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Influence of different carbon sources on *in vitro* rooting of sour cherry cv. Oblačinska

Utjecaj raznih izvora ugljika na *in vitro* ukorjenjavanje višnje cv. Oblačinska

Ines Mihaljević, K.Dugalić, Vesna Tomaš, Marija Viljevac, B.Puškar, T. Čupić, Zorica Jurković

ABSTRACT

The effect of different carbon sources on *in vitro* rooting of sour cherry cv.Oblačinska was investigated at the Agricultural Institute Osijek, Croatia. Sour cherry cv. Oblačinska micropropagated shoots were rooted on half-strength Murashige and Skoog (MS) medium, supplemented with different types and concentrations of sugars, in order to determine the effects of sugar composition and concentration on in vitro rooting. The influence of four carbon sources, sucrose (SU), glucose (G), fructose (F) and sorbitol (SO) on *in vitro* rooting of sour cherry cv. Oblačinska was compared at 0, 20, 30, 40 and 50 g L^{-1} concentrations. Type and concentration of carbohydrates had a significant effect on rooting percentage, mean root number, mean root length and mean fresh and dry root weight. Among the various carbohydrates tested, the best rooting response was obtained with sucrose in terms of rooting frequency, number of roots and root length.

Key words: carbon sources, *in vitro* rooting, micropropagation, sour cherry cv. Oblačinska

SAŽETAK

Na Poljoprivrednom institutu Osijek istraživan je utjecaj različitih vrsta ugljikohidrata na in vitro ukorjenjivanje Oblačinske višnje. Razmnožene biljke su ukorijenjene na Murashige i Skoog podlozi (MS) s različitim vrstama i koncentracijama ugljikohidrata, s ciljem da se utvrdi njihov utjecaj na *in vitro* ukorjenjivanje. Četiri različita ugljikohidrata, saharoza, glukoza, fruktoza i sorbitol su uspoređivani u koncentracijama 0, 20, 30, 40 i 50 g L^{-1}. Vrsta i koncentracija ugljikohidrata imale su znatan učinak na postotak ukorjenjivanja, broj korijenčića, dužinu korijenčića, svježu i suhu tvar korijena. Između testiranih ugljikohidrata najbolji rezultati ukorjenjivanja postignuti su sa saharozom, glede postotka ukorjenjivanja, broja korijenčića i dužine korijenčića.

Ključne riječi: izvor ugljika, *in vitro* ukorjenjivanje, mikropropagacija, višnja cv. Oblačinska

INTRODUCTION

In plants, carbohydrates have various essential functions. They are substrates for respiration, play a role in the synthetic pathways of many compounds and are building blocks of macromolecules (Calamari and De Klerk, 2002). Green cells in culture are not photosynthetically active and require a carbon source. Carbohydrates play an important role in in vitro cultures as an energy and carbon source, as well as an osmotic agent (George et al., 2008). Sugar is a very important component in medium and its addition is essential for in vitro growth and development of plants because photosynthesis is insufficient, due to the growth taking place in conditions unsuitable for photosynthesis or without photosynthesis (Pierik, 1987). Root initiation and growth are high energy requiring processes that could only occur at the expense of available metabolic substrates, which are mainly carbohydrates (Custoido, 2004). Sucrose or glucose at 2-5% is commonly used in cell culture. Other carbohydrate sources, such as fructose, maltose, sorbitol and starch can also be used (Smith, 2005).

Sour cherry cv. Oblačinska is an autochthonous and heterogeneous Serbian cultivar. Oblačinska sour cherry represents a mixture of a great number of clones (genotypes) so problems with its reproduction and exploitation occur (Nikolić et al., 2005). Oblačinska sour cherry is a leading cherry cultivar for the processing industry in Croatia because of its pomological characteristics, suitability for mechanical harvesting, early ripening and good fertility (Jurković et al., 2008). Micropropagation of sour cherry cv. Oblačinska has been developed for growing virus free sour cherry cv. Oblačinska on its own roots. Adventitious root formation is a difficult step in tissue culture propagation for many woody plants. In micropropagation, rooting of microcuttings is often problematic. Losses at this stage have vast economic consequences (De Klerk, 2005).

