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Original Research Article

Albino Regenerants Proliferation of *Dendrocalamus asper in vitro*

Vijay Kumar[1], Shweta Singh[2] and Madhuparna Banerjee[3*]

[1]Research Centre for Plant Growth and Development, University of KwaZulu- Natal, Pietermaritzburg, South Africa
[2]Jharkhand Rai University, Kamre, Ranchi- 835222 Jharkhand, India
[3]College of Biotechnology, Birsa Agricultural University, Kanke, Ranchi-834006, Jharkhand, India
**Corresponding author*

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Dendrocalamus asper, Mass multiplication, Albino, Adenine sulphate

A B S T R A C T

The present study deals with the refinement of micropropagation protocol of *Dendrocalamus asper* for increasing the rate of multiplication. Single nodes of lateral branches were used as explant. Bud breaking and shoot proliferation were induced in solid Murashige and Skoog (MS) basal medium supplemented with BA and Adenine sulphate within 15 days. The rate of multiplication increased 5-10 times in liquid media supplemented with (3.0 mg/l) 6-benzyladenine BA and (50 mg/l) Adenine sulphate. Albino regenerants have been derived from long term cultures in multiplication. The albino regenerants maintained separately showed (21.40 ± 0.73) high rate of multiplication. Maximum rooting (13.96 ± 1.52) as well as growth of green plantlets was observed in MS with (1.0 mg/l) IBA. Rooted plantlets were hardened and after 15-20 days of primary hardening the plantlets were transferred to poly bags for secondary hardening.

Introduction

Bamboo, the largest member of Poaceae family, perhaps is the only natural resource having so many and so varied uses. Bamboo is one of the most important woody shrubs known to mankind. Among the several species of bamboo *Dendrocalamus asper* is an economically and environmentally important ornamental plant belongs to family poaceae. It is native to china and commonly found in Asian countries. Every year US imports 16.8 million of edible bamboo shoots from China, Indonesia, Japan, Taiwan and Thailand (United State Department of Agriculture online service, September 07, 2001) (Banerjee *et al.*, 2011). Bamboo shoots are consumed as food by people all over the world. The mature culms of *D. asper* are consumed in the cooking, construction, handicraft, outriggers on fishing boats, and these are suitable for pulp and paper manufacture also. Indian Council of Forestry Research and Education, Dehradun, India has successfully introduced this exotic bamboo to India in 1994 (Singh *et al.*, 2004). *D. asper* is commonly propagated through seeds and culm cuttings. Small viability period, long flowering cycle and large scale consumption of seed by wild animals are the major drawbacks of large scale propagation of bamboos through seed. Vegetative methods for propagation is also unreliable due to limited availability, low

survival rate and transportation problem (Rao *et al.,* 1985). Protocol for *in vitro* propagation of different species of bamboo has already been established. For production of quality and large number of planting materials at an accelerated pace within a short period of time is necessary. Protocol for *in vitro* propagation of different species of bamboo has already been established. In vitro mass multiplication of *D. asper* was successfully established by several authors (Arya and Arya 1997, Arya *et al.,* 2001, Arya *et al.,* 2008, Arya *et al.,* 1999, Ojha *et al.,* 2009, Singh *et al.,* 2012). Albino regenerants are frequently observed in bamboo tissue culture (Ho and Chang 1998, Lin and Chang 1998), but no reports till date is available for albino regenerants of *D. asper*. To meet the increasing demand much beyond availability, micropropagation offers a better method for rapid production of this valued plant of agro-forestry interest. Therefore, in the present communication, an efficient and reliable protocol was established for high mass multiplication through albino regenerants of *D. asper* using single node of lateral branches in vitro.

