GMR
The Original

SOMATIC EMBRYOGENESIS: AN EFFICIENT ALTERNATIVE TO CONVENTIONAL TISSUE CULTURE (AXILLARY METHOD) METHOD IN *BAMBUSA BALCOOA*

Akanksha Lakra[a*], Kanushree Nandedkar[b], Pragati Baghel[a], Shikha Minj[c], Shailendra Pandey[a] and Zenu Jha[a*]

[a] Department of Molecular Biology and Biotechnology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India
[b] Department of Genetics and Plant Breeding, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India
[c] Naveen Govt. College, Raghunathnagar, Balrampur-Ramanujganj, Chhattisgarh, India
*Corresponding author- akanksha351997@gmail.com, jhazenu@gmail.com

Abstract
The present investigation was undertaken to develop and optimize an efficient somatic embryogenesis (SE) system for large-scale multiplication of *Bambusa balcooa* and to compare its regeneration efficiency and subsequent growth performance with the conventional tissue culture (axillary method) method. Newly emerged *in-vitro* shoots (10–14 days old) were used as explants for somatic embryogenesis, whereas newly emerged culms (3–4 months old) were utilized for conventional axillary propagation. Somatic embryogenesis was successfully induced using Murashige and Skoog (MS) medium supplemented with 3 mg L^{-1} 2,4-D, 2 mg L^{-1} kinetin and 250 mg L^{-1} polyvinylpyrrolidone (PVP). Embryogenic callus formation was observed at 28 days, followed by globular and heart-shaped embryo development and embryo germination at 52 days. The regenerated cultures were subsequently multiplied through liquid MS medium supplemented with 2 mg L^{-1} BAP. Comparative *in-vitro* monitoring revealed that somatic embryogenesis produced a maximum of 430 shoots, compared with 180 shoots through conventional tissue culture (axillary method) method. Earlier root development was observed in SE-derived plantlets (130 days) than in axillary-derived plantlets (145 days). Furthermore, 420 plantlets were obtained at the rooting stage through somatic embryogenesis compared with 170 through the axillary method. Following hardening, 416 plants survived through somatic embryogenesis, whereas 165 plants survived through conventional axillary propagation, representing approximately 2.5-times more plant production. Comparative evaluation of six-month-old hardened plants revealed no statistically significant differences ($p > 0.05$) between the two regeneration methods for any of the seven evaluated growth parameters, including plant height, number of culms, culm thickness, number of nodes, internodal length, leaf length and leaf width These results highlight the potential of the developed somatic embryogenesis method for large-scale commercial micropropagation and mass production of B. balcooa planting material by showing that it offers superior multiplication efficiency while maintaining comparable growth performance to conventionally regenerated plants.

Keywords: *Bambusa balcooa*, Somatic embryogenesis, Tissue culture, Embryogenic callus, *In-vitro* regeneration, Micropropagation

Introduction

Bamboo is an economically and ecologically important renewable forest resource, widely used in construction, furniture manufacturing, paper and pulp industries, handicrafts and other value-added products. Its rapid growth, high biomass production and adaptability make it valuable for sustainable forestry, agroforestry and environmental conservation. Among commercially important bamboo species, *Bambusa balcooa* Roxb. is a large-sized, thick-walled tropical bamboo known for its strong and durable culms. It has considerable industrial and commercial potential; however, its large-scale cultivation requires a continuous supply of healthy, uniform and quality planting material. Recent reviews have emphasized the increasing demand for B. balcooa planting material and the need to improve its propagation systems for sustainable utilization (Jeyachitra *et al*., 2025; Ahmad *et al*., 2025).

