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Somatic embryogenesis of bamboo from root tip explants

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Abstract

With a view to develop and establish a protocol for rapid micropropagation of bamboo, root tip explants of *Bambusa tulda* were used. Root tip explants of *Bambusa tulda* were cultured in MS medium supplemented with different concentrations and combinations of 2,4-D and BAP. 2,4-D at 4 mgL^{-1} in combination with BAP at 3 mgL^{-1} produced calli from root tip explants after 15 days of culture. Subsequently the calli were subcultured in the same medium containing different concentrations and combinations of 2,4-D and BAP. Use of 2,4-D at 3 mgL^{-1} and BAP at 4 mgL^{-1} showed better performance than others. Green shoots were observed after 15 days of subculture and subsequent growth and development were also better in the same combination. After regeneration of shoots a rapid growth rate (1.508 mm day^{-1}) was observed at 3 mgL^{-1} 2,4-D and 4 mgL^{-1} BAP. The developed shoots from root tip explants were then transferred to the same medium containing Indole acetic acid (IAA; 1mgL^{-1}) for rooting. Rooting was absent in that medium.

Keywords: Bamboo, Root tip explants and Somatic embryogenesis

Introduction

Bamboos are tall, perennial and arborescent grasses of Poaceae family. Normally bamboos flower once in its lifecycle and die thereafter. It is found in tropics, subtropics, and even mild temperate regions i.e. strikingly occurrence of bamboos is very high in Central and South America and Eastern Asia. The vegetative phase varies from 3-120 years and its growth in vertiginous. Elite bamboos are recognised on the basis of long and solid culms, long internodes, sparse branching, large number of culms per clump, resistance to disease and pests, drought and frost tolerance and less palatability.

Bamboo is one of the most versatile forest produce and important renewable natural resource having innumerable uses and applications. Different parts of bamboo plant are useful to the mankind. The culm portion is most widely used as structural material for building construction purposes and as raw material for the pulp, paper and products of small cottage industries (Tiwari, 1992) like mats, buskets, toys, hats, wall plates, wall hangers and trays, flutes, fishing rods, etc are made of them. It is known as poor man's timbers. It has become a high value crop for its mass utilization in different paper, pulp and cottage industries (Raste and Bhojwani, 1998). There are several bamboo species for which regeneration of complete plantlets through somatic embryogenesis has been obtained. These includes *Bambusa bamboos* (Mehta *et al.*, 1982), *B. beecheyana* var. *beecheyana* (Yeh and Chang, 1986b), *B. vulgaris* (Rout and Das, 1994), *D. giganteus* (Rout and Das, 1994), and *Sinocalamus latiflora* (Tsay *et al.,* 1990). With the increase of population, the demand of bamboos is getting considerably high. So, the concerted efforts are immediate need to ensure an adequate supply in near future. These increasing demands can be achieved by expanding land area by bamboo cultivation or by augmenting the yields per unit area. Conventional methods for bamboo cultivation by expanding land area is impractical due to many reasons such as poor seed set, long flowering cycle (30-60 yrs), seed damage by rodents, rapid loss of seed viability. Propagation is done by planting rhizomes or offsets, layering, nodal cuttings and marcotting. The conventional methods of cultivation or propagation is time consuming, unpredictable, unreliable and less productive. Therefore, there is an urgent need for devising methods for rapid multiplication of bamboo in near future. *In vitro* regeneration method may solve many of the limitations encountered in conventional methods and can be used for rapid multiplication of elite clones.

Materials and Methods

In vitro regeneration of bamboo was attempted to overcome many of the limitations of the conventional method of propagation. Root tips of *Bambusa tulda* were used in the experiment. Murashige and Skoog (MS) medium was used for somatic embryogenesis. 30 gL^{-1} sucrose was used in the medium as carbon source. Different concentrations and combinations of auxin (2,4-D) and cytokinin (BAP) were used in the culture medium. Proper sterilization of MS medium, test tube, pipette, petriplates, beaker, conical flask, transfer hood, forceps, scalpels and growth chamber were taken by different ways and means. Laminar airflow cabinet was maintained a good aseptic condition. Surface sterilization of root tip explants was done by different concentrations of $HgCl_2$. Minimum contamination was found at 0.15 gL^{-1} $HgCl_2$ treated explants but 0.10 gL^{-1} $HgCl_2$ treated for 10 minutes resulted a good percent of regeneration. Data on callus initiation, callus formation, callus weight, growth rate of calli and shoots were recorded. The observation of cultures started from 7^{th} day of inoculation and continued up to 30^{th} day. Any swelling started in cultures was treated as callusing and recorded as proliferation of cultures. The percentage of explants formed callus was calculated from total number of explants used in culture. The fresh weight of calli developed from explants were recorded by excluding the initial weight of flasks containing media and inoculated explants from the further weight of flasks gained. The calli were subcultured after 30 days of culture. The fresh weight of shoot was also calculated by the same way of callus weight determination. The growth rate of shoots was recorded in mm day^{-1} by dividing the length of developed shoots (mm) with total period of time (day).