Materials and Methods

Plant Source and culture conditions

D. asper plantlets were brought from Institute of Forest productivity (ICFRE), Ranchi, Jharkhand India. The young, actively growing nodal segments (2-3 cm) from lateral branches were collected and washed thoroughly under running tap water and treated in 0.2% Bavistin (systemic fungicide) solution with Tween 20, (5%) solution for 20 min. Finally, the nodal explants were surface sterilized with 0.1% $HgCl_2$ w/v for 20 min and thoroughly rinsed 4-5 times with sterilized distilled water and both the ends were trimmed off. The whole procedure of surface sterilization was performed under laminar air flow cabinet. Sterilized explants were cultured on Murashige and Skoog (1962) medium supplemented with various PGRs such as BA, Kn, Ads with 3% sucrose (w/v), and 0.7% agar. Before autoclaving the medium for 16 min at 121ºC, the pH was adjusted to 5.8. Cultures were maintained in a growth chamber with a 16 h/8 h light/dark photoperiod at 24 ± 1ºC (day) and 20 ± 1ºC (night). Light intensity was 25 µmol $m^{-2}s^{-1}$ by cool-white fluorescent lamps.

Multiple shoot induction and shoot proliferation

For multiple shoot induction and shoot proliferation after 4 weeks of incubation of nodal explants, the sprouted buds were separated from culture and placed in MS medium with various PGRs. Five different concentration (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) of BA and Kn were used for multiple shoot induction, and Ads (50 mg/l) in combination with BA (1-5 mg/l) used to enhance the multiplication frequency. MS medium without any PGR served as a control.

Production of albino regenerants

Few cultures of *D. asper* were kept in growth chamber in same medium without any subculturing. Albino regenerants have been derived from long term cultures in multiplication. These albino regenerants maintained separately in MS liquid medium supplemented with BA (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) in combination with Ads (50 mg/l) for high regeneration frequency.

Rooting and Acclimatization

In vitro regenerated shoots were excised from culture and transferred to liquid MS medium supplemented with IAA (1.0mg/l,

2.0mg/l and 3.0mg/l), IBA (1.0mg/l, 2.0mg/l and 3.0mg/l) and NAA 1.0mg/l, 2.0mg/l and 3.0mg/l) separately. The number of roots developed per shoot was recorded after 4 weeks. Well rooted plantlets were removed from in vitro culture, washed properly with distilled water and transferred to protray containing cocopeat for primary hardening for 15 days. After that plantlets were transferred to polybags containing sand: soil: farmyard manure (1:1:1 v/v) for better survival.

Statistical analysis

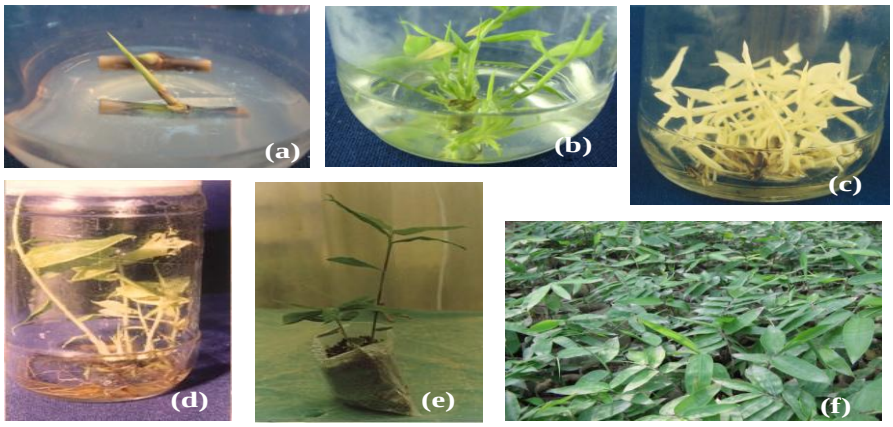
Each treatment consisted of five culture bottle, each containing five explants (n = 25). All experiments were repeated three times. Data obtained from all experiments were presented as the means ± SE of three replications.