Bamboo can be propagated conventionally using seeds, rhizome division, offsets, and culm cuttings, but these methods are sometimes hampered by inconsistent flowering, a shortage of propagules, and comparatively low multiplication rates. An efficient substitute for quick multiplication under carefully monitored circumstances is in vitro micropropagation. Axillary shoot proliferation is one of the most popular methods since it makes use of pre-existing buds and can yield consistent planting material. Negi and Saxena (2011) developed an effective axillary shoot

proliferation micropropagation strategy for B. balcooa. The successful quick in vitro propagation of B. balcooa was recently reported by Waghmare et al. (2024), underscoring the significance of tissue culture in fulfilling the demand for bamboo planting material. However, increasing plant recovery and multiplication efficiency is still a key goal in commercial bamboo micropropagation.

Somatic embryogenesis (SE) is a promising alternative regeneration pathway in which somatic cells develop into embryo-like structures that subsequently germinate and form complete plants. The ability of embryogenic tissues to produce multiple embryos and undergo repeated multiplication offers considerable potential for large-scale clonal propagation. Gillis *et al*. (2007) demonstrated the potential of somatic embryogenesis for mass production of B. balcooa and reported that regenerated plants were morphologically comparable to those obtained through axillary branching. Successful somatic embryogenesis and genetic fidelity assessment have also been reported in Bambusa nutans by Rajput *et al*. (2021). Recent reviews further highlight the potential of SE-based approaches for improving bamboo multiplication and commercial planting material production (Jha *et al*., 2026).

Although both axillary shoot proliferation and somatic embryogenesis have been employed for bamboo regeneration, their comparative performance in terms of shoot multiplication, rooting, hardening and overall plant recovery requires further evaluation. In addition, assessing the morphological characteristics of regenerated plants is important to determine whether increased multiplication through SE is accompanied by detectable changes in subsequent plant growth. Therefore, the present investigation aimed to compare somatic embryogenesis and conventional axillary shoot proliferation in *B. balcooa* with respect to in vitro multiplication, regeneration, rooting response, hardening efficiency and total plant production. Furthermore, six-month-old hardened plants derived from both methods were evaluated for selected morphological growth characteristics to assess their comparative *ex vitro* performance and determine the potential of SE for large-scale production of quality planting material.

Materials and Methods

Experimental location

The present investigation was carried out at Plant Tissue Culture Lab., R.H. Richharia labouratory, College of Agriculture, Raipur with the objective of

Types of Explants

Healthy, disease-free mother plants of 8–10 years of age were selected as the source of explants. Newly emerged culms (3–4 months old) were chosen for conventional axillary production method because of their juvenile nature and high regenerative potential.

Conversely, newly emerged *in vitro* shoots (10-14 days) derived from cultured nodal explants were chosen as explant source for production through somatic embryogenesis method.

Culture conditions

The pH of culture medium was maintained with 5.8. The cultures were incubated in the culture room maintained at 25 ± 2°C, 70–80% relative humidity and 16 h light/8 h dark photoperiod (1000–4000 lux).

Media composition for conventional axillary method's establishment

The trimmed sterilized nodal segments (1.5-2 cm) were inoculated vertically onto culture bottles containing approximately 40 mL of Murashige and Skoog (MS) basal medium supplemented with 3 mgL^{-1} BAP, 30gL^{-1} sucrose, 6gL^{-1} agar and 50 μLL^{-1} PPM (Plant Preservative Mixture). Days to explant responses after inoculation were noted in the record.

After 12–14 days, the cultures were subcultured onto fresh medium to MS basal medium supplemented with 2 mgL^{-1} BAP, 30gL^{-1} sucrose, 6gL^{-1} agar, and 30 μLL^{-1} PPM to promote healthy shoot proliferation. A total of six subcultures were performed at intervals of 12–15 days on the same media composition, and the number of shoots derived from each subculture. After 30 days, the healthy regenerated plantlets were placed in polybags filled with sand:soil:cocopeat (1:2:2).*Media composition for the establishment of somatic embryogenesis method*

The outer 3–4 protective sheaths of *invitro* initiated shoots were carefully removed and the exposed initiated shoots were cut into small segments then inoculated horizontally onto petri plates containing MS medium supplemented with sucrose 30gL^{-1}, gelrite 3gL^{-1}, 2,4-D 3mgL^{1}, Kn 2mgL^{-1} and PVP 250mgL^{-1}. The cultures were initially incubated in darkness for two weeks and then subcultured to MS medium supplemented with sucrose 30gL^{-1} , BAP 3 mgL^{-1}, NAA 1.5 mgL^{-1}, gelrite 3gL^{-1} and 250mgL^{-1} PVP to promote somatic embryo maturation and germination and observations were recorded on average number of days taken for embryos germinated.

