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Micropropagation and in vitro flowering of the bamboo *Dendrocalamus hamiltonii* Munro

S.M. Chambers[1], J.H.R. Heuch[2] & A. Pirrie
Department of Forestry, University of Aberdeen, St Machar Drive, Aberdeen, AB9 2UD, Scotland (*[1]present address: Department of Crop Protection, The Waite Agricultural Research Institute, The University of Adelaide, Glen Osmond SA 5064, Australia; [2]Fiji Pine Commission, PO Box 521, Lautoka, Fiji*)

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Abstract

Multiple vegetative shoots and flowers emerged from the nodes of explants derived from young seedlings of the bamboo *Dendrocalamus hamiltonii* when cultured on a Murashige & Skoog based medium supplemented with the cytokinin 6-benzyladenine (BA). The largest number of shoots produced from intact cultured epicotyl tissue (ca 8 per explant) were obtained after 12 weeks on a medium containing 4.4 μM BA. The first flowers were observed in vitro after only 13 weeks from initial germination of the seedlings. The highest frequency of flowering (47% of nodal explants) was obtained when cultured explants were transferred from 22.2 μM BA to a growth regulator-free medium after 8 weeks.

Abbreviations: BA – 6-benzyladenine, MS – Murashige & Skoog

Introduction

Many bamboo species are of considerable economic and social importance, but are difficult to propagate for a number of reasons. The production of seed is irregular, because in the majority of species including *Dendrocalamus hamiltonii*, flowering occurs on culms over 20 years old (Janzen 1976). Many bamboo species are also known to be monocarpic, flowering once before culm death (McClure 1966). The seed that is available only remains viable for a limited period of time (Nadgir et al. 1984). For these reasons, vegetative propagation has traditionally been used to grow bamboo. Cuttings however, are bulky and therefore difficult to handle and transport, and plantlet survival is usually low (Hasan 1980).

Recently a number of attempts have been made to propagate bamboos using cell and tissue culture techniques, and plants have been recovered in a small number of bamboo species through organogenesis (Huang et al. 1989) and through somatic embryogenesis (Rao et al. 1985; Yeh & Chang 1986a, b and 1987). Tissue culture has the potential to produce a large number of propagules relatively rapidly, and in a form suitable for international transfer.

In vitro flowering in two bamboo species (*Bambusa arundinacea* and *Dendrocalamus brandsii*) has been reported recently (Nadgauda et al. 1990). Flowering was induced on a medium supplemented with coconut milk and the cytokinin BA. Such premature reproductive growth in vitro may prove useful in the study of reproductive development in bamboo. This paper de-

scribes micropropagation and the first recorded incidence of in vitro flowering in the bamboo *Dendrocalamus hamiltonii* on a defined medium containing BA.

Materials and methods

Seeds of *Dendrocalamus hamiltonii* were obtained from the Dhankuta District of Eastern Nepal in January 1990. The husks were removed, and seed surface disinfested in 20% (v/v) Domestos, containing 4% sodium hypochlorite (Lever Ltd, U.K.) for 30 min, rinsed 4–5 times in sterile distilled water, and transferred to MS basal medium (Murashige & Skoog 1962), containing MS vitamins, 3% sucrose and 0.7% Bacto-agar (Difco) with the pH adjusted to 5.6–5.7 with 1N KOH or NaOH before autoclaving at 121°C for 15 min. Seeds were germinated in the dark at 25°C for 7–14 days after which time hypocotyls 3–6 cm in length were used as a source of explants.

Epicotyl tissue of 2-week-old seedlings was either cultured intact or, alternatively, cut into single node sections before placing on a medium containing growth regulators. Six treatments were used, each with the same basal MS medium to that used for seed germination: without BA (MSO control treatment); or supplemented with 4.4 μM BA (MS1B); 22.2 μM BA (MS5B); MS5B for eight weeks, then transferred to MSO (MS5B-MSO); 44.4 μM BA (MS10B); or on a medium previously used by Nadgauda et al. (1990) for two other bamboo species – MS basal medium supplemented with 2% sucrose, 2.2 μM BA and 5% coconut milk (MSB). In a further study of in vitro flowering, four of the above treatments were again used, namely MSO, MS1B, MS5B, and MS5B-MSO media. There were 50 explants for the MSO treatment, and 10–25 explants for each of the other treatments.

Explants were cultured in sterile disposable 8 cm universal vessels (Sterilin) containing 10 ml medium, and maintained at 30°C, with a 16-h photoperiod (140 μmol m^{-2} s^{-1}) provided by cool white fluorescent tubes. Plantlets were transferred to fresh medium every 4 wk. Anthers were removed from flowering cultures and squash mounts were stained with aceto-carmine to test pollen viability.

The number of shoots produced per treatment over 12 wk were compared using a two-way analysis of variance, and 95% confidence intervals were calculated from the means.

