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Research Article

Effect of different concentrations of BAP and constant NAA on tissue-cultured regeneration of manga Bamboo (*Dendrocalamus stocksii*)

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ABSTRACT

The bamboo plant is an important biodegradable raw material that can play an important role in the rejuvenation of the Indian rural economy by positively impacting agricultural, industrial, energy and environmental sectors. In vitro, bud break and aseptic culture establishment were evaluated for large-scale clonal propagation of the commercially important bamboo species *Dendrocalams stocksii* Munro. The experiment was conducted twice using a completely randomized design with 6 replicates per treatment. Among various concentrations of BAP and constant NAA, MS media supplemented with BAP 5 mg/L treatment is most congenial for the initiation and establishment of aseptic cultures. 6 Benzyl aminopurine (BAP) without supplementation of any additive resulted in significant bud breakage in the case of *D. stocksii*. Altered carbon source in MS media resulted in comparatively equal responses, and no significant variation was observed. For multiplication, BAP (5 mg/L) showed a maximum number of shoots (9±2) per explant in *D. stocksii*. For root initiation, further studies are needed. Well-developed plants were successfully acclimatized, and the success rate was 72%.

Keywords: Tissue culture, bamboo, BAP, NAA, *Dendrocalamus stocksii* Munro

INTRODUCTION

Bamboo is a fast-growing multipurpose species that provides benefits equivalent to those of crops. The short rotation, marketability of culms every year, and immediate returns favoured bamboo as a suitable species for plantations and agroforestry (Walter & Köh, 2015). There are approximately 88 genera and 1400 species of bamboo distributed worldwide covering an area of more than 14 million hectares, with 80% of species and area under bamboo confined to south and south-east Asia, largely in China, India, and Myanmar (Tejvir Rathod, 2009). India is the second-largest producer of bamboo after China, having 125 species belonging to 23 genera and producing 4-6 million tons of bamboo annually, out of which 2.2 million tons is used in the paper industry. Other nations with significant bamboo production are Bangladesh, Indonesia, and Thailand.

Bamboos have the largest use in the paper and pulp industry (Chen Zhang et al., 2019), and in India, they constitute nearly 70% of the raw material for this industry. In addition to their application in the paper industry, they are extensively used as timber for house building, furniture making, daily sundry goods, agriculture and fishery tools and crafting materials (Paper, 2014).

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Bamboos are fast-growing multipurpose species that provide benefits equivalent to agricultural crops. Short rotation, marketability of culms every year and immediate returns favored bamboos as suitable species for plantations and agroforestry (Koshe & Bhaskaran, 2005). Under the National Bamboo Mission, steps have been taken to increase the availability of quality planting material by supporting the establishment of new nurseries and strengthening existing ones (Ministry of Agriculture and Farmers Welfare, 2021). In the last few decades, population growth and rapid increases in bamboo-based industries have led to overexploitation of bamboo resources, resulting in severe loss of their natural strands (Dlamini, 2022). Other factors responsible for the rapid depletion of fecundity are high in bamboos, its serendipitous nature of flowering limits continuous availability of planting stock in different regions of the country, and traditional approaches of bamboo propagation are not advocated for large-scale multiplication due to various limitations (Ray & Md. Nasim Al, 2017). Several reports targeting improved and high-efficiency plant production through tissue culture have been published, yet only a few species are multiplied on a commercial scale (Leva et al., 2011). In vitro multiplication of mature bamboo is associated with difficulties in different stages of micropropagation, particularly high-frequency rooting of micro shoots, which remains a bottleneck for large-scale production. Recently, a 20-year-old plant of *B. vulgaris* was used for in vitro propagation, and 46% rooting success was reported (Ramasamy, 2009). Such results obtained in laboratory-scale production are mostly inefficient for large-scale production, as mass propagation aims at 80–90% transplantation success, which has a direct effect on the price and quality of tissue culture-raised plants. In bamboo species, the use of auxin protectors such as coumarin (Ashton, 2020; Ginwal, 2021; Walter & Köh, 2015) and thiadiazuron (TDZ) (Monder, 2018) has enhanced rooting. In tissue culture, shoot and root morphogenesis is strongly dependent on the carbohydrate supply, and it is essential to identify the specific requirements during various phases of micropropagation (Fernando, 2021; Ashton, 2020).