Healthy germinated embryos were transferred aseptically into liquid suspension medium containing MS medium supplemented with sucrose 30gL^{-1} and BAP 2$mgL^{-1}$for multiplication. Total six subcultures were done and observations were recorded on the average number of days required and average number of plantlets developed through SE. Due of their inadequate root development, some germinated embryos were transferred to semi-solid medium containing MS supplemented with sucrose 30gL^{-1} and NAA 3mgL^{-1} for proper root growth and development and after 30 days well-rooted plantlets regenerated through somatic embryogenesis were carefully removed for hardening. Hardening was done similar to conventional axillary method and then observations were recorded on average number of days required for plantlets development and average number of plantlets developed.

Comparative evaluation of growth characteristics of somatic embryogenesis- and conventional tissue culture (axillary method) regenerants

To evaluate the growth characteristics and performance of plants regenerated through somatic embryogenesis, a comparative study was conducted using regenerants obtained through somatic embryogenesis (SE) and conventional axillary shoot proliferation methods. For each regeneration method, 10 replications were maintained. Under *in vitro* conditions, observations were recorded from the initial inoculation of explants until the development of fully regenerated plantlets. The parameters associated with different stages of plant regeneration and development were monitored and recorded for both methods.

Following hardening, six-month-old plants regenerated through both methods were maintained under greenhouse conditions for comparative growth assessment. The observations included plant height (cm), number of culms per plant, culm thickness (mm), number of nodes per culm, internodal length (cm), leaf length (cm), and leaf width (cm). All growth parameters were measured and recorded systematically to assess differences in the growth performance of somatic embryogenesis-derived and axillary-derived plants. The recorded data were statistically analysed using an independent samples t-test to determine the significance of differences between the two regeneration methods.

Results

Comparative analysis of the growth traits of standard tissue culture (axillary method) and somatic embryogenesis regenerants

Comparative *in vitro* monitoring using somatic embryogenesis and conventional tissue culture (axillary method) method showed clear variations in *B. balcooa* growth, multiplication efficiency and regeneration capability. Within 12 days of nodal explant inoculation, bud induction began in both approaches, suggesting a comparable early response. But there were significant differences between the two approaches in the latter phases of development.

Shoot multiplication was started directly from induced buds in the traditional axillary way, yielding around 5 shoots at 28 days. Over the course of the subsequent multiplication cycles at 42, 56, 68, 80, and 90 days, respectively, the number of shoots rose to 10, 30, 60, 120, and 180. The somatic embryogenesis approach, on the other hand, required an extra callus induction and embryogenic development stage. At 28 days, or 16 days following shoot inoculation onto callus-inducing media, callus induction from started shoots was noted. After around 20 to 25 days, globular embryos were seen, and then heart-shaped embryos began to form. At 52 days, embryo germination was seen, and each embryogenic callus (EC) produced four to five shoots.

In terms of subsequent multiplication, somatic embryogenesis-derived cultures generated around 10–12 shoots at 60 days in the first multiplication cycle. This number rose to 20, 65, 170, 240, and 430 shoots at 68, 76, 84, 92, and 100 days in the succeeding cycles. Thus, compared to the traditional axillary approach, the somatic embryogenesis method showed a significantly larger capacity for shoot multiplication. In the somatic embryogenesis approach, each cluster produced around 20–25 new shoots during successive subculturing, while in the standard axillary method, each cluster produced 10–15 new shoots.

