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MASS PROPAGATION OF *DENDROCALAMUS GIGANTEUS* WALL. EX MUNRO THROUGH *IN VITRO* CULTURE

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Abstract

The present experiment was conducted to establish an efficient protocol for mass propagation of *Dendrocalamus giganteus* Wall. ex Munro through *in vitro* culture using nodal segments as explants. The highest percentage of shoot induction (86.67 %) was obtained in the liquid MS medium supplemented with 1.5 mg/l BAP and 1.0 mg/l TDZ with maximum number of shoots (3.91 ± 0.87) per explant. The highest number and length of shoots were 22.83 ± 1.30 shoots/culture and 7.71 ± 0.96 cm, respectively induced on agitated liquid MS medium supplemented with 2.0 mg/l BAP and 1.0 mg/l TDZ. Incorporation of 8 % coconut water in the above mentioned medium resulted satisfactory shoot growth and development. The best response towards root induction (90 %) was observed when 3-4 shoots clump were transferred onto half-strength of gelled MS medium fortified with 4.0 mg/l IBA with 9.31 ± 1.45 roots per unit shoot. Successful acclimatization of well-rooted clumps of 4-5 shoots was achieved in a mixture of soil, sand, and compost (1 : 1 : 1) with 90% survival rate.

Introduction

Bamboos are long-lived, woody, evergreen, monocarp flowering plants belonging to subfamily Bambusoideae under the family Poaceae, which is subsequently divided into three tribes (Clark *et al*. 2015). They are distributed all over the world with 120 genera and 1641 species, but majority of them occur in the tropical, subtropical and temperate zones of the globe (Soreng *et al*. 2015). China is the world leading bamboo producing country in the world (Rahman *et al*. 2017). In Bangladesh there are about 9 genera and 33 species (Banik 1998) of bamboo growing throughout the country. Most of the species belong to *Bambusa* and *Dendrocalamus* while the remaining genera have only 1-2 species each (Rahman *et al*. 2017).

Dendrocalamus, consists of around 30 species (Farrelly 1984), in which the most widely used species is *D. giganteus* Wall. ex Munro, locally known as 'Bhudum Bansh' in Bangladesh. It is considered as the tallest bamboo in the world (Louppe *et al*. 2008). The conventional methods of bamboo propagation are mainly based on seeds and vegetative methods. However, gregarious flowering (Austin and Marchesini 2012) and short viability of seeds (Haile 2008) are the main limiting factors to use seeds as valuable source of propagation. Vegetative propagation methods also have some limitations such as, seasonal dependence, low survival rate, limited rooting (Singh *et al*. 2013) and insufficient number of planting material for large-scale plantation (Mudoi *et al*. 2013). So, as an alternative approach for mass propagation of this species, the present research project was carried to develop an efficient and comprehensive regeneration protocol through direct shoot organogenesis.

Materials and Methods

Nodal segments (3-4 cm) containing pre-existing axillary bud collected from the 3rd to 5th nodes of secondary branches of *Dendrocalamus giganteus* plants were used to initiate and establish the *in vitro* culture. Nodal explants were surface sterilized by following the methodology

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of Raju and Roy (2016) and cultured on liquid MS medium (Murashige and Skoog 1962) fortified with different concentrations of BAP (1.0 - 2.0 mg/l) alone or in combinations with TDZ (0.5 - 1.5 mg/l) for direct shoot induction. For shoot multiplication, induced shoots were excised aseptically from the nodal explants and transferred onto the freshly prepared agitated liquid MS medium supplemented with different concentrations and combinations of cytokinins (BAP, Kn, 2-iP and TDZ) and auxin (NAA), and were maintained by regular sub-culturing at 35 days intervals. In the present investigation, effect of different concentrations of coconut water (CW) (2-10 %) on growth and shoot multiplication was also tested.

For root induction, bunches of shoots (3-4) rather than individual shoots were planted in half-strength of gelled MS medium supplemented with different concentrations of IBA (2.0-5.0 mg/l) alone or in combinations with NAA (0.5-1.5 mg/l). The cultures were exposed to 16 hrs light and 8 hrs dark per day with constant temperature at 24 ± 2°C. The light intensity of the growth chamber was 3000 lux. The plantlets with sufficient roots inside the test tube were then transferred to a soil mixture containing garden soil, sand and compost with (1:1:1) ratio for hardening process. The plantlets were covered with transparent polybag and the inner side of the polybag was sprayed with water at every 8 hrs to maintain high humidity around the plantlets. The polybags were gradually perforated to expose the plantlets with the outer environment and subsequently removed after 14 days. Finally, well established plants were transferred to the outer normal environment.

