IN VITRO CLONAL MULTIPLICATION OF 4-YEAR-OLD PLANTS OF THE BAMBOO, 
DENDROCALAMUS LONGISPATHUS KURZ

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SUMMARY

A complete protocol for micropropagation of 4-yr-old plants of the bamboo Dendrocalamus longispathus is described. Culture initiation was strongly influenced by the nature of the explant and the season. In vitro multiplication was achieved through forced axillary branching. Single node segments from the young lateral branches produced multiple shoots on agar-solidified Murashige and Skoog (MS) medium supplemented with 12 μM benzylaminopurine (BAP) and 3 μM kinetin. The shoots have been multiplied for 15 passages in liquid and thereafter for over 5 passages on semisolid MS + 15 μM BAP + 1 μM indolebutyric acid (IBA) + 10% coconut water at a rate of 3.2- and 2.8-fold, every 4 wk, respectively. The nature of the propagule was a critical factor for shoot multiplication and rooting. Seventy-three percent of the shoots rooted on a modified MS medium (major salts reduced to half strength) containing 1 μM indoleacetic acid, 1 μM IBA, and 68 μM coumarin. Through a simple in vitro hardening step, more than 85% of the tissue culture-raised plants were successfully transferred to soil.

Key words: Dendrocalamus longispathus; bamboo; micropropagation; tissue culture.

INTRODUCTION

Dendrocalamus longispathus, a large tufted bamboo, is one of the 15 commercially important species of bamboos growing in India that have been used in the paper and pulp industries. It is ideally suited for the manufacturing of craft paper. It is also used in a variety of other ways, such as thatching, roofing, construction, basket making, furniture, floats for timber, rafts, fencing, containers, and musical instruments (CSIR, 1952). Young shoots are eaten raw or pickled.

Natural regeneration of D. longispathus occurs both sexually and vegetatively. However, like most other bamboo species, both conventional methods of propagation suffer from certain drawbacks that restrict the large-scale multiplication of this species. The sexual method is unreliable because of long (30 to 60 yr) and often erratic flowering cycle, short viability of seeds (55 days under natural conditions) (Banik, 1987), and large-scale consumption of seeds by wild animals; propagation by vegetative means is difficult on account of fewer and bulky propagules and season specificity.

This paper describes a reproducible and efficient protocol for in vitro clonal multiplication of 4-yr-old plants of D. longispathus. Most of the earlier studies on tissue culture of bamboos deal with seedling material (see Saxena and Bhojwani, in press).

MATERIALS AND METHODS

Initiation of aseptic cultures. Single node segments were excised from young lateral branches of the main culm of 4-yr-old plants of D. longispathus growing at Gwal Pahari in Haryana State. After carefully removing the thin and fibrous leaf sheath that envelops the axillary bud and a part of the upper internode, the stem pieces were given a quick rinse in 95% ethanol, washed with 2% solution of “Cetavlon” (IEL, Limited, India) for 15 min followed by thorough washing under running tap water for 20 min. The nodal segments were then surface sterilized by treating them with 0.1% solution of mercuric chloride for 10 min. After three washings in sterile distilled water, the cut ends of the segments were trimmed, leaving behind 1.0 to 1.5 cm of internodal portions on either side of the node, and planted on different nutrient media. MS (Murashige and Skoog, 1962) and B5 (Gamborg et al., 1968) basal media containing 3% sucrose and 0.8% agar were tried for bud-break. The two media were variously supplemented with cytokinins [benzylaminopurine (BAP) and kinetin], either individually or in different combinations with the auxins indoleacetic acid (IAA) and indolebutyric acid (IBA). The pH of each medium was adjusted to 5.8 before autoclaving. All cultures were initiated in 150-ml glass bottles containing 30 ml of medium.

Multiplication of shoots. The entire clusters of axillary shoots produced by 2-wk-old primary cultures of nodal segments were transferred to liquid MS medium for shoot multiplication. The basal medium was supplemented with BAP alone or in various combinations with other cytokinins (kinetin and 2-ip) and auxins [IAA, IBA, and 1-naphthaleneacetic acid (NAA)]. Unless mentioned otherwise, the pH of the media was adjusted to 5.0 before autoclaving. As with initiation, multiplication of shoots was done in 150-ml glass bottles containing 30 ml of medium.