MATERIALS AND METHODS

Source of material

The nodal explant of bamboo was obtained from a college instructional farm. 20/25 months, and the having plant height 13 ft. explant of bamboo was collected from D-block (field) MGM College of Agriculture biotechnology, Gandheli, Aurangabad, Maharashtra, India

Experimental design

The MS basal medium was supplemented with BAP at variable conc. (1, 2, 3, 4, 5 mg/L) and NAA @ 0.5 mg/L and different sugar sources for the nodal culture of bamboo, the details of the statistical design were applied to the abovementioned objectives.

Table 1: Experimental Design

Statistical design	Completely Randomized Design (CRD)
No. of treatments	06
No. of replications	05
Treatment details	Concentrations of PGR's and Carbon sources

Initiation and shoot proliferation

Collected ex-plant was trimmed up to 2-4 cm and sterilized with commercial bleach containing approximately 5% sodium hypo chloride and thus may be used at a concentration of 10%-20%, which is equivalent to 0.5-1.0% sodium hypo chloride. Shoot initiation in MS media was used for the experiments containing $CaCl_2$, vitamin and sucrose. The pH 5.6-5.8 was adjusted by 1 N NaOH or 1 N HCl, and the volume was made up by autoclaved distilled water. Then, 8 g of agar and 100 microliters of antifungal supplement were added. After that, the media was uniformly mixed with

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a magnetic stirrer and autoclaved at 121°C for 20 min. The plant growth regulators may be destroyed during autoclaving, and such chemicals are therefore sterilized by filtration through a syringe-driven filter membrane with 0.22 µm porosity. After autoclaving, the MS medium was supplemented with different concentrations of BAP (0, 1, 2, 3, 4, 5 mg/L and NAA 0.5 mg/L). In each bottle, MS medium (30 ml) will be supplemented with different concentrations of BAP ranging from 1 mg/L to 5 mg/L along with combinations of 0.5 mg NAA for shoot initiation from nodal explants. Nodal cultures will be maintained at 2500 Lux light density with a photoperiod of 16 hours of light and 8 hours of darkness provided by cool white fluorescence. Additionally, for better shoot initiation, the culture will be kept in the dark at 25°C (±2°C) and 70% relative humidity.

Multiplication

After the first cycle of shoot proliferation, after 21 days of inoculation, the shoots were divided into clumps of 3-4 shoots, and the effect of sugar source per explant was evaluated by inoculating shoot clumps onto multiplication medium supplemented with two different sugar sources. Treatment details are as follows:

1. MS + 4 BAP +) 0.5 NAA + 3% Table Sugar
2. MS + 4 BAP + 0.5 NAA + 3% Sucrose

A total of two treatments with six replications were performed.

Rooting

To achieve rooting of micro shoots, various auxins were tried either singly or in different combinations. Cut ends of the micro shoots in bunches of 2-3 were placed in liquid MS medium supplemented with auxins. The details of the PGR and carbohydrate source combinations used for root induction are given below:

1. MS + IAA + 0 NAA + 0.5 BAP
2. MS + 2 IAA + 0 NAA + 0.5 BAP
3. MS + 2 IAA + 1 NAA+ 0.5 BAP
4. MS + 1 IAA + 2 NAA + 0.5 BAP
5. MS + 0 IAA + 2 NAA + 0.5 BAP
6. MS + 2 IAA + 2 NAA + 0.5 BAP

Acclimatization

For acclimatization, plantlets were transferred to plastic pots containing riverbed sand and covered with plastic pots. Thereafter, they were shifted into a potting mixture containing sand: soil: farmyard manure (1:1:1). The hardened plants were successfully transferred to field conditions.

Statistical analysis

The data obtained on various observations were analysed by the "analysis of variance" method (Panse & Sukhatme, 1985).

RESULTS

Prior selection of the mother plant is a very important step when carrying out micropropagation studies. In the present study, the mother plant was selected by considering the following important parameters: height of bamboo culm, number of culms per clump, and length of the internodes. Explants were taken from the precocious branches of five-year-old field-grown plants.

