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INTRODUCTION, MASS MULTIPLICATION AND ESTABLISHMENT OF EDIBLE BAMBOO *DENDROCALAMUS ASPER* IN INDIA

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Bamboo is an economically important and multi-purpose crop. In India, 126 species of bamboos are reported out of which 25 exotic species are introduced from Myanmar, China, Japan, Malaysia and Thailand. *D. asper* is native to China and is valued economically for its edible tender shoots. A tissue culture protocol was developed for large scale multiplciation of this plant. Succéssful hardening and acclimatization along with field transfer at number of places in India was carried out for its establishment.

Key words : Tissue culture, micropropagation, *Dendrocalamus asper*, exotic species, Bamboo, germplasm.

Bamboos are long lived, woody evergreen grass members of the family Gramineae, tribe Bambuseae. Bamboo is a term used for more than 60 genera and 1500 species of the Gramineae (Soderstrom and Calderon 1979). India is rich in bamboo resources having 19 genera and around 126 species. This diversity is distributed all across from coastal areas in the south to around 10,000 feet above sea level in the Himalayan highlands (Kochhar and Rana 1993). A detailed account of 113 species of bamboos found in India alongwith their distribution has been given by Varmah and Bahadur (1980).

Dendrocalamus asper is one of economically and socially important bamboo species. it is native to China and is valued economically for its edible tender shoots which are consumed by local people as well as sold as canned food all over the world, earning valuable foreign exchange. Moreover, its mature culms are also used for pulp and paper manufacture. The food industry based on young shoots of *D. asper* is already well developed and expanding very fast.

The conventional sexual or vegetative methods for propagation of *D. asper*, pose numerous problems. The production of seeds takes 60 to 100 years. The seed have short viability, high seed sterility and low germinability, resulting in insufficient seedlings for plantation. The offset and rhizome cuttings are bulky and available in less numbers, involving colossal investment for prepara-

tion of planting stock as well as for transportation. Moreover, their survival rate is also very low. Thus, urgent need arose to search a non-conventional tissue culture technique for rapid and large scale micro propagation from survived *D. asper* plants through axillary bud and seed culture.

MATERIALS AND METHODS

In 1993 some (9-12) *D. asper* plants were raised from rhizome and root shoot cuttings were brought from Thailand. The cuttings were tried for establishment at different places in India *viz* - 2 plants each at Tropical Forest Research Institute (TFRI), Jabalpur, Forest Research Institute (FRI), Dehradun, Institute of Forest Genetics and Tree Breeding (IFGTB), Coimbatore, Conservator Forest House (CFH), Chhindwara and 1 at State Forest Research Institute (SFRI), Jabalpur. Out of these only 3 plants (1 each at TFRI, FRI and CFH) survived. Also, 100 g of *D. asper* seeds were obtained from F.A.O. (Food and Agriculture Organization) consultant from Royal Forest Department, Chatuchak, Bangkok.

In vitro axillary bud culture

Nodal segments with axillary buds were collected from young juvenile shoots of mother plant grown at TFRI, Jabalpur and washed with 5% cetavelon (detergent) for 15 min. followed by surface sterlization with 0.1% mercuric chloride solution for 5-10 min. The sterilized buds were rinsed thrice with autoclaved distilled water before transfering them to liquid and semi-solid Murashige and Skoog's 1962 (MS) medium supplemented with different concentrations of benzyl aminopurine (BAP). Explant was placed vertically in medium (Fig. 1-A). The cultures were incubated in the culture room at 26 $\pm$ 1°C with 16 h photoperiod (3000 lux) provided by cool white fluorescent tubes.

In vitro seed culture

Seeds of *D. asper* stored at 4°C were disinfected with 90% ethanol for 1 min. and subsequently in 6% Sodium hypocholorite solution for 25 min. Seeds were rinsed thrice with autoclaved distilled water and cultured on MS medium supplemented with 1.0-10.0 mg/1 BAP. For rooting, shoots (1-3 cm long) were cultured on MS medium supplemented with α-naphthalene acetic acid (NAA) or Indole butyric acid (IBA).

RESULTS AND DISCUSSION

Multiplication of shoots

Axillary bud break was achieved in all healthy cultures within two weeks (Fig. 1-B). The proliferated shoots from axillary buds were excised and

