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FULL LENGTH ARTICLE

Optimization of planting materials for large scale plantation of *Bambusa balcooa* Roxb.: Influence of propagation methods

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Abstract One of the key insufficiencies of the reports on *in vitro* propagation of tree species is that the field performances of the *in vitro* regenerants are not reported in majority of the studies. Although, there are various reports on *in vitro* propagation of *Bambusa balcooa*, no report exists on field growth of *in vitro* regenerants. In the present study we investigated the performance of propagules derived from different *in vitro* and *in vivo* propagation methods. *B. balcooa* Roxb. was propagated through nodal cutting, rhizome splitting and *in vitro* multiple shoot culture and the performance of plantlets was assessed under field condition for two consecutive years. *In vivo* propagation through culm cutting was optimized using coarse sand over soil, soil plus sand (1:1; *v*/*v*) or vermiculite with >95% survival and 8.2-fold multiplication. *In vitro* multiple shoot proliferation from nodal segments was achieved on Murashige and Skoog (MS) medium supplemented with 4 mg l^{-1} N_6-benzylaminopurine and shoots were successfully rooted on MS plus 1 mg l^{-1} indole-3-butyric acid followed by 100% acclimatization on farmyard manure, soil and sand @ 1:1:1 (*v*/*v*) mixture. In the field condition *in vitro* derived plantlets performed better than the plantlets propagated through nodal cutting or rhizome splitting. Considering the consistent two-year field performance based on plant height, culm characters and internode length it can be concluded

Abbreviations: BAP, N_6-benzylaminopurine; IAA, Indole-3-acetic acid; IBA, Indole-3-butyric acid; Kinetin, 6-Furfurylaminopurine; MS, Murashige and Skoog (1962)

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that *in vitro* propagation method would be a better choice instead of *in vivo* nodal cutting or rhizome splitting techniques for large scale plantation of *B. balcooa* Roxb.

1. Introduction

Many South-East Asian countries, India as well, consider bamboo as an essential component of rural economy (TIFAC 2009). Because of the accelerated growth pattern with short developmental phase, bamboo attains an important position in communal agroforestry programs. With the aim to mitigate environmental complications in the form of controlling soil erosion and CO_2 removal, bamboo proves to be an imperative economic resource owing to its typical genetic configuration and ecological significance (Zhou et al., 2005). In the Indian subcontinent *Bambusa balcooa* Roxb., belonging to the family Bambusoideae, stands as an important multipurpose species of bamboo. The dull-green culms of this species are 12–23 m tall, with 18–25 cm circumference and widely scatters up to an altitude of about 600 m in several distinct regions having tropical monsoon, such in Bangladesh, Nepal together with North-East India, tropics in Asia and Africa (Stapleton, 1994; Ohrnberger, 1999). The *B. balcooa* species stands as the best species among the others of genus *Bamboosa* with its thickest and largest exceedingly prized robust culms widely used in construction of houses, other frameworks, basket and mat making etc. Besides, the edible bitter tender shoots of this species, a rich source of phytosterol, serve an imperative source of food and pickle industry as well as a raw material for paper pulp (Tewari, 1992; Ohrnberger, 1999; Sarangthem and Singh, 2003). Nonetheless, with the escalating establishment of bamboo-related trade the agroforestry stocks of bamboo plantation face major loss. In this scenario, sufficient afforestation through replanting of leading bamboo species would improve the stock as well as ensuring the sustainable supply of raw materials to the industries.

Usually the production seed does not ensue following the gregarious flowering in *B. balcooa*. Furthermore, the only succession of flowering, following which the clump perishes exclusive of seed setting, is testified to be as long as 55–60 years (Tewari, 1992). As a result, it can merely be proliferated via alternative asexual methods, for instance, using offsets, culm cuttings, branch cuttings, or rhizomes. Nevertheless, these ways are challenging owing to insufficient and large planting materials, season dependency, desiccation sensitivity, slow and depleted ability in root formation from the culm and branch cuttings (Hassan, 1977; Seethalakshmi et al., 1983; Pattanaik et al., 2004). Hence, bulk number of propagules were made available through both *ex vitro* and *in vitro* adventitious morphogenesis from the axillary buds to meet the commercial requirement. Attempts have been made for *in vitro* propagation of *B. balcooa* using nodal segments (Das and Pal, 2005; Negi and Saxena, 2010, 2011), pseudo-spikelets (Gillis et al., 2007), and culms (Dutta Mudoi and Borthakur, 2009).

Even though some studies are associated with micropropagation of *B. balcooa*, yet each of them carries specific insufficiencies, particularly when it comes to comparing among different propagation methods based on the field performance of planting materials. Moreover, propagation via *in vitro* axillary branching is costly and consequently inapt aiming at supply of quality propagules in rural regions of Asia for agroforestry and silviculture. On the other hand, *B. balcooa*, propagated by culm cuttings, limits the shoot growth including root initiation even after two years of transplanting (Pattanaik et al., 2004). Consequently, it is exceedingly anticipated to establish efficient simple protocol-based approaches, to produce elite bamboo lines and propagules with diminished costs. In agroforestry system extended assessment on field performance of plants is essential to ascertain elite lines from large-scale plantation that is recognized to be challenging. Eventually, till date, no data have been available on prolonged and relative field performance of bamboos propagated via different methods. With this backdrop, our investigation testifies an up-scaling process for large-scale proliferation of this economically imperative bamboo species. Here, we describe the development of bulk number of *B. balcooa* plants by means of different efficient propagation protocols for instance, micropropagation via axillary shoot proliferation, macropropagation by rhizome splitting and nodal/culm cuttings. Furthermore, an attempt was also made to assess the comparative field performance based on morphogenetic competence of propagules obtained from tissue culture, rhizome splitting and nodal cutting of *B. balcooa* for up to two years of transfer.

