Effect of Genotype and Explant Type on Shoot Regeneration in Tomato (*Lycopersicon esculentum* Mill.) *in vitro*

Jozef GUBIŠ[1], Zuzana LAJCHOVÁ[2], Juraj FARAGÓ[2] and Zuzana JUREKOVÁ[3]

[1]Department of Resistance Genetics, Research Institute of Plant Production in Piešťany, Slovak Republic; [2]Department of Cell and Molecular Biology, Research Institute of Plant Production in Piešťany, Slovak Republic; [3]Laboratory of Explant Cultures and Biomolecular Methods, Slovak University of Agriculture, Slovak Republic

Abstract: The regeneration capacity of six types of explants (segments from hypocotyl, cotyledons, epicotyl, leaf, internodes and petiole) was compared in 13 cultivars of tomato (*Lycopersicon esculentum* Mill). Explants were cultured on a regeneration medium containing 1 mg/l zeatin and 0.1 mg/l indole-3-acetic acid. The number of shoot primordia and shoots with 1 or more fully developed leaves was evaluated after 6 weeks. The regeneration capacity was significantly influenced by cultivars and explant types. The total number of shoot primordia produced in all types of explants was highest in the cultivars Hana and Premium and lowest in UC 82 and Money Marker. Cv. Hana also produced the highest number of shoots. The most responsive explants in most cultivars were hypocotyls and epicotyls with up to 100% regeneration and mean production of 6.3 and 6.5 shoot primordia per explant, respectively.

Keywords: culture *in vitro*; organogenesis; Murashige-Skoog medium; indole-3-acetic acid; zeatin

In tomato, genetic transformation with regeneration *in vitro* has been successfully used for genetic improvement (Lindsey 1992). Tolerance to herbicides, resistance to pests and diseases, production of edible vaccines or other novel bioproducts and quality improvement are the most important goals of genetic plant modification. Regeneration of whole fertile plants from appropriate tissues *in vitro* is important in genetic plant transformation. Shoot formation from explants of apical meristems, cotyledons, stems, petioles, leaves, anthers and inflorescences has been reported in tomato (Young *et al*. 1987; Branca *et al*. 1990; Compton & Veilleux 1991).

The most successful procedure up to date is regeneration through adventitious organogenesis (Van Roekel *et al*. 1993; Frary & Earle 1996; Peres *et al*. 2001). *In vitro* plant regeneration has been found to depend on many factors, of which most important are: composition of the basic medium, growth regulators, gelling agent, light intensity and quality, photoperiod, temperature, cultivation vessels and vessel covers (Reed 1999). The purpose of our work was to examine the effect of cultivar and explant type on shoot regeneration *in vitro* in tomato.

MATERIALS AND METHODS

We used 13 cultivars of tomato (*Lycopersicon esculentum* Mill.). Seeds of the cultivars Moldy, Istria, Orbit, Aneta, Denár, Robura, Titan, Premium, Hana, Opál, Red Hunter and UC 82 , were provided by the Research Institute of Vegetables at Nové Zámky (Slovak Republic). The cultivar Money Maker was obtained from the Gene Bank

Supported by the Scientific Grant Agency VEGA of the Ministry of Education, Slovak Republic, Grant No. 1/9075/02 and by the Slovak Academy of Sciences.

