De novo organogenesis in the form of rhizome in *Dendrocalamus asper* and *D. membranaceus*

Bamboos are tall grasses restricted to Asian countries, which are mostly used by the rural people for food, housing and other domestic purposes[1]. Bamboo is commonly propagated by culm cuttings, but shoot development and root induction is limited[2]. Also, vegetative propagation narrows the genetic base and is therefore inadequate for maintaining the genetic diversity. A bamboo propagule should possess a root system, rhizome and shoot for successful growth and establishment. The rhizome is an important propagule from which new culms arise. *In vitro* germinated rhizomes produce shoots and roots, giving rise to complete plantlets and thus act as seeds[3]. Rao and Rao[4] reported rhizome formation in *Dendrocalamus strictus* in plantlets produced by somatic embryogenesis.

Shirgurkar *et al.*[5] observed *in vitro* rhizome formation in *D. strictus* when the plantlets were allowed to proliferate on Murashige and Skoog (MS) medium supplemented with 0.5 mg/l 6-benzylaminopurine (BAP). *In vitro* rhizome formation was reported in *D. hamiltonii* on prolonged sub-culturing of plantlets raised through somatic embryogenesis[6]. Rhizome formation was also induced in *Bambusa bambos* when *in vitro* multiple shoots were transferred to MS medium fortified with 2.5 μM BAP, 0.1 μM gibberellic acid (GA_3), 50 μM naphthaleneacetic acid (NAA) and 5% sucrose[3]. In this communication, we report the effect of growth regulators, and their optimal concentration for *in vitro* rhizome formation in *D. asper* and *D. membranaceus*.

In vitro multiple shoots (Figure 1 *a* and *b*) of *D. asper* and *D. membranaceus*, proliferated for 6–8 months on MS medium supplemented with 7 mg/l BAP for *D. asper* and 5 mg/l BAP for *D. membranaceus* were used for rhizome induction. The experiment was repeated thrice with five explants, bearing 5–8 shoots for each species. Multiplication rate, i.e. the number of shoots produced after subculture divided by the number of shoots inoculated was calculated. The shoot multiplication procedure was repeated several times, which resulted in high multiplication rate. The shoot multiplication rate declined if propagules of less than 3–4 shoots were used for multiplication. Healthy shoots were placed vertically in a test tube containing MS liquid medium supplemented with 3% or 5% sucrose and plant growth regulators (PGRs) in various combinations of NAA (1–3 mg/l) and indole 3-butyric acid (IBA; 1–3 mg/l). The medium was sterilized by autoclaving at 121°C for 17 min. Cultures were kept at 25 ± 2°C and exposed to artificial light 15–40 $\mu Es^{-1} m^{-2}$ with photoperiod 16/8 h. The data collected were subjected to statistical analysis to analyse Analysis of Variance (ANOVA) using SPSS version 17.00. *P*-values < 0.05 were regarded as indicating statistical significance.

Shoots of *D. asper* and *D. membranaceus* proliferated on MS basal medium supplemented with 7 and 5 mg/l BAP respectively. After 12 weeks of subculture on the same medium, the shoot number was 91.60 in *D. asper* and 56.00 in *D. membranaceus* (Table 1). Excised shoots in clusters of 5–8, placed in MS liquid medium containing different PGRs, produced roots and rhizome (Table 2).

The percentage of rhizome formation in *D. asper* was highest (100) in a medium containing 3 mg/l NAA + 2 mg/l IBA + 5% sucrose, whereas the other combinations gave values of 86.7 (3 mg/l NAA + 2 mg/l IBA + 3% sucrose), 73.33 (3 mg/l NAA + 3 mg/l IBA + 5% sucrose) and 66.67 (3 mg/l NAA + 3 mg/l IBA + 3% sucrose). In *D. membranaceus*, the percentage of rhizome formation was highest (86.67) in medium containing 3 mg/l NAA + 3 mg/l IBA + 3% sucrose, whereas the other combination (3 mg/l NAA + 2 mg/l IBA + 3% sucrose) gave 66.67. In contrast to medium supplemented with 3% sucrose, rhizome response in *D. membranaceus* was totally inhibited when 5% sucrose was used (Table 2).

Morphologically, rhizome grown horizontally in liquid MS medium (Figure 1 *e*) showed single node covered with alternating thick green and hairy scale leaves. Rhizomes then turned up to form a culm shoot. Rhizomes were separated from multiple shoots and transferred to agarized MS basal medium for both species (Figure 1 *c* and *i*) under aseptic condition. Roots were profusely formed from nodes of rhizome in MS basal medium without the help of growth regulators within two weeks. Rooted rhizomes (Figure 1 *d* and *j*) were transferred to jars to enhance further growth. Plantlets arising from the rhizomes (Figure 1 *f* and *l*) showed 80% survival rate in *D. asper* and 70% in *D. membranaceus*. In the present study, rhizome formation from multiple shoots was achieved within 4 weeks. Previous workers have used seeds[5] and caryopses[3]. The present study provides a report on rhizome induction in plantlets raised from axillary shoots. PGRs play an important role in this process.

