



# ***In-vitro* Regeneration of Bamboo (*Bambus balcooa*): A Review**

**Radhika Motghare<sup>a++\*</sup>, Kevin Gawali<sup>b#</sup>,  
Shubhankar Tarafdar<sup>a†</sup> and Amol Nagmote<sup>a†</sup>**

<sup>a</sup> Department of Agricultural Botany (GBP), School of Agricultural Sciences, G. H. Rasoni University, Saikheda, Chhindwara (M.P.), India.

<sup>b</sup> School of Agricultural Sciences. H. Rasoni University, Saikheda, Chhindwara (M.P.), India.

## **Authors' contributions**

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## **ABSTRACT**

Bamboos are adaptable, fragrant, perennial, and non-wood forest plants that are extremely significant from an ecological, sociological, and economic standpoint. Bamboo may be propagated using a variety of methods, including rhizome and culm cuttings, clump division, and seed propagation, however these traditional methods have significant limitations when it comes to large- or mass-scale multiplication. These are typically inadequate and ineffective for mass scale dissemination, leaving micropropagation as the sole practical approach. The requirement for bamboo material for cultivation is so high that large-scale multiplying will inevitably necessitate micropropagation. High hopes have been placed in the ability of micropropagation for the mass propagation of bamboo, and a great deal of study has gone into the creation of procedures for large-scale, quick propagation. These include clonal fidelity, somatic embryogenesis, *In vitro* blooming, macro-proliferation, field efficiency as well as the optimisation and development of *in vitro*

<sup>++</sup>M.Sc. Agri Scholar;

<sup>#</sup>Dean;

<sup>†</sup>Assistant Professor;

<sup>\*</sup>Corresponding author: E-mail: [motghareradhika98@gmail.com](mailto:motghareradhika98@gmail.com);

culture procedures. For large-scale micropropagation, which is urgently needed, this paper rapidly presents the most current knowledge on tissue culture mediated biotechnological interventions done in bamboo.

**Keywords:** Bamboos; micropropagation; multiple shoots induction; nodal explants; plantlets regeneration; root induction.

## 1. INTRODUCTION

Bamboo (family: Poaceae), which plays a large part in the world economy, has earned the moniker "Green Gold." [1] (Fernandes, 2017). A current report by Grand View Analysis, Inc. estimates that global marketplace for bamboo would reach USD 98.3 billion by 2025 [2]. Intergovernmental organisations have supported the bamboo growing for its natural as well as commercial advantages since it has been considered as a viable possibility to produce revenue for rural populations (Musau, 2016). Bamboo is used for a variety of purposes, thus many nations support its growing [3], (Takouleu, 2020). North-east India is home to the perennial, drought-resistant bamboo species *Bambusa balcooa* Roxb. (Poaceae: Bambusoideae). Commercial cultivation of it is practised in Australia, India, Bangladesh, Java, and Africa [4,5]. Additionally, grown in a number of South-east Asian nations, southern and central America, tropical Africa, etc. [6]. Along with India, South Asian nations' traditional cuisine has used the plant in a variety of food dishes (Singhal et al., 2013); [7]. It is thought to be one of the outstanding bamboo species for use in construction as well as scaffolding because of its sturdy culms [8,6]. Because of the ban on synthetic materials, bamboo-bottles have become more common by using *B. balcooa* in them [9].

One of the earliest and most widely used bamboo species is for industrial relevance due to its quick development, production capacity, high rate of survival, and variety of utilization [10], [11] (Krishnakumar et al., 2017). Due to its multiple applications, there has been a surge in the need for excellent *B. balcooa* over the past several years, which has encouraged more farmers to grow the plant [12]. The plant is often developed vegetatively, particularly during the summer (Pattanaik et al., 2004) [13]. Nevertheless, adopting vegetative techniques would not be able to satisfy the enormous demand from farmers.

Additionally, there is a significant likelihood of disease transmission via the soil. While the

micropropagation method allows for year-round, extensive production of strong, inherently consistent species. Although past attempts to micropropagate this plant have been made [14], (Gillis et al., 2007) [15,16-20], However, much work is still needed to make it more feasible. Along with optimising the micropropagation process, which can help to improve the environment for plantlets to acclimatise, it is also crucial to investigate the characteristics of plants' leaves [21].

