A simple *in vitro* method of propagation and rhizome formation in *Dendrocalamus strictus* Nees

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Micropropagation technique was developed for mass-scale production of *Dendrocalamus strictus* plantlets using stationary liquid cultures. Seedling cultures were initiated on half strength Murashige and Skoog's basal medium supplemented with 0.5 mg/l 6-benzyl-aminopurine (BA) and 2% sucrose. Further proliferation and production of plantlets was obtained on a simple minimal medium without any growth regulators. *In vitro* rhizome formation was also observed in 80% of the cultures. Plants potted in sand:soil (1:1) showed 90% survival.

BAMBOOS are the most unique and versatile group of forest trees playing an important role in industry and domestic life. Since ancient times, bamboos are used as construction material, weapons, tools, musical instruments, food, etc. However, its main use is in the paper and pulp industry. The principal sources of paper and pulp of acceptable quality in India are *Dendrocalamus strictus* and *Bambusa arundinacea*. It is estimated that out of an annual production of nearly 9.5 million metric tonnes of bamboo in India, about 4.5 million metric tonnes of bamboo are used in paper-making[1]. Due to this rapid industrialization and an increasing population, the natural stands of bamboos are diminishing at a very fast rate. To meet this increasing demand, rapid and large-scale replanting of this natural source is necessary[2].

Conventional methods of propagation of bamboos are mainly by seeds or by vegetative propagation methods. However, gregarious flowering at long intervals followed by the death of clumps[3,4], short viability of seeds, presence of seed-borne fungi and other microorganisms and large-scale consumption of seeds by wild animals rapidly deplete this valuable source of propagation. Vegetative propagation of bamboos is done by offset and clump division, rhizome cuttings, layering, culm cuttings, pre-rooted and pre-rhizomed branch cuttings, and nodal bud chips. Most of these methods have some limitations that restrict the large-scale multiplication of these species. The propagules are bulky, difficult to extract, transport and plant, insufficient in number for large-scale plantation, season-specific and give lower total yield as they flower at the same time as their parents[5,6].

Micropropagation of bamboos offers an alternative method to achieve success in afforestation programmes to overcome the above-mentioned problems[2,7,8].

We describe here an effective method of *in vitro* propagation of *D. strictus* for continuous supply of plantlets on a large scale.

In vitro grown seedlings of *D. strictus* were used as the explants. Seeds of *D. strictus* were collected from 3 different provenances of India, viz. Andhra Pradesh, Karnataka and Maharashtra forest agencies and stored at 10°C till processed. Seeds were surface sterilized using the method described by Nadgir *et al.*[9] The seeds were germinated on White's basal medium[10] containing 2% sucrose (w/v) and 0.42% agar (w/v) (Qualigens India Ltd, Bombay, India) and incubated in dark at 25 ± 1°C. The seeds which germinated within 15 days were selected for further experiments.

Germinated seeds were kept under light till the seedlings were 10–12 cm tall. Each seedling was then dissected and explants were prepared with at least one node and were inoculated on a liquid medium with filter paper support in a test tube (20 mm × 150 mm) containing half strength Murashige and Skoog's salts and vitamins[11], 2% sucrose and BA in the concentration of 0, 0.25, 0.5, 1 and 2 mg/l at pH 5.6 ± 0.2. The cultures were incubated at 16 h photoperiod with 23.34 μmol m^{-2} s^{-1} light intensity. The results were confirmed after 60 days of incubation. MS 1/2 + BA (0.5 mg/l) (multiplication medium – MM) was used for multiplication of shoots.

Rooted shoots from MM were transferred to a production medium (PM) in a test tube containing MS 1/2 salts and vitamins, sucrose 2% with or without filter paper support on liquid medium. After a growth period of 45 days, each culture was subcultured. Elongated (5 to 7 cm) plantlets were removed for planting and small rooted shoots (shoots of the height less than 5 cm) were transferred to a fresh PM for further elongation and multiplication.

All the media were autoclaved at 121°C for 20 min. Temperature for incubation was 25 ± 1°C and photoperiod was maintained at 16 h light, 8 h dark under 23.34 μmol m^{-2} s^{-1} from cool white fluorescent light.

To evaluate the effect of decapitation on plantlet production, two types of subculture procedures were followed in the same seedling culture, keeping other culture conditions constant. In the first method, plantlets of height more than 5 cm were taken out at every subculture while in the second, all the elongated plantlets (height more than 5 cm) were decapitated and again kept on PM for further proliferation. The plantlets were taken out for planting only at the end of the sixth subculture.

Elongated plantlets from PM were transferred to sterile sand:soil (1:1) mixture in polybags (5 × 10 cm) and kept at 25 ± 1°C and 90% humidity under natural light in a polyhouse for hardening.

