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SHORT COMMUNICATION

EFFICIENCY OF THIDIAZURAN ON *IN VITRO* SHOOT REGENERATION FROM COTYLEDONARY NODE EXPLANT IN MUNGBEAN

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Mungbean (*Vigna radiata* (L.) Wilczek) is an important pulse crop and shows recalcitrant behaviour *in vitro* cultures. The present study reports the shoot regeneration of cotyledonary node explants of mungbean grown on thidiazuran (TDZ) enriched MS medium. The shoot bud development was enhanced by 8 days exposure of explants to 2 mM TDZ concentration. The complete loss of regeneration ability and high callus growth of explants was observed at higher concentration of TDZ and with continuous exposure of explants to TDZ.

***Key words*: Explants, mungbean, regeneration, thidiazuran**

Mungbean (*Vigna radiata* (L.) Wilczek) is one of the important pulse crops in India. Various efforts made to produce disease and pest resistant transgenic plants remained in quiescent stage till today (Jaiwal and Gulati 1995, Nagl *et al.*, 1997). The main reason for such failure in production of transgenics in mungbean was lack of an efficient *in vitro* shoot regeneration protocol. Thidiazuron (TDZ), a heterocyclic urea, has been frequently shown to display qualitatively similar biological properties to purine cytokinins (Mok *et al.* 1982). It has been used to induce shoot regeneration in some recalcitrant legumes like peas, chickpea and lentil (Malik and Saxena 1992a, b). Mohamed *et al.* (1993) achieved plant regeneration through successive sub-culture from pedicel derived callus in *Phaseolus vulgaris*. The present experiment was carried out to study the efficiency of TDZ in shoot regeneration of *in vitro* grown cotyledonary node explants of mungbean.

The healthy and mature seeds of mungbean varieties Pusa Bold-I and Pusa-9531 were washed with water containing 5% Teepol, surface sterilized with 0.1% mercuric chloride for 4-5 minutes and rinse with sterile distilled water. The surface sterilized seeds were germinated aseptically on wet cotton in test tubes. The cotyledonary node explants were taken from 4 days old seedlings. The cotyledonary node was cut into parts each with one intact cotyledon called half cotyledonary node.

The explants were first inoculated on MS medium (Murashige and Skoog 1962), supplemented with three different concentrations (1, 2 and 3 μM) of TDZ. The explants were exposed to TDZ enriched MS medium (induction medium) for 4, 8 and 12 days. Later on, these explants were transferred to MS medium (Shoot-bud development medium) without any plant growth regulators (PGRs). The control was without TDZ and the explants were transferred after 4, 8 and 12 days to fresh MS medium without any PGRs. The pH of medium was adjusted to 5.8 and solidified with 0.7% agar-agar. Cultures were incubated at a temperature of 25±2°C under a 16 h photoperiod light provided by fluorescent lamps supplying a light intensity of about 60 $\mu Em^{-2} s^{-1}$. In all the treatments, 50 replications were maintained.

The observations on growth (fresh weight) and regeneration (shoots per explant) were recorded at 30 DAI in both the varieties. The data were statistically analysed using standard method. Completely randomized design (for single factor) and factorial completely randomized design (for more than one factor) were used.

Means were evaluated at $P > 0.05$ level of significance using Duncan's New Multiple Range Test (DMRT).

The results obtained in this study showed that the continuous exposure of explant to TDZ caused significantly higher fresh weight compared to control and the cultures which were exposed to TDZ for 4 and 8 days. The fresh weight of explant enhanced significantly over control with increase in concentration of TDZ under different exposure period. Moreover, the continuous exposure of explant to TDZ resulted in complete loss of regeneration ability of explant at all the concentrations. The regeneration efficiency of explant reduced in both the varieties *viz.* Pusa Bold-I and Pusa-9531 at 3 µM TDZ concentration. The optimum concentration of TDZ and exposure period of explant to TDZ for regeneration was found to be 2 µM for 8 days, in both the varieties (Table 1, 2 and Plate 1, 2). The number of shoots per explant were higher in variety Pusa Bold-I as

Table 1. Effect of TDZ concentration supplemented to MS medium and exposure period to TDZ on *in vitro* growth and regeneration of half cotyledonary node explants of mung bean variety Pusa Bold-I (Data recorded at 30 DAI). Induction medium : MS + TDZ; Shoot bud development medium : MS without TDZ

Treatments	Exposure Period							
	Continuous		4 Days		8 Days		12 Days	
TDZ (µM)	Fw (mg)	Shoot number	Fw (mg)	Shoot number	Fw (mg)	Shoot number	Fw (mg)	Shoot number
0.0 (Control)	410.1^{fg}	1.0^{g}	418.1^{fg}	1.0^{g}	425.1^{fg}	1.0^{g}	453.7^{ef}	1.0^{g}
1.0	525.3^{bc}	0.0	411.3^{fg}	2.6^{d}	410.7^{fg}	4.1^{c}	475.0^{de}	1.3^{f}
2.0	560.6^{ab}	0.0	401.1^{g}	5.1^{b}	425.3^{fg}	9.6^{a}	490.3^{cde}	0.0
3.0	591.4^{a}	0.0	450.7^{ef}	1.7^{e}	477.1^{de}	0.0	510.0^{cd}	0.0

