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Advances and Economical Approaches in Vitro Propagation of Bamboo Cultivars: A Comprehensive Review

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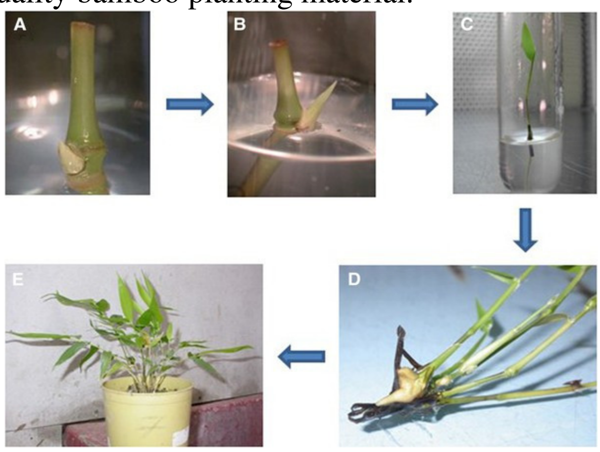
ABSTRACT
Bamboo, a fast-growing, renewable resource, holds significant ecological, economic, and industrial value due to its applications in construction, paper, furniture, bioenergy, and environmental conservation. However, conventional propagation techniques, such as seed germination and vegetative methods, are often hindered by issues like poor seed viability, infrequent flowering, and limited clonal uniformity. In vitro micro propagation offers an efficient and sustainable solution for the mass multiplication of elite bamboo genotypes, ensuring year-round propagation with genetic fidelity. This review highlights recent advancements and cost-effective strategies in the in vitro propagation of major bamboo cultivars, with emphasis on species such as *Bambusa tulda*, *Bambusa balcooa*, and *Melocanna baccifera*. Key stages including explant selection, surface sterilization, culture initiation, shoot proliferation using cytokinins (notably BAP and TDZ), root induction using auxins like IBA, and acclimatization techniques are critically discussed. Incorporation of low-cost supplements, alternative gelling agents, and simplified protocols are presented as viable methods to reduce production costs. Moreover, successful acclimatization protocols significantly enhance survival rates, enabling large-scale field transfer. The review also emphasizes the need for further research on genotype-specific responses, contamination control, and the use of molecular markers for clonal fidelity assessment. The integration of biotechnological tools with traditional forestry practices can facilitate the sustainable utilization and conservation of bamboo resources, particularly in developing regions. This paper aims to serve as a resource for researchers, conservationists, and industries seeking efficient propagation methods for bamboo cultivation.

Keywords: Bamboo micro propagation, *Bambusa tulda*, in vitro culture, shoot multiplication, rooting, acclimatization, cost-effective propagation, plant tissue culture, genetic fidelity, sustainable forestry.

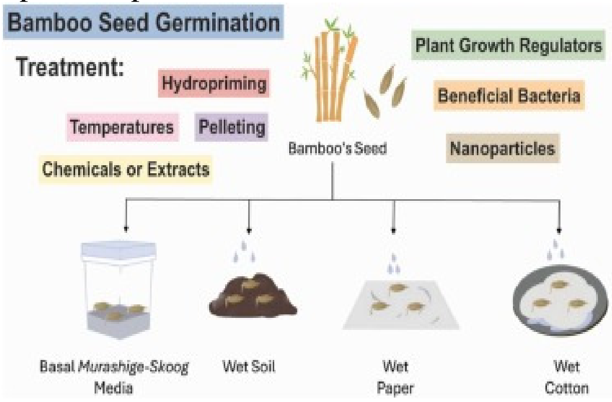
1. INTRODUCTION
Bamboo species are integral to various industries, including construction, paper, and handicrafts, due to their rapid growth and adaptability. Despite their importance, propagation remains a challenge, primarily because of irregular flowering and seed scarcity. In vitro propagation emerges as a solution, allowing for the rapid and large-scale production of uniform plantlets. This review aims to consolidate current knowledge on cost-effective in vitro propagation techniques for bamboo cultivars, highlighting recent advancements and practical approaches.

Bamboo, a fast-growing and highly renewable plant resource, plays a critical role in sustainable development due to its wide range of applications in construction, paper production, and biomass energy. Traditional propagation methods, which rely on seeds or vegetative division, are often inefficient and unpredictable, especially for species like *Bambusa balcooa*, which rarely flower and produce viable seeds. In vitro propagation has thus emerged as a promising technique to meet the growing demand for high-quality bamboo planting material.



Source: Ramesh et al. (2017)

Recent advancements have led to the successful development of protocols using nodal explants and axillary buds, which ensure genetic fidelity and mass multiplication of elite clones. For instance, Ramesh et al. (2017) reported an efficient shoot regeneration and plantlet formation protocol from axillary buds of *Bambusa balcooa*, marking a significant step toward commercial-scale micro propagation and conservation efforts for this economically important species.