Shirgurkar *et al.*[5] described the technique of micropropagation in *D. strictus* by using *in vitro* rhizome formation. Shoots multiplied on stationary MS liquid medium containing 0.5 mg/l BAP and 2% sucrose formed root and rhizome when transferred to MS basal medium supplemented with only 2% sucrose. Kapoor and Rao[3] have reported that using BAP and NAA singly suppressed rhizome formation in *B. bambos*, but

Table 1. Effect of 6-benzylaminopurine (BAP) concentration on shoot proliferation of *D. asper* and *D. membranaceus*

Treatment	Species	
	D. asper	*D. membranaceus*
Shoot number		
Control	$^{a}0.00 \pm 0.00$	$^{A}0 \pm 0.00$
BAP 3 mg/l	$^{a}65.00 \pm 1.58$	$^{A}30.00 \pm 10.12$
BAP 5 mg/l	$^{a}75.60 \pm 1.63$	$^{A}56.00 \pm 6.96$
BAP 7 mg/l	$^{a}91.60 \pm 2.46$	$^{A}5.20 \pm 0.97$

Value are means of five replicates ($n = 5$).
Superscripts on the left side of each column represent significant difference for treatment at < 0.05% probability level.

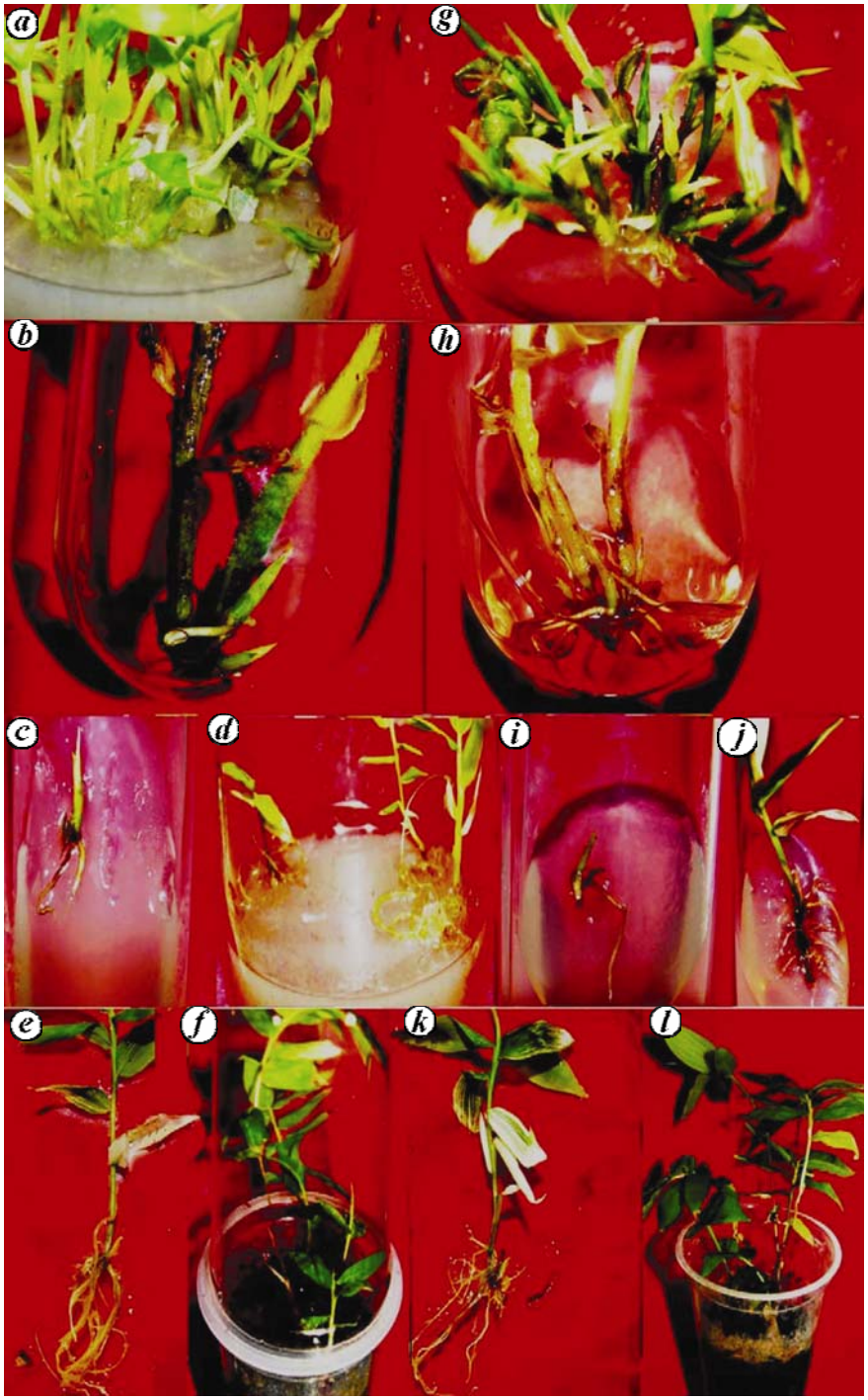

Figure 1. ***a*–*f***, *De novo* organogenesis in *Dendrocalamus asper*. ***a***, Shoot proliferation; ***b***, Rhizome formation; ***c***, Rhizome separation and growth on Murashige and Skoog basal medium; ***d***, Root and plantlet development; ***e***, Plantlet with one shoot and multiple roots, and ***f***, Plantlet acclimatization. ***g*–*l***, *De novo* organogenesis in *D. membranaceus*. ***g***, Shoot proliferation; ***h***, Rhizome formation; ***i***, Rhizome separation and growth on MS basal medium; ***j***, Root and plantlet development; ***k***, Plantlet with one shoot and multiple roots, and ***l***, Plantlet acclimatization.

incorporation of these two hormones together supported multiple shoot proliferation along with *de novo* organogenesis in the form of rhizome. *In vitro* rhizogenesis has been studied in several plant species, which has indicated that rhizogenesis and flowering are two antagonistic phenomena, preceding respective minimum and maximum peroxidase activity[7]. Ansari *et al.*[8] studied peroxidase activity in *in vitro* rhizogenesis and precocious flowering in *Bambusa arundinacea*, and found that the low peroxidase activity reflects early events, such as cell division and elongation in the sequence of *de novo* organogenesis, rather than organ-specific diffrentiation of root or flower. Further, BAP plays a complementary role in the process. Whether such biochemical changes also occur in the presently studied bamboo species is

