Thidiazuron as a promoter of multiple shoots in cotton explants (*Gossypium* hirsutum L.)

Lázara Pereira Campos Caramori*, Simone Fávaro and Luiz Gonzaga Esteves Vieira

*Instituto Agronômico do Paraná, Rodovia Celso Garcia Cid, km 375, 86001-970, Londrina, Paraná, Brasil. *Author for correspondence.*

ABSTRACT. Most cotton *Gossypium hursutum* (Malvaceae) genotypes of commercial interest present problems of *in vitro* regeneration. Aiming at improving regeneration rate, meristems and caulinar apices (region with about 5 mm length, immediately below the meristematic region) of IAC 22 and COKER 312 cultivars were extracted from plants with 2 or 3 primordia leaves and grown in Murashige and Skoog (MS) medium containing thidiazuron (TDZ), with concentrations ranging between 0.02 μM and 5.0 μM, in 3- to 7-day periods, with naphthaleneacetic acid (NAA) and gibberellic acid (GA_3). The number of shoots from meristems was higher in 0.02 μM TDZ concentrations. In the case of caulinar apices, best results were obtained with 0.5 and 1.0 μM of TDZ. Abundant callus formation, length reduction of plant and inhibition of root formation were caused by the addition of TDZ. Propagation rate was comparable to other results for cotton reported in the literature.

Key words: TDZ, tissue culture, *Gossypium hirsutum.*

RESUMO. Thidiazuron como promotor de brotos múltiplos em explantes de algodão (*Gossypium hirsutum* L.). A maioria dos genótipos de interesse comercial de algodoeiro, *Gossypium hursutum* (Malvaceae), apresenta problemas de regeneração *in vitro*. Com o intuito de melhorar a taxa de regeneração dessa espécie, meristemas foliares e ápices caulinares das cultivares IAC 22 e COKER 312, foram extraídos de plântulas com 2 a 3 primórdios foliares e cultivados no meio de cultura Murashige e Skoog (MS), contendo Thidiazuron (TDZ) nas concentrações entre 0,02 μM e 5,0 μM e períodos de exposição de 3 a 7 dias, em associação com NAA e GA_3. O número de gemas axilares regeneradas a partir de meristemas foi maior na concentração de 0,02 μM de TDZ. A partir de ápices caulinares, os melhores resultados foram obtidos com 0,5 e 1,0 μM de TDZ. Formação abundante de calo, redução no comprimento da plântula e inibição radicular foram provocados pela adição de TDZ. A taxa de propagação foi comparável com outros resultados para algodão apresentados na literatura.

Palavras-chave: TDZ, cultura de tecidos, *Gossypium hirsutum*.

One of the most important factors for transformation in plants is the establishment of an efficient regeneration protocol of cells and/or tissues. In the specific case of cotton, most of the genotypes of commercial interest present problems of plant regeneration through callus or cells in suspension (Bayley *et al*., 1992). It has been considered a recalcitrant species to *in vitro* proliferation (Gupta *et al*., 1997). Therefore, improvement of methods of regeneration *in vitro* is necessary to make viable research on transformation and regeneration of this species.

Thidiazuron (TDZ) is one of the various kinds of substituted urea that has been investigated for induction of multiple shoots in plants. Although it has been initially developed as a herbicide, its use as a growth regulator in woody species *in vitro* is considered one of the most positive innovations in tissue culture during the last few years (Huetteman and Preece, 1993).

There are several reports about the positive effects of TDZ in the proliferation of axilary shoots. Huettman and Preece (1993) reported results by several authors from various species, in which positive effects of TDZ to the culture medium have been observed. Bhagwat *et al*. (1996) obtained multiple shoots of cassava from nodal explants by exposition to TDZ with concentrations ranging from 0.11 to 0.22 μM in liquid medium for 6 to 8 days, followed by culture in solid medium supplemented with 6-benziladenine and giberelic acid. High frequency of shoot regeneration from

primary leaf segments of pigeonpea (*Cajanus cajan)*, treated with TDZ only, or in association with indolacetic acid in a culture medium, has been reported by Eapen *et al*. (1998).

The impact of TDZ, in association with other growth regulators, on the proliferation of multiple shoots from different explants of cotton was evaluated in this paper.

