ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA

VOL. XVIII (2) SECTIO EEE 2007

Instytut Roślin Ozdobnych i Architektury Krajobrazu Akademii Rolniczej w Lublinie
ul. S. Leszczyńskiego 58, 20-068 Lublin
e-mail: rosliny.ozdobne@ar.lublin.pl

MAREK DĄBSKI, MARZENA PARZYMIĘS

The influence of type and concentration of carbohydrates on growth and branching of *Clematis integrifolia in vitro*

Wpływ rodzaju i stężenia węglowodanów na wzrost i rozkrzewianie *Clematis integrifolia in vitro*

Summary. The influence of type and concetration of carbohydrates on *in vitro* shoot proliferation of *Clematis integrifolia* was studied. The explants used in the experiment were excised from aseptic cultures incubated on solidifed MS medium. Shoot tips were placed on MS basal medium supplemented with 2 mg 2iP·dm^{-3} and 0.5 mg IAA·dm^{-3}. The aim of the experiment was to estimate the influence of different carbohydrates: sucrose, galactose or fructose at concentration of 0, 10, 20, 30 or 40 g·dm^{-3} on growth and proliferation of *Clematis integrifolia* explants. The obtained results showed that fructose at the concentration of 10 mg·dm^{-3} had the most positive effect on the growth of the main shoot. The highest proliferation rate was observed on the media with sucrose in concentration of 30 g·dm^{-3} and the obtained axillary shoots were in the highest amount, length and weight. Galactose in all concentrations used in the experiment definitely inhibited growth and proliferation and rooting of shoots.

Key words: carbohydrates, *Clematis integrifolia, in vitro*

INTRODUCTION

Genus *Clematis* (*Ranunculaceae*) includes around 300 species of vines, shrubs and herbaceous perennials. Clematises are widely used and valued as garden plants. *Clematis integrifolia* is a perennial of 70–150 cm height. It has large, single leaves and bell-shaped flowers, which are usually dark blue or violet. It flowers from June to September [Matthews 2002].

They are propagated mainly through stem cuttings [Erwin *et al.* 1997, Hlebionek 1999], but many species and cultivars root with difficulty. Micropropagation is a good method of vegetative propagation which allows to increase the number of good quality

and healthy offsprings. There are just a few scientific works concerning clematis micropropagation by direct organogenesis. The research was done by Kreen *et al.* [2002] and Guan-Kai *et al.* [2002]. Mandegaran and Sieber [2002] as well as Luttman *et al.* [1994] obtained *Clematis* microshoots through somatic embryogenesis. However, this type of micropropagation does not guarantee genetic sustainability.

The *in vitro* morphogenic processes are usually affected by physical environment and culture medium such as carbon source [Biahoua and Bonneau 1999, Fuentes *et al.* 2000]. Carbohydrates control morphogenesis by acting as energy source and by altering the osmotic potential of the culture medium, which influences cell wall properties such as extension, hardening and composition, followed by subsequent modification in morphogenesis [Pritchard *et al.* 1991].

The aim of the undertaken experiment was to estimate what type of carbohydrates and in what concentration is the most proper for tissue culture of *Clematis integrifolia* L. *in vitro*.

MATERIAL AND METHODS

The experimental object were 15–20 mm shoots of *Clematis integrifolia* L. excised from aseptically grown shoot clusters. Explants were placed into 300 ml Erlenmayer flasks filled with Murashige and Skoog [1962] basal medium with addition of isopentenyl-adenine (2iP) at the concentration of 2 $mg \cdot dm^{-3}$ and indole-3-acetic acid (IAA) at the concentration of 0.2 $mg \cdot dm^{-3}$. The media was chosen on the basis of previous experiments. In the experiment different carbohydrates: sucrose, fructose and galactose, which were added to the media in concentrations of 0, 10, 20, 30 and 40 $g \cdot dm^{-3}$, were studied. Medium pH was adjusted to 5.8 with NaOH prior to the addition of 0.65% of agar and subsequently autoclaved for 20 min at 120ºC. The cultures were incubated in a culture room at a temperature of 22ºC during the day and 20ºC at night and 16-h photoperiod with irradiance of 35 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Each combination included 21 shoots. Each flask (7 shoots) was treated as a replication. The experiment was repeated twice. After four weeks the experiment was finished and the plant material was analysed. During the experiment the following data were collected: height, number of leaves and weight of the main shoot, number of shoots forming axillary shoots, number, length and the fresh weight of axillary shoots, percentage of rooting, number of roots per shoot, root length and weight, percentage of shoots that callused and callus weight.

The results obtained in the experiment were evaluated statistically with the use of analysis of variance and Tukey t-test at 5% level of significance.

