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Rapid *invitro* Propagation of *Bambusa balcooa* Roxb. (Bamboo)

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A B S T R A C T

Production of bamboo plants by using vegetative propagation is not simple to establish a commercial fields however micropropagation techniques has much ability to achieve the bamboo seedling demands. *In vitro* cultures of *Bambusa balcooa* were achieved by using nodal segment as an explant. Eight different combinations of plant growth regulators with MS basal medium were used for shoot induction and multiplication. The medium with concentrations of BAP 1.5 mg/L + Kin 1.5 mg/L has gave maximum response for shoot induction (5.67) and also for shoot multiplication (13.67). For root induction five different IBA concentrations with MS basal medium ware evaluated. Maximum number of roots (6.34), highest root length (5.67) and more number of leaves (7.68) was obtained from MS basal medium containing 2.0 mg/L IBA. The well rooted *in vitro* regenerated plantlets of *Bambusa balcooa* are further hardened in soil: vermicompost: coco-peat as (1:1:1) proportion medium for primary hardening and riverbed sand: soil: farmyard manure as 1:1:1 proportion medium for secondary hardening. From the total hardened plantlets 90 % plantlets survived well after the secondary hardening stage.

Introduction

Bamboo is a group of the family Poaceae, subfamily Bambusoideae, tribe Bambuseae. Some of its members are giant bamboos, forming by far the largest member of the grass family (David, 2003). It is one of the fastest growing (30-100 cm per day) plant on the planet. It can grow up to 36 meters height with 1-30 cm of a diameter. The bamboo has great ecological importance; its roots absorb more water from soil than other plants and that can helps to reduce the soil erosion. It also absorbs the carbon dioxide (CO_2) from air and produces oxygen in higher concentration. As view of economical aspect, bamboo has immense potential for improving quality of rural and urban life (Ansari *et al.,* 2017). It is good resource for rural economies, structural raw material, fodder and source of fiber for paper industry. In bamboo production China is the top country in the world.

Bamboos are distributed all over the world with 75 genera and 1250 species, but mainly occur in the tropics although, they are found naturally in all subtropical and temperate

zones (Mudoi *et al.,* 2013). *Bambusa balcooa* is a native India subcontinent multipurpose bamboo species with 12-23 meter height, 18-25 cm diameter, and grows up to 600 m altitude.

The flowering cycle of *B. balcooa* is 55-60 years, and the plant dies after flowering without seeds stings (Tewari, 1992). The natural propagation of bamboo is hampered due to short seed dormancy period, high seed sterility, low seed viability, high seed-borne infections and large-scale consumption by wild animals especially rodents (Rajput *et al.,* 2019).

Vegetative propagation of *Bambusa balcooa* is done by using culms, rhizomes or branches but due to bulky and less availability of propagules, seasonal variations, and poor rooting ability making this process inefficient for large scale propagation (Pattanaik *et al.,* 2004).

Micropropagation of *B. balcooa* by using axillary shoots was reported by many authors; Das and Pal (2005), Islam and Rahman (2005), and Ansari *et al.,* (2017). *In vitro* propagation has emerged as a promising technique for mass propagation of elite bamboos and can lead to the production of healthy, disease free plants and the multiplication can continues throughout the year irrespective of season. Stocks of germplasm can be maintained for many years and it also facilitates the international exchange of disease free germplasm (Sun *et al.,* 2008).

The present study focused on establishment of a rapid micropropagation protocol for *in-vitro* regeneration of *B. balcooa* by using axillary shoots. The increasing demand of planting material and inefficient conventional methods of propagation makes more popularity of this topic.

Materials and Methods

Source of ex-plant

Explants were collected from the elite field grown plants of *Bambusa balcooa* growing in the experimental DRS farm Beej-Sheetal Bioscience Foundation (BSBSF), Jalna, Maharashtra state of the India. Elite mother's plant was selected based on the hight, girth, and numbers of culms, or the length of the internodes. Single node segment (2-3 cm long) from healthy culms of *Bambusa balcooa* (3-4 years old) ware collected (Figure 1). The sharp cutter wiped with 70% ethanol was used for cutting of explants.

Sterilization of ex-plant

The leaf sheaths of collected field grown explants are removed carefully; the nodal segments were rinsed with tap water. Explants were given treatment with Bavistin (0.2%) for 10 min, followed by sodium hypo chloride (15%) for 5 min, then mercuric chloride (0.1%) for 5 min and 70% alcohol for 1 minute respectively. After each treatment repeated (2-3 times) washing of explants with sterile distilled water was followed. Bottom portion of nodal explant exposed to sterilants were removed with the help of a scalpel and forceps under laminar air flow.

Shoot induction and shoot multiplication

The explants were prepared for inoculation by removing all debris and bottom of the explants under aseptic condition. The explants were then inoculated aseptically on sterile culture bottles containing MS media along with different combinations of hormones (Table 1). All cultures were stored at 25±2 °C under 16 hour's photoperiod for 2-3 weeks. This time sprouted buds elongated and developed into a multiple shoots.

