In vitro propagation of *Bambusa balcooa* for a better environment

Pratibha Sharma, K. P. Sarma

Abstract —Bamboos are the most productive and fastest growing species on the planet. It covers about 37 million hectors which is just about 1% of the forest world wide. Bamboo has several advantages in terms of sustainability and carbon fixing capacity compared to the other fast growing species. It can play a significant role in linking climate change mitigation to sustainable development of the world. This communication describes for an efficient protocol for large scale production of *Bambusa balcooa. In vitro* propagation was achieved from nodal explants from field grown culms of *Bambusa balcooa* were used to induce multiple shoots on Murashige and Skoog medium supplemented with auxins and cytokinins. *In vitro* axillary shoot formation was successfully made in Murashige and Skoog basal medium supplemented with 1.0 mg/l and 1.5 mg/lof 6-benzyle adenine. Clamps of at least 3 shoots were used for root induction in MS medium with 3.5 and 4.0 mg/l Naphthalene Acetic Acid. Rooted plantlets were successfully acclimatized in green house for 1 month and then were transferred to the polybag. 100% survival rate was recorded after field transfer.

Keywords— Micropropagation, Multiple shoots induction, nodal explants, plantlets regeneration, Root induction,

Abbrebiation-- BA (6- benzyle adenine), NAA (Naphthalene Acetic Acid), IAA (Indole- 3- acetic acid), IBA(Indole-3-butyric acid), Kn(Kinetin)

I. Introduction

BAMBOO is an ancient grass which has celebrated as the High Emperor of all the Grasses. Bamboo is the fastest growing plant on the planet- with a growth rate of up to 1.2 metres a day. Its roots can reduce soil erosion by up to 75 per cent. It can sequester carbon faster than similar other fast growing tree species.

It is a useful resource for local economies and also as structural raw material, fodder and source of fiber for paper manufacture. As bamboos are fast growing plants, recently they are considered as a prime renewable resource for biomass production. The potential of micropropagation for mass scale propagation has raised high hopes and a lot of research has been focused on the development of protocols for large and rapid scale production. It is proposed by Byatriakova *et al.* [1]. that bamboo forests are important potential modulator of global environment. However, Alexander and Rao [2] also reported tissue culture research on bamboo on embryo culture of *Dendrocalamus strictus* and since then starting from Huang and Murashige [3] a good start has been made on tissue culture and a number of laboratories have begun to make plant species.

Bambusa balcooa Roxb., is an indigenous widespread bamboo of North East India. It is tallest, strongest and highly durable and is utilized mostly for structural use and pulping. The species is also valued for its edible tender shoots, mainly for food and pickle industry. It occurs in moist alluvial flat land along water courses upto an altitude of 1500 m. in finer textured soils in the semi evergreen forests in relatively law rainfall areas in Assam.

According to some researcher like Chaturvedi *et al.*[4]; Saxena and Bhojwani [5]; Ramanayake and Yakandawala [6], the clonal multiplication from adult bamboos is very limited. It is reported by Lin and Chang [7] that the maturation of the tree species affects the potentiality of axillary buds and the success of clonal multiplication from adult culm which is restricted by many factors. Maximum rooting developed (92%) occurred in 3 weeks through seed initiation in the previous record. Sexena [8] reported the survival percentage 80-90%. An attempt was made to report for mass multiplication of this commercially important bamboo species in the present study. Survival rate is recorded 100 % after plantation in the field. Commercial production of Bamboo is the first attempt in the North eastern Part of India through tissue culture process. The 100% survival rate is also first record in this type of study.

II. Materials and Method

Field grown healthy nodal segments of *Bambusa balcooa* (1.5 to 2.0 cm in length) were collected from a natural bamboo stands of nearby places of Jagirod (Assam). The nodes containing the axillary buds were used to initiate and establish *in vitro* culture. Clumps of donor bamboos were about 10-20 years old. Leaf sheath of nodal segment were removed sized and were surface sterilized by 70 percent ethanol then disinfected with 0.1 per cent $HgCl_2$ solution for 5 minutes. The disinfected explants were washed thoroughly under running tape water containing 1-2 drops of Tween 20 solution and then with sterile distilled water. Pretreatment of the explants were carried out with aqueous solution of 0.5 per cent of Bavistin a fungicide and bacteriomycine for 15 minutes.

