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Thidiazuron-induced Shoot Multiplication and Plant Regeneration in Bamboo (*Dendrocalamus strictus* Nees)

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Thidiazuron (TDZ) stimulated shoot proliferation from different seedling explants (i.e., shoot, basal node, node and apical segment) of bamboo *(Dendrocalamus strictus)* when incorporated in half-strength Murashige and Skoog (MS) medium having 2% (w/v) sucrose. All the concentrations of TDZ (0.01 to 1.0 mg l^{-1}) tried were effective in shoot proliferation. Maximum shoots (14.8 ± 1.0) were obtained from the shoot explants cultured in 0.5 mg l^{-1} TDZ supplemented half-strength MS liquid medium for 21 days and subsequently transferred to the same medium devoid of TDZ. The longer culture period (i.e. 28 and 35 days) in TDZ medium caused reduction in shoot proliferation. The shoots regenerated with lower concentrations of TDZ treatment (i.e. 0.01 to 0.1 mg l^{-1}) rooted in half-strength MS liquid medium. The shoots formed with 0.5 mg l^{-1} TDZ treatment did not root in basal medium and required auxin supplementation in the medium for rooting and about 55% shoots produced roots in 1.0 mg l^{-1} IBA supplemented medium. The shoots formed with 1.0 mg l^{-1} TDZ did not root even after auxin treatment. The well rooted shoots transplanted to plastic pots filled with sand and garden soil (1:1) mixture showed 98% establishment.

Key words : *Dendrocalamus strictus*, bamboo, seedling explants, shoot proliferation, rooting, vitrification.

Amongst several economically important forest trees, bamboos have a long history of traditional use and a wide spectrum of applications in daily life. However, their main use is in the paper and pulp industry. Bamboos *(Dendrocalamus strictus* and *Bambusa arundinacea)* are the principal raw materials for paper and pulp industry in India (1). Extensive use of bamboo resources has led to a decline in natural bamboo cover and created a wide gap between demand and supply. Thus, to narrow this gap, their mass propagation is an urgent need. The conventional methods are not efficient in mass propagation of bamboos. However, *in vitro* regeneration offers viable alternative for large scale production of bamboo plants and has been successfully used in regeneration of some bamboo species (2-4). To date bamboos have been propagated *in vitro* through somatic embryogenesis (3,5-7) as well as by shoot multiplication (1,2,4,8-11).

Thidiazuron (TDZ), a cytokinin, has been known for stimulating strong shoot proliferation activity in several plant species (12-14). It can stimulate shoot multiplication either alone in some plant species (12,14,15) or in combination with other growth regulators (16,17). The potentiality of TDZ to stimulate efficient shoot forming system is more common in dicotyledonous plants (14,15,18), while in monocots only a few plants are known where TDZ has been used in regeneration (2,19). Therefore, the present work was undertaken to study the influence of TDZ on the regeneration potential of a monocot *Dendrocalamus strictus.*

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Abbreviations : IAA – Indole-3-acetic acid; IBA – Indole-3-butyric acid; NAA - α-Naphthalene acetic acid; TDZ – N-phenyl N´-1,2,3-thiadiazol-5-yl-urea; MS – Murashige and Skoog.

Materials and Methods

Seed germination and shoot proliferation — Seeds were surface sterilized by agitating in 1.0% (v/v) sodium hypochlorite solution for 10 min and after rinsing under running tap water, they were again sterilized with 0.05% (w/v) mercuric chloride solution for 4 to 5 min and finally washed 3 times with autoclaved double distilled water. The surface- sterilized seeds were aseptically inoculated and germinated on half-strength Murashige and Skoog (20) medium containing 2% (w/v) sucrose and 0.8% (w/v) agar. In all the experiments in the present study, half-strength MS medium was used. After 10 to 15 days of seed inoculation, 2 to 4 cm long shoots were excised aseptically from the developing seedlings and were cultured either in the liquid medium containing 2% sucrose and supplemented with 0.01, 0.05, 0.1, 0.5 and 1.0 mg l^{-1} of TDZ or on 0.8% (w/v) agar solidified medium

containing 2% sucrose and supplemented with 0.1, 0.5 and 1.0 mg l^{-1} TDZ. Media without TDZ served as control in both the experiments. After 3 weeks of culture initiation, all the cultures were transferred to liquid medium devoid of TDZ.