In contrast to axillary-derived plantlets (145 days), somatic embryogenesis-derived plantlets showed root initiation sooner (130 days). Interestingly, rooted in the somatic embryogenesis procedure was place without the use of plant growth regulators (PPM, according to the experimental record). At this point, the axillary technique produced 170 plantlets, whereas somatic embryogenesis produced 420 plantlets.

The plantlets were moved to the hardening stage after they had successfully undergone in vitro regeneration and rooted. The axillary-derived plantlets took 210 days to complete the process, whereas the somatic embryogenesis-derived plantlets finished in about 195 days. Plants were kept in net-house and shade-house settings for around 1.5 months during the hardening process. Individual plantlets were then placed in polybags with one plantlet per bag, and after 20 to 25 days, two to three additional shoots or plants were seen. Compared to 165 plants using the conventional axillary approach, a total of 416 plants survived using the somatic embryogenesis method.

In comparison to the conventional axillary approach (165 plants), the somatic embryogenesis method produced around 2.5 times more hardened plants (416 plants) due to its overall greater regeneration and multiplication efficiency. Additionally, it promoted early root formation, increased shoot proliferation, and a shorter total regeneration time. These results show that somatic embryogenesis is a viable and effective method for commercial micropropagation and large-scale *B. balcooa* multiplication.

Table 1: *In-vitro* comparative monitoring of conventional tissue culture (axillary method) and somatic embryogenesis method.

Stages	**Axillary method**		**Somatic Embryogenesis (10 replications)**		**Remarks**
	Days (Average)	**Observations (Average)**	**Days (Average)**	**Observations (Average)**	

Inoculation	0	Inoculation of node	0	Inoculation of node	
	12	Bud induction	12	Bud induction	
			28	Callus induction from the initiated shoots	*16 days from the inoculation of shoot segments in callus inducing media. *Globular shaped (20-25 days) and heart shaped embryo were observed.
			52	Embryo germination	*4-5 shoot induces from each EC.
Multiplication I	28	5 shoots	60	10-12 shoots	20-25 new shoots develop from each cluster in each subculturing in Somatic Embryogenesis (SE) method. *10-15 new shoots develop from each cluster in each subculturing in Axillary conventional (AC) method.
Multiplication II	42	10 shoots	68	20 shoots	
Multiplication III	56	30 shoots	76	65 shoots	
Multiplication IV	68	60 shoots	84	170 shoots	
Multiplication V	80	120 shoots	92	240 shoots	
Multiplication VI	90	180 shoots	100	430 shoots	
Rooting	145	170 plantlets	130	420 plantlets	* Roots develop earlier in SE method (no ppm was used)
Hardening	210	165 Plants survived	195	416 Plants survived	*For Hardening Plants were kept for 1.5 months in Net and Shade house for 20-25 days that time 2-3 new plants grown in polybag (having 1 plantlet).

Comparative Evaluation of Growth Performance of Somatic Embryogenesis- and Axillary-Derived Plants under Greenhouse Conditions

When six-month-old hardened plants were examined, somatic embryogenesis (SE)-derived plants showed somewhat higher mean values for plant height (43.31 cm), culm thickness (2.253), internode length (4.73 cm), and leaf width (2.10) than axillary-derived plants. On the other hand, axillary-derived plants had somewhat higher mean values for leaf length (10.51) and number of nodes (5.1), but the mean number of culms (2.7) was the same for both approaches. Nevertheless, for all seven growth characteristics during ten replications, the independent samples t-test did not show any statistically significant differences ($p > 0.05$) between the two regeneration techniques. These findings indicate that somatic embryogenesis-derived plants are suitable for creating morphologically consistent planting material of Bambusa balcooa, since they retained similar ex-vitro growth performance to conventionally regenerated axillary plants.

