



©Biotechnology Society



[www.bti.org.in](http://www.bti.org.in)  
ISSN 0974-1453  
Research Article

---

## **RAPID *IN VITRO* PROPAGATION OF THE SOLID BAMBOO, DENDROCALAMUS STRICTUS NEES, THROUGH AXILLARY SHOOT PROLIFERATION**

Shatakshi Kapruwan, Meena Bakshi, and Manpreet Kaur\*  
Forest Botany Division, Forest Research Institute Deemed University, Dehradun  
\*Corresponding author: [manpreetchinna@gmail.com](mailto:manpreetchinna@gmail.com)

### **ABSTRACT**

*Dendrocalamus strictus*, also known as Solid Bamboo or Calcutta Bamboo is a native to Southeast Asia. The demand of *D. strictus* is increasing day by day as it contains 85% cellulose and used for the paper and pulp industry. It is also drought tolerant bamboo so it is extremely suitable for low rainfall areas and soil. We have developed an accelerated protocol for large-scale propagation of *D. strictus*. For initiation of aseptic cultures various concentrations of mercuric chloride for different time period was studied and it was found that 0.2%  $\text{HgCl}_2$  was found to be effective. The axillary shoots of *D. strictus* containing single axillary bud were inoculated in semisolid Murashige and Skoog (MS) medium fortified with different concentration of growth regulator, 6-benzylaminopurine (BAP). Maximum bud break and multiple shoot formation were observed in 5mg/l BAP. A maximum of 100 % shoots were effectively rooted when transferred to liquid MS medium supplemented with 2.5mg/l 6-benzylaminopurine (BAP) and 5 mg/l 5-indole acetic acid (IAA). Effect of Phenyl-N'-(1, 2, 3-thiadiazol-5-yl) (Thidiazuron/TDZ) was also studied. The maximum bud break and multiple shoot formation were observed in MS media supplemented with 4mg/ l BAP and 0.25mg/ l TDZ which was greater than the resulted obtained in MS media having 5mg/l BAP only. The increase in TDZ concentration had a negative impact on bud break and multiple shoot formation.

**Key words:** *D. strictus*, axillary shoots, MS media, growth regulator, TDZ

## INTRODUCTION

Bamboo grows naturally in many types of forests and also cultivated in many areas of India. About 50% of the annual production of bamboo in our country is used by various industries like pulp, paper, rayon, mat boards, besides agricultural implements. It is also used for making baskets, bridges, coffins, beds, toys and weapons (Reddy, 2006). The leaves of bamboo have good quality of forage and are used as an alternative to the traditional sources to fulfill the demand of ever increasing cattle population. The total digestive nutrients and digestive crude protein in the leaves of *Dendrocalamus strictus* and *Bambusa arundinacea* are worked out to be 48.9% and 9.3%, and 46.5% and 13.5% respectively (Chobey and Sharma, 2011). Estimates regarding future use of bamboo also indicate that there will be a huge shortage of bamboo planting material in long terms (Subramaniam, 1994; Nadgauda *et al.*, 1997). The main species used for papermaking in India is *Dendrocalamus strictus* (Singh *et al.*, 1991; Saxena and Dhawan, 1999). In different parts of North-east India, the young shoots of *D. strictus* also used for eating purpose because of its high nutritive values (Goyal *et al.*, 2010). It is assumed that bamboo leaves provide the medicinal benefits and help to get rid of certain diseases due to its antioxidant capacity (Mee *et al.*, 2001). Leaf powder has cut and wound healing property (Mahapatra *et al.*, 2008). According to literature, in some places, the leaf decoction alone is used as abortifacient (Sharma and Borthakur, 2008) and when mixed with turmeric powder (*Curcuma longa*) is used to treat cold, cough and fever (Kamble *et al.*,

2010). *D. strictus* has long and erratic flowering cycle (30–45 years) that's why the regeneration through seeds is quite difficult. In addition, as in other bamboos, there are several other factors that restrict large-scale multiplication of this species through sexual or vegetative means (Saxena and Bhojwani, 1993). To overcome these problems and fulfill the required demand it has necessitated restoring the productivity of plants by the use of plant tissue culture (Bhattacharjee, 2006), as *in vitro* micropropagation includes the rapid vegetative multiplication of valuable plant material for agriculture and forestry.

