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An Efficient Technique for in Vitro Propagation of Dendrocalamus Brandisii Kurz using Nodal Segments

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Abstract

Bamboos are propagated by Vegetative propagation method which take long time and requires bulk quantity of planting material. Hence Plant tissue culture offers the unique opportunity to produce quality planting material in large volumes using micropropagation technique. An efficient technique for in vitro propagation of Dendrocalamus brandisii has been standardized by using nodal shoot segments. The parameters for shoot initiation, shoot multiplication, rooting of shoots and hardening were optimized by using Murashige and Skoog's (MS) medium (both liquid and agar gelled form) supplemented with suitable cytokinins and auxins in varying concentrations. To enhance the shoot growth and girth, MS medium is enriched with additives such as Ascorbic acid, Citric acid, Cysteine and Glutamic acid. The regenerated plantlets of *D. Brandisii* were successfully hardened in the green house by transferring them to sand, soilrite, and cocopeat mixture in the ratio (2:1:1).

Keywords: *D. Brandisii*, nodal explants, MS medium, additives.

1. Introduction

Micropropagation is a distinct approach of Biotechnology which plays a key role in the improvement of propagation through nodal segments and somatic embryogenesis which could help the regeneration of large numbers of plants in a relatively short time

(Chambers et al., 1991; Woods et al., 1995). For large scale production, efficiency of axillary shoot proliferation method is safe with less risk of aberration and basic research allows identifying endogenous and exogenous factors for rapid shoot induction, multiplication and rooting (Geilis and Oprins, 1998).

Dendrocalamus is a tropical genus of giant clumping bamboo having 29 species, which is similar to the genus *Bambusa*. This genus is found from the Indian subcontinent throughout Southeast Asia. *Dendrocalamus brandisii* is a plant having thick-walled culms with dark green velvety leaves similar to *D.asper*. Thick walled culms are used for construction and craft purposes and young shoots are edible. *Dendrocalamus brandisii* is distributed in Manipur and Andamans Islands and is introduced in Karnataka (Coorg) as well as in Kerala. It is found growing in the tropical forests, chiefly on calcareous rocks up to an altitude of 1300 m.

Dendrocalamus brandisii are versatile plants with enormous eco-sociological importance. Hence to produce quality planting material in large volumes, micropropagation using nodal shoot segments from selected mature plants was undertaken.

2. Material and Methods

2.1 Explant Preparation:

Mother plants were collected from the germplasm nursery of Institute of Wood Science and Technology, Bangalore as well as from its nursery of Gottipura and were preserved in the green house at Dayananda Sagar Institutions, Bangalore.

Nodal segment of 1.5 to 2cm in length was collected from selected mother plant and were treated with 5-6 drops of Tween 80 for 5mins and thoroughly washed with distilled water. Then they were rinsed in a solution of 0.1-0.2% bavistin for 5-6mins to avoid fungal contamination. Buds were treated with 70% alcohol for 20-30secs followed by mercuric chloride of 0.75%-0.1% for 4-5mins and subsequently washed with sterile distilled water and inoculated into nutrient medium.

2.2 Media Preparation

Analytical grade chemicals obtained from Himedia laboratories and hormones and vitamins from Sigma-Aldrich chemicals were used for preparing stock solutions and subsequent media preparation. Murashige and Skoog's (1962) medium with 3% (w/v) sucrose was used as basal medium. Growth regulators and additives (Ascorbic acid-25mg/l, Citric acid-12.5mg/l, Cysteine-12.5mg/l and Glutamic acid-50mg/l) are added to the basal medium. pH of the medium was adjusted to 6.2 and autoclaved at 121ºC for 15-20mins.

2.3 Incubation conditions

The cultures were incubated at 28±2°C in a growth room with 16hrs daylight regime at about 2500lux which is provided by cool-day fluorescent lamps.

2.3 Subculture
The shoot clumps were transferred to fresh media under aseptic conditions within two weeks period. Delay in sub-culturing leads to deterioration of cultures.