Establishment of aseptic culture

Surface sterilisation with Sodium hypochlorite 0.5-1.0% prolonged exposure (15 minutes) reduces contamination and improves explant survival. In our experiment Out of 30 inoculum, nearly 26 entered the shoot proliferation cycle, with only 4 eliminated due to fungal contamination. In another experiment in our lab, we discovered that more than 2% sodium hypochlorite is lethal and that short-term exposure did not function to sterilise the culture treated. This clearly shows that surface

Table 2: Biometric Observation of *Dendrocalamus stocks on* in vitro Shoot Multiplication

Treatment	Symbol	Day of Initiation	No. of leaves per explant	Height of explant	Number of shoots
Treatment 1	T1	21	1.6	23.0	2
Treatment 2	T2	22	2.3	35.6	2
Treatment 3	T3	19	3	43.6	2.6
Treatment 4	T4	19	4	50.6	3
Treatment 5	T5	17	4	47.6	2.3
Treatment 6	T6	16	5	57.3	4.3
MEAN		19	3.3	42.9	2.7
SE		2.09	1.1	11.1	0.79
CD @ 0.05		1.79	1.1	6.6	4.5
CV			18.04	8.5	5.9

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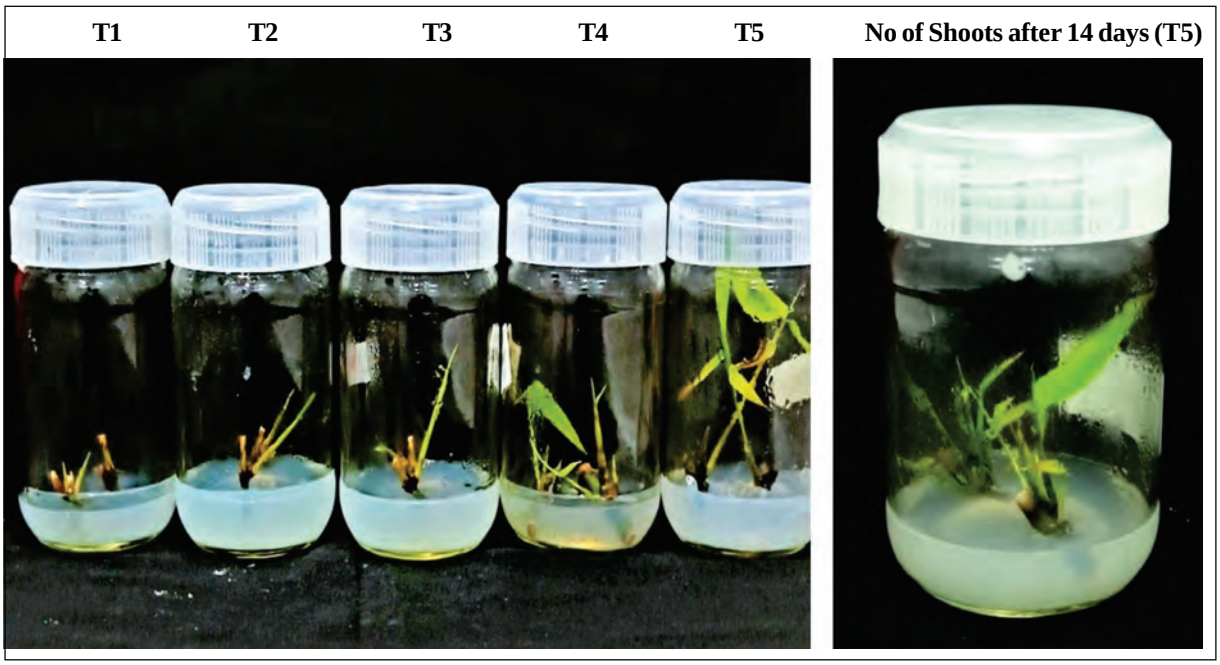


Figure 1: Effect of different concentration of BAP and NAA on shoot Proliferation of Bamboo

sterilisation was successful in decreasing contamination (Figure 1). MS medium supplemented with (0, 1, 2,3,4,5 mg/L BAP and constant NAA 0.5 mg/L) with 5 replications was developed for nodal proliferation of *B. stockisi* (Yasodha et al., 1997). All treatments of various concentrations of BAP and constant NAA were efficient for establishment and shoot proliferation. Sprouting occurred within an average of 19 days (Table 2 and Figure 2,3), and multiple shoots were produced from the node in a period of 12 weeks (Table 2 & Figure 2,3). However, the nodal explants sprouted within 10 days of inoculation on MS medium supplemented with BAP and constant NAA.