	Fresh Weight			Shoot number		
	ED	T	EDxT	ED	T	EDxT
SEm±	7.91	6.85	13.71	0.044	0.038	0.076
CD at 5%	22.62	19.59	39.18	0.12	0.11	0.22

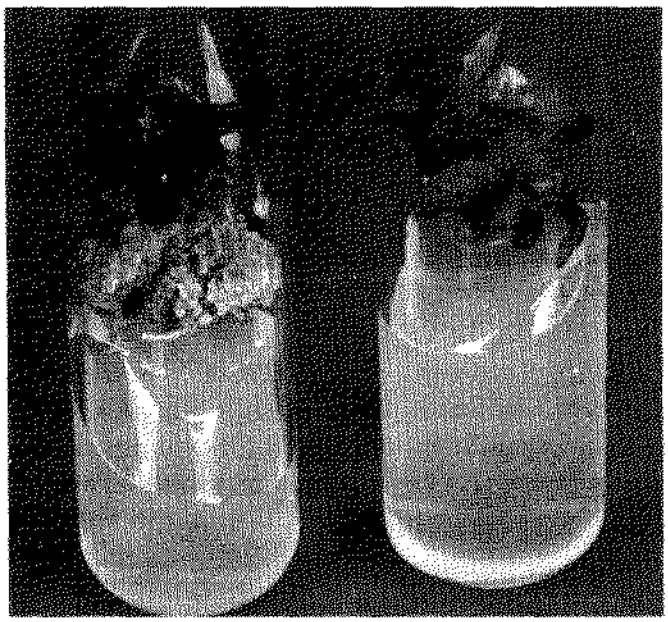
ED = Exposure days; T = Treatment; Mean values followed by similar letters are non significant at 5% level.

Table 2. Effect of TDZ concentration supplemented to MS medium and exposure period to TDZ on *in vitro* growth and regeneration of half cotyledonary node explants of mungbean variety Pusa 9531 (Data recorded at 30 DAI). Induction medium : MS + TDZ; Shoot bud development medium : MS without TDZ

Treatments	Exposure Period							
	Continuous		4 Days		8 Days		12 Days	
TDZ (µM)	Fw (mg)	Shoot number	Fw (mg)	Shoot number	Fw (mg)	Shoot number	Fw (mg)	Shoot number
0.0 (Control)	395.0^{f}	1.0^{g}	402.1^{f}	1.0^{g}	415.0^{ef}	1.0^{g}	425.1^{def}	1.0^{g}
1.0	475.1^{bc}	0.0	390.3^{f}	3.3^{d}	401.7^{f}	5.5^{c}	452.3^{cde}	1.6^{f}
2.0	510.7^{ab}	0.0	410.6^{ef}	5.9^{b}	425.3^{def}	6.7^{a}	475.7^{bc}	1.0^{g}
3.0	545.3^{a}	0.0	436.1^{def}	1.5^{f}	460.7^{cd}	2.9^{e}	515.1^{ad}	0.0

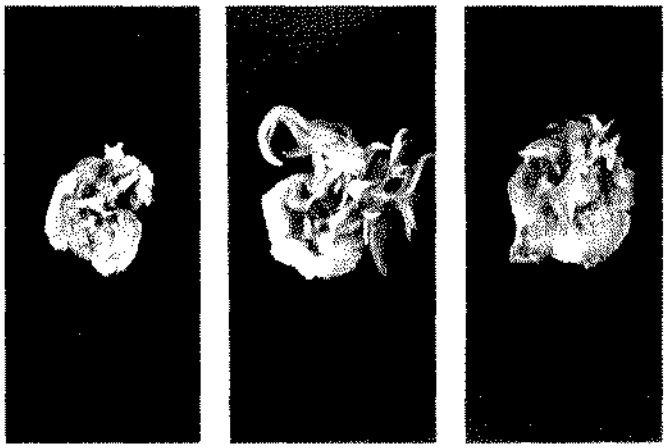
	Fresh Weight			Shoot number		
	ED	T	EDxT	ED	T	EDxT
SEm±	9.26	8.02	16.05	0.06	0.05	0.010
CD at 5%	26.48	22.43	45.86	0.17	0.15	0.29

ED = Exposure days; T = Treatment; Mean values followed by similar letters are non significant at 5% level.



A B

Plate 1. The effect of exposure days of explant to TDZ on regeneration in mungbean var. Pusa Bold-1.
(A) Continuous exposure, (B) 8 Days exposure



A B C

Plate 2. The effect of TDZ concentration on *in vitro* shoot differentation of half cotyledonary node explant in mungbean var. Pusa Bold-1.
A: 1 µM; B: 2 µM; C: 3 µM

compared to Pusa 9531. TDZ has been used to induce shoot regeneration in several recalcitrant legumes *viz. Cicer arietinum, Pisum sativum, Lens culinaris* and *Phaseolus vulgaris* from cotyledonary node and intact

Plate 3. The microshoots induced by TDZ at 45 DAI in mungbean var. Pusa Bold-1.

seedlings (Malik and Saxena 1992 a and b). Some reports indicate that higher concentration (5-10 µM) and continuous exposure to TDZ may cause loss of regeneration *in vitro* cultures (Malik and Saxena 1992 b and Eapen *et al.* 1998). Hence, a precaution is necessary while using the TDZ for regeneration in *in vitro* culture. In mugbean the TDZ with 2 µM concentration and an exposure period of 8 days was found to be optimum for maximum *in vitro* shoot regeneration.

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