Results

When intact epicotyls were used as explants, multiple shoots arose from nodes cultured in the presence of the cytokinin BA after only 4 wk and increased in number over a 12-wk period. The MS1B treatment produced the greatest number of shoots after 12 wk, while explants on MSO usually produced 1 or sometimes 2 shoots (Fig. 1). Flowering was also observed in these cultures, occurring first in the MS1B and MSB treatments (after 13 and 15 wk respectively). Some cultures in all cytokinin treatments produced roots, but rooting of plantlets using specific growth regulators was not attempted.

Where epicotyl tissue was divided into nodal sections prior to culture, multiple shoots were produced on media supplemented with BA in numbers similar to those from whole hypocotyl explants. Flowering appeared first in the MS1B and MS5B treatments (after 11 wk), and then in the MS5B-MSO treatment (after 12 wk). No flowering occurred in the MSO control treatment. The greatest number of flowering cultures (47%) occurred in the MS5B-MSO treatment and an intermediate level (24%) in the MS1B and MS5B treatments.

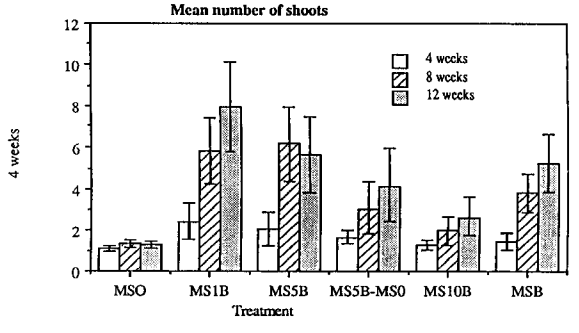

Fig. 1. Mean number of shoots (±95% confidence interval) produced from epicotyl tissue explants of *Dendrocalamus hamiltonii* on MS medium supplemented with BA over 12 wk (MSO = without BA; MS1B = 4.4 μM BA; MS5B = 22.2 μM BA; MS5B-MSO = MS5B for 8 wk then transferred to MSO; MSB = MS supplemented with 2% sucrose, 2.2 μM BA and 5% coconut milk (Nadgauda et al. 1990)).

Fig. 2. In-vitro flowering in *Dendrocalamus hamiltonii* Munro. Flowers were produced from single node explants on MS basal medium supplemented with 22.2 μM BA. Stigmas were exserted first from lemma and palea (*A*), followed several days later by stamens (*B*). Scale bar = 1 mm.

Flowers were mainly produced as buds from the original excised node, and consisted of a lemma and palea, containing either five or six stamens, one to three plumose stigmas, and a hairy ovary. The stamens and style were pink-purple in colour. During flower development the stigmas were exserted first from the palea and lemma (Fig. 2A), followed several days later by stamens that hung pendulously from the flower (Fig. 2B). Pollen, which appeared viable when stained with aceto-carmine, was obtained from anthers. Controlled cross-pollinations were attempted, however no fertilization or seed set were observed. Flowering plants were subcultured, and more flowers were produced from the nodes over 28 wk. Not all of the nodes from an individual seedling produced flowers.

Discussion

The initial aim of these experiments was to establish conditions under which this species of bamboo could be propagated in vitro. The presence of BA in the culture medium stimulated the growth of multiple shoots from the nodes of cultured hypocotyls; the MS1B treatment (4.4 μM BA) produced the greatest number of shoots. This method may allow large numbers of plants to be produced from a limited supply of original seedling material, which in the future could prove useful in the economic mass production of plants.

Previously, Nadgauda et al. (1990) used low concentrations of BA (2.2 μM) with coconut milk (5%) in an MS basal medium to induce flowering in two species of bamboo, *Bambusa arundinacea* and *Dendrocalamus brandsii*. In this study on *Dendrocalamus hamiltonii*, flowering was observed to occur in vitro 13 wk after seed germination. Flowering only occurred in treatments containing BA in the tested range, and not in growth regulator-free controls. In contrast to the results reported by Nadgauda et al. (1990), *D. hamiltonii* flowering was induced on a defined medium, without the addition of complex organic additives such as coconut milk.

The pattern of floral development, with the stigmas exserted first, followed by the stamens several days later, implies that the stigma is

receptive before pollen is produced from the anthers (protogyny) (Bell & Woodcock 1978), and suggests that out-crossing probably occurs in this species. The structure of the flowers produced in vitro was found to be similar to that of flowers observed in the field (Gamble 1896).

The greatest frequency of cultured explants giving rise to flowers occurred when the nodal explants were transfered to a growth regulator free culture medium, after a period of time of 22.2 μM BA. The cytokinin BA may merely facilitate the growth of pre-existing floral meristems. However, this appears unlikely, because optimum levels of BA for vegetative growth are higher than those needed to induced flowering in our experiments (see Fig. 1 and Table 1). The role of BA in floral induction remains unclear, and is the subject of further investigation.

Flowering does not usually occur in vivo until *D. hamiltonii* has grown vegetatively for around 20 years (Janzen 1976). This paper is the first report on promotion of plants of this species from the juvenile to the mature growth phase, where they are capable of flowering. The induction of flowering in vitro may provide a useful model to study flowering in the bamboos, and may lead to a greater understanding of their reproductive biology.

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