Results and Discussion

In the present study, complete regeneration was successfully achieved from node segment of *D. asper* (Fig. 1). Node segment collected from wild grown of *D. asper* produced varied number of multiple shoots in MS medium supplemented with PGRs such as BA (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l) and Kn alone and Ads in combination with BA (1-5 mg/l). The type and concentration of PGRs influenced the differentiation and average number of shoots per explants as well as mean length of shoots. The highest number (12.84 ± 0.44) of multiple shoots was obtained within 30 days of culture (Fig. 1b) when the initiated buds were transferred to MS liquid medium supplemented with BA (3.0 mg/l) in combination of Ads (50.0 mg/l). Different concentration of BA and Kn were used for the establishment of multiple shoot induction (Fig. 2). BA alone proved to be more effective for induction of axillary shoot buds compare to KN. Similar results were found on *Gigantochloa atroviolaceae* (Bisht *et al.,* 2010) and *D. asper* (Banerjee *et al.,* 2011). The explants did not respond well when Kn alone was used. Ads play a vital role for the mass multiplication as PGR. The simulative role of Ads in shoot multiplication has been emphasized from time to time in various plants (Dhar and Upreti 1999, Husain *et al.,* 2008). Among all PGRs used, BA (3.0 mg/l) in combination with Ads (50 mg/l) was the most effective (12.84 ± 0.44) for multiple shoot induction. Few cultures of *D. asper* were kept under same condition and in same growth medium in a growth chamber. Albino regenerants of *D. asper* were found after long term of culture in 45 days of time (Fig. 1c).

These albino regenerants were transferred to MS liquid medium supplemented with BA (1-5 mg/l) in combination with Ads (50 mg/l). The rate of multiple shoot induction of *D. asper* is significantly high with yield a cluster of highest numbers of shoots (21.40 ± 0.73) per explants (Fig 3.) with a maximum length (52.93 ± 0.67 mm) (Fig. 4). Using the method described here, we were able to obtain a maximum number of albino *D. asper* regenerants in vitro, which will serve as experimental material for future studies. Two albino mutants have been derived from long term shoot proliferation of *Bambusa edulis* by Liu *et al.,* (2007). According to them, chloroplast genome aberrations might be involved in albino shoot generartion. I*n* contrast to previous studies (Rout and Das 1994, Ho and Chang 1998), few albino-mutants were obtained among the regenerated shoots. Albino inflorescence proliferation in *Dendrocalamus latiflorus* is also reported (Lin and Chang 1998). Banerjee *et al.,* (2011) reported 14 shoots per explants when axillary buds were transferred to liquid MS medium with BA (5.0 mg/l) in combination with Ads (40 mg/l).

Fig.1 *In vitro* regeneration of *D. asper* from nodal explants



(a) Shoot bud initiation from a single node explant taken from field grown lateral branch on MS medium after 7 days (b) Multiple shoot proliferation after subculture in liquid MS medium supplemented with 3.0 mg/l BA in combination with 50.0 mg/l Ads after 30 days (c) Multiple albino shoots proliferation after 45 days (d) In vitro rooting in liquid MS supplemented with 1.0 mg/l IBA after 28 days (e) primary acclimatization of *D. asper* (f) Plantlets after secondary acclimatization in sand:soil:farmyard manure (1:1:1 v/v)

Fig.2 Effects of various concentrations of 6-benzyladenine (BA) and Kinetin (Kn) alone on percent response of *D. asper*. Values represent means ± SE of three replications

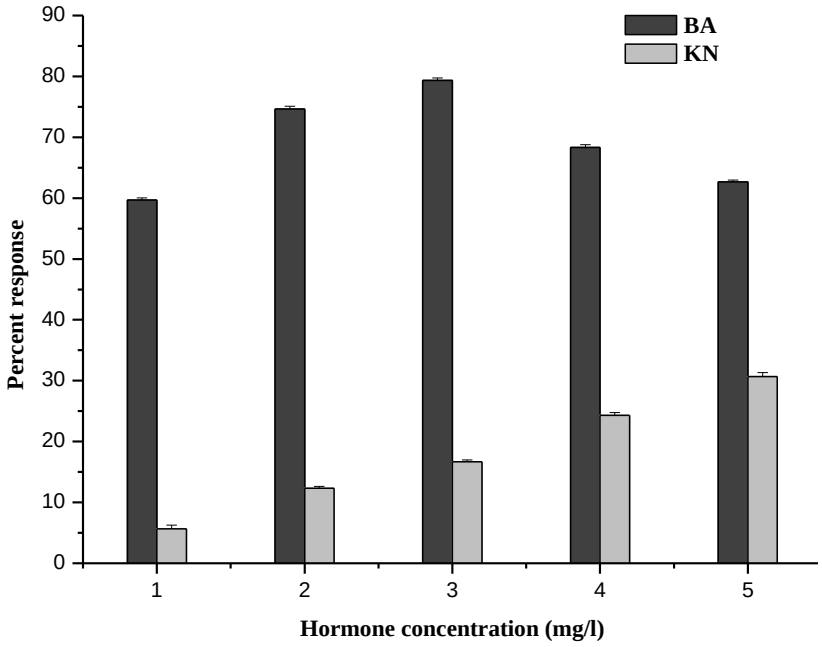


Fig.3 Effects of various concentrations of 6-benzyladenine (BA) alone, in combination with Adenine sulphate (Ads) and Kinetin (Kn) alone on number of shoots per explants of *D. asper*. Values represent means ± SE of three replications