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MS medium (Murashige & Skoog [9]) supplemented with 100mg/l myo-inositol, sucrose (3%) and 2.5 per cent Gelrite were used for culturing the explants. The medium was supplemented with different concentrations of plant growth regulators like BA, IBA, IAA, NAA and kinetin singly or in combinations.

The p^H of the medium adjusted to 6.0. The media was sterilized at 15 lb pressure at 121^0 C for 15 minutes. The sterilized nodal buds were cultured in the semi solid MS medium in culture tubes. Selected buds were sprouted within three to four weeks. The sprouted buds were again transferred to culture bottles in same media. By this time sprouted buds elongated and developed into a number of multiple shoots. The later shoots were used as explants either as a single or a cluster of two- three shoots for production of additional multiple shoots and root induction. An aseptic condition was maintained throughout the whole operation. Cultures were incubated at 25 ± 2^0C under illumination with florescent light (approx.45 μmol $m^{-2}S^{-1}$) for 16 hr photo period/ day.

Plantlets with well developed roots were removed from culture medium and after washing the roots gently under running tape water were transferred to root trainer containing cocopeat and vermicompost into 3:1 ratio. They were irrigated with water and kept it in close condition maintaining the humidity at green house for 20-30 days. The plantlets were acclimatized at green house before transferring to shade net house and then to natural condition. Within a period of one to two months the plantlets transferred to the propagation bed produced mini clumps of shoots in abundance. The rooted shoots of mini clumps proliferated further when individual mini clumps were separated and planted in the specially prepared planting bags containing soil: sand: cowdung mixture (1:1:2). This procedure is found most suitable for large scale production. These poly bags are ready for distribution or sale.

III. Result and Discussion

MS medium with solidifying agent Gelrite either alone or supplemented with different concentrations of plant growth regulators like BA, IBA, IAA, NAA and kinetin were prepared. The sterilized nodal explants collected in the month of October were cultured in those media concentrations. After one to three weeks axillary bud break was noticed but those, which did not sprout remained green for a longer period and dried up. Breaking of nodal buds and sprouting of shoot depend on the condition of explants, season of the year and culture condition. *In vitro* bud breaking of two bamboos (*Dendrocalamus giganteus* and *Bambusa vulgaries*) were studied by Ramanayake *et al*. [10] from April, 1994 to April, 1995 and found seasonal effect on bud breaking. Similar observations were also made by Saxena and Dhawan [11] on *D. longispathus*. After the bud break, the elongated shoots were separated from nodes by sharp scalpel and transferred in the same fresh medium. Initially the sprouted nodal buds produced thick shoots. The clusters of shoots are of varied number. The excised shoots (either single or two- three together) clusters, established from nodal buds of parent bamboo were used as explants bamboo were used as explants.

Arya and Arya [12] and also Chambers *et al*. [13] reported that the effectiveness of BA in different concentrations in a number of bamboo species. However, in the present study BA (0.25 $mg^{-1}$and 0.50 mg^{-1}) proved to be ideal for healthy shoot initiation. It is followed by BA (1.0 mg^{-1}) and BA (1.5 mg^{-1})

Table I:
Morphogenic response of nodal explants of *Bambusa balcooa* at different concentration of cytokinins supplemented to MS medium.