2. Materials and methods

2.1. Propagation by culm cuttings *in vivo*

One-year old culms were collected, cutting them just above the first node, from healthy bamboo clumps. Collected culms were trimmed approximately to 30 cm and side branches were clipped without injuring the axillary buds. Further, the supple, slender terminal portions of culm carrying leaves with shoots were removed. Furrows were made at a depth of 10–15 cm with a space of 40–50 cm throughout the nursery beds. Culm cuttings were horizontally placed in furrows across the nursery beds and covered with a 3–5 cm layer of substrates in such a manner that the buds are placed laterally (Fig. 1a). The substrates being used were coarse sand, soil, sand plus soil (1:1; *v*/*v*), and vermiculite (Grade IV). Raised nursery beds of 5 m × 1.5 m size were prepared by profound digging and filled with substrates. The beds were drenched separately with Bavistin® (20 l of 0.05% (a.i.) readied with addition of 1 g Bavistin 50 WP l^{-1} water) one week prior to planting. Subsequent sprouting, rhizome development and new shoot initiation occurred on each substrate. After 90 days, well-rooted cuttings could be excavated, overflowing the beds with water and loosening the soil. The cuttings budded and rooted from both nodes were detached by splitting them prudently at the midmost of each internode to develop two plantlets. The rooted plantlets were separated from their source and transferred to

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Figure 1 *In vivo* propagation of *Bambusa balcooa* Roxb. through culm cuttings. a. Placement of culm cuttings on substrates (*Bar*, 15 cm), emergence of plantlets from b. soil (*Bar*, 10 cm), c. coarse sand (*Bar*, 10 cm), d. sand-soil mixture (1:1; *v*/*v*) (*Bar*, 10 cm), e. vermiculite (*Bar*, 10 cm), f. shoot and root formation from nodes (*Bar*, 10 cm), g. isolation of plantlet from culm explants (*Bar*, 10 cm), h. transferred plantlets in polythene packs containing a balanced blend of sand and soil and FYM (1:1:1; *v*/*v*/*v*) (*Bar*, 10 cm).

polypackets (15 cm in diameter) filled with sand, soil and farmyard manure (FYM) (1:1:1; *v*/*v*/*v*) for further growth and acclimatization. These were watered two times daily and maintained in shadow for about a month before they could be transferred to the field. However, following 3–4 months of propagation, plants developed from the most favorable substrate were used for the field assessment experiment.

2.2. Propagation by rhizome splitting in vivo (macropropagation)

During the acclimatization process, the *in vivo* formation of rhizomes was observed after an approximate 90 or more days of growth during which the tissue culture raised saplings were maintained in poly-buckets (10 cm diameter). Each plantlet was again multiplied into two/three by splitting of *de novo* formed rhizomes (Fig. 2a) and then grown in a balanced blend of sand and soil and FYM (1:1:1; *v*/*v*/*v*) as mentioned earlier, with ensured water supply, devoid of greenhouse condition followed by the method of Banerjee et al. (2011). Finally, 180 days old plants were used for the field experiment.

2.3. Propagation via in vitro nodal culture

2.3.1. Initiation of aseptic cultures with multiple shoot

Nodal segments (2.5–3 cm) were harvested from profusely growing culms of a natural *B. balcooa* stands (around 10 years old with profuse shoot growth) growing at the greenhouse premises of West Bengal State Council of Science and Technology, Salt Lake City, Kolkata, India. Initial collections of nodal segments as explant material were accomplished throughout March. Leaf sheaths covering upper internodes and the axillary buds were carefully detached without injuring the underlying bud. The explants were washed in 75% ethanol for 15 s, followed by treating the same in 0.1% (*w*/*v*) mercuric chloride for 6–8 min depending on the thickness of the explant. After three further washes with sterile distilled water, the exposed terminals of the segments were cropped and cultured

