RESEARCH ARTICLE

Protocol for Induction of Multiple Shoot through Nodal Explants Culture of *Bambusa bambos* for Biomass Production

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ABSTRACT- Aim of the present study was biomass production by induction of multiple shoots from *Bambusa bambos*. In general, the efficient and reproducible procedure for the propagation of bamboo can be achieved by seed propagation, clump division, and rhizome for small scale. In the case of mass-scale propagation, this technique would be highly insufficient and inefficient. For efficient production of bamboo, micropropagation technique was used in large scale production. Nodal segment from fields grown clumps was used as the explants to develop a method of *in vitro* Micropropagation in bamboo. Plant growth hormone BAP (benzyl amino purine), KIN (kinetin), NAA (1- naphthalene acetic acid), IBA (indole-3 butyric acid), IAA (indole-3 acetic acid) was studied on *in-vitro* Micropropagation of the effective shoot and roots of bamboo. Effective axillary bud breaking was achieved in Murashige and Skoog (MS) media. Nodal explants culture was inoculated in both solid (0.8%) and liquid MS media and observed the maximum proliferation of shoot in solid MS medium (4/ nodal explants). The concentration of sucrose was varied and their growth was examined. The sucrose was optimized (3%). Under the optimized sucrose condition, the hormone was varied and growth was examined. Under this condition, BAP response was high. Thus the concentration of BAP was varied for further studies. The response was high in 3 mg/l of BAP concentration. This review briefly provided state-of-the-art information on tissue culture mediated biotechnologically interventions made in bamboo for large scale micropropagation. The established protocol will be of help to stakeholders in edible bamboo trade to conserve gene-pool and increase productivity.

Key-words- Bamboo, Benzyl amino purine, Micropropagation, Multiple shoots, Tissue culture

INTRODUCTION

Bamboo is a rhizomatous plant. It is a non-wood forestry product. It is one of the most important agriculture plants. It's belonging to family Poaceae with woody culms growing uprightly. Bamboos assume a greater significance in the Indian context because after China, India has the second largest bamboo genetic resources in the world (23 genera and 125 species). The mass utilization of bamboo resources for hand craft industries, construction, paper and pulp industries, fishery, and human consumption. The biomass production is incomparable in bamboo plant. In the recent years, extensive research regarding micropropagation of bamboos has been done [1-3]. For carrying out *in vitro* propagation, different explants have been employed by different workers but nodal explants and seeds are the most commonly used ones. Initially, Successful multiplication of shoots derived from nodal explants from the adult plants of *B. bambos*, *B. vulgaris*, and *Dendrocalamus strictus* [4]. Although, the establishment of micropropagation protocol through forced axillary branching in *Dendrocalamus longispathus* on MS medium supplemented with BAP and Kn [5]. Similarly, In-vitro micro propagation of *B vulgaris* by inter-node explants [20]. Apart from mass production and cultivation, various bamboos based fermented foods were produced by Galo (Sub-tribe) of Arunachal Pradesh, India [21].

Several workers have reported higher rates of shoot multiplication and improved growth in liquid medium [6,7]. Induction of multiple shoots (Shoot clumps) rather than single shoot was found to be effective for multiplication of bamboo plants [2,8]. Similarly, propagules containing a minimum of three to four shoots proliferated at a maximum rate whereas single shoots proliferated at a much slower rate [9]. Protocols for plantlets regeneration were developed for *B. tulda* through seeds [1] and nodal explants [10], previously reported. The aim of the present study to initiate

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multiple shoots (Shoot clumps) from the nodal explants culture of Bamboo sp. under *in-vitro* condition.

MATERIALS AND METHODS

Sources of plant materials- Adult nodal cuttings of *Bambusa bambos* were used as plant materials in the study. The explants were collected from a garden of Kumaraguru College of Technology, Coimbatore, Tamil Nadu, India in the duration of 2017.