Growth Regulator (mgl^{-1})	Shoot numbers/clumps	Shoot Length (Cm)	Total Leaf number
MS 0	0	0	0
Kn 0.25	1.5±0.05	1.1±0.1	2.3±1.2
Kn 0.50	1.0±1.6	1.0±0.5	3.8±0.9
Kn 1.00	2.0±2.7	1.5±0.4	5.0±2.0
Kn 1.50	2.3±1.6	1.4±0.4	3.8±0.9
Kn 2.00	5.0±2.9	1.5±0.3	6.0±1.9
BA 0.25	5..3±1.6	1.5±0.1	5.3±1.4
BA 0.50	7.6±0.6	3.5±1.2	5.3±1.4
BA 1.00	15.0±3.8	3.3±0.2	15.0±1.0
BA 1.50	**20.5±3.9**	**1.5±0.4**	**15.3±5.2**

Percentage of culture response, time taken for bud break, number of shoot initiated and shoot lengths were also found to vary in the different concentrations of cytokinins. All explants did not produce equal number of new shoots within the same period of time in the same species. The variation of new shoot emergence may be due to size, age or other condition of explants.

Table II: Effect of NAA in rooting

NAA(mgl^{-1})	No. of roots	Root length (cm)	Rooting percentage	Root growth*
0	0	0	0	-
0.25	0	0	0	-
0.50	0	0	0	-
1.00	0	0	0	-
1.25	1.2±0.2	1	20	+
2.00	1.1±0.3	1-1.5	30	+
2.50	3.3±0.2	2-3	40	+++
3.00	5.0±1.0	3-5	45	+++
3.50	7.6±1.5	5-7	85	++++
4.00	10.6±1.5	7-8	90	++++

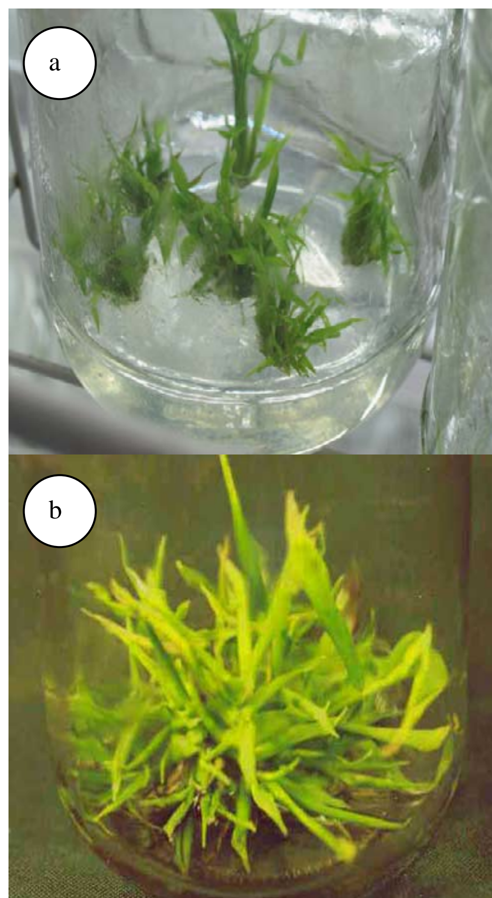
* indicates number of root found

+: Single root; ++: Two roots; +++: Three roots; ++++: Four or more roots.

It is also observed by Saxena and Dhawan [11] that shoots induced from nodes of *B. valgaries* multiplied at a slower rate than those from *D. longispathus* If once the bud break was achieved, shoot proliferation could be increased and maintained by regular subculturing at 4 weeks interval on MS medium with concentration of plant growth regulator. Subculturing at lower concentration of Kn (0.25 mg^{-1}, 0.50 mg^{-1} 1.0 $mg^{-1}$and 1.50 mg^{-1}) fortified medium caused stagnant and unhealthy growth of the shoots, leading to ultimate death of the cultures. Among the three cytokinin tested, BA was selected as the most suitable hormone to induce shoot multiplication.