Micropropagation is the process of vegetative growth and multiplication from plants tissues or seeds. It is carried out in aseptic and favourable conditions on growth media, using various plant tissue culture techniques. Since in last few decades many researchers and companies around the world have performed research to develop efficient micropropagation technology for tropical and temperate bamboos. (Alexander and Rao, 1968) were the first to germinate the seeds in test tubes for bamboo tissue culture. In 20th century successful protocols had been worked out based on somatic embryogenesis of tropical bamboos from seeds of *Dendrocalamus strictus* and *Bambusa bambos* (Metha *et al.*, 1982). For the same species propagation by axillary branching was successful (Nadgir *et al.*, 1984). In the present investigation we have established a rapid high regeneration protocol for *D. strictus* that could be used in future to enrich the plywood industry.

## MATERIAL AND METHODS:

### Explants and culture initiation

The explants used to initiate aseptic cultures were axillary shoots, 2.5 to 3.0 cm in length. They were collected from Plant Physiology lab, FRI Dehradun, India. The actively growing shoots were collected and nodal segments were prepared with the help of sharp secateurs and washed thoroughly under running tap water to remove the dust particles followed by washing in Tween 20 solution for 5 min and then pre-sterilized in a mixture of fungicides viz. 1% (w/v) Bavistin and 1% (w/v) Blitox for 15 min. Then after, they were disinfected for 10 to 15 min in 0.1% or 0.2% (w/v) mercuric chloride for getting best aseptic cultures and washed in sterile distilled water four to five times. The sterilized explants were subsequently cultured singly in culture tubes (25x150 mm) containing shoot initiation medium, that is, 15 ml of MS (Murashige and Skoog, 1962) medium supplemented with various combinations of BAP and auxins.

#### **Culture condition**

Murashige and Skoog (MS) basal medium (consisting of salts, vitamins) was used for inoculation. Different plant growth regulators (PGRs) viz. BAP, and NAA were added at various concentrations to MS medium before the pH of the medium was adjusted to 5.6. Media were autoclaved at  $1.06 \text{ kg cm}^{-2}$  and  $121^\circ \text{C}$  for 15 min. Cultures at all growth stages were incubated under artificial conditions:  $25 \pm 2^\circ \text{C}$ , 60% RH and a 16 h photoperiod (using white fluorescent tubes) under a photosynthetic photon flux density of  $40 \mu \text{mol m}^{-2} \text{s}^{-1}$ .

#### **Axillary shoot proliferation and multiplication**

The explants were inoculated in solid MS medium with different concentrations (1-5mg/ l) of BAP along with NAA (0.5mg/ l) and also with

different concentrations of TDZ (0.25 & 0.5mg/ l) in tissue culture tubes. A minimum of 24 replicates were maintained for each treatment. Within 10 days, buds were induced in incubated cultures. Elongated axillary buds after 10 days were separated and cultured for multiple shoot proliferation on the same medium.

#### ***In vitro* rhizogenesis**

Roots were induced during multiplication when media were supplemented with auxin Indol-3-acetic acid (IAA 5mg/ l). The plantlets with well developed root system were advanced for acclimatization.

#### **Hardening and field transfer of plantlets**

Rooted shoots from cultures were taken out from the flasks carefully, without causing any damage to the root system, and the roots were gently washed under running tap water. The plants with washed and cleaned root systems were transferred to autoclaved vermiculite. These plantlets were supplied with half-strength MS medium (without organics) once a week. After two weeks, the pots were shifted to a mist-chamber with a relative humidity of 80 to 90% and a temperature of  $30 \pm 2^\circ \text{C}$ . The polythene covers were removed and the plantlets were allowed to remain in the pots for 3 to 4 days before they were transferred to pots containing a mixture of sand, farmyard manure and soil in a ratio 1:1:1 (by volume).

#### **Results:**

##### **Culture initiation**

The sterilization procedures used yielded 40% or 50% aseptic cultures by giving 10 or 15 min 0.1% mercuric chloride treatment. An increase in the concentration of mercuric chloride (0.2%) at the duration of the sterilization treatment (10 or 15 min) helped to obtain a higher frequency of aseptic cultures. Treatment of

mercuric chloride 0.2% for 10 and 15 minutes yielded 60% and 70% aseptic cultures respectively (Table. 1).