The *in vitro* developed shoots obtained from initiation ware transferred for multiple shoots formation. The explants were prepared for inoculation by cutting top portion and removing all debris and brown part at the bottom of the explants under aseptic condition. The explants were then inoculated aseptically on sterile culture bottles containing MS media along with same combinations of hormones evaluated for the initiation (Table 1).

Rooting

The *in vitro* cultured shoots were prepared for inoculation by separating the clumps and removing the black debris under aseptic condition. The explants were then inoculated aseptically on sterile culture bottles containing MS rooting media supplemented with 0.6% agar, 3% sucrose and 50 mg/L charcoal and evaluated along with 5 different concentrations of IBA (0.5, 1.0, 1.5, 2.0 and 2.5 mg/L).

Acclimatization

Primary hardening of *in vitro* grown rooted plants of *B. balcooa* Roxb

Well rooted plantlets were transferred for primary hardening. Rooted plantlets were subjected to stepwise acclimatization and hardening. The leads of bottles were loosened and then removed 5 hours before transferring the plants. Then the plants were washed thoroughly with distilled water to remove agar. Then ware treated by the solution of Bavistin to avoid fungal growth. The healthy plants were transferred to plastic tray containing soil: vermicompost: coco-peat as (1:1:1) proportion or then covered the tray by using polythene bag to maintain the humidity level and kept in polythene tunnel for primary hardening.

Secondary hardening of *in vivo* grown primary hardened plants of *B. balcooa* Roxb

After the emergence of new leaves, plants were transferred to the potting mixture containing riverbed sand: soil: farmyard manure in 1: 1: 1 ratio and shifted to greenhouse condition for secondary hardening.

Results and Discussion

Effect of sterilants

Failure of surface sterilization procedure to produce aseptic cultures is a main problem in bamboos and plays a major role in initiation of cultures. Sterilization of explants was done by fallowing the procedure as per mentioned in methods and achieved superior culture without any sign of microbial growth.

Different strategies have been employed by different workers to counter/eliminate microbial contamination. Sodium hypochloride (NaOCL) has been used as an effective sterilant. 0.2% Bavistin, 10% Sodium hypochloride and 0.2% Mercuric chloride solution for each and finally rinsed in 70% ethanol was used by Das and Pal (2005) in *B. balcooa* and *B. tulda.*Sodium hypochloride (NaOCL) at a concentration of 10% for 10 min followed by 0.1% Mercuric chloride ($HgCl_2$) for 8 min was used by Brar *et al.,* (2012) in *Dendrocalamus membranaceus*. Similarly the mercuric chloride ($HgCl_2$) was used as 0.2% for 25 min in *Bambusa vulgaris* by Rout and Das (1994).

Effect of plant growth regulators on shoot initiation and multiplication

For auxillary bud break, nodal explants were inoculated on MS basal medium with eight different combinations of hormones where

sprouting occurred after 4-5 days of inoculation. Sprouting of nodal explants were occurred on all of the combinations of MS medium, but when the MS basal medium supplemented with BAP 1.5 mg/L + Kn1.5 mg/L produced maximum number of shoots (5.67) after 21 days of culture. Effect of different combinations of plant growth regulators on shoot induction is depicted in (Table 2 and Figure 2a).

For more shoot proliferation, the sprouted buds were transferred for multiplication on to the same MS medium used for the initiation. Auxillary shoot proliferation occurred in all of the combinations of MS medium, the MS basal medium supplemented with the concentration of (BAP 1.5 mg/L + Kn 1.5 mg/L) produced maximum number of shoots (13.67) after 30 days of culture. The effect of different combinations of plant growth regulators on shoot proliferation is depicted in (Table 2 and Figure 2b). The shoots formed were further subdivided into smaller clumps each having 2-3 shoots and sub cultured onto fresh medium for shoot proliferation and to avoid shoot necrosis.

Similar harmone combinations was used by Das and Pal 2005 or Brar *et al.,* 2012 and reported the highest shoot induction and shoot multiplication in *Bambusa balcooa.*

Table.1 Different cytokinin combinations for shoot induction and shoot multiplication

Treatment name	Hormone combinations (mg/L)
T1	BAP 0.5 + Kinetin 0.5
T2	BAP 1.0 + Kinetin 1.0
T3	BAP 1.5 + Kinetin 1.5
T4	BAP 2.0 + Kinetin 2.0
T5	Kinetin 0.5 + NAA 0.5
T6	Kinetin 1.0 + NAA 1.0
T7	Kinetin 1.5 + NAA 1.5
T8	Kinetin 2.0 + NAA 2.0

Table.2 Effect of different plant growth regulators on *in vitro* shoot induction and multiplication in *Bambusa balcooa* Roxb

