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Micro Propagation and Development of High Yielding New Bamboo Variety *Bambusa Balcooa* BB1 of Bangladesh

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ABSTRACT

In vitro protocol of *Bambusa balcooa* BB1 was developed from branch nodal bud culture of the parental stock *Bambusa balcooa* a thick wall village bamboo of Bangladesh. Multiple shoot production of a single shoot was optimized and observed a vigorous growth in different culture media. Each shoot produced a mini clump within 6-8 weeks with maximum shoots (40 > nos/ culture) in the culture vessels. The rooted shoots were transferred in soil for hardening under green house and nursery. The hardened plantlets were produced profuse multiple shoots and grew luxuriantly in polybags. Selection was made among the plantlets for their field evaluation. Field trails were done at different locations of the country in 2005 and the subsequent years. Performance of seedlings was observed at different locations and it was found promising. A three years old clump of *Bambusa balcooa* BB1 produced maximum number of culms which was recorded as 30.4 nos/clump in the field. This value is at least three times higher than the rhizome produced clump of the parental stock. Average culm height and diameter was recorded as 19.3 meter and 7.7 cm respectively which is also higher than the rhizome produced clump. With the proper management, each seedling formed a full clump within 3-4 years of planting with maximum numbers of new bamboo. Seedling survivality in the field level was found 100%. No infection of diseases was observed. Later on the developed bamboo variety was multiplied for mass production and wider distribution among the farmers. The new variety was conserved at BFRI bambusetum and farmers field as a source of germplasm for future use.

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Introduction

Bamboo is emerging as one of the most important 21st century crop, since it produces food and wood. It is one of the fastest growing, annually renewable and harvestable plants with highest productivity and short harvesting cycle. It has a great potential in poverty reduction, industrial and sustainable development in rural areas. It also playing important roles for preserving our environment i.e. soil conservation and flood control, soil health, river bank protection, wind break etc. Bamboo plantation can act as wind breaks and help in soil conservation and flood control. Therefore, in Japan bamboo cultivation has been recommended since sixteenth century for the protection of river bank. Due to the extensive root and rhizome system bamboo effectively bind the top 33cm (1 feet) of soil, critical for soil health. Because of the dense surface roots, bamboos provide protection against sheet and gully erosion more effectively than most tress. An expansion of bamboo plantation, especially on the exposed and degraded slopes can help prevent many potential disasters in mountain areas. In China vas areas of hilly regions are covered by bamboos, which form a green protective cover. This resulted in minimal erosion, and streams and rivers flowing in these regions remain clear of suspended silt. The rural people in Bangladesh, on the basis of their indigenous knowledge have been cultivating bamboos on the canal and pond bank for stabilizing the soil and also a wind break or shelterbelts. A single bamboo plant could bind up to 6.0 m3 soil. Besides a bamboo clump can protect up to 12m^3 of river embankment. Bamboos efficacy as soil binder successfully used in Puerto Rico. *Bambusa vulgaris* planted at certain strategic points along the course of the river, especially at points where the river is curved, solved the problem for ever. It has been proved that in China temperate bamboos stands and leaf litter can intercept up to 25% of rainfall- values much higher than those for conifers and pines. Two rivers in China one in Yunnan Province and the other Fujian Province where succeeded in protecting river banks after soil rock engineering efforts and planting of other trees failed to yield results. Bamboos along road sides can reduce the surface erosion and the small landslides that fill the drains or blocks. In combination with selected trees that can penetrate root to a greater depth bamboo provide a low cost means of slope stabilization in Bhutan. Putero Rican researchers found bamboo to be one of the most effective in controlling landslides. There are more than 1500 species in 75 genera of bamboos in the world. The total area of bamboo forest in the world amounts to 14 million ha distributed mainly in bamboo zones of Asia, Pacific, Americas and Africa. However, East Asia and Southeast Asia have the largest bamboo forest areas including some 80% of the species of the world. China is the world leading country both in terms of bamboo species and the growing area. It ranks first in the world. There are more than 500 species of bamboos belonging to 40 genera in China, nearly half of the globe's total. The number of species and genera are more than in any other country or region in the world.