### **Bud break and development of lateral shoots**

The different concentration of BAP has different impact on bud break. At 1mg/ l concentration of BAP only 50 % of the explants sprouted within 6 days, while lowest sprouting rates (40%) within 9 days were obtained with 3 mg/ l. A further increase in the concentration of BAP caused an increment in bud sprouting. Maximum bud sprouting 80% was recorded at BAP 5mg/ l within 7 days (Fig. 1b). The media supplemented with 0.25 and 0.5 mg/ l TDZ has 70% bud sprouting. Maximum bud sprouting 80% was recorded at BAP 4 mg/ l with TDZ 0.25 mg/ l within 4 days (Fig. 1a), whereas increase in the concentration of TDZ at 0.5mg/ l has 50% bud sprouting within 6 days in best combination of bud sprouting (4mg/l BAP and TDZ 0.25mg/l), firstly, there was an increase in bud volume followed by development of sheaths (starting usually 4 d after culture initiation). After 15 days in culture, the growing axes reached 3 cm in height. Development of lateral shoots started after 15 days in culture (Fig. 2a & 2b). When the growing axes reached 8 cm in height (approximately at day 30) and continue growing with the lateral shoots.

### **Plant multiplication**

The same combination of BAP, TDZ and BAP alone were then evaluated for further plant multiplication on explants. The effect of the BAP concentration on formation of new shoots was evaluated and there was a slight positive linear correlation i.e., higher BAP concentrations induced formation of more lateral shoots (Fig. 3), up to the highest concentration

tested (5 mg/ l). The results showed that the mean shoot number in 1mg/ l BAP was  $3.80 \pm 0.74$  which reduced to  $2.75 \pm 0.82$  at 3mg/ l BAP where in single BAP treatment the 5mg/ l was found best as it was having  $5.12 \pm 0.92$  number of shoots (Table 2). TDZ alone has positive effect on multiplication as the number of shoots increased with increase in TDZ concentration from 0.25mg/ l to 5.0mg/ l whereas in combination of 4mg/ l BAP with 0.25mg/ l TDZ maximum shoot number i.e.  $6.12 \pm 0.78$  was observed which decline to  $4.80 \pm 1.16$  when the TDZ concentration was raised to 0.5mg/ l. The maximum shoot length  $6.87 \pm 1.61$  cm was also measured in the same combination i.e. 4mg/ l BAP and 0.25 mg/ l TDZ. The shoot length  $5.87 \pm 0.78$  cm was observed in 5mg/ l BAP (Table. 3).

### **Rooting and acclimatization**

When the media supplemented with 2.5mg/ l 6-benzylaminopurine (BAP) and 5 mg/ l 5-indole acetic acid (IAA), rooting occurred spontaneously in 100% of the explants that developed lateral shoots (Fig. 4). From approximately 30d after culture initiation, a continuous and exponential development of new roots was observed (Figs. 4). The well-developed root explants were transferred to pot filled with sterilised vermiculite pre-soaked with half strength MS medium (sucrose free). After two weeks they were transferred to pots containing a mixture of sand, farmyard manure and soil in a ratio 1:1:1 (Fig. 5).

### **DISCUSSION:**

Establishment of aseptic culture is priority requirement in micro propagation. Commonly Mercuric chloride, ethanol and commercial bleach are used for control of contamination (Huarte and Garcia, 2009; Liu *et al.*, 2011). We have used 0.1% and

0.2% HgCl<sub>2</sub> for 10-15 minutes for standardisation of initiation of aseptic culture. For *in vitro* shoot induction MS medium was used. The use of MS basal medium for micro propagation of *D. strictus* has been reported in earlier reports (Ravikumar *et al.*, 1998; Saxena and Dhawan, 1998; Reddy, 2006; Pandey and Singh, 2012).

For micropropagation most of the workers have used nodal segments from seedlings (Nadgir *et al.*, 1984; Saxena, 1990; Das and Rout, 1991; Ansari *et al.*, 1996; Maity and Ghosh, 1997; Yashoda *et al.*, 1997). Seedling explant was also used for multiple shoot induction in *D. brandisii* (Vongvijitra, 1988); *B. tulda* (Saxena, 1990); *Thamnocalamus spathiflorus* (Zamora, 1994); *D. strictus* (Shirgurkar *et al.*, 1996); *B. nutans* and *D. membranaceus* (Yasodha *et al.*, 1997) and *D. hamiltonii* (Sood *et al.*, 2002).