of the Czech Republic at the Research Institute of Crop Production, Prague-Ruzyně. The seeds were surface-sterilised by immersion of seeds into a 4% (v/v) solution of sodium hypochlorite for 15 min and rinsed four times with in sterile distilled water. The seeds were then germinated in glass containers with 25 ml of a half-strength medium of Murashige and Skoog (1962) (abbreviated further as MS), 100 mg/l *myo*-inositol, 2 mg/l thiamine.HCl, 0.5 mg/l pyridoxine.HCl, 0.5 mg/l nicotinic acid, 1% (w/v) sucrose and 0.6% (w/v) agar. The cultures were initially kept for two days in the dark at 27 ± 1°C and then maintained under a 16 h photoperiod at 50 μmol/m^2/s, with day/night temperature of 25°C/20°C. From the seedlings, grown *in vitro*, hypocotyl, cotyledon, epicotyl, leaf, petiole and internode segments were cut. Nodal explants and shoot tips of seedlings were placed into bacteriological tubes with a modified MS medium. In it the concentration of three salts was changed to 380 mg/l KNO_3, 330 mg/l NH_4NO_3 and 74 mg/l $MgSO_4.7H_2O$, as suggested by Frary and Earle (1996). The hypocotyls were cut into a lower, middle and upper segment. Each cotyledon was transversally cut into a proximal and a distal half-segment. Segments of epicotyls, internodes, and leaves were isolated from three weeks old nodal explants regenerating on the modified MS medium. Epicotyls, petioles and internodes were transversally cut into 4–7 mm segments and leaf-blades into pieces of 30–40 mm^2. The explants were placed horizontally on the medium surface, cotyledon and leaf-blade explants with the adaxial surface in contact with the medium. Regeneration was induced by a MS medium supplemented with 1 mg/l zeatin (ZEA) and 0.1 mg/l indole-3-acetic acid (IAA) (Ichimura & Oda 1995). The media were adjusted to pH 5.8 prior to autoclaving. Petri dishes with 25 ml of medium were used. The regeneration ability of explants was assessed six weeks later. The following parameters were evaluated: frequency of regeneration (percents of regenerating explants), the number of shoots per explant and the number of shoot primordia per explant.

The experiment was repeated two times with 20–40 explants per variant (depending on the explant type). Significance of differencies between the results was estimated by Analysis of Variance (ANOVA) with data transformed by ln (x + 1). Variation among means was analysed using Tukey's procedure.

RESULTS

In total, 1483 tomato explants were cultivated on a MS medium supplemented with 1 mg/l ZEA and 0.1 mg/l IAA. On the cutting edges developed within two weeks yellow-green calli, on which shoot primordia and later shoots appeared. The cultivars and also the explant types differred significantly in regeneration (Table 1). Hypocotyls and epicotyls

Table 1. Regeneration frequencies (%) in 13 cultivars of different tomato explants on MS medium (Murashige & Skoog 1962) supplemented with 1 mg/l zeatin and 0.1 mg/l indole-3-acetic acid

Cultivar	Percentage of regenerating explants					
	hypocotyl	cotyledon	epicotyl	leaf	petiole	internode
Moldy	86	83	83	86	0	10
Istria	100	58	100	71	10	11
Orbit	100	67	100	25	0	20
Aneta	100	67	100	51	0	60
Denár	100	92	100	52	0	40
Robura	100	92	100	72	10	40
Titan	100	92	65	76	0	50
Red Hunter	100	100	83	30	10	10
Premium	100	100	100	100	0	80
Hana	100	75	100	90	60	20
Opál	100	92	100	58	0	10
UC 82	100	67	65	66	10	40
Money Maker	100	92	59	3	5	25

regenerated by 100% in nearly all cultivars. The regeneration was frequent also in cotyledons and leaf explants, while petioles showed no or poor response. Among the cultivars Premium showed the highest regeneration frequency on all types of explants except from petioles. The cultivars Hana and Robura showed also a good regeneration capacity.

