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In-vitro Regeneration of *Bambusa balcooa* (Bamboo) through Nodal Segments

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A B S T R A C T

Bambusa balcooa (a commercial bamboo) are the most important and fastest growing species. *Bambusa balcooa* micro propagation protocol was established for a consistent supply of quality plants. The surface sterilization of nodal explants using 0.1% mercuric chloride followed by initiation in solid Murashige and Skoog (MS) media supplemented with BA 0.5-1.5 mg/L as growth regulator. Shoots were multiplied using MS medium with different concentrations of BA 0.5-1.5 mg/L. In the present study we are reported that when we gradually increases BA concentration from 0.5-1.5 mg/L the number of shoots were also increases and maximum shoots were reported in BA concentration 1.5 mg/L. Then 3-4 shoots were transferred for rooting media with NAA 0.5-1.5mg/L for 2-3 weeks. During the rooting we noticed that when we increases NAA concentrations from 0.5-1.5 mg/L there are gradually increase in number of roots per plantlets and the maximum rooting were observed in NAA concentration 1.5 mg/L. After root development hardening were done using a mixture of coco-peat and vermi-compost.

Introduction

Bamboo is a perennial woody grass of family *Poaceae*. It is the fastest growing plant on the earth with a growth rate ranging from 30-100 cm per day in growing season. It can grow to a height of 36 meters with diameter of 1-30 cm. a culm can reach its full height in matter of two to three months.

Its roots can reduce soil erosion by up to 75 percent. It is use full resource for local economies and also as structural raw material, fodder and source of fiber for paper manufacture. India is one of the leading countries in the world in bamboo production. Bamboos are also planted on private lands particularly in homesteads, field bunds and other marginal lands available. India is also very rich in bamboo diversity.

*Bambusa balcooa*is a native India subcontinent multipurpose bamboo species height of 12-23 meters, diameter of 18-25 cm, and grows up to 600 m altitude in various parts of India (Tewari, 1992). The flowering cycle of *B. balcooa* is 55-60 years, and the plant dies after flowering without seeds stings (Tewari, 1992). Vegetative propagation is difficult though culm, branch or rhizomes due to few and bulky propagules, seasonal variations, and poor rooting potential making

this process inefficient for large scale propagation of *B. balcooa* (Pattanaik *et al.*, 2004).

In vitro propagation method allow large scale production of the *Bambusa balcooa* there are few some research on use of axillary shoot portion for micro propagation of *B. balcooa* (Das and Pal 2005, Islam and Rahman, 2005)

This study reported on micro propagation protocol for *in-vitro* propagation of *B. balcooa* through axillary shoot. This is useful for land reclamation through bamboo plantation and help for rural economy and livelihood promotion.

Materials and Methods

Collection of ex-plant

The field grown healthy nodal segments of *Bambusa balcooa* (1.5-2.0 cm in length) were collected from 3-4 years old and disease free plant from Campus of ShriMukund biotech Lab Jabalpur. Leaf sheath tissues and dead parts of the upper internodes were removed through scalpel (Sharma *et al.*, 2011 and Patel *et al.*, 2015)

Surface sterilization of ex-plant

The nodal parts were thorouly washed under running tap water for 20 minutes.

They were then washed in 5% labolene solution and 1% bavistin for 10-15 minutes and rinse with distilled water followed by 70% isopropyl alcohol.

Further treatment was done under laminar air flow. The ex-plant was treated with 0.2% mercuric chloride solution for 5-10 minutes and then thorouly washed with sterile distilled water (Jimenez *et al.*,. 2006 and Pandey *et al.*, 2012)

Initiation

Initiation was carried out in Murashige and Skoog (MS) solid media with 3% sugar, 6% agar and different concentration of BA (0.5-1.5 mg/l). 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.

Shoot multiplication

Newly sprouted axillary shoots containing 3-5 shoot propagules were sub-cultured at regular intervals of 3-4 weeks in fresh multiplication media with BA (0.5-1.5 mg/ l) for induction of multiple shoots.

Rooting

Developed shoots having 8-10 shoot propagules, measuring about 3.5-6 cm were transferred to rooting media after completion of 4 multiplication cycles. The MS basal media with different concentrations of NAA (0.5-1.5 mg/l) were used for rooting. All cultures were transferred in plant tissue culture chamber for 2-3 weeks.

