Influence of growth regulators on plant regeneration in tomato

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ABSTRACT: We studied the effect of different plant growth regulators on *in vitro* regeneration and plant growth of three cultivars of tomato (*Lycopersicon esculentum* Mill.) from explants derived from hypocotyls and cotyledons of aseptically grown seedlings. The regeneration capacity was significantly influenced by cultivar and explant type. The highest number of shoots regenerated in both types of explants was recorded on MS medium supplemented with 1.0 mg/dm^3 zeatin and 0.1 mg/dm^3 IAA. The cultivar UC 82 showed the best regeneration capacity on all types of used media. The most responsive explants were hypocotyls with 90–92% regeneration in dependence on the used cultivars and mean production from 0.18 to 0.38 shoots per explant.

Keywords: culture *in vitro*; organogenesis; BAP; IAA; TDZ; ZEA; *Lycopersicon esculentum*

Tomato is one of the frequently studied higher plants because it is an important crop species with several advantages for genetic, molecular and physiological studies (McCormick et al. 1986). Several protocols for tomato regeneration *in vitro* are nowadays referred to. The most successful procedure is regeneration through adventitious organogenesis (Van Roekel et al. 1993; Frary, Earle 1996; Peres et al. 2001).

The *in vitro* morphogenetic responses of cultured plants are affected by different components of culture media, and so it is important to evaluate their effect on plant regeneration. Growth regulators used in regeneration media affect the formation of adventitious buds and shoots. Ohki et al. (1978) achieved the shoot differentiation ratio ranging from 14% to 30% when they used 6-benzylaminopurine (BAP), isopentenyladenosine (IPA) and kinetin as supplementary growth regulators. They obtained an optimal shoot regeneration rate from hypocotyl segments using IAA and IPA. However, shoot formation on hypocotyl segments was observed in the presence of naphthaleneacetic acid (NAA) combined with BA (Yakuwa et al. 1973). Better results were achieved using zeatin (ZEA) (McCormick et al. 1986; Hamza, Chupeau 1993) or zeatin riboside (Pfitzner 1998).

The purpose of our study was to evaluate the effects of different plant growth regulators on plant regeneration in tomato.

MATERIALS AND METHODS

Three cultivars of tomato Premium, Hana and UC 82 (*Lycopersicon esculentum* Mill.) were used in an experiment. Seeds were supplied by the Research Institute of Vegetables at Nové Zámky (Slovak Republic). The seeds were surface-sterilised by immersion into a 4% (v/v) solution of sodium hypochlorite for 15 min and rinsed four times with sterile distilled water. The seeds were then germinated in glass containers with 25 cm^3 of a half-strength medium

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Table 1. Composition of culture media

	T 1, T 4, T 7, T 10[b]	T 2[c]	T 3[d]	T 5[c]	T 6[d]	T 8[c]	T 9[d]	T 11[c]	T 12[d]
MS[a]	+	+	+	+	+	+	+	+	+
Sucrose (g/dm^3)	30	30	30	30	30	30	30	30	30
Agar (g/dm^3)	7	7	7	7	7	7	7	7	7
ZEA (mg/dm^3)		0.5	1					0.05	0.1
IAA (mg/dm^3)		0.05	0.1	0.1	0.2	0.05	0.1	0.05	0.1
BAP (mg/dm^3)				1.5	3			1	2
TDZ (mg/dm^3)						0.25	0.5		