Material and methods

Seeds of *Gossypium hirsutum* L. (Malvaceae) from IAC 22 and COKER 312 cultivars, provided by the Instituto Agronômico do Paraná (IAPAR), were quickly soaked in concentrated H_2SO_4 and superficially decontaminated in a commercial solution of sodium hypochlorite (2% of active chloride) for 30 minutes, followed by 3 washes in sterilized water. The seeds were transferred to a basal medium consisting of macro- and micronutrients of MS medium (Murashigue and Skoog, 1962), B5 vitamins (Gamborg *et al*., 1968), 3% of sucrose and 0,3% of phytagel. The pH of medium was adjusted to 5.8 before autoclaving. Two types of explants, shoot tip meristems and caulinar apices, were evaluated:.

a) *Meristem* – Meristems of the cultivar IAC22 were excised with 2 or 3 primordia leaves (approximately 5mm from the apex). Explants were maintained for 3 days in basal medium containing 0.01 μM of NAA and 0.1μM of GA_3, with the following concentrations of TDZ: 0, 0.05, 0.02, 0.01 and 0.1 μM. Explants were then transferred to basic medium plus 0.46 μM of kinetin. Shoots produced by each explant were evaluated for an additional period of 30 days.
b) *Caulinar apices* - Explants from cultivars COKER 312 and IAC 22 with about 5mm of caulinar apices below the apical meristematic region, were excised and transferred to the basic medium with the following concentrations of TDZ: 0; 0.5; 1; 2 and 5.0 μM. Explants were kept in the medium with TDZ for periods of 3, 5 and 7 days. After the exposition to TDZ, explants were transferred to basic medium supplemented with 0.46 μM of kinetin, where they remained for 30 days until evaluation.

In both experiments, the density of 5 explants per 50 ml of medium was used. Seven replications were used for each treatment. The experiments were carried out at 28°C and fluorescent light intensity of 70 $\mu M.m^{-2}.s^{-1}$, with photoperiod of 16 hours of light and 8 hours of dark. The following parameters were evaluated: survival of explants exposed to TDZ, number of shoots, length of shoots, and callus formation. Callus formation was evaluated through a comparative numeric scale varying from 0 to 5, in which 0 corresponded to absence of callus and 5 to abundant callus formation.

Results and discussion

The rate of meristem survival was very high, varying between 95% and 100% (data not shown), independent of the treatment applied to both cultivars. Nevertheless, a generalized chlorosis of the explants was observed. It revealed a phytotoxic effect caused by the exposition to TDZ.

Tables 1 and 2 give results obtained for meristematic explants and caulinar apices, respectively. The number of shoots per explant, shoot length and callus formation were stimulated by the addition of TDZ (Table 1). The number of shoots from meristems (Table 1) was higher at concentration 0.02 μM of TDZ associated with 0.1 μM of NAA and 0.1 μM of GA_3. Shoot length showed higher average values at concentrations 0.05 and 0.02 μM of TDZ, followed by 0.01 μM. The amount of callus formation increased in all treatments with TDZ. This effect is well known in the literature and has been reported in several papers (Huetteman and Preece, 1993).

Table 1. Effect of different concentrations of TDZ on *in vitro* development of cotton, cultivar IAC 22, obtained from meristematic explants in the presence of 0.1 μM of NAA and 0.1 μM of GA_3. Values between brackets represent the standard deviations of mean

Concentration of TDZ (μM)	Number of shoots per explant	Shoot length (cm)	Callus formation
0.10	1.95 (0.68)	0.56 (0.26)	4.3 (0.49)
0.05	0.80 (0.39)	1.48 (0.50)	4.5 (0.36)
0.02	5.80 (0.85)	1.38 (0.36)	3.8 (0.76)
0.01	2.00 (1.03)	1.00 (0.30)	2.9 (1.30)
Control	0.25 (0.43)	0.15 (0.06)	1.2 (0.25)

Table 2. Average number of shoots of cotton cultivars COKER 312 and IAC 22, obtained from caulinar apices explants exposed to different TDZ concentrations for 3, 5 and 7 days. Values between brackets represent the standard deviations of the mean

Concentration of TDZ (μM)	Exposition time					
	COKER 312			IAC 22		
	3 days	5 days	7 days	3 days	5 days	7 days
0.0	1.20 (0.69)	1.00 (0.87)	0.97 (0.72)	0.66 (0.44)	0.87 (0.13)	0.35 (0.18)
0.5	1.46 (0.41)	1.16 (0.74)	1.46 (0.76)	3.60 (1.56)	1.28 (0.25)	3.45 (0.60)
1.0	1.68 (0.92)	1.07 (0.67)	1.72 (0.36)	4.08 (1.53)	2.15 (1.18)	2.60 (0.56)
2.0	0.86 (0.50)	0.91 (0.44)	1.34 (0.37)	2.94 (1.41)	1.14 (0.85)	1.16 (0.65)
5.0	1.00 (0.58)	0.42 (0.16)	0.67 (0.30)	3.12 (0.97)	0.17 (0.08)	0.43 (0.21)

For caulinar apices explants (Table 2), concentrations 0.5 and 1.0 μM of TDZ provided higher regeneration rate of axilary shoots for all

exposition times and on both cultivars. Cultivar IAC 22 was more responsive compared to COKER 312. The effect of exposition time was not consistent, except for 5 μM TDZ, which presented higher number of shoots with 3 days and had a deleterious effect with exposition time of 5 and 7 days. Positive results of TDZ have also been reported by other authors such as Huetteman and Preece (1993) for several species, Bhagwat *et al*. (1996) for cassava and Eapen *et al*. (1998) for pigeonpea.