Results and Discussion

Direct shoot initiation from the nodal explants was observed in most of the media composition used. Among the media component used, the combined effect of 1.5 mg/l BAP and 1.0 mg/l TDZ was found to be most effective (86.67%) for direct shoot induction with an average 3.91 ± 0.87 shoots per explant and average 4.16 ± 1.24 cm shoot length after 16-25 days of inoculation (Table 1). Similarly, Raju and Roy (2016) reported that liquid MS medium in conjugation with 2.0 mg/l BAP and 1.0 mg/l TDZ was good enough for direct shoot induction in case of *Banbusa bambos*. However, a lower concentration of TDZ was found to be suitable in some other previous studies for micropropagation of *D. strictus* (Singh *et al*. 2000), *B. edulis* (Lin *et al*. 2004) and *B. oldhamii* (Lin *et al*. 2007).

Table 1. Effect of different concentrations of BAP alone or in combination with TDZ in liquid MS medium on direct shoot induction from the nodal explants of *Dendrocalamus giganteus*.

Concentrations of growth regulators (mg/l)		Percentage of responding explant	Number of shoots produced per explant (Mean ± SE)	Shoot length (cm) (Mean ± SE)
BAP	TDZ			
1.0	-	35.00	0.96 ± 0.78	1.54 ± 0.91
1.5	-	43.33	2.32 ± 1.22	1.83 ± 0.69
2.0	-	40.00	2.51 ±1.07	1.66 ± 2.20
1.0	0.5	45.00	1.57 ± 0.80	1.77 ± 2.01
1.0	1.0	65.00	1.60 ± 0.94	1.70 ± 1.03
1.0	1.5	60.00	1.48 ± 0.34	1.95 ± 1.36
1.5	0.5	63.33	1.97 ± 1.60	2.05 ± 0.55
1.5	**1.0**	**86.67**	**3.91 ± 0.87**	**4.16 ± 1.24**
1.5	1.5	73.33	3.16 ± 2.03	3.32 ± 1.29
2.0	0.5	68.00	2.37 ± 0.92	2.34 ± 1.36
2.0	1.0	72.00	2.84 ± 1.44	3.21 ± 0.39
2.0	1.5	56.00	2.73 ± 2.59	2.93 ± 1.22

Values are means (± SE, Standard error of mean) obtained from four independent experiments.

Table 2. Effect of different concentrations and combinations of cytokinin (BAP, TDZ, 2-iP and Kn) and auxin (NAA) on shoot multiplication of *Dendrocalamus giganteus*.

Growth regulators (mg/l)	Percentage of shoot proliferation	Number of regenerated shoots per culture (Mean ± SE*)	Shoot length (cm) (Mean ± SE*)
BAP			
1.0	40.00	4.43 ± 0.78	1.94 ± 1.04
2.0	55.00	4.60 ± 1.88	2.91 ± 0.82
3.0	70.00	9.53 ± 0.81	4.62 ± 0.94
4.0	50.00	8.05 ± 2.11	4.32 ± 1.39
5.0	35.00	4.94 ± 0.35	2.85 ± 0.26
BAP+Kn			
1.0+1.0	45.00	4.61 ± 0.25	2.18 ± 0.97
1.0+2.0	60.00	4.45 ± 0.70	2.00 ± 0.65
2.0+1.0	65.00	7.56 ± 0.69	4.22 ± 0.40
2.0+2.0	75.00	13.02 ± 0.33	4.70 ± 0.82
3.0+1.0	50.00	5.03 ± 2.12	4.37 ± 0.50
3.0+2.0	40.00	3.86 ± 0.57	3.54 ± 1.54
BAP+2-iP			
2.0+1.5	64.00	6.26 ± 1.12	3.94 ± 0.84
2.0+2.0	60.00	6.30 ± 0.54	3.82 ± 1.17
2.5+1.5	52.00	5.04 ± 1.23	3.45 ± 2.10
2.5+2.0	68.00	8.41 ± 0.67	4.11 ± 0.84
BAP+TDZ			
1.0+1.0	48.00	7.23 ± 0.70	3.83 ± 0.57
1.0+1.5	56.00	7.06 ± 0.52	4.67 ± 1.02
2.0+1.0	**84.00**	**22.83 ± 1.30**	**7.71 ± 0.96**
2.0+1.5	72.00	17.20 ± 1.37	6.17 ± 1.04
3.0+1.0	60.00	7.43 ± 0.92	5.19 ± 0.98
3.0+1.5	40.00	5.06 ± 2.21	3.50 ± 1.83
BAP+NAA			
1.0+0.2	55.00	5.42 ± 0.81	4.05 ± 1.12
1.0+0.5	40.00	4.04 ± 0.22	3.18 ± 0.77
2.0+0.2	70.00	9.27± 0.48	5.21± 1.06
2.0+0.5	60.00	5.51 ± 0.44	3.53 ± 0.67
3.0+0.2	50.00	3.87 ± 0.32	2.65 ± 0.82
3.0+0.5	30.00	2.84 ± 0.59	2.41 ± 1.07
BAP+TDZ+NAA			
1.0+1.0+0.2	50.00	4.23 ± 0.60	3.69 ± 1.31
1.0+1.0+0.5	35.00	2.87 ± 2.03	3.02 ± 0.90
2.0+1.0+0.2	65.00	6.29 ± 0.54	3.92 ± 0.36
2.0+1.0+0.5	45.00	4.30 ± 0.85	3.11 ± 1.48
3.0+1.0+0.2	55.00	3.47 ± 0.67	2.21 ± 0.50
3.0+1.0+0.5	35.00	2.60 ± 2.33	1.96 ± 1.24