Hardening

In-vitro plantlets were removed from culture bottles, washed thoroughly under running water to remove traces of medium from roots. Plantlets were transferred to hardening trays consist of 3:1 ratio of coco peat and vermicompost. The plants were grown under greenhouse conditions maintained at 25-30 °C temperature and relative humidity of 75% for 3-4 weeks (Beena *et al.*, 2015).

Results and Discussion

MS media (solid media) were used for the development of nodal ex-plant (*B. balcooa*) with different concentration of plant growth

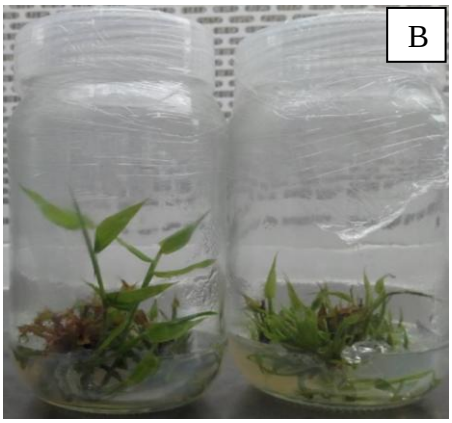
regulators (BA 0.5-1.5 and NAA 0.5-1.5). The treated nodal cultures inoculated in the solid MS media.

After 2-3 weeks highest shoot development is found in MS media with BA concentration 1.5 mg/L i.e. 8.2±2.4 shoots per explant and highest shoot length 3.8±0.4 were recorded. Similar observation were also made by Sharma *et al.,* 2011 and Patel *et al.,* 2015.

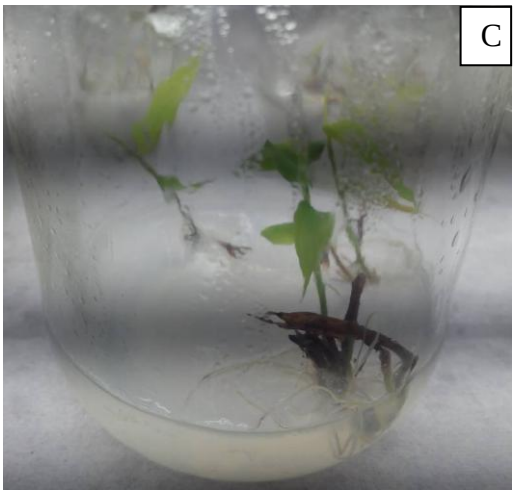
Mature buds were then transferred in multiplication media for 2-3 weeks at controlled conditions.

A. Shoot initiation from axillary buds

B. Shoot multiplication [Effect of BA (1.5 mg/L)]



C. Effect of NAA (1.5 mg/L) in rooting



D. Plantation at Green House

Table.1 Effect of BA in MS media on Shooting

S. No.	Media	Concentration (mg/l)	Number of Shoot	Shoot length
1	MS + BA	0.5	3.8 ± 0.2	1.2 ± 0.1
2	MS + BA	1.0	5.0 ± 1.2	2.6 ± 1.3
3	MS + BA	1.5	8.2 ± 2.4	3.8 ± 0.4

Table.2 Effect of NAA in MS media on Rooting

S. No.	Media	Concentration (mg/l)	Number of Shoot	Shoot length
1	MS + BA	0.5	1 ± 1	0.1 ± 0.2
2	MS + BA	1.0	3 ± 1	1.5 ± 0.5
3	MS + BA	1.5	4 ± 1	2.3 ± 0.3

Best shoot multiplication was found in media containing BA 1.5 mg/l. The newly developed shoots were recoreded in 5-8 numbers whose shoot length were 3.8±0.4 observed. Best period for recycling of multiplication shoots was recorded i.e. 18 days old culture. Same result was observed by Mudai and Borthaker 2009. For root induction developed shoot bunch (3-4 shoots) were placed in the media containing different concentration of NAA (0.5-1.5 mg/l). Best result was recorded (4±1 roots) in media containing 1.5 mg/l NAA whose length was 2-3 cm recorded after 2-3 weeks. Same results were observed by Arya *et al.*, 2002 and Pratibha and Sharma, 2011. The developed rooted plants were washed under running tap water and plants were transferred to root trainer with mixture contain coco-peat and vermin-compost into 3:1 ratio then add to some water and kept in close condition and maintaining the humidity at green house for 20-30 days. The 90% survival rate was recorded from developed rooted *B. balcooa*.

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