[a]Murashige and Skoog (1962), [b]MS without plant growth regulators, [c]MS medium containing half concentration of plant growth regulators, [d]MS medium containing full concentration of plant growth regulators
ZEA – zeatin, IAA – indole 3-acetic acid, BAP – 6-benzylaminopurine, TDZ – thidiazuron

of Murashige and Skoog (1962) (hereinafter abbreviated as MS), 100 mg/dm^3 *myo*-inositol, 2 mg/dm^3 thiamine.HCl, 0.5 mg/dm^3 pyridoxine. HCl, 0.5 mg/dm^3 nicotinic acid, 1% (w/v) sucrose and 0.7% (w/v) agar. The cultures were initially kept in the dark at 27 ± 1°C for two days and then maintained under a 16 h photoperiod at 50 μmol m^2 s, with day/night temperature of 25°C/20°C. Hypocotyl and cotyledon segments were cut from the seedlings grown *in vitro*. The hypocotyls were cut into three segments. Each cotyledon was transversally cut into two segments. Hypocotyls were transversally cut into 4–7 mm segments and leaf-blades into pieces of 30–40 mm^2. The hypocotyl explants were placed horizontally on the medium surface and cotyledon explants with the adaxial surface in contact with the medium. Regeneration was induced on MS medium supplemented with different concentrations of cytokinins (ZEA, BAP, TDZ) and auxin (IAA) (Table 1). After 3 weeks cultured explants were transferred on: 1) MS without plant growth regulators (PGRs), 2) MS medium contains half concentration of PGRs and 3) MS medium with full concentration of PGRs (Fig. 1). The media were adjusted to pH 5.8 prior to autoclaving. Glass containers with 25 cm^3 of medium were used.

Six weeks later the regeneration capacity of explants was assessed. The following parameters were evaluated: the frequency of regeneration (percent of regenerating explants) and the number of shoots per explant. Data on regeneration frequency (%) were transformed by arcsin√x prior to statistical analysis. The experiment was repeated twice using 30 explants per variant. Significance of difference

Table 2. Effect of growth regulators on shoot regeneration of tomato explants and cultivars – the number of shoots/plated explant are means of 30 explants; data were taken after 6 weeks of culture

Media	Hypocotyl				Cotyledon			
	Premium	Hana	UC 82	mean	Premium	Hana	UC 82	mean
T 1	0.00	0.03	0.20	*0.08*^{a}	0.00	0.00	0.07	*0.02*^{ab}
T 2	0.30	0.47	0.53	*0.43*^{e}	0.00	0.03	0.10	*0.04*^{ab}
T 3	0.17	0.40	0.50	*0.36*^{cde}	0.17	0.10	0.37	*0.21*^{c}
T 4	0.47	0.00	0.30	*0.26*^{bcd}	0.00	0.03	0.10	*0.04*^{ab}
T 5	0.10	0.23	0.37	*0.23*^{abcd}	0.03	0.00	0.20	*0.08*^{b}
T 6	0.00	0.07	0.53	*0.20*^{abc}	0.03	0.00	0.20	*0.08*^{b}
T 7	0.37	0.10	0.67	*0.38*^{de}	0.00	0.00	0.07	*0.02*^{ab}
T 8	0.43	0.17	0.30	*0.30*^{bcde}	0.00	0.00	0.03	*0.01*^{ab}
T 9	0.37	0.30	0.33	*0.33*^{bcde}	0.00	0.00	0.00	*0.00*^{a}
T 10	0.37	0.03	0.10	*0.17*^{ab}	0.00	0.00	0.00	*0.00*^{a}
T 11	0.40	0.27	0.37	*0.34*^{cde}	0.03	0.07	0.07	*0.06*^{ab}
T 12	0.07	0.10	0.33	*0.17*^{ab}	0.07	0.00	0.03	*0.03*^{ab}
Cultivar class means	*0.25*^{a}	*0.18*^{a}	*0.38*^{b}		*0.03*^{a}	*0.02*^{a}	*0.10*^{b}	

Media 1–12 see Table 1; different letters mean significant differences

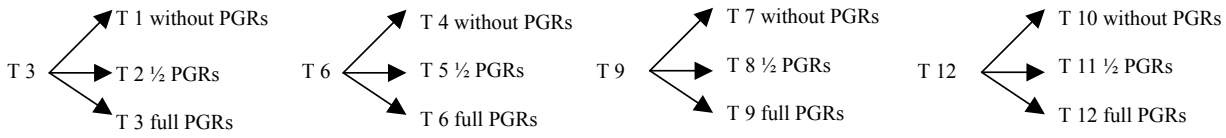

Fig. 1. Scheme of cultivation

between the results was estimated by analysis of variance (ANOVA). Variation among means was analysed using LSD ($P \leq 0.05$) procedure.

