Thidiazuron-induced *in vitro* flowering in *Dendrocalamus strictus* Nees

Bamboos are one of the most useful groups of plants and have the peculiar behaviour of flowering and seeding at the end of a very long vegetative growth phase, the length of which is considered to be species-specific[1]. The induction of *in vitro* flowering could be an option for *in vitro* improvement through selective breeding method as well as in the study of reproductive biology of bamboos. Initially *in vitro* flowering was reported by Nadgauda *et al.*[2] in *Bambusa arundinacea*, and *Dendrocalamus brandisii* and subsequently in a few more species of bamboos[3–5]. In most of these studies, flowering was induced in medium supplemented with 6-benzyl adenine (BA). Rout and Das[4] have used adenine hemisulphate, indole 3-butyric acid (IBA) and gibberellic acid for flower induction in *B. vulgaris*, *D. giganteus* and *D. strictus*. Many other cytokinins tested were ineffective[6].

Thidiazuron (TDZ, *N*-phenyl-*N'*-1,2,3-thiadiazol-5-yl urea), a cytokinin, has been used in tissue culture since 1982 (ref. 7); it greatly stimulates shoot regeneration[8–11] as well as somatic embryogenesis[13,14]. The present work was undertaken to study the role of TDZ for induction of *in vitro* flowering in *D. strictus* using seedling explant.

The dehusked seeds were surface disinfected by agitating in 1.0% (v/v) sodium hypochlorite solution for 10 min, followed by 10 to 12 min washing under running tap water. It was followed by second sterilization in laminar flow hood with 0.05% (w/v) mercuric chloride solution for 4 to 5 min and finally washed 3 to 4 times with sterile double-distilled water. These surface sterilized seeds were cultured on half strength Murashige and Skoog[15] (MS) medium containing 2% (w/v) sucrose and gelled with 0.8% (w/v) agar. The medium was adjusted to pH 5.8 ± 0.02 before autoclaving at 121°C for 15 min. After 10 to 15 days of culture, 2 to 4 cm long shoots were excised aseptically from the germinated seeds and were cultured in half strength MS liquid medium containing 2% (w/v) sucrose and different concentrations (0, 0.01, 0.05, 0.1, 0.5 and 1.0 mg/l) of TDZ. The cultures were incubated under 16 h photoperiod (at 50–70 $\mu E^{-2} S^{1}$) at 25 ± 2°C. After 21 days all the cultures were transferred to half strength MS liquid medium without TDZ; the newly formed shoots were maintained on this medium for about three months (with regular transfer at 15 days) for induction of flowering. Observations were recorded after 12 weeks of transfer of cultures to the medium without TDZ. To study pollen morphology, anthers were squashed and stained in acetocarmine.

Each treatment had twelve replicates and each experiment was repeated thrice. Data were analysed using one way Analysis of Variance (ANOVA) and difference between the treatment means was compared using Least Significant Difference (LSD) test.

The shoot explant initially cultured in TDZ-supplemented medium showed shoot bud formation (on the second day) from the basal node of the shoot explant. The conversion of shoot buds into shoots was seen after a week of culture. At 0.01 mg/l TDZ-supplemented medium about 94 per cent cultures were found to be responsive for shoot multiplication, while at other concentrations 100 per cent explants showed shoot multiplication. In control (without TDZ) about 11 per cent cultures showed regeneration by producing a single shoot bud and occasionally these buds grew into shoots. The flower induction was observed after about two months of transfer of newly formed shoots from TDZ medium to the medium without TDZ. Among different concentrations tried, the shoots regenerated with 0.5 and 1.0 mg/l TDZ concentrations produced spikelets (Figure 1). No flower induction was seen in control cultures. Spikelets usually developed in clumps (Figure 2 *a*) or sometimes singly (Figure 2 *b*) at the nodes of the regenerated shoots. The number of florets in a culture varied; a maximum of 12 florets could be counted in a culture initially treated with 0.5 mg/l TDZ (Figure 1). The length of the floret ranged from 0.4 to 1.6 cm. Each floret was green and consisted of lemma, palea, lodicules, androecium and gynoecium.

Anthesis of the florets was not synchronized and it occurred at any time during the day or night. In some florets lemma and palea did not separate fully