Table 2. Effect of plant growth regulators on rhizome formation from multiple shoots and their number

Treatment	*D. asper*			*D. membranaceus*		
	Rhizome formation (%)	Rhizome number	Remarks	Rhizome formation (%)	Rhizome number	Remarks
Control (MS)	$^{a}0.00 \pm 0.00$	$^{a}0.00 \pm 0.00$	No roots formed	$^{A}0.00 \pm 0.00$	$^{A}0.00 \pm 0.00$	No roots formed
MS + 2 mg/l NAA + 1 mg/l IBA + 3% sucrose	$^{a}0.00 \pm 0.00$	$^{a}0.00 \pm 0.00$	No roots formed	$^{A}0.00 \pm 0.00$	$^{A}0.00 \pm 0.00$	No roots formed
MS + 2 mg/l NAA + 2 mg/l IBA + 3% sucrose	$^{b}33.33 \pm 6.67$	$^{b}0.73 \pm 0.177$	Roots as well as rhizome formed	$^{B}26.67 \pm 6.67$	$^{B}0.40 \pm 0.12$	Roots as well as rhizome formed
MS + 2 mg/l NAA + 3 mg/l IBA + 3% sucrose	$^{a}0.00 \pm 0.00$	$^{a}0.00 \pm 0.00$	Only roots were formed	$^{A}0.00 \pm 0.00$	$^{A}0.00 \pm 0.00$	Only roots were formed
MS + 3 mg/l NAA + 1 mg/l IBA + 3% sucrose	$^{a}0.00 \pm 0.00$	$^{a}0.00 \pm 0.00$	Only roots were formed	$^{A}0.00 \pm 0.00$	$^{A}0.00 \pm 0.00$	Only roots were formed
MS + 3 mg/l NAA + 2 mg/l IBA + 3% sucrose	$^{c}86.67 \pm 6.67$	$^{c}2.33 \pm 0.24$	Rhizome only	$^{C}66.67 \pm 6.67$	$^{C}1.27 \pm 0.18$	Rhizome only
MS + 3 mg/l NAA + 3 mg/l IBA + 3% sucrose	$^{dh}100.00 \pm 0.00$	$^{dg}4.47 \pm 0.67$	Rhizome only	$^{D}86.67 \pm 6.67$	$^{D}3.47 \pm 0.77$	Rhizome only
MS + 2 mg/l NAA + 1 mg/l IBA + 5% sucrose	$^{a}0.00 \pm 0.00$	$^{a}0.00 \pm 0.00$	No roots formed	$^{A}0.00 \pm 0.00$	$^{A}0.00 \pm 0.00$	Only roots were formed
MS + 2 mg/l NAA + 2 mg/l IBA + 5% sucrose	$^{eb}46.67 \pm 6.67$	$^{ebh}0.67 \pm 0.67$	Rhizome only	$^{A}0.00 \pm 0.00$	$^{A}0.00 \pm 0.00$	Rhizome only
MS + 2 mg/l NAA + IBA 3 mg/l + 5% sucrose	$^{a}0.00 \pm 0.00$	$^{a}0.00 \pm 0.00$	Only roots were formed	$^{A}0.00 \pm 0.00$	$^{A}0.00 \pm 0.00$	Only roots were formed
MS + 3 mg/l NAA + 1 mg/l IBA + 5% sucrose	$^{a}0.0000 \pm 0.00$	$^{a}0.00 \pm 0.00$	No roots formed	$^{A}0.00 \pm 0.00$	$^{A}0.00 \pm 0.00$	No roots formed
MS + 3 mg/l NAA + 2 mg/l IBA + 5% sucrose	$^{fci}66.67 \pm 6.67$	$^{ei}1.60 \pm 0.12$	Rhizome only	$^{A}0.00 \pm 0.00$	$^{A}0.00 \pm 0.00$	No roots formed
MS + 3 mg/l NAA + 3 mg/l IBA + 5% sucrose	$^{gcjf}73.33 \pm 6.67$	$^{fde}1.87 \pm 0.24$	Rhizome only	$^{A}0.00 \pm 0.00$	$^{A}0.00 \pm 0.00$	No roots formed