Results and Discussion

Callus induction/development from root tip explants

Root tip explants of *Bambusa tulda* produced callus when cultured on medium supplemented with different concentrations and combinations of 2,4-D and BAP. Maximum callus induction was achieved on the medium supplemented with 4 mgL^{-1} 2,4-D and 3 mgL^{-1} BAP. Reports of Ravikumar *et al.* (1998) described that multiple shoots were induced from seedling and axillary buds of mature *Dendrocalamus strictus* plants on medium supplemented with BA and Kinetin.

Fresh weight of calli developed from root tip explants

Initiation of calli were observed after 15 DAI. Different concentrations and combinations of 2,4-D and BAP were used for the growth of the callus. The combination of 4 mgL^{-1} 2,4-D and 3 mgL^{-1} BAP resulted better growth of calli. The fresh weight of 0.093, 0.155, 0.210, 0.300 g were obtained at 15, 20, 25 and 30 DAI respectively. The initial weight of explants was 0.05 g where as after 30 DAI the explants attained a weight of 0.300 g. The best average increase of weight of 0.013 g day^{-1} was recorded with the same combination (Table 1, Fig. 1. A-B). Chang and Lan (1995) achieved embryogenesis and subsequent formation of plantlets from calli of root explants of *Bambusa beecheyana* var. beecheyana.

Fresh weight of shoot obtained from calli of root tip explants

Shoot initiation was recorded after 15 days of subculture. Different concentrations and combinations of 2, 4-D and BAP were used for the growth of the shoot derived from the callus of root tip explants. Among the concentrations, 3 mgL^{-1} 2,4-D and 4 mgL^{-1} BAP had better growth resulting fresh weight of shoots to be 0.450, 0.985, 1.822 and 2.511 g after 15, 20, 25 and 30 days of subculture respectively. Average fresh weight of shoot increased at the rate of 0.110 g day^{-1} at this combination (Table 2).

Table 1. Effects of plant growth regulators on the fresh weight of callus developed from root tip explants

Concentration and combination of PGRs (mgL^{-1})		Fresh weight of explant (g) inoculated	Fresh weight of callus (g) at days after culture				Average weight of callus (g) initiated day^{-1}
2,4-D	BAP		15	20	25	30	
2	1	0.05	0.052b	0.120b	0.151b	0.234b	0.005b
3	2	0.05	0.051c	0.080c	0.120c	0.169c	0.003c
4	3	0.05	0.093a	0.155a	0.210a	0.300a	0.013a
$LSD_{0.05}$			0.001	0.001	0.001	0.039	0.001
CV (%)			1.61	6.92	5.11	27.20	48.26

- In column, figures with same letter(s) do not differ significantly at 5% level of significance

Table 2. Effects of plant growth regulators on the fresh weight of shoot developed from calli of root tip

Concentration and combination of PGRs (mgL^{-1})		Fresh weight of callus(g) during subculture	Fresh weight of shoot (g) at days after subculture				Average weight (g) of shoot gained day^{-1}
2,4-D	BAP		15	20	25	30	
1	2	0.30	0.385c	0.588c	1.290c	1.997c	0.084c
2	3	0.30	0.401b	0.812b	1.672b	2.130b	0.091b
3	4	0.30	0.450a	0.985a	1.822a	2.511a	0.110a
$LSD_{0.05}$			0.001	0.001	0.001	0.001	0.001
CV (%)			1.42	0.13	0.37	0.26	6.14

- In column, figures with same letter(s) do not differ significantly at 5% level of significance

Growth rate of shoots developed from calli of root tip explants

The rate of growth of shoots initiated from the calli was recorded at 15 days after subculture. Different combinations and concentrations of 2,4-D and BAP were used in the culture media. 3 mgL^{-1} 2,4-D and 4 mgL^{-1} BAP produced healthy shoots and simultaneously, highest growth rate of the shoots was also observed on the same medium (Table 3).

Table 3. Effects of plant growth regulators on the growth rate of shoot developed from calli of root tip

Concentration and combination of PGRs (mgL^{-1})		Initial stage of shoot (mm)	Length (mm) of developed shoots at days after subculture				Growth rate of shoot (mm day^{-1})
2,4-D	BAP		15	20	25	30	
1	2	0.0	6.50c	13.25c	24.50c	37.25c	1.240c
2	3	0.0	7.00b	16.50b	27.25b	39.75b	1.325b
3	4	0.0	8.50a	18.25a	29.50a	45.25a	1.508a
$LSD_{0.05}$			0.178	0.109	0.167	0.006	0.006
CV (%)			1.21	0.36	0.30	0.02	0.43

- In column, figures with same letter(s) do not differ significantly at 5% level of significance

Initiation of new leaf

During the process of subculturing first leaf appeared at 15 days of subculture. Gradually all other leaves initiated during the development of the shoot and after 30 days of subculture six leaves were counted and total height of the shoot was 45.25 mm (Fig.1. C-G). At this stage, the shoots were transferred to rooting medium supplemented with 1 mg^{-1} IAA for root initiation. Rooting was absent on this medium.

