

# *In Vitro* Propagation of *Arundinaria callosa* Munro- an Edible Bamboo from Nodal Explants of Mature Plants

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**Abstract:** A procedure for *in vitro* propagation of *Arundinaria callosa* Munro has been developed. The method allows bud-break from nodal segments containing single axillary bud on Murashige and Skoog (1962) medium supplemented with different concentrations of 6- benzylaminopurine (BAP). The early bud-break was obtained in 8.9-13.3  $\mu\text{M}$  BAP, within 8-15 days. The position of the node on the culm of lateral branches also affected bud-break percentage and multiplication, mid-culm nodes are the most suitable. The optimal concentration of 13.3  $\mu\text{M}$  BAP is found significant for shoot multiplication. Addition of 1.0  $\mu\text{M}$  3- indolebutyric acid (IBA) enhances the shoot multiplication rate. *In vitro* rooting was induced when 15  $\mu\text{M}$  IBA was incorporated for three subcultures in the shoot proliferation medium, was transferred to  $\frac{1}{2}$  strength MS containing 25 $\mu\text{M}$  IBA and 0.05  $\mu\text{M}$  BAP, and finally withdrawn from the rooting medium. The regenerants were successfully transplanted into a soil mixture for acclimatization before field planting.

## 1. INTRODUCTION

*Arundinaria callosa* Munro, is an evergreen thorny bamboo which grows abundantly in dense foggy areas of the hill peaks in Manipur (at 2,800-9,000 ft altitude) situated in the north-eastern India. The culms are 20-40 ft high and 0.6-3 inch in diameter, upper portions are reduced in size and bent downwards, short thick conical spines present at the nodes. The young juvenile shoots are edible and regarded as one of the common vegetables of the local people. These bamboo shoots are rich sources of nutrient and other minerals [1]. Another important constituent of bamboo shoots are the phytosterols which serve as precursor to many pharmaceutically active steroids found in plants [2]. Most bamboos grow naturally in mountainous areas where neither chemical fertilizers nor pesticides are applied. Therefore, bamboo shoots provide clean vegetables without pollution. In Manipur, the fermented shoot is one of the popular indigenous food items prepared by using traditional methods. Fermented young shoots of *A. callosa* (locally known as 'soidon') are used in the preparation of many traditional food items. Indiscriminate exploitation of this natural resource to meet the ever increasing demand, and insufficient attempts for replenishment and cultivation have resulted in severe depletion of wild stock, resulting in critically low population levels of this bamboo species. The conventional propagation is associated with many problems such as unpredictable flowering cycle, seed remaining viable for limited period and difficult vegetative propagation [3, 4]. Hence, the present study has been undertaken to conserve the wild stocks using *in vitro* propagation techniques for this important edible bamboo species. This paper describes an efficient and rapid method of *in vitro* propagation of *A. callosa* from nodal explants of adult grown clump.

## 2. MATERIALS AND METHODS

Nodal segments (1.0-1.5 cm) with unsprouted buds of *Arundinaria callosa* Munro were collected from the lateral branches of young culm growing in the natural forest of Leimaram hill located in the Bishnupur District of Manipur. The lateral branches for experiments were collected at two different seasons i.e. May-July (rainy season) and September-November (after raining season). Nodal segments, containing axillary buds were washed with Exalin (Merck) and rinsed thoroughly with double-distilled water. Then, nodes containing lateral spines were removed by scraping carefully with scapel before undergoing surface sterilization. These explants were surface-sterilized with 0.1% solution of mercuric chloride ( $\text{HgCl}_2$ ) for 10 mins followed by washing with autoclaved double-distilled water for 3-4 times. The sterilized nodal buds were cultured in liquid Murashige and Skoog's (MS) [5] medium supplemented with various concentrations of BAP. The pH of the medium was adjusted to 5.7 prior to autoclaving at 121°C for 15 mins. In all the cases, pH of the media was adjusted by using 1N NaOH and 1N HCl. The plant growth regulators (PGRs) were purchased from Sigma chemical Co., USA. The proliferated axillary shoots were excised and cut into 3-5 shoot clusters and cultured in liquid MS medium supplemented with various concentrations of BAP or in combination of 13.3  $\mu\text{M}$  BAP with various concentrations of IBA or  $\alpha$ -naphthalene acetic acid (NAA). After first shoot multiplication, the shoot cultures were cut into 3 shoot clusters and transferred to liquid MS medium containing PGRs. The shoots in liquid cultures were supported by using filter paper bridges and regularly transferred to fresh medium to check the influence of phenolic exudation. The number of propagules cultured and propagules derived at the end of subculture is regarded as the rate of multiplication.