The effect of growth hormones on bud breaks and shoots multiplication in bamboos including *D. strictus* has been studied by different scientists. Induction of shoot proliferation in several bamboos viz. *B. arundinacea* and *Bambusa vulgaris* (Nadgir *et al.*, 1984); in *B. arundinacea* (Nadguada *et al.*, 1990); in *B. tulda* (Saxena, 1990) ; *Dendrocalamus hamiltonii* (Chambers *et al.*, 1991); in *B. nutans* and *D. membranaceus* (Yasodha *et al.*, 1997); in *D. Strictus* (Nadgir *et al.*, 1984; Nadguada *et al.*, 1990; Ravikumar *et al.*, 1998; Maity and Ghosh, 1997); in *D. asper* (Arya *et al.*, 1999, Banerjee *et al.*, 2011) and *Guadua angustifolia* (Jimenez *et al.*, 2006) using cytokinins, especially 6-benzylaminopurine (BAP) either singly or in combination with auxin and complex additives has been reported. Moreover, half strength of MS with BAP was responsible for the same in *D. strictus*

(Reddy, 2006). We have examined the effect of different concentration of BAP (1-5mg/ l) on bud break and shoot proliferation.

In our experiment, we have applied two different concentrations of TDZ alone and in combination with BAP and observed that the MS media supplemented with TDZ showed better results. Thidiazuron (TDZ) is a cytokinin and it has been used in micro propagation since 1982 (Mok *et al.*, 1982). It was also found suitable in previous studies on *D. strictus* (Singh *et al.*, 2000).

#### ACKNOWLEDGEMENT:

The authors are thankful to Department of Science and Technology, Government of India, for providing financial assistance the under DST INSPIRE Fellowship Scheme. We are also thankful to the Director, Forest Research Institute Deemed University, Dehradun for providing Laboratory and Infrastructure.

#### REFERENCES:

- Alexander, M. P., Rao, T.C. (1968). *In vitro* culture of bamboo embryo. Current Science. 415, 37.
- Ansari, S.A., Kumar, S., Palaniswamy, K. (1996). Peroxidase activity in relation to *in vitro* rhizogenesis and precocious flowering in bamboos. Current Science. 71,358-359.
- Arya, S., Sharma, S., Kaur, R., Arya, I.D. (1999). Micropropagation of *Dendrocalamus asper* by shoot proliferation using seeds. Plant Cell Rep. 18,879-882.
- Banerjee, M., Gantait, S., Pramanik, B.R. (2011). A two step method for accelerated mass propagation of *Dendrocalamus asper* and their

- evaluation in field. *Physiol Mol Biol Plants*. 17(4),387–393.
- Bhattacharjee, S.K. (2006). *Advances in Ornamental Horticulture*. Pointer, Jaipur. 6, 2065.
- Chambers, S.M., Heuch, J.H.R., Pirrie, A. (1991). Micropropagation and *in vitro* flowering of bamboo *Dendrocalamus hamiltonii* Munro. *Plant Cell Tiss. Organ Cult.* 27,45-48.
- Chobey, O.P., Sharma, A.K. (2011). Capacity Building- Cultivation of bamboo to Enterprise development. National conference on Cluster Development of Bamboo Products – a way to inclusive growth. pp1-8
- Das, M. and Pal, A. (2005). *In vitro* regeneration of *Bambusa balcooa* Roxb.: factors affecting changes of morphogenetic competence in the axillary buds. *Plant Cell Tiss. Organ Cult.* 81,109-112.
- Das, P. and Rout, G. R. (1991). Mass multiplication and flowering of bamboo *in vitro*. *Orissa J. Hort.* 19, 118-121.
- Goyal, A.K., Middha, S.K., Usha, T., Chatterjee, S., Bothra, A.K., Nagaveni, M.B., Sen, A. (2010). Bamboo-infoline: A database for North Bengal bamboo's. *Bioinformation*. 5(4),84-5.
- Huarte, R., García, M. D. (2009). *Tripsacum dactyloides* (L.) L. germination responses to hydrogen peroxide, fluridone, gibberelic acid and abscisic acid. *Seed Science & Technology*. 37:544-553.
- Jiménez VM, Castillo J, Tavares E, Guevara E, Montiel M (2006). *In vitro* propagation of the neotropical giant bamboo, *Guadua angustifolia* Kunth, through axillary shoot proliferation. *Plant Cell Tiss. Organ Cult.* 86,389-395.
- Kamble, S.Y., Patil, S.R., Sawant, P.S., Sawant, S., Pawar, S.G., Singh, E.A. (2010). Studies on plant used in traditional medicine by Bhilla tribe of Maharashtra. *Indian Journal of Traditional Knowledge*. 9(3),591-8.
- Liu, D., Yu, H.L., Li, F.L., Guo, H.H. (2011). An analysis of dormancy and dormancy release in *Taxus chinensis* var *mairei* seeds. *Seed science and technology*. 39, 29.
- Maity, S., Ghosh, A. (1997). Efficient plant regeneration from seeds and nodal segments of *Dendrocalamus strictus* using *in vitro* technique. *Indian Forester*. 123,313-318.
- Mee-Hyang, K., Han-Joon, H., Ha-Chin, S. (2001). Identification and Antioxidant Activity of Novel Chlorogenic Acid Derivatives from Bamboo (*Phyllostachys edulis*). *J Agric Food Chem*. 49,4646-55.
- Mehta, U., Ramanuja Rao, I.V., Mohan, R.H.Y. (1982). Somatic embryogenesis in bamboo. In: Fujiwara A (ed) *Plant tissue culture Japanese Association of Plant Tissue Culture*, Tokyo, pp 109–110.
- Mohapatra, S.P., Prusty, G.P., Sahoo, H.P. (2008). Ethnomedicinal observations among forest dwellers of the Daitari Range of hills of Orissa India. *Ethanobotanical leaflets*. 12,1116-23.

