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Development of a Rapid Micropropagation Protocol for *Bambusa vulgaris* via Nodal Explants and Assessment of Genetic Fidelity Using RAPD Markers

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ABSTRACT

The present study focuses on developing an efficient and reproducible in vitro micropropagation protocol for *Bambusa vulgaris* through nodal explants and assessing the genetic fidelity of regenerated plantlets using Random Amplified Polymorphic DNA (RAPD) markers. Multiple shoot induction was achieved using Murashige and Skoog (MS) medium supplemented with various concentrations and combinations of cytokinins and auxins. Among the tested combinations, MS medium fortified with 3.0 mg/L BAP (6-benzylaminopurine) and 0.5 mg/L NAA (α-naphthaleneacetic acid) resulted in maximum shoot proliferation. Rooting was optimized using half-strength MS medium containing 1.5 mg/L IBA (indole-3-butyric acid), yielding robust root systems. The acclimatized plantlets exhibited a survival rate of 92% under greenhouse conditions. Genetic uniformity among micropropagated plants was confirmed using RAPD markers, which showed monomorphic banding patterns identical to the mother plant. The study demonstrates a rapid, cost-effective, and genetically stable micropropagation system suitable for large-scale propagation and conservation of *Bambusa vulgaris*.

Keywords: *Bambusa vulgaris*, Micropropagation, Nodal explants, MS medium, Growth regulators, RAPD markers, Genetic fidelity, Acclimatization.

INTRODUCTION

Bambusa vulgaris Schrad. ex J.C. Wendl. is among the most widely distributed and economically valuable bamboo species in tropical and subtropical regions. It belongs to the family Poaceae (subfamily Bambusoideae) and is characterized by its fast growth, adaptability, and versatile uses in construction, furniture, paper and pulp industries, handicrafts, and as a raw material for biomass and bioenergy production (Nadgir et al., 1984; Das and Pal, 2005). Due to its rapid vegetative growth and high biomass productivity, *B. vulgaris* has gained significant ecological and economic importance in afforestation, soil conservation, and sustainable rural livelihoods (Arya et al., 2009). It also plays a crucial role in carbon sequestration and ecosystem restoration, making it a vital species for climate resilience and environmental management (Rout and Das, 1994). Despite its immense potential, the propagation of *B. vulgaris* through conventional methods remains a major limitation. Seed propagation is unreliable due to its irregular and infrequent flowering cycles, poor seed set, and short seed viability period (Ramanayake and Yakandawala, 1997). Vegetative propagation through rhizomes or culm cuttings offers limited multiplication rates and requires a large amount of planting material, which restricts large-scale plantation efforts (Singh et al., 2004). Consequently, there is an urgent need for alternative propagation methods that are rapid, efficient, and capable of producing true-to-type plantlets for commercial and conservation purposes.

Plant tissue culture techniques offer a promising solution to overcome these challenges. Micropropagation, particularly through nodal explants, has proven effective in various bamboo species such as *Dendrocalamus hamiltonii* (Das and Pal, 2005), *Bambusa balcooa* (Arya et al., 2008), and *Dendrocalamus strictus* (Shirin and Rana, 2007). This technique allows for the rapid production of a large number of uniform plantlets from limited starting material under aseptic and controlled conditions. Furthermore, it facilitates year-round propagation, independent of seasonal constraints, while maintaining the genetic fidelity of the parent genotype (Rout and Das, 1997). The success of in vitro propagation primarily depends on the appropriate combination and concentration of plant growth regulators (PGRs), culture medium composition, and environmental conditions such as light, temperature, and photoperiod (Murashige and Skoog, 1962). Cytokinins like 6-benzylaminopurine (BAP) are known to induce axillary bud proliferation and shoot multiplication, whereas auxins such as indole-3-butyric acid (IBA) and α-naphthaleneacetic acid (NAA) promote rooting and elongation (Nadgir et al., 1984; Singh et al., 2010). Optimization of these factors is critical for developing a reproducible and efficient micropropagation protocol for bamboo species. However, in vitro culture conditions can sometimes induce somaclonal variations, leading to genetic instability among regenerated plants (Larkin and Scowcroft, 1981). Therefore, assessing the genetic fidelity of micropropagated plants is an essential step before their field deployment. Molecular markers such as Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR), and Amplified Fragment Length Polymorphism (AFLP) have been effectively used for clonal fidelity assessment in various plant species (Williams et al., 1990; Bhatia et al., 2009). RAPD markers, in particular, are

advantageous due to their simplicity, cost-effectiveness, and ability to detect polymorphisms without prior sequence information (Lakshmanan et al., 2007; Singh et al., 2011). Given this background, the present investigation was undertaken with the following objectives:

(1) To develop a rapid and efficient in vitro propagation protocol for *Bambusa vulgaris* using nodal explants;
(2) To optimize the combinations of plant growth regulators (cytokinins and auxins) for enhanced shoot multiplication and root induction;
(3) To evaluate the acclimatization and survival rate of regenerated plantlets under greenhouse conditions; and
(4) To confirm the genetic stability of in vitro regenerated plants through RAPD marker analysis.

