



Micropropagation of *Dendrocalamus asper* through Inter Nodal Segment

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ABSTRACT

An efficient and reproducible procedure for the large-scale propagation of *Dendrocalamus asper* is described. Direct shoot proliferation was induced in aseptically inter node cultures of *D. asper* on modified Murashige and Skoog's (1962) medium supplemented with 0.5 mg/l benzyladenine (BA). Multiple shoots (1-25) were formed within 4 weeks of internode culture without root formation. The shoot-forming capacity of inter node was influenced by the BA concentration in the medium. Proliferating shoot cultures were established by repeatedly subculturing shoots in propagules of 3 shoots each. A multiplication rate of 15-16 fold was achieved on MS medium +2.0 mg/l BA. Roots were formed on excised propagules. Callus formed when inter node cultured on medium containing 0.5 kin +3.0 mg/l 1-naphthaleneacetic acid (NAA). Plantlets were hardened, acclimatized and established in soil, where they exhibited normal growth.

Key words Micropropagation · Tissue culture · *Dendrocalamus asper* · Bamboo

Abbreviations: MS Murashige and Skoog (1962) medium ·

BA N6-benzyladenine · kinetin · NAA 1-naphthaleneacetic acid

INTRODUCTION

Dendrocalamus asper, a bamboo species, is valued for its edible tender shoots. The food industry based on these young shoots is well-developed and expanding rapidly. However, the available methods for its propagation are slow and difficult for a number of reasons. The production of seeds is irregular as flowering occurs on culms of 100-year-old plants. Like other bamboo species it is also known to be monocarpic, i.e. flowering once before culm death. The result is a limited number of seeds available for plantation purposes. Vegetative propagation is commonly practised in bamboo cultivation but the plants developed will all be as old as their stock and will tend to flower and die simultaneously as the actual age is the same in every part of the bamboo. Also, vegetative propagation through cuttings and rhizomes is undependable due to the bulky size of the propagules and the non-availability of propagules in the required number. They are difficult to handle and transport, and plantlet survival in such cases is usually low [1,2]. The present paper describes a simple and efficient procedure for the *in vitro* propagation of *Dendrocalamus asper* by internode.

Bamboo belongs to the sub-family Bambusoideae of the grass family-Poaceae. These comprise the herbaceous and the woody bamboos. Woody bamboos that belong to the tribe Bambusoideae, have played an integral role in the social, cultural and economic development in Asia, throughout history [3]. Bamboo contributes to the livelihood of the rural poor in these countries. Its industrial use in the manufacture of paper pulp dates back to over 2000 years while the use of bamboo strips for writing, dates back to the Han dynasty of China (206 BC - 220AD) [4]. In the recent past a number of bamboo based wood substitutes has been developed [4,5,6,7]. At present this natural resource is overexploited and diminished requiring large-scale replanting [8]. Rare seeding in bamboo requires propagation by vegetative methods. Conventional methods of vegetative propagation cannot cater to the present scale of demand for propagules and the alternative is the use of tissue culture techniques. This is now applied in commercial propagation of bamboo species [9]. Selection of propagules for plantation establishment and management of plantations. The plant is native to China and is commonly planted in and is commonly planted in India, Vietnam, Malaysia (Peninsular and East), Indonesia and Philippines.

METHODOLOGY AND DISCUSSION

Internodal segment of *Dendrocalamus asper* were obtained from the Forest of Raipur C.G in 2009, The inter nodal segment were surface-sterilized with sodium hypochlorite (4%) for 20 min followed by three to four rinses with sterile distilled water. Disinfected internodal segment 1-2 cm were

germinated in 100-ml Borosil flasks and culture bottles containing 35 ml of germination medium [MS medium supplemented with 100 mg/l myo-inositol (Sigma), 30 g/l sucrose (Qualigens), 7 g/l agar (Loba, India) and +.5mg/l BA (Sigma), pH 5.8]. Cultures were maintained at 25°C±1°C under a 16-h photoperiod with a light intensity of 30 mmol m⁻² s⁻¹ provided by cool-white 40 W fluorescent tubes (Philips). Shoots developed on inter nodal segment with in 3-4 weeks. And callus formed with in 3-4 weeks. after this explant were subcultured in MS Supplimented with different conc.of BAP. After 3-4 subculuring the plants were transplanted into green house in co co pits..