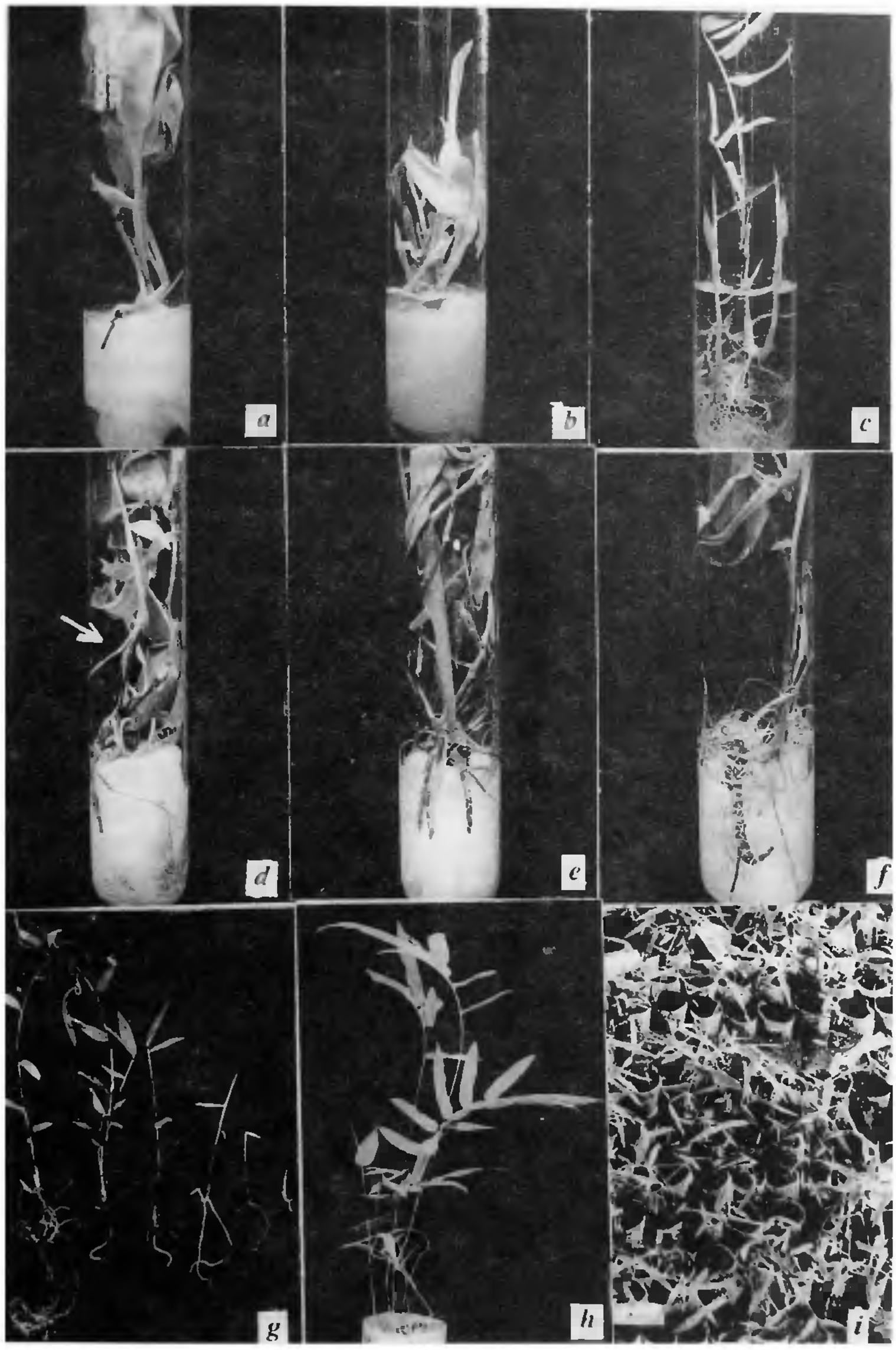

Figure 1. *a*, Multiple shoot formation from axillary node on MM; *b*, Multiple shoot formation from coleoptile region on MM; *c*, Elongation of shoots and roots on PM; *d*, New shoot and root formation from different nodes on PM; *e* Rhizome formation on PM; *f*, Shoot formation from rhizome on PM; *g*, Different sizes of shoots formed on PM; *h*, Plant in polybag; *i*, Plants in polyhouse.

All the experiments were done in triplicates and repeated three times for confirmation of results.

Germination of seeds started after fifth day of incubation and continued till at least 30 days. However, the seeds germinating after 15 days were found to be very slow growers in further experiments. Therefore only the seeds which germinated in the first 15 days were selected for production.

When nodal and coleoptile regions of germinated seedlings were transferred to MM for multiple shoot formation, it was observed that about 80% of these segments showed initiation of shoots in 10–15 days. The average shoot formation on 0.5 mg/l BA concentration was 2–3 shoots per axillary node while the coleoptile region initiated an average of 4–5 shoots in 20 days of incubation (Figure 1 *a*, *b*). With 0.25 mg/l of BA, no new shoots formed from axillary nodes while only 1–2 shoots were produced from the coleoptile. Further incubation for up to 60 days did not increase the total number of shoots. Concentrations above 0.5 mg/l, however, induced 4–5 shoots from axillary nodes as well as coleoptile regions initially. Shoots turned brown after 20 days of incubation. As a result 0.5 mg/l BA was therefore added to medium for production of multiple shoots and plantlets. The response of root formation on MM was observed in 50% of the cultures. These cultures were further subcultured for production of plantlets.