There are about 9 genera and 33 species of bamboos growing throughout the country. It has 5 lac hectares bamboo plantations both in village and forest lands. Seven bamboo species are grown in the forest land covering 2 lac hectares and others 26 species are found as the cultivated bamboos in the village land covering 3 lac hectares [1]. Among them *Bambusa balcooa*, local name (Borak bans) is one of the important thick wall village bamboo.

The high utility of this bamboo has made it vulnerable to the rural communities for making houses and paper manufacturing units where it is consumed as a basic raw material. It is also used in furniture manufacturing as an alternative of timber. So demand for this bamboo is increasing day by day of its diversified use with the increasing of population in our country for which it vastly exceeds supply. Therefore, the gap between the supply and demand is to be narrowed by large-scale production of bamboo. The greatest problem in the cultivation of this renewable resource is the difficulty in raising propagules every year because of long and often unpredictable flowering cycle 25-80 years [2]. Conventionally bamboos are propagated through rhizome, seed, branch cutting, culm cutting, ground layering, etc. in different parts of the world but no one method of propagation of bamboos is universal and effective for all the species. Vegetative propagation methods have limitation for mass propagation because propagules are difficult to extract, bulky to transport and planting materials are insufficient in number for large-scale plantation [3,4]. Seasonal dependence, low survival rate and limited rooting of the propagules are another limitation [5]. In fact, vegetative propagation by rhizome or offset is an age-old method but it is unsuitable for large scale plantation for due to limited availability rhizome and offsets along with the bulkiness, difficulties, in extraction and transportation. The saplings have also been found to grow slowly in plantation [2,6]. Besides, the survival potential of rhizome saplings is not always satisfactory and clumps lose their regenerative potential if more rhizomes (3-4) are extracted from a single clump. Considering problems encountered in both sexual and asexual propagation as well as the growing interest of countries economy and ecological benefit a method for large scale production of bamboos highly desirable. So the present research has therefore been design to develop an efficient and reproducible regeneration protocol for the new bamboo variety *Bambusa balcooa* BB1 using nodal bud culture and subsequent field evaluation of the regenerated plants.

Materials and Methods

Sources of Explants

The study was conducted in Plant Tissue Culture Laboratory under Silviculture Genetics Division of Bangladesh Forest Research Institute (BFRI), Bangladesh and different locations at field level. The parentage of *Bambusa balcooa* BB1 i.e. *Bambusa balcooa* (Borak bans) BFRI- BB LC 34/1-8 was centralized at BFRI bambusetum in 1975. Potentials of in vitro regeneration of *Bambusa balcooa* BB1 were investigated using branch nodal bud explants. Branches were collected from 1-2 years old healthy culm as a source of explant.

Explants Preparation and Sterilization

Each single node with bud was dissected from the branch with a length of 4.0-5.0 cm for further processing. The explants were taken in a bottle and washed with detergent and rinsed under running tap water for 30 minutes. Then it was carried under laminar air flow. The surface sterilization was started with one drop of tween 20 for 7-10 minutes with frequent shaking. Later on washed with sterilized distilled water for 2-3 times. After washing, the explants were immersed in 70% ethanol for 1 minute and then surface sterilized with 20% Clorox® for 15 minutes followed by rinsing with sterilized distilled water for three times. Finally, sterilization was done with 0.1% $Hgcl_2$ treatment twice for 10 and 5 minutes subsequently followed by rinsing with sterilized distilled water for four to five times to remove the $Hgcl_2$ traces.

Culture Media Preparation

The sterilized nodal buds were inoculated onto MS medium comprising 3% sucrose as carbon source and 2.8 gm/L gelrite as solidifying agent [7]. Various plant growth regulators such as; cytokinins (BAP & Kn) and auxins (IBA & NAA) were used to prepare MS medium for culture establishment, multiple shoots production and root induction from the base of excised new shoots. The pH of the medium was adjusted to 5.8 using 0.1N NaOH or 0.1N HCl before addition of gelrite and sterilized by autoclaving at 1.08 kg/cm^2 pressure and 121°C for 20 minutes.

Culture Conditions

The cultures were incubated at 25±2°C under cool white and fluorescent light of 2000-2500 lux, relative humidity about 60-80% and 16/8 hours photo and dark period were maintained in growth chamber, respectively. These culture conditions were used in all the experiments mentioned below unless otherwise stated. Observations were made at regular intervals and recorded the responses of explants at every step.

