Direct regeneration of shoots from immature inflorescences in *Dendrocalamus asper* (edible bamboo) leading to mass propagation

Sarita Arya, Richa Satsangi and I. D. Arya

Tissue Culture Discipline
Division of Botany, Forest Research Institute, Dehradun-248006, INDIA.
Email: aryaid@gmail.com

ABSTRACT

Flowering in bamboos is a catastrophic event that leads to a number of post-flowering consequences and is hazardous for plants, which are monocarpic in nature. Direct shoot regeneration from immature inflorescence explants will help in overcoming the potential loss caused by unpredictable flowering in bamboos. Plant regeneration from cultured immature inflorescences of *Dendrocalamus asper* was obtained by direct shoot regeneration on Murashige & Skoog's 1962 (MS) medium supplemented with 7mg/l BAP. Best-regenerated shoots were obtained when 0.5-1.0 cm sized immature inflorescences were used. Regenerated shoots were grown on MS medium supplemented with 3mg/l BAP for further multiplication and development. Twelve to fifteen fold shoot multiplication rate was observed on MS medium. *In vitro* rooting was observed in 90-95% shoots on MS medium supplemented with 10mg/l IBA. After hardening and acclimatization plantlets were transferred to field and showed a 80-90% survival rate.

**Keywords:** Shoot-regeneration, immature inflorescence culture, *Dendrocalamus asper*, micropropagation, bamboo.

**Abbreviations:** ABA- Abscisic acid, BAP-6-Benzylaminopurine, 2,4-D- 2,4-Dichloro phenoxyacetic acid, GA3 - Gibberellic acid, IAA-Indole-3-Acetic acid, IBA-Indole-3-butyric acid, NAA -1-Naphthalene acetic acid, FYM-farmyard manure, RH-relative humidity, CRD- Complete randomized design, SPSS-Statistical package for social science.

INTRODUCTION

Bamboo is a very important plant affecting the livelihood of millions of people around the world. However, research efforts on bamboos are very small compared with that on agricultural crops. *Dendrocalamus asper* is a sympodial tropical bamboo growing in large, dense clumps of a single genotype, and it is known for its edible tender shoots, which are also used for building material. It is commonly called "sweet bamboo" and is one of the best tropical bamboos in Asia in terms of its shoot quality. It grows up to 20-30m tall and individual stems are 8-20 cm in diameter. The species is heavily exploited as a food source and currently demand outstrips supply. The flowering of bamboo is poorly understood. Bamboo is monocarpic, i.e. a plant flowers only once and then dies. The flowering of bamboo needs to be controlled to avoid the losses in agroforestry resulting from the death of monocarpic bamboos.

*Dendrocalamus asper* flowers gregariously after 60-100 years, although some sporadic flowering has been reported (Anantachote 1998, Wang 1995, Satsangi et al. 2001). Nevertheless, diffuse sporadic flowering suffers from poor seed set and low viability. Flowering in vegetatively propagated plants, like those from micro propagation remains in synchrony with that of the mother plant (John et al. 1995). Consequently, vegetative propagation does not normally overcome the problem of monocarpy. However, it has been suggested / reported (Gielis et al. 1999) that shoot regeneration from pseudospikelets cultures from the inflorescence can produce plants that will be start of a new vegetative
generation. Regeneration from inflorescence tissue also offers the opportunity to overcome the problems associated with mass gregarious clump mortality after a flowering event.


**MATERIALS AND METHODS**

Inflorescences at different developmental stages were collected from flowering branches of culms. These clumps were two-year-old tissue culture raised plants growing at experimental fields of Forest Research Institute, Dehradun, India. Explants (flowering buds) from immature inflorescences (Pre-anthesis buds of 0.5-2.5 cm) and of mature inflorescences (Post anthesis buds of 2.5-3.5 cm) were harvested from flowering clumps. They were cleaned with 70% alcohol soaked cotton and then sterilized with 0.1% mercuric chloride for 8-15 min. Finally they were washed three times in sterilized distilled water. Inflorescence tissues at different developmental stages were cultured (Table 1). Immature inflorescences (Figs.1&2) of six different bud sizes (0.1; 0.5; 1.0; 1.5; 2.0 and 2.5 cm) were cultured on MS medium supplemented with BAP. The explants were plated on MS medium supplemented with 1, 2.5, 3.0, 4.0, 5.0, 7.0 and 10 mg/l BAP. In all the experiments, data collected were computed using SPSS ver 8.0 software packages and analyzed by one-way ANOVA using CRD design of experiments. For each experiment five replicates were taken with six explants (in two flasks) per replicate and each experiment was repeated three times.