Parameters	**Method**	**Mean**	**t-value**	**pvalue**	**N/ NS**
Plant height	Axillary	42.21	-1.60	0.07	NS
	SE	43.31			
Number of culms	Axillary	2.7	0	1	NS
	SE	2.7			
Culm thickness	Axillary	2.166	-0.48	0.63	NS
	SE	2.253			

Number of nodes	Axillary	5.1	0.24	0.80	NS
	SE	5			
Length of internode	Axillary	4.54	-2.98	0.07	NS
	SE	4.73			
Leaf length	Axillary	10.51	0.98	0.33	NS
	SE	10.23			
Leaf width	Axillary	2.09	-0.26	0.79	NS
	SE	2.1			

*No significant differences (NS), ($p > 0.05$) between axillary propagation and somatic embryogenesis for all evaluated growth parameters

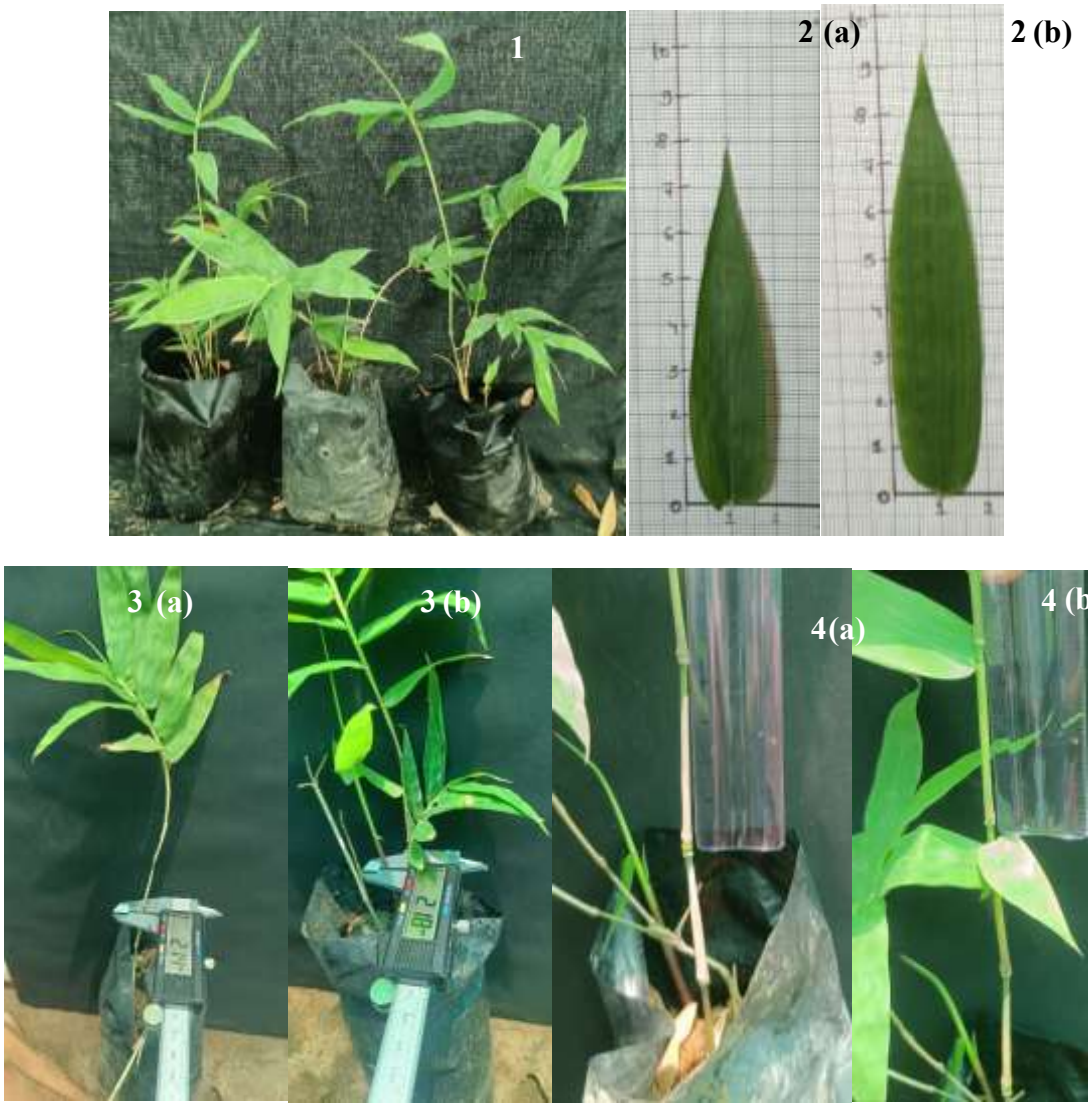


Fig.1: Regenerants obtained from micropropagation technique. (1) 6 months old regenerants obtained from somatic embryogenesis method; 2. Leaf length and width of (a) regenerants of conventional tissue culture-axillary method and (b) regenerants of somatic embryogenesis; 3. Culm thickness of (a) regenerants of conventional tissue culture-axillary method and (b) regenerants of somatic embryogenesis; 4. Length of internodal of (a) regenerants of conventional tissue culture-axillary method and (b) regenerants of somatic embryogenesis.

Discussion

The present study demonstrated that somatic embryogenesis (SE) was more efficient than conventional axillary shoot proliferation for the large-scale multiplication of *Bambusa balcooa*. Although bud induction occurred within 12 days in both methods, their subsequent developmental pathways and multiplication capacities differed considerably. Axillary propagation proceeded through direct shoot proliferation, whereas SE involved additional callus induction and embryogenic development before shoot regeneration. Despite this additional developmental phase, SE produced

substantially more shoots and hardened plants within a shorter overall regeneration period. This distinction is important because the advantage of SE in the present investigation was not faster initial bud induction, but its greater multiplication capacity and higher final plant recovery. Recent reviews have emphasized the importance of developing efficient *in vitro* propagation systems to meet the increasing demand for quality bamboo planting material.

