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In Vitro Micropropagation of Himalayan Weeping Bamboo, *Drepanostachyum falcatum*

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Abstract

Plant growth hormone BAP (benzyl amino purine), KIN (kinetin), NAA (1-naphthalene acetic acid) and IBA (indole-3 butyric acid) effect was studied on *in vitro* multiplication of shoots and rooting of *Drepanostachyum falcatum*. *In vitro* micropropagation of himalayan weeping bamboo is explained by *in vitro* shoot induction and proliferation. Excised explant with axillary bud is surface sterilized with 0.1% $HgCl_2$ for 10 - 12 minutes, cleaned with 90% ethanol and inoculated on liquid Murashige and Skoog (MS) culture medium supplemented with different concentrations of BAP/KIN. Effect of BAP/KIN on shoot induction is with different rate and number of shoots produced by explants with axillary bud cultured on MS media supplemented with 0.0 mg/L BAP/KIN - 5.5 mg/L BAP/KIN. Shoot multiplication with highest rate is achieved on MS medium supplemented with 3.5 mg/L BAP after 4th sub-culturing. The most effective with highest rate and number of root induction combination is 6.5 mg/L IBA after 5 weeks. The roots produced by 6.5 mg/L IBA is best compared with other combination of auxin NAA (1-naphthalene acetic acid).

Keywords

***In Vitro*, *Drepanostachyum falcatum*, Himalayan Weeping Bamboo, Plant Growth Hormone, Micropropagation**

1. Introduction

Drepanostachyum falcatum is economically important bamboo commonly known as Himalayan weeping bamboo are the most harvested species for making baskets, mats, flowerpots etc. and other commercial purposes in

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district Rudraprayag, Garhwal region, Uttarakhand. It is also used in compounding many medicines, making lotion for cleaning wounds [1] and used as soil and water conservation tool [2].

This species is introduced in Nilgiri Hills, Europe, South and North America, Laos and Entebbe Botanic Garden, Uganda. It is found in the hills areas of North India—Garhwal and Kumaon hills and is also marketed as "blue bamboo" due to the cold hardy plant nature. It grows at altitudes up to 2100 m in high slopes, high terraces in moist, sheltered, shady conditions. It grows well in sheltered shady spot into a 3 - 4 meter plant with masses of small drooping leaves and prefers rich sandy loam to clayey loam soil often mixed with stones. It can be trimmed for a good indoor bamboo plant. *Drepanostachyum falcatum* flowers irregularly at times, gregarious over large areas, while few culms may be found in flowers almost every year. This belongs to the irregularly flowering group with the flowering and seedling cycle of 28 - 30 years [3]. Recorded flowering was in Shimla in 1858, 1916; Mussoorie in 1916, Jaunsar in 1868, 1916; Tehri Garhwal in 1916, Kew, England in 1908. The importance of this species is highlighted by the diverse uses; it is put to by the locals.

Looking to the tremendous economical importance of *D. falcatum* due to its multifarious uses, there is always a shortage of planting stock material of this bamboo. Due to over exploitation and large scale indiscriminate cutting of natural stands of ringal bamboo, the plant is now facing threat of its existence and survivability in the hilly areas of Uttarakhand. Availability of seed is also reduced drastically for raising planting stocks. Since the bamboos is of commercially importance and provides livelihood and useful material to the rural areas of the hills therefore it is in huge demand for its plantation which may be for reforestation, a forestation and for commercial plantation purpose. Hence the existing method of plant propagation through seed, rhizomes and through cuttings is in sufficient to provide required planting stock. Hence the non-conventional method of plant propagation *i.e.* plant tissue culture technology is to be adopted for large scale multiplication in shorter duration. To date, very little work has been done for its fast propagation technique.

The conventional means is only limited to seed sets which take a long time and are limited through rhizome propagation. Efficient *in vitro* propagation can be a reliable and useful method for establishment of new bamboo plantations and they offer an attractive alternative to conventional methods for mass propagation of bamboo species [4]-[7]. The indiscriminate extraction from natural populations coupled with large-scale habitats loss has seriously endangered the dwarf bamboo (Himalayan Weeping Bamboo) genetic resource. Thus, here was a need to develop its propagation technology through tissue culture technique for rapid and large-scale propagation. In this context, the aim of this study is to develop *in vitro* micropropagation of *Drepanostachyum falcatum* through tissue culture.

