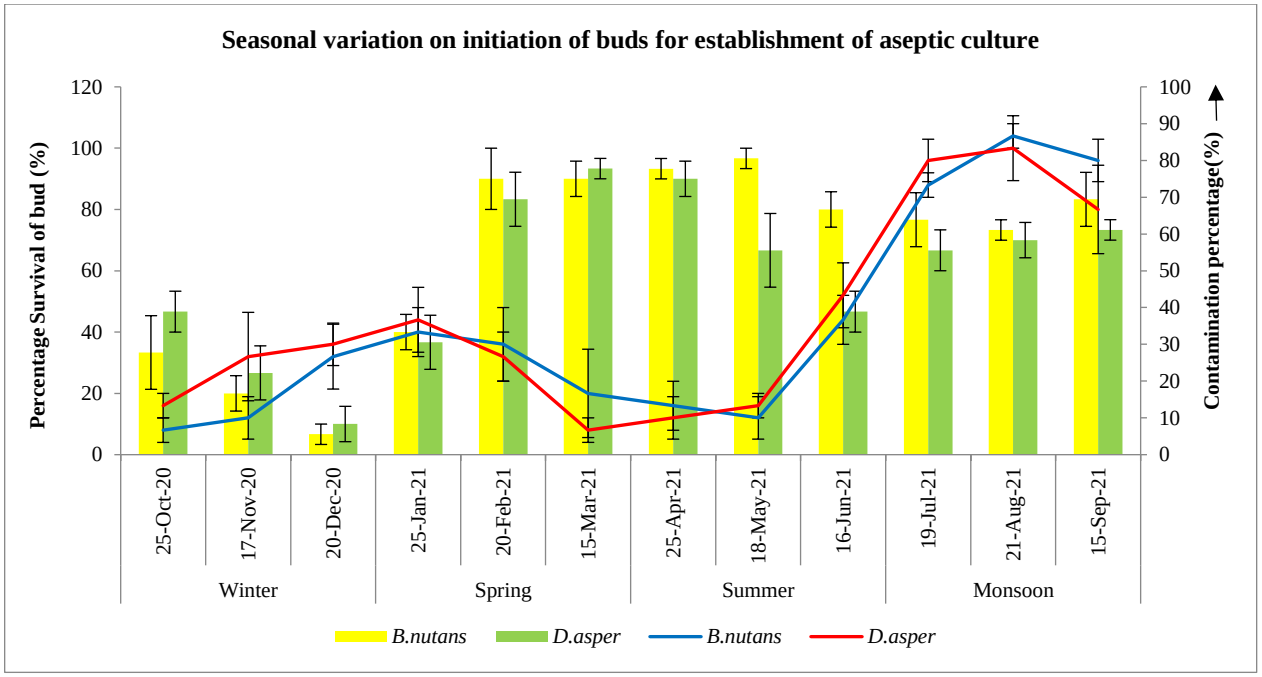


Fig 1: Influence of seasons on bud initiation establishment of aseptic culture

The survival response of nodal explants in relation to different sterilization procedures (P1–P6) varied significantly in both *B. nutans* and *D. asper* (Fig- 2). Out of the five Procedures tested, procedure P4 (Tween 20+Dettol (1-2 drops) + $HgCl_2$ (0.025%) and Bavistin 1%) for 10 min was the most suitable treatment for elimination of contamination and for establishing aseptic cultures. In this treatment there was **the highest shoot initiation both the species** 96.67%

and **93.33% in *D. asper* and *B. nutans***, respectively with reduced microbial load **(6.67 to 10.57%).** However, treatment P_5 and P_6 showed efficient regeneration (83.33 to 93.33%) but there also exhibited comparatively higher microbial contamination. Sterilization treatments P_4 to P_6 achieved optimal disinfection, Yielding both high explant viability and asepsis. In contrast, NaOCl-based treatments (P1– P3) had poor viability (43.33 to 63.33%) high contamination (36 to 40%).These findings suggested that in $HgCl_2$ based sterilization protocols significantly enhanced explant survival and minimized contamination in both the species, *B. nutans* and *D. asper*. It has been reported earlier that compared to NaOCL, $HgCl_2$ has higher antimicrobial penetration efficiency and broad-spectrum activities (Arya & Sharma, 2009; Sharma *et al*., 2021, Choudhary *et al*., 2017; Kumari, 2022). The high efficacy of the Treatment P4 can be attributed to its **two-stage $HgCl_2$ application** consisting of a mild pretreatment (0.025%) outside the laminar airflow followed by a higher concentration (1%) inside laminar air flow. This addition of a non-ionic surfactant (**$HgCl_2$)** reduced surface tension, allowing for better contact between the sterilant and the explant surface and ultimately improving the microbial load removal (Shirin & Rana, 2007; Das *et al*., 2019). Before the chemical sterilization, the pretreatment of explants with Dettol (chloroxylenol-based antiseptic) at the initial wash enhanced the removal of dust, debris, and surface-borne pathogens as it has broad-spectrum antibacterial and antifungal activity (Singh *et al.,* 2020). Additionally, the ethanol treatment **(70%)** as a rapid surface disinfectant, denaturing surface proteins and disrupting lipid membranes, thereby reducing microbial load before the final $HgCl_2$ treatment (Banerjee *et al*., 2022). Though NaOCl is widely used sterilants in tissue culture sterilization due to its easy availability and low cost, however, its oxidative action is sometimes insufficient against persistent endophytic microbes in woody plants (Purkayastha *et al*., 2017; Banerjee *et al*., 2022. Kumar *et al*., 2020). High NaOCl exposure can cause tissue necrosis, specially of tender nodal meristems. The present findings revealed that $HgCl_2$, when used in combination with surfactants (Tween 20), antiseptics (Dettol), fungicide (Bavistin) and ethanol pre-treatment, was the most effective for sterilization of *B. nutans* and *D. asper*.