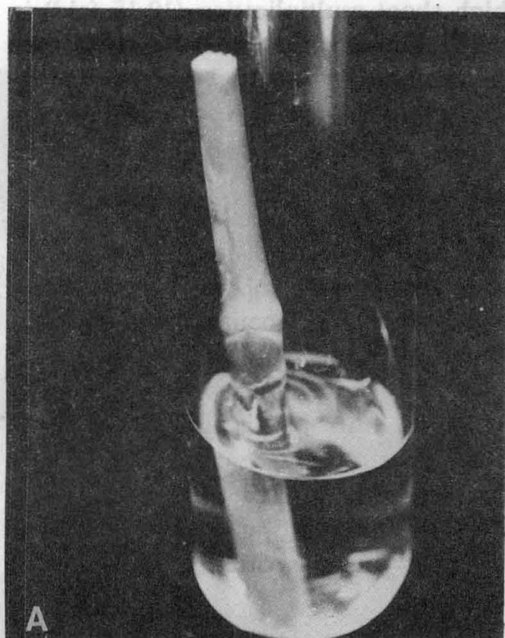

Fig. 1a. Axillary bud cultured on liquid MS + 5.0 mg/1 BAP

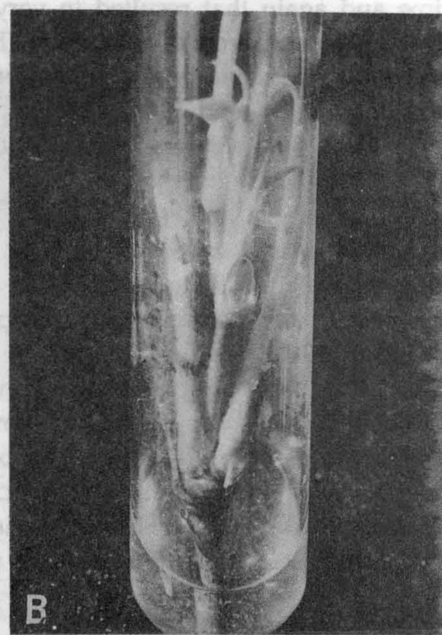

Fig. 1b. Axillary bud proliferation in 2 weeks

subcultured on MS medium supplemented with 2.0-5.0 mg/1 BAP for further shoot multiplication. Multiple shoots 1-20 were formed immediately after seed germination. Number of shoots developed from the single seed was directly correlated to BAP concentration used in the medium.

After 3-4 weeks of subculture, the *in vitro* shoots further multiplied (Table 1). The cluster of shoots were carefully excised (clusters of three shoots) and

Table 1. Micropropagation and survival of plants of *D. asper* through axillary bud and seed culture

Source	Shoot multiplication after				Survival (%) of plants after	
	I subculture	III subculture	X subculture	Rooting (%)	Hardening	Field transfer
Single Axillary bud (TFRI plant)	1-2	1-3 fold	15 fold	100	95	70-80
Seed (Ten lines)	1-20	5-7 fold	10-12 fold	100	95	80-90

again subcultured on shoot multiplication medium. The procedure was repeated time and again that resulted in very high shoot multiplication (10-15 fold) in every 3-4 week cycle of subculture (Fig. 1C). Similarly, shoots were maintained and multiplied by repeated subculture for large scale shoot production. Thorpe and Patel (1984) and Ahuja (1991) have reported *in vitro* asexual multiplication by axillary bud breaking and production of adventitious buds in tree species. The recommendations of the third International bamboo workshop held in India (Ramanuja Rao *et al*. 1990) on the conservation of bamboo resource gave emphasis on the collection of gene pools and the germplasm exchange. The FRI's Collections which include 25 exotic species of bamboos, introduced in the past from Myanmar, China, Japan, Malaysia and Thailand (Vermah and Bahadur, 1980) is now increased to 26 exotic species with the addition of *D. asper*. Thomas *et al* (1990) have reviewed the strategies for conservation of bamboos which also gave due emphasis on micropropagation. The major difficulty for active exchange of bamboo germplasm among countries/continents is the remote availability of seed due to long flowering cycles and the vulnerability of bulky rhizomes which get damaged during transportation but in comparison tissue culture-raised plants can be transported easily.

In vitro rooting

Rooting was achieved in shoots (3-5) cultured on MS medium supplemented with 3.0 mg/1 NAA or 10.0 mg/1 IBA in 98-100% culture. The root initiation was observed after 2 weeks from culture on rooting medium. Usually 8-10 roots developed from each shoot clumps when cultured on MS + 2.0-3.0 mg/1 NAA and 10-20 roots on MS + 8.0-10.0 mg/1 IBA medium (Fig. 1D).

Hardening and acclimatizaton of tissue culture raised plants

Four-week-old cultures on rooting medium developed healthy root and shoot system. These plantlets were first transferred to polybags containing soilrite: sand (2:1 v/v) mixture and were kept in the mist chamber under 80% RH and 30± 2°C temperature for hardening (Fig. 1E). The plants were irrigated with half strength macro- and micro-nutrients of MS salts. After 20-25 days, the hardened plants were transferred to polybags containing a mixture of soil: sand : organic manure (1:1:1) and kept under high density agronet shade house for acclimatization (Fig. 1F). Plants wrre ready for field transfer in one month. Till date, some 18,000 plants have been propagated from single axillary bud culture and around 5,000 plants through seed culture. Field transfer of tissue culture raised plants was carried out during June-December at Jabalpur, Baster and Chhindwara regions of Madhya Pradesh and other states of the country (Table 2). The field plants developed rhizomes within two months,