For studies on *in vitro* rooting, the shoots were cut into 3-4 shoot clusters and placed on  $\frac{1}{2}$  strength MS medium incorporated with 15  $\mu\text{M}$  IBA and 13.3  $\mu\text{M}$  BAP for two subcultures. These shoots were then transferred to  $\frac{1}{2}$  strength

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MS medium supplemented with 10-25  $\mu\text{M}$  IBA before transfer to PGR-free  $\frac{1}{2}$  strength MS medium for further root growth and development. After 8 weeks of culture, the number of roots per propagule and rooting percentage was recorded.

All computations were made using SPSS (Version 14, SPSS Inc. Chicago, USA). The statistical significance was calculated by one-way ANOVA followed by Tukey's comparison tests at 5% probability test.

Field transfer of *in vitro* rooted plantlets was carried out by transferring the plantlets to pots containing sand: soil: FYM (farm yard manure) in 1:1:1 ratio, maintained at  $30\pm 2^\circ\text{C}$  and relative humidity of  $80\pm 5\%$ . These plants were supplied with half-strength MS medium (without organics) twice a week.

### 3. RESULTS

#### 3.1. Culture Initiation

Treatment with 0.1%  $\text{HgCl}_2$  did improve the frequency of aseptic cultures without adversely affecting the overall frequency of the bud-break. Considering the incidence of browning and the frequency of bud-break, 0.1%  $\text{HgCl}_2$  for 10 min was regarded as the best treatment to initiate aseptic cultures of nodal segments from mature plants. The explants collected during rainy season showed high rate of bacterial contamination. As a result, the percentage of *in vitro* bud-break was found to be low. However, the contamination rate was reduced to 30% with good response of bud-break in all the explant cultures collected after monsoon. On basal medium, the frequency of bud-break was very low. Statistical analysis showed that in the presence of BAP, there was significant increase in the percentage of bud-break (Table 1). Early bud-break response was observed in 8.9-13.3  $\mu\text{M}$  BAP within 8-15 days. In all the experiments, 1-3 shoots proliferated as a result of axillary bud-break (Fig. 1a). About 62 % cultures showed bud-break within 7-10 days, with over 3 shoots per explants in MS medium containing BAP at an optimum level of 13.3  $\mu\text{M}$  BAP. The position of the nodal

buds on the culm of lateral branches did affect bud-break (Fig. 2). Nodal explants taken from the top showed low bud-break compared to the basal and mid-culm nodes of the secondary branches.

#### 3.2. Shoot Multiplication

The axillary shoots produced from primary cultures of nodal segments were transferred to various shoot multiplication media (Fig. 1b). To study the effect of cytokinin, BAP was tested alone at different concentrations. The BAP treatments were carried out at six different concentrations ranging from 4.4-31.1  $\mu\text{M}$  (Table 2). Of these, treatment with 13.3  $\mu\text{M}$  BAP showed significant increase in shoot multiplication (Fig. 1c). Increased concentrations (22.2-31.1  $\mu\text{M}$  BAP) showed steady decline in shoot multiplication rate as well as shoot length. To improve the rate of shoot multiplication, IBA or NAA, each at different concentrations (1.0, 2.0 & 3.0)  $\mu\text{M}$  were added to liquid MS containing 13.3  $\mu\text{M}$  BAP. Of all the auxin treatments, 1.0  $\mu\text{M}$  IBA proved to be the most effective with respect to the rate of shoot multiplication and shoot growth (Fig. 1d). NAA proved to be inhibitory at all the concentrations used. The presence of NAA caused yellowing and browning of leaves.

