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Short Communication

THIDIAZURON-INDUCED ANATOMICAL CHANGES AND DIRECT SHOOT MORPHOGENESIS IN *DENDROCALAMUS STRICTUS* NEES

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ABSTRACT

Thidiazuron (TDZ) has potential for *de novo* multiple shoot induction without any callusing at any stage in *Dendrocalamus strictus* Nees. Longitudinal section through basal node region of the explants cultured in half strength Murashige and Skoog (MS) medium with 2.3µM TDZ showed shoot bud initials within a day of culture initiation while those cultured in TDZ free half strength MS medium did not show any evidence for the presence of shoot bud. The organization of shoot bud initial was tunica corpus type and in 3-4 days a short cylinder of provascular tissue is differentiated as the initial stage of vascular differentiation. After about 8 days of culture the newly differentiated bud began to elongate and new shoot bud initials were originated successively from actively dividing cells at the base of elongating shoots. The above process of regeneration continued even after transfer of explants into medium without TDZ.

Keywords: Thidiazuron, shoot bud, peripheral zone, vascular tissue, safranin.

INTRODUCTION

Thidiazuron (TDZ) has been known for stimulating strong shoot proliferating activity in several plant species (Dhavala and Rathore, 2010; Hutteman and Preece, 1993; Singh *et al*., 2001). It is a substituted phenylurea (N-phenyl-N′-1,2,3-thidiazol-5-ylurea) and has been found more active than other phenylurea compounds in tissue culture systems. Although, structurally it is different from both the auxins and the purine-based cytokinins, but exhibits the unique property of mimicking both auxin and cytokinin effects on growth and differentiation of cultured explants (Murthy *et al*., 1986). Responses of TDZ induced *in vitro* shoot multiplication varies from species to species, thus needs a thorough study in case of each and every plant species. It can stimulate shoot multiplication either alone in some plant species (Lata *et al*., 2009; Kumaria *et al*., 2012) or in combination with other growth regulators (Haddadi *et al*., 2013; Thomas and Philip, 2005). Despite the extensive literature on shoot multiplication in dicotyledonous plants (Dhavala and Rathore, 2010; Hussain *et al*., 2007), little attention has been paid on monocotyledonous plants (Singh *et al*., 2001). Further investigations are needed to confirm the initial cell division pattern and shoot bud morphogenesis which is so far has not been demonstrated under the influence of TDZ in any *in vitro* regeneration system. Moreover, the anatomical details could also be evidence for direct shoot regeneration and the shoots regenerated directly from the cultured explants could be exploited as true clone. In a previous study, we demonstrated that TDZ could induce multiple shoots from different seedling explants in a bamboo (*D. strictus*). The *D. strictus* has a long history of widely used resource (Singh *et al*., 2001) and thus, in order to confirm the mode of direct clonal propagation under the influence of TDZ, the present study was under taken to trace the initial anatomical changes of shoot initiation from the basal node explants of seedlings of *D. strictus*.

MATERIALS AND METHODS

The dehusked seeds were surface sterilized by agitating in 1.0% (v/v) sodium hypochlorite solution for 10 min, followed by 10-12 min washing under running tap water. The seeds were sterilized again with 0.05% (w/v) mercuric chloride solution for 4 to 5min and finally washed 3 times with autoclaved double distilled water. The surface sterilized seeds were aseptically germinated on half strength Murashige and Skoog (1962, MS) medium containing 2% (w/v) sucrose and gelled with 0.8% (w/v) agar. The pH of the medium was adjusted 5.8 ± 0.02 before autoclaving at 1.06 kg cm^{-2} for 15 min. After 10 to 15 days of seed inoculation 2 to 4cm long shoots having 2 or 3 nodes were excised from the seedlings and cultured in half-strength MS liquid medium containing 2% (w/v) sucrose, supplemented with 2.3µM TDZ for 21 days and subsequently transferred into half strength MS basal liquid medium devoid of TDZ. The cultures were incubated at 25 ± 2ºC and under 16h photo

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period with intensity 20-30 µmoles m^{-2} s^{-1} provided with cool white fluorescent tube-lights.

To prepare permanent histological slides, at different intervals after the explants inoculation, a small portion of the proliferating region (basal node) of explants were excised and fixed in formalin-acetic acid-alcohol (formalin, glacial acetic acid and 50% ethyl alcohol in a ratio of 5:5:90 by volume) for 24h. The fixed materials were dehydrated using different ratios of absolute alcohol and chloroform (Sass, 1958). For cutting the serial sections dehydrated tissues were infiltrated and embedded in paraffin wax (melting point 58-60ºC). Ten to fifteen micron thick serial sections were cut with the help of rotary microtome and the ribbons of the sections were fixed on the surface of the glass slides using haupt's adhesive. The section were deparaffinised in xylol and hydrated following the reverse way of dehydration schedule used before embedding. The dehydrated sections were stained with 1.0% (w/v) alcoholic safranin solution prepared in 70% alcohol for 4-5h. The safranin stained sections were dehydrated and counterstained with fast green for about 30 seconds and then dehydrated through alcohol and xylol series and mounted with DPX mounted. Observations were carried out under light and inverted microscope.