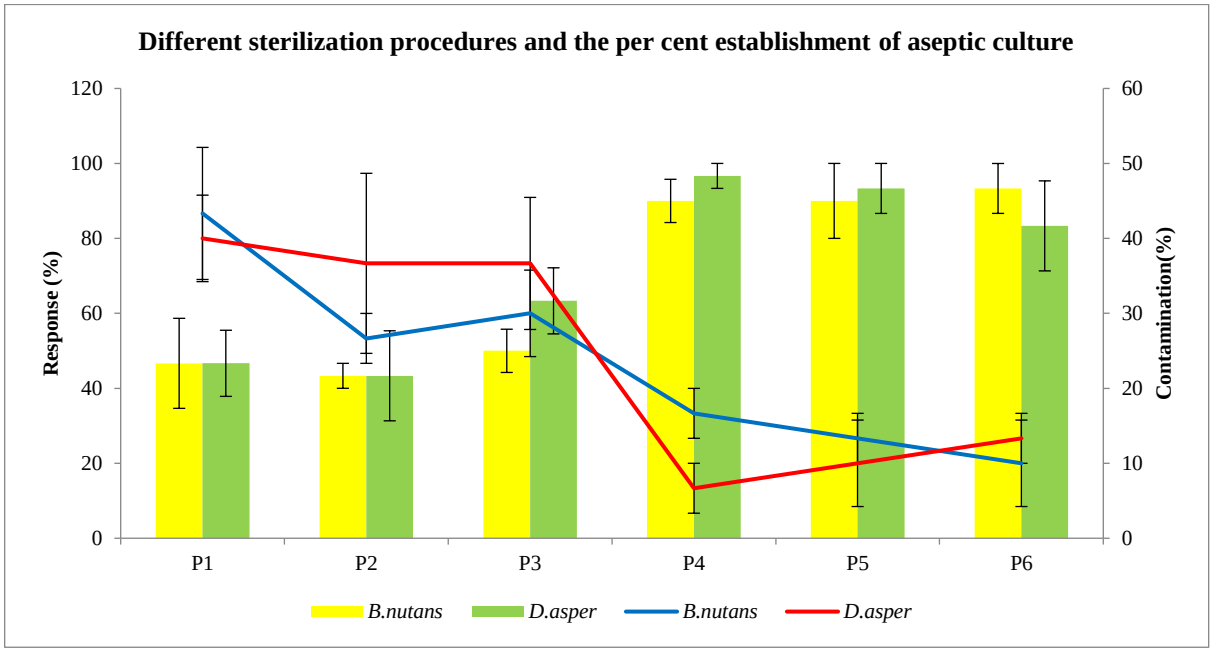


Fig 2 : Different sterilization procedures and the per cent establishment of aseptic culture

Influence of cytokinins (BAP, Kn, and TDZ) supplemented in MS media at varying concentrations was observed in semi-solid Vs liquid media for both the species of bamboos *B. nutans* and *D. asper*. Disinfected explants when applied in MS medium (without hormones), there was significantly low level of induction of shoots or had a negligible response of initiation indicating the crucial role of cytokinins in shoot morphogenesis (Fig- 3-4). Compared to different cytokinin's BAP proved to be the most effective cytokinin for inducing shoot formation and promoting shoot length and its effect was significantly high. In case of *B. nutans*, in semi-solid media concentration of 5.0 mg/l BAP resulted in the maximum number of shoots (4.27 ± 0.18), followed by concentration of 2.5 mg/l BAP (3.70±0.11). However, the shoot length was significantly high (13.13±9.2cm) at 2.5 mg/l of BAP, suggesting this concentration as better suited for both multiplication and elongation. In case of D. asper, as at BAP 5.0 mg/l there was the maximum number of shoots (4.10±0.07) with a mean shoot length of 5.17 ±0.82cm, however, the shoot length was the highest (8.62 ±1.33cm) with 3.81±0.26 number of shoots at the BAP level 2.5mg/l in semi-solid media. Our findings are in consistent with the previous reports highlighting the superiority of BAP compared to other cytokinins working on diverse bamboo species, *B. ventricosa* (Wei *et al*., 2015), *B. arundinacea* (Kalaiarasi *et al*., 2014), *D. asper* (Arya and Arya, 1997), *B. bambos* (Arya and Sharma, 1998), *B. balcooa* (Choudhary *et al*., 2017), *D. hamiltonii* (Arya *et al*., 2012), *D. strictus* (Goyal *et al*., 2015), *B. nutans* (Choudhary *et al*., 2016) and *B. tulda* (Choudhary *et al* 2020a; Choudhary *et al*., 2025a). BAP's has promoting role in shoot organogenesis in bamboos due to its high efficiency for cytokinin receptors and ability to stimulate cell division in meristematic tissues (Choudhary *et al*., 2017; Kumari, 2022; Li *et al*., 2023). TDZ treatments resulted moderate shoot induction in *B. nutans* as well as in *D. asper*. 0.25 mg/l of TDZ produced 2.93 ± 0.18 and 3.04 ± 0.17 shoots in *B. nutans and D. asper*, respectively. However, shoot elongation was moderate in both the cases in these treatments. Another cytokinin, Kinetin-supplemented media showed significantly low levels of shoot induction and shoot lengths compared to BAP and TDZ. TDZ is known to act both as a shoot initiator and modulator of endogenous hormone metabolism, resulting enhanced axillary bud break with reducced apical dominance (Gupta and Kothari, 2020; Banerjee *et al.,* 2022). Kinetin, though capable of initiating shoot formation, consistently yielded lower shoot initiation. Kn showed lower efficacy in bamboo micropropagation of *B. tulda* and *B. nutans* compared to BAP, possibly due to its slower uptake and weaker interaction with cytokinin receptors (Singh *et al.,* 2016; Mehta *et al.,* 2022). The higher concentration of cytokinins adversely affected shoot length resulting in stunted shoots, however, the rate of proliferation was high. In this study we evaluated semi-solid and liquid media with various cytokinins and found that liquid media resulted in a significantly higher shoot induction producing healthier and more vigorous cultures. The maximum number of shoots (4.8 ± 0.28 in *B. nutans* and 5.04 ± 0.05 in *D. asper)* were obtained in MS medium enriched with 5.0 mg/l BAP. However, shoot length elongation was significantly high in BAP 2.5 mg/l, reaching 13.80 ± 0.33 cm in *D. asper* and 14.82±1.66 cm *B. nutans*. Liquid media promoted better shoot growth and quality then agar- solidified media, mainly attributed to enhanced nutrient uptake uniform hormone distribution, proper gas exchange, decreased vitrification and improved physiological functioning of the shoots (George *et al*., 2008; Dewir *et al.,* 2018; Bhattacharya *et al.,* 2017; Ahmad *et al*., 2020; Choudhary *et al*., 2020b; Lestari *et al.,* 2023). These findings indicate that optimizing cytokinin type, concentration and culture type is crucial for maximizing shoot initiation and achieving better shoot length in *B. nutans* as well as in *D. asper*.