The basal medium used was solidified with 0.8% agar (Hi-media laboratories, Bombay, India) and supplemented with 2% (w/v) sucrose. The pH was adjusted to 5.8 prior to autoclaving. The shoots regenerated from these explants (immature-spikelets) were excised in clusters of 3-5 shoots called propagule. Three propagules per cultured flask were subcultured for shoot multiplication on MS medium supplemented with 1-10 mg/l BAP. Ninety shoots were cultured using CRD design per experiment on each media. Once the *in vitro* shoots were established the shoots were excised into groups of 3-4 shoots (propagule), which were subcultured and maintained on fresh MS medium at an interval of four weeks. The rate of multiplication was determined from the number of propagules at the end of each subculture divided by total number of propagules cultured.

Table 1: Effect of developmental age and size of explants for induction of shoots. MS medium was used. Data were collected after 4 weeks.

<table>
<thead>
<tr>
<th>Explants</th>
<th>Size of explants (cm)</th>
<th>BAP (mg/l) of shoots</th>
<th>% Response</th>
<th>Average Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immature inflorescence tissue</td>
<td>0.5</td>
<td>7</td>
<td>36.67</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>7</td>
<td>52.67</td>
<td>4.0</td>
</tr>
<tr>
<td></td>
<td>2.0</td>
<td>7</td>
<td>56.67</td>
<td>5.5</td>
</tr>
<tr>
<td>Mature inflorescence tissue</td>
<td>2.5</td>
<td>7</td>
<td>40.00</td>
<td>2.5</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>7</td>
<td>33.33</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>3.5</td>
<td>7</td>
<td>15.00</td>
<td>1.0</td>
</tr>
<tr>
<td>Significance level ‘F’</td>
<td></td>
<td></td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>CD at 5%</td>
<td></td>
<td></td>
<td>9.72</td>
<td>0.904</td>
</tr>
<tr>
<td>S.E ±</td>
<td></td>
<td></td>
<td>4.36</td>
<td>0.405</td>
</tr>
</tbody>
</table>
Rooting was induced in regenerated in vitro shoots on full strength MS medium supplemented with 10 mg/l IBA as described by Arya et al. (2002). Cultures were maintained at a temperature of 25 °C + 2°C under 16/8hrs (light/dark) photoperiod at a high light intensity (60-70 Em² s⁻² of cool white light) provided by fluorescent lamps (Philips, India).

Rooted plantlets were transferred to polybags containing vermiculite and kept under green house at 30°C temperature and 90% RH through misting for hardening. These plants were fed with half strength MS salts without organics twice a week for 1 month. Subsequently these plants were transferred to polybags containing sand: soil: FYM in 1:1:1 ratio. After 1 month, plants were acclimatized in a shade house for the next 4 weeks before they were ready for field planting.

RESULTS AND DISCUSSIONS

Mercuric chloride proved to be the best sterilant. Surface sterilization with 0.1% HgCl₂ for 8 min yielded 60% results for inflorescence sterilization. An increase in treatment duration to 10 min resulted in necrosis in buds. Inflorescences if cleaned with ethyl alcohol...
(70%) swabbed cotton prior to surface sterilization with mercuric chloride increased the percentage of explants free of contamination by 10%. 50% of the explants cultured on MS medium supplemented with 7mg/l BAP regenerated and therefore this was considered as the optimal medium. On MS medium with 1-4 mg/l BAP only 1-2% of the explants responded and up to 20% on 5-6 mg/l BAP medium. At 8-10mg/l of BAP their generation frequency of shoots was also 50% but the shoots were condensed and small in size. Shoots regenerated from the explants after 10-14 days of culture depending on their size. At the optimal medium with 7mg/l BAP, 52% and 56% explants of 1 cm and 2 cm respectively responded by producing an average 4-6 shoots (Fig. 3). As the explants matures, the shoot regeneration response decreased from 40% to 15% (Table 1). In the present investigations inflorescences were cultured during 3-4 months of their development and their response as explants for the induction of direct shoot regeneration (Fig. 4 & 5) in culture were studied (Table 1&2).