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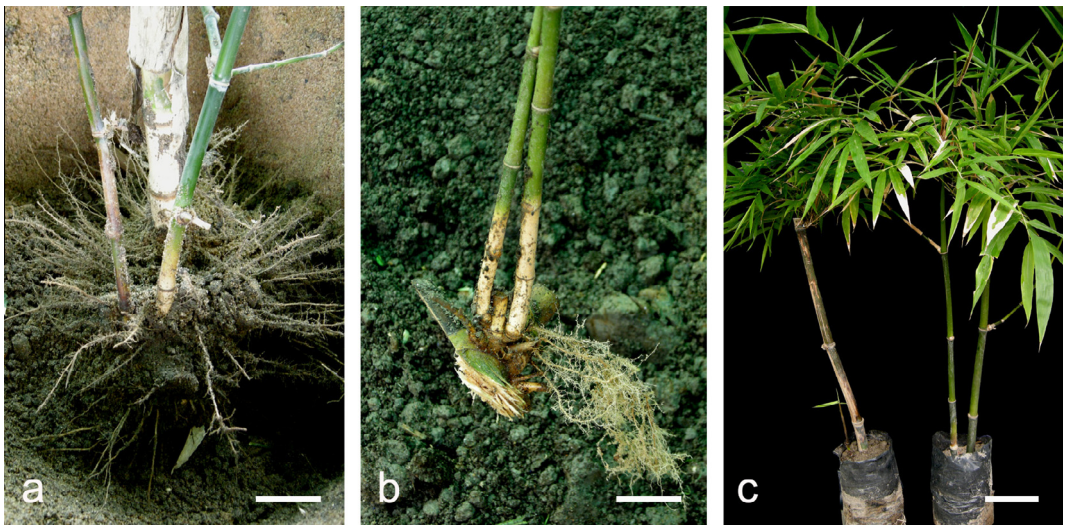

Figure 2 *In vivo* propagation of *Bambusa balcooa* Roxb. via rhizome splitting (*Bars*, 10 cm).

on Murashige and Skoog (1962) (MS) basal medium fortified with different cytokinins (Sigma Chemicals, St. Louis, MO) *i.e.* 1, 2, 3, 4 or 5 mg l^{-1} either of N_6-benzylaminopurine (BAP) or 6-furfurylaminopurine (Kinetin), and 3% (*w*/*v*) sucrose, gelled with 0.8% (*w*/*v*) agar (HiMedia), and dispensed into 2.5 × 10 cm culture vessels (Borosil, Chennai, India). Before autoclaving the medium at 121 °C with 1 kg cm^{-2} for 15 min the pH of the same was amended to 5.8 either with 1 N NaOH (Qualigens, Mumbai, India) or 1 N HCl (Qualigens, Mumbai, India). Later, the inoculated cultures were maintained under a 16 h photoperiod with a of 60 µmol m^{-2} s^{-1} light intensity specified by cool, white fluorescent tubes (Phillips India Ltd., India) at a growth room temperature of 25 ± 2 °C. After axillary shoots were initiated, the explants were transferred for multiple shoot proliferation on to liquid MS medium (without agar) supplemented with the best performing cytokinin concentration during shoot initiation. Assessment of multiplication rates were carried out based on the number of shoots regenerated per inoculant. Rotten shootlets with necrosis were excluded from multiple shoot clumps prior to transferring on new root initiation media.

2.3.2. Rooting

Propagules with two/three shoots of 3–5 cm in length, derived following the multiplication phase were shifted to liquid MS media fortified with diverse auxins (SRL, India) namely, indole-3-acetic acid (IAA) or indole-3-butyric acid (IBA) individually, at a concentration of 1, 2 and 3 mg l^{-1}.

2.3.3. Acclimatization

Plantlets obtained through *in vitro* regeneration system were removed from culture vessels, and washed exhaustively under water flow to eradicate the left over media from roots. Primary hardening was achieved on net pots fitted within pro-trays containing sterile soil plus coco-peat (1:1; *v*/*v*). Following four weeks after primary hardening, plantlets were shifted to polythene packets (16 × 8 cm) each filled with the sand, soil and farmyard manure (1:1:1; *v*/*v*) for their secondary hardening. The plantlets were acclimatized in greenhouse maintaining 27 ± 1 °C temperature and 200 µmol m^{-2} s^{-1} light intensity for next four weeks. The plants were then shifted from shaded zone to an exposed nursery where they were retained for two months until their plantation in the field.

2.4. Field transplantation and growth

For each experiment, 100 well-acclimatized plants were transferred to the field, during the end of June at Gossairhat Forest beat, Moraghat Range of Jalpaiguri Division of West Bengal, India. Prior to field transference, pits (0.6 × 0.6 × 0.6 m) were dug and packed with 10 kg FYM per pit and 500 g of Neem oil cake initially and planted maintaining a gap of 3 × 3 m. Subsequently, N:P:K (20:10:10; *v*/*v*/*v*) was added as the only fertilizer dose at four month of growth phase to augment the *ex vitro* growth. Furthermore, Furadan 3G @ 10 g/plant was applied to prevent the contamination of soil-borne pathogen, at initial phase. The plant population was provided with an entirely unirrigated situation in field. Commencing from two-month growth phase, the survival rate was evaluated till two year. Various field growth attributes, for example number of culms/bush, length and circumference of culms were monitored and continued more than two years.

2.5. Statistical analysis

The experiments were carried out based on complete randomized design. Each of the experiments excluding field performance study was executed in five replicates having 20 samples per replication. For *in vitro* culture, every single explant was treated as an experimental unit. The percentage of survival was assessed from the number of plants revived out of 100, subjected to field growth. The composed data were subjected to one-way analysis of variance (ANOVA), and the effects of the different treatments during micropropagation along with influence of different planting materials (raised from *in vivo* propagation methods) were enumerated, and the significant differences among the treatments were tested by Tukey's test at $P = 0.05$ through SPSS (Version 11, SPSS

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Inc. Chicago, USA) software package. The percent data were transformed using arc sine before carrying out ANOVA and were then converted back to the original scale (Compton, 1994).