- Mok, Mc, Mok, D.W.S., Armastrong, D.J., Shudo, K., Isogoi, Y., Okamoto T. (1982). Cytokinin activity of N-phenyl-N'-1,2,3-thiadiazol-5-ylurea (thidiazuron). *Phytochemistry*, 21, 1509-1511.
- Nadgauda, R.S., John, C.K., Joshi, M.S., Parasharami, V.A., Mascarenhas, A.F. (1997). A comparison of in vitro with in vivo flowering in bamboo: *Bambusa arundinacea*. *Plant Cell Tiss. Organ Cult.* 48,181-188.
- Nadgauda, R.S., Parasharami, V.A., Mascarenhas, A.F. (1990). Precocious flowering and seeding behaviour in tissue-cultured bamboos. *Nature*. 344,335-336.
- Nadgir, A.L., Phadke, C.H., Gupta, P.K., Parsharami, V.A., Nair, S., Mascarenhas, A.F. (1984). Rapid multiplication of bamboo by tissue culture. *Silvae Gene*. 33,219-223.
- Pandey, B.N., Singh, N.B. (2012). Micropropagation of *Dendrocalamus strictus* nees from mature nodal explants. *Journal of Applied and Natural Science*. 4 (1), 5-9.
- Ravikumar, R., Ananthakrishnan, G., Kathiravan, K., Ganapathi, A. (1998). In vitro shoot propagation of *Dendrocalamus strictus* Nees. *Plant Cell Tiss. Organ Cult.* 52,189-192.
- Reddy, G. M. (2006). Clonal propagation of bamboo (*Dendrocalamus strictus*). *Current Science*. 11, 1462-1464.
- Saxena, S., Bhojwani, S.S. (1993). In vitro clonal multiplication of 4- year-old plants of the bamboo, *Dendrocalamus longispathus* Kurz. *In Vitro Cell Dev Biol* 29,135-142.
- Saxena, S. (1990). In vitro propagation of the bamboo (*Bambusa tulda* Roxb.) through shoot proliferation. *Plant Cell Rep.* 9,431-434.
- Saxena, S., Dhawan, V. (1999). Regeneration and large-scale propagation of bamboo (*Dendrocalamus strictus* Nees) through somatic embryogenesis. *Plant Cell Rep.* 18,438-3.
- Sharma, T.P., Borthakur, S.K. (2008). Ethnobotanical observations on bamboos of Adi tribes in Arunachal Pradesh. *Indian Journal of Traditional Knowledge*. 7(4),594-7.
- Shirgurkar, M.V., Thengane, S.R., Poonawala, I.S., Jana, M.M., Nadgauda, R.S., Mascarenhas, A.F. (1996). A simple in vitro method of propagation and rhizome formation in *Dendrocalamus strictus* Nees. *Current Science*. 70,940-943.
- Singh, S.V., Rai, A.K., Dhawan, R. (1991). Advances in pulp and paper research in India. *ICFRE* 15.
- Singh, M., Jaiswal, U., Jaiswal, V.S. (2000). Thidiazuron - induced in vitro flowering in *Dendrocalamus strictus* Nees. *Current Science*. 79,1529-1530.
- Sood, A., Ahuja, P.S., Sharma, O.P., Godbole, S. (2002). In vitro protocols and field performance of elites of an important bamboo *Dendrocalamus hamiltonii* Nees et Arn. Ex. Munro. *Plant Cell Tiss. Organ Cult.* 71,55-63.