#### 3.3. Root Induction and Plantlet Development

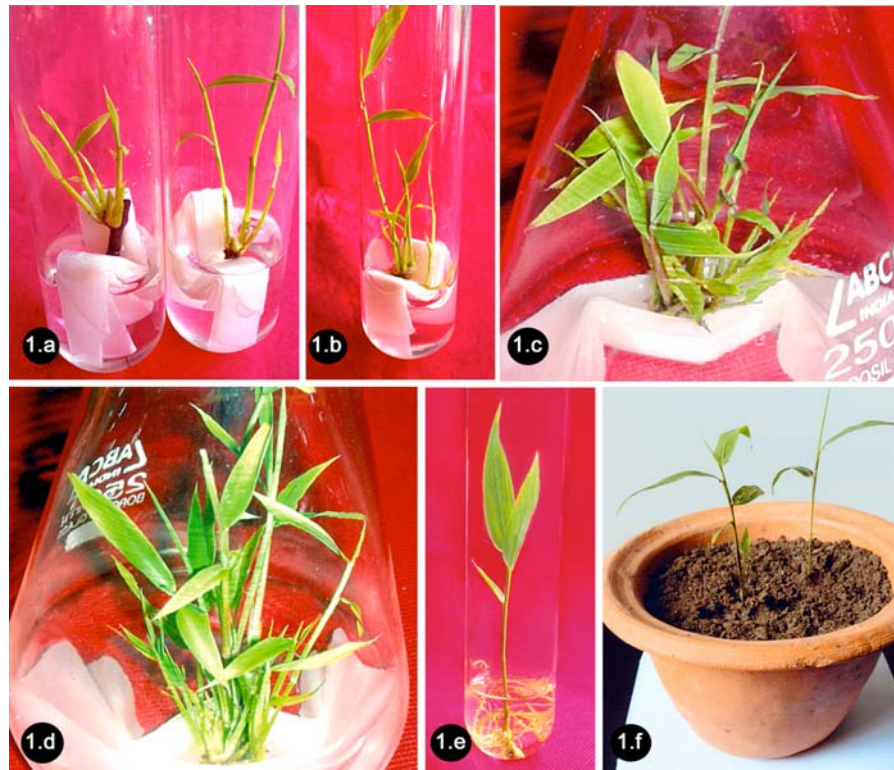
The root induction experiment was done by incorporating 15  $\mu\text{M}$  IBA to the shoot multiplication medium. Subsequently, a cluster of 3-5 shoots (propagules) were transferred to the rooting medium. No rooting occurred when 10  $\mu\text{M}$  IBA was incorporated in  $\frac{1}{2}$  strength MS medium (Table 3). 30-40% rooting was obtained in MS medium supplemented with 15-25  $\mu\text{M}$  IBA. The rooting and rooting percentage significantly increased when 0.05  $\mu\text{M}$  BAP was added to 10-25  $\mu\text{M}$  IBA supplemented medium. The maximum roots per propagules and rooting percentage obtained were 3.8 and 75% respectively on  $\frac{1}{2}$  strength MS liquid medium supplemented with 25  $\mu\text{M}$  IBA and 0.05  $\mu\text{M}$  BAP (Fig. 1e). The transfer of *in vitro* rooted shoots to PGR-free  $\frac{1}{2}$  strength MS

**Table 1. Effect of BAP Supplemented MS Medium on Bud-Break Percentage and Number of Shoots per Explant from Nodal Explants of *Arundinaria callosa*. Data Scored after 2 weeks in Culture**

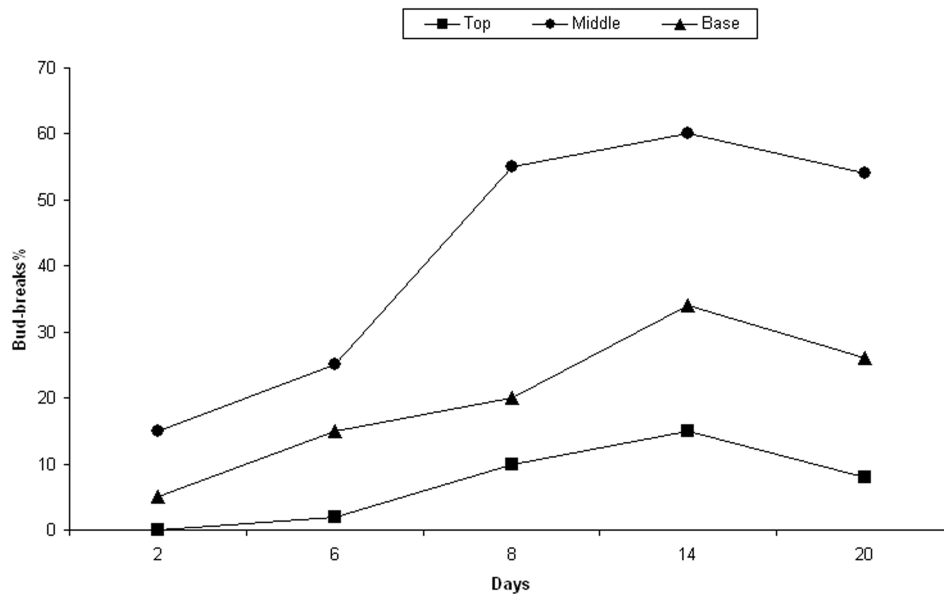
BAP Concentration ( $\mu\text{M}$ )	Bud-Break Frequency (%)	No. of Shoots/Explant*
0	34.0	$0.6 \pm 0.2^a$
2.2	52.0	$1.0 \pm 0.3^{ab}$
4.4	60.0	$1.6 \pm 0.4^{abc}$
8.9	66.0	$2.4 \pm 0.7^{abc}$
13.2	62.0	$3.0 \pm 0.6^{bc}$
22.2	58.0	$3.4 \pm 0.2^c$
26.6	54.0	$2.2 \pm 0.6^{abc}$
31.1	50.0	$1.8 \pm 0.5^{abc}$
44.4	46.0	$1.6 \pm 0.4^{abc}$

\*Mean of 10 replicates  $\pm$  S.E.