Highest shoot multiplication is found in corporation with BA (1.0 mg^{-1} and 1.5 mg^{-1}). The shoot multiplication rate are 15.0±3.8 and 20.5±3.9 with shoot length 3.3±0.2 and 1.5±0.8 (Table:1)

with highest and maximum shoot generation. Shoot regeneration was also achieved in the MS medium containing Kn (1.0, 1.5 and 2.0 mg^{-1}). But the shoot multiplication was low. Highest multiplication is observed in the concentration of Kn (2.0 mg^{-1}). The shoot multiplication rate is 5.0±2.9 with shoot length 1.5±0.3 cm. Multiplication potentiality were observed in the cluster having more than 2-3 shoots. Best period for recycling of multiplying shoots is 3 week old culture. Delaying of sub-culturing period resulted in gradual browning re of the shoots. Subculturing period was recorded as the most crucial factor for obtaining optimal and desired level of regeneration of shoots. Same result was observed by Mudai and Borthakur [14] in *Bambusa balcooa*. For root induction shoot clumps (one/Two shoots) were placed vertically in the medium containing different concentration of auxins. Among the three auxins treated, NAA is found to most effective for normal and maximum number of root formation. Rooting started after 15-20 days of transfer in the media. Almost 85-90 percentage root formation observed in different concentration of NAA. No root formation was observed in the medium containing IAA and IBA. Higher concentration of IBA shows gradual degradation of growth of the shoots and resulted dryness. NAA induced maximum no. of roots in concentration of 3.5 mg^{-1}(7.6±1.5). The rooting percentage is 85 percentage (Table:II). This result was followed by NAA (4.0mg^{-1}) with 10.6±1.5 shoot numbers and 90 percent rooting percentage Low rate of rooting observed in NAA (1.25, 2.00 and 2.50 mg^{-1}). Arya *et al*. [15] reported favorable response of NAA for *in vitro* rooting of *B. arundinaceae*. Effectiveness of IBA for root initiation was also reported in *B. balcooa* by Das and Pal [16]. But this report is not included in our study. After attaining a height of 4-5 cm, rooted shoots were thoroughly washed under running tape water to adhere Gelrite from the roots and were transferred to root trainer with a mixture containing cocopeat and vermicompost into 3:1 ratio. They were irrigated with water and kept it in close condition maintaining the humidity at green house for 20-30 days. 100% survival rate was recorded from well established rooted of *B. balcooa*. Thereafter, the plantlets of root trainer of green house were transferred to the propagation beds sheltered from direct sunlight and maintained there for another 20 to 25 days. There also 100% plantlets were survived in the propagation beds. Beds are prepared with Forest top soil, river sand and cowdung. Successful plantlets in the propagation beds produced profuse multiple shoots and grew luxuriantly. Young mini clumps with two-five shoots each were divided into two-three parts and each are carefully planted for further proliferation in the proliferated beds and then planted in poly bags filled with soil: sand: cowdung mixture (1:1:2) for storage. It may be mentioned that Zamora and Gruezo [17] found micro- cloned mini-clumps suitable as macro-propagation technology of bamboos and field planting. In general, rooting and transplantation of plantlets to the field is the most important and difficult task in micropropagation (Murashige.T, [18]). Except for a few reports of bamboo, viz. *D. strictus* (Nadgir *et al*.[19] and Saxena [8]), all the others involved a callus phase which could lead to loss of genetic fidelity. The poly bags were distributed to the local farmers and although no intensive care was taken, the propagules were in good condition throughout the period under observation and almost 100% survival was reported in general field condition. Macro proliferation can increase the production upto minimum three times. Economic/commercial viability of this protocol was estimated for production in a well established plant tissue culture laboratory. At present, the costing of per plant through this process is considered as Rs.8 to Rs. 10. The total production for a year was targeted as 3.8 lakhs and successfully achieved the same. However, the laboratory has the capacity to produce 2 million plants per year. In this paper, a continuous mass multiplication protocol of *B. balcooa* was described, which is cost effective, easy to raise, economic to adopt, easy to transport for selling purpose.



