The effect of carbon source on plant regeneration in tomato

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ABSTRACT: The effect of different tomato cultivars and different sugar types (sucrose, glucose and maltose) and concentrations (1.0, 2.0 and 3.0%) on shoot regeneration from aseptically grown hypocotyl and cotyledon explants were studied. Among sugar types, sucrose at a concentration of 3.0% induced the highest number of shoots from both types of explants. In hypocotyl explants, cv. Premium showed the best regeneration capacity (0.23 shoots per explant), and in cotyledon explants, cv. Hana produced the maximal number of shoots (0.43 or 0.37 for media with 2.0% or 3.0% sucrose, respectively).

Keywords: *in vitro* culture; MS medium; sugars; organogenesis

In vitro regeneration of cultivated tomato (*Lycopersicon esculentum* Mill.) has been a subject of research because of the commercial value of the crop and its amenability for further improvement via genetic manipulation (Evans 1989). 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 influenced by several different components of the culture media and it is important to evaluate their effect on plant regeneration. The purpose of our study was to evaluate the influence of carbon source on plant regeneration in tomato.

Three cultivars of tomato (*Lycopersicon esculentum* Mill.), Premium, Hana and UC 82, were used. The seeds were surface-sterilised by immersion into a 4% 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 of Murashige and Skoog (1962) (abbreviated hereinafter as "MS"), 100 mg/m^3 *myo*-inositol, 2 mg/dm^3 thiamine.HCl, 0.5 mg/dm^3 pyridoxine.HCl, 0.5 mg/dm^3 nicotinic acid, 1% sucrose and 0.7% agar. The cultures were initially kept in the dark at 27 ± 1°C for two days and then maintained under a 16h 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 4–7mm 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. The effect of different concentrations of sucrose, glucose and maltose (1.0, 2.0 and 3.0%) that were added to the MS basal medium supplemented with 1 mg/dm^3 zeatin (ZEA) and 0.1 mg/dm^3 indole-3-acetic acid (IAA) (Ichimura, Oda 1995) on regeneration capacity of explants was studied. The media were adjusted to pH 5.8 prior to autoclaving. Glass containers with 25 cm^3 of medium were used. The regeneration capacity of explants was assessed 6 weeks later. The following parameters were evaluated: frequency of regeneration (percents of regenerating explants) and the number of shoots per explant. Significance of differences between the results was estimated by analysis of variance (Statgraphics Version 5.0). Variation among means was analysed using LSD ($P \leq 0.05$) procedure according to the method described by Snedecor and Cochran (1956).

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Table 1. Influence of different concentrations (1, 2 and 3%) of sugars on plant regeneration in tomato – the number of shoots/plated explant are means of 30 explants; data were taken after 6 weeks of culture. Different letters mean significant difference at $P \leq 0.05$

Sugar (%)		Hypocotyls				Cotyledons			
		Premium	Hana	UC 82	mean	Premium	Hana	UC 82	mean
Sucrose	1.0	0.03	0.00	0.00	*0.01^{a}*	0.07	0.10	0.03	*0.07^{ab}*
	2.0	0.00	0.07	0.00	*0.02^{a}*	0.03	0.43	0.07	*0.18^{b}*
	3.0	0.23	0.03	0.03	*0.10^{b}*	0.03	0.37	0.13	0.18^{b}
Glucose	1.0	0.00	0.00	0.00	*0.00^{a}*	0.07	0.33	0.03	*0.14^{ab}*
	2.0	0.00	0.03	0.03	*0.02^{a}*	0.07	0.30	0.13	*0.17^{ab}*
	3.0	0.10	0.03	0.00	*0.04^{a}*	0.10	0.17	0.03	*0.10^{ab}*
Maltose	1.0	0.00	0.00	0.00	*0.00^{a}*	0.10	0.20	0.00	*0.10^{ab}*
	2.0	0.13	0.00	0.00	*0.04^{a}*	0.27	0.23	0.00	*0.17^{ab}*
	3.0	0.07	0.07	0.00	*0.05^{a}*	0.00	0.13	0.03	*0.05^{a}*
Cultivar class means		*0.06^{b}*	*0.03^{a}*	*0.01^{a}*		*0.08^{a}*	*0.25^{b}*	*0.05^{a}*	

The use of different tomato cultivars and different sugar types and concentrations for shoot regeneration from aseptically grown hypocotyls and cotyledons resulted in significant effects of both the genotypes and the sugars. A large variability in the number of shoots was observed across cultivars and across the different sugar concentrations (Table 1). Cultivar mean comparisons (LSD, $\alpha = 0.05$) showed only two classes differing in the induction potential. The cultivar that produced the highest number of shoots per hypocotyl (0.06) was cv. Premium while cv. Hana produced the highest number of cotyledon explants (0.25). However, the lowest number of shoots per explant in both explant types was induced in the cv. UC 82. The highest induction class of cotyledon explants included two different concentrations (2.0 and 3.0%) of sucrose, which produced an average number of regenerated shoots per explant 0.18 (Table 1). Contrary to sucrose, the medium supplemented with 2.0% glucose or maltose also showed a good regeneration capacity (0.17) on cotyledon explants. For hypocotyl explants the highest number of shoots was regenerated on the medium with 3.0% sucrose.

Normally for the cells, tissues or organ cultures it is essential to add a carbon source into the growth medium. Sucrose is almost universally used for micropropagation purposes as it is readily utilisable by cells. The optimum concentration of sucrose required induction of morphogenesis or growth differs among genotypes. Sucrose seems to be essential for the healthy growth of tomato cultures, and most researchers have used it as the sole source of 'carbon' (Chen et al. 1999; Costa et al. 2000; Venkatachalam et al. 2000).

Sucrose concentration of 30 g/dm^3 (compared to 5, 10, or 20 g/dm^3) was found to be optimal for the microplant growth of tomato (Schnapp, Preece 1986). The majority of researchers have used this sucrose concentration in their initiation and multiplication media (Chen et al. 1999; Costa et al. 2000; Venkatachalam et al. 2000).

El-Bakry (2002) compared the effect of carbon source on shoot induction and plant regeneration in tomato. He used glucose, fructose, maltose and sucrose at a 3% concentration. In his experiment maltose gave the highest number of shoots in the genotypes he used (Castlerock, UC97-3 and Peto 86).

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Vplyv zdroja uhlíka na regeneráciu rastlín rajčiaka

ABSTRAKT: Bol študovaný vplyv rozdielnych odrôd rajčiaka jedlého, typov cukru (sacharóza, glukóza a maltóza) a ich koncentrácie (1, 2 a 3%) na regeneráciu výhonkov z asepticky naklíčených hypokotylových a klíčnolistových explantátov. Spomedzi rôznych typov cukrov 3% sacharóza indukovala najvyšší počet výhonkov pri oboch typoch explantátov. Najlepšiu regeneračnú schopnosť (0,23 výhonku na explantát) pre hypokotylové explantáty vykazovala odroda Premium a maximálny počet výhonkov (0,43 alebo 0,37 pre médium s 2 % alebo 3 % sacharózy) pri klíčnolistových explantátoch produkovala odroda Hana.

Kľúčové slová: *in vitro* kultúra; MS médium; cukry; organogenéza

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