The rate of regeneration obtained in the present paper is within the range presented in the literature (Morre *et al*., 1998). Specifically for cotton, Gupta *et al*. (1997) reported shoot proliferation from several Indian cultivars of cotton, cultured on modified MS medium supplemented with cytokinins. Morre *et al*. (1998) obtained an average of 3.4 shoots per embryonic axis, for explants cultured on medium containing 13.3 μM of 6-benzyladenine (BA). Hemphill *et al*. (1998) demonstrated that explants with pre-existing meristems excised from several germlines, could be regenerated when cultured in MS medium supplemented with 0.3 μM of BA.

A reduction on plantlet size was evident in all experiments, due to the increase of TDZ concentration, for both cultivars evaluated. This occurred because there was a reduction of internode size proportional to the period of exposition to TDZ.

Inhibition of root development was also reported in the presence of TDZ, mainly under concentrations 2.0 μM and 5.0 μM (data not shown). This inhibition apparently varies with plant species. For example, Yusnita *et al*. (1990), working with *Acer saccharinum*, did not detect any inhibitory effect of TDZ on adventitious root formation, while Gray and Benton (1991) detected inhibitory effect of TDZ on *Vitis rotundifolia*.

Results presented in this paper indicate the feasibility of *in vitro* regeneration of cotton germplasms. The use of 0.02 μM of TDZ for meristematic explants and 0.5-1.0 μM of TDZ for caulinar apices enhanced propagation rate. The combination of TDZ with other growth regulators such as BA (Morre *et al*., 1998) may provide further increase on the number of regenerated explants. This will open new perspectives for plant transformation programs.

Acknowledgements

The authors would like to thank CNPq for the financial support given to this project.

References

BAYLEY, C. *et al*. Engineering 2,4-D resistance into cotton. *Theor. Appl. Genet.*, Berlin, v. 83, p. 645-649,1992.

BHAGWAT, B. *et al*. Simulation of *in vitro* shoot proliferation from nodal explants of cassava by thidiazuron, benzyladenine and gibberellic acid. *Plant Cell Tiss. Org. Cult.*, London, v. 46, p. 1-7, 1996.

EAPEN, S. *et al*. Thidiazuron-induced shoot regeneration in pigeonpea (*Cajanus cajan* L.). *Plant Cell Tiss. Org. Cult.*, London, v.53, p. 217-220, 1998.

GAMBORG, O. *et al*. Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res.*, Orlando, v. 150, p. 151-158, 1968.

GRAY, D.J.; BENTON, C.M. *In vitro* micropropagation and plant establishment of muscadine grape cultivars (*Vitis rotundifolia*). *Plant Cell Tiss. Org. Cult.,* London, v. 27, p. 7-14, 1991.

GUPTA, S.K. *et al*. *In vitro* proliferation of shoots and regeneration of cotton. *Plant Cell Tiss. Org. Cult.*, London, v.51, p. 149-152, 1997.

HEMPHILL, J.K. *et al*. Rapid in-vitro plant regeneration of cotton (*Gossypium hirsutum* L.). *Plant Cell Reports*, Berlin, v. 17, p. 273-278, 1998.

HUETTEMAN, C.A.; PREECE, J.E. Thidiazuron: a potent cytokinin for woody plant tissue culture. *Plant Cell Tiss. Org. Cult.,* London, v. 33, p. 105-119, 1993.

MORRE, J.L. *et al*. Multiple shoot induction and plant regeneration from embryonic axes of cotton. *Plant Cell Tiss. Org. Cult.,* London, v. 54, p. 131-136, 1998.

MURASHIGUE, T.; SKOOG, F. A revised medium for rapid growth and bioassay with tobacco cultures. *Physiol. Plant.,* Elmont, v. 15, p. 473-497, 1962.

YUSNITA, S. *et al*. Micropropagation of white flowering eastern redbud (*Cercis canadensis* var. *alba* L.). J. *Environ. Hort.*, Washington D.C., v. 8, p. 177-179, 1990.

Received on January 31, 2001.
Accepted on June 01, 2001.