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Somatic embryogenesis and regeneration of plants in the bamboo *Dendrocalamus strictus*

I. Usha Rao, I. V. Ramanuja Rao, and Vibha Narang

Department of Botany, University of Delhi, Delhi 110 007, India

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Abstract

Somatic embryogenesis leading to plant regeneration has been achieved in the bamboo, Dendrocalamus strictus, by culturing seeds (caryopses) on B_5 basal medium supplemented with 2,4-dichlorophenoxyacetic acid. Callus cultures obtained from the embryonal end of the seeds differentiated chlorophyllous embryoids. On transfer to a germination medium (B_5 liquid, sucrose, indolebutyric acid, and α-naphthaleneacetic acid) 40% of the embryoids developed into plantlets. Further development of the plantlets occured on B_5 liquid medium (half strength) + sucrose (1%) + IBA (5×10^{-7}M) + NAA (10^{-7}M).

Abbreviations

2,4-D = 2,4-dichlorophenoxyacetic acid; IBA = Indolebutyric acid; NAA = α-naphthaleneacetic acid.

Key words: Bamboo, Dendrocalamus strictus, Regeneration, Somatic Embryogenesis.

Introduction

There is an urgent need for developing a method for large scale propagation of bamboos. This has become essential because of severe paucity of planting material. Vegetative propagation through conventionally used offsets is beset with problems such as bulkiness and non-availability of the propagules and difficulties in transport over long distances. Propagation through seeds is also undependable on account of the long intervals between seeding (48 years in Dendrocalamus strictus), the large scale consumption of seeds by wild animals, seed sterility and poor seed set during off-season flowering. In comparison, in vitro propagation of bamboos if adequately developed can prove to be a reliable method for establishment of new bamboo plantations.

Somatic embryogenesis has recently been reported in cereals and millets (Wernicke and Brettell 1980, Lu and Vasil 1981, Vasil and Vasil 1981, Ozias-Akins and Vasil 1982, Ammirato 1983, Lu et al. 1983, Ho and Vasil 1983, Boyes and Vasil 1984). In bamboos, apart from aseptic development of embryos excised from mature seeds (Alexander and Rao 1968), protoplast release in Bambusa (Tseng et al. 1975) and initiation of callus cultures of Bambusa, Phyllostachys and Sasa from leaves of the shoot tips (Huang and Murashige 1983), limited headway has been made in their in vitro culture. Recently, however, Mehta et al. (1982) reported somatic embryogenesis and regeneration of plantlets in Bambusa arundinacea.

In this communication we report the production of somatic embryos from callus formed from the embryonal end of cultured caryopses and their development into plantlets in Dendrocalamus strictus. Economically this is the most important bamboo species in India, accounting for 66.6% of the raw material used for paper production.

Material and Methods

Seeds of Dendrocalamus strictus Nees were obtained from the Chanderpur forest near Nagpur, India. After dehusking, the mature bold seeds were washed in 2% Teepol (Shell, India) solution on a magnetic stirrer for 5 min. The Teepol solution was removed by washing in running tap water for 15-20 min followed by a rinse

Offprint requests to: I. Usha Rao

in distilled water. Sterilization of the material was effected by a 5 min immersion of seeds in chlorine water (saturated chlorine water diluted 5 times with distilled water) followed by thorough washing in sterile distilled water. Surface moisture was removed with a sterilised filter paper and the seeds were implanted in tubes containing nutrient medium under aseptic conditions.

The nutrient medium consisted of salts and vitamins of B_5 basal medium (Gamborg et al. 1968), sucrose (2%), 0.8% agar (w/v) supplemented with various concentrations of 2,4-D (10^{-5}M, 3 x 10^{-5}M and 10^{-4}M). The pH of the medium was adjusted to 5.8 prior to autoclaving at 15 psi for 15 min. Cultures were maintained at 27 ± 2°C under continuous illumination (2,500 lx) provided by cool white daylight fluorescent tubes. Forty-eight tubes were inoculated for each treatment. Germination of the embryoids and plantlet formation was carried out on B_5 (liquid) medium supplemented with 2% sucrose, IBA (5 x 10^{-7}M) and NAA (10^{-7}M). Plantlets were transferred to B_5 liquid medium (half strength), sucrose (1%), IBA (5 x 10^{-7}M) and NAA (10^{-7}M) for further development.

