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Rapid Multiplication of Bamboo by Tissue Culture*)

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(Received 7th March 1984)

Abstract

Multiple shoots of *Dendrocalamus strictus* from seedlings were obtained in shake flasks on liquid medium containing Murashige & Skoog's medium supplemented with BAP and coconut milk. Rooting of these shoots was obtained on Murashige & Skoog's half strength liquid medium after treatment with IBA for 48 hours in dark. The rooted plantlets were transferred to field. Multiple shoots from nodal segments of mature trees of *Dendrocalamus strictus, Bambusa arundinacea* and *Bambusa vulgaris* were obtained on Murashige & Skoog's medium supplemented with coconut milk, kinetin and BAP. Rooting of the shoots of *Dendrocalamus strictus* was obtained on Murashige & Skoog's half strength medium with activated charcoal after treatment with IBA for 96 hours in dark. Fifteen subcultures have been carried out from seedling explants without any diminution in the capacity for shoot or root formation. By this method of subculture it can be estimated that 10,000 plantlets can be obtained from one single seedling in a year.

Key words: Tissue culture, plantlets, seedling, mature nodal segments, *Dendrocalamus strictus, Bambusa arundinacea, Bambusa vulgaris.*

Zusammenfassung

Die Vermehrung von Sproßtrieben aus Sämlingen von *Dendrocalamus strictus* wurde im Schüttelkolben mit einem flüssigen Medium erreicht, welches aus Murashige & Skoog's Medium, angereichert mit BAP und Kokosmilch, bestand. Die Bewurzelung solcher Triebe wurde mit Murashige & Skoog's halbkonzentriertem Flüssigmedium nach 48stündiger Behandlung mit IBA im Dunkeln erzielt. Die bewurzelten Plantlets wurden danach auf die Freifläche verpflanzt. Die Vermehrung von Sproßteilen aus nodalen Segmenten von älteren Mutterbäumen von *Dendrocalamus strictus, Bambusa arundinacea* und *Bambusa vulgaris* werden mit dem Medium von Murashige & Skoog erzielt, ergänzt durch Kokosmilch, Kinetin und BAP. Die Bewurzelung von *Dendrocalamus strictus* Trieben wurde mit dem halbkonzentrierten Murashige & Skoog's Medium mit Aktivkohle nach einer 96stündigen Behandlung mit IBA im Dunkeln erzielt. Es wurden 15 Subkulturen mit Sämlingsexplantaten angelegt, ohne Verringerung der Kapazität der Sproß- oder Wurzelbildung. Mit dieser Methode der Subkulturen können in einem Jahr schätzungsweise 10.000 Plantlets von einem einzelnen Sämling erzielt werden.

Introduction

Bamboo is a versatile multipurpose forest tree which plays a vital role in the world's industrial and domestic

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Table 1. — Multiple shoot induction from seedling and mature tree explants of *D. strictus*

Sr. No.	Medium	Seedling shoots/seedling		Mature tree	
		Solid	Liquid	Shoots/ nodal segment	Opening of buds %
1.	Kn (0.2 - 1.0) or BAP (0.2 - 1.0)	-	1	-	-
2.	Kn (0.2) + CM (5%)	1	2 - 3	1	20
3.	BAP (0.2) + CM (5%) [MS-1]	4 - 5	8 -10	1	25
4.	BAP (0.2) + CM (5%) + IAA (0.05) or NAA (0.05)	2 - 3	6 - 7	1	20
5.	BAP (0.5) + CM (5%)	2	7 - 8	1 - 2	30
6.	BAP (0.2) + CM (10%)	3 - 4	6 - 8	1 - 2	40
7.	BAP (0.2) + Kn (0.2) + CM (5%)	3 - 4	7 - 8	1 - 2	50
8.	BAP (0.5) + Kn (0.2) + CM (10%) [MS-2]	2 - 3	4 - 5	2 - 3	80
9.	BAP (1.0) + Kn (0.2) + CM (10%)	1 - 2	2 - 3	1 - 2	55
10.	BAP (0.5) + Kn. (0.5) + CM (10%)	1 - 2	2 - 3	1 - 2	60
11.	BAP (0.5) + Kn (0.2) + CM (10%) + IAA (0.05) or NAA (0.05)	1 - 2	2 - 3	1	40
12.	BAP (0.5) + Kn (0.2) + CM (15%)	1	1 - 2	1 - 2	45