RESULTS

The effect of different tomato cultivars and different PGRs concentrations on shoot regeneration from aseptically grown hypocotyls and cotyledons showed significant variation in both the genotype and PGRs. A large variability in the number of shoots was observed between cultivars and between the different PGRs concentrations (Table 2). Cultivar mean comparisons (least significant difference, LSD, $P \leq 0.05$) showed only two classes differing in the induction potential. The cultivar which produced the most shoots on hypocotyl and cotyledon explants was UC 82. The lowest number of shoots was induced from cultivar Hana on both types of explants.

PGR mean comparisons (LSD, $P \leq 0.05$) gave five hypocotyl and three cotyledon different classes. The highest induction class for hypocotyl explants included two different ZEA – IAA combinations: 1.0–0.1 mg/dm^3 and 0.5–0.05 mg/dm^3, which gave 0.36 and 0.43 shoots per explant, respectively, and TDZ – IAA combination: 0.5–0.1 mg/dm^3 transferred after three weeks on MS without PGRs, which gave 0.38 shoots per explant. However, the highest induction class for cotyledon explants was observed only in ZEA – IAA combinations: 1.0–0.1 mg/dm^3, which gave 0.21 shoots per explant. The lowest numbers of shoots, 0.08, were produced when hypocotyl explants were transferred on MS without PGRs. No regeneration was observed on T 9 and T 10 medium (Fig. 1) on cotyledon explants (Table 1).

The frequency of regeneration calculated as the percentage of responding explants showed statistically significant differences between the media. The best responding media were T 2 (99%) and T 3 (96%) for hypocotyl and T 3 (94%) for cotyledon explants (Table 3). ZEA – IAA concentration treatments gave the same trend as with the mean number of shoots. The media which gave minimally 90% response on both explants were T 3, T 6, T 11 and T 12.

For cotyledon explants, cultivar UC 82 showed the highest regeneration frequency. All cultivars showed good regeneration capacity on hypocotyl explants. Statistically significant differences between the cul-

Table 3. Regeneration frequencies (%) of tomato explants and cultivars on MS media supplemented with different concentrations of growth regulators (percentages of explants regenerated after 6 weeks are means of 30 explants)

Media	Hypocotyl				Cotyledon			
	Premium	Hana	UC 82	mean	Premium	Hana	UC 82	mean
T 1	79	93	89	*87^{ab}*	44	64	76	*61^{a}*
T 2	100	96	100	*99^{c}*	70	90	96	*85de*
T 3	96	96	97	*96^{c}*	97	88	96	*94^{e}*
T 4	86	97	70	*84^{ab}*	77	70	70	*72^{abc}*
T 5	87	100	90	*92^{abc}*	86	87	88	*87de*
T 6	84	100	96	*93^{bc}*	100	77	100	*92^{e}*
T 7	80	87	87	*85^{ab}*	76	58	66	*67^{ab}*
T 8	100	87	93	*93^{bc}*	86	58	90	*78^{bcd}*
T 9	100	83	91	*91^{abc}*	90	74	97	*87^{cde}*
T 10	87	83	80	*83^{a}*	70	60	58	*63^{a}*
T 11	94	96	89	*93^{bc}*	83	97	91	*90de*
T 12	96	87	100	*94^{bc}*	90	89	96	*92de*
Cultivar class means	*91^{a}*	*92^{a}*	*90^{a}*		*81^{ab}*	*76^{a}*	*85^{b}*	

Media 1–12 see Table 1; different letters mean significant differences

tivars were observed only on cotyledons (Table 3). The cultivar × explant interaction was also statistically significant.