Squash preparations of calli were made in acetocarmine (1%) and mounted in glycerine (10%). The cultures were photographed in a Zeiss SV8 Stereozoom Microscope.

Results and Discussion

Callusing from the embryonal end of the seeds occured on B_5 basal medium containing 2,4-D at a concentration of 10^{-5}M and 3 x 10^{-5}M. Both friable and compact, white to creamish calli were obtained within 10-12 days of culture. The compact callus showed a lobed or nodular appearance. Subsequently, localised development of chlorophyll occurred and several small whitish to green embryoids, some with well developed scutella, were observed in the compact calli by 30 days (Fig. 1A). An average of 8.45 embryoids at the chlorophyllous stage of the callus were observed after dissection in a 30-day-old culture. In several embryoids the scutellar region proliferated and gave rise to a second generation of embryoids (Fig. 1B). Through this mechanism it has been possible to continually obtain somatic embryoids. This property is not lost on subculture of the differentiating callus.

Two distinct types of cells could be seen in acetocarmine squash preparations of the callus. The small, generally rounded cells (at times elongated and slightly thick walled) were rich in cytoplasm and had prominent nuclei and starch grains. These cells were embryogenic. Further stages leading to organized filamentous and globular structures were also observed (Fig. 1C,D). The second type of cells were thin-walled, long and highly vacuolated with little cytoplasm and few starch grains. About 67 per cent of the callus cultures formed green and well-differentiated somatic embryos. In the remaining cultures, only undifferentiated calli were observed although filamentous embryogenic structures could be seen in the histological preparations.

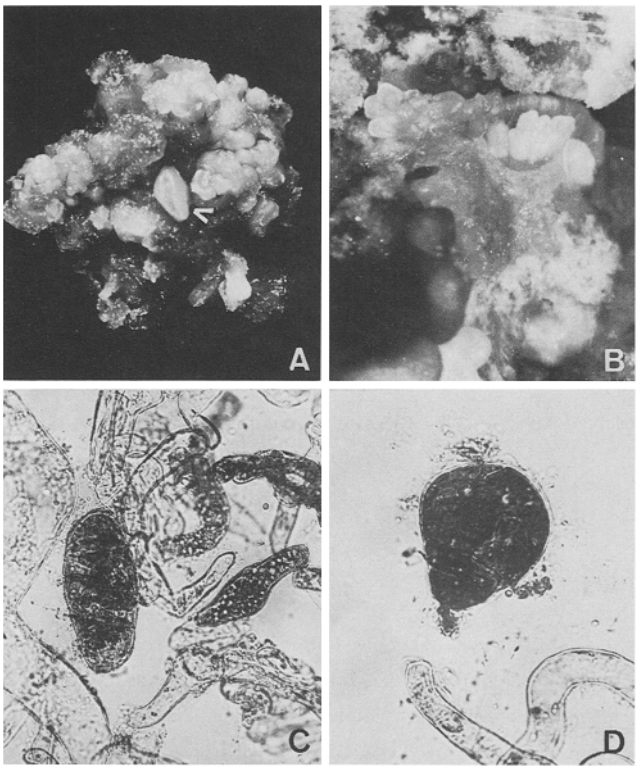
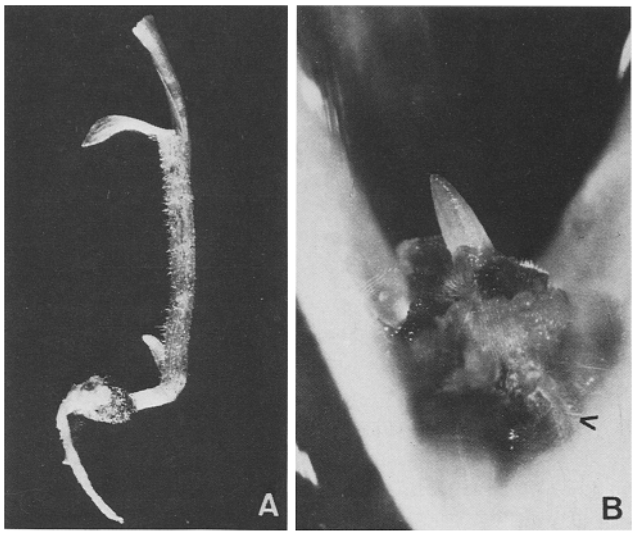


Fig. 1A. Callus with chlorophyllous embryoids 30 days after culture on B_5 + 2,4-D (3 x 10^{-5}M). Arrow indicates the well-developed scutellum in a mature embryoid. X 2.