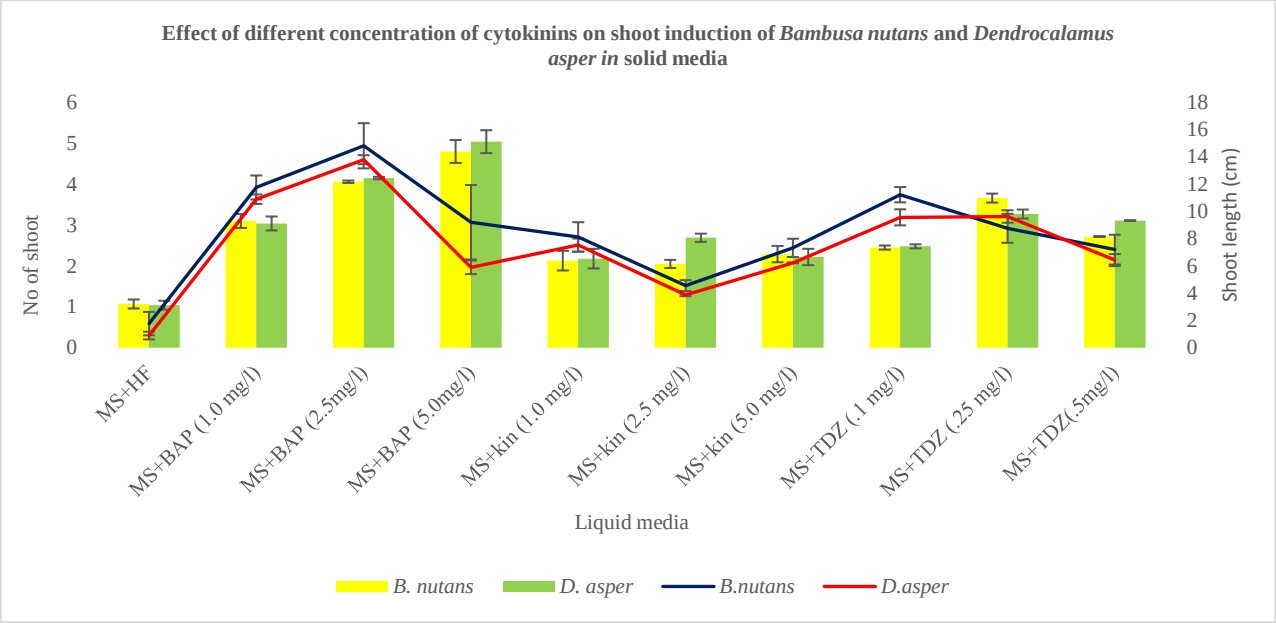


Fig 3: Effect of different concentration of cytokinins on shoot induction in solid media.