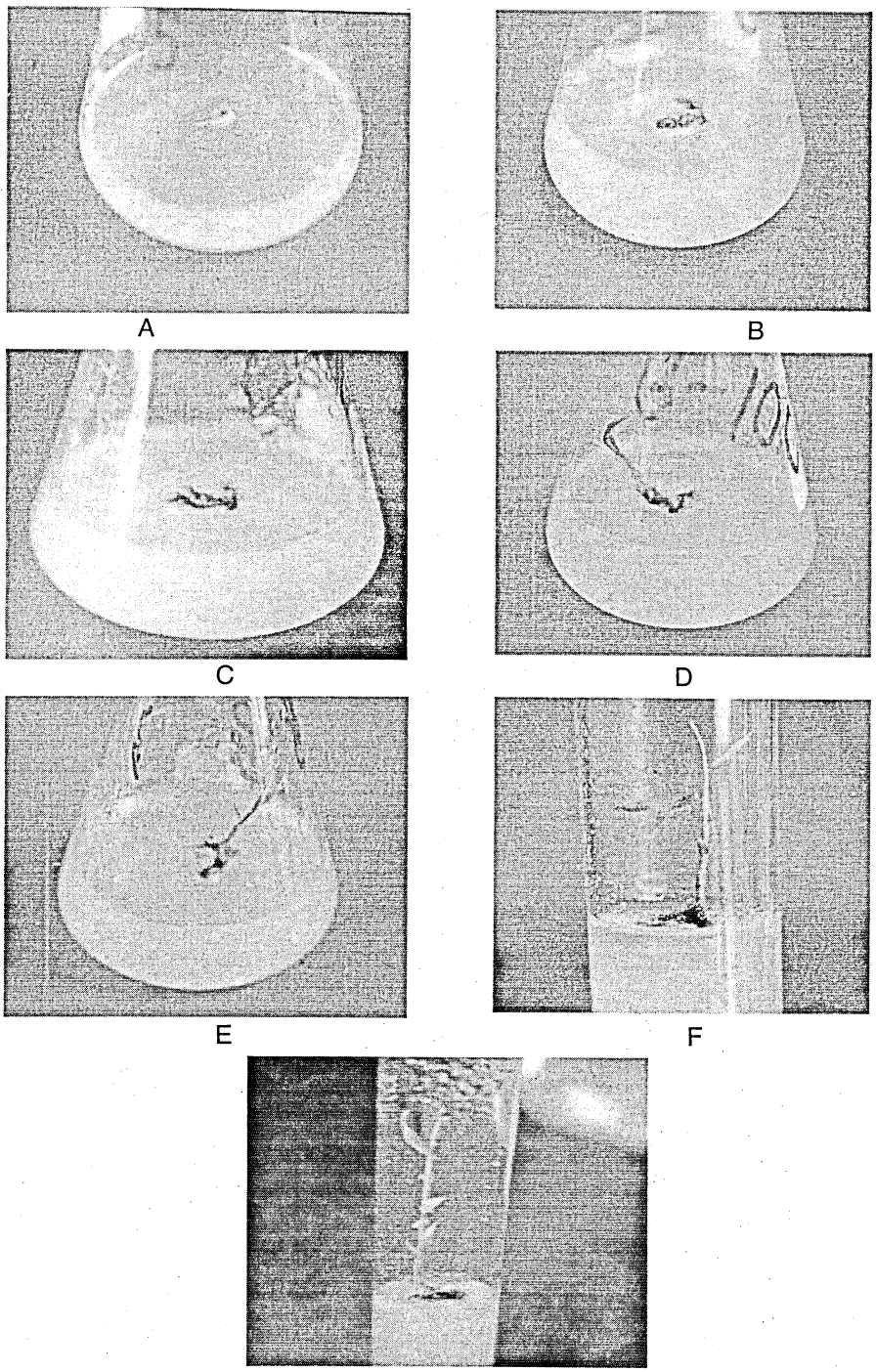

Fig. 1 **A-B.** Rgeneration through root tip explants culture in MS medium supplemented with 4mgL^{-1} 2,4-D and 3mgL^{-1}BAP. **A.** Root tip explants culture. **B.** Developmental stage of callus after 30 days of culture.

Fig. 1. **C-G.** Subculturing of callus in MS medium supplemented with 3mg L^{-1} 2,4-D and 4mg L^{-1} BAP. **C.** Developmental stage of shoot after 15 days of subculture. **D.** after 20 days **E.** after 25 days. **F.** after 30 days of subculture (development of six leaves). G. Shoot transferred to rooting medium.

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