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Given the challenges associated with bamboo propagation, such as irregular flowering cycles and low seed viability, this review focuses on various species and the innovative techniques developed to overcome these limitations. The authors discuss methods including pre-sowing treatments, controlled environmental conditions, and biotechnological interventions that have shown promise in improving germination rates and seedling vigor. By consolidating current research, the article provides valuable insights for researchers and practitioners aiming to optimize bamboo cultivation through improved seed germination practices.

2. EXPLANT SELECTION AND SURFACE STERILIZATION

Explant selection and surface sterilization are foundational steps in the in vitro propagation of bamboo species, directly affecting the efficiency and success of tissue culture protocols. The selection of an appropriate explant type is critical, as it determines the regenerative potential and responsiveness of the culture. Among various plant parts, nodal segments are widely recognized as the most suitable explants for bamboo micro propagation. This preference stems from their inherent high regenerative capability and the presence of pre-existing meristematic tissues, which facilitate direct shoot induction without the intermediate callus phase, thereby preserving genetic fidelity (Ramesh et al., 2017).

However, the high susceptibility of bamboo tissues to microbial contamination presents a significant challenge during in vitro culture initiation. Therefore, effective surface sterilization protocols must be employed to eliminate external and surface-adhering microbes while minimizing tissue damage. Typically, explants are first subjected to a brief treatment with 70% ethanol for about 30 seconds to one minute. This step acts as a primary disinfection measure and helps to dislodge surface contaminants. Following the ethanol wash, a more rigorous sterilization step is conducted using chemical agents such as mercuric chloride ($HgCl_2$), usually at concentrations ranging from 0.1% to 0.2%, for durations of 5 to 10 minutes. The exposure time is carefully controlled to strike a balance between microbial eradication and explant viability. After chemical sterilization, explants are rinsed thoroughly—often three to five times—with sterile distilled water to remove any residual sterilant, which could otherwise be toxic to plant tissues (Sharma et al., 2018; Negi & Saxena, 2020).

For instance, in the case of *Bambusa tulda* and *Melocanna baccifera*, researchers successfully employed a two-step sterilization process involving initial immersion in 70% ethanol followed by treatment with 0.1% mercuric chloride. This method was effective in minimizing contamination and resulted in a high percentage of viable, clean cultures ready for further growth in vitro (Das & Pal, 2011). These optimized sterilization techniques have proven essential for ensuring reproducibility and success across different bamboo species and laboratories. Thus, the meticulous selection of nodal segments as explants, combined with a carefully calibrated surface sterilization protocol, plays a vital role in initiating clean and responsive cultures for the efficient micro propagation of bamboo cultivars.

3. CULTURE MEDIUM OPTIMIZATION

3.1 Basal Media

Murashige and Skoog (MS) medium is widely utilized for bamboo tissue culture due to its comprehensive nutrient profile. Studies have shown that half-strength MS medium can be more effective for certain species, promoting better shoot proliferation and reducing costs.

3.2 Carbon Sources

Sucrose is the standard carbon source in culture media, typically at concentrations of 2-3%. However, replacing sucrose with commercial table sugar has been explored as a cost-saving measure. In Dendrocalamus asper and D. hamiltonii, the use of table sugar did not adversely affect shoot multiplication, suggesting its viability as a cheaper alternative

3.3 Gelling Agents

While agar is traditionally used to solidify media, its high cost has prompted the exploration of alternatives like gellan gum. Liquid media, supported by filter paper bridges, have also been employed to reduce expenses and enhance nutrient uptake, though care must be taken to prevent hyperhydricity.

4. PLANT GROWTH REGULATORS (PGRS)

4.1 Cytokinins

Cytokinins such as 6-benzylaminopurine (BAP) and kinetin (Kn) are pivotal in inducing shoot proliferation. Optimal concentrations vary among species; for example, a combination of 3 mg/L BAP and 2 mg/L Kn yielded the highest shoot multiplication rates in Bambusa tulda and Melocanna baccifera.

4.2 Auxins

Auxins like indole-3-butyric acid (IBA) and naphthaleneacetic acid (NAA) are essential for root induction. In Bambusa arundinacea, the combination of 3.0 mg/L IBA with 2.0 mg/L silver nitrate ($AgNO_3$) achieved an 85% rooting rate, demonstrating the effectiveness of auxin-supplemented media.

5. SHOOT MULTIPLICATION AND SUBCULTURING

Shoot multiplication and subculturing are crucial stages in the micro propagation process, as they determine the overall success of mass propagation protocols and the quality of regenerated plantlets. Efficient shoot multiplication is not only essential for increasing the number of propagules but also for maintaining the physiological vigor and morphological integrity of the cultures over successive passages. In the case of *Bambusa balcooa*, one of the most commercially important bamboo species, the application of cytokinin-rich media has proven particularly effective. Specifically, the use of 1.5–2.0 mg/L of 6-Benzylaminopurine (BAP) has been reported to significantly enhance axillary shoot proliferation. Under these conditions, cultures exhibit robust shoot development with minimal signs of vitrification or necrosis. To sustain active growth and avoid phenolic-induced browning, which is a common issue in bamboo tissue culture, regular subculturing at intervals of 2 to 3 weeks is recommended. These intervals ensure the timely replenishment of nutrients and hormones while diluting the accumulation of toxic exudates (Ramesh et al., 2017).