Statistically significant differences in the number of shoots and shoot primordia per explant were

Table 2. Regeneration of adventitious shoot primordia in different tomato explants and cultivars on Murashige and Skoog (1962) medium supplemented with growth regulators. The data were taken after 6 weeks of culture

Cultivar/explant	Number of shoots primordia/planted explant ± SE*						
	hypocotyl	cotyledon	epicotyl	leaf	petiole	internode	mean
Moldy	4.56 ± 1.03	4.17 ± 0.77	3.83 ± 1.70	5.57 ± 0.92	0.0 ± 0.0	0.1 ± 0.1	3.04^{ab}
Istria	7.11 ± 0.35	2.83 ± 0.83	9.46 ± 0.31	6.25 ± 0.91	0.3 ± 0.3	0.22 ± 0.22	4.36^{bc}
Orbit	5.67 ± 0.75	4.17 ± 0.94	6.0 ± 0.96	1.44 ± 0.41	0.0 ± 0.0	1.2 ± 1.0	3.08^{ab}
Aneta	6.89 ± 0.11	3.42 ± ±0.86	8.5 ± 1.0	3.62 ± 0.51	0.0 ± 0.0	4.8 ± 1.50	4.54^{abc}
Denár	6.89 ± 0.11	4.5 ± 0.65	8.17 ± 1.05	2.04 ± 0.57	0.0 ± 0.0	0.7 ± 0.40	3.72^{abc}
Robura	6.89 ± 0.11	4.58 ± 0.68	8.4 ± 0.87	5.03 ± 0.74	0.8 ± 0.8	0.5 ± 0.22	4.37^{abc}
Titan	5.78 ± 0.60	4.33 ± 0.58	2.0 ± 0.46	5.31 ± 0.56	0.0 ± 0.0	1.4 ± 0.50	3.14^{ab}
Red Hunter	6.22 ± 0.66	6.55 ± 0.21	7.83 ± 1.64	1.86 ± 0.45	0.4 ± 0.4	0.2 ± 0.2	3.84^{abc}
Premium	6.33 ± 0.55	4.42 ± 0.81	9.27 ± 0.26	8.23 ± 0.51	0.0 ± 0.0	4.8 ± 1.50	5.51^{c}
Hana	6.67 ± 0.24	4.08 ± 0.87	9.8 ± 0.2	6.46 ± 0.68	6.0 ± 1.63	1.5 ± 1.07	5.75^{c}
Opál	6.11 ± 0.35	4.5 ± 0.48	5.0 ± 1.51	3.91 ± 0.72	0.0 ± 0.0	0.1 ± 0.1	3.27^{ab}
UC 82	6.0 ± 0.5	1.75 ± 0.51	2.43 ± 0.58	2.7 ± 0.41	0.2 ± 0.2	1.1 ± 0.55	2.36^{a}
Money Maker	6.96 ± 0.04	3.5 ± 0.49	3.67 ± 0.70	0.13 ± 0.05	0.33 ± 0.26	0.4 ± 0.14	2.50^{ab}
Mean	6.31^{d}	4.06^{c}	6.50^{d}	4.04^{c}	0.62^{a}	1.31^{b}	

*values followed by the same letters are not significantly different at α = 0.05

Table 3. Adventitious shoot regeneration of tomato explants and cultivars cultured on Murashige and Skoog (1962) medium supplemented with growth regulators. The data were taken after 6 weeks of culture

Cultivar/explant	Number of shoots/planted explant ± SE*						
	hypocotyl	cotyledon	epicotyl	leaf	petiole	internode	mean
Moldy	0.89 ± 0.20	0.33 ± 0.19	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.22^{bc}
Istria	0.56 ± 0.24	0.17 ± 0.11	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.12^{ab}
Orbit	0.0 ± 0.0	0.08 ± 0.08	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.03^{a}
Aneta	0.33 ± 0.17	0.42 ± 0.15	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.21	0.17^{ab}
Denár	0.22 ± 0.15	0.5 ± 0.19	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.12^{abc}
Robura	0.22 ± 0.15	0.08 ± 0.08	0.1 ± 0.1	1.36 ± 0.16	0.0 ± 0.0	0.0 ± 0.0	0.29^{c}
Titan	0.33 ± 0.17	0.5 ± 0.19	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.14^{ab}
Red Hunter	0.33 ± 0.17	0.72 ± 0.24	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.18^{abc}
Premium	0.89 ± 0.26	0.42 ± 0.19	0.18 ± 0.08	0.09 ± 0.05	0.0 ± 0.0	0.0 ± 0.0	0.26^{bc}
Hana	0.78 ± 0.22	1.0 ± 0.30	1.6 ± 0.51	0.03 ± 0.03	0.6 ± 0.27	0.1 ± 0.1	0.68^{d}
Opál	0.44 ± 0.18	0.08 ± 0.08	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.09^{ab}
UC 82	0.0 ± 0.0	0.58 ± 0.29	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.13	0.13^{ab}
Money Maker	0.59 ± 0.11	0.25 ± 0.08	0.12 ± 0.12	0.0 ± 0.0	0.0 ± 0.0	0.05 ± 0.03	0.17^{bc}
Mean	0.43^{b}	0.40^{b}	0.15^{a}	0.11^{a}	0.05^{a}	0.07^{a}	