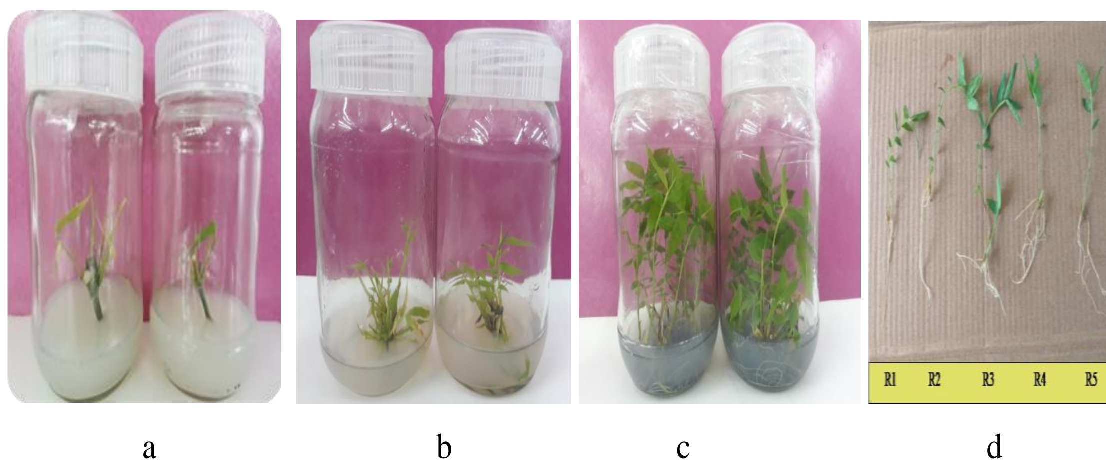
Hormone supplements	No. of Shoots induced/ explant during initiation	No. of shoots formed / clump during multiplication
0.5 BAP + 0.5 KIN	3.92	9.50
1.0 BAP + 1.0 KIN	4.65	10.84
1.5 BAP + 1.5 KIN	5.67	13.67
2.0 BAP + 2.0 KIN	4.17	10.42
0.5 KIN + 0.5 NAA	3.47	7.84
1.0 KIN + 1.0 NAA	3.92	8.67
1.5 KIN + 1.5 NAA	3.54	11.34
2.0 KIN + 2.0 NAA	4.45	10.42
C.D. (0.05)	1.093	0.738
SE (m)	0.372	0.251

Table.3 Effect of different IBA concentrations on rooting characteristics of *Bambusa balcooa* Roxb

Treatment name	Different concentrations of IBA	No. of roots/ plantlet	Length of roots/ plantlet	No. of leaves/ plantlet
R1	0.5 IBA	0.84	1.24	4.92
R2	1.0 IBA	2.42	2.51	6.76
R3	1.5 IBA	4.26	3.84	7.09
R4	2.0 IBA	6.34	5.67	7.68
R5	2.5 IBA	3.84	4.17	6.84
C.D. (0.05)		0.617	0.972	1.286
SE (m)		0.203	0.320	0.423

Fig.1 Collection of explant

Fig.2 Micropropagation of *Bambusa balcoaa*
(a: shoot induction, b: multiplication, c: rooting, d: rooted plantlets)



Effect of IBA on root induction

The shoots obtained after multiplication were inoculated onto MS medium for induction of rooting. Since *in vitro* raised shoots failed to develop root on a hormone free basal medium, use of different concentrations of IBA was attempted. Medium supplemented with 2.0 mg/L IBA forming 6.34 roots per shoots having a root length of 5.67cm as depicted in (Figure 2c) and 7.68 number of leaves per plantlet. The effect of different concentrations of IBA on rooting has been compiled in (Table 3 and Figure 2d).

The similar results were obtained by Choudhary *et al.,* (2016) reported 2.0 mg/l IBA was better for root induction and achived 100% root induction with 6.22 number of roots and root length of 7.16 cm in *B. nutans.*

These results are in line with earlier reports on several bamboos such as *D. asper* and *D. falcatum* (Arya and Arya 2015), *Melocanna baccifera* (Kant *et al.,* 2009) and *Bambusa balcooa* Roxb. (Patel *et al.,* 2015).

Hardening and acclimatization

Plantlets with well-developed roots were removed from the culture medium and washed under running tap water and then treated with solution of Bavistin. The treated plantlets were planted onto plastic tray containing soil: cocopeat: vermicompost as 1:1:1 proportion for primary hardening and were covered with transparent polythene cover for maintaining high relative humidity for 1 week. After the emergence of new leaves, plants were transferred to the potting mixture containing riverbed sand: soil: farmyard manure in 1:1:1 ratio and shifted to greenhouse for secondary hardening. Among the total secondary hardened plantlets 90 % plantlets were survived well.

According to Ray and Ali (2016) reported the healthy rooted plantlets was removed from the rooting medium and transferred to pot containing growth supporting materials like soil, sand, soilrite, perlite, vermiculate, compost, farm yard manure etc either alone or in various ratios. Out of several substrate used, soil: sand: farmyard manure (1:1:1) was mostly used. This treatment is reported in almost all bamboo species namely, *B. bambos* (Anand *et al.,* 2013), *B. balcooa* (Gantait *et al.,* 2016).

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