To study the effect of duration of TDZ treatment on shoot multiplication, the shoots were cultured in 0.5 mg l^{-1} TDZ supplemented liquid medium for 0, 7, 14, 21, 28 and 35 days before being transferred to liquid medium without TDZ.

The shoot multiplication from the basal node, node, internode and apical segment explants excised from 15-day-old seedlings were studied with 0.5 mg l^{-1} TDZ supplemented liquid medium. In this medium explants were cultured for 21 days and then transferred to the medium having the same composition but without TDZ.

In all the experiments, the pH of the medium was adjusted to 5.8 ± 0.02 before autoclaving at 1.06 kg cm^{-2} for 15 min. All the cultures were incubated under 16 h photoperiod (at 50-70 $\mu E\ m^{-2}\ s^{-1}$) at 25 ± 2°C.

Rooting of shoots and acclimatization of plantlets — The shoots regenerated with different concentrations of TDZ were initially transferred to half-strength MS liquid medium for root induction. Since the shoots regenerated with 0.5 and 1.0 mg l^{-1} TDZ treatment did not root in half-strength MS liquid medium, rooting of such shoots was studied in auxin supplemented medium. Such shoots (2.0 cm or >2.0 cm in length) were harvested in a group of 3 shoots and cultured in half-strength MS liquid medium supplemented with different auxins (IAA, IBA and NAA) in different concentrations (0.01, 0.1, 1.0 and 5.0 mg l^{-1}). The well rooted shoots in 1.0 mg l^{-1} IBA supplemented medium were washed thoroughly under running tap water and transplanted in plastic pots filled with sand and garden soil (1:1) mixture and covered with glass beakers. Initially the potted plants were kept under laboratory conditions (at 25-30°C and about 70 $\mu E\ m^{-2}\ s^{-1}$ light intensity) and were irrigated with tap water. After 2 to 3 weeks of potting, the glass beakers were removed gradually and the survival percentage was recorded.

Data record and statistical analysis — For all the experiments, each treatment had twelve replications and each experiment was repeated thrice. Data for the experiment performed to study the effect of duration of TDZ treatment were recorded 3 weeks after transfer of cultures to medium without TDZ, and in all the other experiments performed on shoot multiplication data were recorded after 3 or 6 weeks of culture initiation. For rooting experiments data were recorded after 4 weeks of culture of newly formed shoots in the rooting medium. Data were analysed using one way Analysis of Variance (ANOVA) and comparisons between the mean value of treatments were made by the Least Significant Difference (LSD) test (21).

Results

The shoot buds began to initiate from the basal node of the shoot explants on the second day of culture initiation at all the concentrations of TDZ tested in liquid culture initiation medium (Fig. 1A). After 4 or 5 days of inoculation, shoot bud initiation was also seen at the first or second node (above the basal node) of the explant. In the liquid medium without TDZ (control) the initiation of shoot bud was observed on the 6th day of culture from the basal node of the shoot explant and no shoot bud formation was observed from the other nodes of the explant during the entire culture period. A significant difference was observed in per cent explants showing shoot multiplication between the control (about 11.1%) and TDZ (94-100%) supplemented medium (Table 1). In the shoot explants cultured in liquid medium without TDZ (control) only single shoot bud was produced and occasionally these buds grew into shoots. Incorporation of TDZ in the medium caused a significant increase in shoot bud and shoot formation as compared to the control (Table 1). The shoot bud and shoot production increased with increase in the concentration of TDZ up to 0.1 mg l^{-1} and decreased with further increase in concentration of TDZ in the medium. The increase recorded at different level of TDZ containing medium was not significant (Table 1). Since in TDZ supplemented medium shoot bud and shoot formation was slow (Fig. 1B; Table 1), all the cultures were transferred to the liquid medium without TDZ. The potentiality of shoot bud and shoot formation increased after the transfer of cultures from liquid TDZ medium to medium without TDZ (i.e. in the half-strength MS liquid medium). In the medium without TDZ, the production of shoot buds and shoots were dependent on the concentration of TDZ used during the initial culture period (Table 1). The shoot bud and shoot production increased significantly with increase in the concentration of TDZ and maximum shoots (14.8 ± 1.0) were obtained when the shoot explants were initially cultured in 0.5 mg l^{-1} TDZ supplemented medium (Fig. 1C). The concentration of TDZ higher than 0.5 mg l^{-1} (i.e. 1.0 mg l^{-1}) showed a slight decrease in shoot bud and shoot production. Besides the decrease in shoot bud and shoot production the culture of shoot explants in 1.0 mg l^{-1} TDZ supplemented medium resulted