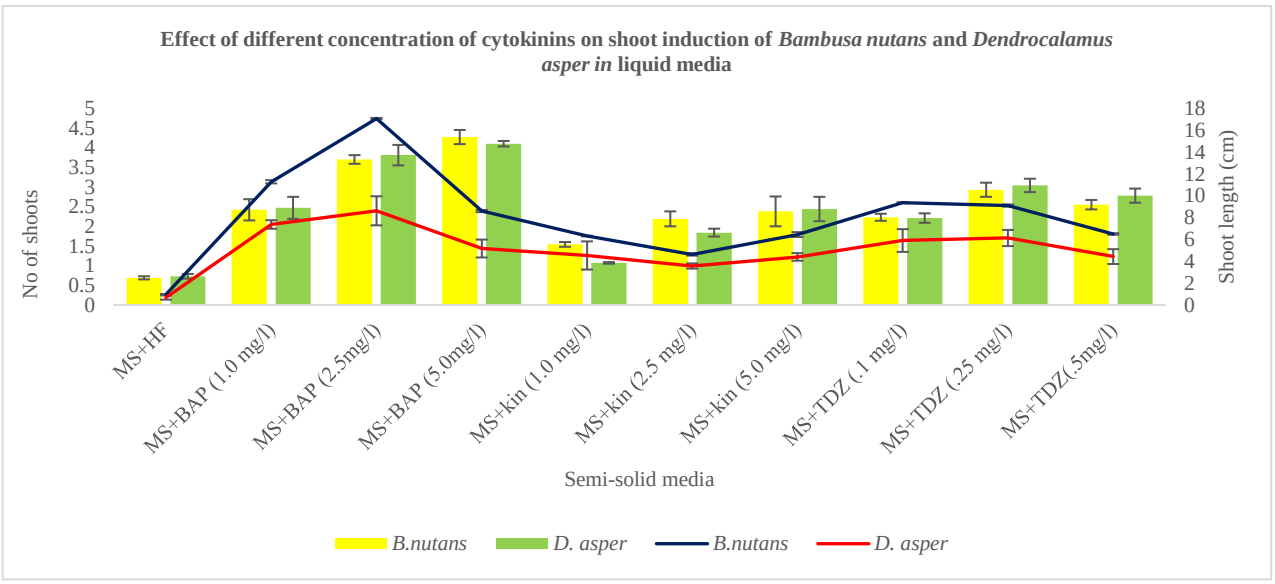


Fig 4: Effect of different concentration of cytokinins on shoot induction in liquid media.

MS basal medium with of 3% sucrose, devoid of Plant growth regulators (PGRs) resulted to the cell necrosis and death. This necessitated the addition of suitable hormones (alone or in combination) in the culm multiplication media (Table- 3). To achieve the high rate of Multiplication of shoot and its growth in both the species, varied concentrations of BAP (1.0,2.5,5.0mg/l) with or without the combination of NAA and additives were tested. In *B. nutans*, the highest shoot proliferation (11±1.21) was observed with 5.0 mg/l BAP + 0.1 mg/l NAA+ Additives, followed by 2.5 mg/l BAP+0.1 mg/l NAA + additives, which was statistically comparable to 5.0 mg/l BAP+0.1 mg/l NAA without additives. *D. asper*, had the highest shoot number (12.80 ± 0.37) in 5.0 mg/l BAP+ 0.1 mg/l NAA+ Additives, followed by 2.5mg/l BAP +0.1mg/l NAA+ Additives, where shoot number (10.23 ± 0.91. In terms of shoot elongation, *B.*

nutans showed maximal shoot length (7.63±0.14) with 2.5 mg/l BAP +NAA(0.01mg/l)+ Additives followed by 1.0 mg/l BAP+NAA (0,1mg/l) + Additives where the length was 6.86±0.12^{cm}. *D. asper* had and also shoot elongation when BAP was combined with NAA and additives. The maximum shoot length (8.77 ± 0.13 cm) of plantlets was recorded in 2.5 mg/l BAP along with 0.1 mg/l NAA and Additives, followed by BAP 1.0 mg/l+ NAA (0.1mg/l) + Additives where shoot length was 8.17 ± 0.87 cm (Fig - 5). Treatments with BAP alone showed comparatively shorter shoots (2.87±0.23 to 5.6± cm). NAA and additives had positive influence in shoot elongation in both the species. We observed optimal multiplication rate of *both species in* 5.0 mg L^{-1} BAP combined with 0.1 mg L^{-1} NAA and additive, indicating a synergistic effect between high cytokinin concentrations with low auxin supplementation. The addition of lower level of NAA at lower concentrations further improved shoot quality and vigour compared to BAP alone. This might be due to role of NAA in promoting cell elongation, vascular differentiation, and sustained apical dominance (Rao *et al*., 2017; Haque *et al*., 2020). On the other hand, high concentration of NAA has been reported to suppress the shoot induction in bamboo by diverting cellular metabolism towards callus formation and rooting (Rout & Das, 1994; Thapa *et al.,* 2023). The supplementation with additives, ascorbic acid (50 mg L^{-1}) along with cysteine (25 mg L^{-1}), and citric acid (25 mg L^{-1}) significantly enhanced shoot proliferation and elongation in both the species. Ascorbic acid is a potent non-enzymatic antioxidant which reduces phenolic oxidation, prevent tissue browning and thus helps to maintain cellular integrity during active shoot development (Gupta *et al*., 2019; Ramesh *et al*., 2020). Whereas, Cysteine is a sulfur-containing amino acid, which contributed to improved cell division and morphogenesis by serving as a precursor for glutathione synthesis (GHS), which played a critical role in redox homeostasis and protection against oxidative stresses (Bhatia & Ashwath, 2008; Faisal *et al*., 2018). Citric acid supplementation further enhanced explant health by chelating free metal ions which catalyzed phenolic oxidation thereby, preserving culture viability and chlorophyll stability of the cultures (Pati *et al*., 2006; Hassan *et al*., 2022).

Table 3: Effect of different Phytohormonal concentration on rate of multiplication of *B. nutans* and *D. asper* in MS supplemented liquid media.