Culture Establishment, Multiple Shoots Production and Optimization

The aseptic nodal buds were cultured on MS medium supplemented with 0.0 (MS0/control), 0.5, 1.0, 1.5 and 2.0 mg/L of BAP alone for bud breaking and shoot production. Number of explants induced shoots through bud breaking and their morphological responses were observed periodically. The shoots were separated from the node and cultured on shoot producing medium. To optimize the shoot production, single shoot was cultured on MS medium supplemented with different concentrations of BAP (0, 1.0, 2.0, 3.0 4.0 and 5.0 mg/L) and Kn alone and/or in combination. Similarly, effect of sub-culturing and the strength of sucrose level and culture periods were evaluated. Rate of multiplication of shoots and their growth were recorded up to 3-8 weeks of each culture.

Development of Roots at the Base of the Shoot, Hardening and Acclimatization of Plantlets

In vitro elongated shoots (6-7 cm) with at least 3-4 nodes were taken out from the culture vessel and transferred to half strength MS medium with different concentrations (0.0, 0.5, 1.0, 2.0 mg/L) of IBA for root induction. The rooted shoots were taken out from the culture vessels, washed thoroughly under running tap water to remove the debris gelling agent with care and transferred to a pot (10 cm x 9 cm) filled with 2:1 garden soil and compost. The potted plants were kept inside the green house for adaptation and maintained the humidity and temperature through misting. Within 10-15 days each plant produced new leaves with shoots and resumed its growth. The tissue culture plants were brought out from the greenhouse and putted under full sunlight in the nursery for further growth up to the planting season. About 95% potted plants were established successfully.

Field trail and Evaluation of Regenerated *Bambusa Balcooa* BB1

Field trails and evaluation of the newly introduced tissue culture saplings were done at different locations of the country in 2005 and subsequent years. Four hundred saplings were planted per hectare with the spacing of 17' X 17' (plant to plant distance.). The standard hole size for each plant was 1.5' X 1.0' (depth X wide) and initial fertilizer like as 10 kg Cowdung, 10 gm Urea 10 TSP and 5 gm MoP were putted inside the hole for each seedlings before planting. Usually the planting season was June to August every year. Rhizome saplings of parental stock (check) B. *balcooa* were also planted for control. Different morphological characteristics were recorded from planting to harvesting period of each plant and analyzed in compare to the check plants.

Statistical Analysis

All experiments were performed as Completely Randomized Design (CRD). Data were analyzed using statistical analysis system (SAS v9.3) and means were statistically compared using LSD test. The significance level was set up at $p < 0.05$. Three replications were considered for each treatment and repeated thrice.

Results

In vitro Culture Initiation and Axillary Shoot Formation

In vitro culture initiation of *Bambusa balcooa* BB1 was started with nodal bud breaking through axillary shoot formation from the parental stock *Bambusa balcooa*. The nodal explants were cultured on full strength MS medium supplemented with or without growth regulator adding vitamin B5. In the present study, experiments were conducted on MS medium supplemented with lower concentrations of BAP like as 0.5, 1.0, 1.5 and 2.0 mg/L and without any growth regulator as a control for bud breaking and shoot production. The influence of growth regulator on bud breaking and shoot formation was observed. It was found that addition of growth regulators in culture medium resulted in faster growth and maximum bud breaking of explants while the culture without growth regulator (MS0) takes longer time to initiate the culture and further growth. The highest 90% explants established and produced axillary shoots in MS medium supplemented with 1.0 mg/L BAP followed by 1.5 mg/L BAP (68%) and the lowest 20% in the PGRs free MS0 medium (Table 1, Fig. 1A). Besides, it takes 7 days only to initiate the culture in BAP supplemented media whereas more than 15 days in BAP free media. The growing shoots with 2.0 cm in length were separated from each node and transferred in shoot producing medium for multiple shoot production and optimization.