Because of that, research on root formation is very important from the practical point of view. The type of carbon source and its concentration affects rooting in many plant species. The basic purpose of this study was to determine the influence of different carbon sources and various concentrations on in vitro rooting of sour cherry cv. Oblačinska.

MATERIALS AND METHODS

The experiment was conducted in the Plant tissue culture laboratory, Agricultural Institute Osijek, Department for fruit-growing, during February and March of 2012. Shoot tips (explants) were collected from actively growing trees of sour cherry cv. Oblačinska, clone OS in the orchard of the Agricultural Institute Osijek. Aseptic cultures of sour cherry cv. Oblačinska were established from axillary buds. Stock cultures of sour cherry cv. Oblačinska were maintained on medium consisting of DKW (Driver and Kuniyuki, 1984) macro elements, MS (Murashige & Skoog, 1962) micro elements and supplemented with DKW (Driver and Kuniyuki, 1984) vitamins, 0.8 mg L^{-1} 6-benzylaminopurine (BAP), 0.01 mg L^{-1} indole -3-butyric acid (IBA), 6.5 g L^{-1} agar and 30 g L^{-1} sucrose.

Proliferated shoot tips about 2 cm long from stock cultures were transferred to half-strength MS media consisting of MS macro, micro elements and vitamins (Murashige & Skoog, 1962), supplemented with 1 mg L^{-1} IBA, 6 L^{-1} g agar, Fe sequestrene 200 mg L^{-1} and with different concentrations of carbon sources. Sucrose, glucose, fructose and sorbitol, were used in concentrations 0, 20, 30, 40 and 50 g L^{-1} to evaluate the effect of these carbon sources on rooting (Table 1). The pH of all media was adjusted to 5.8 before autoclaving the media at 121 ºC and 1.5 atm for 20 min. The cultures were kept in a growth chamber at 25ºC, with 16 hours photoperiod and 3500 lux of light intensity.

Table 1. Treatments with different type and concentrations of carbohydrates for in vitro rooting of sour cherry cv. Oblačinska

Tablica 1. Tretmani sa različitim vrstama i koncentracijama ugljikohidrata za in vitro ukorijenjavanje oblačinske višnje

Treatments	Carbon sources	Treatments	Carbon sources
K1	0 g L^{-1} sucrose	K3	0 g L^{-1}sorbitol
T1	20 g L^{-1} sucrose	T9	20 g L^{-1}sorbitol
T2	30 g L^{-1} sucrose	T10	30 g L^{-1}sorbitol
T3	40 g L^{-1} sucrose	T11	40 g L^{-1}sorbitol
T4	50 g L^{-1} sucrose	T12	50 g L^{-1}sorbitol
K2	0 g L^{-1} glucose	K4	0 g L^{-1} fructose
T5	20 g L^{-1} glucose	T13	20 g L^{-1} fructose
T6	30 g L^{-1} glucose	T14	30 g L^{-1} fructose
T7	40 g L^{-1} glucose	T15	40 g L^{-1}fructose
T8	50 g L^{-1} glucose	T16	50 g L^{-1}fructose

The experiment was CRD (Completely Randomized Design) consisting of three replications per treatment and ten shoots per treatment, placed in a 200 mL culture jar containing 50 mL of medium. The experiment lasted 30 days. Data was taken on the following parameters: percentage of rooting, root number, root length, root fresh weight and root dry weight per shoot. Root dry weight was obtained by drying root in a forced air oven at 75°C for 72 hours. Root lengths were determined by measuring the longest root in each shoot.

Statistical analysis of the data was carried out by using analysis of variance (ANOVA). The assumptions of variance analysis were tested by insuring that the residuals were random, homogenous, with a normal distribution about a mean of zero. Analysis of variance was conducted to determine differences between types of carbohydrates and the influence of various concentrations on rooting and other examined traits of root. All data were analyzed using PROC GLM procedure with software SAS 9.1.3 (SAS Institute, 2003). Means were separated using the Dunnett's t test for comparisons with control at 0.05 and 0.01 levels of significance.

RESULTS

The influence of carbon source and concentrations was significant by analysis of variance (Table 2). Data showed that type and concentration of carbohydrates had a significant effect on rooting percentage, root number, root length, fresh and dry mass of root.

