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Effect of growth regulators on shoot induction and plant regeneration in tomato (*Lycopersicon esculentum* Mill.)

Jozef GUBIŠ[1], Zuzana LAJCHOVÁ[1], Juraj FARAGÓ[1]* & Zuzana JUREKOVÁ[2]

[1]*Division of Applied Genetics and Breeding, Research Institute of Plant Production in Piešťany, Bratislavská cesta 122, SK-92168 Piešťany, Slovakia, phone: ++ 421 33 7722 311, fax: ++ 421 33 7726 306, e-mail: farago@vurv.sk*
[2]*Laboratory of Explant Cultures & Biomolecular Methods, Slovak University of Agriculture, Tulipánová 7, SK-94901 Nitra, Slovakia*

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The effect of different growth regulators on *in vitro* growth and plant regeneration of tomato (*Lycopersicon esculentum* Mill.) explants, derived from hypocotyls and cotyledons of aseptically grown seedlings, was studied. With regard to the regeneration frequency, number of shoot primordia and shoots per explant, the best regeneration medium was the Murashige-Skoog (MS) medium supplemented with 1 mg L^{-1} of zeatin and 0.1 mg L^{-1} of indole-3-acetic acid. In all genotypes studied, 100% frequency of regeneration was observed when hypocotyl explants were used.

Key words: cytokinin, genotype, organogenesis, shoot primordium, explant type.

Abbreviations: BAP, 6-benzylaminopurine; IAA, indole-3-acetic acid; MS, Murashige & Skoog (1962) medium; NAA, 1-naphthaleneacetic acid; ZEA, zeatin.

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is considered to be an important vegetable crop and a model species for introduction of agronomically important genes into dicotyledonous crop plants (WING et al., 1994). Nevertheless, morphogenesis in cultivated tomato has been achieved less frequently compared to other members of the family *Solanaceae*, such as *Nicotiana* spp., *Petunia* spp., and *Solanum* spp., known for their amenability to *in vitro* culture. The most frequently used way of regeneration in tomato is via shoot organogenesis from callus from leaf or cotyledon explants or directly from thin cell layers of the inflorescence (COMPTON & VEILLEUX, 1991).

In vitro regeneration through organogenesis and somatic embryogenesis can be used for multiplication of genetically identical clones and it is an integral part of genetic transformation procedures. The *in vitro* morphogenetic responses of cultured plants are affected by different components of the culture media and it is important to evaluate their effect on plant regeneration. Although advances

* Corresponding author

are being made toward better understanding of metabolic processes correlated with regeneration (Lambé et al., 1997; Cairney et al., 2000), determining of the conditions for *in vitro* plant regeneration is still largely an empirical process. Thus, *in vitro* regeneration can be difficult to achieve for some plant species or particular genotypes within a species.

Among the *Lycopersicon* species, *L. peruvianum* is considered to be highly organogenetic and regeneration of shoots from roots has already been documented (Koornneeff et al., 1993). Other genotypes were also described for their ability to form shoots on calli derived from hypocotyls (*L. pimpinellifolium* WV700; Faria & Illg, 1996), cotyledons (*L. esculentum* cv. UC82 B; Hamza & Chupeau, 1993), suspension cells (*L. esculentum* cv. VFNT; Meredith, 1979) and protoplasts (*L. esculentum* cv. Lukullus; Morgan & Cocking, 1982).

In this paper we compare the effect of different growth regulators on shoot induction and plant regeneration of tomato.

Material and methods

Seeds of tomato (*Lycopersicon esculentum* Mill.) cultivars Hana and Premium, used in the experiments, were provided by the Research Institute of Vegetables (Nové Zámky, Slovakia) and those of the cultivar Money Maker by the Gene Bank of the Czech Republic at the Research Institute of Crop Production (Prague, Czech Republic). Surface-sterilization was performed by immersion of seeds into a solution of 4% (v/v) sodium hypochlorite for 15 min and then by four rinses in sterile distilled water. Thereafter, the seeds were allowed to germinate in glass containers containing 25 mL of half-strength MS medium containing the MS salts (Murashige & Skoog, 1962), 100 mg L^{-1} *myo*-inositol, 2 mg L^{-1} thiamine · HCl, 0.5 mg L^{-1} pyridoxine · HCl, 0.5 mg L^{-1} nicotic acid, and 1% (w/v) sucrose. The regeneration medium was solidified with 0.6% (w/v) agar. Cultures were cultivated initially for two days in dark at 27±1 °C temperature and then they were maintained under photoperiod of 16 h illumination with light intensity of 50 μmol m^{-2} s^{-1} (25 °C) and 8 h dark (20 °C).