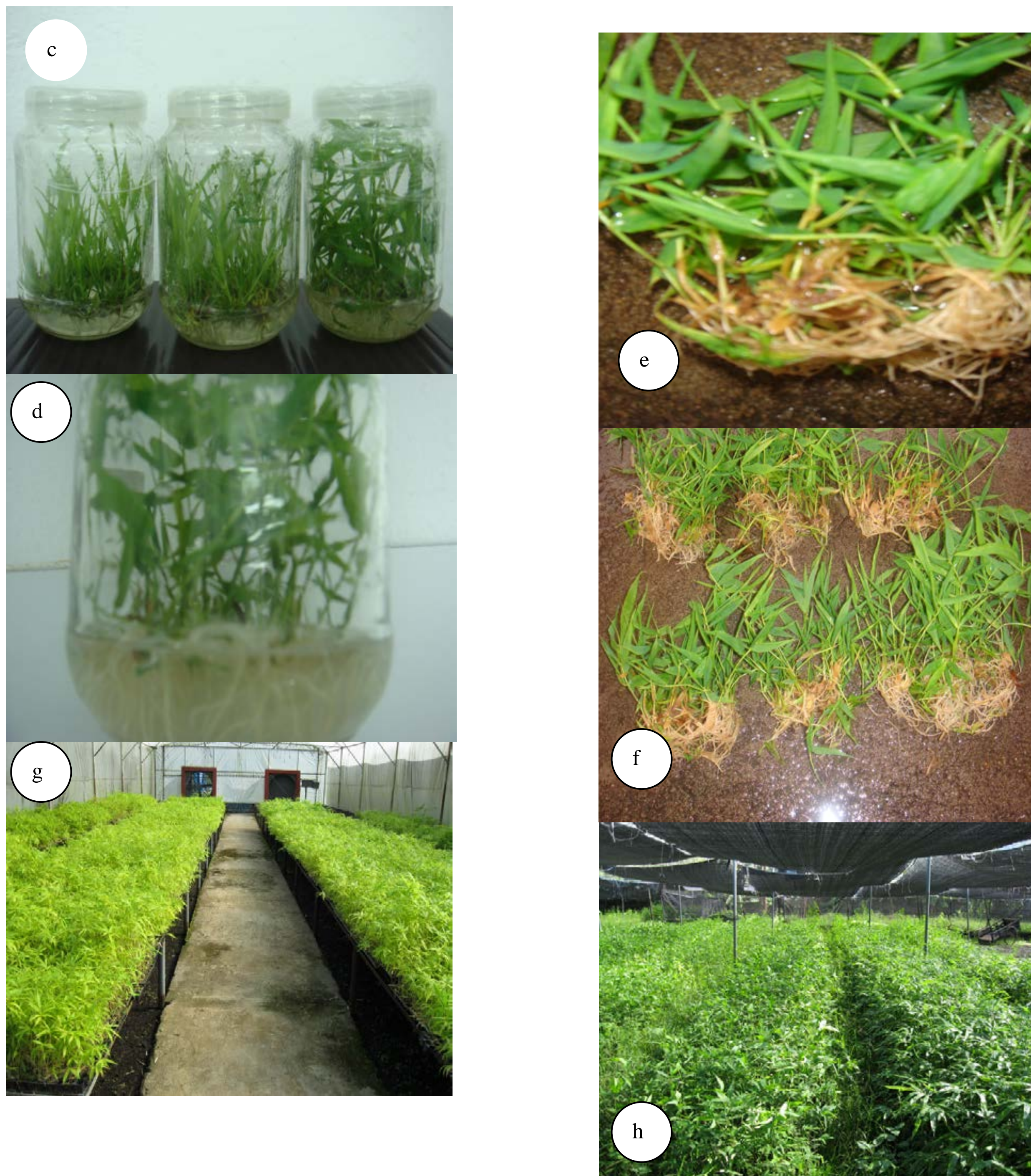


Fig1: Stages of micropropagation of *Bambusa tulda*.

a. Shoot initiation from axillary bud breaking and shoot multiplication [BA (1.0 mg^{-1})]
b. Shoot multiplication [Effect of BA (1.0 mg^{-1})]
c. Effect of NAA (5.0 $mgl-^{1}$) in rooting
d. Well developed roots.

e-f. Plantlets after removal of Gelrite in the Culture Bottle before plantation in root trainer

g. Plantation at Green House

h. Plantation in Poly bages at Shade Net House ready for sale.

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