3. Result and discussion

3.1. Propagation by culm cuttings

The technique incorporated treatment of culm cuttings with plant growth regulators to induce roots. Large-scale multiplication of elite varieties is promising via this technique. Out of four substrates used in our study, soil resulted in earliest emergence of axillary shoot buds within ~9 days. On the other hand, soil plus sand or vermiculite took around or more than two weeks to initiate shoot buds from culms. Nonetheless, considering overall performance, coarse sand proved to be the most favorable (with 96.5% survival) substrate for propagation via culm cutting in comparison with the other three substrates employed in the present study (Table 1). Culm cuttings planted in soil transformed into well-developed plantlets as earliest as within ~90 days (Fig. 1b), whereas soil-sand mixture took ~104 days (Fig. 1d); however, soil born disease infestation was high in both of these substrates. The maximum of ~119 and ~124 days was required to attain optimum plantlet growth from coarse sand and vermiculite, respectively. In terms of root initiation, coarse sand proved to be the quickest presumably due to its highest aeration property than soil and vermiculite that resulted in root formation at 50–60 days of plantlet growth. Moreover, it is evident from the Table 1 that multiple shoot formation frequency was recorded highest (8.2 fold) from culm cutting raised under coarse sand (Fig. 1c). However, frequent water supply was necessary to retain the moisture content in case of coarse sand unlike the other three substrates where vermiculite showed best water retention capacity resulting in 4.2 fold multiple shoots (Fig. 1e).

3.2. Propagation by rhizome splitting

Production of new plants through rhizome is one of the conventional practices since long. However, the rate of propagation is very slow which was evident in the present study. Emergence of new shoots (two to three in number) were recorded from single rhizome (Fig. 2a and b) which then further split and raised as two well developed plantlets (Fig. 2c). However, due to limited availability of rhizomes this method is not effective for large-scale propagation.

3.3. In vitro propagation: axillary bud, multiple shoot and root development

Two sets of plant growth regulator (PGR) formulations were employed during establishment of *B. balcooa in vitro* organogenesis. BAP at five concentrations (1–5 mg l^{-1}) was tested in opposition to five similar levels (1–5 mg l^{-1}) of Kinetin (Fig. 3a). Following ~5 (4.6) days of inoculation, axillary bud break/shoot initiation was observed in 4 mg l^{-1} BAP (Fig. 3b). A highest rate of explants showed shoot multiplication in this media formulation (97.2%) wherein for each inoculated nodal segment explant 6.67 cm long ~11 shoots were observed (Table 2). Interestingly, as the cytokinin source BAP was detected as favorable one when compared to Kinetin for initiation and multiplication of shoots which supports the earlier reports on *B. balcooa* (Sharma and Sarma 2011) as well as other bamboo species such as *Gigantochloa atroviolaceae* (Bisht et al., 2010) and *Dendrocalamus asper* (Banerjee et al., 2011). In the present experiment, the higher level of BAP (more than 4 mg l^{-1}) resulted in decline of multiplication rate of shoots where it dropped down from 11.2 to 9.1. However, establishment of explant was best observed in semisolid medium while liquid MS media executed superior results (data not presented) than semisolid MS medium during proliferation of shoots and rooting of shoots. More than 11 well grown multiple shoots per explant were recorded following 40 days of axillary bud inoculation (Fig. 3c).

Regenerated clump of shoots were separated, each having 2–3 shootlets, and transferred to the rooting media. Within ~11 days after transfer, first root initiation was recorded on liquid MS medium complemented with 1 mg l^{-1} IBA wherein highest rooting frequency of 94.1% was obtained. Around 4 (4.4) roots with average root length of 4.2 cm (Table 3) per inoculated shoot were scored after 30 days of culture (Fig. 3d). It is worth mentioning that frequency of spontaneous rooting of the shoots observed was less in this species in comparison with other species of bamboo.

In our study, semisolid MS medium with optimized PGR level for culture establishment, liquid MS medium for multiplication shoot and initiation of roots was apposite to standardize the protocol for effective large-scale propagation *in vitro*. The current procedure comprising the switch of MS medium from semisolid to liquid state proved to be effectual for *in vitro* organogenesis which endorse the former statement of Negi and Saxena (2011) on micropropagation of *B. balcooa* commencing with nodal explants. In the present study, the lone use BAP enthused the shoots development and proliferation whereas the collective manifestation of multiple cytokinins

Table 1 Comparative assessment of different substrates during propagation through culm cutting of *Bambusa balcooa* Roxb.