2.4 Shoot initiation
For initial multiple shoot induction, the explants were cultured on Murashige and Skoog's medium supplemented with different concentrations of BAP and TDZ (0.00mg/l-5.0mg/l) in combination with NAA (0.25mg/l). MS medium both in liquid and agar gelled form were used to study the high rate of multiple shoot induction in *Dendrocalamus brandisii.*

2.5 Shoot multiplication
In vitro shoots were used in clump of 2-6 shoots/clump for better multiplication and clump formation and sub culturing was carried out within 2weeks into fresh MS medium. In order to increase the number of shoots further, shoot clumps were excised from mother explant and sub-cultured to fresh MS medium.

2.6 In vitro rooting
Shoot clumps (2-3shoots) with 3-4cm in length were used for rooting. The various auxins such as NAA, IAA, IBA and NOA and their concentration at 1.0mg/l in a liquid and agar gelled form of a modified MS medium (i.e., MS½ and MS¾) were tested for *In vitro* rooting.

2.7 Hardening of plantlets
In vitro rooted plants were successfully transplanted into containers (polybags) consisting of rooting mixture and placed within the polytunnels/polyglobules inside green house. Relative humidity at 75-80% and temperature 28±2°C was provided for 2-3weeks period for achieving high rate of survival of plants at hardening stage.

3. Results and Discussion
3.1 Shoot initiation
D. Brandisii, in MS liquid media supplemented with NAA (0.25mg/l) and TDZ (0.25mg/l) was shown to be best for shoot initiation from nodal bud explants compare to MS agar medium. The bud:shoot ratio was found to be 1-6 after two weeks period**(Table 1, Fig. 1).** After a period of three weeks, 5-6 elongated shoots/node was observed **(Fig. 2)**.

These results are in contrast to report by Ratan Lal Banik et al., (1993) who had observed higher concentration of BAP (5mg/l) for shoot proliferation in *D.brandisii.* Saxena (1990) has also reported that higher concentration BAP ($8X10^{-6}$ M) and Kn ($4X10^{-6}$M) in MS liquid medium was favourable for multiple shoot induction from three weeks old *in vitro* grown seedlings of *B.tulda.*

Table 1: Shoot initiation in MS liquid medium with different concentrations of BAP and TDZ.

Sl. No	Media	Total no of buds	No of shoots/ clump*
1.	MS+additives**	8	0-1
2.	MS+additives+NAA-0.25 mg/l+BAP-1.0mg/l	8	0-3
3.	MS+additives+NAA-0.25 mg/l+BAP-2.5mg/l	8	1-3
4.	MS+additives+NAA-0.25mg/l +BAP-5.0mg/l	8	1-3
5.	MS+additives+NAA-0.25mg/l+TDZ-0.25mg/l	8	1-6
6.	MS+additives+NAA-0.25mg/l+TDZ-0.5mg/l	8	1-3
7.	MS+additives+NAA-0.25mg/l+TDZ-1.0mg/l	8	1-3

*: Data scored after two weeks period.
**: Hormone free.

Additives-Ascorbic acid-25mg/l, Citric acid-12.5mg/l, Cysteine-12.5mg/l, Glutamic acid-50mg/l.

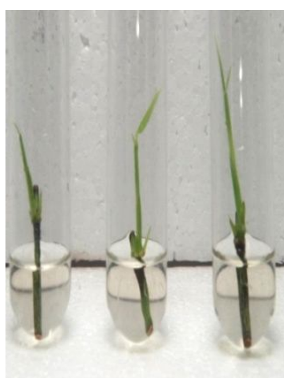

Fig. 1: Shoot initiation from nodal segments (one week after incubation)

Fig. 2: Shoot elongation (after three weeks)

Table 2: Shoot initiation in MS Agar gelled medium with different concentrations of BAP and TDZ.