DISCUSSION

The *in vitro* morphogenetic responses of cultured plants are affected by different factors, and so it is important to evaluate their effects on plant regeneration. The most important factors are as follows: composition of basal medium, explant type, cultivar and growth regulators. So the effect of genotype on regeneration has been documented in a variety of crops. Although regeneration in tomato was successfully performed for a variety of cultivars for many years (Fillatti et al. 1987; Lu et al. 1997; Chen et al. 1999; Moghaieb et al. 1999), further studies on factors that may enhance the process as well as extend the genotypes amenable to *in vitro* manipulation are needed.

In our experiment, culture media with different PGRs commonly used for tomato regeneration were tested on Premium, Hana and UC 82 cultivars. The results of this experiment confirmed the positive effects of growth regulator addition on the number of shoots regenerated from tomato cotyledons and hypocotyls. Among the eight formulations tested, zeatin-supplemented media induced the highest mean shoot number per explant in all cultivars, and it was significantly different from all other formulations (Table 2). The presence of both ZEA and IAA in the culture medium was reported to promote shoot organogenesis in many papers (Ichimura, Oda 1995; Moghaieb et al. 1999; Nogueira et al. 2001; Arrillaga et al. 2001).

In this study, except the combination of ZEA and IAA in the culture medium the combination of TDZ and IAA also showed positive effects on shoot regeneration (Table 2). Krasnyanski et al. (2001) also studied the influence of TDZ on shoot organogenesis of cotyledon explants. In their experiment the mean number of shoots per explant was significantly increased by using the combination of BAP and TDZ, and by elevating the concentration of TDZ from 2.27 μM to 4.54 μM.

Analogously, ZEA addition to shoot regeneration media had a positive effect on the frequency of regeneration (Table 3). In our experiment, the frequency of regeneration was in the range from 83% to 99% on hypocotyl explants and from 61% to 94% on cotyledon explants. Both we and Moghaieb et al. (1999) studied regeneration of three tomato cultivars using hypocotyls and cotyledons as explants. The highest regeneration frequency in their experiment was 70.2% for hypocotyls and 35.3% for cotyledons while Nogueira et al. (2001) reported higher regeneration frequency (when using MS medium supplemented with 1.0 mg/dm^3 ZEA and 0.1 mg/dm^3 IAA), 92% or 85%, on cotyledon explants of the genotype Santa Clara or its natural mutant Firme, respectively.

Concluding remarks. This study confirmed our previous results that combining ZEA and IAA in the tissue culture medium had favourable effects on shoot organogenesis in tomato hypocotyl and cotyledon explants (Gubiš et al. 2003). The transfer of hypocotyl explants onto a medium with half concentration of ZEA and IAA showed positive effects on shoot organogenesis. Generally, on all media we observed the positive effects of explant transfer to a medium without PGRs or medium containing a half concentration of PGRs. The regeneration capacity of explants mainly depends on the cultivar and explant type. The highest regeneration capacity was observed in the cultivar UC 82 on both explant types.

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Vplyv rastových regulátorov na regeneráciu rastlín rajčiaka jedlého

ABSTRAKT: Bol študovaný vplyv rozdielnych rastových regulátorov na *in vitro* regeneráciu a rast hypokotylových a klíčnolistových explantátov 3 odrôd rajčiaka jedlého (*Lycopersicon esculentum* Mill.), odvodených z asepticky naklíčených klíčencov. Regeneračná kapacita bola signifikantne ovplyvnená odrodou a typom explantátu. Najvyšší počet výhonkov regenerujúcich na oboch typoch explantátov bol zaznamenaný na médiu doplnenom 1,0 mg/dm^3 zeatínom a 0,1mg/dm^3 IAA. Najlepšiu regeneračnú kapacitu vykazovala odroda UC 82 na všetkých použitých typoch médií. Najlepšie reagujúcim explantátom boli hypokotyly s 90–92% regeneráciou v závislosti na použitej odrode a s priemernou tvorbou výhonkov 0,18–0,38 na explantát.

Kľúčové slová: *in vitro* kultúra; organogenéza; BAP; IAA; TDZ; ZEA; *Lycopersicon esculentum*

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