Substrate	Days taken to bud emergence	Days taken to complete plantlet formation	Survival (%)	Days to root	Multiplication rate
Coarse sand	12.2 ± 0.4c	119.6 ± 2.9a	95.8 ± 0.6a	41.8 ± 1.2c	8.2 ± 0.7a
Soil	9.2 ± 0.3d	89.6 ± 1.4c	86.9 ± 0.9c	53.0 ± 1.4b	3.4 ± 0.5c
Soil + sand (1:1)	14.6 ± 0.5b	104.6 ± 2.4b	91.2 ± 0.7b	44.6 ± 0.8c	6.2 ± 0.6ab
Vermiculite (Gr. IV)	18.5 ± 0.7a	124.8 ± 2.1a	84.4 ± 0.9c	62.6 ± 1.6a	4.2 ± 0.4bc

Data represent (mean ± standard error) 20 samples per treatment in five replicated experiments. Mean values with the same *letter* are not significant at $P = 0.05$ based on one-way analysis of variance (ANOVA) followed by Tukey's test.

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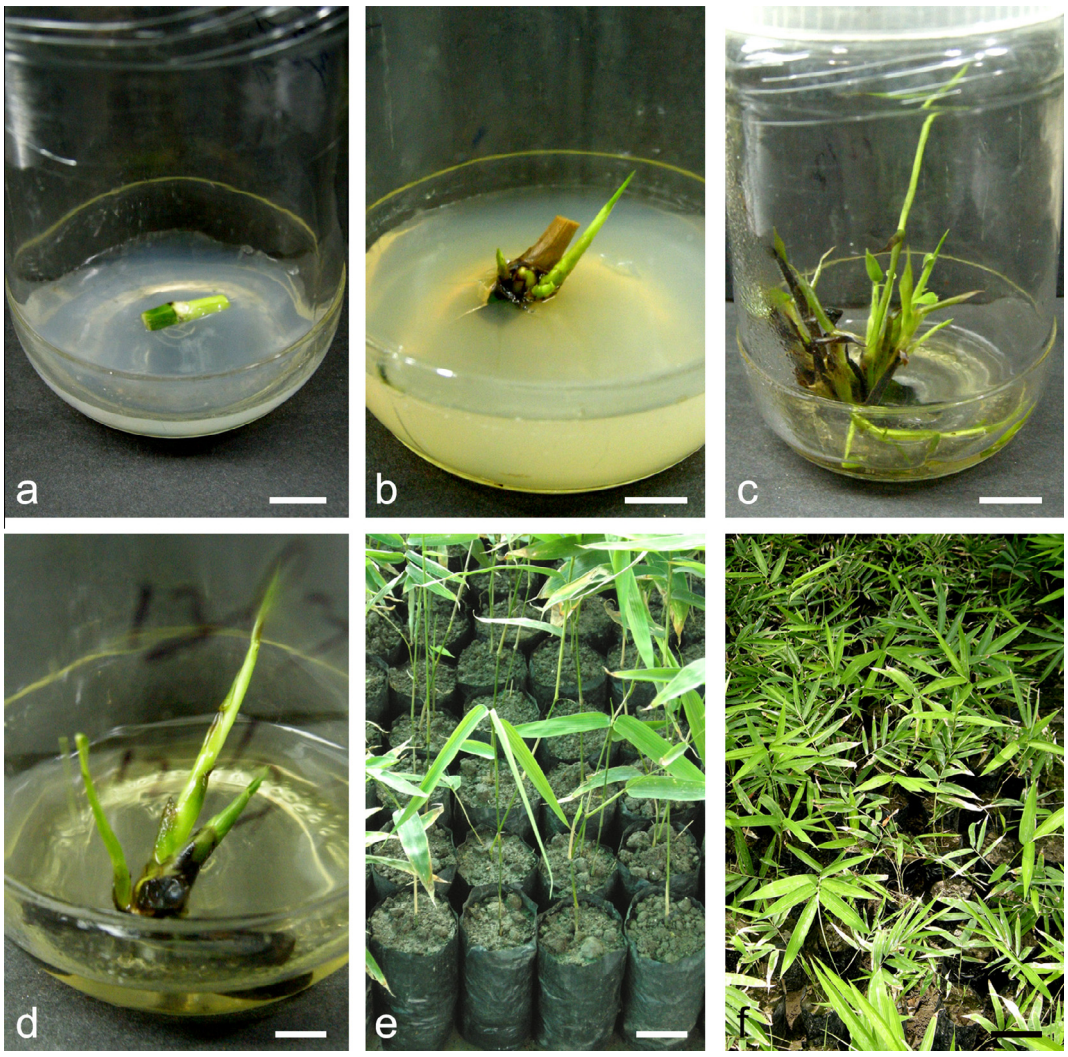

Figure 3 *In vitro* propagation of *Bambusa balcooa* Roxb. using nodal segment explants. (a) Inoculation of explant in MS semisolid media with variable levels of BAP or Kinetin (*Bar*, 1.5 cm), (b) initiation of axillary buds in MS plus 4 mg l^{-1} BAP (*Bar*, 1 cm), (c) proliferation of multiple shoots in liquid MS plus 4 mg l^{-1} BAP (*Bar*, 1.5 cm), (d) rooting of multiple shoots in liquid MS fortified with 1 mg l^{-1} IBA (*Bar*, 1.5 cm), (e) primary acclimatization of *in vitro* regenerated plantlets in potting mixtures containing cocopeat and soil (1:1; *v*/*v*) under green house (*Bar*, 5 cm), and (f) secondary acclimatization of plantlets in sand, soil and farmyard manure (1:1:1; *v*/*v*) under poly house (*Bar*, 10 cm).