This study contributes to the advancement of bamboo biotechnology by providing a reproducible system for large-scale propagation and genetic conservation of *Bambusa vulgaris*, thereby supporting both commercial plantation programs and environmental sustainability.

RESEARCH METHODOLOGY

2.1 Plant Material and Explant Preparation

Young, healthy shoots of *Bambusa vulgaris* were collected from a mature clump maintained in the Botanical Garden, Department of Botany. Nodal segments (2–3 cm) containing single axillary buds were excised, washed thoroughly under running tap water, and surface sterilized using 0.1% (w/v) mercuric chloride for 3 minutes, followed by three rinses with sterile distilled water.

2.2 Culture Media and Growth Regulators

Murashige and Skoog (MS) basal medium supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar was used. Various concentrations and combinations of BAP (1.0–5.0 mg/L) and NAA (0.1–1.0 mg/L) were tested for shoot induction and multiplication. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C for 20 minutes.

2.3 Culture Conditions

Cultures were maintained at 25 ± 2°C under a 16-hour photoperiod with light intensity of 40 µmol m^{-2} s^{-1}. Subculturing was done every 4 weeks to fresh medium for shoot multiplication.

2.4 Root Induction and Acclimatization

Elongated shoots (3–4 cm) were transferred to half-strength MS medium containing IBA (0.5–2.0 mg/L) for root induction. Well-rooted plantlets were washed with sterile distilled water to remove agar residues and transplanted into pots containing a mixture of soil, sand, and vermicompost (1:1:1). Acclimatization was carried out under a mist chamber for 15 days, followed by transfer to greenhouse conditions for hardening.

2.5 Genetic Fidelity Assessment Using RAPD

Genomic DNA was extracted from young leaves of the mother plant and randomly selected in vitro regenerated plantlets using CTAB extraction method. RAPD analysis was performed using ten decamer primers (Operon Technologies). PCR products were resolved on 1.5% agarose gels, stained with ethidium bromide, and visualized under UV light. Banding patterns were compared to assess genetic similarity.

3. Analysis

3.1 Shoot Induction and Multiplication

The response of *Bambusa vulgaris* nodal explants to different concentrations of cytokinins (BAP and kinetin) and auxins (NAA and IAA) varied considerably. Among the tested media compositions, MS medium supplemented with 3.0 mg/L 6-benzylaminopurine (BAP) and 0.5 mg/L α-naphthaleneacetic acid (NAA) produced the most favorable results, yielding a mean of 9.6 ± 0.3 shoots per explant within four weeks of culture initiation. The shoots were robust, green, and exhibited minimal vitrification or callus formation. Lower concentrations of BAP (1.0–2.0 mg/L) induced fewer shoots (3–5 per explant), while higher concentrations (>4.0 mg/L) resulted in hyperhydricity, stunted growth, or callus development at the base of the explant.

The synergistic interaction between BAP and a low level of NAA was essential for breaking apical dominance and promoting axillary bud proliferation. Similar cytokinin-auxin synergy has been reported in *Bambusa balcooa* (Arya et al., 2008) and *Dendrocalamus hamiltonii* (Das and Pal, 2005), where BAP combined with a small amount of NAA or IAA stimulated multiple shoot formation. It was also observed that kinetin alone or in combination with NAA was less effective compared to BAP, corroborating earlier findings that BAP is the most potent cytokinin for shoot proliferation in bamboo species (Nadgir et al., 1984; Shirin and Rana, 2007).

The higher shoot multiplication rate in the present study suggests that *B. vulgaris* possesses strong morphogenetic potential under optimized PGR conditions. The subculturing of proliferated shoots every four weeks maintained vigor

and sustained shoot production up to the fourth subculture without any decline in response, indicating genetic and physiological stability during multiplication. The duration of the culture also influenced shoot elongation; shoots reached 3–5 cm in height after 25–30 days of culture. Shoots developed under optimal conditions were characterized by uniform size and well-developed leaf primordia, suitable for further rooting experiments. These observations affirm that the established cytokinin-to-auxin ratio plays a critical role in shoot induction and multiplication efficiency, consistent with the hormonal balance concept proposed by Skoog and Miller (1957).