Mean followed by the same letter did not differ significantly at  $q < 0.05$ , according to Tukey's comparison test.



**Fig. (1).** Micropropagation of *Arundinaria callosa* (a) axillary bud-break in liquid medium (b) axillary shoot proliferation in MS medium (c) multiple shoots formation on MS + 13.3  $\mu\text{M}$  BAP (d) multiple shoots formation on MS + 1.0  $\mu\text{M}$  IBA + 13.3  $\mu\text{M}$  BAP (e) root formation in  $\frac{1}{2}$  MS + 25  $\mu\text{M}$  IBA + 0.05  $\mu\text{M}$  BAP (f) a micropropagated plant established on soil.



**Figure2:** Effect of bud-break percentage on the position of the node on the culm of lateral branches

**Fig. (2).** Effect of bud-break percentage on the position of the node on the culm of lateral branches.

medium produced good quality root and growth within 7-10 days. The above rooting procedure, i.e., initial exposure to IBA, subsequent transfer to medium containing IBA and BAP, and finally withdrawal of PGR from the rooting medium was found effective for overall root development. The shoots turned green and elongated after hardening. All

the plants were found to be established with 60-70% survival (Fig. 1f).

#### 4. DISCUSSION

Bamboo shoots are greatly demanded in the international market for their shoot-based food industry. Among the

**Table 2. Effect of Growth Regulators on Shoot Growth and Multiplication of *Arundinaria callosa***

Growth Regulators( $\mu\text{M}$ )	No. of Shoot Produced*	Multiplication Rate*	Shoot Length(cm)*
4.4 BAP	$6.3 \pm 1.8^a$	$1.8 \pm 0.5^a$	$3.7 \pm 0.5^a$
8.9 BAP	$13.0 \pm 2.2^{ab}$	$4.2 \pm 0.8^{ab}$	$3.5 \pm 0.3^a$
13.3 BAP	$15.7 \pm 0.9^b$	$5.2 \pm 0.3^b$	$3.3 \pm 0.3^a$
22.2 BAP	$15.8 \pm 1.5^b$	$5.0 \pm 0.5^b$	$3.3 \pm 0.3^a$
26.6 BAP	$11.7 \pm 1.9^{ab}$	$3.8 \pm 0.7^{ab}$	$3.2 \pm 0.3^a$
31.1 BAP	$11.5 \pm 2.0^{ab}$	$3.7 \pm 0.8^{ab}$	$3.1 \pm 0.3^a$
1.0 IBA + 13.3 BAP	$16.5 \pm 0.5^b$	$5.2 \pm 0.2^b$	$3.9 \pm 0.3^a$
2.0 IBA + 13.3 BAP	$15.0 \pm 1.2^{ab}$	$4.5 \pm 0.3^{ab}$	$3.8 \pm 0.4^a$
3.0 IBA + 13.3 BAP	$13.2 \pm 2.1^{ab}$	$4.0 \pm 0.7^{ab}$	$3.6 \pm 0.4^a$
1.1 NAA + 13.3 BAP	$12.3 \pm 1.8^{ab}$	$3.8 \pm 0.6^{ab}$	$3.5 \pm 0.4^a$
2.2 NAA + 13.3 BAP	$11.2 \pm 2.0^{ab}$	$3.5 \pm 0.7^{ab}$	$3.5 \pm 0.2^a$
3.3 NAA + 13.3 BAP	$8.2 \pm 1.9^a$	$2.3 \pm 0.7^a$	$2.9 \pm 0.3^a$

\* Mean of 10 replicates  $\pm$  S.E.Mean followed by the same letter did not differ significantly at  $q < 0.05$ , according to Tukey's comparison test.**Table 3. Effect of Treatments on Rooting of *Arundinaria callosa* on  $\frac{1}{2}$  Strength MS Medium**