(BAP and Kinetin) and their synergistic effect was essential for multiple shoot proliferation in *B. balcooa* as reported by earlier studies (Das and Pal, 2005). Despite the fact that, impulsive root formation was recorded during multiple shoot proliferation in way of plantlet development *in vitro*; nevertheless, the use of apposite PGR shortened the root formation span necessarily and commencement of rooting was achieved quickly. In correspondence to our study, a similar report on promotional influence of IBA on *in vitro* root formation was published earlier on *B. balcooa* (Das and Pal, 2005). The influence of IAA and IBA was examined on rooting, wherein performance-wise IBA was a superior choice over IAA. The present result is in sustenance to the previous reports of Saxena and Bhojwani (1993) and recently Banerjee et al. (2011) in other genus of bamboo (*Dendrocalamus longispathus* and *D. asper*, respectively).

3.4. Acclimatization

Sterile cocopeat was observed to provide optimal anchorage to the saplings and accelerated initiation of fresh roots during initial stage of acclimatization (Fig. 3e) in two weeks. Supply of water in mist form and covering of translucent polyethylene sheet retained favorable moisture besides raising the temperature that markedly influenced the formation of new roots and boosted the acclimatization process. However, altering the acclimatization substrates in the form of sand, soil and FYM (1:1:1; *v*/*v*) during the subsequent four weeks resulted in 100% acclimatized plantlets (Fig. 3f). Maintenance of humidity was an important factor of this successful acclimatization (Banerjee et al., 2011). Apart from intermittent water spraying, coverage with polyethylene sheet (Thomas and Ravindra, 1997) along with the usage of FYM during the final phase of

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Figure 4 Comparative field assessment of *Bambusa balcooa* Roxb. plantlets obtained through *in vitro* or *in vivo* propagation methods. (a) Growth and development of *in vitro* generated plants after one year (*Bar*, 0.5 m), (b) growth and development of culm cutting generated plants after one year (*Bar*, 0.5 m), (c) growth and development of rhizome-derived plants after one year (*Bar*, 0.25 m), (d) growth and development of *in vitro* generated plants after two years (*Bar*, 1 m), (e) growth and development of culm cutting generated plants after two years, (f) growth and development of rhizome-derived plants after two years (*Bar*, 0.5 m).

Table 2 Effect of BAP and kinetin on shoot multiplication of *Bambusa balcooa* Roxb.

Kinetin (mg l^{-1})	BAP (mg l^{-1})	Response (%)	Days to shoot initiation	No. of shoots	Shoot length (cm)
0	0	11.7 ± 0.4 g	34.3 ± 0.9a	1.3 ± 0.3f	1.4 ± 0.2f
0	1	24.3 ± 1.6f	16.1 ± 0.9c	3.8 ± 0.3e	2.9 ± 0.2e
0	2	51.7 ± 2.4e	11.9 ± 0.4d	4.8 ± 0.3de	4.4 ± 0.4 cd
0	3	79.7 ± 1.6b	7.2 ± 0.2 fg	8.2 ± 0.3bc	5.1 ± 0.2bcd
0	4	97.2 ± 1.0a	4.6 ± 0.5 g	11.2 ± 0.8a	6.8 ± 0.3a
0	5	66.3 ± 1.5 cd	11.2 ± 0.9de	9.1 ± 0.6ab	5.7 ± 0.3abc
1	0	32.2 ± 1.9f	20.6 ± 0.5b	2.9 ± 0.4ef	2.7 ± 0.3ef
2	0	45.1 ± 1.6e	17.1 ± 0.5c	3.7 ± 0.4e	4.1 ± 0.4de
3	0	62.7 ± 1.9d	13.9 ± 0.7 cd	6.7 ± 0.3 cd	5.1 ± 0.7bcd
4	0	70.7 ± 1.7 cd	8.5 ± 0.7ef	8.9 ± 0.5bc	6.2 ± 0.2ab
5	0	73.03 ± 1.1bc	12.1 ± 0.7d	7.3 ± 0.3bc	5.7 ± 0.3abc

Data represent (mean ± standard error) 20 nodal segment explants per treatment in five replicated experiments. Mean values with the same *letter* are not significant at $P = 0.05$ based on one-way analysis of variance (ANOVA) followed by Tukey's test.

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Table 3 Effect of IBA and IAA on *in vitro* rooting of *Bambusa balcooa* Roxb.

IBA (mg l^{-1})	IAA (mg l^{-1})	Response (%)	Days to root	No. of roots	Root length (cm)
0	0	61.9 ± 2.3c	29.7 ± 0.9a	2.0 ± 0.2c	1.7 ± 0.2d
0	1	48.4 ± 1.4e	26.4 ± 0.8ab	2.4 ± 0.2bc	1.6 ± 0.1d
0	2	58.4 ± 1.2 cd	23.4 ± 0.8b	2.7 ± 0.3bc	2.7 ± 0.2 cd
0	3	52.9 ± 2.4de	16.7 ± 0.6c	3.7 ± 0.2ab	3.7 ± 0.2bc
1	0	94.1 ± 1.1a	11.1 ± 0.4d	4.4 ± 0.6a	4.2 ± 0.2a
2	0	89.1 ± 1.2ab	13.4 ± 0.5 cd	2.8 ± 0.2bc	4.0 ± 0.3a
3	0	83.6 ± 1.2b	15.7 ± 0.7c	2.5 ± 0.3bc	3.4 ± 0.4bc

Data represent (mean ± standard error) 20 shoots per treatment in five replicated experiments. Mean values with the same *letter* are not significant at $P = 0.05$ based on one-way analysis of variance (ANOVA) followed by Tukey's test.