Sl. No	Media	Total no of Buds	No of Shoots/ Clump*
1.	MS+additives**	8	0-1
2.	MS+additives+NAA-0.25 mg/l+BAP-1.0mg/l	8	0-2
3.	MS+additives+NAA-0.25 mg/l+BAP-2.5mg/l	8	1-3
4.	MS+additives+NAA-0.25mg/l +BAP-5.0mg/l	8	1-3
5.	MS+additives+NAA-0.25mg/l+TDZ-0.25mg/l	8	1-3

6.	MS+additives+NAA-0.25mg/l+TDZ-0.5mg/l	8	1-2
7.	MS+additives+NAA-0.25mg/l+TDZ-1.0mg/l	8	1-3

*: Data scored after two weeks period.
**: Hormone free.

Additives-Ascorbic acid-25mg/l, Citric acid-12.5mg/l, Cysteine-12.5mg/l, Glutamic acid-50mg/l.

3.2 Shoot multiplication

Initial subculture was carried out on MS liquid medium enriched with additives, NAA (0.25mg/l), TDZ (0.25mg/l) for 2-3 passages to increase the stock. Since TDZ is a highly active cytokinin its further use induced pseudoshoots. Hence MS liquid medium with BAP was found to be suitable for further shoot multiplication and maintenance.

In order to analyse the effect of MS media in liquid and agar form, BAP at different concentrations was used. The experimental results revealed that the MS liquid medium with NAA(0.25mg/l) +BAP(2.5mg/l) +additives(**Table 3, Fig. 3)** proved the best for inducing higher shoot multiplication with maximum shoot growth whereas agar gelled medium induced less number of shoots and poor shoot growth.

In vitro shoots were used in clumps of 2-6 shoots/clump for better multiplication and clump formation and subculturing was carried out within 2weeks period into fresh medium. Shoot multiplication rate was increased by 2-3 folds and delay in sub-culturing lead to deterioration of cultures

In contrast to our findings, Mukunthakumar et al., (1999), reported that higher concentration of BAP (5mg/l) on MS medium was better for shoot multiplication and their maintenance.

Table 3: Effects of BAP at different concentrations on shoot multiplication in MS liquid medium.

Sl No	Media	No of shoots/ clump	No of shoots/ clump*
1	MS+additives**	5	3
2	MS+additives+NAA-0.25 +BAP-0.5	5	5
3	MS+additives+NAA-0.25+BAP-1.0	5	7
4	MS+additives+NAA-0.25+BAP-2.0	5	6
5	MS+additives+NAA-0.25+BAP-2.5	5	10
6	MS+additives+NAA-0.25+BAP-3.5	5	13
7	MS+additives+NAA-0.25+BAP-4.0	5	8
8	MS+additives+NAA-0.25+BAP-4.5	5	12
9	MS+additives+NAA-0.25+BAP-5.0	5	9

*: Data scored within two weeks period.
**: Hormone free.

- Concentrations of growth substances in mg/l.

Additives-Ascorbic acid-25mg/l, Citric acid-12.5mg/l, Cysteine-12.5mg/l, Glutamic acid-50mg/l.

Fig. 3: Shoot multiplication (after two weeks)

Table 4: Effects of BAP at different concentrations on shoot multiplication in MS agar gelled medium.

Sl. No	Media	No of Shoots/ Clump	No of Shoots/ Clump*
1	MS+additives**	5	3
2	MS+additives+NAA-0.25 +BAP-0.5	5	6
3	MS+additives+NAA-0.25+BAP-1.0	5	8
4	MS+additives+NAA-0.25+BAP-2.0	5	10
5	MS+additives+NAA-0.25+BAP-2.5	5	18
6	MS+additives+NAA-0.25+BAP-3.5	5	10
7	MS+additives+NAA-0.25+BAP-4.0	5	13
8	MS+additives+NAA-0.25+BAP-4.5	5	14
9	MS+additives+NAA-0.25+BAP-5.0	5	16

*: Data scored within two weeks period.
**: Hormone free.

- Concentrations of growth substances in mg/l.

Additives-Ascorbic acid-25mg/l, Citric acid-12.5mg/l, Cysteine-12.5mg/l, Glutamic acid-50mg/l.