Table 1. Effect of different concentrations of TDZ on shoot multiplication

Physical state of medium	Concentration of TDZ (mg l^{-1})	Per cent responsive explant	[a]Number of shoot buds produced	[a]Number of shoots produced	[b]Number of shoot buds produced	[b]Number of shoots produced
Liquid culture initiation medium	0.00	11.1±5.6	0.7±0.3	0.3±0.3	0.7±0.3	0.5±0.3
	0.01	94.5±2.8	4.0±0.1	1.5±0.5	5.2±0.4	2.2±0.1
	0.05	100.0	5.5±0.3	2.6±0.2	10.1±2.5	5.9±1.4
	0.10	100.0	7.0±0.8	3.1±0.3	16.0±3.2	9.0±1.8
	0.50	100.0	6.7±0.6	1.9±0.2	36.0±4.9	14.8±1.0
	1.00	100.0	5.8±1.1	1.9±0.5	29.4±7.7	11.4±2.7
LSD (P<0.05)		7.82	1.87	1.09	12.38	8.43
Agar solidified culture initiation medium	0.00	8.3±4.8	1.0±0.0	0.0	0.7±0.3	0.3±0.3
	0.10	78.5±6.8	2.8±0.3	1.1±0.3	6.8±1.6	3.5±1.0
	0.50	80.6±10.0	2.9±0.2	1.3±0.4	14.9±2.7	10.6±2.4
	1.00	70.8±4.2	2.1±0.3	0.7±0.3	11.9±2.1	4.8±0.9
LSD (P<0.05)		22.35	0.92	0.89	6.18	3.55

[a]Data recorded after 3 weeks of culture of shoot explants in TDZ supplemented medium.

[b]Data recorded after 6 weeks of culture i.e., 3 weeks culture in TDZ supplemented medium and 3 weeks without TDZ.

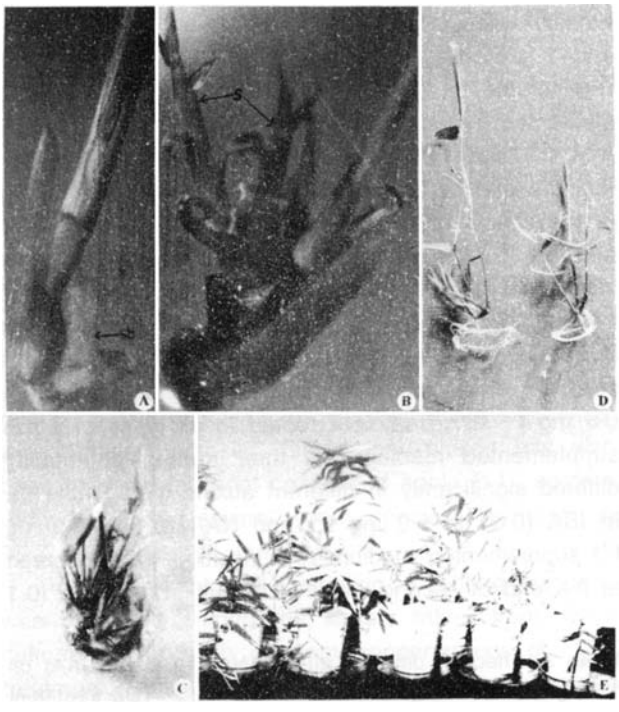

Fig. 1. A & B - Shoot bud and shoot formation from the shoot explants cultured in 0.5 mg l^{-1} TDZ containing half - strength MS liquid medium; **A** - Two-day-old culture showing shoot bud (sb), and **B** - Two-week-old culture showing shoots (s). **C** - Shoots formed in medium containing 0.5 mg l^{-1} TDZ for 3 weeks followed by 2 weeks culture in TDZ devoid half-strength MS liquid medium. **D** - Well rooted shoots after two months of culture in 1 mg l^{-1} IBA containing half - strength MS liquid medium. **E** - Hardened plants after about 8 months of transplantation to soil.