Values are means (± SE, Standard error of mean) obtained from three independent experiments.

Directly induced axillary shoots were excised from the node and cultured on agitated liquid MS medium supplemented with different concentrations of BAP alone or in combinations with Kn, 2-iP, TDZ and NAA. Among the different types of cytokinins tested, BAP was found to be the best cytokinin for shoot multiplication of *D. giganteus*. It was found that, increase in the concentration of BAP up to 3.0 mg/l enhanced the rate of shoot multiplication (Table 2). Similarly, Arya *et al.* (2006) showed 5.44-fold increase of shoot multiplication rate in case of *D. giganteus* on MS medium supplemented with 20 µM BAP. In case of *D. giganteus*, Ramanayake *et al.* (2001) also achieved continuous shoot proliferation in MS medium fortified with 6.0 mg/l BAP and 2% sucrose.

The combined effect of BAP with other cytokinin like TDZ, Kn and 2-iP, was found to be more effective particularly on the MS liquid medium supplemented with 2.0 mg/l BAP and 1.0 mg/l TDZ for axillary shoot multiplication of *D. giganteus* than using BAP singly (Table 2). In this combination the number of shoots per propagule and shoot length was 22.83 ± 1.30 and 7.71 ± 0.96 cm, respectively. These results are in agreement with the findings of Raju and Roy (2016) in case of *B. bambos*. In contrary, Arya *et al*. (2006) found that addition of BAP in the Kn supplemented medium enhanced the shoot multiplication rate to 6.35-fold in case of *D. giganteus*. Combined effect of BAP with TDZ and NAA in shoot multiplication was also tested in the present study, in which a moderate multiplication rate was found (Table 2). There are some reports that addition of auxins (IAA or NAA) has either no effect on multiplication or decreased the multiplication of bamboo (Saxena 1990, Das and Rout 1991).

A significant increase in the shoot multiplication rate with healthy shoot was observed, when 8% (v/v) coconut water was added with 2.0 mg/l BAP and 1.0 mg/l TDZ. This medium combination gave a maximum rate of multiplication with an average 33.17 ± 0.84 shoots per culture which were 9.24 ± 1.08 cm long (Fig. 1). Similarly, Ramanayake and Yakandawala (1997) in case of *D. giganteus* used 8% (v/v) coconut water in the proliferation medium. However, in case of *D. giganteus*, Saxena and Bhojwani (1993) and in case of *B. bambos*, Raju and Roy (2016) reported that 10% coconut water enhanced shoot proliferation.

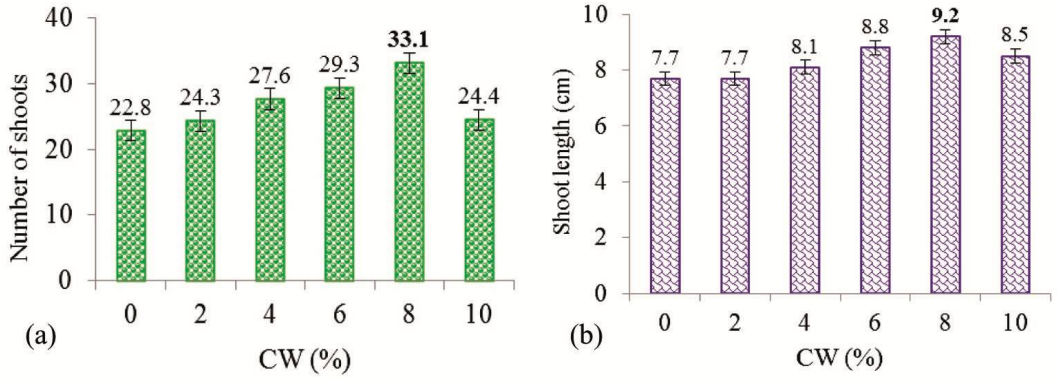

Fig. 1. Effect of different concentrations of CW along with constant 2.0 mg/l BAP, 1.0 mg/l TDZ and 3% sucrose on average (a) number and (b) length of regenerated shoots per culture.

