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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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