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Short Communication

Thidiazuron Induced Regeneration in *Cuminum cyminum* L

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In this communication we report shoot organogenesis from hypocotyl explants in cumin *(Cuminum cyminum)* genotype RZ-19 by the use of thidiazuron (TDZ). Various levels of TDZ were incorporated in MS basal medium to induce regeneration. Regeneration was achieved with a frequency up to 30% on 0.5 and 0.1 mg l^{-1} concentration of TDZ. Shoots once produced could be multiplied on 0.5 mg l^{-1} kinetin (KN) at the rate of approximately 8 shoots per regenerated shoot. These multiplied shoots could go through 3-4 multiplication cycles after which they root on 1.0 mg l^{-1} IAA.

Key words *:* cumin, regeneration, thidiazuron.

Cumin (*Cuminum cyminum* L) is an economically important cash crop of Rajasthan with great deal of producion and export potential. It is, however, highly susceptible to *Fsarium* wilt and resistance genes are not available in the existing germplasm pool. In the absence of a natural source of resistance, exploitation of somaclonal variation could be one of the options (1). However, availability of a reproducible regeneration protocol is an essential prerequisite before such exploitation. The proposed research work was thus aimed to develop a reproducible system of regeneration in cumin.

Regeneration has been achived successfully in various members of Umbelliferae *viz., Daucus carota* (2) *Foeniculum vulgare* (3) *Eryngium* (4) and among these *D. carota has become a classical example of in vitro* regeneration especially through somatic embryogenesis. Cumin, however appears to have limited potential for *in vitro* manipulaton. Treatment of explants with various cytokinins (BAP, KN, Zeatin) and auxins (IAA / NAA / 2,4-D) used singly or in cmbinations could not induce organogenetic events in cumin. (5)

Thidiazuron (TDZ) a substituted phenyl urea, basically used as a defoliant, also produces high cytokinin like activity in *in vitro* cultivated cells (6-8). The mechanism of action of TDZ is partly related to the inhibition of the cytokinin degradation by cytokinin oxidase, resulting in increased levels of endogenous cytokinin (9). TDZ has been reported to induce shoot morphogenesis in several plant species (10-12).

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Seeds of agronomically superior and released variety RZ19 were obtained from SKN College of Agriculture, Jobner. Seeds were surface sterilized in 0.1% (w/v) $HgCl_2$ for 3 min, then rinsed several times with sterile distilled water. Seeds were germinated aseptically on paper bridges dipped in sterile distilled water at 22°C in dark. After seven days germinated seedlings were shifted in light. Hypocotyl explants from 14-d-old seedlings were placed on MS medium (13) supplemented with a range of concentrations of TDZ. Explants were placed horizontally on mdium surface. All cultures were incubated in 35-µmol m^{-2} s^{-1} light intensity, 16-h photoperiod at 27°C temperature. The frequency of regeneration was measured as percentage of explants producing shoot buds and number of shoots per explant after 30d.

Hypocotyl explants produced large amounts of green compact callus (Fig.1A) on al the tested levels of TDZ. Callus initiatin took place ithin 10 days of inoculation. Shoot buds appeared after 30d (Fig. 1A). Both, the frequency of regeneration and total number of shoots per explant were higher on 0.1 mg mg l^{-1} concentratin (Table 1). Subculture of regenerating callus on 0.1 mg l^{-1} TDZ irrespective of the callus induction medium, enhanced the number of shoot buds. Similar observations were made by Kim *et al* (14) in *Liquidambar* where organogenesis was observed at 0.1 mg l^{-1} TDZ from hypocotyl explant. On the contrary Magioli *et al* (12) have reported high frequency (80%) of shoot regeneration in hypocotyl explant of *Solanum melongena* at micromolar concentration (0.05 µM) of TDZ, while in *Glycine max* shoot regeneration

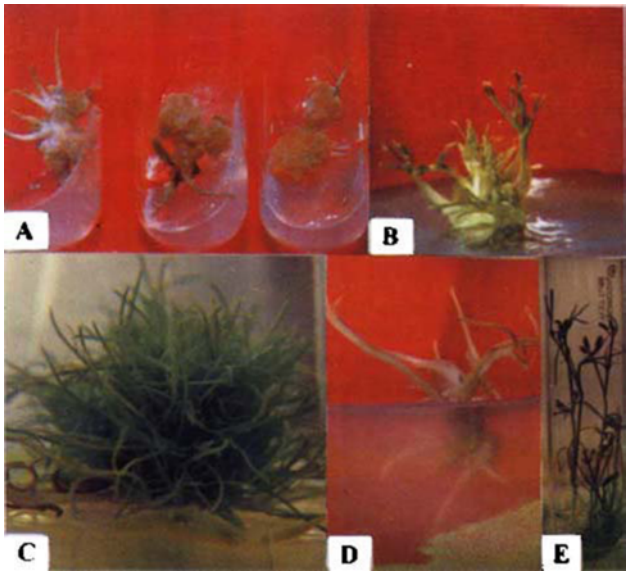

Fig. 1. Shoot bud regeneration, multiplication and *in vitro* flowering in *Cuminum cymimum* L hypocotyl cultures. (**A**) compact green callus showing emergence of shoots on MS medium with 0.1 mg l^{-1} TDZ; (**B-C**) Multiplication of regenerated shoot buds on MS medium with 0.5 mg l^{-1} kinetin; (**D**) Rooting in regenerated shoot; (**E**) Elongated and flowering shoot on MS basal medium.

Table 1. Effect of thidiazuron (TDZ) levels on shoot bud regeneration from hypocotyl explants of *Cuminum cyminum*

TDZ (mg l^{-1})	% Organogenic response	Mean no. of shoots/explant
0.001	27.7	4.6 ± 2.40
0.005	11.1	8.0 ± 00
0.01	21.4	4.3 ± 1.15
0.05	11.8	10.5 ± 2.12
0.1	27.7	7.6 ± 4.5
0.5	25	6.25 ± 2.06
1.0	0	0

was obtained on hypocotyl explant at very high level (2 mg l^{-1}) of TDZ (15). Wide variation in effective levels of TDZ, inducing organogenesis in different plant systems, may reflect per-se hormonal requirement of a genotype.

Although in cumin, shoot regeneration was low, large number of shoots (8 shoots/explant) could be multiplied on MS medium supplemented with 0.5 mg l^{-1} KN (Fig. 1 B, C). The multiplication cycle could be maintained for 3 to 4 subcultures, after which shoots started showing hyperhydricity. Shoots thus regenerated were subcultured on various root inducing media consisting of ½ MS medium with auxins. Maximum frequency of rooting was found on 1 mg l^{-1} IAA (89%) followed by NAA (55%) and IBA (44%) (Fig. 1D). Requirement of high IAA conc may be due to the enhanced activity of cytokinin oxidase by TDZ (9). Shoots grew very well on MS basal medium and flowered *in vitro* as well (Fig. 1E).

This is the first report of successful *in vitro* regeneratin in *Cuminum cyminum* by the use of TDZ, where 0.1 mg l^{-1} concentration was found appropriate for shoot bud induction.

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