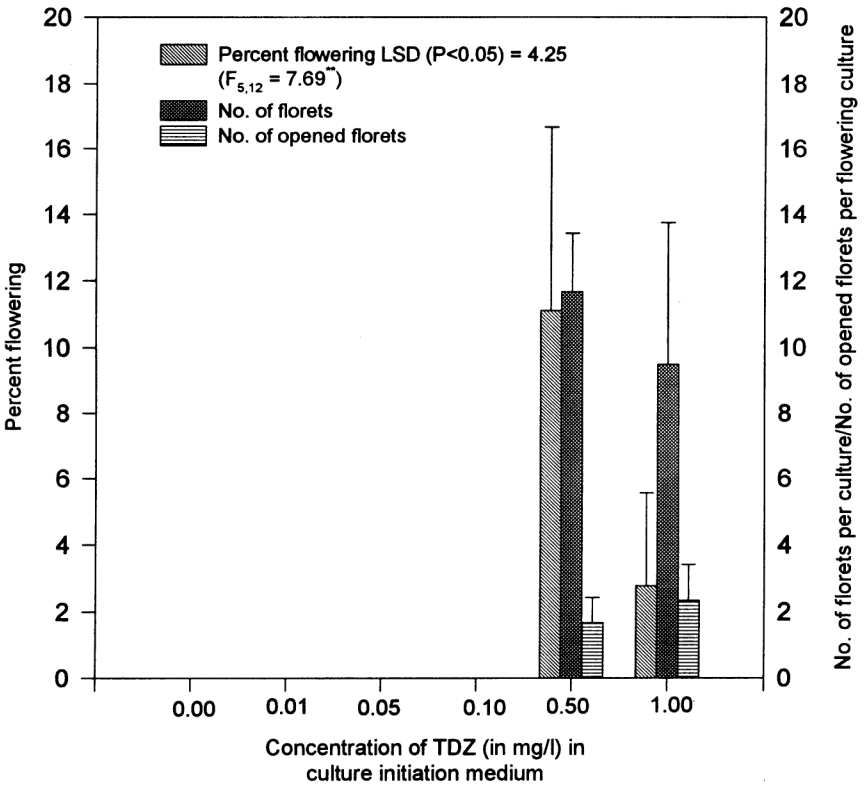

Figure 1. Effect of TDZ on *in vitro* flowering (**, highly significant).

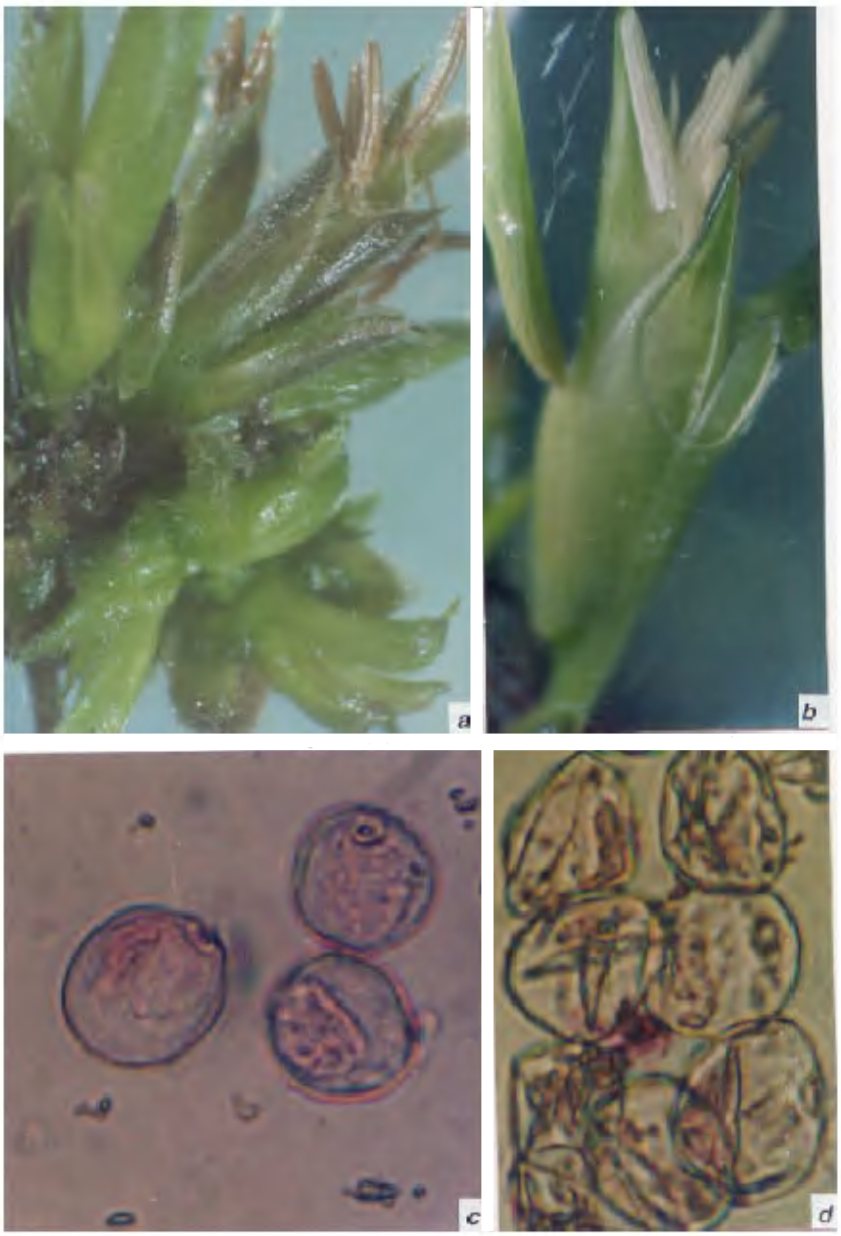

Figure 2. Spikelets produced in clump (***a***) or singly (***b***) at the nodes of the regenerated shoots (× 9.0); Acetocarmine stained normal (***c***) and empty (***d***) pollen grains (× 175).

and the anthers protruded only partially, while the gynoecium remained within. Anthers failed to dehisce. Anther squashes showed about 20% normal (Figure 2 *c*) and 80% empty (Figure 2 *d*) pollen grains.

It is noteworthy that in all the reports on *in vitro* flowering in bamboo, the media contained cytokinin. In the present study also, initial culture on TDZ-supplemented medium was found to be necessary for flower induction. In *B. edulis*, Lin and Chang[16] were able to induce flowering from the nodal explant of mature tree on both TDZ-supplemented medium as well as after the transfer of cultures from TDZ-supplemented medium to the same medium but without TDZ. The pattern of floral development observed in the present work was similar to reports by other workers[17,18]. The absence of seed set may be due to the lack of anther dehiscence though a few normal pollen grains were recorded.

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