Fig. 1B. Callus cultures depicting the proliferating scutellar region in several embryoids which has given rise to further embryoids. X 3.

Fig. 1C,D. Acetocarmine squash preparations of callus showing highly cytoplasmic and densely stained multicellular embryogenic structures. Note also the presence of starch-filled cells and highly vacuolated cells. C X 1333; D X 920.



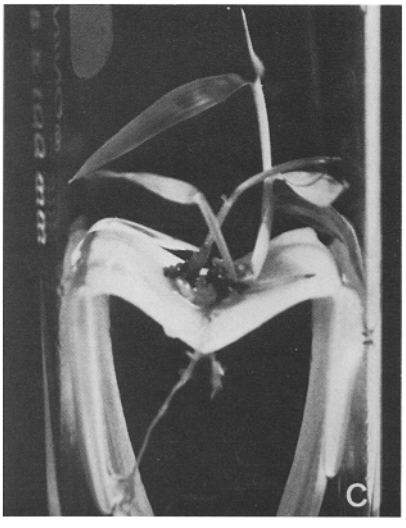


Fig. 2A. Stage in the development of plantlets from embryoids after 50 days of culture having a shoot and root axis. X 2.

Fig. 2B. Embryoids from a 50-day-old culture induced on B_5 + 2,4-D (3×10^{-5}M) medium and transferred to liquid medium (B_5 full-strength) + IBA (5×10^{-7}M) + NAA (10^{-7}M) for further growth into plantlets. Note presence of root (see arrow). X 3.

Fig. 2C. Embryoids germinated to form well developed plantlets with root and shoots on filter bridge in liquid medium (B_5 half-strength) + IBA (5×10^{-7}M) + NAA (10^{-7}M). Lateral root formation is also visible. X 2.

Some of the embryoids germinated while in the callus and gave rise to well-developed plantlets (Fig. 2A). Mature somatic embryos were otherwise separated from the callus and placed for germination on filter paper bridges in 30 ml screw cap tubes containing B_5 liquid medium supplemented with sucrose (2%), IBA (5×10^{-7}M) and NAA (10^{-7}M) (Fig. 2B). Within 15-20 days, 40% of the embryoids germinated and formed plantlets with roots and shoots (Fig. 2C). Of the remaining, 25% formed only roots and 35% turned brown and failed to germinate. Normal bamboo plantlets with one or two leaves were formed in 20 days. Lateral root growth was observed. Transfer of plantlets to B_5 (liquid) medium (half-strength), sucrose (1%), IBA (5×10^{-7}M) and NAA (10^{-7}M) produced further growth with an average shoot length of 6.8 cm by 50 days with four to six leaves. The root system was well-developed at this stage with long roots and a few lateral roots.

Somatic embryogenesis is now recognised as a superior method for plant propagation in vitro because it enables the rapid production of a large number of complete uniform plants within a relatively short period of time. This is mainly due to the production of bipolar somatic embryos as compared to other methods in which axillary shoots formed first must be later rooted, or where adventitious shoot and root formation from callus are induced sequentially. The present investigation demonstrates that mature embryos retain the potential to form somatic embryos from which plantlets can be obtained. Work done on cereals and millets to date has principally emphasised the use of immature embryos for somatic embryogenesis (Ozias-Akins and Vasil 1982) although somatic embryos have been obtained from cultured leaves and inflorescences as well (Wernicke and Brettell 1980, Vasil and Vasil 1981, Lu and Vasil 1981). The results obtained in the present work indicate that for bamboos somatic embryogenesis can provide a convenient and dependable source for obtaining plants.

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