2. Materials and Methods

2.1. Plant Material Source

Explants in the form of nodal segments (2 - 3 cm) were collected from mature field grown clumps of healthy, disease free plants of *Drepanostachyum falcatum* for axillary bud induction and proliferation. Nodal segments with single axillary buds were used as source material for micropropagation.

2.2. Explants, Media Preparation and Culture Initiation

The axillary buds were first washed with 5% cetrimide solution (ICI Ltd. India) for 5 minutes and then cleaned with ethanol 90% swabbed cotton followed by surface sterilization with 0.1% $HgCl_2$ solution for 10 - 12 minutes and rinsed 3 - 4 times with sterilized distilled water. The surface sterilized axillary buds were cultured on semi-solid and liquid MS (Murashige and Skoog, 1962) medium supplemented with different concentrations of cytokinin (0.0 - 10.0 mg/l BAP/KIN) [8]. The pH of the medium was adjusted to 5.6 prior to autoclaving the medium at 121˚C for 15 minutes. Cultures were maintained at 25˚C ± 2˚C temperature with 16 hours illumination with a photon flux density of 2500 lux from white fluorescent tubes (Philips, India).

2.3. Establishment and Multiplication of Shoot Cultures

Axillary buds cultured on liquid and semisolid MS medium supplemented with cytokinin, proliferated number of axillary shoots. These axillary shoots were excised and sub-cultured on fresh liquid as well as semisolid MS medium for further shoot multiplication. In 3 - 4 weeks these shoots were further multiplied and cut into shoot clusters of 3 - 6 shoots and were again subcultured on semi-solid MS medium supplemented with 0.0 - 5.5 mg/l

BAP/KIN. These subcultured shoots were multiplied after every 3 - 4 weeks. Different sets of experiments were conducted to obtain maximum shoot multiplication rate. For this, multiplied shoots were subcultured in propagules consisting of 1 to 6 shoots. Observations were recorded after an interval of 4 - 5 weeks. The number of propagule cultured and number of propagule derived at the end of subculture gave the multiplication rate.

2.4. Rooting, Hardening and Acclimatization

The *in vitro* regenerated shoots (2 - 3 cm long) produced were cultured on MS medium containing various concentrations of auxins 1.5 - 9.5 mg/l IBA/NAA in the medium for root induction. Three propagules (Shoot clusters of different sizes of shoots) were cultured per conical flask (100 - 150 ml). Rooting response was recorded in terms of rooting percentage, average number of roots produced and average root length.

Rooted shoots from four week old cultures were transferred to soil under shade house either directly or after *in vitro* hardening the plantlets were taken out from the flasks, washed to remove adhered agar and then transferred to autoclaved 250 ml screw cap glass bottle containing 1/3 volume of autoclaved vermiculite. These plantlets were supplied with half strength MS solution (without organics) thrice a week for two weeks. After two weeks, these bottles were shifted to mist chamber having relative humidity of 60% - 80% with a temperature of 35˚C ± 2˚C. The caps of bottles were removed and plantlets were allowed to remain in the bottle for 3 - 4 days before they were transferred to polyethylene pots containing a mixture of sand, farmyard manure and soil. In the mist chamber, the plants were kept for four weeks and were irrigated with half strength MS medium. Later, these polyethylene pots were shifted to green house for acclimatization and kept for two months. After one month in shade house the plants were transferred to polyethylene pots containing same soil composition.

2.5. Statistical Analysis

Data collected was analyzed using CRD design of experiments as it is one of the most widely used design in case of tissue culture experiments (controlled set of conditions and for homogenous materials) and gives best result. Degree of variations was shown by standard error and critical difference at 5%. Significance level was tested at 1%, 0.5% and 0.1% level.

3. Results and Discussion

3.1. Culture Establishment, Shoot Formation and Shoot Multiplication

In *Drepanostachyum falcatum* incorporation of BAP into the medium was found to improve the incidence of bud break and promoted multiple shoot formation. Maximum bud break (90% - 95%) in *D. falcatum* was obtained on MS medium supplemented with 4.5 mg/l BAP. The explant response cultured in MS media supplemented with BAP and KIN are shown in (**Table 1**) (**Figure 1(a)**, **Figure 1(b)**). These results are in line with those of other workers, indicating the efficiency of BAP for shoot culture initiation and multiplication in several bamboos. Similar results with BAP were also reported in different species of bamboo [7] [9]-[13]. Ramanayake and Yakandwala [14] used 2.0 mg/l BAP + 0.1 mg/l Kn in *D. giganteus*. Though in present study BAP when used alone gave better bud break as compared to BAP and Kn used. Hirimburegama and Gamage [15] found cytokinin to be essential for bud break. Huang and Huang [16] used 4.44 μM BAP for initiation of cultures of *Bambusa ventricosa*.