The type and concentration of carbon sources in the culture medium showed no significant effects on survival rate of shoots (Table 2). Shoot survival with all carbohydrates was >75%. (Figure 1). Rooting percentage varied significantly with the type and concentration of different carbohydrates. The highest average rooting percentage (57.33 %) was obtained with sucrose as carbon source, although results with glucose were also satisfactory. The results (Table 3.) indicated that rooting percentage on medium with sucrose and glucose was not significantly different at the 5% level but they were significantly different compared to medium with fructose and sorbitol. On medium with fructose, glucose and sucrose rooting percentage significantly increased as sugar concentration increased. The highest mean root number was observed with sucrose (2.70) and significantly different results were only on medium with sorbitol, with average number of root (1.10) (Table 3) Similar results, related to the highest number of roots were obtained with concentrations (40 g L^{-1}) of sucrose, fructose and glucose, except that fructose was not significantly

Table 2. Analisys of variance for influence of different type and concentration of carbohydrates on survival percentage, rooting percentage, mean number of roots per shoot, mean length of roots, fresh and dray weight of the root.

Tablica 2. Analiza varijance za utjecaj različite vrste i koncentracije ugljikohidrata na postotak preživljenih pupova, zakorijenjenih pupova , prosječni broj korijena, prosječnu dužinu korijena, svježu i suhu tvar korijena

Source	df	Survived plants [%]	Rooting [%]	Root number	Root length [mm]	Root dry weight [g]	Root fresh weight [g]
Rep.	2	224.8 n.s	721 n.s	1,0 n.s	15,4 n.s	0,001 n.s	0,007 n.s
Sugar	3	47.6 n.s	4535**	7,2**	74,0**	0,002n.s	0,06**
Concentration of sugar	16	170.9 n.s	2317**	5,6**	61,2**	0,004**	0,041**
Fructose	4	93,3 n.s	85,6 n.s	5,5 n.s	128,6 n.s	0,001 n.s	0,022 n.s
Glucose	4	190,0 n.s	2766**	5,8**	64,4**	0,004**	0,109**
Sucrose	4	106,7 n.s	3856**	7,5**	17,9**	0,009 n.s	0,02**
Sorbitol	4	364,7 n.s	1790 n.s	3,6 n.s	33,8*	0,001 n.s	0,002 n.s
Error	35	129.5	329	1,7	17,2	0,001	0,001

** indicate significant differences at 0.01 level of probability.

Non significant differences at 0.05 level of probability are indicated as n.s

different from the control. The best result regarding the length of roots was observed with fructose (7.30 mm), where fructose produced the longest root in the concentration of 50 g L^{-1} (Table 3). In the same concentration glucose also resulted in longest root formation. Between these two carbohydrates there were no significant differences, but they were statistically significant in comparison with sucrose and sorbitol media. Among all carbohydrates, sorbitol produced the smallest roots. Fresh root weight was significantly affected by carbohydrate type and concentrations (Table 3). Data showed that glucose had a significant effect on fresh root weight in comparison with fructose, sucrose and sorbitol. Glucose produced the highest amount of fresh root weight under 40 g L^{-1} concentration. The lowest fresh root weight was observed in the medium with sorbitol. The type of sugar had no significant effect on root dry weight but concentration of carbohydrates had significant effect. Only concentrations within glucose significantly improved root dry weight and at a higher concentration of (40 g L^{-1}) glucose produced significantly more dry weight with regard to the control (Table 3).

Table 3. Mean values of survival percentage, rooting percentage, number of roots per shoot, length of roots, fresh and dry mass of the roots with different type and concentrations of carbohydrates in media

Tablica 3. Srednje vrijednosti postotaka preživljenih biljaka, zakorijenjenih biljaka, brojeva korijena po biljci, dužine korijena, svježe i suhe tvari korijena sa različitim vrstama i koncentracijama ugljikohidrata u mediju.