Table 1: Effect of BAP Fortified Basal Media* on Bud Breaking and Axillary Shoot Formation from Branch Nodal Explant After 28 days of Culture

Treatment	% of Explant Induced Shoots	Mean no. of Shoots	Mean Length of Shoots (cm)	Days till Initiation
MS + BAP (Mg/L)				
0.0	20 ±0.57	1.00 ±0.28	1.52±0.002	15 ±0.28
0.5	51 ±0.28	3.00 ±0.28	2.80±0.05	10 ±0.28
1.0	**90 ±0.76**	**6.20 ±0.05**	**4.95±0.002**	**7 ±0.5**
1.5	70 ±0.28	4.30 ±0.1	3.76±0.07	8 ±0.28
2.0	60 ±0.57	3.45 ±0.005	3.55±0.01	8 ±0.5

Medium: MS+additives, Mean ±SE, n=3 replicates

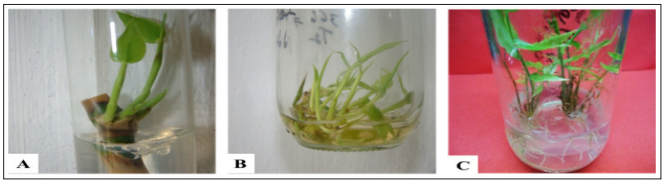

Figure 1: *In Vitro* Culture Establishment, Multiple Shoot and Root Induction of *Bambusa Balcooa* BB1 (A-C). Nodal Bud Breaking and Culture Initiation on MS + 1.0 mg/L BAP+ 3% sugar (A). Multiple Shoot Production on MS + 3.0 mg/L BAP + 1.0 mg/L Kn +4% Sucrose After 28 days of Culture (B). Root Induction on the *in Vitro* Grown Shoot. ½ MS + 1.0 mg/L IBA+ 2% Sucrose after 28 days of Culture

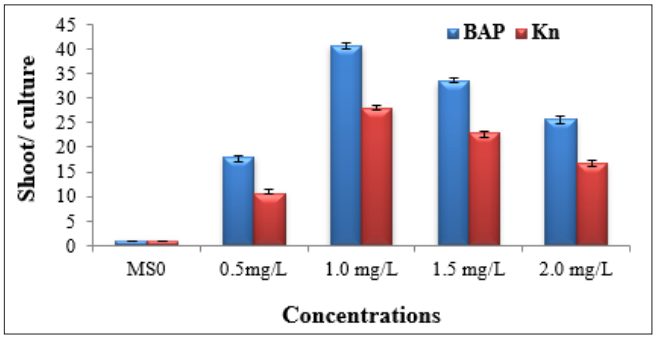

Figure 2: Effect of different Concentrations of BAP and Kn Supplemented with MS Medium on Multiple Shoot Production of *Bambusa Balcooa* BB1. The Vertical Bar Represents the Standard Error

Shoot Multiplication and Optimization

The effect of plant growth regulators on multiple shoot formation and optimization of *in vitro* grown single shoot were tested on MS medium supplemented with different concentrations (MS0, 1.0, 2.0, 3.0, 4.0 and 5.0 mg/L) of BAP and Kn. The results showed that MS medium without plant growth regulators induced little number of shoots whereas the supplementation of plant growth regulators enhanced shoot formation rate. Between the two cytokinins BAP was found more potential than Kn for new shoot formation. The maximum number of shoots produced per culture in MS medium supplemented with 3.0 mg/L BAP, followed by 3.0 mg/L Kn. The mean number of shoots was found 40.66 and 28.0 per culture respectively after 12 weeks of culture initiation (Figure 1B, Figure 2).

The multiple shoots production rate increased per culture for the both cytokinins BAP and Kn between the ranges of 1.0 mg/L to 3.0 mg/L. However, both the cytokinins BAP and Kn alone enhanced the shoot proliferation but the regenerated shoots remain stunted in their growth. To enhance the shoot growth the combined effect of BAP and Kn was evaluated. The best concentration of BAP (3.0 mg/L) was supplemented with different concentrations of Kn (0.0, 1.0, 2.0, 3.0 and, 4.0 mg/L) in the same medium. A positive effect was observed in both shoot proliferation and shoot growth as well. The average highest shoot number per culture (25.33) and average shoot length was recorded as (6.00 cm) on medium containing MS + 3.0 mg/L BAP + 1.0 mg/L Kn + 4% sugar after 28 days of culture (Table 2).