MS medium proved to be the best medium for the establishment of shoot cultures in bamboos. In earlier reports on bamboos MS medium has been successfully used for shoot initiation and establishment of bamboo cultures [10]-[13] [16]-[18]. In bamboos bud proliferation and induction was better in liquid medium as compared to semisolid medium. Similar results have been reported in other bamboos [7] [10] [13] [19]-[21].

Shoot multiplication is the major criterion for successful commercial micropropagation. Cytokinins are essential for axillary bud proliferation and shoot multiplication in bamboos. A high rate of shoot multiplication was obtained due to BAP in the medium, which stimulated the growth of multiple shoots during shoot multiplication cycle. In *Drepanostachyum falcatum*, 7 - 9 fold shoot multiplication was achieved on MS medium supplemented with 3.5 mg/l BAP (**Table 2**, **Figure 1(c)**, **Figure 1(d)**). These results are supported by earlier reports on *in vitro* propagation of bamboos, where BAP had invariably been used for shoot multiplication [7] [11]-[13] [18]-[20] [22]-[27].

Shoot multiplication rates obtained for bamboo species (*D. falcatum*) on Kn supplemented medium was lower

Table 1. Effect of plant hormones, cytokinin BAP/KIN in MS medium on axillary bud induction from nodal segments of *Drepanostachyum falcatum* after 35 days of culture.

Plant Hormone Concentration (mg/l)	Response %	Mean Shoot Number	Mean Shoot Length (cm)
BAP			
0.0	33.33 ± 0.57	1.25 ± 0.13	1.61 ± 0.14
1.5	45.00 ± 0.32	1.75 ± 0.20	1.78 ± 0.11
3.5	68.75 ± 0.56	4.15 ± 0.23	1.98 ± 0.12
4.5	90.70 ± 0.40	11.08 ± 0.17	1.99 ± 0.14
6.5	85.25 ± 0.55	8.08 ± 0.23	1.85 ± 0.10
8.5	74.80 ± 0.59	6.90 ± 0.19	1.60 ± 0.09
10.0	56.25 ± 0.40	4.00 ± 0.22	0.84 ± 0.06
KIN			
0.0	29.16 ± 0.57	1.33 ± 0.14	0.92 ± 0.09
1.5	35.49 ± 0.55	1.32 ± 0.10	1.31 ± 0.09
3.5	81.31 ± 0.56	6.31 ± 0.22	1.75 ± 0.07
4.5	73.00 ± 0.55	5.17 ± 0.23	1.96 ± 0.11
6.5	67.73 ± 0.50	5.97 ± 0.19	1.92 ± 0.13
8.5	64.56 ± 0.53	4.75 ± 0.22	1.22 ± 0.09
10.0	45.10 ± 0.52	3.57 ± 0.17	0.86 ± 0.06