RESULTS AND DISCUSSION

The present histological investigation revealed that TDZ has high potential for inducing *de novo* shoot multiplication without any callusing at any stage from the seedling explants of *D. strictus.* No any histological abnormalities were noticed during shoot bud differentiation. The shoot bud initiation observed from the basal node of the shoot explants on the second day of culture initiation and initiation of new buds was continued even after transfer of regenerating explants from TDZ containing medium to medium free of TDZ. The permanent histological slides prepared in order to trace the early differentiation showed that the anatomical differentiation begins within a day of culture initiation on the medium having TDZ (Fig. 1B) and no such differentiation was noticed in the explants cultured in medium without TDZ (Fig. 1A), thus, confirming that TDZ could induce and enhance shoot bud formation. The shoot bud initial was hemispherical consisted of few layers of small isodiametric cells. The two outermost darkly stained layers were considered to constitute the tunica in which anticlinal divisions predominated. The tunica corpus organization of shoot bud initials in the process of organogenesis has also been demonstrated in Passionfruit (Da gloria *et al*., 1999) and Black pepper (Sujatha *et al*., 2003). The cells of these two layers were more or less similar in size (Fig. 1C) and in a few cases those of the second layer were slightly larger. The outermost layer was continuous with the protoderm basipetally. In the hemisphere, the cells of the central zone were slightly larger than those of the peripheral zone and were less intensely stained with safranin-fast green (Fig. 1C). McArthur and Steeves (1972) showed that the meristematic cylinder or provascular tissue represented the initial stage of vascular differentiation, which developed under the sole influence of the apical meristem. In the present study also the vascular tissues were found initiated in the form of procambium beneath the peripheral zone, the cells of which assume a rather narrow elongated form may be because of the predominance of longitudinal division (Fig. 1C). This agrees with the description provided by a number of previous workers in various angiosperm species (Esau, 1965; Sujatha *et al*., 2003; Xia and Steeves, 1999). In longitudinal view the provascular tissue was continuous with the peripheral zone of the apex above and with the typical procambium below (Fig. 1C). Similar observation that there was no sharp boundary between provascular tissue and peripheral zone of the apex has been reported in carrot, where the histochemical evidence of the presence of carboxylesterases has been used to distinguish it from the peripheral zone of the apical meristem (Xia and Steeves, 1999). In seed plants it has long been noted that there is a recognizable tissue just under the peripheral region of the apical meristem and in the path of differentiation of procambium to the leaf primordial. Some workers reported that this was a pre or pro vascular tissue because the procambial traces arise from it (Esau, 1965; Xia and Steeves, 1999).

Further growth of the shoot apical meristem was similar to that of the normal shoot apex and the median longitudinal section of an 8 days old culture showed completely differentiated shoot bud (Fig. 1D). Similar pattern of shoot bud differentiation was reported in carrot (Xia and Steeves, 1999), and Eucalyptus (Azmi *et al*., 1997). Once the shoot bud differentiation completed, it began to elongate and grow up into small shoot (1cm long) and considerable increase in shoot length was noticed after the transfer of regenerating shoots from TDZ containing medium to medium devoid of TDZ. Similar observation has been shown in *R. tetraphylla* that MS medium supplemented with TDZ was favourable for induction of maximum number of shoots and that transfer to hormone-free medium was essential for shoot elongation (Faisal *et al*., 2005). The new shoot buds were formed successively from actively dividing cells at the leaf axils of elongating shoots or newly emerging shoots buds. The above process of the regeneration of new shoots was observed even after transfer of cultures to medium without TDZ (Fig. 1F) and thus, confirming the *de novo* initiation of shoot buds in medium devoid of TDZ. This is also in confirmation to earlier reports, where a significant increase in shoot multiplication rate has been reported after the transfer of explants from medium supplemented with TDZ to TDZ-free medium (Singh *et*