Preparation and sterilization of nodal explants- After collection of shoots with internodes of bamboo cultivars, these were thoroughly washed with the running tap water for 5 to 6 times, explants were washed in 1% sodium hypochlorite solution for 2–3 min. The time duration varies with different kinds of explants. The 1% sodium hypochlorite solution was decanted in empty beaker and the explants were washed 2–3 times using sterile distilled water. Finally, the explants were rinsed with 70% ethanol for 1–5 min. Transfer the surface sterilized explants into a sterile Petri dish.

Culture media, carbon source, hormone preparation, and sterilization- Murashige and Skoog (MS) medium was used as the basal medium for shoot induction of the collected sample of explants. The energy source for the micropropagation of shoots was reagent grade sucrose. MS medium supplemented with different concentration of sucrose (10–50% Data not shown) with PGR in various combinations. After 8 weeks of culture the number of explants responding to various treatment, a rate of shoot multiplication were recorded. All experimental studies triplicate.

Effect of media on auxiliary bud proliferation of nodal explants- For induction of multiple shoots, two different types of media had taken for this study. Both liquid and solid media (MS) were used at the same concentration of all macro and micronutrients supplemented with PGR.

Effect of PGR on auxiliary bud proliferation of nodal explants- The explants (nodal cutting) were inoculated in MS Medium, which was supplemented with different PGR (IAA, IBA, BAP, IAA+IBA and IBA+BAP) at 3% used for multiple shoot initiation from auxiliary bud from nodal explants.

Effect of BAP concentration on auxiliary bud proliferation of nodal explants- The explants (nodal cutting) were inoculated in MS Medium, which was supplemented with different concentration of BAP (1,2,3,4, and 5 mg/l) used for multiple shoot initiation from auxiliary bud from nodal explants.

Shoot development- The cultures were carefully observed for shoot regeneration and when any bud initiation observed it was recorded carefully and percentage of direct shoot development was calculated by the following formula.

$$\text{Direct shoot development (\%)} = \frac{\text{No. of nodal explants cultures with direct shooting}}{\text{No. of nodal explants cultures inoculated}} \times 100$$

RESULTS AND DISCUSSION

Bud sprouting from nodal segment- The explants (nodal cutting) was inoculated in MS medium, which was supplemented with different concentration and the combination of carbon source and hormone for shoot initiation, which was successfully developed. At first, the response was compared by supplementing same concentration of nutrients and hormones in both solid and liquid medium as shown in Table 1 & Fig. 1.

From the below results, we proceeded with a solid medium because of its high and effective response. Next concentration of carbon source was optimized along with combinations of hormones. Varying sucrose concentration (carbon source) has a great impact on the number of shoots initiated in each nodal cutting. This was done to get an optimum concentration of carbon source and the suitable hormone.

Effect of PGR concentration (%) on auxiliary bud proliferation of nodal explants- In these studies, various PGR studied on auxiliary bud proliferation of nodal explants under *in-vitro* conditions. 80% response was recorded in IAA (3 mg/l) and IAA (3 mg/l)+IBA (3 mg/l) with low number of shoots initiated (Fig. 2) 100% response was shown in three hormones BAP (3 mg/l), IBA (3 mg/l) and BAP (3 mg/l)+IBA (3 mg/l) but the number of shoots formed in each explant was high in BAP (5 shoots) while others two have 2 and 3 shoots respectively. This concludes that BAP as an effective hormone. Previously study reported that optimal shoot growth was obtained on Modified Murashige and Skoog (MMS) medium supplemented with 2 mg/l of BAP [11]. Previously various research reports supported that onventional method as well as *in-vitro* production of plants can be achieved through the selection of desirable explants for large scale multiplication of lite bamboo [12-15].

We observed number of shoot initiation in the medium supplemented with BAP. Hence, the concentration of BAP was varied and the optimal concentration was determined.