Treatments	Cytokinin treatment	No of shoot (Mean±S.E)		Shoot length (cm) (Mean±S.E)	
		B. nutans	***D. asper***	***B. nutans***	***D.asper***
1	MS+HF	0.18±0.06^{ij}	0.57±0.09^{hij}	1.11±0.11^{j}	0.86±0.06^{j}
2	MS+BAP(1.0mg/l)	2.83±0.40^{defghi}	2.99±0.40^{efghi}	3.3±0.26^{efghi}	2.87±0.23^{ghi}
3	MS+BAP(2.5mg/l)	4.96±0.14^{de}	5.27±1.94^{ef}	5.06±1.16^{abcde}	4.97±0.37^{cdef}
4	MS+BAP(5.0mg/l)	4.63±1.28^{def}	9.97±1.10^{abcd}	4.93±0.37^{cdef}	3.8±0.40^{fgh}
5	MS+BAP(1.0mg/l)+NAA(0.1mg/l)	3.7±0.32^{defgh}	3.05±1.22^{efgh}	3.96±0.39^{efgh}	4.03±0.30^{efg}
6	MS+BAP(2.5mg/l)+NAA(0.1mg/l)	3.9±0.95^{defg}	5.63±1.05^{e}	6.23±0.33^{abc}	6.33±0.30^{bcd}
7	MS+BAP(5.0mg/l)+NAA(0.1mg/l)	8.2±1.53^{abc}	10.03±0.23^{abc}	4.03±0.31^{efg}	5.83±1.21^{cde}
8	MS+BAP(1.0mg/l)+NAA(0.1mg/l) +Add	5.5±0.62^{bcd}	4.28±0.55^{efg}	6.86±0.12^{ab}	8.17±0.87^{ab}
9	MS+BAP(2.5mg/l)+NAA(0.1mg/l) +Add	8.2±1.1^{ab}	10.23±0.91^{ab}	7.63±0.14^{a}	8.77±0.13^{a}
10	MS+BAP(5.0mg/l)+NAA(0.1mg/l) +Add	11±1.21^{a}	12.8±0.37^{a}	6.1±0.80^{abcd}	6.5±0.76^{bc}

Values are expressed in mean (n= 10). Means of with different superscript letters within the column differ significantly (p≤0.05) according to Duncan's multiple range (DMR) test.