**IN VITRO SHOOT MULTIPLICATION**

Regenerated shoots from inflorescences were excised from explants and were maintained separately on MS medium supplemented with BAP (1-5 mg/l). A 12 to 15 fold shoot multiplication rate was observed in inflorescence-derived shoots on MS medium supplemented with 3mg/l BAP (Table 3). Shoot multiplication rate decreased at reduced BAP levels (1-2mg/l) in MS medium. Also at increased BAP level (5-10 mg/l) condensation of shoots was observed resulting in decline in the multiplication rate. Thus, 3mg/l BAP supplemented MS medium proved the best for shoot multiplication (Fig. 6).

During initial cycles of shoot multiplication 3-6 folds multiplication was observed for the first to the fourth subculture. After subculture

Figures 6-8: Plant propagation through shoot regeneration from immature inflorescence.
Fig.6: In vitro shoot multiplication on MS + 3 mg/l BAP.
Fig.7: Tissue culture plantlet on MS + 2 mg/l NAA.
Fig.8: Hardened and acclimatized plants ready for field transfer.
Table 3: Effect of BAP concentration in MS medium on in vitro shoot multiplication and development. Propagules of three shoots were cultured and data were recorded after 4 weeks.

<table>
<thead>
<tr>
<th>BAP mg/l</th>
<th>No. of shoots developed</th>
<th>Shoot length (cm)</th>
<th>Size of leaves (width in mm)</th>
<th>Multiplication Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>–</td>
<td>4.5±1.0</td>
<td>9.0±2.1</td>
<td>0.0</td>
</tr>
<tr>
<td>1.0</td>
<td>14.1±1.0</td>
<td>3.3±0.7</td>
<td>6.2±1.0</td>
<td>5.6</td>
</tr>
<tr>
<td>2.5</td>
<td>33.4±3.2</td>
<td>2.3±0.2</td>
<td>4.2±0.6</td>
<td>12.2</td>
</tr>
<tr>
<td>3.0</td>
<td>34.0±3.8</td>
<td>2.2±0.4</td>
<td>3.0±0.1</td>
<td>12.6</td>
</tr>
<tr>
<td>4.0</td>
<td>30.3±3.6</td>
<td>1.2±0.5</td>
<td>2.3±0.4</td>
<td>11.3</td>
</tr>
<tr>
<td>5.0</td>
<td>30.2±2.5</td>
<td>1.2±0.2</td>
<td>2.0±0.1</td>
<td>10.2</td>
</tr>
<tr>
<td>7.5</td>
<td>16.3±2.5</td>
<td>1.0±0.1</td>
<td>2.0±0.5</td>
<td>6.2</td>
</tr>
<tr>
<td>10.0</td>
<td>17.0±4.0</td>
<td>0.8±0.2</td>
<td>1.6±0.2</td>
<td>7.0</td>
</tr>
</tbody>
</table>

cycle 5-6 multiplication rate increased to 10-16 folds and then became constant 12 to 15 fold. When MS medium was used without a supplement of BAP, the cultured in vitro shoots increased in length but without shoot multiplication. Uses of BAP for in vitro shoot multiplication in bamboos have been reported by many researchers ( Arya et al. 2003, Arya and Sharma 1998, Arya and Arya 1997, Prutpongse and Gavinlertvatana 1992, Chambers et al. 1991, Saxena 1990). Also, for in vitro shoot multiplication clusters of three shoots (propagule) were subcultured together. The shoot multiplication rate declined sharply if propagules of less than 3 shoots were cultured. The multiplication rate also declined if propagules of more than 3 shoots were used for multiplication. Liquid as well as semisolid medium showed significantly similar shoot multiplication rate. However, on long subculture cycles in the liquid medium shoots become brittle and vitrified. Hence semisolid medium was used for multiplication cycles.