Table 1: Comparison of liquid and solid medium for bud sprouting and induction multiple shoots from nodal explants

Media	Time(days) required to initiate shoots	No. of buds / nodal explants	No. buds breaking / nodal explants	No. of shoots initiated / buds / per nodal culture	% of success rate*
Solid (0.8%)	6	1	1	4	100
Liquid	10	2	2	2	50

***Based on number shoots formed after bud breaking**

Fig. 1: Effect of media on bud breaking and induction of multiple shoots (After - 10th day of incubation
A- Liquid media; **B-** Solid media

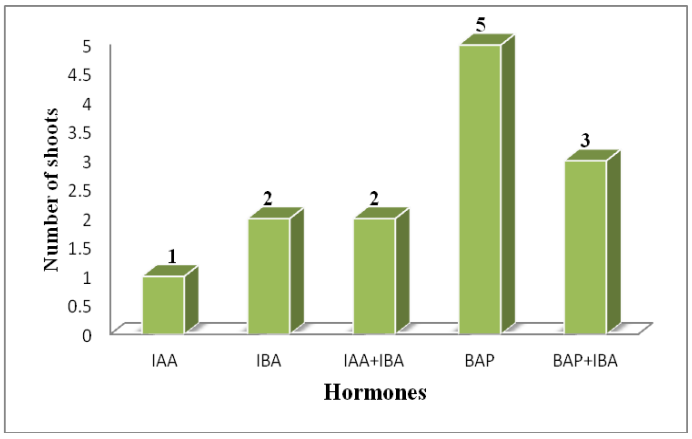

Fig. 2: Effect of various PGR and their interaction on auxiliary bud proliferation of nodal explants

Effect of BAP concentration (%) auxiliary bud proliferation of nodal explants- In this study, concentration of BAP (%) was varied in the range of 1 (mg/l)–5(mg/l) in that higher response was observed in 3 (mg/l). Our results are shown in Fig. 3 & Fig. 4. The maximum number of shoots (4) was observed in at 3 (mg/l). For remaining concentration, 2 shoot for both 1 and 2 mg/l and 3 shoots for concentration of 4 and 5 mg/l. Nodal explants (1 to 5 years) of various species of bamboo were being previously studied for mass production bamboo under *in-vitro* micropropagation.

Species such as *B. balcooa, B. nutans, Bambusa salarkhanii, B. vulgaris, B. vulgaris* var *striata, Thyrsostachys oliveri* [16]; *Bambusa bambos* [17]; *D. hamiltonii* [18,19]; *Guadua angustifolia* [7] was cultured on BAP fortified MS medium for shoot proliferation.

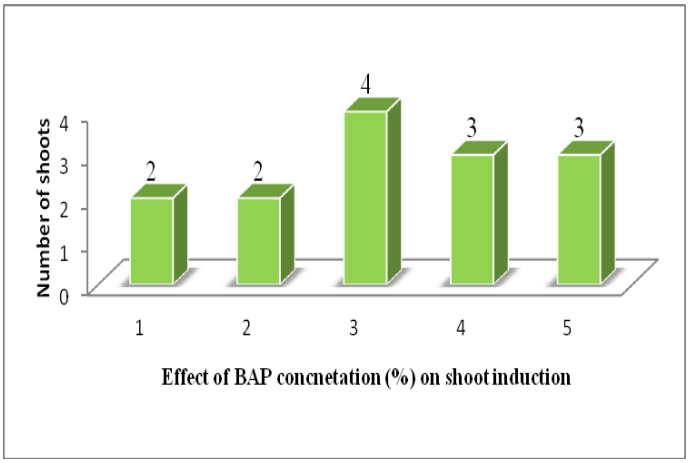

Fig. 3: Effect of BAP concentration (%) and their interaction on auxiliary bud proliferation of nodal explants