Basal Medium: Murashige and Skoog's (MS)
Incubation : Culture tubes were incubated at 25° C in light (1500 lux, 16 h photoperiod). Shake flasks containing liquid medium were kept on shaker rotating at 120 rev/min. under continuous light (1000 lux)

economics. India's natural resources of bamboo, consisting of a large number of species, constitute one of the world's largest reserves of this commodity. In India, out of a total forest area of 75 million hectares (VARMAH and PANT, 1981), bamboos, natural and planted, occupy 10 million hectares. It is estimated that out of an annual production of nearly 9.5 million tonnes of bamboo in India (VARMAH and PANT, 1981), about 4.9 million metric tonnes are being presently utilised for paper making, producing roughly 6,00,000 tonnes of paper pulp per year. This falls much below the country's demand. Out of a total of nearly 1,000 known species the world over (excluding bambusoid grasses), (VARMAH and PANT, 1981) about 100 have so far been found and recorded in India. *Dendrocalamus strictus* NEES, *Bambusa arundinacea* WILLD, and *Bambusa vulgaris* SCHRAD are among the important species found in India. The flowering (The Wealth of India, 1948) cycle of these 3 bamboo species can vary from 25—60 years when whole forests die after seeding. Bamboo seeds have a peculiar property that they have no dormancy period and also have a short viability. Bamboo is propagated mostly by seeds, offsets or rhizomes and also sometimes by cuttings and layers. The latter methods are not very successful. The rate at which the existing bamboo reserves are being utilised is far beyond the present replenishment of these forests since flowering and seeding are very unpredictable and vegetative methods are not very reliable.

Tissue culture offers an alternative method for rapid multiplication of bamboo species (KONDAS, 1982). By this technique success has already been achieved for some forest tree species such as teak (GUPTA et al., 1980) *Eucalyptus* species (MASCARENHAS *et al.*, 1982), sandalwood (RAO and BAPAT, 1980; SITA *et al.*, 1979) aspen (AHUJA, 1983; AHUJA and MUHS, 1982) and conifers (VERMA and EINSPAHR, 1983). Recently isolation of callus cultures (HUANG and MURASHIGE, 1983) and embryogenesis and plantlet formation (MEHTA *et al.*, 1982) has been reported in bamboo. This paper describes our studies on plantlet regeneration from seedlings and mature trees of *Dendrocalamus strictus* and of multiple shoot induction from mature trees of *Bambusa arundinacea* and *B. vulgaris*.

Materials and Methods

Bamboo seeds *(Dendrocalamus strictus)* were supplied by the Silviculturist, Maharashtra State, Pune. They were surface sterilized by standard methods (MASCARENHAS *et al.*, 1975) and kept for germination in dark on White's basal medium (WHITE, 1963) with 2% sucrose. After 4—5 days, cultures were transferred to light to enable a healthy growth of seedlings.

Mature trees: Nodal segments (10—15 mm) from the lateral branches of fresh twigs were collected from 10—15 year old trees of *Dendrocalamus strictus, Bambusa arundinacea* and *B. vulgaris* growing at Maharashtra State Forest Department, Hadapsar, Pune. These segments were surface sterilized with 0.1% $HgCl_2$ for 10 minutes and explants containing one node were inoculated in different media.