Effect of carbon source on multiplication rate

To study the effect of different carbon sources on shoot multiplication, six shoots per propagate were cultured on MS medium containing BAP (4 mg/L) supplemented with either 3% table sugar or 3% sucrose. Sucrose was

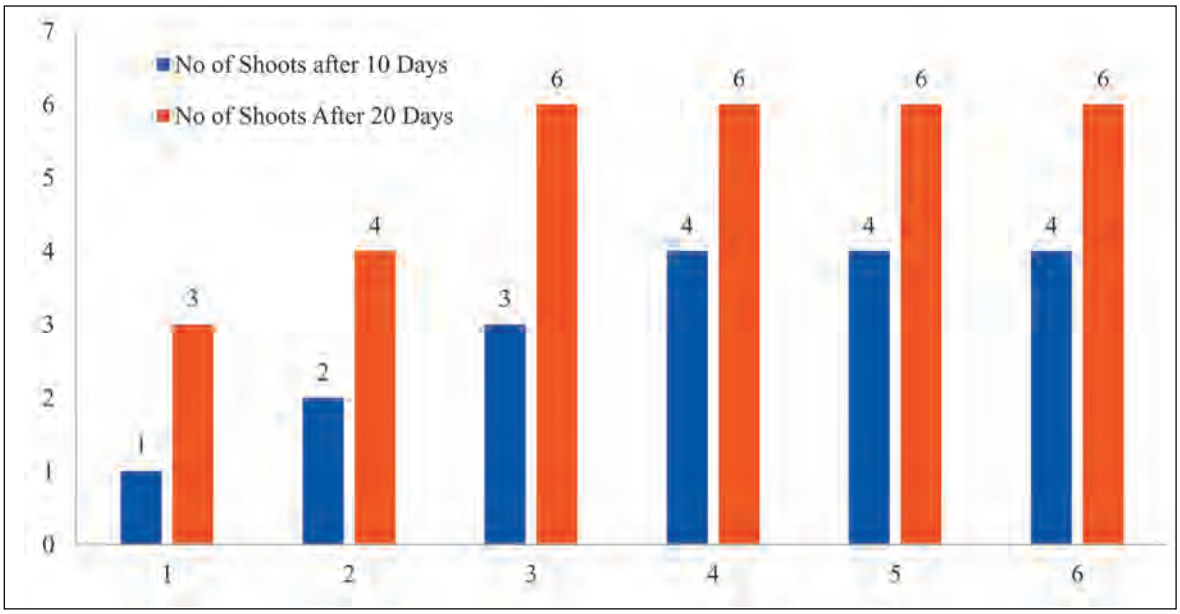


Figure 2: Number of days Required For Shoot Initiation

Table 3: Effect of carbon source on multiplication rate

Treatment	Symbol	Days	Leaves	Height	Number of shoots
Treatment 1	T1	21	1.6	33	7
Treatment 2	T2	22	2.3	55.66	9
MEAN		21.5	1.95	44.33	8
SE		0.5	0.35	6.33	0
CD @ 0.05		1.79	1.13	6.68	4.5

found to be the most suitable carbon source, as on average, 8-10 shoots were obtained after 3 weeks of culture (Table 3 and Figure 3). The length of shoots was also maximum (55 mm) (Table 3 and Figure 3) in sucrose-supplemented medium. However, replacement of sugar with less expensive table sugar did not affect the multiplication rate significantly; instead, it considerably reduced the cost of production.

Rooting

Rooting is a major bottleneck when carrying out in vitro multiplication of woody species. Clumps of 3 to 4 shoots were inoculated onto MS medium supplemented with different concentrations and combinations of auxins. Shoots failed to root even after 45 days of culture on MS medium even after supplementation with different concentrations of auxin. After 15 days of transfer to root induction medium, the propagules turned brown in all treatments. Hence, a regular transfer to fresh media was performed every 15 days to ensure the development of healthy shoots and roots.

