

Artificial seed production in the male bamboo *Dendrocalamus strictus* L.

S. Mukunthakumar* and Jaideep Mathur

Plant Tissue Culture Laboratory, Department of Botany, University of Gorakhpur, Gorakhpur 273009 (India)

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Artificial seeds of *Dendrocalamus strictus* were prepared by encapsulating somatic embryos that had been obtained on MS medium containing 3.0 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.5 mg l^{-1} kinetin (Kin), in calcium alginate beads. A germination frequency of 96% and 45% was achieved in vitro and in soil, respectively. The in vivo plantlet conversion frequency was increased to 56% following an additional coating of mineral oil on the alginate beads. Germinated artificial seeds could be raised into plantlets.

Key words: artificial seeds; bamboo; *Dendrocalamus strictus*

Introduction

Artificial seeds, comprising somatic embryos enclosed in a nutritive and protective coating, have been proposed as a low-cost, high-volume propagation system [1]. In addition such seeds may also be used for the large scale propagation of plants where the natural seed formation takes place after very long intervals or where a state of constant physiological sterility exists. Amongst such plants, are the bamboos with their long vegetative periods and a low pollen and seed viability [2]. The bamboos are usually propagated through vegetative means [3], but recent researches have utilized tissue culture methods for their propagation [4,5]. Thus protocols are now avail-

able for somatic embryogenesis in a number of bamboo species [6].

The present study, therefore utilized in vitro regenerated somatic embryos of *Dendrocalamus strictus*, commonly called the male bamboo due to its nearly solid culms, for artificial seed production and assessed their in vitro and in vivo germination and development.

Materials and Methods

Seeds of *Dendrocalamus strictus* were obtained from the Forest Research Institute, Dehradun. Following earlier reports on regeneration from bamboo tissue cultures [7,8], somatic embryos were obtained from the seed derived callus developed on Murashige and Skoog's medium [9], containing 3.0 mg l^{-1} 2,4-D and 0.5 mg l^{-1} Kin. The embryoids developed into plantlets upon transfer to medium containing 1.0 mg l^{-1} NAA and 0.5 mg l^{-1} Kin and therefore the encapsulating matrix (6% sodium alginate and $100 \text{ mM CaCl}_2 \cdot 2\text{H}_2\text{O}$) contained these growth regulators in addition to the usual MS salts and 3% sucrose.

Correspondence to: Jaideep Mathur, Plant Tissue Culture Laboratory, Department of Botany, University of Gorakhpur, Gorakhpur 273009, India.

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; Kin, kinetin; MS, Murashige and Skoog's medium (1962); NAA, α -naphthaleneacetic acid.

*Present address: TBGRI, Palode 695 562, Thiruvananthapuram, Kerala, India.

However, for in vivo experiments the sucrose was omitted. Encapsulation was carried out using single embryoids and clumps of 2–4 embryoids following previously standardized procedure [10]. The calcium alginate beads containing the embryoids were placed in vitro on 0.7% plain agar (prepared in tap water) and in trays/pots containing sterilized soil, according to the method given by Slade et al. [11]. Observations on germination and subsequent development were taken from the

fifth day onwards until 35 days after placement. In later experiments on in vivo germination, the alginate beads were blotted dry with sterilized Whatman paper and then coated with autoclaved, heavy grade mineral oil (liquid paraffin, Loba Chemie, India) before placing in pots covered with polythene sheets/beakers and kept under greenhouse conditions. Beads sown in soil were irrigated every second day either with dilute (1/10) MS inorganic nutrients or plain tap water.