In another study involving *Bambusa tuldoides*, a synergistic effect of BAP and Thidiazuron (TDZ) was observed when used in combination in liquid Murashige and Skoog (MS) medium. The optimized hormonal combination—3.0 mg/L BAP with 1.5 mg/L TDZ—led to a remarkable enhancement in shoot proliferation, producing an average of 15.40 ± 1.21 shoots per explant. Liquid media are often favored for such protocols due to their improved nutrient uptake and aeration, which further contribute to higher multiplication rates (Zhao et al., 2014). TDZ, known for its high cytokinin activity, stimulates cell division and morphogenesis, while BAP aids in shoot elongation and stability of the shoot clumps.

6. ROOTING AND ACCLIMATIZATION

Rooting and acclimatization represent the final but critically important stages of the micro propagation cycle, ensuring the successful conversion of in vitro cultured shoots into self-sustaining plants that can survive ex vitro conditions. Root induction is essential for establishing a robust root system, which supports nutrient and water uptake once the plantlets are transferred to soil or potting substrates. In bamboo micro propagation, rooting is often considered a challenging phase due to species-specific responses to auxins and the physiological recalcitrance of some genotypes.

In studies involving *Bambusa tulda* and *Melocanna baccifera*, effective root formation was achieved using half-strength Murashige and Skoog (MS) medium supplemented with 3.0 mg/L Indole-3-butyric acid (IBA) and 10 mg/L coumarin. The use of half-strength MS medium is intentional, as lower salt concentrations reduce osmotic stress and better mimic natural rooting conditions, thereby enhancing root initiation and elongation. IBA is a widely preferred auxin for rooting due to its high efficacy and relatively low toxicity. It promotes the differentiation of root initials and elongation of root primordia. The addition of coumarin, a phenolic compound known to synergize with auxins, further stimulates root induction and improves rooting frequency by modulating enzymatic activities involved in cell differentiation and hormone signaling (Das & Pal, 2011; Devi & Sharma, 2023).

Following successful in vitro rooting, the acclimatization phase is critical for ensuring plantlet survival under natural environmental conditions. During this stage, rooted plantlets are gradually transitioned from the aseptic, high-humidity, and nutrient-rich in vitro environment to the more variable and often harsher external conditions. Acclimatization typically occurs in two phases. Initially, plantlets are transferred to a high-humidity growth chamber or polyhouse with controlled temperature and light conditions. A substrate such as a mixture of sterile soil, sand, and vermiculite or cocopeat is commonly used to support root development and moisture retention. After 2–4 weeks, once the plantlets show signs of active growth and strengthened physiology, they are moved to open nurseries or greenhouse conditions for further hardening before field planting.

Proper acclimatization significantly enhances the survival rate of bamboo plantlets, often exceeding 80–90% when protocols are optimized. This step ensures the development of functional stomata, lignified cell walls, and an adaptive root system capable of withstanding soil-borne pathogens and fluctuating moisture levels (Negi & Saxena, 2020).

7. COST-EFFECTIVE STRATEGIES

Implementing cost-saving measures is essential for the commercial viability of bamboo micro propagation. Strategies include:

- **Media Optimization**: Utilizing half-strength MS medium and replacing expensive components like sucrose with table sugar.
- **Alternative Gelling Agents**: Employing cheaper gelling agents or liquid media with support structures to reduce costs.
- **Efficient PGR Use**: Determining optimal concentrations of PGRs to minimize usage without compromising efficacy.
- **Sub culturing Intervals**: Establishing appropriate sub culturing schedules to maximize shoot proliferation and minimize resource expenditure.

8. CONCLUSION

The micro propagation of bamboo cultivars presents a sustainable and cost-effective alternative to traditional propagation methods, addressing the growing global demand for bamboo as an ecological, economic, and industrial resource. The success of in vitro propagation largely depends on the careful optimization of each stage—from explant selection, sterilization, shoot initiation, multiplication, rooting, to acclimatization. Nodal explants have proven to be reliable sources for culture initiation, while cytokinins like BAP and TDZ significantly enhance shoot multiplication when applied in appropriate concentrations and combinations. Rooting efficiency is greatly improved by the use of IBA in combination with additives such as coumarin, and the acclimatization process ensures the survival of plantlets in ex vitro environments.

While numerous protocols have been developed and refined for species such as *Bambusa tulda*, *Bambusa balcooa*, and *Melocanna baccifera*, challenges still remain—particularly regarding contamination control, somaclonal variation, and cost reduction of media components. Future research should focus on integrating low-cost substrates, organic growth additives, and automation technologies to enhance scalability. Furthermore, molecular tools for assessing genetic fidelity and optimizing hormone pathways can greatly advance the reliability of micro propagation systems. Overall, the integration of advanced biotechnological methods with traditional silvicultural practices will pave the way for efficient bamboo cultivation and large-scale conservation.

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