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Research article

Enhanced rhizome induction and fast regeneration protocol in liquid culture of *Dendrocalamus longispathus* Kurz: A single step culture

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Abstract: The present experimental study was aimed for *in-vitro* regeneration through nodal culture of *Dendrocalamus longispathus* an important bamboo species of north-eastern region. Single auxillary buds were cultured in different concentration of BAP and Kn incorporated media for bud breaking and shoot regeneration. Effect of collection period and type of explants is a major impact on bud breaking. Single step plantlet regeneration has been achieved in the liquid basal medium Murashigs and Skoogs (MS) with BAP 1.0 + Kn 1.0 shows best regeneration of 6 to 8 numbers of shoots within 3 weeks of culture. Both inoculated intact node and cut node cultures produced shoots and rhizomes during subcultures. Increased incubation period up to 11 weeks with serial sub culture produced simultaneous roots and rhizomes in the cultured media containing BAP 1.0 + Kn 1.0. Culture response of 90% healthy rooted plantlets has been established outside the lab condition. The whole experiment completed within 12 weeks of culture incubation. Good growth of established *in-vitro* plantlets in field of FRCBR, Aizwal is observed after one year.
Keywords: Tissue culture - Bamboo - Auxillary bud - Intact node - Cut node.

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INTRODUCTION

Bamboos are the tallest and largest member of the grass family having several commercial applications which are widely distributed in India and abundantly occur in northeast region. Demand for bamboo is rising in all over the world and are considered as one of the most economically important plants for their utility in handicraft industry, construction, paper making, fishery, human consumption etc. (Scurlock *et al.* 2000). *Dendrocalamus longispathus* (Kurz) is a long-sheath bamboo grows up to 20 m tall and locally known as '*rawnal*' in Mizoram (Fig. 1). Native place of the species are Bangladesh, Myanmar and Thiland and widely distributed across the South and Southeast Asia, particularly in India (northeastern state of Assam, Manipur, Meghalaya, Mizoram, Tripura and Nepal). The conventional method of propagation of bamboo through seed possesses several problems like long flowering cycle up to 120 years, poor seed set, as well as low seed viability etc. *In vitro* rhizomes are produce shoots and roots giving rise to complete plantlets act as seeds and help in the early establishment of plants in the field and culm production for both the commercial production and germplasm conservation (Kapoor & Rao 2006). Hence, considering the importance of rhizome in bamboo many researchers like Shirgurkar *et al.* (1996) observed *in vitro* rhizome formation and micropropagation in *Dendrocalamus strictus* (Roxb) Nees. *In vitro* rhizome formation was also reported in *Dendrocalmus hamiltonii* Nees & Am. ex Munro on prolonged sub-culturing of plantlets raised through somatic embryogenesis (Godbole *et al*. 2002). Rhizome formation was also induced in *Bambusa bambos* (L.) Voss by Kapoor & Rao (2006). In the present study we report an easy method of micropropagation and *in vitro* rhizome induction of *D. longispathus* in a single step on cut and uncut node cultures in the liquid basal medium of Murashigs & Skoogs (1962).

MATEIALS AND METHODS

The experimental study was conducted with the nodal segment of lateral branches collected from the

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bamboo nursery of Advanced Research Centre for Bamboo and Rattan, Aizwal, Mizoram, during the month of January to June. The average temperature of the site is 15 to 30°C, RH 84 to 91 %, rainfall 182 mm and lies between 23° 30' N to 92° 43' E. Nodal segments with axillary buds covering with leaf sheaths were carefully removed and placed under slow running tap water to wash out dust particles and prepared in larger size explants for bud breaking and initiation of culture (Fig. 2). Collected explants are washed in 2% Teepol solution and shake for 15to 20 min in 500 ml Erlenmeyer conical flask very carefully to prevent from rupturing of the tender, soft auxilary bud. The teepol solution is removed by washing with several times in tap water followed by rinse in distilled water. Surface sterilization is done with 0.1% v/v mercuric chloride solution for 7 to 8 min and washed in sterile distilled water until to remove the traces. BA has been found to be superior over other cytokinins for shoot regeneration in a number of earlier reports in different bamboo species. These sterilized nodal segments were cultured in three different basal liquid media *viz* Murashiges and Skoogs (MS), ½ strength of MS and SH (Schenk & Hildebrandt 1972) with 30 gm l^{-1} sucrose for bud breaking and shoot regeneration with BAP five different concentration (1.0, 2.0, 3.0, 4.0 and 5.0 mg l^{-1}) alone and in combination with BAP 1.0 mg l^{-1} and Kn (1.0, 2.0 and 3.0 mg l^{-1}). pH of the medium adjusted to 5.6 to 5.8 prior to autoclaving at 121°C in 15 psi for 15 min and the cultures are maintained at 25±2°C under continuous illumination of 3000 lux provided by daylight cool white fluorescent tubes maintaining 16-hr photoperiod. Proliferated 3–5 axillary shoots with intact node were subculture in the same medium. Multiplied shoots were supported by sterile filter paper bridges and shoots were transferred regularly at the interval of 7 days into fresh medium. After shoot initiation, culture incubated for 3 weeks and divided into two parts *i.e.* pre cultured uncut node left intact with differentiated shoots and pre- cultured cut node with differentiated shoots. Culture continued in the same medium and multiplied shoots were counted after 30 days to evaluate the multiplication rate and rhizome production in each culture medium. Control of browning for the species is the best methodology as to adopt for frequent sub culturing after 7 days of culture so as to maintain healthy cultures. Longer sub-culture durations usually lead to longer and pale shoots which gradually turn brown to black instead of enhancing the multiplication rate further (Mudoi & Borthakur 2009, Singh *et al.* 2012). All the experiments were repeated twice with five replicates each. Later, regenerated plants were divided into several parts and planted in the poly bags for field plantation.