*values followed by the same letters are not significantly different at α = 0.05

found between cultivars and also between explants (Tables 2 and 3). Statistical cultivar x explant interactions were also significant. The highest number of shoots and shoot primordia was obtained in the cultivar Hana (Tables 2 and 3). The cultivar Premium showed a similar number of shoot primordia, but a significantly lower number of shoots per explant.

The highest number of shoot primordia regenerated on hypocotyl and epicotyl explants (Table 2), while the highest number of shoots formed on hypocotyl and cotyledon explants (Table 3). The shoot production on different explant types was variable. Most cultivars produced shoots from cotyledon or hypocotyl explants. A few cultivars regenerated shoots also from explants of epicotyls (4 cultivars), leaves (3), internodes (6) or petioles (1). The highest number of shoots per explant regenerated in the cultivar Hana on epicotyl explants (1.6) and in the cultivar Robura on leaf-blade explants (1.36). The regeneration ability of cotyledon explants ranged from 0.08 to 1.0 shoots per explant and of hypocotyl explants from 0–0.89 shoots per explant (Table 3).

DISCUSSION

In vitro culture is used in tomato in different biotechnological applications, such as clonal propagation, production of virus-free plants (Moghaieb *et al*. 1999), genetic transformation (Frary & Earle 1996; Ling *et al*. 1998) and in many fundamental research programes (Hanus-Fajerska 2000; Arrillaga *et al*. 2001). Most of the reports about adventitious regeneration in tomato deal with induction of regeneration in hypocotyl or cotyledon explants (Asakura *et al*. 1995; Ichimura & Oda 1995; Moghaieb *et al*. 1999). To our knowledge, complex work on regeneration ability of 6 various types of explants in different tomato cultivars has not yet been described. In our work we compared the regeneration ability of 6 seedling-derived explant types in 13 tomato cultivars.

Money Maker is reported as the most frequently used cultivar for regeneration and transformation of tomato (Van Roekel *et al*. 1993; Frary & Earle 1996; Ling *et al*. 1998; Bertram & Lercari 2000; Hanus-Fajerska 2000). Thus we decided to use it as a reference cultivar to compare the regeneration capacity of 12 tomato cultivars of Slovak or Czech origin. The best capacity of shoot primordia regeneration was observed in the cultivars Hana, Premium and Robura, while Money Maker showed one of the lowest regeneration capacity. Only regeneration from hypocotyls was similar to the regeneration ability of the 3 cultivars mentioned above.

Moghaieb *et al*. (1999) studied regeneration of 3 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. Their results were in agreement with results of others (Philips & Collings 1984; Aly *et al*. 1994). But in our work the regeneration capacity of hypocotyls was much higher, 100% in 12 out of 13 cultivars. Nogueira *et al*. (2001) observed high regeneration frequency 92% and 85% on cotyledon explants of the cultivars Santa Clara and its natural mutant Firme, respectively, but the average number of shoots regenerated per explant was lower than 2. Moghaieb *et al*. (1999) obtained about 7.2 shoots per explant using 1 mg/l zeatin in the medium. In our experiment, the cultivars Hana and Premium regenerated 11.4 and 9.45 shoots and shoot primordia per epicotyl explant.