in vitrification and about 11.1% cultures showing regeneration became vitrified. The trend for shoot bud and shoot production was broadly similar for liquid and agarized TDZ supplemented media but gelling of the medium reduced the shoot bud and shoot production at each tested concentration of TDZ in agar-gelled medium as well as after the transfer of cultures from the TDZ supplemented agar-gelled medium to the liquid medium devoid of TDZ as compared to the experiment performed with the liquid medium (Table 1). Vitrification was not observed on gelling of the TDZ supplemented medium with agar.

Since shoot multiplication was high in liquid culture initiation medium and among different concentrations of TDZ tried 0.5 mg l^{-1} TDZ treatment showed maximum shoot multiplication, all other experiments on shoot multiplication were performed with 0.5 mg l^{-1} TDZ supplemented liquid medium.

Shoot explants cultured for different time periods (i.e. 0, 7, 14, 21, 28 and 35 days) in 0.5 mg l^{-1} TDZ supplemented medium and subsequently transferred to medium without TDZ showed 100% response for shoot multiplication at each culture period except for the 0 day (i.e. control). Of these responsive explants all of them survived with 7, 14 and 21 days of culture period in TDZ medium, whereas culture period longer than 21 days in TDZ medium showed a decrease in per cent survival of responsive explants (Table 2). The shoot bud and shoot production increased with the increase in duration of the culture period in TDZ medium. Maximum shoots (13.1 ± 0.9) were produced with 21 days of culture period and it decreased significantly

Table 2. Effect of duration of TDZ (0.5 mg l^{-1}) treatment on shoot multiplication

Treatment period (in days)	Per cent response	Per cent survival of responsive explants	Average number of shoot buds produced per responsive explant	Average number of shoots produced per responsive explant
0	11.1±5.6	80.6±2.3	0.7±0.3	0.0
7	100.0	100.0	10.8±0.3	5.4±0.8
14	100.0	100.0	14.9±1.8	6.8±0.6
21	100.0	100.0	31.0±1.3	13.1±0.9
28	100.0	91.7±3.9	11.0±2.6	5.3±0.9
35	100.0	73.2±2.3	4.9±0.2	3.1±0.2
LSD(P<0.05)	6.97	18.02	4.07	2.09
ANOVA	$F_{5,12}$=256.53**	$F_{5,12}$= 4.12*	$F_{5,12}$= 54.45**	$F_{5,12}$= 39.15**

*Significant at P<0.05
**Significant at P<0.01.

(P<0.05, Table 2) after 28 and 35 days of culture period in TDZ. Most of the regenerated shoots with 35 days of culture period in TDZ medium were fasciated and finally necrosed.

Regeneration was observed from all types of explants except for the internode when the explants were cultured for 21 days in 0.5 mg l^{-1} TDZ supplemented medium and subsequently grown in the half-strength MS liquid medium. The internodal explant did not respond, became brown and finally died within 2 or 3 weeks of their inoculation.The per cent response for shoot multiplication, 94.5, 83.3 and 69.5%, respectively for the excised portion of seedlings like basal node, node and apical segment explants was lower as compared to the entire shoot explant cultured in liquid TDZ medium (Table 1). Average number of shoot buds produced per responsive plant was 24.9, 16.4 and 15.6 in comparison to shoot number of 8.1, 8.8 and 8.6, respectively from basal node, node and apical segment. However, no significant variation was recorded in the number of shoot buds and shoots produced per responsive explant among different responsive explant types.