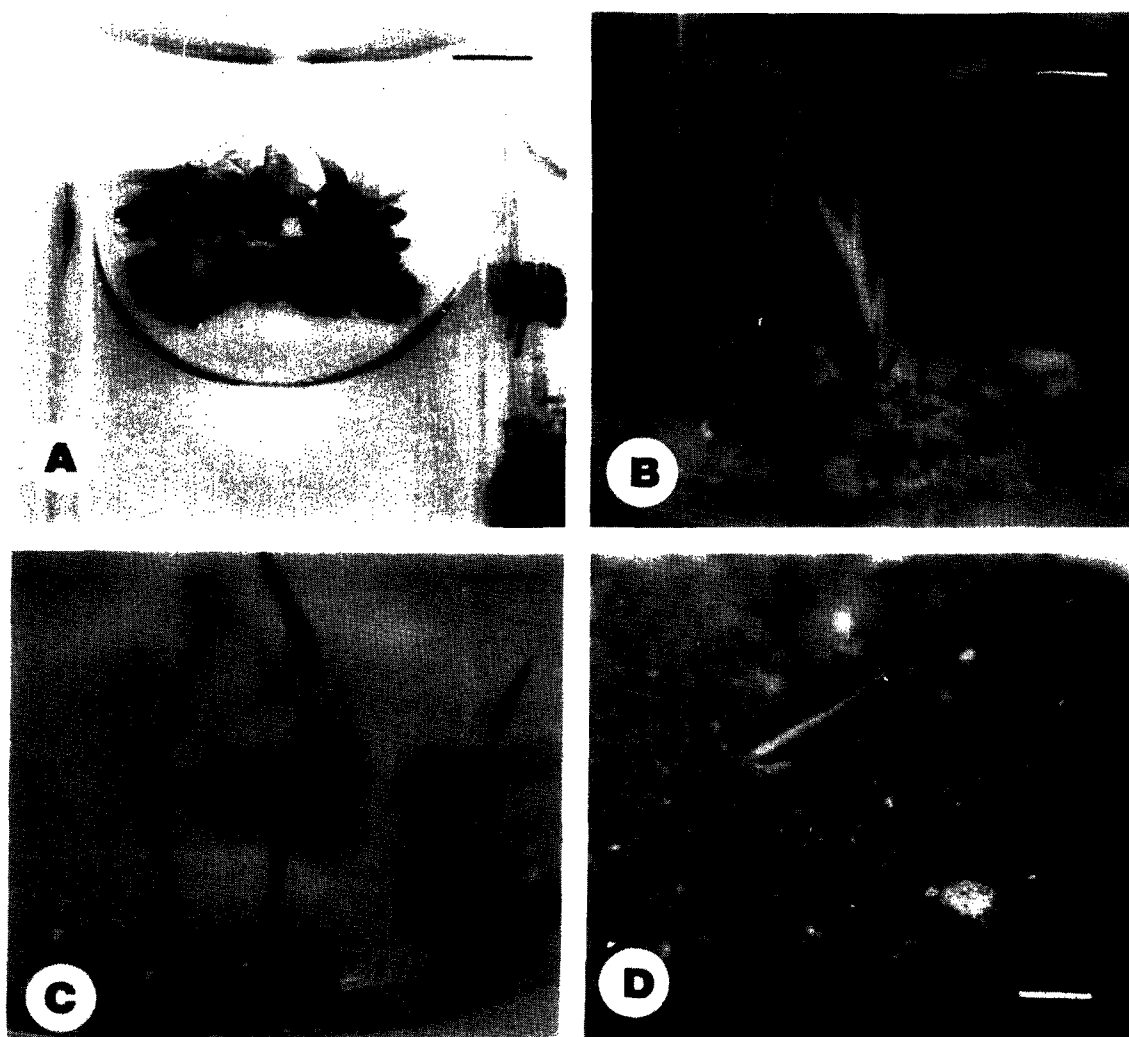


Fig. 1. Artificial seed production in *Dendrocalamus strictus*. (A) Somatic embryogenesis in callus cultures obtained on MS medium containing 3.0 mg l^{-1} 2,4-D and 0.6 mg l^{-1} Kin (Bar = 0.5 cm). (B) Plantlet development from somatic embryos on medium containing mg l^{-1} NAA and 0.5 mg l^{-1} Kin (Bar = 1.0 cm). (C, D) Artificial seeds germinating in vitro on plain agar (C) and on soil (D) 10 days after placing on the substratum. (Bar = 0.5 cm).

Table I. Germination and developmental response of uncoated and mineral-oil coated artificial seeds of *Dendrocalamus strictus* on different substrata.

Substratum	No. seeds placed	No. seeds germinated	Days for emergence of		No. plantlets obtained
			Root	Shoot	
<i>Uncoated</i>					
Plain agar (in vitro)	100	95	7-9	3-5	95
Soil	180	82	6-8	3-8	80
<i>Mineral-oil coated</i>					
Plain agar (in vitro)	250	235	8-12	5-10	230
Soil	250	144	15-20	10-15	138