CARBOH-YDRATES	Conc.	Survival plants [%]	Rooting [%]	Number of root	Length of root [mm]	Dry root Mass [g]	Fresh root mass[g]
FRUCTOSE	0	100.00	0.00	0.00	0.00	0.00	0.00
	20	93.33	23.33	2.78	7.44	0.03	0.20
	30	93.33	16.67	1.97	3.39	0.01	0.06
	40	86.67	40.00	3.53	8.24	0.05	0.18
	50	100.00	40.00	2.83	17.40	0.03	0.16
Average		**94.67**	**24.00^{B}**	**2.22^{A}**	**7.30^{A}**	**0.02**	**0.12^{B}**
GLUCOE	0	100.00	0.00	0.00	0.00	0.00	0.00
	20	80.00	46.67**	2.60**	4.72n.s	0.01n.s	0.07n.s
	30	96.67	60.00**	2.56**	9.85**	0.03n.s	0.22*
	40	86.67	80.00**	3.48**	9.58**	0.10**	0.50**
	50	90.00	63.33**	3.26**	11.11**	0.04*	0.19*
Average		**90.67**	**50.00^{A}**	**2.38^{A}**	**7.05^{A}**	**0.04**	**0.20^{A}**
SUCROSE	0	100.00	0.00	0.00	0.00	0.00	0.00
	20	86.67	46.67**	2.70**	5.44**	0.01	0.06*
	30	93.33	80.00**	3.17**	3.36**	0.01	0.05n.s
	40	90.00	70.00**	4.03**	3.61**	0.02	0.08*
	50	100.00	90.00**	3.57**	6.37**	0.13	0.22**
Average		**94.00**	**57.33^{A}**	**2.70^{A}**	**3.76^{B}**	**0.03**	**0.08^{BC}**
SORBITOL	0	100.00	0.00	0.00	0.00	0.00	0.00
	20	83.33	56.67	2.58	7.12*	0.03	0.06
	30	66.67	36.67	1.48	5.96	0.01	0.09
	40	66.67	26.67	1.46	1.83	0.01	0.07
	50	50.00	0.00	0.00	0.00	0.00	0.00
Average		**73.33**	**24.00^{B}**	**1.10^{B}**	**2.98^{B}**	**0.01**	**0.04^{C}**
Total average		88.17	38.83	2.10	5.27	0.03	0.11
Carbohydrates $Dunetti_{0,05}$		-	13,4	0,75	2,91	-	0,06

Numbers followed by the same letter are not significantly different at the 5% level.

*, ** indicate significant differences at 0.05 and 0.01 level of probability.

No significant differences at 0.05 level of probability are indicated as n.s

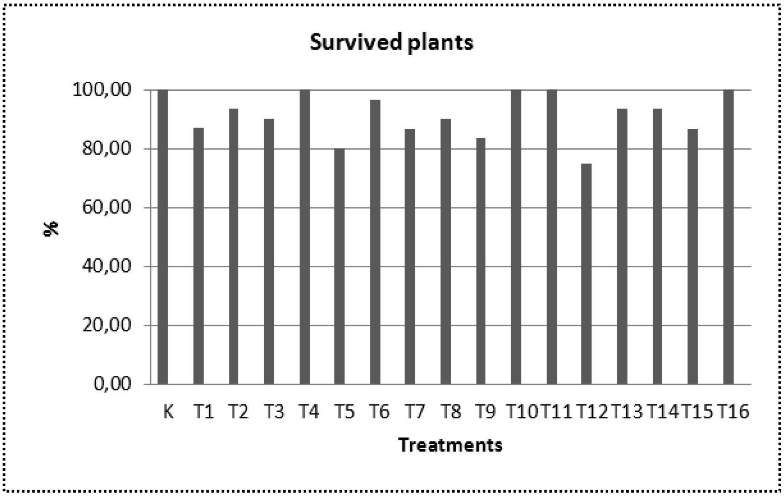

Figure 1. Influence of different type and concentration of carbohydrate on survival of plants

Slika 1. Utjecaj različite vrste i koncentracije ugljikohidrata na preživljavanje biljaka

DISCUSSION

Among the many available carbon sources, sucrose has been the major one (Petersen et al., 1999). All carbon sources do not sustain rooting equally. For most plant species including Prunus, sucrose has been used for promoting rooting of shoots (George, 1996).