Foliar anatomy investigations are used to identify physiological modifications for the plant's capacity to adapt to an ex vivo environment and increase photosynthetic efficiency [22,23]. The plantlets' physiological as well as morphological adjustments make them more suitable to their environment and enable them to carry out all vital physiological tasks [24]. In recognition of *B. balcooa*'s versatility, farmers have been increasingly turning to its cultivation in recent years, which has boosted the need for high-quality planting material. Thus, the purpose of the current study was to:

1. Standardization of surface sterilization protocol for ex-plant initiation.
2. Standardization of *in-vitro* shoot proliferation media by using different concentration and combination of cytokines and auxins.
3. Standardization of *in-vitro* root induction in developed shoots by using different concentration and combination of auxins

## 2. *In-vitro* PROPAGATION OF BAMBOO

The sole cutting-edge method that can be used to overcome the difficulties of rapid and widespread bamboo species multiplication is plant tissue culture [25]. The successful propagation of is a substitute technology that offers quick bulk bamboo proliferation combined with healthy plants along with a clone, helping to preserve bamboo and satisfy market demand that is expanding. In addition to routinely

providing high-quality planting materials, micropropagation aids in the preservation of bamboo's genetic diversity (Arya et al, 2008). For bamboo micro-propagation, a variety of explants are employed, including seeds, seedlings, inflorescences, roots, matured nodes, meristem domes, clusters, or foliage. Both young as well as mature plant in bamboo explants [26]. Because they include repurposed dietary ingredients, nodal segments are regarded as more efficient explants for *in vitro* cultivation. It grows into new plantlets as a result of the nodal segments' extremely active meristematic tissue [27]. An Explant responses are influenced by the plants' physical condition as well as the mother plants' wellbeing, the time of year explants were harvested from the field, their size, and their placement inside the mother plants. Nodal explant minimise possibility of soma clonal variation during organogenesis [25]. Numerous studies have been done on bamboo micropropagation using nodal explants. The created procedures, however, are either insufficient or inapplicable because they are only applicable for research and not for industrial mass production. It aims to investigate the most appropriate micropropagation procedures for large-scale bamboo growth. This study is thorough in providing a summary of current literature findings that highlight the value of micropropagation employing nodal segments of bamboo species as well as the variables that affect it.

### 3. COLLECTION AND STERILIZATION OF EXPLANT

The major goal of disinfecting explants is to eliminate bacterial and fungal contamination without impairing the explants' biological function. Bleaching, ethanol, sodium hypochlorite (NaOCl), and mercuric chloride (HgCl<sub>2</sub>) are some of the frequently used sterilants. The species and kind of explant determine the sterilant's concentration and exposure period (Razdan M K 2003). Explants (nodals) of *Bambusa balcooa* was sterilized in fungicide and antibiotic i.e 1% Bavistin + 0.5% Ampicillin solution (explant was dipped in the solution for 25 mins) then 3 times of autoclaved distilled water cleaning. Then explant nodal was treated with different concentration of HgCl<sub>2</sub> followed by 3 time washed with autoclaved distilled water. Out of which 0.3% of HgCl<sub>2</sub> for 15 min's gave 80% survival rate which was the highest. Same treatment was conducted by Thapa N, Gauchan DP et al. [28].

### 4. MEDIA AND CONDITION CULTURE

The effective development and differentiation of excised plant tissues and organs depends heavily on media. MS media (Murashige T & Skoog F 19962) medium was used extensively in the regeneration of bamboos. Tsay and his co-workers [29] used N6 For the regeneration of *Sinocalamus latiflora*, medium coupled with MS medium was successful in producing embryonic calli, while (Ndiaye et al 2006) employed three distinct media—MS medium, Gamborg medium, and Lloyd and Crown medium—for *Bambusa vulgaris*' quick spread. Modified MS medium, one of the three media studied, improved axillary bud formation and shoot development. For more than just obtaining the most auxiliary bud breaking, but MS [30]. In contrast to other media like SH, medium has also been frequently used for bamboo cultivation with superior response bud proliferation and subsequent multiplication. [31], B5 [32] as well as NN [33], WP Media (Lloyd and Crown 1980) [34]. Likewise, Shirgurkar et al. [35], Singh et al. [36], Ogita et al. (2009), and Negi and Saxena [28] observed in order to achieve effective *In vitro* cultivation in bamboo, half strength MS medium were preferable to full strength MS media [28]. Another element that affects the ability to produce plant tissue *in vitro* is the condition of the culture medium physically. According to several researches, invitro production on semi solid/solid MS medium allowed for the effective proliferation and shoot multiplication of bamboo [37].