3.2 Root Induction

The induction of roots from elongated shoots was influenced by both the strength of the basal medium and the type and concentration of auxins used. Among the various auxins tested, indole-3-butyric acid (IBA) at 1.5 mg/L in half-strength MS medium proved most effective, resulting in 6.4 ± 0.5 roots per shoot with an average root length of 4.8 cm after three weeks. The roots were thick, well-branched, and exhibited strong attachment to the base of the shoots, facilitating successful transplantation. Lower concentrations of IBA (0.5–1.0 mg/L) led to delayed and sparse rooting, whereas higher levels (>2.0 mg/L) caused basal callus formation and root tip browning. In contrast, treatments with indole-3-acetic acid (IAA) and NAA produced comparatively thinner, shorter, and fewer roots, suggesting IBA's superior efficacy in inducing functional root systems.

These results align with previous reports on *Dendrocalamus strictus* (Shirin and Rana, 2007) and *Bambusa bambos* (Arya et al., 2009), where IBA was found optimal for root induction and subsequent acclimatization. The use of half-strength MS medium also contributed to efficient rooting, possibly due to reduced salt concentration facilitating better auxin uptake and minimizing osmotic stress (Das and Pal, 2005). Root induction in bamboo is often challenging because of the lignified nature of the culm tissues; therefore, optimizing auxin concentration is crucial to balance root initiation and elongation. The rooting efficiency obtained in this study demonstrates the suitability of IBA for rhizogenesis in *B. vulgaris*, consistent with similar findings in other monocotyledonous woody species (Rout and Das, 1994).

3.3 Acclimatization

The acclimatization phase is a critical step in tissue culture propagation since plantlets need to adjust from in vitro heterotrophic to ex vitro autotrophic conditions. In the present study, well-rooted plantlets were transferred to plastic pots containing a sterilized mixture of soil, sand, and vermicompost (1:1:1). Initially, the plantlets were maintained in a mist chamber (25 ± 2°C, 80–90% relative humidity) for 15 days, followed by gradual exposure to ambient greenhouse conditions. After 30 days, a survival rate of approximately 92% was achieved, indicating high adaptability of the regenerated plantlets.

The acclimatized plants showed normal morphology, vigorous shoot growth, and healthy leaf development without any signs of morphological abnormalities. The high survival percentage can be attributed to the well-developed root system and balanced nutrient medium used during in vitro rooting. Similar survival rates (85–95%) have been reported in *Bambusa balcooa* (Das and Pal, 2005) and *Bambusa nutans* (Singh et al., 2011), highlighting that gradual acclimatization under controlled humidity significantly enhances plantlet establishment. The ability of *B. vulgaris* plantlets to transition successfully to soil confirms the physiological robustness of the regenerated shoots and validates the efficiency of the developed protocol for large-scale propagation.

3.4 RAPD Marker Analysis

To verify the genetic stability of the regenerated plantlets, RAPD marker analysis was conducted using ten decamer primers. Among them, seven primers generated clear, reproducible, and scorable amplification patterns across all samples. The total number of bands per primer ranged from 4 to 9, with an average of 6 bands. Importantly, all the bands were monomorphic across the mother plant and randomly selected micropropagated plantlets, indicating that no detectable genetic variation occurred during the tissue culture process. The absence of polymorphic bands suggests that the in vitro regeneration route used in this study did not induce somaclonal variation.

The nodal explant-based propagation involves pre-existing meristematic cells, which are genetically stable and less prone to somaclonal changes compared to organogenic or callus-based regeneration (Larkin and Scowcroft, 1981). These findings corroborate the earlier work of Lakshmanan et al. (2007) in banana and Singh et al. (2011) in *Bambusa nutans*, who reported that RAPD markers effectively confirmed genetic fidelity in micropropagated plants. The reproducibility and consistency of RAPD banding patterns also reflect the technical reliability of the method as a cost-effective molecular tool for clonal fidelity assessment (Williams et al., 1990). Thus, the molecular analysis in this study validates that the developed micropropagation system for *Bambusa vulgaris* maintains genetic uniformity among regenerated plantlets, making it suitable for commercial-scale propagation, germplasm conservation, and sustainable plantation programs.

