Large scale propagation of an exotic edible bamboo, *Phyllostachys pubescens* Mazel ex H. De Lehale (Moso Bamboo) using seeds†

Anil Sood*, Harleen Kaur Nadha, Sangita Sood1, Shivani Walia1 & Om Parkash

Division of Biotechnology, CSIR-Institute of Himalayan Bioresource Technology (IHBT) Palampur 176 061, India

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For *ex vitro* propagation, seeds of *P. pubescens* were treated with different concentrations of gibberellic acid (GA3) and germination of seeds was tested both in plastic pots as well as by direct sowing in the nursery beds. Maximum seed germination was achieved when treated with 200 mgL⁻¹ (w/v) GA3. For *in vitro* propagation, an exposure of nodal explants from *in vitro* raised seedlings to 0.2 mgL⁻¹ 1–phenyl–3–(1,2,3–thiadiazol–5–yl) urea and 1 mgL⁻¹ kinetin supplemented medium for 30 days and thereafter to hormone free Murashige and Skoog basal medium resulted in axillary shoot proliferation. For rooting, *in vitro* raised shoots were exposed to MS medium containing 2 mgL⁻¹ indole-3-butyric acid for 15 days and then shifted to hormone free medium. On an average, 2.8 shoots were obtained in 75% of the cultures within 4 weeks. Such *in vitro* raised plants were successfully hardened and shifted to field conditions.

**Keywords:** Bamboo, Edible shoots, *Ex vitro* propagation, *In vitro* propagation, *Phyllostachys pubescens*

**Materials and Methods**

Promotion of *in situ* seed germination—After manually dehusking the seeds of *P. pubescens* procured from China, were soaked in water for 24 h while rejecting the floaters. After wash, these were treated with different concentration of gibberellic acid (GA3; Hi-Media, Mumbai) for 2 h and planted in plastic pots containing a mixture of sand:soil:farm yard manure in equal proportions and covered with cling film. Twenty pots of four different sizes each i.e. 6,8,10 and 12 inches in replicates of three were used alongwith Hikko trays for seed germination. The germination percentage was recorded after 20-25 days. Similarly, 20 seeds per treatment were sown in raised beds during February in a grid spacing of 5 × 5 cm at a depth of 10 cm inside a poly house. The beds were sprayed with water 2-3 times a week. The average temperature and humidity inside the poly-tunnel were maintained at 20-25 °C and 40-50% respectively.

*In vitro* propagation—For raising aseptic cultures, the dehusked seeds were surface sterilized using standard procedures and inoculated on basal Murashige and Skoog medium containing 0.25% (v/w) Phytage (Hi-Media) and incubated in the culture lab having 16:8 h L:D photoperiod of 70 μ Mol m⁻² s⁻¹ light intensity at 25±2 °C. During *in vitro* seed germination, occurrence of fungal contamination was a major issue.

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*Correspondent author
Telephone: 01894-233337, 9418042984
Fax: 91-1894-230433/230428
E-mail: asood@ihbt.res.in

1Department of Food and Nutrition, CSK, HPKV, Palampur 176 061, India

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which would appear 4-5 days after inoculation. This could be checked to a large extent by soaking seeds in Bavistin (1%) and keeping the vessel on a horizontal shaker overnight. About 43.05% aseptic cultures could be obtained showing 95% germination. Nodal explants from 3 week old seedlings were used to initiate cultures.

Shoot multiplication was attempted using cytokinins like benzyl aminopurine (BAP), kinetin (Kn), -6-(y,y-dimethylallylamino) purine (2ip) and 1-phenyl-3-(1,2,3-thiadiazol-5-yl) (TDZ) for 15, 30 and 45 days. The pulse treatment with TDZ (0.2 mg L\(^{-1}\)) and Kn (1 mg L\(^{-1}\)) for 15 days and then shifting to hormone-free medium resulted in axillary shoot proliferation. For root induction, three auxins viz. indole-3-acetic acid (IAA), indole-3-butyric acid (IBA) and \(\alpha\)-naphthalene acetic acid (NAA) were used either individually or in combinations and even pulse treatment was attempted. The rooted plants after washing in lukewarm water were hardened in moist river bed sand while keeping covered with inverted jars.