Growth regulators for plants (PGR) are the chemical compounds that have an impact on how the plant grows, either positively or negatively. The morphological structure of plants can alter when PGR levels are low. In tissue culture, both natural and synthetic phytohormones are often utilised. For bamboo callogenesis and histogenesis, cytokinins and auxin are mostly employed. Various concentrations of the 6-Benzyl aminopurine, 6-Benzyl adenine, Napthalene Acetic Acid, Indole 3-Butyric Acid, Indole Acetic Acid, Zeatin, kinetin, Thidiazurn. On micropropagation of the bamboo, 3% sucrose along with 100 mg/L myo-inositol were employed as supplements. The hormones employed by researchers as growth regulators are listed in Table 1.

After aseptic inoculation of the explants is completed, the incubation conditions are extremely important in plant tissue culture. In

**Table 1. Micropropagation from Nodal segments/Explants of *Bambusa balcooa* (bamboo)**

| S. No. | Species    | Explant used        | Basal medium | Surface sterilization  | PGR'S   | Result  | Reference                                |
|--------|------------|---------------------|--------------|--|---|---|--|
| 1.     | B. balcooa | Nodal segments      | MS           | 0.1% Gentamycine 0.5% Bacteriomycline for 15 minutes   | BAP (11.25 µM) + Kin (4.5 µM) + ½ MS + IBA (1.0µM)  | SHOOT INIATION ( <i>In-vitro</i> regeneration ) | Das and Pal [38]                         |
| 2.     | B. balcooa | Nodal buds          | MS           | 0.1% Gentamycine 0.5% Bacteriomycline for 15 minutes   | ** BAP (1.0 mg l-l) *** BAP (1.0-5.0 mg l-l) + ½ MS + NAA (1.0-3.0 mg l-l) + IBA (1.0-5.0 mg l-l)   | Mass propagation                                | Islam and Rahman [39]                    |
| 3.     | B. balcooa | Nodal segment       | MS           | teepol solution and bavistin (0.1% w/v)  | BAP (4.4 µM/L)+NAA (0.53 µM/L) MS + BAP (4.4 µM/L)+ NAA (0.53 µM/L) MS + NAA (16.11 µM/L)   | Mass multiplication and Rooting                 | Anand M, Brar J, Sood A. [40]            |
| 4.     | B. balcooa | Nodal segment       | MS           | Tween 20 for 5 min 1% Bavistin for 10 min 70% isopropyl and disinfection with 0.1% MgCl2 solution for 5 min. | citric acid (25 mg/L) + ascorbic (50 mg/L) +BAP (3.5 mg/L)+ BAP (3 mg/L)+ NAA (0.5 mg/L) +NAA(4 mg/L)   | Mass multiplication and Rooting                 | Patel B, Gami B, Patel N, Bariya V. [41] |
| 5.     | B. balcooa | Nodal buds          | MS           | 2% sodium hypochlorite with sonication for 30 min.   | MS+TDZ (0.1 mg/L)+ Gelrite (2g/l) MS + TDZ (0.1 mg/L) MS + TDZ ( 0.01 mg/L) + 2,4-D (0.5 mg/L).   | Mass multiplication and rooting                 | Lin CS, Kalpana K, Chang WC, Lin NS [42] |
| 6.     | B. balcooa | Nodal segment       | MS           | 0.1% mercuric chloride and Gentamycin (3.0-8.0 mg/L)   | MS + BAP (1 mg/L) MS+ BAP (1 mg/L) MS+ BAP (1 mg/L) + NAA (3mg/L)   | Mass multiplication and Rooting                 | Mudoi KD, Borthakur M. [43]              |
| 7.     | B. balcooa | Nodal bud           | MS           | -----  | MS+ BAP (4 mg/L) Liquid MS + BAP (4 mg/L) MS Liquid+ IBA (1mg/L)  | Mass multiplication and Rooting                 | Gantait S, Mandal N, Nandy S. [44]       |
| 8.     | B. balcooa | Nodal buds          | MS           | HgCl2 (0.1%) and a few drops Tween 20 (Polyxyethylene sorbitan Monolaurate)                                  | Liquid MS + BAP (1 mg/L) MS+BAP (1.0-5.0 mg/L) ½ MS+ NAA (3 mg/L)/ IBA (5 mg/L)   | Mass multiplication and Rooting                 | Islam SA, Rahman M [39]                  |
| 9.     | B. balcooa | Nodal segment       | MS           | 0.25 ml Folin phenol reagent + 0.75 ml saturated Na2CO3 solution   | Liquid MS + BAP (11.25 µM/L) + KN (4.5 µM/L) Liquid MS + IBA (1 µM/L) ½ MS Liquid) + IBA (1 µM/L)   | Mass multiplication and Rooting                 | Das M, Pal [38]                          |
| 10.    | B. balcooa | Single node segment | MS           | Teepol (4–5 drops ) and four to five drops of a germicide for 15 min   | MS+ BAP (4.4 µM/L) + KN (2.32 µM/L)+ Gelrite (0.2% w/v) Liquid MS + BAP (6.6 µM/L)+KN ( 2.32 µM/L)+ Coconut water (2.5% (v/v) 1/2 MS +IAA (5.71 µM/L)+ IBA (4.9 µM/L)+NAA (5.37 µM/L) | Mass multiplication and Rooting                 | Negi D, Saxena S [37]                    |