Figure 1. Mature *Dendrocalamus longispathus* in natural habitat.

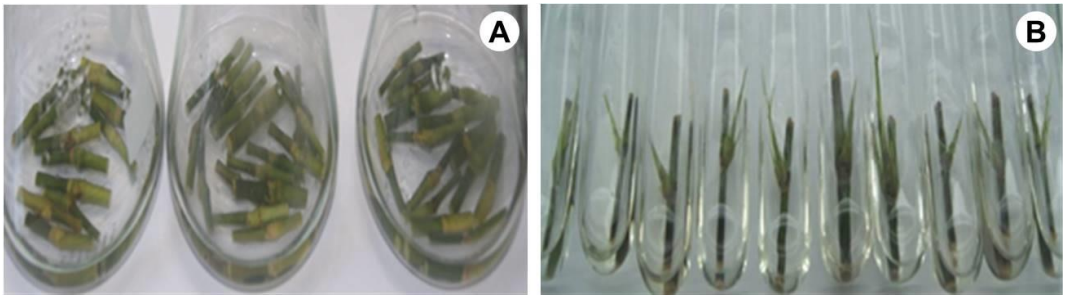

Figure 2. A, Aseptic explants before inoculation; **B,** Bud breaking and shoot initiation.

RESULTS

Figure 3. Rhizome induction and plant regeneration of *Dendrocalamus longispathus* (Kurz): **A,** Shoot multiplication; **B,** Rooting of regenerated shoots; **C,** Number rhizome buds and rooting; **D–G,** Rooted rhizome of intact node explants; **H–I,** Rooted rhizomes of cut node explants; **J,** Deflasked plantlets after 11weeks of culture in the shoot regeneration media; **K,** Hardening under shaded area; **L,** Plants after field establishment.

Results of the study reveals single step plantlet regeneration in the liquid basal medium of MS along with BAP 1.0 + Kn 1.0 with optimum 6 to 8 numbers of shoots within 3 weeks of culture in the pre-cultured node left intact. Both the explants produced rhizomes along with healthy shoots. Shoots initiated from the axillary buds of the explants, with a maximum number of shoots after a period of 30 days. Increased incubation period up to 11 weeks with serial subculture produced simultaneous roots and rhizomes in the same medium from pre-cultured cut node with differentiated shoots. Both the explants response for root initiation but differs in regeneration time

and percentage of rooting and produced rhizomes along with the regenerated shoots. All regenerated shoots were nicely green and healthy in appearance and small roots were visible from the basal part of the regenerated shoots after 14 days of culture on the shoot multiplication medium without changing any hormones. Cultures were produced 2 to 4 numbers of rhizomes from the base of the regenerated shoots and later developed into shoots. After 60 days of incubation, the shoots at the nodes were grown to 2.5 to 5.0 cm in length with roots. Rooted shoots were isolated and maintained in the same medium until fully developed plant lets about to thirty days. To minimize the level of contamination we have success in simple initial steps of surface sterilization. For acclimatization, the rooted plants were transferred to paper cups containing sterilized soil and covered with a plastic film to retain moisture. The plastic cover was gradually opened day by day during the acclimatization period 8 days. These plants were transplanted to garden pots, containing a mixture of sand and soil in 1: 1 and watered regularly. After 15 days acclimatization plantlets were established. 90% of the culture possesses healthy and plantlet establishment outside the laboratory has been completed within 12 weeks.