Culture media: The following media were used: (W) White's medium (WHITE, 1963) supplemented with 0.25 ppm each of Na_2MoO_4 and $CoCl_2$; (MS) Murashige and Skoog's basal (MURASHIGE, 1962) medium from which edamin, kinetin and IAA were omitted and sucrose added at 2%: (MS-1) MS medium containing 5% CM (coconut milk) 0.2 ppm BAP; (MS-2) MS medium containing 10% CM, 0.2 ppm kn (kinetin) and 0.5 ppm BAP (6-benzylaminopurine); (MS-3) MS medium added at half the concentration. The pH of all media were adjusted to 5.8. Either semisolid medium (20 ml containing 0.8% Difco agar in 20 mm × 150 mm Corning brand test tubes) or liquid medium (50 ml in 250 ml Erlenmeyer flasks) were used for multiple shoot induction. Test tubes containing liquid medium were provided with a filter paper support. All the chemicals used were of analytical grade (British Drug House, E. Merck, Sigma or Difco).

Culture conditions: Cultures were incubated at 25° C for 16 hours under light (illuminated by 40 Watt fluorescent tubes, 1500 lux and at 23° C in dark for 8 hours.

Five replicates were used for shoot initiation studies and ten replicates for determining the conditions for rooting. All experiments were repeated at least twice and were found reproducible. Data are presented as the average number of shoots per culture tube or flask and results of rooting experiments are presented as the percentage of cultures showing a rooting response .

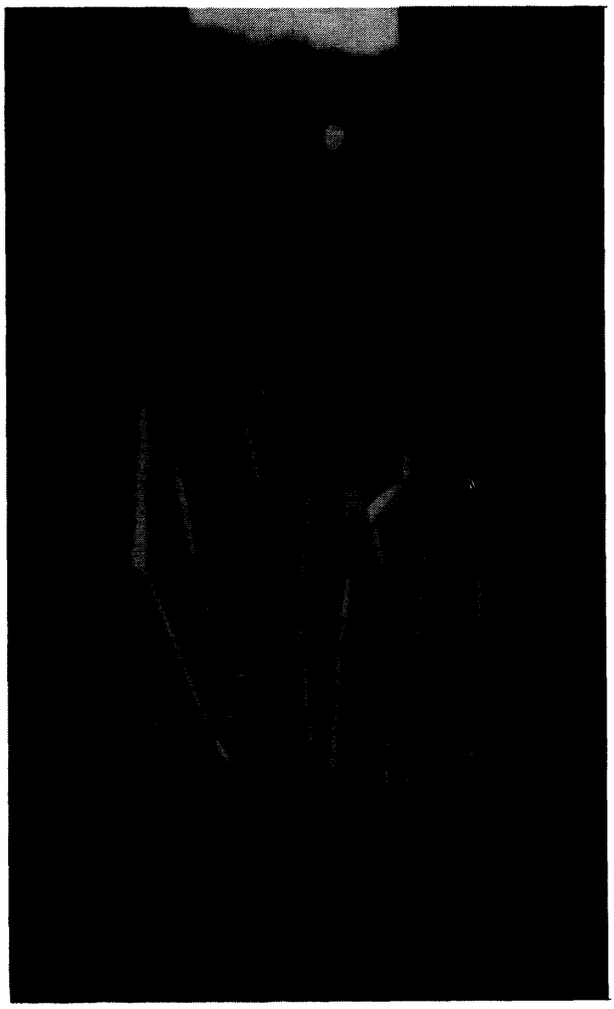

Fig. 1. — Multiple shoots of *D. strictus* (seedling) on (MS-1) medium.

Results and Discussion

Seedlings: When seedlings had attained a height of about 40—50 mm after 15—20 days they were transferred to solid and liquid (MS) basal medium both supplemented with different concentrations and combinations of Kn, BAP, NAA, IAA and CM. Maximum number of shoots (8—10) were obtained within 6—7 weeks in liquid shake flask (MS-1) medium rotating at 120 rev/min under continuous illumination (1000 lux) *(Table 1, Fig. 1).* Root formation was also observed from some of the shoots (40%) in liquid shake flasks (MS-1) medium after 15 days, which was not observed on solid medium of the same composition.