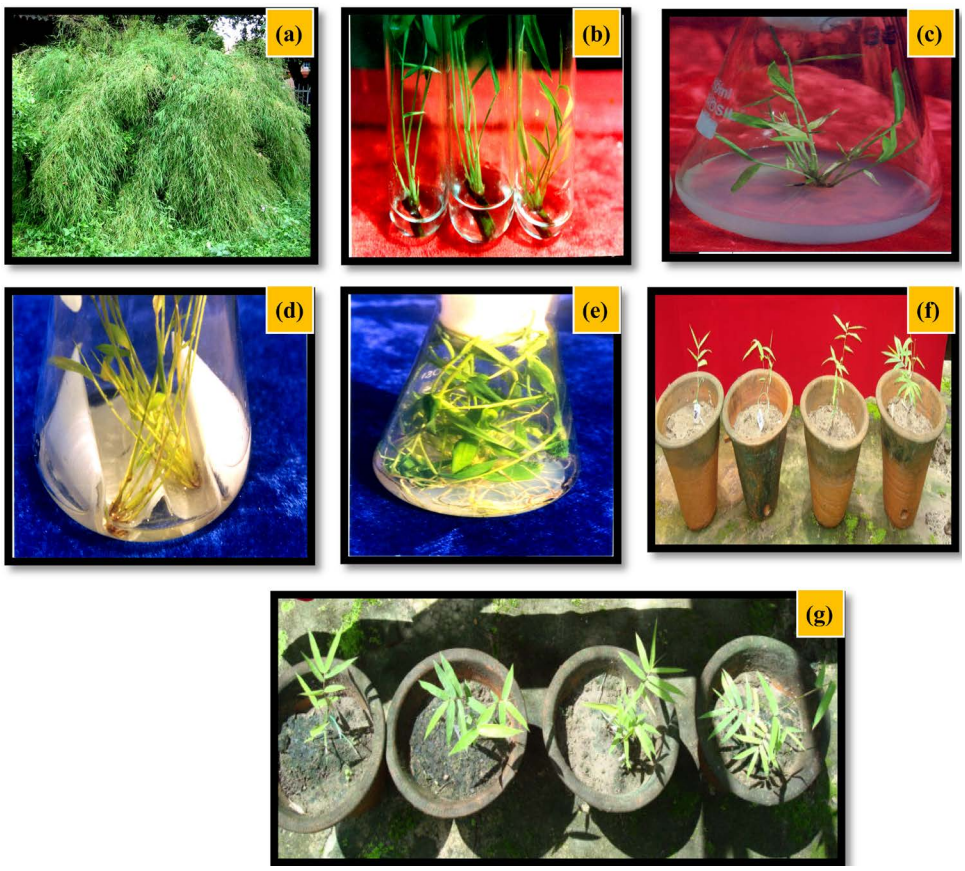

Figure 1. Direct plant regeneration of *in vitro* cultured *Drepanostachyum falcatum*. Mother plant of *D. falcatum* at FRI campus (a); Axillary shoot proliferation in *Drepanostachyum falcatum* on MS + 4.5 mg/l BAP supplemented medium (b); *In vitro* shoot multiplication in *D. faclatum* on MS medium supplemented with 3.5 mg/l BAP (c); *In vitro* shoot multiplication after 4th subculture (d); *In vitro* rooting in *Drepanostachyum falcatum* on MS + 6.5 mg/l IBA after 4 weeks (e); Hardened and acclimatized *in vitro* plantlets of *D. falcatum* in pots in poly house (f) (g).

Table 2. Effect of cytokinins (BAP/KIN) in MS medium on shoot multiplication rate. Data recorded after 4 weeks.

Plant Hormone Concentration (mg/l)	Mean Shoot Number	Mean Shoot Length (cm)	Multiplication Rate
BAP			
0.0	10.08 ± 0.23	0.55 ± 0.01	2.52 ± 0.06
1.5	12.89 ± 0.51	1.00 ± 0.01	2.47 ± 0.11
2.5	23.00 ± 0.50	1.98 ± 0.01	5.00 ± 0.12
3.5	41.49 ± 0.60	2.15 ± 0.02	09.87 ± 0.14
4.5	37.89 ± 0.36	2.29 ± 0.02	8.96 ± 0.08
5.5	29.81 ± 0.30	2.20 ± 0.01	6.69 ± 0.07
KIN			
0.0	11.33 ± 0.33	0.76 ± 0.02	2.83 ± 0.08
1.5	22.56 ± 0.73	0.96 ± 0.02	5.14 ± 0.17
2.5	34.96 ± 0.69	1.53 ± 0.01	7.72 ± 0.17
3.5	29.05 ± 0.33	1.95 ± 0.01	6.50 ± 0.07
4.5	23.40 ± 0.95	2.10 ± 0.01	5.10 ± 0.14
5.5	16.21 ± 0.82	1.76 ± 0.31	3.46 ± 0.19

than the shoot multiplication rates obtained on BAP supplemented medium. Nadgir *et al.* [28], Das and Rout [9] also reported reduced multiplication on Kn supplemented medium. Superiority of BAP over Kn has been reported and discussed in relation to shoot multiplication of trees [29]. Bhati *et al.* [30] also reported BAP to be more superior than Kn for shoot differentiation and proliferation in *Aegle marmelos.* Similar results have also been obtained in case of *Prunus serotina* [31] and *Syzygium cuminii* [32]. Arya and Sharma [10] obtained 5 fold multiplication by subculturing after 4 weeks in *Bambusa bambos*. Bag *et al.* [18] reported 6 - 11 fold shoot multiplication after 8 weeks of subculturing. Arya *et al.* [13] reported 5 fold shoot multiplication in *Dendrocalamus giganteus* after every 4 weeks of subculturing. The earlier reports on micropropagation of bamboo [22] [33]-[36] involved a callus phase which may lead to genetically aberrant plants.