The *in vitro* grown seedlings were used as source of two types of explants: hypocotyl and cotyledon segments. Shoot tip explants with apical meristem were planted into bacteriological tubes containing modified MSOm medium, which contained modified MS salts (380 mg L^{-1} KNO_3, 330 mg L^{-1} NH_4NO_3 and 74 mg L^{-1} $MgSO_4 \cdot 7H_2O$ (all other components were at the usual levels), as suggested by Frary & Earle (1996). Hypocotyls were cut into three segments (lower, middle and upper), and these explants were placed horizontally on the surface of regeneration medium. Each cotyledon was transversally cut into two half-segments (proximal and distal), which were placed with the adaxial surface in contact with the regeneration medium. For induction of regeneration, five different media were used: (i) MS1 – MS medium without growth regulators (control); (ii) MS2 – MS medium + 1 mg L^{-1} ZEA + 0.1 mg L^{-1} IAA (Ichimura & Oda, 1995); (iii) MS3 – MS medium + 1 mg L^{-1} BAP + 0.1 mg L^{-1} NAA; (iv) MS4 – MS medium + 2 mg L^{-1}BAP + 0.2 mg L^{-1} NAA; and (v) MS5 – MS medium + 4 mg L^{-1} BAP + 0.4 mg L^{-1} NAA; where ZEA stands for zeatin, BAP 6-benzylaminopurine, IAA indole-3-acetic acid and NAA 1-naphthaleneacetic acid.

The media were adjusted to pH 5.8 prior to autoclaving and 25 mL of these media were dispensed into each of Petri dishes. Regeneration of explants was assessed after six weeks. The following parameters were evaluated: the frequency of regeneration (No. of regenerating explants/No. of plated explants) × 100 and the number of shoots and shoot primordia/explant plated. The experiments were repeated two times and data were analysed at 5% significance level using analysis of variance (ANOVA).

Results and discussion

The *in vitro* morphogenic responses of cultured plant tissues are affected by the different components of the culture media, especially by concentration of growth hormones, and it is therefore important to evaluate their effects on plant regeneration. Tomato is one of the most studied higher plants because of its importance as a crop species, and of several advantages for genetic, molecular and physiological studies (McCormick et al., 1986).

Two explant types, derived from cotyledons and hypocotyls, were isolated from seedlings of 3 tomato cultivars. Fifty segments from each type of explant were cultured on each type of MS medium supplemented with growth regulators. Previous studies demonstrated that 8 to 10 day-old cotyledons of tomato were superior to other sources of explants, including hypocotyls, stems and leaves for promoting shoot organogenesis of tomato (Hamza & Chupeau, 1993; Van Roekel et al., 1993; Ling et al., 1998). In our experiments 8-day-old *in vitro* seedlings were used as source of explants.

The frequency of adventitious shoot regeneration differed depending on the type of explants and both the type and concentration of growth regulators added to the regeneration medium (Fig. 1). The medium supplemented with 1 mg L^{-1} ZEA and 0.1 mg L^{-1} IAA (MS2 medium) was the most effective (100%) in induction of adventitious shoots from hypocotyl explants in all cultivars and

Table 1. Adventitious shoot regeneration of tomato explants and cultivars cultured on MS medium supplemented with growth regulators. The data were taken after 6 weeks of culture.