Superscripts on the left side of each column represent significant difference for treatments at < 0.05 level.
MS, Murashige and Skoog medium; NAA, Naphthaleneacetic acid; IBA, Indole 3-butyric acid.

yet to be ascertained. In date palm (*Phoenix dactylifera*), rhizogenesis and callogenesis were studied; it was found that these two pathways are initiated from the same cell type, the ultimate developmental fate depending upon auxin concentration[9]. A study on the effect of growth regulators and rhizogenesis of *Dianthus henteri*, a Romanian endemic species was carried out[10]. The best results of rhizogenesis were obtained in a medium containing 1 mg/l NAA and 0.1 mg/l BAP. In the present study, we found that NAA and IBA when used in combination are most efficient in rhizome induction in both species. The method of rhizome induction in *D. asper* and *D. membranaceus* described in this study has potential for mass-scale production of these two commercially important bamboos, as *in vitro* rhizome development ensures high survival rate and early establishment in the field.

1. Ohrnberger, D., *The Bamboos of the World*, Elsevier, Amsterdam, 1999.
2. Surendran, T. and Seethalakshmi, K. K., *KFRI Res. Rep.*, 1985, **30**, 47.
3. Kapoor, P. and Rao, U., *Plant Cell Tissue Organ Cult.*, 2006, **85**, 211–217.
4. Rao, I. V. R. and Rao, I. U., In Proceedings of the International Bamboo Workshop, 14–18 November 1988, Cochin (eds Rao, I. V. R., Gnanaharan, R. and Sastry, C. B.), Kerala Forest Research Institute, Peechi and IDRC, Canada, 1990.
5. Shirgurkar, M. V., Thengane, S. R., Poonawala, I. S., Jana, M. M., Nadgauda, R. S. and Mascarenhas, A. F., *Curr. Sci.*, 1996, **70**, 940–942.
6. Godbole, S., Sood, A., Thakur, R., Sharma, M. and Ahuja, P. S., *Curr. Sci.*, 2002, **83**(7), 885–889.
7. Gaspar, T., In *Aspect and Prospects of Plant Growth Regulators, Monograph 6* (ed. Jeffecoat, B.), British Plant Growth Regulators Group, Wantage, 1981, pp. 39–49.
8. Ansari, S. A., Kumar, S. and Palanisamy, K., *Curr. Sci.*, 1996, **71**(5), 358–359.
9. Gueye, B. *et al.*, *Plant Cell Tissue Organ Cult.*, 2009, **98**, 47–58.
10. Cristea, V., Brummer, A., Jarda, L. and Miclăus, M., *Rom. Biotechnol. Lett.*, 2010, **15**(1), 25–33.

Received 17 August 2009; revised accepted 2 December 2010

C. NIRMALA*
ABDULMINAM HUSSIEN ALI
T. BADAL

Department of Botany,
Panjab University,
Chandigarh 160 014, India
**For correspondence.*
e-mail: cnirmala10@yahoo.com