Subculture: For subculture, individual shoots growing on (MS-1) liquid medium in shake flasks were excised and separated from the base and transferred to fresh (MS-1) liquid medium and incubated on shakers as described earlier. By this procedure, 15 subcultures have already been carried out at intervals of 6—7 weeks. Six to seven shoots were found to develop from each shoot at each subculture.

Rooting: In a small percentage (40%) of shoots grown on (MS-1) medium in shake flasks, roots developed. The remaining shoots which did not show rhizogenesis were excised at the base of the explant when they had attained a height of 40—50 mm and the cut ends treated with dif-, ferent auxins (IAA, IBA, IPA & NAA) single or in combination at different concentrations (0.05—5.0 ppm) in (MS-3) liquid medium in test tubes. Treatments were given for different intervals of time (24, 48, 72 and 96 hours) in dark. 80% shoots rooted within 4 weeks, when treated with IBA (0.1 ppm) for 48 hours in dark followed by transfer to (MS-3) liquid medium in light *(Fig. 2).*

Transfer to pots

When the plantlets attained a height of about 50—60 mm they were transferred to pots containing a sterile soil: sand (1:1) mixture and covered with glass beakers to maintain humidity. These pots were incubated at 25° C (16 h light and 8 h dark). After 15—20 days when new leaves had emerged they were transferred to the green house *(Fig. 3)* and then to the field. The survival of rooted plantlets in the field was 70—80%. 15 plantlets were actually obtained from one seedling which are now undergoing field trials. No morphological abnormalities have been observed in the tissue culture raised plants which are now 15 months old. By this procedure it can be estimated that about 10,000 viable plantlets can be produced from one seedling in a year with subcultures every 6—7 weeks.

Mature trees: In preliminary experiments, explants from mature trees of *D. strictus* were inoculated under the same conditions using either (MS-1) agar or liquid medium in shake flasks. These conditions were however ineffective. The effect of different concentrations and combinations of Kn, BAP, IAA and CM were therefore tested using (MS) basal medium both in liquid and agar media. Of the different media combinations tested, 80% of the nodal segments from mature trees of *D. strictus, B. arundinacea* and *B. vulgaris* were found to develop shoots (2—3) *(Table 1)* within 3—4 weeks on (MS-2) semisolid medium containing 0.2 ppm Kn, 0.5 ppm BAP and 10% CM. The explants did not respond to shake flask conditions for multiple shoot induction as was found beneficial for seed-

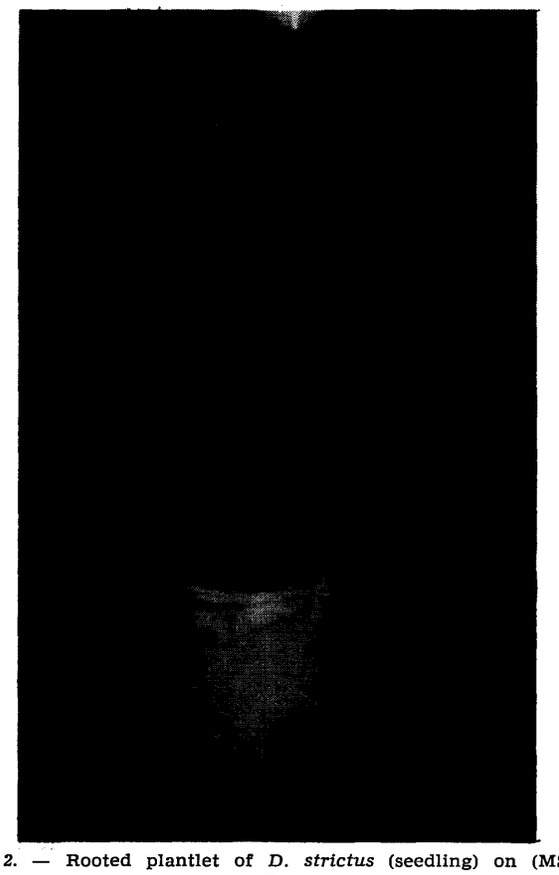

Fig. 2. — Rooted plantlet of *D. strictus* (seedling) on (MS-3) medium.