Since there is no study on in vitro propagation of sour cherry cv. Oblačinska, we compared our results with those reported for the other plant species. In our experiment among fructose, glucose, sucrose and sorbitol the best results for in vitro rooting were obtained with sucrose while sorbitol was the least effective. The highest percentage of rooting and the highest number of roots was achieved in the medium with sucrose (Figure 2; 3). With sorbitol the percentage of rooting, mean number of roots and root length were very low (Figure 2; 3; 4).

These results are in accordance with Bahmani et al., 2009 who reported that among different types and concentrations of carbohydrates, sucrose had the highest percentage of rooting and by increasing the concentration of sucrose in the medium, the rooting percentage in apple rootstock MM.106 increased. Sorbitol was the least effective sugar in terms of rooting frequency in this work.

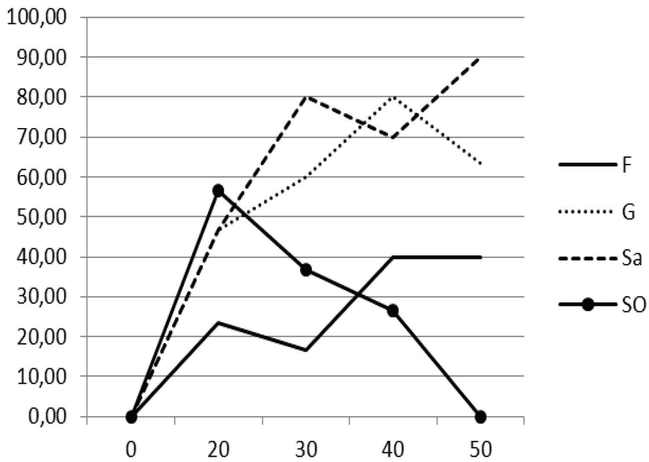

Figure 2.Influence of different carbohydrates on rooting

Slika 2. Utjecaj različitih ugljikohidrata na ukorijenjavanje

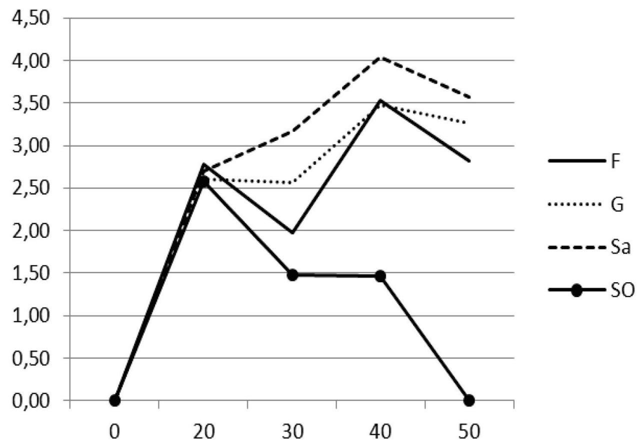

Figure 3. Influence of different carbohydrates on root number

Slika 3. Utjecaj različitih ugljikohidrata na broj korijena

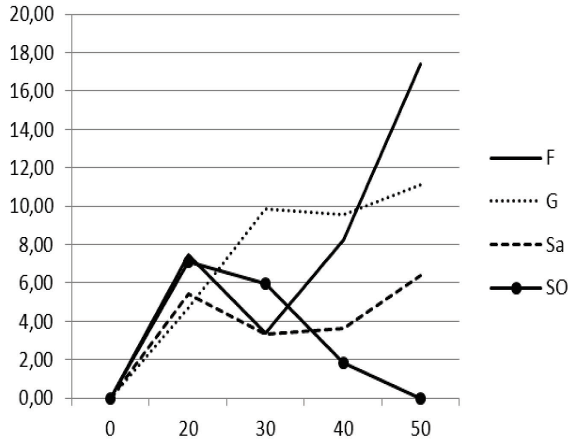

Figure 4. Influence of different carbohydrates on root length

Slika 4. Utjecaj različitih ugljikohidrata na dužinu korijena

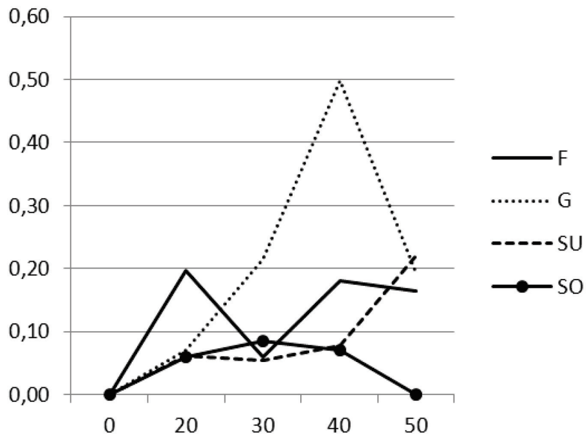

Figure 5. Influence of different carbohydrates on fresh root weigth

Slika 5. Utjecaj različitih ugljikohidrata na svježu tvar korijena

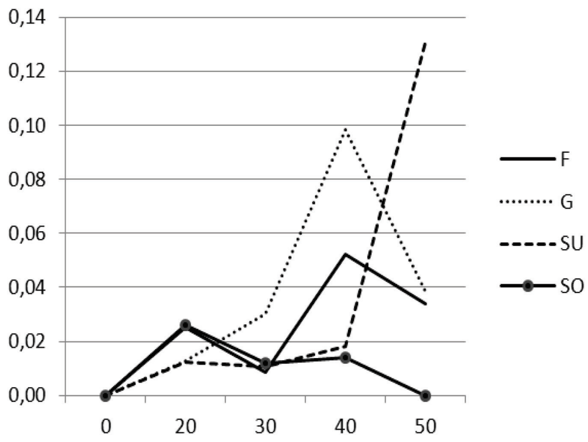

Figure 6. Influence of different carbohydrates on dry root weigth

Slika 6. Utjecaj različitih ugljikohidrata na suhu tvar korijena

The negative results obtained with sorbitol showed that it was not efficiently metabolized by some species, or sorbitol in the medium is not used as carbon source at all, but regulates osmotic potential only (Bahmani et al., 2009). Yaseen (2009) stated in his work opposite results for apple rootstock M9 and M26 and from these results it is evident that there are significant differences among carbohydrates in terms of their interaction with apple rootstocks. Custodio et al., (2004) also reported similar results to ours with in vitro rooting of carobe tree when sucrose had the highest percentage of rooting, the