In stationary liquid cultures, submerged shoots died, whereas in shake cultures they invariably became vitrified. To prevent the shoots from getting submerged into the liquid medium, a small piece of rubber foam (4.5 × 4.5 × 1.0 cm) was placed inside each bottle and the base of the shoot was fixed on top of it. Before using, the foam pieces were washed with 5% Teepol solution for 10 min followed by thorough washing under running tap water for 20 min. The foam pieces were then autoclaved in 2000-ml conical flasks containing distilled water (just enough to keep the foam pieces soaked). After autoclaving, the foam pieces were manually squeezed to remove the water containing leachouts, if any. After three washings with...
distilled water, the foam pieces were dried in an oven at 80 °C and stored until use. The shoots were subcultured at 4-wk intervals. Inasmuch as individual shoots failed to survive in subcultures, at the end of each passage the proliferated shoot clusters were cut into discrete units, each containing three to five shoots, hereafter called a propagule (Fig. 1 D), and transferred to fresh medium. The multiplication rates have been calculated on the basis of the number of propagules derived from one culture at the end of each passage. All experiments were repeated twice with at least 12 cultures per treatment. Wherever possible, the effect of different treatments was quantified on the basis of percent cultures showing the response and the degree of response per culture. For the latter, standard error of means was calculated, which is presented in the tables as “±” and as bars in the graphs (Figs 4, 5).

Rooting. Rooting of the shoots was attempted both under in vivo and in vitro conditions. For in vivo rooting, the entire cluster of multiple shoots, obtained at the end of a 4-wk cycle of shoot multiplication, was used. Each cluster was given a pulse treatment with IAA (280 μM, 570 μM) or IBA (250 μM, 490 μM) alone or in combination with coumarin (340 μM, 680 μM) in liquid MS medium for 1 to 4 days. During treatment the cultures were maintained on a rotary shaker at 140 rpm. The treated shoots were directly transferred to a potting mix consisting of peat moss and soilrite in a 1:1 ratio (vol/vol) and irrigated with the inorganic nutrients of MS basal medium (pH 5.0).

For in vitro rooting, the propagules bearing three to five shoots were used. Initially, liquid and semisolid MS media were tried. Subsequently, modified MS media with the major inorganic salts reduced to half strength (MS1) or quarter strength (MS2) were also tested. The media were variously supplemented with IAA, IBA, NAA, activated charcoal (AC), coumarin, and boric acid. The liquid media (pH 5.0) were tested with and without rubber foam and the semisolid media (pH 5.8) were gelled with 0.25% gelrite (Scott Laboratories, Carson, CA). Medium (25 to 30 ml) was poured into 150-ml glass bottles. Each hormonal combination was tested 3 times with 12 cultures per experiment. However, with modified MS media, 50 cultures were used in each treatment.

Transplantation. Rooted shoots from 4-wk-old cultures on MS1 + IAA (10 μM) + IBA (10 μM) + coumarin (60 μM) were transferred to a potting mix comprised of equal quantities of soil, soilrite, and farmyard manure (vol/vol) in black polythene bags, either directly or after in vitro hardening. For in vitro hardening, the plantlets from the rooting medium were transferred to 200-ml screw-cap glass bottles one-third filled with soilrite (Fig. 1 F) and irrigated with 40 ml of inorganic salt solution of MS medium (major salts reduced to half strength) with the pH adjusted to 5.0. After 7 to 8 days, the caps of the bottles were removed (Fig. 1 G) and the plantlets allowed to grow in the glass bottles for another 2 days. During this period the plantlets were irrigated as before. The in vitro hardened plantlets were transferred to the polythene bags. The potted plants (Fig. 2 A) were reared for 3 mo. inside a glasshouse, maintained at 28°C ± 2°C and relative humidity 75 to 85%, before transfer to field conditions. The media and soilrite were steam sterilized at a pressure of 1.06 kg/cm² for 15 min.

Culture conditions. All cultures were maintained at 26°C ± 2°C under a 12-h photoperiod with light intensity of 4000 lux provided by cool, white fluorescent tubes of 40 W (Philips, Calcutta, India).