Treatment ( $\mu\text{M}$ )	Average Root no*	Rooting Percentage
10.0 IBA	0	0
15.0 IBA	$1.2 \pm 0.6^a$	30.0
25.0 IBA	$1.4 \pm 0.2^a$	40.0
10.0 IBA + 0.05 BAP	$1.8 \pm 0.6^{ab}$	55.0
15.0 IBA + 0.05 BAP	$2.0 \pm 0.5^{ab}$	66.7
25.0 IBA + 0.05 BAP	$3.8 \pm 0.6^b$	75.0

\*Mean of 10 replicates  $\pm$  S.E.Mean followed by the same letter did not differ significantly at  $q < 0.05$ , according to Tukey's comparison test.

north-eastern States of India, the maximum production of fresh shoots comes from Manipur with production averaging 2,188 tonnes per annum [6]. Tremendous socio-economic pressures besides jhum cultivation in the natural habitats have often compelled over-exploitation of raw bamboo resources to a critical level. Tissue culture techniques offer an effective strategy for rapid propagation and mass multiplication of edible bamboo species keeping in view of their sustainable development and utilization.

The initial aim of these experiments was to establish aseptic culture conditions under which bamboos could be propagated *in vitro*. The present investigation shows that explants collected during rainy season show high bacterial contamination because collection of nodal explants are possible only during the warm rainy season (May-August) when bacterial contaminations are high. On the contrary, the successful aseptic culture initiation and frequency of bud-break are achieved when explants are collected after the monsoon. Mascarenhas *et al.* [7] reported that microbial culture contaminants obstructed *in vitro* sprouting of nodes in *D. strictus* from adult field culms. The present work on *Arundinaria callosa* shows the importance of cytokinin,

i.e., BAP for bud-break. This can be expected in general as cytokinin is known to overcome apical dominance resulting in the growth of axillary buds. Similar observations have been reported in *Bambusa bambos* [3] and *Dendrocalamus strictus* [8]. The relative position of the buds on the nodes also influenced the frequency of bud-break in the present experiments. Similar findings have also been reported in *Dendrocalamus longispatus* [9] and *Bambusa vulgaris* [10] that the mid-culm nodes of secondary branches are the potential explants for axillary shoot initiation. The occurrence of phenolic exudation at the cut ends of explants was the main problem faced during the present study. Due to this exudation, the explants could not survive and eventually died without giving any response. This problem was, however, overcome by proper removal of brown leaf sheaths and quick transfer to fresh medium whenever browning appeared. Researches carried out on micropropagation of bamboo showed variation in species in response to levels of BAP for shoot multiplication as in the case of *Dendrocalamus longispatus* [9], *Dendrocalamus giganteus* [11] and *Bambusa bambos* [3]. The present investigation shows that 13.3  $\mu\text{M}$  BAP significantly induces shoot multiplication. The

combined effect of cytokinins and auxins proves to be useful in increasing shoot multiplication. Synergistic effect of BAP and IBA shown is in agreement with the findings in *Thamnocalamus spathiflorus* [12]. A serious problem preventing successful micropropagation of bamboo and other woody species is their reluctance to form root [13, 14], and *Arundinaria callosa* is not an exception. The induction of rooting in many bamboo species require many additives such as coumarin, phloroglucinol and TDZ, particularly when the explant is taken from the nodal segments of adult plants [11, 15]. Saxena and Bhojwani [9] achieved 73% rooting in *Dendrocalamus longispatus* in a medium supplemented with coumarin along with auxins (IAA and IBA). In the present study, coumarin was not necessary for the development of roots when supplemented with IBA in the rooting medium. A three-step rooting procedure followed in the present investigation resulted in excellent rooting. The transfer of plantlets in PGR-free medium overcomes this problem, thereby giving rise to healthy growth of roots. Bag *et al.* [12] reported that when microshoots were transferred to PGR-free medium following initial culture in medium supplemented with IBA, 100% rooting efficiency could be achieved.

## 5. CONCLUSION

*In vitro* bud-break was enhanced by supplementation of BAP. MS medium with 13.3  $\mu$ M BAP was optimal for shoot multiplication and synergistic interaction with 1.0  $\mu$ M IBA enhanced its multiplication rate. The three-steps rooting procedure increased the root number and rooting percentage. The plant survival was about 70% when acclimatization was carried out by transferring the plantlets to potting mixture. In conclusion, the present study presents a detailed protocol for successful rapid micropropagation of *Arundinaria callosa*, an edible bamboo from nodal explants of adult plant.

## ABBREVIATIONS

BAP	=	6-benzylaminopurine
IBA	=	indole-3-butyric acid
NAA	=	$\alpha$ - naphthalene acetic acid
PGR	=	Plant growth regulator

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