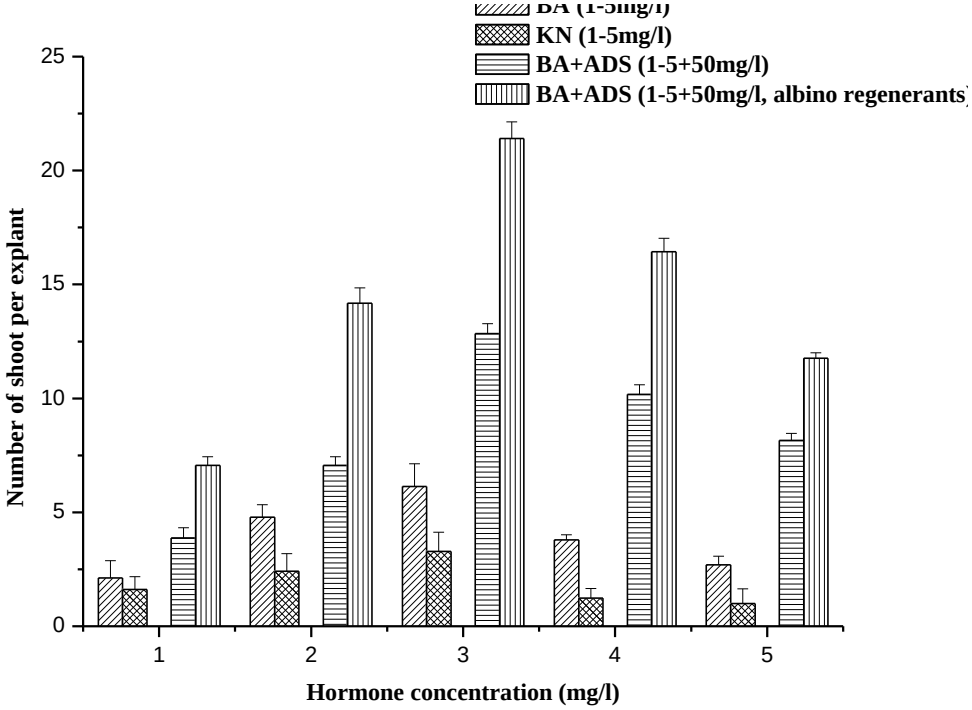


Fig.4 Effects of various concentrations of 6-benzyladenine (BA) alone, in combination with adenine sulphate (Ads) and Kinetin (Kn) alone on mean shoot length of *D. asper*. Values represent means ± SE of three replications