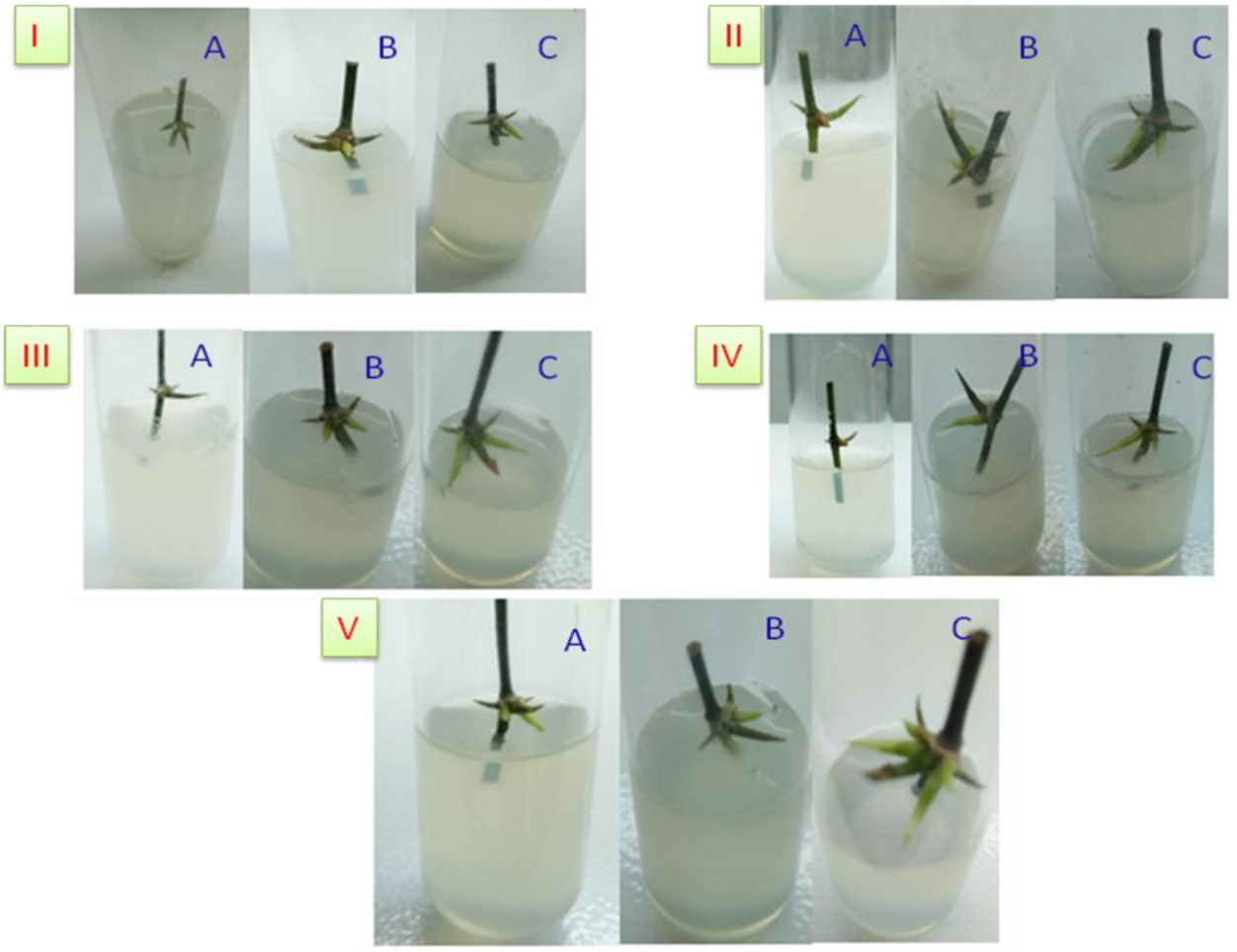

Fig. 4: Effect of BAP concentration (%) on induction of shoot: **I-** 1%; **II-** 2%; **III-** 3%; **IV-** 4%; **V–** 5%; **A-** after 5 days; **B-** after 10 days; **C-** After 15 days

CONCLUSIONS

We were concluded that the micropropagation *Bambusa bambos* was effectively influenced by carbon source (Sucrose) and hormone (BAP). The optimum concentration of carbon source was found to be 3%, which was 3 g/l by the heightened response of nodal cutting. Also, the hormone BAP shown effective shoot induction due to its bud breaking ability other hormones showed comparatively low responses. The effective concentration of BAP was 3 mg/l at which no of shoot induced was more than other concentration.

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