**IN VITRO ROOTING**

Attempts for in vivo rooting of in vitro raised shoots were only partially (15%) successful during rainy season, and the rest of the shoots turned pale in 5-7 days and eventually died. In vitro rooting was highly successful. For in vitro rooting shoot clusters of 3-5 shoots were transferred on MS medium supplemented with IBA or NAA. A success rate of 95% of in vitro rooting of in vitro shoots was obtained both in liquid and agar-gelled MS medium. However, in the liquid medium root initiation occurred 4 days earlier. Shoots rooted readily within 8-12 days on MS medium. Maximum in vitro rooting (80-95%) was achieved on MS medium supplemented with 10mg/l IBA or 3mg/l NAA. The root induction effect of IBA and NAA in the present case is similar to earlier reports on Morus indica (Chand et al. 1995), Bambusa tulda (Saxena 1990), Bambusa bambos (Arya and Sharma 1998). A propagule of 3 shoots of 1-2 cm in length developed 10-15 roots in 30 days.

**HARDENING AND ACCLIMATIZATION**

Four-week-old plantlets with well-developed root systems (Fig. 7) were hardened in Soilrite for 20 days. Plants were fed with half strength macro- and micronutrients of MS medium thrice a week in the mist chamber under RH 85-90% and at 30° ± 2°C. Acclimatization of these plants was carried out in an open agronet shade house in polybags containing a mixture of sand: FYM: soil in a ratio of 1:1:1 for 2 months. Rhizome formation occurred within two months and the plants eventually were established in the soil (Fig. 8).

Thus, present study shows that inflorescence culture is amenable to high frequency shoot regeneration and opens up the possibility of using this for further research in bamboos. The studies have shown that inflorescence explants could differentiate into plants under in vitro conditions. Since pseudospikelets are modified
vegetative buds, it is quite likely that immature floral buds have the capacity to revert to vegetative buds under appropriate in vitro conditions (Gielis et al. 1997; George and Sherrington, 1984; McClure, 1966). Determination of the appropriate stage of explant from the clump after onset of flowering may be an ineffective marker because the size of inflorescence and its developmental stages differed even between branches of the same clump. Inflorescence size is the best parameter for explant selection. Regeneration of plants from inflorescence culture has been previously reported in several plants such as wheat, rye, triticale (Eapen and Rao, 1985), ginger (Babu et al., 1992), Amaranthus (Arya et al. 1993) and C. alismatifolia (Wannakairoj, 1997). In D. asper inflorescence segments (0.5-2 cm) with intact floral buds were cultured on MS medium supplemented with 7mg/l BAP. At the end of 4 weeks shoot primordia emerged from the surface of the explants (Fig. 4). Later with the passage of time number of shoot primordia differentiated from complete surface of the cultured inflorescence and developed into clusters of well-developed shoots in four to five weeks of culture (Fig. 5). Explants cultured on 7mg/l BAP gave optimal results as on this medium maximum number of shoot buds and shoots per explant were scored. The inflorescence is composed of many meristems which develop buds larger than 2 cm had mature stages of floral primordia, which did not regenerate into in vitro shoots, when cultured on MS medium supplemented with 7mg/l of BAP.

Thus in the present case it was found that subculture of the proliferating explants on a medium with BAP enhanced the formation of additional meristematic centers, which continued to grow and developed into buds. Regeneration occurred, as long as the tissue is young and actively growing. However, it is also reported in some species that regeneration of shoots occurred even when the flowers were still enclosed by the bracts. The inflorescence is composed of many meristems, which develop sequentially to form the florets. These meristems can develop into vegetative buds in several species if they are isolated at the appropriate develop-

mental stage and cultured in vitro on suitable medium. The shoot buds often form clusters that can be used for subsequent mass propagation. Ziv and Kipnis (2000) reported in geophytes that at appropriate developmental stage under optimal growth hormones, reversion of meristems is possible, which later developed sequentially to form florets. The inflorescence can be reversed from flowering to vegetative states (Gielis and Goetzheheur, 1997), and buds proximal to the inflorescence can develop into innovation shoots. Size of explants at different developmental age of the spikelets plays an important role. Tanimoto and Harada (1979) in their studies on Torenia reported that 10-12 mm inflorescence segments produced vegetative buds.

Arya et al. (1993) developed in vitro plantlets from inflorescence cultures of Amaranthus cultured on MS medium supplemented with BA. Similarly, Salvi et al. (2000) supported similar findings that BAP at higher concentrations formed direct shoots from inflorescence tissues in case of Cucurma species. They also found cytokinins have the ability to stimulate flowering but tend to be more supportive of vegetative growth.

REFERENCES