Table 2: Combined Effect of BAP and Kn on Shoot Multiplication of *Bambusa Balcooa* BB1 After Four Weeks of Culture

Hormonal conc. (mg/L)	Mean No. of Shoot/ culture	Mean no. of Shoot length (cm)
3.0 BAP + 0.0 Kn	12.00 ± 0.28	3.30 ± 0.18
3.0 BAP + 1.0 Kn	25.33 ± 0.5	6.00 ± 0.35
3.0 BAP + 2.0 Kn	20.00 ± 0.5	5.56 ± 0.01
3.0 BAP + 3.0 Kn	15.33 ± 0.28	4.00 ± 0.16
3.0 BAP + 4.0 Kn	10.00 ± 0.28	3.95 ± 0.48

Medium: MS+ additives, mean ± SE, n= 3 replicates

Effect of Different Strength of Sucrose on Multiple Shoot Formation

The sucrose level of cultures was optimized in MS medium containing 10, 20, 30, 40 and 50g/L. The number of shoots per culture increased in the media having sucrose level from 10 to 40g/L supplemented with growth regulators. The media having MS+ 3.0 mg/L BAP + 1.0 mg/L Kn + 40g/L sucrose produced the maximum shoots with a mean of 40.66 per culture after 8 weeks. Meanwhile media containing 50g/L sucrose produced less number of 33.00 shoots per culture after the same period of time. (Figure 1B, Figure 3).

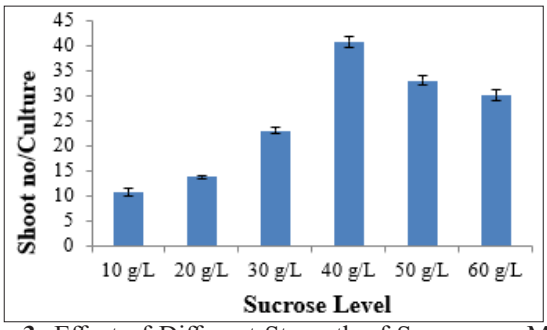

Figure 3: Effect of Different Strength of Sucrose on Multiple Shoot Production of *Bambusa Balcooa* BB1 in MS Medium. The Vertical Bar Represents the Standard Error

Effect of Sub Culture on Multiple Shoot Production

The effect of sub culturing on multiple shoot production was also evaluated. Every 4 weeks of interval sub-cultures were maintained for multiple shoot formation in the shoot producing media. It was observed that the new shoots were regenerated in each sub-culture without loss of morphogenic responses. In the first sub-culture, the mean of shoots per culture was 15.66 and it increased up to the 3rd sub-culture as 37.66 shoots/culture. However, in fourth sub-culture the shoot number decreased as 27.66 shoots/ culture subsequently (Figure 4).

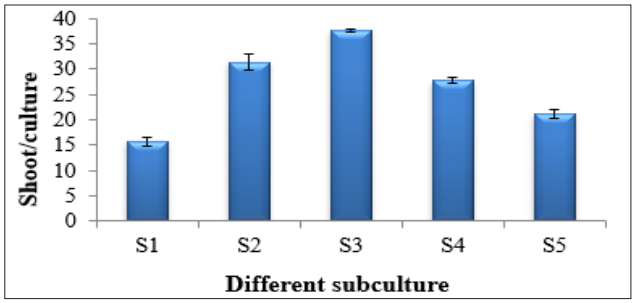

Figure 4: Effect of Different Sub Culturing on Multiple Shoot Production of *Bambusa Balcooa* BB1 in MS Medium. The Vertical Bar Represents the Standard Error

Effect of Different Concentrations of Auxin on *in Vitro* Rooting

Optimization of *in vitro* rooting of excised shoots were carried out in ½ MS medium supplemented with different concentrations of IBA viz. 0.0, 0.5, 1.0, 2.0, 3.0 and 4.0 mg/L. It was observed that no roots were induced in the auxin free MS medium. The average number of root formation was significantly higher on hormone supplemented medium. However, medium containing 1.0 mg/L IBA induced fastest rooting and supported a higher number of roots per culture. It was about 90% culture induced roots on the base of excised shoots in this medium. Among the different concentrations of IBA, the maximum mean number of roots 12.00 per shoot was recorded after 4 weeks of culture. (Figure 1C, Figure 5).