3.3 In vitro rooting

Rooting is the most crucial step particularly from the shoots originating from mature woody plants including bamboo species. Success of the protocol also depends on

rooting frequency. Various factors such as auxins source, its concentration and incubation conditions have significant role on *in vitro* rooting.

Among all the auxins tested, NAA was found to be the best in terms of rooting frequency, root number, and root length. In comparison with MS½ strength and MS¾ strength media, MS½ strength liquid media was found to be suitable for inducing more number of roots than MS½ strength solid media **(Table 5, Fig. 4)**. In MS¾ strength media, browning of shoots was noticed.

Root clump formation was significantly better in MS½ liquid medium supplemented with NAA (1mg/l) than other auxins in *D.brandisii* within 4 weeks period. Comparatively significant difference was noticed in different auxins treatments and NAA was found to be most effective by inducing 80-90% rooting followed by IBA, NOA, and IAA.

Contrary to our reasults, Arya and Sharma (1998) have reported 80-85% rooting from *in vitro* shoots of *B.bambos* on MS medium (3.0mg/l).

Prutpongse and Gavinlertvatana (1992) tested various auxins in MS, MS½ strength, and MS¾ strength medium and reported that MS medium with NAA (1mg/l) proved best in most of the species originated from about 10 year old culms, which is in concurrence with our findings.

Table 5-Effect of different auxins in MS½ liquid medium on *in vitro* rooting.

Sl. No	Media	No of Roots/ Clump*	Root Length (cm)*
1	MS½ +additives**	1	3.1
2	MS½ +additives+NAA-1.0mg/l	5	10.4
3	MS½ +additives+IAA-1.0mg/l	2	1.6
4	MS½ +additives+IBA-1.0mg/l	3.5	7.2
5	MS½ +additives+NOA-1.0mg/l	2	6.8

*: Data scored after four weeks period.
**: Hormone free.

Additives-Ascorbic acid-25mg/l, Citric acid-12.5mg/l, Cysteine-12.5mg/l, Glutamic acid-50mg/l

Fig. 4: Rooting (after four weeks)

Table 6: Effects of different auxins in MS½ agar gelled medium on *in vitro* rooting.

Sl. No	Media	No of Roots/ Clump*	Root Length (cm)*
1.	MS½ +additives**	1	2.4
2.	MS½ +additives+NAA-1.0mg/l	4	5.1
3.	MS½ +additives+IAA-1.0mg/l	2	1.8
4.	MS½ +additives+IBA-1.0mg/l	4	4.2
5.	MS½ +additives+NOA-1.0mg/l	1	3.6

*:Data scored after four weeks period.
**: Hormone free.

Additives-Ascorbic acid-25mg/l, Citric acid-12.5mg/l, Cysteine-12.5mg/l, Glutamic acid-50mg/l

4. Hardening of Plantlets

Rooted shoots under *in vitro* conditions are partially autotrophs, having poor development of epicuticular wax and weak regulation of stomata. Therefore gradual hardening is essential for high rate of survival.

Plantlets were directly transferred from rooting medium to rooting/potting mixture. Rooting mixture plays a crucial role by providing physical support, retention of moisture, providing drainage and good aeration to *in vitro* rooted plantlets.

After 3-4 weeks **(Fig. 5)** plantlets were placed outside the polytunnel and were watered everyday. *In vitro* rooted shoots of *D.brandisii* produced through nodal shoot proliferation were hardened for two months, which resulted in 80-90% survival rate.

Fig. 5: One month old hardened plantlets.

Chaturvedi et al., (1993) have also recorded 80% success rate in micropropagated plantlets originated through nodal segments from the culm of *D.strictus* using the potting mixture.

5. Conclusion

In conclusion, this study clearly demonstrates high frequency multiplication and enhanced rooting in *D.brandisii*. These features are necessary for increased production of true-to-type plants for large-scale multiplication of this species. Tissue culture method allows the production of a large number of plantlets identical to the mother plant, is less labour intensive and cheaper once the protocols has been standardized and hence offers distinct advantages over conventional methods of multiplication of different species of elite bamboo clones.

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