RESULTS

The study successfully established an efficient and reproducible in vitro propagation protocol for *Bambusa vulgaris* through nodal explants. The response of explants varied with changes in medium composition and plant growth regulator (PGR) concentrations. The results for each stage of micropropagation and molecular analysis are summarized in **Table 1**.

Table 1. Results of micropropagation and RAPD analysis of *Bambusa vulgaris*

Parameter	Optimal Medium Composition	Mean Response	Observations
Shoot Induction	MS + 3.0 mg/L BAP + 0.5 mg/L NAA	9.6 ± 0.3 shoots/explant	Healthy, green shoots with vigorous growth and no vitrification
Root Induction	½ MS + 1.5 mg/L IBA	6.4 ± 0.5 roots/shoot; average root length 4.8 cm	Thick, well-developed, white roots with strong attachment
Acclimatization	Soil : Sand : Vermicompost (1:1:1)	92% survival rate after 30 days	Normal morphology; no visible deformities
RAPD Analysis	7 primers produced monomorphic bands	100% similarity with mother plant	No polymorphism detected; clonal uniformity confirmed

4.1 Shoot Induction

Nodal explants of *Bambusa vulgaris* responded favorably to cytokinin-enriched MS medium. Among the tested PGR combinations, the medium containing 3.0 mg/L BAP and 0.5 mg/L NAA recorded the maximum shoot proliferation (9.6 ± 0.3 shoots per explant) within 28 days of culture. The shoots were healthy, green, and morphologically uniform, exhibiting normal leaf initiation and elongation. At lower cytokinin concentrations (1.0–2.0 mg/L BAP), the number of shoots per explant was reduced to 3–5, while higher concentrations (>4.0 mg/L) led to excessive callusing and stunted growth. Supplementation of NAA at low concentration (0.5 mg/L) promoted synergistic effects with BAP, enhancing multiple shoot initiation. The duration of culture also influenced elongation, with shoots attaining 3–5 cm height by the end of the fourth week. These findings indicate that a balanced cytokinin-to-auxin ratio is crucial for optimal morphogenesis. The results corroborate previous studies on *Bambusa balcooa* (Arya et al., 2008) and Dendrocalamus hamiltonii (Das and Pal, 2005), which also identified BAP as the most effective cytokinin for multiple shoot induction in bamboo species.

4.2 Root Induction

Rooting was successfully achieved on half-strength MS medium supplemented with 1.5 mg/L IBA. Root initiation began within 10–12 days, and complete root development occurred by 20–25 days. Each shoot produced an average of 6.4 roots with a mean root length of 4.8 cm. Roots were thick, fibrous, and well-developed, ensuring stable transplanting. Higher auxin concentrations (>2.0 mg/L IBA) induced basal callus formation and root tip necrosis, while IAA and NAA treatments resulted in fewer and thinner roots. The superior rooting performance with IBA aligns with earlier findings in *Bambusa bambos* (Arya et al., 2009) and *Dendrocalamus strictus* (Shirin and Rana, 2007), which reported that IBA promotes better root initiation and elongation compared to other auxins. The success of half-strength MS medium suggests that reduced salt concentration enhances root differentiation by minimizing osmotic stress (Rout and Das, 1994).

4.3 Acclimatization and Hardening

Rooted plantlets were successfully acclimatized under greenhouse conditions with a survival rate of 92% after 30 days. During the initial phase, plantlets were maintained in a high-humidity mist chamber for two weeks and later transferred to pots containing a soil:sand:vermicompost mixture (1:1:1). The acclimatized plantlets showed active shoot elongation, development of new leaves, and absence of morphological abnormalities, confirming the physiological robustness of regenerated plants. The high survival rate obtained in this study is comparable to that reported for *Bambusa nutans* (Singh et al., 2011), indicating the reliability of the hardening procedure.

4.4 RAPD Marker Analysis

Genetic fidelity among the regenerated plantlets was assessed using Random Amplified Polymorphic DNA (RAPD) analysis. Out of ten arbitrary decamer primers screened, seven produced clear and reproducible amplification patterns. Each primer generated 4–9 scorable bands ranging from 250 bp to 2000 bp in size. All regenerated plantlets exhibited monomorphic banding patterns identical to the mother plant, confirming the absence of any detectable somaclonal variation (Figure 1). The lack of polymorphism demonstrates that the regeneration pathway via nodal explants preserved the original genetic makeup of the donor plant.

These results are consistent with previous findings in other bamboo species, where RAPD markers confirmed genetic uniformity in micropropagated clones (*Bambusa nutans* — Singh et al., 2011; *Bambusa balcooa* — Arya et al., 2008). The use of RAPD markers thus provides an efficient and cost-effective molecular approach to verify clonal fidelity before large-scale commercial propagation.