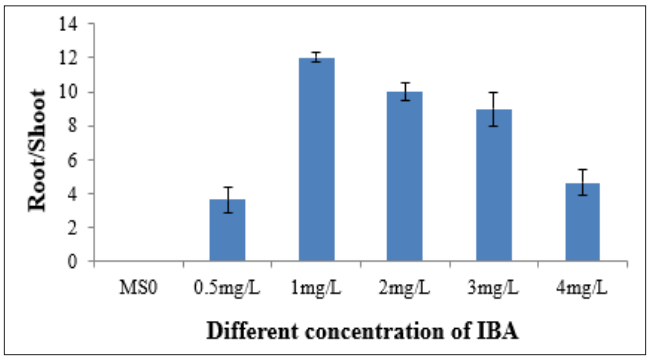

Figure 5: Effect of Different Concentrations of IBA on Root Induction of *in Vitro* Grown Excise Shoots of *Bambusa Balcooa* BB1

Acclimatization of Plantlets

In vitro rooted plantlets with culture bottles were brought to the green house for initial hardening. After 2-3 days, the plantlets were removed from the culture tubes and planted in small plastic pot/poly bag containing forest soil and compost (3:1) and kept in the green house followed by transfer to net house after another 2–3 weeks of hardening. Finaly the acclimatized and hardened plants (1–2 feet height) were transferred to the open sun in natural conditions. The survival rate was found 90 -95% after 60 days of hardening of the tissue culture produced *Bambusa balcooa* BB1seedlings are in the nursery (Figure 6A).

Figure 6: Plant Production and Field Performance of *Bambusa Balcooa* BB1 (A-C). Transplanted Rooted Plantlets in Poly Bag and Hardened in Nursery (A). Growing Clump of BB1 in the Field, a 3 Years Old Clump (B) and 4 Years Old Clump (C)

Field Trail and Evaluation of New Bamboo Variety *Bambusa Balcooa* BB1

The hardened saplings were brought out for field trail and evaluated considering the different morphological characteristics expressed in field condition. Rhizome saplings were used as control treatment in comparison with saplings of *Bambusa balcooa* BB1. In June 2004 planted the saplings at different locations of the country. Initial data were collected on April 2005 after eleven months of the planting. Data on survivality and the plant growth rate showed highly satisfactory results. About 100% saplings were survived at different plantation sites with an excellent growth

rate producing a large number of new shoots. It was observed that each plant produced maximum number of new culm and formed a complete clump within 3-4 years with a number of mature harvestable bamboos. Whereas Rhizome producing sapling of the parental stock produced a minimum number of new culms with slow growing. It was observed that a3 years old clump of the new bamboo variety BB1 produced maximum mean number of culms 30.4 nos which was at least three times higher than the parental stock rhizome saplings. Average culm height and diameter was recorded as 19.3m and 7.7 cm respectively which was also higher than the parental stock (Table 4, Figure 6B&C).

Table 4: Location wise Performance of different Characters of Proposed Bamboo Line in Compare to Parental Stock after 3 Years of Plantation

Parameters	Proposed Bamboo Line						Parental Stock Rhizome (check) *B. Balcooa*					
	Location											
	Faithong, Bandarban		CU Campus		BSCRI, Ishwardi		Faithong, Bandarban		CU Campus		BSCRI, Ishwardi	
	Ave.	Max	Ave.	Max	Ave.	Max	Ave.	Max	Ave.	Max	Ave.	Max
Culm no. / clump	10.13	13.40	16.33	24.0	17.03	30.40	5.00	6.60	5.50	6.00	5.80	6.30
Culm ht. (m)	11.50	13.20	10.63	14.5	14.60	19.30	9.80	12.30	9.70	13.0	12.40	17.30
Culm dia (cm)	6.80	7.70	5.80	7.0	6.03	7.60	5.02	6.80	4.80	6.90	5.00	6.20
Internode no.	60.60	70.00	58.40	73.0	69.60	72.00	55.60	65.00	55.00	70.50	65.00	67.00
Internode length(cm)	22.94	34.5	19.67	31.0	25.50	39.5	17.45	20.50	17.60	19.90	23.5	27.35
Wall thickness (cm)	1.23	3.30	1.00	2.10	2.00	4.50	1.33	1.95	1.13	1.75	2.23	3.80

* CU= Chittagong University, BSCRI= Bangladesh Sugar Crop Research Institute

The new bamboo variety formed a full clump within 3-4 years of planting with maximum numbers of new culm. The survivalist of seedlings in the field level was found 100%. Usually, the bamboo seedlings were disease resistant. No infection of diseases was observed. The field evaluation was continued for the next 3 to 5 years of new variety.