| S. No. | Species   | Explant used   | Basal medium | Surface sterilization   | PGR'S   | Result                                 | Reference   |
|--------|-----------|----------------|--------------|---|---|--|---|
| 11.    | B.balcooa | Nodal segments | MS           | HgCl <sub>2</sub> treatment for (9 min ) was given after treating with bavistin (25 min). | BAP (3 mg/L) MS + BAP (5 mg/L) MS +NAA (4.5 mg/L)   | Mass multiplication as well as Rooting | Thapa N, Gauchan DP, Suwal MM, Bhujju S, Upreti A, Byanju B, Lamichhane J. [28] |
| 12.    | B.balcooa | Nodal cutting  | MS           | 5 % teepool+ 0.1 % (w/v) Bavistin (for 5 min  | 4.0 mg L <sup>-1</sup> BAP, 50 mg L <sup>-1</sup> ascorbic acid and 25 mg L <sup>-1</sup> each of L-arginine, citric acid and adenine sulphate. | Mass multiplication along with rooting | Bharat S. Rajput a , Minal Jani et.al [45]                                      |
| 13.    | B.balcooa | Nodsl segment  | MS           | (Tween 20) for 5 mins + (Bavistin 1%) for 10 min+ o.1 Hgcl <sub>2</sub> for 5 min         | BAP (1.5 µg/L and 2 µg/L)   | Mass multiplication and rooting        | Anbuselvi, S., Priyanka, P.S. ( 2022)   |

order to produce good clones, an ideal temperature is needed. Additionally, certain tissues may develop in the dark, but others favour light. The amount of light has a significant impact on tissue regeneration as well. For instance, numerous researchers recorded photoperiods as high as 16 h, while Alexander and Rao [27] observed 12 h photoperiodism in *Bambusa spp* [46]. Light intensity varies across species as well. We previously stated that *D. strictus* may thrive under cool white fluorescent light between 2000 and 3000 lux [47].

### 5. *In-vitro* SHOOT MULTIPLICATION

The propagates size and quantity play a critical role in the shoot multiplication. For each culture to proliferate, three to four propagules were shown to be successful [48] than individual propagule cultured [49]. Additionally, BAP were widely utilised in the *In vitro* proliferation of several bamboo shoot [50]. The quantity and length of shoots decreased as BAP concentration increased. MS media (solid medium) was used at different levels to the nodal ex-plant (*B. balcooa*) throughout growth and combinations of BAP + NAA. After being inoculated on the medium MS, nodal explants displayed shoot beginning after 7 to 10 days later at various BAP levels. Rathore et al. [16] is the only researcher who combined the impact of NAA and BAP as well as it was effective for *B. balcooa* as well as *B. bambos* shoot proliferation.