All rooted shoots in clumps were then transferred to PM where elongation of shoots and roots was observed in 15 days (Figure 1 *c*). Incubation on this medium was continued for further 40–50 days where new shoot formation along with rooting was observed from all the nodes (Figure 1 *d*). About 80% of these cultures were found to form rhizomes. In all the cultures, at least 2 propagules were obtained in 40–50 days of incubation, each containing 2–3 rooted shoots and/or rhizomes (Figure 1 *e*). Each culture after 45 days of incubation gave shoots of different sizes varying from 2 to 15 cm (Figure 1 *g*). Elongated plantlets of 5 cm and above were removed for planting and small newly formed young rooted shoots and/or rhizomes were transferred to fresh PM for further elongation, multiplication and rooting where again new shoot formation was observed from all the nodes of these shoots or rhizomes (Figure 1 *f*). All the shoots obtained gave rise to root formation. Thus the process of subculture and multiplication was continued. The cultures are now in 25th subculture without any difference in multiplication ratio. No callusing has been observed at any of these steps so far.

Table 1. Effect of decapitation on total number of plant production

Subculture no.	Before subculture	After subculture	No. of plants
Method I			
S-3	10	16	14
S-4	16	25	20
S-5	20	35	35
S-6	35	50	45
		Total	114
Method II			
S-3	10	27	–
S-4	27	70	–
S-5	70	175	–
S-6	175	50	450
		Total	450

Subculture period 45 days.

Table 1 indicates the results of the experiments carried out for evaluation of effect of decapitation on number plantlet production. In method 1, where elongated plantlets were removed for plantation during every subculture, only 114 plantlets could be produced as against the 2nd method where the total number of plantlets was 450. This increase in number of plantlets was due to decapitation which induced sprouting of new shoots from basal as well as axillary nodes along with roots and new rhizome formation as a result of decapitation. All these plantlets produced by using the second method were of uniform size.

Banik[12] described the technique of macropropagation by using aerially rooted and rhizomed branches as cuttings in some bamboo species. According to him, about 2–3 years are required for the formation of rhizomes and sufficient rooting.

Kumar *et al.*[13,14] and Kumar[15] reported a macroproliferation technology where mother stock of *D. strictus* seedlings having culms, rhizomes and roots could be multiplied 6 times in a period of 8 months. For best survival of these plants, the average length of the propagule needed was 15–20 cm.

Based on these reports we suggest here a term 'microproliferation' to describe the present *in vitro* micropropagation technique for bamboo plants. In the method described here, even a very small plantlet of the height of 10–15 mm with rhizomes can be used for repeated multiplication in a period of 45 days. This method can be used for an uninterrupted plant supply throughout the year, as against macroproliferation technology, where plants can be produced only twice in a year[13–15].

Micropropagation of *D. strictus* has been reported by Nadgir *et al.*[9] on a complex liquid MS medium supplemented with cytokinin, coconut milk and the use of rotary shakers. A separate rooting procedure has also been reported by them. In the method described by us, the 2-step process has been converted into a single step where multiplication, elongation and rooting take place on a single and very simple medium without using any rotating conditions.

Kumar[16] could obtain plantlets of *D. strictus* from seedling cultures on LS basal medium supplemented with auxins and cytokinins. According to him, continuous multiplication of cultures becomes difficult and results in high casualty due to lack of meristematic tissue at the base of the explant. He could develop *in vitro* rhizomes only in the presence of auxins. In the present protocol, elongation of plantlets and rhizome formation were simultaneous processes. 80% of cultures showed

rhizome formation from all the nodes of the initial plantlets. Such *in vitro*-grown rhizomes sprout and form plantlets when transferred to fresh production medium. *In vitro* rhizome formation increased the survival percentage of plants as well as early culm formation. Saxena and Bhojwani[17] observed vitrification of *D. longispathus* shoots on a liquid medium after 15 passages. However we have not observed any such vitrification or lower growth rate even after 25 subcultures. The cultures are still in the multiplication stage.

The plantlets grown by this method being prerooted on simple liquid medium needed very little care during hardening. These plants showed 90–95% survival in polyhouse (Figure 1 *h*, *i*). So far we have produced 15,000 plants by this method which have been supplied to various locations in India for field trials.

The method described here has a tremendous potential for mass-scale production of *D. strictus* plants and has many advantages because of the use of simple minimal medium, a stationary liquid culture, no special rooting treatment and *in vitro* development of rhizome which ensures high survival percentage with early culm formation. This method is very useful for batch production of uniform and identical plants.

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