Discussion

In vitro shoot multiplication and mass production of *B. balcooa* BB1 was initiated through branch nodal bud culture in MS medium supplemented with different concentrations of BAP (0.0, 0.5, 1.0, 1.5 and 2.0 mg/L) and without BAP. The influence of plant growth regulator was evaluated on bud breaking and axillary shoot formation in compare to the control treatment. It was observed that the explant responded faster to bud breaking and shoot formation in medium having growth regulator than the medium devoid of plant growth regulators [8]. They stated that the supplementation of plant growth regulators was positively influenced the shoot proliferation of *Phyllanthus embolic.* The apical shoot tip of *P. embolic* enabled to produce shoots in MS medium supplemented with different concentrations of cytokinin BAP to initiate the culture. The germination competence of the seed explants seems to be markedly influenced by different growth regulators in nutrient media [9]. MS with or without growth regulators was found to be effective for the germination of immature seeds [10]. BAP concentration significantly affected the seed germination and shoot initiation ($p<0.001$), i.e., days required for germination, number of shoot and shoot length of *D. giganteas* [2]. It was also observed that the concentrations of cytokinin had a crucial effect on shoot formation. BAP and Kn at higher concentrations significantly reduced the number of shoots formed per explants. These findings were consonance with the findings in *Opuntia ficusindica* and *Zingiber petiolatum* [11,12]. The result was supported and mentioned that the shoot initiation percentage was greatly influenced by the type and concentrations of BAP in *D. asper* and *D. Hamiltonian* respectively [13,14]. The requirement of exogenous plant growth regulators for *in vitro* regeneration depended on the endogenous level of the plant tissue, which varied with organs, plant genotype and the phase of plant growth [8,15]. The regeneration efficiency depended on plant growth regulator concentrations and combinations [16,17]. Explants cultured on MS medium without cytokinin's developed 1-2 shoots indicating that the explants had enough endogenous cytokinin's to induce limited number of shoots upon culturing. While explants cultured on MS medium supplemented with higher concentration of BAP induced a number of shoots without roots. This might be due to the enhanced level of cytokinin's and auxin ratio favors only shoot regeneration in absence of equivalent level of auxins inside the plant. The micro shoots produced in lower levels of BAP and Kn were green, taller having bigger leaves than those produced at higher concentration of cytokinin's. In MS medium containing BAP, the plantlets were slightly taller than those produced in MS medium supplemented with Kn. Cytokinin types had a strong effect on the quality of the shoots produced [18]. The growth of plantlets was retarded at higher concentration of BAP. It was reported that cytokinin's commonly stimulate shoot proliferation in *Capsicum sp*. and *P. embolic* which inhabit the shoot elongation [8,19]. The treatments of *Prunus sp*. with BAP concentrations promoted the shoot numbers per explants but decreased the shoot length and negatively affected shoot development [20]. The highest shoot length was recorded as in the same media composition. BAP was the most widely used cytokinin for multiple shoot formation [21]. The superiority of