In the axillary method, shoot production increased progressively from approximately 5 shoots at 28 days to 180 shoots at 90 days. In comparison, SE-derived cultures produced 10–12 shoots at 60 days and increased to 430 shoots by 100 days. Thus, the final recorded shoot production through SE was approximately 2.4 times that of the axillary method (430 versus 180). The higher multiplication response may be associated with the formation of embryogenic callus, which provides a pathway for the development of multiple embryos and subsequently several shoots from embryogenic tissue. The observation that each embryogenic callus produced approximately four to five shoots, followed by repeated multiplication during subculturing, supports the usefulness of this pathway for mass propagation. (Salaun *et al*., 2021) Somatic embryogenesis is widely recognized as an effective regeneration system for clonal propagation because embryogenic tissues can undergo proliferation and produce multiple regenerated plantlets (Sivanesan *et al.,* 2022).

The greater multiplication response observed in the present study is consistent with recent research on bamboo micropropagation. Rajput *et al.* (2020) developed a large-scale clonal propagation protocol for *B. balcooa* and reported approximately 62 shoots per node per culture bottle under their optimized multiplication conditions. Waghmare et al. (2021) also established an in vitro propagation protocol for *B. balcooa* and reported successful shoot multiplication and 90% survival after secondary hardening. More recently, Shirin *et al*. (2026) demonstrated that genotype and plant growth regulator combinations significantly influenced shoot multiplication and rooting in *B. balcooa*. These studies confirm that shoot multiplication and plant recovery are important determinants of efficient commercial bamboo propagation.

The advantage of SE was also evident during the rooting and plant recovery stages. Root initiation occurred at 130 days in SE-derived cultures, compared with 145 days in axillary-derived cultures. At this stage, 420 rooted plantlets were obtained through SE, whereas the axillary method produced 170 plantlets. The greater number of rooted plantlets indicates that the multiplication advantage achieved during earlier culture stages was retained during plantlet regeneration. The earlier root initiation may also have contributed to the shorter time required to complete the regeneration process. However, the difference in rooting time should be interpreted in the context of the respective culture protocols, since the two methods followed different developmental pathways and subculture schedules. Shirin *et al*. (2026) also demonstrated that rooting response in *B. balcooa* was strongly influenced by auxin combinations and genotype, highlighting the importance of optimizing the rooting stage for successful plant recovery.