Subramaniam, K.N. (1994). Bamboo-demand and supply of planting stock. INBAR Newslett. 5,24-25.

T. Murashige and F. Skoog.(1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15,473-497.

Vongvijitra, R. (1988). Traditional vegetative propagation and tissue culture of some Thai bamboos. In: I.V.R. Rao, R. Gnanaharan, C.B. Shastri (eds) Bamboos Current Research, Proc. Intl. Bamboo Workshop Cochin, India. pp 159-166.

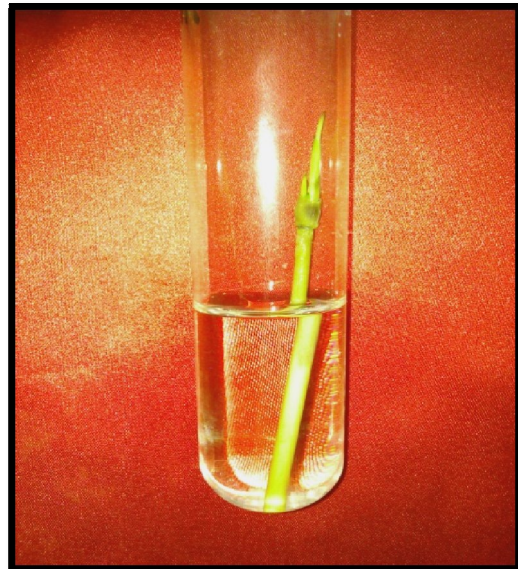
Yashoda, R., Sumathi, R., Malliga, P. and Gurumurthi, G.(1997). Genetic

enhancement and mass production of quality propagules of *Bambusa nutans* and *Dendrocalamus membranaceus*. Ind For., 4: 303-306.

Zamora, A.B. (1994). Review of micropropagation research on bamboos. In: Constrains to Production of Bamboo and Rattan, INBAR Technical Report No. 5, New Delhi, International Network for Bamboo and Rattan. pp. 45-1000.



(a) In 0.25(mg/l) TDZ+ 4.0(mg/l) BAP



(b). In 5.0(mg/l) BAP

**Fig 1. Bud Induction**





(a) In 0.25(mg/l) TDZ+ 4.0(mg/l) BAP



(b) In 5.0(mg/l) BAP

**Fig 2. Shoot proliferation:**



**Fig. 3 (a & b) Subculturing after 3-4 weeks of bud proliferation.**



**Fig 4. (a&b) Rooting in *D. strictus* using 5mg/l IAA**



**(a) *In vitro* raised plants in sterilised vermiculite (b) *In vitro* raised plant in pot**  
**Fig 5. Hardening and Acclimatization**

**Table 1: Effect of sterilants on explants and bud break in *D. strictus***

S. no.	Treatment HgCl <sub>2</sub> concentration	Time (minutes)	No. of explants survived (%)	No. of explants contaminated (%)
1.	0.1%	10	40	60
2.	0.1%	15	50	50
3.	0.2%	10	60	40
4.	<b>0.2%</b>	<b>15</b>	<b>70</b>	<b>30</b>

**Table 2: Effect of BAP concentration in MS medium on *in vitro* axillary bud and shoot proliferation from explants of *D. strictus***

S. No	BAP (mg/l)	NAA (mg/l)	Response %	Mean shoot number	Mean shoot length(cm)
1	1 mg/l	-	50	3.80±0.74	3.60±0.80
2	3mg/l	-	40	2.75±0.82	3.0±0.71
3	4 mg/l	-	70	4.28±1.03	5.14±0.83
4	<b>5 mg/l</b>	-	<b>80</b>	<b>5.12±0.92</b>	<b>5.87±0.78</b>
5	4 mg/l	0.5 mg/l	60	4.03±0.12	3.0±75

Data represent mean±SE of 20 replicates per treatment in three repeated experiments

**Table 3: Effect of TDZ concentration in MS medium on *in vitro* axillary bud and shoot proliferation from explants of *D. strictus***

S.No	BAP (mg/l)	TDZ (mg/l)	Response %	Mean shoot number	Mean shoot length(cm)
1	<b>4 mg/l</b>	<b>0.25</b>	<b>80</b>	<b>6.12±0.78</b>	<b>6.87±1.61</b>
2	4 mg/l	0.5	50	4.80±1.16	4.80±0.74
3	-	0.25	70	5.0±0.92	5.14±0.98
4	-	0.5	70	5.62±0.99	5.71±0.69

Data represent mean±SE of 20 replicates per treatment in three repeated experiments