The concentrations of TDZ used in shoot production during the initial culture period influenced greatly the rooting ability of regenerated shoots. The shoot explants inoculated in the control medium showed root initiation within a week of culture initiation and data recorded after 7 weeks of culture initiation showed rooting in about 63.9 ± 14.4% cultures. However, in TDZ supplemented medium shoot explants did not root, and produced shoot buds and shoots. The newly formed shoots with 0.01 to 0.1 mg l^{-1} TDZ treatment rooted after one or two weeks of their transfer to medium without TDZ and data recorded after 4 weeks of transfer of cultures (i.e. after 7 weeks of culture initiation) to medium without TDZ showed 66.7 ± 16.7, 20.8 ± 12.6 and 12.5 ± 4.2 % rooted cultures when shoots were regenerated in 0.01, 0.05 and 0.1 mg l^{-1} TDZ supplemented medium, respectively. The shoots produced with 0.5 and 1.0 mg l^{-1} TDZ did not root even after their transfer to medium without TDZ. Thus, for rooting of the shoots regenerated with 0.5 or 1.0 mg l^{-1} TDZ treatment, different auxins in different concentrations were tried. The shoots produced with 1.0 mg l^{-1} TDZ treatment did not root even after their culture in various auxins in different concentrations supplemented to half-strength MS liquid medium. The shoots produced with 0.5 mg l^{-1} TDZ treatment rooted in all types of auxin supplemented medium but their rooting potentiality differed significantly in different auxins tried (Table 3). In IBA (0.01 to 5.0 mg l^{-1}) and NAA (0.1 to 5.0 mg l^{-1}) supplemented medium root initiation was observed at the end of second week of culture and in IAA (0.1

Table 3. Effect of different auxins (NAA, IBA and IAA) on rooting of shoots regenerated with 0.5 mg l^{-1} TDZ treatment

Concentration (mg l^{-1})	Per cent rooting (Mean ± SE)		
	NAA	IBA	IAA
0.00	0.0	0.0	0.0
0.01	0.0	17.7 ± 0.5	0.0
0.10	11.6 ± 6.4	15.3 ± 2.5	8.5 ± 4.3
1.00	25.8 ± 7.3	55.2 ± 2.9	23.9 ± 4.9
5.00	22.7 ± 6.0	46.7 ± 4.6	25.0 ± 5.6
LSD P<0.05	16.09	8.29	12.10

to 5.0 mg l^{-1}) containing medium 18 to 20 days were needed for root initiation. The time needed by different auxins to induce root was found to be independent on the concentrations of auxin used in the medium but the percentage of rooted shoots depended on the concentrations and type of auxin used in the medium (Table 3). Among all the treatments maximum rooting was observed in IBA supplemented medium and lowest was observed in IAA supplemented medium (Table 3).

One- to two-month old well rooted shoots in 1.0 mg l^{-1} IBA supplemented half- strength MS liquid medium (Fig. 1D) transplanted to garden soil and sand (1:1) mixture showed 98% establishment (Fig. 1E). Such plants could be successfully transplanted to field conditions in the botanical garden of Banaras Hindu University, where they are showing luxuriant growth.

Discussion

The present investigation revealed that TDZ has high potentiality for inducing shoot multiplication without any callusing at any stage from the seedling explants of *D. strictus*. A two stage culture procedure for shoot multiplication was found to be useful as reported in apple (14). The reduction in shoot multiplication rate on solidification of TDZ supplemented medium as compared to liquid medium observed in the present study has also been reported in *Bambusa tulda* (4). Our observation of vitrification of cultures in liquid medium is in conformity with observation of Viseur (22) in pear.

The use of TDZ for a longer duration, i.e. 35 days, resulted in formation of fasciated or distorted shoots, which finally necrosed. Similar observation has also been reported in shoot cultures of apple (14), eastern redbud (23) and several woody species (18) raised in TDZ supplemented medium.

In the present work, variation in rooting response was observed among the shoots taken from shoot cultures raised with different concentrations of TDZ treatment. Similar observation has also been recorded in eggplant (15). Use of IBA for root induction from the shoots regenerated with TDZ has also been reported in eastern redbud (23) and *Liquidambar styraciflua* (24).

The most important outcome of the present study has been the initiation of shoot bud on second day of culture from shoot explants and formation of shoots from all (100%) shoot explants in the liquid medium enriched with 0.01 to 1.0 mg l^{-1} TDZ. This is in contrast to the earlier reports in *D. strictus* where not only per cent response for shoot formation was lower but also the minimum time taken for initiation of shoot was about 7 days (1,10,11,25). Thus the protocol established in the present study is more efficient and can be commercially exploited for the mass propagation of this economically important bamboo species.

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