For root induction, among the various concentrations of IBA investigated, it was found that 4.0 mg/l IBA in absence of NAA was the most effective for root induction and the percentage of rooting was 90% with maximum number of roots (9.31 ± 1.45) per propagule and maximum root length (9.13 ± 0.20 cm). However, further increase in the concentrations of IBA above 4.0 mg/l

caused reduction in the rooting percentage, number and length of root (Table 3). In the present experiment, combined effect of IBA and NAA was also tested, but did not provide satisfactory results. However, in most of the treatments, root induction was started within 10-14 days after inoculating them in rooting media. These findings are in accordance with the findings of Hossain *et al*. (2018) and Devi *et al*. (2012) in case of *D. giganteus*; and Arya *et al*. (2012) in case of *D. hamiltonii*. In contrary, Raju and Roy (2016) in *B. bambos* and Singh *et al*. (2012) in *D. asper* observed maximum number of rooting by using the combination of IBA and NAA.

Table 3. Effect of different concentrations and combinations of IBA and NAA on rooting of *in vitro* raised shoots of *Dendrocalamus giganteus*.

Concentrations of IBA and NAA (mg/l)		Percentage of rooting	Number of roots per propagule (Mean ± SE*)	Root length (cm) (Mean ± SE*)
IBA	NAA			
2.0	-	35.00	2.21 ± 0.37	2.84 ± 0.56
3.0	-	60.00	4.60 ± 0.94	3.88 ± 0.65
4.0	**-**	**90.00**	**9.31 ± 1.45**	**8.76 ± 0.92**
5.0	-	65.00	5.06 ± 2.43	4.78 ± 1.96
2.0	0.5	20.00	1.94 ± 2.62	2.27 ± 2.11
2.0	1.0	25.00	2.35 ± 0.76	2.32 ± 0.89
3.0	0.5	40.00	3.38 ± 0.09	3.25 ± 0.46
3.0	1.0	55.00	4.23 ± 2.04	3.71 ± 1.34
4.0	0.5	70.00	5.50 ± 1.68	5.92 ± 1.43
4.0	1.0	50.00	4.18 ± 1.30	4.06 ± 0.69
5.0	0.5	40.00	2.96 ± 0.84	3.08 ± 0.92
5.0	1.0	15.00	1.56 ± 0.55	2.73 ± 0.70

Values are means (± SE, Standard error of mean) obtained from three independent experiments.

In vitro grown plantlets were gradually acclimatized to the external environmental condition. For this, *in vitro* rooted *D. giganteus* plantlets were successfully acclimatized in polybag containing garden soil, sand and compost mixture with 1:1:1 ratio. In this soil mixture the survival rate was 90% after 2 weeks of acclimatization. Similar results were reported by Ansari *et al*. (2009), Devi *et al*. (2012) and Hossain *et al*. (2018) using the same soil mixture in case of *D. giganteus*. Using the same potting mixture Anand *et al*. (2013) and Raju and Roy (2016) reported 80-100% survival rate in case of *B. bambos*. Finally, the acclimatized plants, after a month, were transferred to the larger pots containing garden soil and compost with 1:1 ratio for sufficient growth and transplanted to the field. Different stages of *in vitro* regeneration of *D. giganteus* are presented in Fig. 2.

The present research work provides an efficient and cost effective micropropagation protocol for *Dendrocalamus giganteus* Wall. ex Munro from nodal explant without any significant changes to its macroscopic characteristics. This protocol will be helpful for high frequency plant regeneration of *D. giganteus* at a faster rate than any conventional method of propagation. This study can also serve as a baseline for germplasm conservation of this economically important bamboo species.

Fig. 2: *In vitro* regeneration of *Dendrocalamus giganteus*. a. Surface sterilized nodal segments inoculate in liquid MS medium containing 1.5 mg/l BAP+1.0 mg/l TDZ, b. Direct shoot induction after 20 days of inoculation, c. Multiplication of *in vitro* raised shoot in agitated liquid MS medium containing 2.0 mg/l BAP+1.0 mg/l TDZ, d. Rapid multiplication with elongated shoots in agitated liquid MS medium containing 2.0 mg/l BAP+1.0 mg/l TDZ+8% CW, e. *In vitro* root induction on half-strength of gelled MS medium containing 4.0 mg/l IBA, f. Complete plantlets, g. Acclimatization of the regenerated plantlets in poly bags, h. 2-weeks old plants in soil, i. Fully acclimatized plant in clay pot.

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