Table 4 Year-wise field performance of *Bambusa balcooa* Roxb. plantlets obtained through *in vitro* or *in vivo* propagation methods.

Propagation method	First year				Second year			
	Plant height (cm)	Shoot (culm) number	Culm circumference (cm)	Internode length (cm)	Plant height (cm)	Shoot number	Culm circumference (cm)	Internode length (cm)
Tissue culture	445.1 ± 9.94a	4.2 ± 0.4a	12.7 ± 0.3b	20.9 ± 0.6a	868.0 ± 16.6a	11.2 ± 0.7a	23.9 ± 1.0a	29.2 ± 0.9a
Culm-cutting	368.6 ± 5.15b	2.6 ± 0.2b	9.7 ± 0.5c	18.3 ± 0.5b	751.8 ± 6.2b	5.8 ± 0.4b	17.6 ± 0.5b	27.8 ± 1.2ab
Rhizome-derived	265.8 ± 5.16c	1.4 ± 0.2b	14.2 ± 0.3a	15.7 ± 0.6c	504.2 ± 8.5c	3.4 ± 0.2c	25.4 ± 0.8a	24.7 ± 1.0b

Data represent (mean ± standard error) 10 plants per treatment in five replicated experiments. Mean values with the same *letter* are not significant at $P = 0.05$ based on one-way analysis of variance (ANOVA) followed by Tukey's test.

acclimatization, additionally contributed a noteworthy share in preserving the dampness quotient (Gantait, 2009; Gantait et al., 2009).

3.5. Comparative field assessment of in vitro and in vivo propagated plants

Plantlets, obtained via different propagation methods were established in the field to compare their field performance in order to identify the best method of propagation. Bamboo *de novo* shoot (culm) development in micropropagated plants was detected within six months in comparison with 6–8 months in culm-regenerated or rhizome-derived plants. It was observed for two consecutive years in the present study that in case of plantlets originated from *in vitro* culture, the plant height, the shoot (culm) number per plant, circumference of the main culm (at the second internode), and length of the internode were significantly higher than those of the plants derived from the other two propagation methods (Table 4 and Fig. 4), which corroborates the former statement of Mascarenhas et al. (1989) in *Dendrocalamus strictus*, another important bamboo species. However, there was no flowering recorded, since bamboo rarely flowers, occasionally following an interval of 12–120 years. Plants obtained by the way of splitting of rhizome, showed severe lodging and once lodged in the field the plant growth stunted in some instances. Uniformity in plants derived from *in vitro* propagation was higher than the others especially during juvenile stage. Presumably, this can attribute to diversity among propagules during *in vivo* propagation compared with the single micropropagated clone (Gupta et al., 1991). During field trial, plants propagated via culm cutting or rhizome-splitting (other than *in vitro* culture) grew significantly slower which might be attributed to the fact that saplings derived through any extended propagation system grew in a slow pace, similarly (McCown and McCown, 1987).

The methods of propagation described in the current study are simple in comparison with the methods stated by earlier reports. Furthermore, in no case the performance of the plants, produced through *in vivo* or *in vitro* approach, in field was reported earlier. Also, a comparative study as stated in this article provides the opportunity to select proper methodology of propagation and subsequent large-scale field trials.

Authors' contribution

M. Banerjee, S. Gantait, and B. Ray Pramanik conceived the idea and designed the experiments; S. Gantait and B. Ray Pramanik executed the experiments and analyzed the data; S. Gantait and M. Banerjee wrote the manuscript. All authors discussed the results and implications, and commented on the manuscript at all stages.

Conflict of interest

The authors of this article declare that there is no conflict of interest and do not have any financial gain from it.

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References

Banerjee, M., Gantait, S., Pramanik, B. Ray, 2011. A two step method for accelerated mass propagation of *Dendrocalamus asper* and their evaluation in field. Physiol. Mol. Biol. Plants 17, 387–393. http://dx.doi.org/10.1007/s12298-011-0088-0.

Bisht, P., Pant, M., Kant, A., 2010. *In vitro* propagation of *Gigantochloa atroviolaceae* Widjaja through nodal explants. J. Am. Sci. 6, 1019–1025.

Compton, M.E., 1994. Statistical methods suitable for the analysis of plant tissue culture data. Plant Cell Tiss. Organ Cult. 37, 217–242. http://dx.doi.org/10.1007/BF00042336.

Das, M., Pal, A., 2005. *In vitro* regeneration of *Bambusa balcooa* Roxb.: factors affecting changes of morphogenetic competence in the axillary buds. Plant Cell Tiss. Organ Cult. 81, 109–112. http://dx.doi.org/10.1007/s11240-004-3017-x.