3.2. Formation of Roots and Acclimatization

In *D. falcatum* the shoots obtained from axillary bud and multiplied enormously were successfully rooted. The ability of plant tissue to form roots depends on interaction of many endogenous and exogenous factors. During the studies on *D. falcatum*, it was recorded that the shoot multiplication was obtained in medium with cytokinins alone. These shoots were rooted on auxin supplemented (IBA and NAA) MS medium, where they produced roots. These shoots also showed simultaneous shoot elongation, which is due to "cytokinin-carry over effect" in the shoots. A varied effect of auxins (IBA, NAA) was observed by incorporating them in MS medium at different concentration (1.5 mg/l - 9.5 mg/l) (**Table 3**). The best results obtained in *Drepanostachyum falcatum* where 100% rooting was achieved on MS medium supplemented with 6.5 mg/l IBA (**Figure 1(e)**). In the present case full strength MS medium with auxin yielded best rooting response. Effective role of IBA in bamboos for rooting has also been reported [12] [14] [15] [18] [37] [38].

In present case 90% - 95% survival of plantlets was observed after their hardening and acclimatization. Hardened and acclimatized *in vitro* plantlets of *D. falcatum* in pots in polyhouse (**Figure 1(f)**, **Figure 1(g)**) for 1 - 2 months before their field transplantation that improved the survival percentage of plants in the field. So far, only 80% - 90% of transplantation success is reported [10] [12] [18] [19] [22]-[27] [39]. Use of vermiculite or soilrite like inert substance for hardening has been reported by many workers in bamboos [19] [37]. Vermiculite is an inert material and absorbs large quantity of water. It has a relatively high cation exchange capacity and thus can hold the nutrients in reserve and later release them. It contains enough magnesium and potassium to supply most of plants. Rooted plantlets were shifted to sand: soil: FYM mixture for hardening and enriched with 1/2 × nutrient solution for few weeks and is reported in bamboos [7] [10] [12] [19] [39]. Acclimatization and hardening depends on the relative humidity and temperature therefore misting is preferred which maintains temperature of 30˚C and relative humidity of 80% - 85%. It has been reported by many workers that survival percentage

Table 3. Effect of auxins IBA/NAA on rooting of *in vitro* shoots in MS medium. Data recorded after 4 - 5 weeks.

Plant Hormone Concentration (mg/l)	Response %	Mean Root Number	Mean Root Length (cm)
IBA			
1.5	43.81 ± 0.45	2.16 ± 0.21	1.29 ± 0.03
3.5	85.81 ± 0.58	6.00 ± 0.21	1.28 ± 0.01
4.5	89.64 ± 0.36	8.62 ± 0.26	2.16 ± 0.02
6.5	99.00 ± 0.52	11.34 ± 0.22	2.18 ± 0.02
8.5	99.00 ± 0.52	9.16 ± 0.28	1.18 ± 0.17
9.5	89.62 ± 0.36	6.43 ± 0.32	1.96 ± 0.02
NAA			
1.5	73.00 ± 0.27	3.23 ± 0.17	0.75 ± 0.02
3.5	99.00 ± 0.01	5.13 ± 0.25	1.95 ± 0.02
4.5	99.14 ± 0.01	8.13 ± 0.34	2.16 ± 0.02
6.5	89.14 ± 0.35	6.63 ± 0.19	1.35 ± 0.03
8.5	81.00 ± 0.16	6.15 ± 0.28	0.72 ± 0.02
9.5	68.48 ± 0.43	3.31 ± 0.24	0.65 ± 0.03

increases if the plants are transplanted to soil in rainy season [10] [12]. Hardened and acclimatized plants on transfer to field conditions survived and grew to normal plants.

4. Conclusion

The present investigation was undertaken to develop appropriate tissue culture technology as a non-conventional method for mass multiplication of economically important bamboo *Drepanostachyum falcatum*. MS culture medium supplemented with 3.5 mg/L BAP gave the highest rate of shoot multiplication using nodal explants. The highest rate of rooting was obtained with 6.5 mg/L IBA. The obtained plantlets of *D. falcatum* survived and grew normally in polyhouse.

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