DISCUSSION

Considering the basal media for optimum result in the present investigation, similarly in several bamboo species MS is the most widely used medium for bud breaking as reported in *Dendrocalamus hamiltonii* (Sood *et al.* 1994, Agnihotri *et al.* 2009); *D. giganteus* Munro (Ramanayake & Yakandawala 1997); *D. asper* (Schult) Backer (Arya *et al.* 2008); *Bambusa vulgaris* Schrad (Rout & Das 1997); *B. edulis* Carrière, *Bambusa odashimae* Hutus. ex D. Z. Li & Stapleton (Lin & Chang 1998); *B. balcooa* Roxb (Das & Pal 2005, Negi & Saxena 2011) etc. Again, Singh *et al.* (2011, 2012), reported that MS was found to be as better in his study when compared with MS, B_5 (Gamborg *et al.* 1968) and NN (Nitsch & Nitsch 1969). Simultaneously the size of the explants plays an important role in the regeneration of shoots, which was supported by Anand *et al.* 2013 in *Bambusa bambos* that larger explants are more suitable than smaller one to initiate the culture within a short time because of its high endogenous hormonal effect. Some researchers also reported that nodal bud sprouting for shoot formation is generally depends on their physiology of the explants tissue, collection of time and year and culture (Saxena & Dhawan 1994, Ramanayake *et al.*1995, Ramanayake & Yakandawala 1997, Singh *et al.* 2011, 2012). In the case of present study, stems collected after February showed a low frequency of bud-break and gradually decrease to July. It was observed that the nodal segments from the upper portion of stem showed higher percentage of bud break. According to the report of Saxena & Bhojwani (1993) bud break frequency in *D. longispathus* was strongly influenced by the juvenility stem showed the higher percentage of bud break. According to the report of Saxena & Bhojwani (1993) bud break frequency in *D. longispathus* was strongly influenced by the juvenility of lateral shoots, the position of axillary bud on the branch and the season in which cultures were initiated. Similarly, our result of bud breaking was a response from (January to April) and gave the best response in terms of decreased contamination. Early shoot initiation and increased the percent of bud breaking with the higher number of shoots in *D. asper* is reported by Singh *et al.* (2011) while, Singh *et al.* (2012) reported that the early summer *i.e.* April–June was best for explants collection period and the establishment of *D. hamiltonii* with low rate of contamination. Incorporation of BAP into the medium improved the axillary bud proliferation was reported by Nadgir *et al.*(1984), Dekkers & Rao (1989), Hirimburegama & Gamage (1995) and Arya *et al.* (2006); while, Kn alone was found to be less effective by Ramanayake & Yakandawala (1997), Arya *et al.* (2006) and Singh *et al.* (2011). Synergistic effect of the two cytokinins BA and Kn was reported best for shoot multiplication in *D. giganteus* reported by Arya *et al.* (2006) and *B. bambusa, glaucescens* (Wolld.) Merr., *Bambusa multiplex* (Lour.) Raeusch. ex Schult. by Shirin & Rana (2007).

The response in the liquid medium of bamboos, higher rates of shoot multiplication and improved growth was observed by several workers (Saxena & Bhojwani 1993, Sood *et al.* 2002, Das & Pal 2005, Arya *et al.* 2006, Shirin & Rana 2007, Ogita *et al.* 2008). Alternatively, use of the liquid medium is more economical as compared to solid. Several authors reported on good growth and shoot multiplication rate in liquid medium than agar gelled such as in *Dendrocalamus hamiltonii*, *Bambusa tulda* Roxb. by Saxena (1990) and Sood *et al.* (2002); Somashekar *et al.* (2008) in *Pseudoxytenanthera stocksii* (Munro.) T.Q. Nguyen; Kabade (2009) in *Bambusa bambos* and *Dendrocalamus strictus* (Roxb.) Nees; Negi & Saxena (2011) in *Bambusa nutuns* Wall. ex Munro. This may be due to easy and faster uptake of nutrients and growth regulators from the liquid medium. Precultured cut node explants compared to decapitated *in vitro* seedlings of *Ochlandra wightii* (Munro) C.E.C. Fisch. where explants cultured onto half-strength of MS liquid supplemented with various concentrations of sucrose for the induction of *in vitro* rhizomes studied by Bejoy *et al.*(2012). In rooting, similar to our study Shirqurkar *et al.* (1996) observed in *Dendrocalamus strictus* where spontaneously during the rooting phase *in*

vitro rhizome formation when plantlets were allowed to proliferate on MS medium supplemented with the low concentration of BAP. Simultaneously, *Bambusa bambos* showed the highest multiplication that can be obtained in the medium without kinetin but the lowest rooting was observed in medium without Kn by Nayak *et al.*, (2010). Krishnamurthy *et al.* (2001) showed the longest root with the application of 0.5 mg L^{-1} BAP in *Polianthes tuberosa* L. Here, we have successfully developed efficient plant regeneration and rhizome induction in low hormonal concentration which may be a helpful study for the future course of the investigation.

CONCLUSION

Here we have successfully developed efficient plant regeneration for *Dendrocalamus longispathus* using single step culture of the nodal segment along with rooting. The findings may lead the further studies of the commercial production through liquid culture.

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