Fig. 3. — One month old *D. strictus* plant (seedling) in pot.

ling segments. When these shoots on (MS-2) agar media elongated to 20—30 mm, they were separated from the base of the explants and subcultured to fresh media of the same composition. Within 4—5 weeks 2—3 shoots developed from each shoot.

Rooting: When these shoots on (MS-2) medium had attained a height of about 30—40 mm, they were excised and given the same treatments and conditions used for rooting of seedling shoots but rooting was not observed. However 20% of the shoots from mature trees of *D. strictus* rooted within 4—5 weeks if treated with 1.0 ppm IBA for 96 h in dark followed by transfer to (MS-3) semisolid medium with 0.25 activated charcoal in light *(Fig. 4).* By this procedure the plantlets turned pale yellow and appeared very unhealthy. This could be avoided by transferring the rooted plantlets to (MS) liquid medium containing 0.1 ppm each of Kn and BAP with 5% CM. All the conditions tested were however ineffective for rooting of the shoots developed on (MS-2) medium from the mature tree explants of the other two bamboo species.

Experiments are in progress to increase multiplication on subculture and percentage of rooting from mature trees of *D. strictus* and also to induce rooting of shoots of mature trees of *B. arundinacea* and *B. vulgaris* and for transfer of these to field. The present results indicate that the method used for the propagation of seedlings was not applicable to tissues from mature trees and required several modifications. This has also been observed earlier in mature trees of teak (Gupta *et al.*, 1980) and *Eucalyptus citriodora* (Gupta *et al.*, 1981).

Explants from mature trees of the 3 species of bamboo *D. strictus, B. arundinacea* and *B. vulgaris* required a semisolid medium, a higher concentration of CM, BAP and also an additional cytokinin Kn (MS-2) as compared to the medium (MS-1) for seedling explants of *D. strictus* which grew better under liquid shake flask conditions, For root induction of shoots from mature trees of *D. strictus,* IBA at a 10 times higher level for 96 hours was required as compared to seedling shoots which rooted by treatment with 0.1 ppm IBA for 48 hours. Activated charcoal was found essential for root initiation from mature shoots, which was not essential for seedling shoots. These results could indicate that shoots from mature trees may contain some rooting inhibitors which are adsorbed by activated charcoal. A similar observation was reported earlier for some *Eucalyptus* species (Mascarenhas *et al.*, 1982).

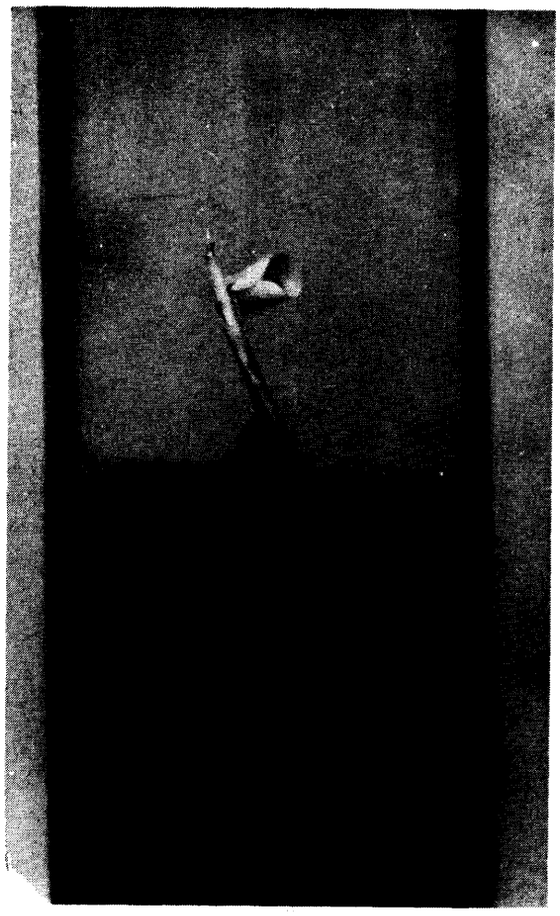

Fig. 4. — Rooted plantlet of *D. strictus* (mature tree) on (MS-3) medium with 0.25% activated charcoal.