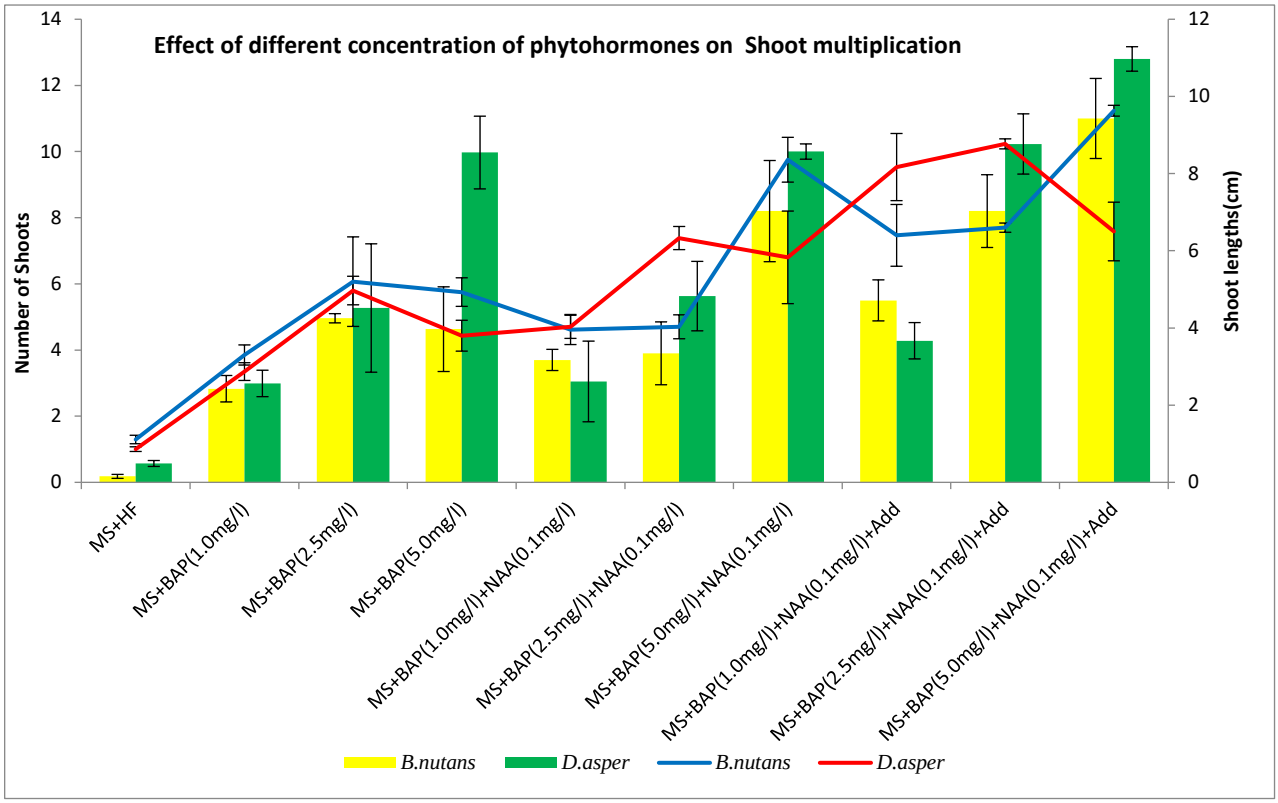
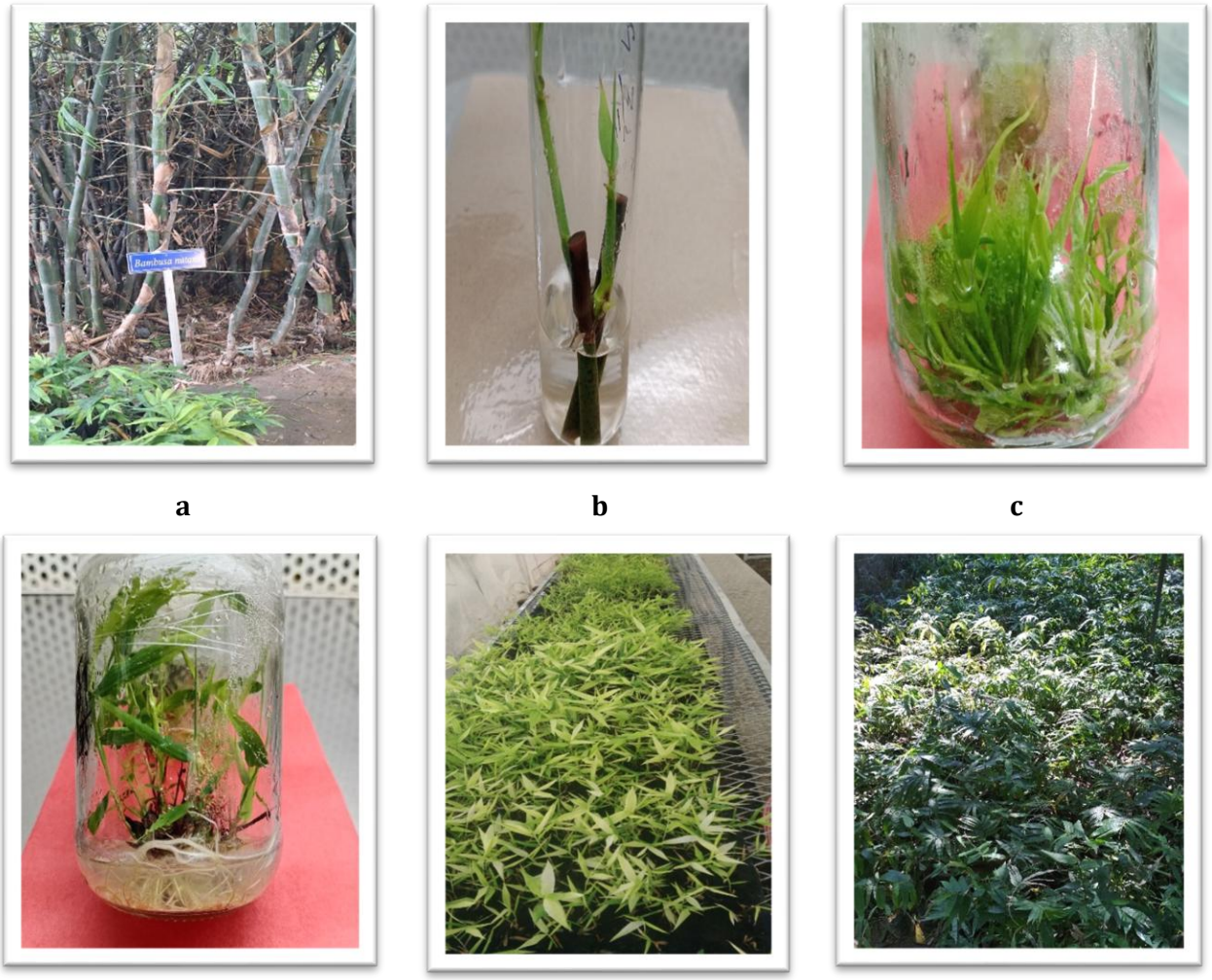


Fig 5: Effect of different concentration of phytohormones on Shoot multiplication

In vitro rooting is the limiting factor in many species of bamboos, however, auxins play a vital role in rhizogenesis. To achieve the high rate of root induction and growth in *B.nutan and D.asper,* influence of Auxins (IAA, NAA, IBA) at different concentrations (1.0, 2.5 and 5.0 mg/l) supplemented in half strength of MS based media (MS/2) was observed. Among the tested auxins (IAA, NAA, and IBA **NAA), NAA at 5.0 mg/l** most effectively in promoted root formation in both species. In *B. nutans*, the maximum number (10.07 ± 0.33) of root with larg. Root length (6.75± 0.44) was recorded in MS/2 basal medium +5.0 mg/l NAA supplemented with. D. asper also exhibited the highest root number (12.61) and root length (4.78cm) at the same NAA concentration (5mg/l) in half strength MS media. **IBA showed intermediate effectiveness, 5.0** IBA inducing **8.61 ± 0.05** number of roots in *B. nutans* and **6.43 ± 0.07** in *D. asper*, although root elongation was less pronounced compared to NAA. In contrast, **IAA had comparatively low rooting performance** in both the species. Among all the auxins tested, **NAA at 5.0 mg/l** (added in MS/2) was found as most effective in both species for **rhizogenesis due to its high stability, prolonged activity, ability to stimulate adventitious root initiation through cell elongation and division** (Choudhary *et al.,* 2016; Kumar *et al*., 2021; Singh and Bhatnagar, 2023). In contrast to our findings some researchers working on *D. strictus* have reported that full strength MS medium outperformed half strength MS for *in vitro* rooting. **IAA consistently showed poor rooting efficiency** in both species (*B. nutans* and *D. asper*), supporting previous findings in other monocotyledonous species where IAA's is rapidly instability and susceptibility to enzymatic oxidation limits its effectiveness in promoting rooting (Das *et al*., 2020; Sharma *et al*., 2024). *B. nutans* **showed more efficient response to auxin treatments compared to** *D. asper*, indicating species-specific genotypic and physiological variation and hormone sensitivity that influence rooting abilities (Table- 4, Fig- 6 & 7)