(A)	number of shoots primordia/regenerating explant ±SE*					
medium	Premium		Hana		Money Maker	
	hypocotyl	cotyledon	hypocotyl	cotyledon	hypocotyl	cotyledon
MS1	3.33±1.86	0	5.00±2.00	0	5.00±0.40	0
MS2	6.33±0.55	5.89±0.35	6.67±0.24	5.44±0.67	6.96±0.04	5.25±0.40
MS3	2.40±0.68	4.13±0.48	3.50±0.57	5.44±0.60	2.96±0.27	5.79±0.33
MS4	3.50±0.85	4.33±0.82	3.50±0.65	4.64±0.53	4.25±0.47	5.30±0.36
MS5	3.17±0.87	3.11±0.75	5.60±0.87	4.64±0.65	4.00±0.47	5.10±0.35
mean	3.75^{a}	3.49^{a}	4.85^{b}	4.03^{ab}	4.63^{ab}	4.29^{b}

(B)	number of shoots primordia/regenerating explant ±SE*					
medium	Premium		Hana		Money Maker	
	hypocotyl	cotyledon	hypocotyl	cotyledon	hypocotyl	cotyledon
MS1	0	0	2.00±0.00	0	1.08±0.08	0
MS2	1.33±0.21	1.25±0.25	1.17±0.17	1.71±0.26	1.07±0.07	1.13±0.13
MS3	0	1.00±0.00	0.50±0.50	0	0.50±0.50	1.20±0.20
MS4	0	0	0.50±0.50	0.50±0.50	0	0
MS5	0	0	0	0	0	0
mean	0.27^{a}	0.45^{a}	0.83^{c}	0.44^{a}	0.53^{b}	0.47^{a}

[a] For each parameter (mean number of shoot primordia and shoots), values followed by the same letters are not significantly different at $\alpha = 0.05$. The composition of the media is explained in the Materials and methods section.

from cotyledon explants in cultivar Premium. No regeneration was observed on MS1 medium for cotyledons. Regeneration frequency of cotyledons was from 67% to 100% on the media supplemented with different concentration of BAP and NAA and from 75% to 100% on the medium supplemented with ZEA, depending on cultivars. Nogueira et al. (2001) observed high regeneration frequency 92% or 85% on cotyledonary explants of genotype Santa Clara or its natural mutant Firme, respectively.

Results of this experiment confirmed the positive influence of growth regulator addition on the number of shoots regenerated from tomato cotyledons and hypocotyls. For all cultivars, zeatin-supplemented media tended to give the higher number of shoots primordia and shoots per explant (Tab. 1). Differences between the types of explants were statistically significant in cultivars Hana and Premium. We observed weaker effect of BAP on adventitious shoot regeneration in tomato compared to ZEA and this corresponded with the frequency of regeneration for particular cultivars, too. Our experiments supported the results of other authors (Ichimura & Oda, 1995; NoGueira et al. 2001) who found that the most efficient medium for *in vitro* regeneration of tomato being induction medium supplemented with a cytokinin zeatin. The feasibility of using the MS2 medium in our laboratory for adventitious shoot induction in 13 cultivars of tomato was recently proved by Gubiš et al. (2003).

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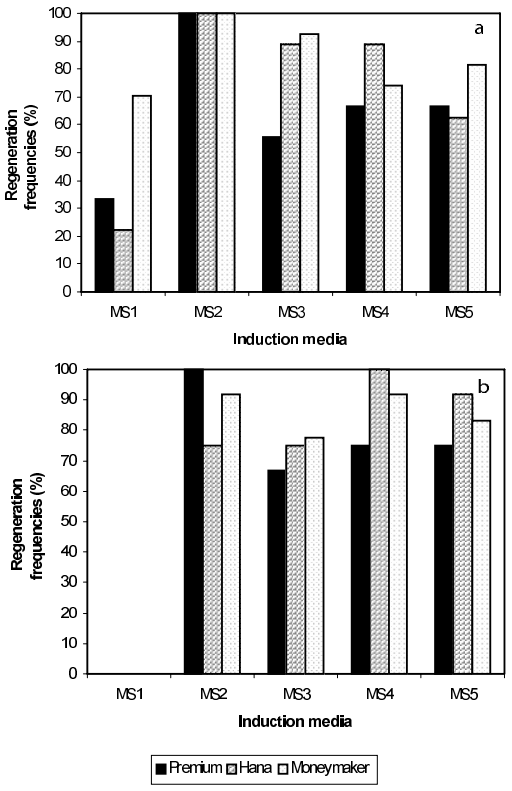

Fig. 1. Regeneration frequency from hypocotyl (a) and cotyledon (b) explants of tomato on MS medium supplemented with different growth regulators. The composition of the media is explained in the Materials and methods section.

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