Dutta Mudoi, K., Borthakur, M., 2009. *In vitro* micropropagation of *Bambusa balcooa* Roxb. through nodal explants from field-grown culms and scope for upscaling. Curr. Sci. 96, 962–966.

Gantait, S., Mandal, N., Bhattacharyya, S., Das, P.K., Nandy, S., 2009. Mass multiplication of *Vanilla planifolia* with pure genetic identity confirmed by ISSR. Int. J. Plant Dev. Biol. 3, 18–23.

Gantait, S., 2009. Accelerated cloning in vitro and induction of variation in six plants having medicinal, aromatic or aesthetic importance. PhD Thesis, Bidhan Chandra Krishi Viswavidyalaya, West Bengal, India.

Gillis, K., Gielis, J., Peeters, H., Dhooghe, E., Oprins, J., 2007. Somatic embryogenesis from mature *Bambusa balcooa* Roxb. as basis for mass production of elite forestry bamboos. Plant Cell Tiss. Organ Cult. 91, 115–123. http://dx.doi.org/10.1007/s11240-007-9236-1.

Gupta, P.K., Timmis, R., Mascarenhas, A.F., 1991. Field performance of micropropagated forestry species. In vitro cell. Dev. Biol. – Plant 27, 159–164. http://dx.doi.org/10.1007/BF02632210.

Hassan, S.M., 1977. Studies on vegetative propagation of bamboos. Bano Biggyan Patrika 6, 64–71.

Mascarenhas, A.F., Khuspe, S.S., Nadgauda, R.S., 1989. Biotechnological application of plant tissue culture to forestry in India. In: Dhawan, V. (Ed.), Application of Biotechnology in Forestry and Horticulture. Plenum Press, New York, pp. 73–86.

McCown, D.D., McCown, B.H., 1987. North American hardwoods. In: Bonga, J.M., Durzan, D.J. (Eds.), . In: Cell and Tissue Culture in Forestry, vol. 3. Martinus Nijhoff, Dordrecht, pp. 247–260.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. Physiol. Plant. 15, 473–497. http://dx.doi.org/10.1111/j.1399-3054.1962.tb08052.x.

Negi, D., Saxena, S., 2010. Ascertaining clonal fidelity of tissue culture raised plants of *Bambusa balcooa* Roxb. using inter simple sequence repeat markers. New For. 40, 1–8. http://dx.doi.org/10.1007/s11056-009-9182-3.

Negi, D., Saxena, S., 2011. Micropropagation of *Bambusa balcooa* Roxb. through axillary shoot proliferation. In Vitro Cell Dev. Biol. – Plant 47, 604–610. http://dx.doi.org/10.1007/s11627-011-9403-2.

Ohrnberger, D., 1999. The Bamboos of the World. Elsevier.

Pattanaik, S., Das, P., Borah, E., 2004. Vegetative multiplication of *Bambusa balcooa* Roxb. using branch cuttings. J. Bamboo Rattan. 3, 365–374.

Sarangthem, K., Singh, T.N., 2003. Microbial bioconversion of metabolites from fermented succulent bamboo shoots into phytosterols. Curr. Sci. 84, 1544–1547.

Saxena, S., Bhojwani, S.S., 1993. *In vitro* clonal multiplication of 4-year-old plants of the bamboo *Dendrocalamus longispathus* Kurz. In Vitro Cell. Dev. Biol. – Plant 29, 135–142. http://dx.doi.org/10.1007/BF02632285.

Seethalakshmi, K.K., Venkatesh, C.S., Surendran, T., 1983. Vegetative propagation of bamboos using growth promoting substances. *Bambusa balcooa* Roxb. Indian J. For. 6, 98–103.

Sharma, P., Sarma, K.P., 2011. In vitro propagation of Bambusa balcooa for a better environment. In: International Conference on Advances in Biotechnology and Pharmaceutical Sciences (ICABPS'2011) Bangkok Dec. 2011, pp. 248–252.

Stapleton, C.M.A., 1994. The bamboos of Nepal and Bhutan, Part I, Bambusa, Dendrocalamus, Melocanna, Cephalostachyum, Teinostachyum and Pseudostachyum (Gramineae: Poaceae, Bambusoideae). Edinb. J. Bot. 51, 1–32. http://dx.doi.org/10.1017/S0960428600001682.

Tewari, D.N., 1992. A Monograph on Bamboo. International Book Distributors, Dehradun, India.

TIFAC (Technology Information, Forecasting and Assessment Council), 2009. National Missions on Bamboo Applications (NMBA), India. www.bambootech.org.

Thomas, P., Ravindra, M.B., 1997. Effect of pruning or removal of *in vitro* formed roots on *ex vitro* root regeneration and growth in micropropagated grapes. Plant Cell Tiss. Organ Cult. 51, 177–180. http://dx.doi.org/10.1023/A:1005928615179.

Zhou, B., Fu, M., Xie, J., Yang, X., Li, Z., 2005. Ecological functions of bamboo forest: research and application. J. For. Res. 16, 143–147. http://dx.doi.org/10.1007/BF02857909.