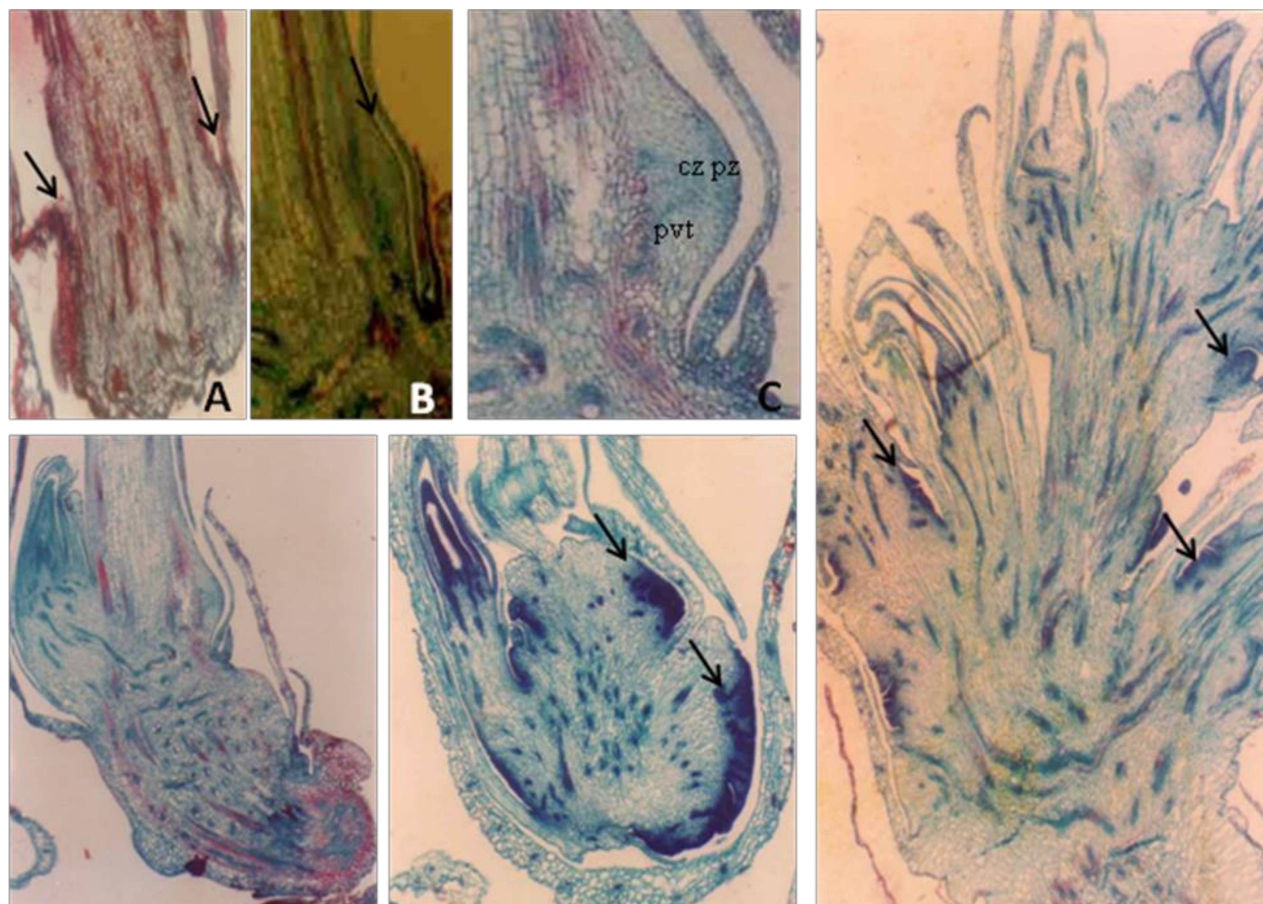

Fig. 1. TDZ induced shoot bud organogenesis from Basal node explants of seedling shoots of *Dendrocalamus strictus*.
A and B: Longitudinal Section of basal node explants after a day of culture initiation. (A) in half-strength MS basal liquid medium (X 87.5). (B) in MS liquid medium with 2.3 µM TDZ (X 87.5).
C: Median Longitudinal Section of shoot bud initials showing distinct zonation pattern. pz-peripheral zone, cz-central zone, pvt-provascular tissue (X 87.5).
D: An 8-day old culture showing completely differentiated shoot bud (X 35).
E: L.S. showing shoot buds differentiation in superficial layer of the cultured explants (X 35).
F: Longitudinal Section a 28-days old culture showing elongation of shoot buds and differentiation of new buds in the leaf axil (X 35).

al., 2001). In the present study, origin of shoot meristem was noticed on superficial layer and in leaf axil only (Fig. 1E). Thus, this histological study is showing that TDZ could be used for clonal multiplication without any callusing at any stage and the development of shoot bud and shoot was similar to that of seed plant. The addition of TDZ into induction medium was necessary for *de novo* shoot bud regeneration.

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