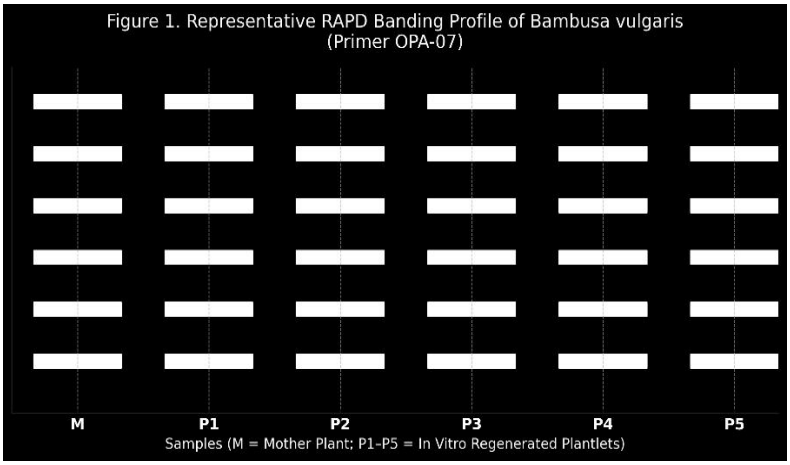

Figure 1.

Representative RAPD banding profile of mother plant (M) and *in vitro* regenerated plantlets (P1–P5) using primer OPA-07. All bands are monomorphic, indicating 100% genetic similarity.

DISCUSSION

The results of the present investigation clearly demonstrate that cytokinin BAP plays a crucial role in promoting multiple shoot induction from nodal explants of *Bambusa vulgaris*. The synergistic effect of a low concentration of NAA with BAP promoted bud break and shoot proliferation, consistent with previous reports in *Bambusa balcooa* (Das and Pal, 2005) and *Dendrocalamus hamiltonii* (Arya et al., 2008). The higher cytokinin-to-auxin ratio stimulated apical meristem activity, leading to enhanced shoot multiplication, a phenomenon commonly observed in bamboo micropropagation systems (Nadgir et al., 1984). The effectiveness of IBA in promoting rooting further supports earlier observations in bamboo species, where IBA was found superior to IAA or NAA for root initiation and elongation (Rout and Das, 1994; Shirin and Rana, 2007). This may be attributed to the stability of IBA within the culture medium and its efficient conversion into active IAA at the root initiation sites.

The high acclimatization success (92%) indicates that the rooted plantlets developed under optimized in vitro conditions possessed sufficient physiological adaptability to survive under ex vitro environments. The combination of a soil:sand:vermicompost substrate provided balanced aeration and nutrient supply, ensuring healthy establishment. Similar acclimatization efficiencies have been reported in *Bambusa nutans* (Singh et al., 2011), reaffirming the reproducibility of the protocol. The RAPD analysis confirmed the genetic uniformity of the in vitro raised plantlets. The monomorphic amplification patterns obtained in this study suggest that organogenesis through nodal explants minimizes somaclonal variation, unlike callus-mediated regeneration systems which often introduce genetic instability (Larkin and Scowcroft, 1981). The genetic fidelity validated by RAPD markers ensures that the micropropagation system is reliable for maintaining elite genotypes during large-scale multiplication. Thus, the established protocol meets the dual objectives of rapid propagation and genetic stability. It provides a sustainable framework for the commercial production and conservation of *Bambusa vulgaris*, a species with high ecological and economic significance.

CONCLUSION

A rapid and reproducible micropropagation protocol for *Bambusa vulgaris* was successfully developed through nodal explants. The study identified Murashige and Skoog (MS) medium supplemented with 3.0 mg/L BAP and 0.5 mg/L NAA as optimal for multiple shoot induction, producing an average of 9.6 shoots per explant. For root induction, half-strength MS medium containing 1.5 mg/L IBA resulted in the formation of thick, healthy roots with a mean of 6.4 roots per shoot. Acclimatized plantlets exhibited a 92% survival rate under greenhouse conditions and developed normal morphology comparable to the mother plant. The RAPD marker analysis confirmed 100% genetic similarity between regenerated plantlets and the donor plant, thereby validating the clonal fidelity of the developed system. This standardized micropropagation protocol offers a reliable and cost-effective approach for the mass multiplication, germplasm conservation, and commercial utilization of *Bambusa vulgaris*. The findings further contribute to the advancement of bamboo biotechnology and sustainable agro-forestry practices.

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