highest mean number of roots, while sorbitol had the smallest percentage and the smallest mean number of roots. Ahmad et al. (2007) reported that sorbitol was a better carbon source than sucrose for rooting of peach rootstock GF677. In our experiment the 50 g L^{-1} fructose treatment induced the longest roots (Figure 4). Custodio et al., (2004) in their work stated that the root elongation of carobe tree was the greatest on fructose, and reported (Kagami, 1994) similar results on Japanese persimmon. Other authors (Bahmani et al., 2009, Kadota, 2004) reported that fructose did not achieve good results with in vitro rooting and had no effect on rooting. In our study root fresh and dry root weights were increased when we used glucose and sucrose in concentration of 40, 50 g/ L^{-1}; this result might be due to increased of number and length of roots (Figure 5;6). These results are similar to earlier reports on peach rootstock (Fotopoulos, 2004) and on data palm (Al- Khateeb, 2001). Comparing the results from our study with results of other authors, we can see that different plant species under the influence of different carbohydrates react differently to in vitro rooting. In many plant species the response of shoot cultures to different carbohydrate treatments appears to be, to some extent genotype dependent (Cuenca, 2001).

Since media devoid of carbohydrates did not produce roots, this study points to the fact that selection of carbon source and their concentrations does play an important role in rooting.

In this study we have shown effects of different type and concentrations of carbohydrates on rooting of sour cherry cv. Oblačinska. The results show that sucrose is the best carbon source for rooting of sour cherry cv. Oblačinska with optimal rooting percentage, optimal number and length of root for commercial production. This is the first report on the effect of carbon source, type and concentration, on sour cherry rooting and provides a basis for the improvement of sour cherry cv. Oblačinska micropropagation.

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Authors adress – Adresa autora:
Ines Mihaljević, corresponding author: ines.mihaljevic@poljinos.hr
Krunoslav Dugalić,
Vesna Tomaš,
Marija Viljevac,
Boris Puškar,
Tihomir Čupić,
Zorica Jurković
Agricultural institute Osijek, Department for fruit growing,
Južno predgrađe 17, 31000 Osijek, Croatia