For field performance, well-established plants from the nursery were planted in the fields in pits of the size 0.6 x 0.6 x 0.6m :: L:D:W at a distance of 6 meters apart.

Statistical analysis—The effect of different treatments was determined by analysis of variance (ANOVA) using STATISTICA data analysis software v7 (Stat Soft Inc., Tulsa, OK). Significance differences between the means were assessed by Duncan’s Multiple Range Test (DMRT) at \(P=0.05\).

Results and Discussion

Among different treatments of GA\(_3\) viz. 50, 100, 150, 200 mgL\(^{-1}\) to the seeds and their sowing in different sized plastic pots, Hikko trays and open beds, germination percentage varied from 15-100% in 15-20 days. Hundred percent germination was seen in the seeds treated with 200 mgL\(^{-1}\) of GA\(_3\) and sown in 30 x 30 cm plastic pots. There was not much difference in seed germination when sown in Hikko trays or open beds. A large number of seedlings were raised this way in poly-houses as shown in Fig. 1. Although Singh et al.\(^3\) had reported such beneficial effect of GA\(_3\) treatment on seeds of Banj Oak trees, there is no such report in case of bamboos and this is particularly important in view of scarcity of bamboo seeds coupled with low viability. Fungal contamination in the seeds during germination was effectively checked by treatment with 1% bavistin.

Earlier, Woods et al.\(^4\) had reported high level of contamination (30%) but contamination rates declined by using excised embryos which is not convenient in bamboos.

The seedlings grew to 6-7 cm length in 6 weeks and nodal explants were then excised and used as explants for raising multiple shoots (Fig. 2a). Among the cytokinins viz BAP, Kn and TDZ, there was no multiple shoot formation in BAP or Kn containing MS medium whereas, TDZ at concentrations of 0.05-0.2 mgL\(^{-1}\) showed formation of 2.40-5.00 shoots in 30 days. However, best response was discerned in a combination of TDZ (0.2 mgL\(^{-1}\)) and Kn (1 mgL\(^{-1}\)) where 8.60 shoots were obtained and even the roots were found to be longer (5.24 cm). The results are shown in Table 1. The TDZ concentrations beyond 0.2 mg L\(^{-1}\) proved inhibitory. TDZ has proved an effective cytokinin for shoot proliferation in long term cultures of Bambusa oldhamii\(^5\). Similarly positive results of TDZ were also reported in Bambusa edulis using nodal explants\(^6\) at low concentrations. Differential response of TDZ supplementation has also been observed in Bambusa vulgaris\(^7\) and Dendrocalamus giganteus\(^8\). The pulse treatment of exposing in vitro raised shoot explants having 3-4 shoots in bunches to TDZ and then shifting to hormone-free medium did not show any appreciable increase in proliferation. Single shoot explants turned necrotic both when used for shoot multiplication or rooting. Such observations were recorded in Dendrocalamus hamiltonii\(^9\), and by Bag et al.\(^10\) and

Fig. 1—Young Moso seedlings growing in polyhouse
For root induction, 3-4 cm long shoot bunches were transferred to MS medium containing three different auxins viz. IBA, IAA and NAA at concentrations of 0.5-3.0 mg L$^{-1}$. No rooting was seen in either IAA or NAA at the concentrations used. Rooting was achieved only in presence of IBA (1.0-3.0 mg L$^{-1}$) when a two step procedure was followed. Best rooting response (up to 75%) in shoots of *P. pubescens* was observed in MS media supplemented with IBA (2.0 mg L$^{-1}$), with 15 days exposure and subsequently withdrawing IBA from the medium completely. Continuous culture on medium with higher doses of IBA for relatively longer periods adversely affected growth and the shoots turned necrotic. Full strength of MS medium gave best response and up to 80% survival of the rooted plantlets thus generated were noticed when these were shifted to plastic pots containing moist river bed sand and covered with glass jars initially for 10-12 days before transferring to poly-sleeves containing a mixture of garden soil:sand:FYM in equal proportions (Fig. 2b).

After successful hardening and rearing the plants for 5-6 months under shade net, 384 plants were transferred to the fields with a survival percentage of 77%. New culms emerged after 3 months of field transfer indicating their establishment in soil.

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**References**