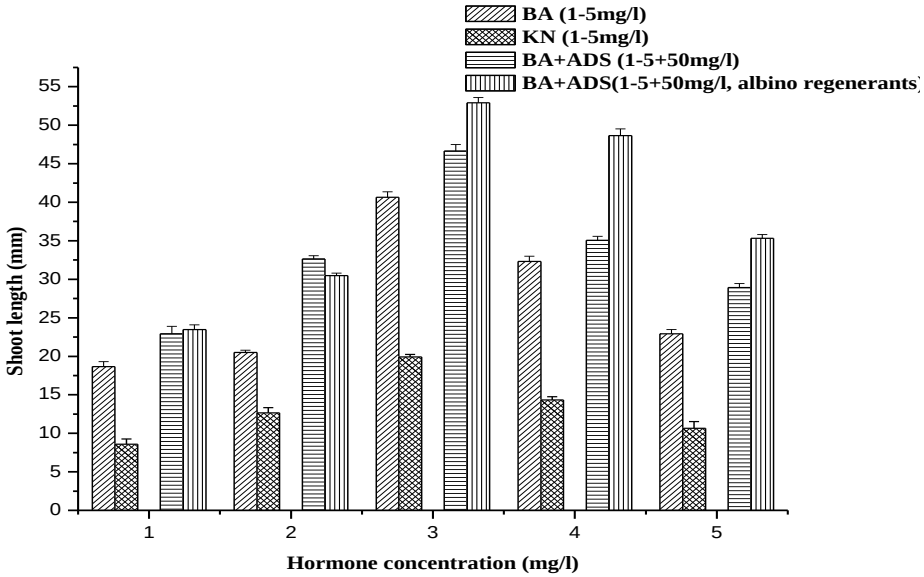
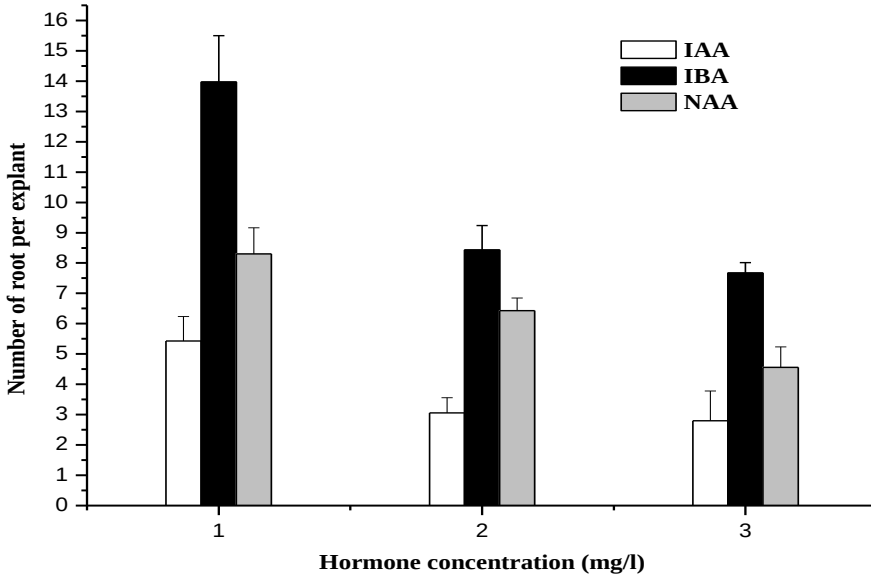


Fig.5 Effects of different auxins (IAA, IBA and NAA) on number of roots per shoot of *D. asper*. Values represent mean ± SE of three replications



But in our study decreased concentration of BA in combination with increased concentration of Ads showed maximum multiple shoot induction. Three different auxins (indole acetic acid (IAA), indole butyric acid (IBA) and napthalene acetic acid (NAA) were used to study the effect of root induction in *D. asper.* Among the three auxins tested (1.0 mg/l) IBA was found to be more effective for root induction.

The highest number of roots (13.96 ± 1.52) per shoot (Fig. 5) was produced on liquid MS medium containing (1.0 mg/l) IBA in 4 weeks of time. Maximum 7 roots per explants were observed on MS liquid medium supplemented with (1.0 mg/l) IBA in *D. asper* (Banerjee *et al.*, 2011). Similarly same concentration of IBA was found to be best for rooting in our present study. MS medium supplemented with higher concentration (10 mg/l) of IBA was found to be best (10-20) for rooting (Arya *et al.*, 2001). But in our study least concentration of (1.0 mg/l) IBA was found to be optimal for best rooting.

After 4 weeks of root induction, the plantlets with fully expanded leaflets were successfully transferred to cocopeat and hardened in green house under shade net for 15 days.

After that plantlets were transferred to poly bags containing potting mix sand: soil: farmyard manure (1:1:1 v/v) for secondary hardening. Plantlets exhibited up to 95% survival rate after 30 days. The present study is efficient protocol which can be adopted commercially for mass multiplication of edible bamboo, *D. asper*. Our protocol is simple and reliable and can be used to produce a maximum number of albino shoots of *D. asper* within a short period of time and raising them on a mass scale for plantations and forestation purposes.

The significance of this mass multiplication from albino variant regenerants studies becomes crucial as they provide maximum number of shoots and also fulfil the demand in global market of these economically important plants.

Acknowledgement

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