BAP over Kn and other cytokinin's for multiple shoot formation was also demonstrated *Salix pseudolasiogyne* [22]. The results showed that the addition of lower level of BAP and Kn to the medium enhanced the shoot regenerative ability in *D. giganteas* [2]. However, the number of multiple shoots reduced at higher concentration of BAP and Kn. *In vitro* grown higher plants are fully autotrophic [23]. Therefore, plant tissues culture required an exogenous carbon source and generally sucrose, is an essential ingredient of all culture media [24]. This is because in the culture vessels, photosynthesis was insufficient due to the growth taking place in conditions unsuitable for photosynthesis or without photosynthesis (in darkness) and the concentration of carbon dioxide (CO_2) was limited for photosynthesis. Specific carbohydrate may have different effects on morphogenesis *in vitro*, thus the carbohydrate requirements must be defined and optimized for each propagation system [25]. The effect of carbohydrate type and concentration on shoot proliferation were genotype dependent. In this study 4% sucrose was a most optimum carbon source for *in vitro* multiple shoot formation in *B. balcooa* BB1. Sucrose concentration in culture medium had significant effect on shoot and root regeneration [26,27]. Higher concentration of sucrose was deleterious to shoot growth and caused decrease in dry matter accumulation due to decrease in osmotic potential of the medium [23]. Increasing sucrose levels more than 7% in the medium caused osmotic stress which significantly inhabited the growth of *Parthenium argentum* [28]. In this study, no shoot proliferation was observed in the carbohydrate free medium. Sub culture exercised an important role on the multiplication of cultures [29]. The duration of culture depended on plant species, growth rate, physical and physiological condition as well as the development stage of the plant [30]. However, sub-culturing performed at 2 weeks interval did not enhance the production of multiple shoots but produced bigger shoot which was dark green in color. Plant tissue might have a chance to develop mutation due to repeated sub culturing, or it might produce callus, became abnormal and reduced the proliferation rate. The result revealed that *Bambusa balcooa* BB1 did not show morphological changes after repeated sub-culturing. Likewise, it was reported that the long-term culture of *Digitalis obscura* did not affect the genetic stability *in vitro* [31]. The shoot production ability varied greatly among different species. In this study, the number of shoots and growth declined by repeated sub-culturing after four sessions. Repeated sub-culturing caused shoots reduction in *Zingier officinal*, *Curcuma domestic, Alpinia galanga, and Kaempferia galanga* [32]. In contrast, repeated sub culturing of *in vitro* shoot of *Spilanthes acmella* increased the multiple shoots formation by three hold [33]. *In vitro* rooting of shoots was tested in half strength MS with different concentrations of IBA and NAA. All media responded positively for root formation but no rooting was observed in the hormone free medium. However, in the present study, IBA was found to be more effective than NAA for *in vitro* rooting of *Bambusa balcooa* BB1. No significant result was found in the media supplemented with different concentrations of NAA. In woody plants usually, low level of salt concentration is sufficient for rooting of shoots. Similar observation was made by [34]. Superiority of IBA over NAA for *in vitro* root induction on *B. balcooa* was reported [35]. *In vitro* developed plantlets have morphological and physiological abnormalities due to the *in vitro* culture conditions [36]. Direct transfer of *in vitro* plantlets to *ex vitro* condition may result in rapid wilt and death [37]. Therefore, acclimatization is essential for the survival and successful establishment of plantlets [38]. Plants grown *in vitro* were gradually acclimated to the external environment in the greenhouse and the nursery. Addition of compost to the soil increases the porosity resulting in better aeration of roots and thus better growth of the plantlets. Season was found to influence the survival rate and growth of the plantlets in the field. High humidity in the environment during the rains provides optimum conditions for the survival of new bamboo seedlings in the field. Seedling number required per hectare 400 with the spacing of 17' X 17' (plant to plant distance). The standard hole size is 1.5' X 1.0' (depth X wide) for each seedling. Fertilizers requirement will vary depending upon the fertility status of the soil. However, fertilizer should be applied @ 10 kg cowdung, 10 gm urea, 10 gm TSP, and 5 gm MoP in each hole before planting the seedlings. Planting season is June to August and the harvesting season November to January each year. Well drained, sandy loam to clay loam except saline soil, with pH ranging from 5 to 8 was found suitable for cultivation of *Bambusa balcooa* BB1. During dry season depending on soil moisture content, two to three irrigations should be applied. In rainy season, irrigation is usually not necessary except during prolonged drought. More than 3 months continuous water logging at the planting stage can cause the damage of seedlings. The yield of the new bamboo variety was found 6800 culm/ha after three years of cultivation. This production is at least three times higher than that of any other bamboo species in Bangladesh. Large scale cultivation of this bamboo variety will create the opportunity to minimize the demand and supply gap of bamboos in *Bangladeshi.*

Conclusion

The results of this study allow the establishment of a protocol for *in vitro* mass propagation of *Bambusa balcooa* BB1 nodal bud culture. MS medium supplemented with 1.0 mg/L BAP with 4.0 g/L sugar may be recommended for maximum multiple shoot production. Half the strength of MS medium with 0.5 mg/L IBA found best combination for *in vitro* root induction on the micro-shoots. This protocol is an efficient means for the large-scale propagation of *Bambusa balcooa* BB1 which may be applicable for other bamboo species. The distinct characteristics of *Bambusa balcooa* BB1 especially the high yield production, short duration of harvesting time, field survivality and disease free make it attractive to the planters.

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