RESULTS

Initiation of shoot cultures. The sterilization procedure described in Materials and Methods yielded 70% aseptic cultures. Bud-break frequency was strongly influenced by the juvenility of lateral shoots, position of axillary bud on the branch, and season in which cultures were initiated. Only the young lateral branches showed bud-break. Older branches with partially sprouted axillary buds did not respond in culture. The first five to six nodal cuttings from the shoot tip and one to three cuttings from the base of the branch (leaf number was assumed as the corresponding node number) did not develop shoots. Therefore, for all subsequent experiments, nodal cuttings were taken from the middle of the young lateral branches.

On basal media the frequency of bud-break was very low. Incorporation of BAP (1, 3, 6, 9, 12, or 15 μM) to the basal media improved the incidence of bud-break and promoted multiple shoot formation (Table 1). The frequency of bud-break and the number of shoots developed on an explant were much higher on MS than on B5. Consequently, in all subsequent experiments only MS basal medium was used. On the optimum MS medium, containing 12 μM BAP, 58% cultures showed bud-break within 2 wk with over four shoots per explant. Subsequently, two other cytokinins (kinetin and 2-ip) and auxins (IAA and IBA) were tested, at different concentrations (1, 3, and 6 μM), in conjunction with 12 μM BAP. Of all the treatments, BAP combined with 3 μM kinetin induced maximum bud-break (75%) as well as shoot yield per explant (Table 2) and shoot growth (Fig. 1 A, B). The addition of either IAA or IBA was inhibitory. In all the combinations the incidence of bud-break was much lower than that with BAP alone (Table 2).

The results described above refer to the experiments conducted during the monsoon season (July to September). However, during other months the bud-break frequency varied considerably. To study the effect of season on bud-break, fresh cultures of nodal segments derived from plants growing under irrigated conditions were raised on MS medium containing 12 μM BAP and 3 μM kinetin, at regular intervals throughout the year. The cultures initiated during the rainy season (July to September) showed best response not only in terms of the frequency of bud-break (Fig. 3) but also in the vigor of the shoots. Another advantage of initiating the cultures during July to September is the availability of large number of explants.

Shoot multiplication. During the first four cycles of shoot multiplication, 85% of the cultures showed browning of the medium. Addition of 1000 mg/liter of AC or soluble polyvinyl pyrrolidone (PVP) to liquid MS + 15 μM BAP + 1 μM IBA + 10% coconut water (CW) checked browning to some extent, but the presence of AC also inhibited shoot growth and multiplication. Fortnightly transfer of shoots to fresh medium proved most effective to circumvent the browning problem. By Passage 5, the incidence of browning was reduced to 42% (Fig. 4), and thereafter regular four weekly subcultures could be made.

On basal medium the shoots started turning pale and necrotic from Day 4 and finally died after 10 to 15 days of culture. Incorporation of BAP (1, 5, 10, 15, or 20 μM) to MS basal medium induced shoot multiplication, the frequency of which varied with the concentration of the cytokinin (Table 3). At its optimum level (15 μM), 1.8-fold multiplication occurred every 4 wk. The presence of kinetin or 2-ip in conjunction with 15 μM BAP improved the multiplication rates marginally. The most effective combination of BAP (15 μM) and kinetin (3 μM) yielded a proliferation rate of 2.1-fold in 4 wk. Higher concentrations of kinetin proved inhibitory (Table 4).

Of the different auxins (IAA, IBA, and NAA) tested in conjunction with 15 μM BAP, at three concentrations (1, 3, or 6 μM), 1 μM IBA proved to be most effective, resulting in a multiplication rate of 2.4-fold. Although IAA was promotory only at the lowest concentration (1 μM), NAA proved inhibitory at all the tested concentrations (Table 5).

Addition of 10% CW to MS + 15 μM BAP + 1 μM IBA enhanced the rate of shoot multiplication to 3.2-fold every 4 wk (Fig. 1 C). On this medium, even the shoot growth appeared more vigorous. At higher concentrations (15 and 20%), CW proved inhibitory and often caused vitrification of shoots.

Gelling of the multiplication medium (MS + 15 μM BAP + 1 μM