### 6. *In-vitro* ROOT MULTIPLICATION

For root initiation, NAA, IAA, and IBA are often utilised individually or in combination. All three of these hormones worked better for *D. asper* rooting (Pratibha S, Sarma KP 2014). 3-5 shoot clusters worked well for transplanting into rooting medium [41]. For invitro rooting, full-strength of MS along with half-strength of MS medium with addition of root promoting hormone was often used. Arya et al. [51] After dealing with *D. asper* and *D. falcatum*, it was found that 80–90% of the roots were found in MS media with the addition of NAA or Indole butyric acid following five weeks after the transfer. *Bambusa balcooa* produced shoot bunches were planted in the 12 MS media containing different conc. as well as combinations of NAA along with IBA for root initiation. Islam and Rahman [39] have also reported using IBA and NAA together for rooting. Arya et al. [51] and Rathore et al. [16] in

numerous significant bamboo species. However, in half-strength MS medium treated with NAA, strong roots and healthy shoots were seen. Negi and Saxena [37] have proven that in *B. nutans*, the maximum rooting were found on media supplied with Indole acetic acid, Indole butyric acid as well as NAA and the exact same outcome was also attained by Kapoor and Rao [52] they claimed that *B. bambos* had 100% rooted on 12 MS medium with the right amount of IBA and NAA. Singh et al. (2012) demonstrated a synergetic result for rooting in *D. asper* when IBA and NAA were used together rather than separately. Again, numerous researchers found that in a variety of bamboo species, half-strength MS medium treated with NAA along with Indole butyric acid performed better than full-strength MS media. Islam and Rehman [39] It was determined that pairing of NAA along with Indole butyric acid was appropriate for bamboo germination.

### 7. HARDENING AND ACCLIMATIZATION

Another broad definition of micro-propagation is the shifting of invitro generated plantlet from a laboratory to the ground [53]. Despite having well-developed roots, the plant grown in a lab is unable to thrive in the wild due to a lack of adaptability and adequate hardening [54]. Researchers have used a variety of hardening techniques to get around the hardening bottleneck. The healthy as well as well rooted plantlet are often rinsed to remove them from the rooting media and moved to a container with growth supportive materials as soil, sand, soil-rite, perlite, coco-peat, agro-peat, vermiculite, compost, etc., either used alone or in different proportions [53]. The majority of studies have utilised or changed a 1:1:1 substrate ratio. To produce the most plantlets possible, several scholars have characterised main hardening along with secondary hardening. Then, when plantlets were transplanted to polybags made from a 1:1:1 sand composition: Farmyard waste: The soil from which they grew high-rate plantlets. Because *in vitro* grown plantlets are unable to withstand biotic as well as abiotic challenges, death percentage of plants increases when they're immediately sent to an unfamiliar surroundings. (Bag N, Chandra S, 2000). However, Negi and Saxena [37] immediately hardened in a 2:1 mixture of soil: agro peat in *B. nutans* and had a 95.83% success rate. In a similar way several researchers revealed on the hardening of invitro plants in following combinations: soil-sand-compost cocopeat

(1:1:1) in *B. nutans* [55]; soil: sand: cow dung (1:1:2) in *B. balcooa* [51] and soil: sand: coco-peat along with vermi-compost (3:1) in *B. balcooa*. *In vitro* saplings was effectively acclimated in *B. bambos* without the need of other substrates or soil [56].

## 8. CONCLUSION

The country's economy is developing quickly, and the demand for bamboo has increased the bamboo's rapidly decreasing rootstock. Bamboo can store a lot of carbon, which helps to weaken climate change and protect the environment. It serves as a different source of the forest in a similar manner. Consequently, this has an important impact on conservation biology showed has emerged as a top priority. Despite their awareness of biology and environmental protection, individuals must nevertheless satiate the vast market demands and utilise the finite resources.

Harvesting from the resources necessitates the micropropagation of numerous bamboo plantlets on a huge scale to make up for the loss in plant stocks. The review discusses mass yield manufacturing techniques and the variables that affect them. Several studies have documented a number of procedures. With the optimum season for explant collection, adequate development agents in MS medium under purification conditions, and the proper location of the nodal segment in mother plant stocks, nodal explants are superior explants for micro-propagation methods. BA/BAP is the most efficient cytokine for the bud initiation and shoot multiplication of bamboo species. Specific to bamboo species is *in vitro* rooting. Comparison of NAA and Indole acetic acid, shows the latter being the most potent rooting hormone for bamboo. However, multiple investigations revealed that a combination of IBA along with NAA is equally useful for *in vitro* rooting. According to reports, several bamboo species can root well with the addition of NAA, IBA, and IAA. Only sand as a medium is unable to support the plant in the hardening stage. However when combined with other substrates, the ratio may be significantly increased for *in vitro* rooted plants. It is necessary to appropriately address such challenges in order for more applicable procedures to emerge. The goal of future research should be to produce bamboo plants on a vast scale.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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