Results

Somatic embryogenesis from callus cultures

Seeds of *Dendrocalamus strictus* started callus-ing within 15 days of inoculation on MS medium containing 3.0 mg l⁻¹ of 2,4-D and 0.5 mg l⁻¹ of Kin. Over 45 days, the callus became compact and developed small greenish-white structures which progressively differentiated into embryoids (Fig. 1A). At this stage, subculture of the embryogenic callus on to fresh 2,4-D-Kn- medium resulted in further proliferation of the callus, while leaving it on the old medium resulted in the development of 6.3 ± 1.34 embryoids per gram fresh wt. of callus. However shoot growth in this case was invariably faster than root development. A more balanced growth of roots and shoots was achieved when the embryoids were transferred to medium containing 1.0 mg l⁻¹ of NAA and 0.5 mg l⁻¹ of Kin (Fig. 1B). Encapsulation experiments were therefore carried out using the latter growth regulator combination added to both the sodium alginate and calcium chloride solutions.

Germination of the artificial seeds

The calcium alginate beads containing the bamboo embryoids had a diameter ranging between 0.5-0.8 cm. Nearly 95% artificial seeds germinated within 7-10 days (Table I) of placing on the plain agar substratum (Fig. 1C). Depending upon the content of the beads, single shoots or clumps of

shoots developed, while 3-5 roots (1.2-1.5 cm long) also developed per 'seed'.

The artificial seeds when placed in soil and watered every second day with dilute MS salts showed 45% germination within 10 days of placing (Fig. 1D and Table I). Seeds that did not germinate by this time exhibited browning and died. Germinated seeds, however, continued growth even after the alginate coat showed intense microbial growth. Since microbial infestation was the major limiting factor in achieving a higher germination frequency in vivo the subsequent experiments used an additional protective coating of mineral oil on the alginate beads. Following this coating the germination frequency in soil was found to increase to 56%. Though all the alginate beads remained free from microbial infestation the emergence of both root and shoot was delayed until 15-20 days after placement on soil following the mineral oil coating (Table I).

Plantlet formation from germinated artificial seeds

Under in vitro conditions 7-10 cm long plantlets with 2-5 leaves could be obtained from the germinated seeds in 30-45 days. However, in most cases, clumps of 4-6 shoots developed and could be picked up as individual units for planting in the soil. Seeds that had germinated on soil also remained on the surface and the roots had to be covered with soil for their normal growth and plantlet establishment.

Discussion

Much of the success of any plant tissue culture endeavour depends upon the nature and state of the plant material being used. Such is also the case with artificial seeds, where high germination and plantlet conversion frequencies are achieved in vitro but which fail to provide as many plantlets in soil. Nevertheless, plantlet conversion frequencies of 20% have been routinely obtained in soil-less media under mist, from alfalfa synthetic seeds [12] and a maximum of 64% plantlet conversion under a humidity tent watering system has also been reported for the same crop [13]. Similarly, the growth and development of 64% of shoot buds encapsulated in alginate containing antimicrobial substances was observed in vermiculite, under glass-house conditions in *Valeriana wallichii* [10]. In the present study, however, 45% germination occurred in covered pots containing sterilized soil, in spite of heavy microbial infestation on the alginate coat. Apparently, the anatomy and properties of bamboos [14], even at an early stage of development are able to resist the microbial invasion and continue growth. Moreover, following our earlier experiments with *Valeriana wallichii* [10], sucrose had been omitted from the encapsulation matrix in order to remove unnecessary complications of microbial infestation arising due to its presence. While its absence did not inhibit germination in vitro or on soil, a lowering in the germination percentage had been expected in mineral oil coated beads due to their prolonged germination time. On the contrary, after the coating the germination percentage increased, ostensibly, due to a reduced metabolic rate [15], to meet which the small amounts of cellular reserves may have been utilized.

An additional coating on the alginate capsule, like the Elvax polymer coating has been used [12], to minimise tackiness of artificial seeds and facilitate their sowing using a Stanhay seed planter [1]. For artificial seeds of *Dendrocalamus strictus*, however, the commonly available mineral oil was used to provide the additional protection against desiccation, tackiness, leakiness and invading microbes and was effective in increasing the germination frequency to 56%. The mineral oil

may even be used to store synthetic seeds for 6 months or more [15].

The use of a single embryoid per 'seed' has been advocated in most of the studies on artificial seeds. However, a clump of bamboo embryoids [2–4] stood a better chance of survival and establishment in soil as compared to single units and did not produce an unmanageable seed size. These results have a direct bearing in artificial seed production in a physiologically sterile bamboo, *Bambusa vulgaris*, where nodal callus has given rise to deep seated somatic embryos that cannot be excised as single units (unpublished results).

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