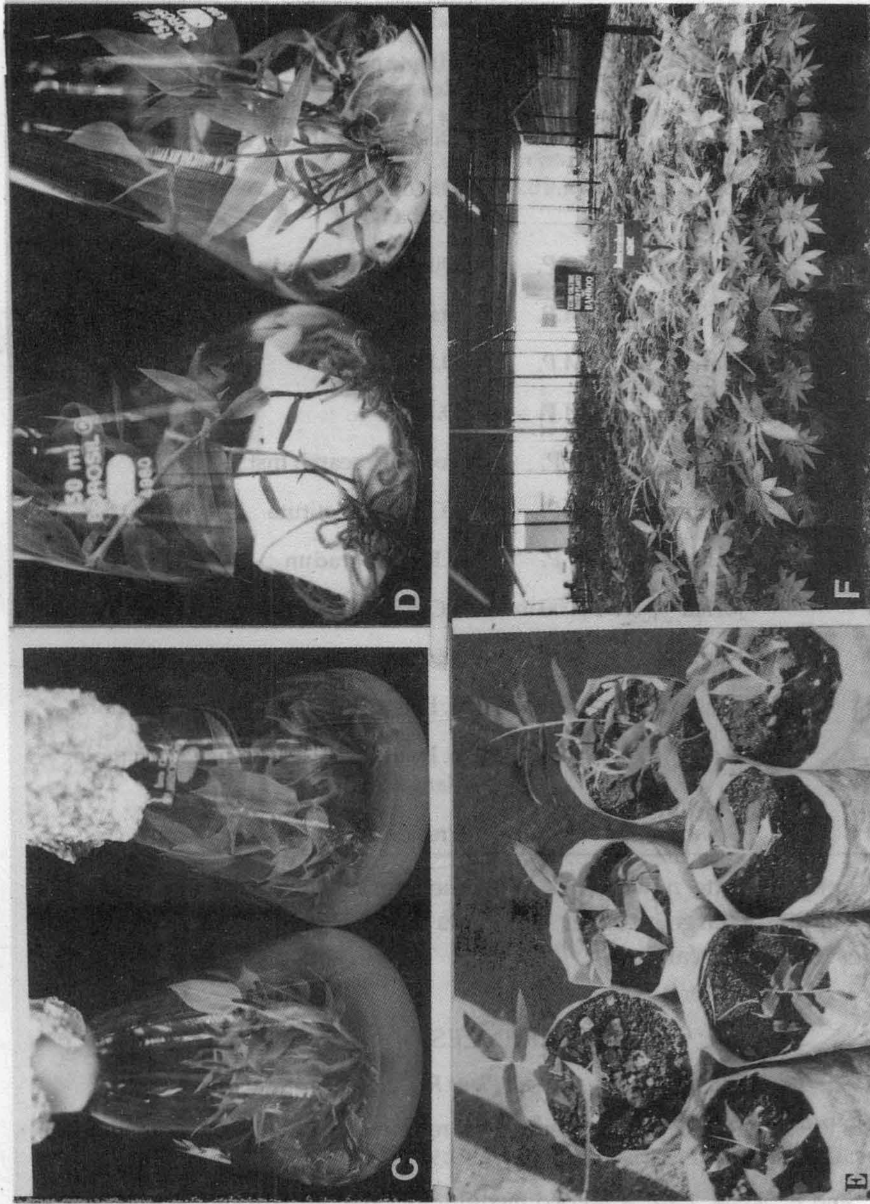

Fig. 1c. Shoot multiplication on MS + 3.0 mg/l BAP, d. *In vitro* rooting on MS + 3.0 mg/l NAA, e. Hardened plants in polybags and f. Acclimatization in shade house

Table 2. Distribution and field plantation of *D. asper* in 1995

S. No.	No. of Plants	Place	State	Address
1.	2,000	T.F.R.I.	M.P.	T.F.R.I., P.O. R.F.R.C., Jabalpur
2.	700	Chhindwara	M.P.	Human Resource Development Research Institute, Chhindwara
3.	200	Bastar	M.P.	Distributed among farmers
4.	400	Korba	M. P.	District Forest Officer (D.F.O) Korba Div.
5.	1200	Kanker	M.P.	Distributed to farmer
6.	450	SFRI, JBP	M.P.	State Forest Research Institute, Jabalpur
7.	100	Mandla	M.P.	D.F.O.
8.	40	Seonui	M.P.	D.F.O.
9.	20	Kanour	U.P.	Forest Research Inst., Kanpur
10.	500	Allahabad	U.P.	I.C.F.R.E. Institute
11.	10	Dehradun	U.P.	F.R.I., Dehradun
12.	100	Jorhat	Assam	I.C.F.R.E. Institute
13.	700	Nagpur	M.S.	Divisional Manager, F.D.C.M., Nagpur
14.	715	Banglore	Karnataka	a. Forest Research Centre, Bangalore
				b. Institute of Wood Science technology, Bangalore
15.	300	Delhi	Delhi	President House, Delhi

which later sprouted into new culms. With the present efforts of multiplication of *D. asper* plants, a large number of plants have been supplied to various user agencies including farmers.

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