Arrillaga *et al*. (2001) reported in wild tomato *Lycopersicon cheesmanii* regeneration of 5.8 and 6.0 shoots using leaf and cotyledon explants, respectively, when zeatin was added to the culture medium. Ichimura and Oda (1995) used the same medium as we did, but the aim of their study was to study the effect of different agar concentrations and different gelling agents on shoot regeneration in cultivated tomato. They used only one cultivar (Zuiken) and obtained 0–0.8 shoots per cotyledon explant on agar media.

Cultivar, explant type and medium composition are considered the three main factors affecting *in vitro* plant regeneration in many plant species. In this work, we observed statistically significant differences in regeneration capacity between genotypes and between explant types, expressed as frequency of regeneration and average number of shoot primordia and shoots per explant.

Concluding remarks. We were able to achieve plant regeneration in 13 tomato cultivars using 6 different explant types. The regeneration capacity of explants strongly depended on the cultivar and explant type. The highest regeneration capacity was observed in the cultivars Hana and Premium and with hypocotyl explants. Among the explant types cotyledon segments showed the highest shoot regeneration ability.

Acknowledgements: We thank Doc. M. Valšíková from the Research Institute of Vegetables Nové Zámky for supplying seeds of 12 cultivars and the Gene Bank of Czech Republic at the Research Institute of Crop Production, Prague-Ruzyně, for supplying seeds of the cultivar Money Maker.

References

Aly M.A.M., Khali M.F.M., Moghaieb R.E.A., Sawsan S.Y., El-Sharkawy A.M.A. (1994): Somatic embryogenesis in berseem (*Trifolium alexanderinum* L.): the influence of genetic background. Egypt. J. Genet. Cytol., **23**: 131–141.

Arrillaga I., Gisbert C., Sales E., Roig L., Moreno V. (2001): *In vitro* plant regeneration and gene transfer in the wild tomato *Lycopersicon cheesmanii*. J. Hortic. Sci. Biotechnol., **76**: 413–418.

Asakura N., Misoo S., Kamijama O., Sawano M. (1995): High frequency regeneration of diploids from apical end of cultured hypocotyl tissue in tomato. Breeding Sci., **15**: 455–459.

Bertram L., Lercari B. (2000): Phytochrome A and phytochrome B1 control the acquisition of competence for shoot regeneration in tomato hypocotyl. Plant Cell Rep., **19**: 604–609.

Branca C., Bucci G., Domiano P., Ricci A., Bassi M. (1990): Auxin structure and activity on tomato morphogenesis *in vitro* and pea stem elongation. Plant Cell Tiss. Org. Cult., **24**: 105–114.

Compton M.E., Veilleux R.E. (1991): Shoot, root and flower morphogenesis on tomato inflorescence explants. Plant Cell Tiss. Org. Cult., **24**: 223–231.

Frary A., Earle E.D. (1996): An examination of factors affecting the efficiency of *Agrobacterium*-mediated transformation of tomato. Plant Cell Rep., **16**: 235–240.

Hanus-Fajerska E. (2001): Studies on the reaction in tissue culture of tomato genotypes under biotic stress. Acta Soc. Bot. Pol., **70**: 5–10.

Ichimura K., Oda M. (1995): Stimulation of shoot regeneration from cotyledon segments of tomato (*Lycopersicum esculentum* Mill.) by agar and its extract. J. Jpn Soc. Hortic. Sci., **64**: 135–141.

Lindsey K. (1992): Genetic manipulation of crop plants. J. Biotechnol., **26**: 1–28.