The hardening results further confirmed the higher plant recovery efficiency of the SE method. A total of 416 SE-derived plants survived hardening, compared with 165 plants obtained through conventional axillary propagation. Thus, SE produced approximately 2.52 times more hardened plants than the axillary method. In addition, the SE-derived plants completed the reported regeneration and hardening process in approximately 195 days, compared with 210 days for axillary-derived plants. These findings indicate that the additional callus and embryo-development stages did not prevent successful acclimatization under the conditions used in this study. Waghmare *et al*. (2021) reported 90% survival of micropropagated B. balcooa after secondary hardening, while Shirin et al. (2026) reported 100% acclimatization under their optimized substrate conditions. Grzelak *et al*. (2024) emphasized that acclimatization is a critical stage of micropropagation, particularly in woody plants, because regenerated plantlets must adapt to changes in humidity, light intensity and environmental conditions.

The greater capability of somatic embryogenesis for mass propagation is likewise compatible with the increased plant recovery seen by SE. Based on ISSR analysis, Rajput et al. (2021) effectively regenerated Bambusa nutans via somatic embryogenesis and showed the value of this method for creating plantlets with high genetic fidelity. The possibility of in vitro somatic embryogenesis for bamboo mass production was also reviewed by Jha et al. (2026), who emphasised its significance as a substitute for traditional vegetative propagation. When taken as a whole, these studies demonstrate the value of SE-based strategies for expanding the supply of bamboo planting material.Evaluation of six-month-old hardened plants revealed no statistically significant differences ($p > 0.05$) between SE- and axillary-derived plants for any of the seven morphological parameters examined. SE-derived plants showed slightly higher mean values for plant height (43.31 cm), culm thickness (2.253 mm), internode length (4.73 cm) and leaf width (2.10 cm), whereas axillary-derived plants showed marginally higher values for leaf length (10.51 cm) and number of nodes (5.1). Both propagation methods produced an equal mean number of 2.7 culms per plant. These findings indicate that, under the experimental conditions, the higher multiplication capacity of SE was not accompanied by a detectable reduction in the measured vegetative growth characteristics of regenerated plants.

Comparable findings have been reported by Rajput *et al*. (2020), who developed a large-scale clonal propagation protocol for B. balcooa and reported no detectable genetic or morphological variation in micropropagated plants. Their findings provide direct support for the maintenance of morphological uniformity in tissue-culture-derived B. balcooa. Similarly, Khare *et al*. (2021) reported genetic fidelity in micropropagated Dendrocalamus strictus plantlets using ISSR markers. Rajput *et al.* (2021) also demonstrated high genetic fidelity in SE-derived B. nutans plantlets using

ISSR analysis. These findings support the importance of evaluating both morphological performance and molecular stability in regenerated bamboo plants.

Recent studies on other woody plants also emphasize the importance of assessing regenerated plants after transfer to ex vitro conditions. Grzelak *et al*. (2024) discussed the morphological, anatomical and physiological changes that can occur during acclimatization of micropropagated woody plants, highlighting the need to assess their subsequent establishment and growth. In addition, the review by Jeyachitra *et al*. (2025) specifically emphasized the importance of efficient micropropagation systems for producing quality planting material of B. balcooa. The comparable growth characteristics observed in the present investigation therefore provide useful evidence that SE-derived plants can maintain satisfactory early ex vitro growth while offering substantially higher multiplication potential.

Taken together, the present results demonstrate that somatic embryogenesis is a promising alternative to conventional axillary shoot proliferation for the large-scale multiplication of *B. balcooa*. The production of approximately 2.4 times more shoots and 2.5 times more hardened plants, along with earlier rooting and a shorter overall regeneration period, highlights its multiplication advantage. Furthermore, the absence of statistically significant differences in the seven measured growth parameters indicates comparable early ex vitro growth performance between the two regeneration methods. Therefore, the SE protocol developed in this study offers considerable potential for the commercial production of quality *B. balcooa* planting material.