a **b** **c**

d **e** **f**

Fig 6: *In vitro* Culture of *B.nutans* (a) Source of explants (b) Initiation (c) Multiplication (d) *In vitro* rooting (e) Primary hardening in Greenhouse (f) Secondary hardening

a **b** **c**

d **e** **f**

Fig 7: *In vitro* Culture of *D. asper* (a) Source of explants (b) Initiation (c) Multiplication (d) *In vitro* rooting (e) Primary hardening in Greenhouse (f) Secondary hardening

Table: 4 Effect of varied concentrations of Auxins on number of roots per culm and root length of *B. nutans* and *D. asper*

Treatments No.	Auxin Treatments (mg/l)	No. of Root (Mean±S.E)		Root length (cm) (Mean±S.E)	
		B. nutans	***D. asper***	***B.nutans***	***D. asper***
1	MS/2+IAA(1.0mg/l)	1.20 ± 0.03^{i}	0.71 ± 0.03^{hi}	1.50 ± 0.11^{fgh}	0.67 ± 0.04^{ghi}
2	MS/2+IAA(2.5mg/l)	2.34 ± 0.05^{fg}	1.41 ± 0.06^{fgh}	1.54 ± 0.09^{fg}	0.74 ± 0.03^{gh}
3	MS/2 + IAA(5mg/l)	2.16 ± 0.21^{fgh}	1.64 ± 0.09^{fg}	0.73 ± 0.01^{i}	1.05 ± 0.20^{fg}
4	MS/2+NAA(1.0mg/l)	6.66 ± 0.29^{e}	4.94 ± 0.49^{de}	6.33 ± 0.37^{ab}	2.92 ± 0.40^{cd}
5	MS/2+NAA(2.5mg/l)	9.33 ± 0.16^{b}	10.53 ± 0.12^{b}	5.36 ± 0.26^{c}	5.42 ± 0.03^{a}
6	MS/2 + NAA(5mg/l)	10.07 ± 0.33^{a}	12.61 ± 0.25^{a}	6.75 ± 0.44^{a}	4.78 ± 0.81^{ab}
7	MS/2+IBA(1.0mg/l)	2.52 ± 0.10^{f}	2.28 ± 0.23^{f}	3.41 ± 0.04^{d}	1.6 ± 0.12^{ef}
8	MS/2+IBA(2.5mg/l)	7.59 ± 0.25^{d}	5.57 ± 0.17^{cd}	2.92 ± 0.04^{de}	2.43 ± 0.08^{cde}
9	MS/2 + IBA(5mg/l)	8.61 ± 0.05^{c}	6.43 ± 0.07^{c}	2.13 ± 0.01^{f}	2.95 ± 0.19^{c}

Values are expressed in mean (n= 10). Means of with different superscript letters within the column differ significantly ($p\leq0.05$) according to Duncan's multiple range (DMR) test.

Survival rate during acclimatization varied substantially depending on transplanting in various transplanting media. The **cocopeat and vermicompost (2:1)** yielded the **highest survival rates for** both the species, with **96.67%** for *B. nutans* and for **93.33% *D. asper* (Fig- 8).** This was followed by **sand : soil (1:1)** and **sand: soilrite: cocopeat (1:1:2)** mixtures, which showed considerably high survival rate of *B. nutans,* 93.33% and 90%, respectively. In contrast, **sand alone** resulted in **poor survival**, especially for *D. asper* (33.33 %), highlighting its inadequacy as a sole medium for acclimatization. Soilrite, when used alone, did not match the effectiveness of organic-rich mixture, however, it was better than sand. Comparatively *B. nutans* adapted more readily across all the treatments. The results emphasize the importance of **organic amendments like cocopeat and vermicompost** in promoting successful acclimatization, especially for *D. asper*. The significantly higher survival rate of *B. nutans* and *D. asper* in cocopeat and vermicompost combination (2:1) highlight the crucial role of substrates that retain **moisture provide, aeration, and supply nutrients availability** during acclimatization. Cocopeat has been widely been reported to enhance acclimatization success by maintaining

optimal water and air balance, while vermicompost supplies readily available macro- and micronutrients that promote rooting efficiency (Acharya *et al.,* 2024). Comparable results have been observed in some woody perennials plants, where performance of cocopeat–based mixtures showed 90 per cent curvival during primary hardening (Kumari, 2022). Transplanting mixtures cpmprising of vermicompost, peat, rice husk (3:1:1) significantly enhanced the acclimatization success, with the survival rate upto 92 % in case sweet potato, mainly by to maintaining favourable vapour pressure conditions during early hardening (Pérez-Pazos *et al*., 2023). The poor performance of sand and soilrite alone in the present study aligns with our previous reports that inert substrates lack the necessity of nutrient buffering and moisture-holding capacity for fragile *in vitro* plantlets (Choudhary *et al.,* 2025a). Our results also confirm that composite organic media, particularly cocopeat and vermicompost, can markedly improve the *in vivo* raised efficiency of tissue cultured raised plantlets (Acharya *et al*., 2024).