Ling H-Q., Kriseleit D., Ganal M.W. (1998): Effect of ticarcillin/potassium clavulanate on callus growth and shoot regeneration in *Agrobacterium*-mediated transformation of tomato (*Lycopersicon esculentum* Mill.). Plant Cell Rep., **17**: 843–847.

Moghaieb R.E.A., Saneoka H., Fujita K. (1999): Plant regeneration from hypocotyl and cotyledon explant of tomato (*Lycopersicon esculentum* Mill.). Soil Sci. Plant Nutr., **45**: 639–646.

Murashige T., Skoog F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plantarum, **15**: 473–497.

Nogueira F.T.S., Costa M.G., Figueira M.L., Otoni W.C., Finger F.L. (2001): *In vitro* regenerations of "Santa Clara" tomato plantlets and its natural mutant "Firme". Sci. Agrotec., Lavras, **25**: 36–71.

Peres L.E.P., Morgante P.G., Vecchi C., Kraus J.E., van Sluys M-A. (2001): Shoot regeneration capacity from roots and transgenic hairy roots of tomato cultivars and wild related species. Plant Cell Tiss. Org. Cult., **65**: 37–44.

Philips G.C., Collins G.B. (1984): Red clover and other forage crops. In: Sharp W.R., Evans D.A., Ammirato P.V., Yanada Y. (eds): Hand Book of Plant Cell Culture. Macmillan Publ. Co., New York: 169–210.

Reed B.M. (1999): Desing a micropropagation system: Workshop presentation from the 1998 SIVB Congr. on i*n vitro* Biology. In Vitro Cell Dev. Biol.-Plant, **35**: 275–284.

Van Roekel J.S.C., Damm B., Melchers L.S., Hoekema A. (1993): Factors influencing transformation frequency of tomato (*Lycopersicum esculentum*). Plant Cell Rep., **12**: 644–647.

Young R., Kaul V., Williams E.G. (1987): Clonal propagation *in vitro* from immature embryos and flower buds of *Lycopersicon peruvianum* and *L. esculentum*. Plant Sci., **52**: 237–242.

Received for publication October 25, 2002
Accepted after corrections February 10, 2003

Abstrakt

Gubiš J., Lajchová Z., Faragó J., Jureková Z. (2003): **Vliv genotypu a typu explantátu na regeneraci výhonů rajčete jedlého** (*Lycopersicon esculentum* Mill.) ***in vitro***. Czech J. Genetic. Plant Breed., **39**: 9–14.

Byla porovnávána regenerační schopnost šesti typů explantátů (hypokotyly, klíční listy, epikotyly, listy, řapíky a internodia) 13 odrůd rajčete jedlého (*Lycopersicon esculentum* Mill.). Explantáty byly kultivovány na regeneračním Murashige a Skoog (1962) médiu s přídavkem 1 mg/l zeatinu a 0,1 mg/l kyseliny indolyl-3-octové. Počet

základů výhonů a počet výhonů s alespoň jedním vyvinutým listem byly hodnocené po šesti týdnech kultivace. Regenerační kapacita byla signifikantně závislá na odrůdě a typu explantátu. Celkový počet základů výhonů při všech typech explantátů byl nejvyšší u odrůdy Hana a Premium a nejnižší u UC 82 a Money Maker. Odrůda Hana tvořila také nejvyšší počet výhonů. Nejlépe odpovídající explantáty u většiny odrůd byly hypokotyly a epikotyly s téměř 100% frekvencí regenerace a průměrnou tvorbou základů výhonů 6,3 a 6,5 na explantát.

Klíčová slova: *in vitro* kultura; organogeneze; Murashige-Skoog médium; kyselina indolyl-3-octová; zeatin

Corresponding author:

Ing. Jozef Gubiš, Výskumný ústav rastlinnej výroby, oddelenie genetiky rezistencie, Bratislavská cesta 122, 921 68 Piešťany, Slovenská republika
tel.: + 421 337 722 311, fax: + 421 337 726 306, e-mail: gubis@vurv.sk