Table 4: Effect of IBA on rooting

Treatment	Symbol	10 DAI	20 DAI
Treatment 1	T1	0±1	0±1
Treatment 2	T2	0±1	0±1
Treatment 3	T3	0±1	0±1
Treatment 4	T4	0±1	0±1
Treatment 5	T5	0±1	0±1
Treatment 6	T6	0±1	0±1

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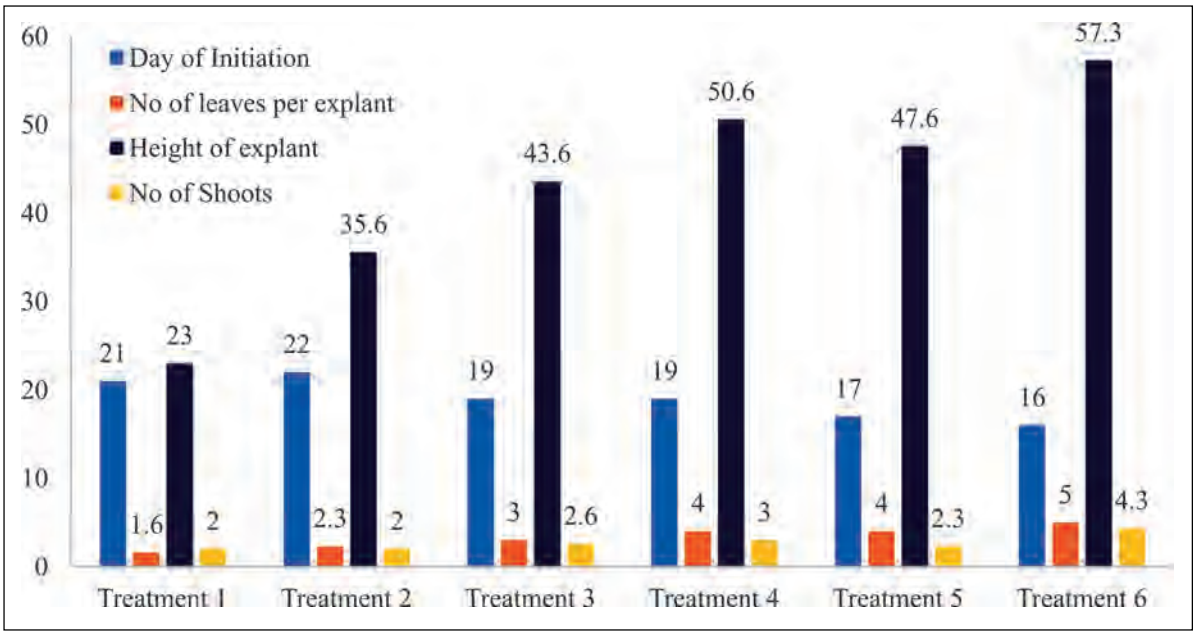


Figure 3: Biometric observation of *Dendrocalamus stockisi in vitro* shoot initiation

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DISCUSSION

To develop a rapid in vitro cloning protocol for *B. Stockisi* studies were conducted on the following aspects to increase the rapid in vitro cloning protocol for *B. Stockisi* studies were performed on the subsequent aspects: In vitro studies of shoot initiation, the effect of various PGRs, various combinations of cytokinins and constant auxin were examined for shoot initiation and their subsequent increase from nodal shoot segments on MS liquid medium. The effect of the carbon source on shoot multiplication of bamboo was examined using sucrose and table sugar with six replications. Among all the treatments with PGRs made in the investigated bamboo species, the medium including MS + NAA 0.5 mg/l + BAP 5 mg/l + additives was excellent for high frequency multiple shoot formation (6-7 shoots/explant) within 14 days of culturing at 28±2°C temperature and 2500 Lux intensity light for a 16-hour photoperiod. Sucrose was found to be the most suitable carbon source, as on average, 8-10 shoots/explant were observed after 3 weeks of culture. However, replacement of sugar with less expensive table sugar did not affect the multiplication rate significantly; instead, it considerably reduced the cost of production. Rooting is a major bottleneck when carrying out in vitro multiplication of woody species. Clumps of 3 to 4 shoots were inoculated onto MS medium supplemented with different concentrations and combinations of auxins. Shoots failed to root even after 45 days of culture on MS medium even after supplementation with different concentrations of auxin.

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