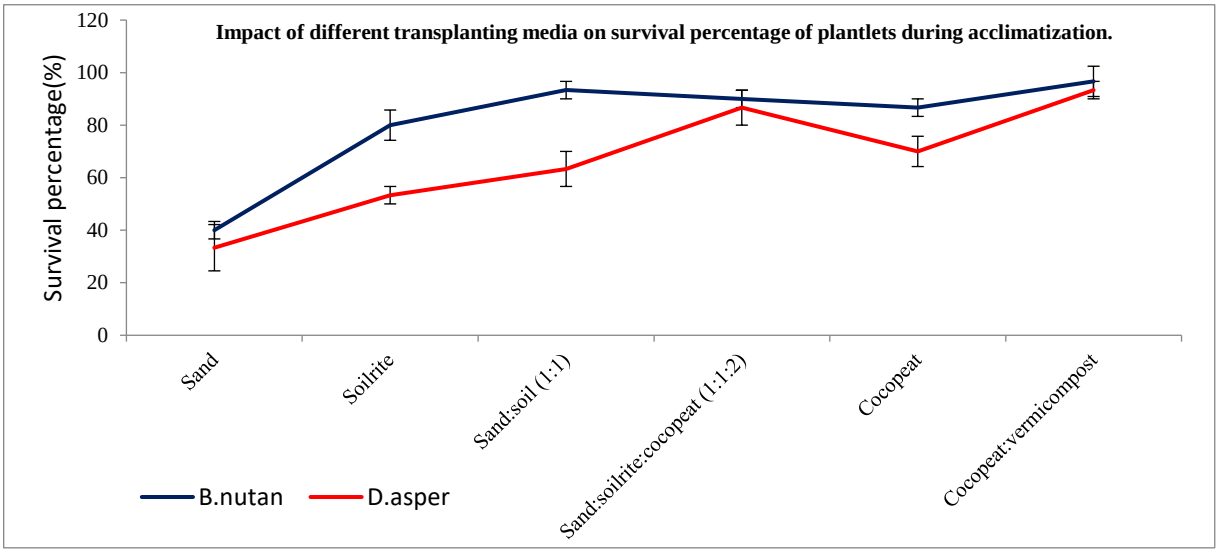


Fig 8: Effect of different transplanting media on per cent survival of plantlets during acclimatization

The survival rate of *B. nutans* and *D. asper* plantlets tissue culture (TC) raised during secondary hardening was significantly influenced by potting mixture used (Table - 5). Among all treatments, the combination of sand, soil and vermicompost (1:1:1) had the highest survival rate 93.33 % for *B. nutans* and 96.67 % *D. asper* followed by sand and cow dung (1:1) and sand, soil and FYM (1:1:1), in which the survival varied from (83.32 to 90.57%) for both the species. *B. nutans* and *D. asper* showed moderate survival rates in 1:1 mixture of sand and soil or sand and vermicompost, while soil (alone) resulted the lowest survival rate 56.67% and 53.33% respectively. Our finding suggest the crucial role, of aeration and organic matter in secondary hardening. The highest survival achieved with sand, soil, vermicompost (1:1:1) combination highlights the synergistic benefits of improved aeration, optimal moisture retention and enhanced nutrient availability provided by vermicompost. Vermicompost has rich components of macro and micronutrients, humic substances and beneficial microbial populations, which promote root proliferation, enhance stress tolerance and accelerate acclimatization of micropropagated raised plantlets (Atiyeh *et al*., 2002; Lazcano and Dominguez, 2011). Positive effect of vermicompost on survival high during secondary hardening has been reported in other

bamboo species, including *Bambusa balcooa* and *Dendrocalamus hamiltonii* (Negi and Saxena, 2011; Singh *et al*., 2012; Choudhary *et al.,* 2025a).The moderate survival observed in sand and cow dung (1:1) and sand and soil with farmyard manure (FYM) (1:1:1) mixtures suggested that the addition of organic amendments supports the gradual nutrient release and improves the soil texture and thereby aiding root establishment (Bhojvaid and Timmer, 1998). However, vermicompost is generally more effective than FYM or cow dung in promoting growth of TC raised seedlings due to its higher beneficial microbial nutrient mineralization activities (Edwards et al., 2010).In contrast, soil (alone) resulted in the lowest survival rates likely due to poor aeration, compaction and drainage capacity, which can lead to hypoxic conditions in the rhizosphere with the increased risk of root rot (Hazarika, 2003; Arya *et al*., 1999; Islam *et al*., 2005).

Table 5: Influence of various potting mixtures on per cent survival per cent of plantlets during Secondary hardening.

S.N.	Potting Mixture	Survival (%) (Mean±S.E.)	
		B.nutan	*D.asper*
1	Soil	56.67 ± 6.67^{f}	53.33 ± 3.33^{f}
2	Sand+soil (1:1)	80 ± 5.77^{abcde}	70 ± 5.77^{bcde}
3	Sand+cowdung	90 ± 5.77^{abc}	86.67 ± 6.67^{abc}
4	Sand+soil+FYM (1:1:1)	90 ± 5.77^{ab}	83.33 ± 3.33^{abcd}
5	Sand+ Soil +vermicompost (1:1:1)	93.33 ± 3.33^{a}	96.67 ± 3.33^{a}
6	Sand+vermicompost (1:1)	86.67 ± 6.67^{abcd}	86.67 ± 6.67^{ab}

Values are expressed in mean (n= 10). Means of with different superscript letters within the column differ significantly ($p\leq0.05$) according to Duncan's multiple range (DMR) test.

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