OBSERVATION TABLE

Table 1 Effect of BA concentration in MS medium on shoot formation from seeds of *D. asper*

| BA concentration (mg/l) | Number of shoots after 6 weeks ^b | Shoot length ^a (cm) | Root formation |
|-------------------------|---|--------------------------------|----------------|
| 0.0 | 1 | 3.4±0.4 | ++ |
| 1.0 | 1-2 | 2.8±0.6 | + |
| 2.0 | 4-8 | 2.1±0.4 | - |
| 3.0 | 8-10 | 1.8±0.2 | - |
| 5.0 | 10-15 | 1.2±0.3 | - |
| 7.0 | 18-20 | 0.7±0.2 | - |
| 10.0 | 25-30 | <0.5 | - |

^a Mean of 30 replicates ±SD

^b The shoots of 0.5 cm and above were counted

Table 2 Effect of BA concentration in MS medium on shoot and leaf development of *D. asper*. A propagule of three shoots was cultured and data recorded after 4 weeks

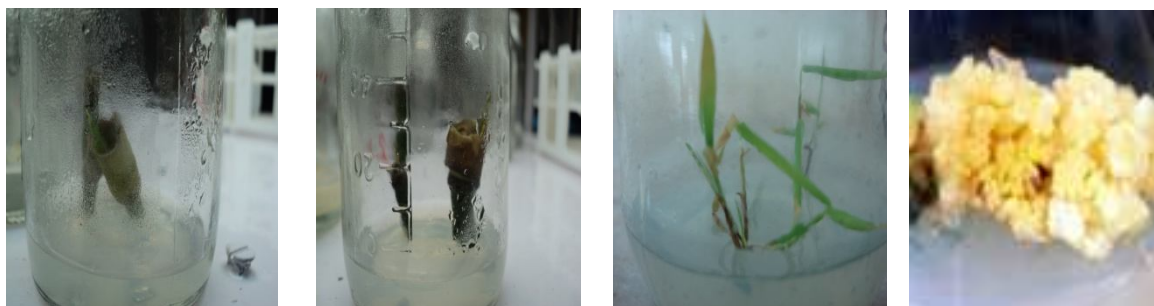
| BA concentration (mg/l) | Number of shoots ^a | Shoot length ^a (cm) | Leaf width ^a (mm) | Multiplication rate |
|-------------------------|-------------------------------|--------------------------------|------------------------------|---------------------|
| 0.0 | - | 4.5±1.0 | 9.0±2.1 | 0 |
| 1.0 | 14.1±2.9 | 3.3±3.7 | 6.2±1.0 | 5.6 |
| 2.0 | 33.4±3.2 | 2.3±0.2 | 4.2±0.6 | 12.2 |
| 2.5 | 41.0±5.1 | 2.2±0.4 | 3.0±0.1 | 15.3 |
| 3.0 | 46.5±5.1 | 1.8±0.5 | 3.0±0.6 | 17.2 |
| 4.0 | 36.5±3.6 | 1.2±0.5 | 2.3±0.4 | 13.3 |
| 5.0 | 30.2±6.5 | 1.2±0.2 | 2.0±0.1 | 12.2 |
| 7.5 | 16.3±2.3 | 1.0±0.1 | 2.0±0.5 | 6.2 |
| 10.0 | 17.0±4.0 | 0.8±0.2 | 1.6±0.2 | 7.0 |

^a Mean of 30 replicates ±SD

Table 3 Effect of the size of the *D. asper* propagule on the development of the number of shoots and propagules and shoot multiplication rate, when cultured on MS+3.0 mg/l BA. Data recorded at the tenth subcultured cycle

| Size of propagule | Average no. of shoots produced | Average no. of propagules obtained (3 shoots each) | Multiplication rate after 4 weeks |
|-------------------|--------------------------------|--|-----------------------------------|
| One shoot | 6.54 | 1.5 | 6.3±0.8 |
| Two shoots | 20.88 | 5.23 | 10.4±1.5 |
| Three shoots | 47.23 | 12.33 | 14.7±2.0 |
| Four shoots | 48.74 | 10.29 | 12.3±1.8 |
| Five shoots | 46.84 | 11.78 | 9.5±1.6 |

^a Mean of 30 replicates ±SD



A

B

C

D

A.&B. Initiation of explants when cultured on MS Media supplemented with .5mg/l BAP

C. Observation after 4 weeks of Culture

D. Formation of Callus MS +.5 kin+3.0mg/INAA



E

F

G

H

E. Multiplication of shoots in explants in MS+ MS+3.0 mg/l

F. Subculturing and root formation of explants

G. 3rd Subculturing and commercial multiplication

H. Three months old plants field Transfer Of explants in MS+Activated Charcoal+3.0mg/l BA.

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