Flowering in bamboo is gregarious and unpredictable (The Wealth of India, 1948) and may take anywhere from 25 to 60 years. After seeding the whole forests die. Moreover viability of bamboo seeds (DOGRA, 1981) persists for a short period, but can be extended if stored at lower temperatures. Because of these major drawbacks, any vegetative method of propagation even from seedlings would be very important if very high multiplication rates can be achieved. This would bypass the unpredictable flowering and seeding problems associated with many bamboo plantations. Furthermore by this method seedlings would always be available for planting. The method we have developed for *D. strictus* seedlings by which over 10,000 viable plantlets can be obtained from one seedling in a year could be an asset for plantation and social forestry programmes. To our knowledge, this is the first report on the rapid micropropagation of *D. strictus* where high rates of multiplication and survival of plants in the field have been achieved by tissue culture of seedlings.

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Results of 5- to 6-Year-Old Provenance Trials of Pinus oocarpa Schiede on Eight Sites in Puerto Rico

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(Received 14th March 1984)

Summary

Four of fifteen *Pinus oocarpa* provenances, three from Nicaragua and one from Mt. Pine Ridge, Belize, outperformed all others in Puerto Rico at five and six years from planting. Mean annual height and diameter increments for the four were respectively 2.0 m and 2.7 cm, compared to 2.0 m and 2.6 cm for a fast-growing Alamicamba, Nicaragua *Pinus caribaea* var. *hondurensis* tester provenance. Survival averaged 70% for the top *P. oocarpa* provenances and 76% for the *P. caribaea* tester. Detailed form and volume assessments at one site showed that, compared to *P. caribaea* provenances in adjacent, similar-age trials, *P. oocarpa* provenances had finer branching, flatter branch angles, greater forking, higher overbark volumes, and less foxtailing.

All *P. oocarpa* provenances suffered wind or rain damage from two tropical storms in 1979. Highest blow-down mortality averaged 16 to 20% and included two of the top *P. oocarpa* performers. The *P. caribaea* tester had only 3% blow-down and the least non-mortality damage, 17%. All *P. oocarpa* provenances had inferior wind resistance to the *P. caribaea* tester, indicating potential problems in reforesting large wind-prone areas with *P. oocarpa*.

Key words: Provenance test, *Pinus oocarpa, Pinus caribaea* var. *hondurensis*, hurricane damage.

Zusammenfassung

Vier von fünfzehn *Pinus oocarpa* Herkünften, drei aus Nicaragua und eine vom Mt. Pine Ridge in Belize, übertrafen in Puerto Rico 5 und 6 Jahre nach der Pflanzung alle anderen. Der mittlere jährliche Höhen- und Durchmesserzuwachs der vier Herkünfte betrug 2,00 m und 2,7 cm im Vergleich zu 2,00 m und 2,6 cm bei einer schnellwachsenden *Pinus caribaea* var. *hondurensis* Kontrollherkunft aus Alamicamba, Nicaragua. Das durchschnittliche Überlebensprozent betrug für die Spitzenherkünfte von *Pinus oocarpa* 70% und für die *Pinus caribaea* Kontrolle 76%. Detaillierte Form- und Volumenerhebungen an einem Standort zeigten, daß, verglichen mit *Pinus caribaea* Provenienzen, in benachbarten gleichaltrigen Versuchsflächen, *Pinus oocarpa* feinere Äste, flachere Astwinkel, eine größere Gabelung, ein höheres Stammvolumen mit Rinde und weniger Foxtails aufwies.