RESULTS

The growth of the main shoot was statistically similar in all used combinations of sugar studied in the experiment. However, on the basis of the obtained results it can be stated that fructose at the concentration of 10 $mg \cdot dm^{-3}$ had the most positive effect on the growth of *Clematis integrifolia* main shoots in the presence of which they were the highest (17.4 mm). Good results were also observed while sucrose in all concentrations used was added to the medium (12.9 to 15.2 mm). Significantly smaller shoot growth was on the media supplemented with galactose in all studied concentrations (4.2 to 4.8 mm) – Tab. 1, Fig. 1.

Table 1. The effect of carbohydrates and their concentration on *Clematis integrifolia* growth and development *in vitro*

Type of carbohydrates	K	Sucrose				Fructose				Galactose			
Concentration ($g \cdot dm^{-3}$)	0	10	20	30	40	10	20	30	40	10	20	30	40
Shoot height (mm)	10.5_a	13.3_a	14.4_a	15.2_a	12.9_a	17.4_a	11.6_a	7.2_a	6.5_a	4.2_a	4.8_a	4.5_a	4.2_a
Mean	10.5_a *	14.0_a				10.7_a				4.4_b			
No. of leaves	5.9_{a-d}	6.6_{ab}	6.6_{ab}	6.4_{ab}	6.6_{ab}	7.3_a	6.1_{abc}	5.1_{bcd}	4.5_{cd}	3.4_d	3.4_d	3.4_d	3.5_d
Mean	5.9_b	6.6_a				5.8_b				3.4_c			
Shoot weight (mg)	21.2_a	21.7_a	19.0_a	19.7_a	18.0_a	24.4_a	24.8_a	15.2_a	14.4_a	7.5_a	12.0_a	9.6_a	7.9_a
Mean	21.2_a	19.6_a				19.7_a				9.2_b			
Percentage of branched shoots	37_{abc}	14_{bc}	40_{ab}	63_a	48_{ab}	43_{ab}	17_{bc}	11_{bc}	0_c	0_c	0_c	0_c	0_c
Mean	37_a	41_a				18_b				0_c			
No. of axillary shoots	1.9_a	1.5_a	1.7_a	2.1_a	1.6_a	1.6_a	0.9_a	0.6_a	0_a	0_a	0_a	0_a	0_a
Mean	1.9_a	1.7_a				0.8_b				0_c			
Length of axillary shoots (mm)	1.9_a	2.4_a	3.2_a	4.3_a	3.6_a	3.2_a	4.1_a	1.0_a	0_a	0_a	0_a	0_a	0_a
Mean	1.9_a	3.4_a				2.1_a				0_b			
Weight of axillary shoots (mg)	1.6_a	1.5_a	2.4_a	3.2_a	2.1_a	2.8_a	3.6_a	1.0_a	0_a	0_a	0_a	0_a	0_a
Mean	1.6_a	2.3_a				1.8_a				0_b			
Percentage of callused shoots	3_a	6_a	26_a	43_a	31_a	28_a	28_a	40_a	20_a	0_a	0_a	0_a	0_a
Mean	3_b	26_a				29_a				0_b			
Callus weight (mg)	0.2_a	0.6_a	2.6_a	2.5_a	1.6_a	2.4_a	3.1_a	3.1_a	2.4_a	0_a	0_a	0_a	0_a
Mean	0.2_b	1.8_{ab}				2.8_a				0_b			

K – Control

* Means followed by the same letter do not differ significantly

The number of leaves differed significantly depending on the type of sugar used in the research. The most leaves were obtained with the use of fructose at the concentration of 10 $g \cdot dm^{-3}$ (7.3 leaves per shoot) in comparison to the concentration of 30 and 40 $g \cdot dm^{-3}$ of the same sugar (5.1 and 4.5 leaves per shoot, respectively) and galactose in all concentrations (3.4 to 3.5 leaves per shoot).

The weight of the main shoot did not differ significantly depending on the sugar added to the medium. On the basis of the obtained results it was observed that the main shoot of the biggest weight was on the media supplemented with fructose at the concentration of 20 $mg \cdot dm^{-3}$.

Sucrose added to the media promoted branching of the main shoot. In the presence of this sugar at the concentration of 30 mg·dm^{-3} 63% shoots formed axillary shoots. Statistically similar results were obtained with sucrose at the concentrations of 40 and 20 mg·dm^{-3} (48 and 40%, respectively), fructose at the concentration of 10 mg·dm^{-3} (43%) and without sugar (37%). Fructose at the concentrations 20–40 mg·dm^{-3} and galactose at all concentrations used inhibited branching significantly. Addition of glucose or fructose at the concentrations of 40 mg·dm^{-3} completely stopped proliferation (0%, Tab. 1).

The number of axillary shoots did not significantly differ depending on the sugar added to the medium. On the basis of the obtained results it can be stated that the highest number of axillary shoots was obtained while sucrose at the concentration of 30 mg·dm^{-3} was used (2.1 mm). The sucrose at the concentration 30 mg·dm^{-3} also promoted shoot length in the highest degree (4.3 mm), although there were no differences statistically. Carbohydrates used in the experiment did not have any effect on axillary shoots weight.

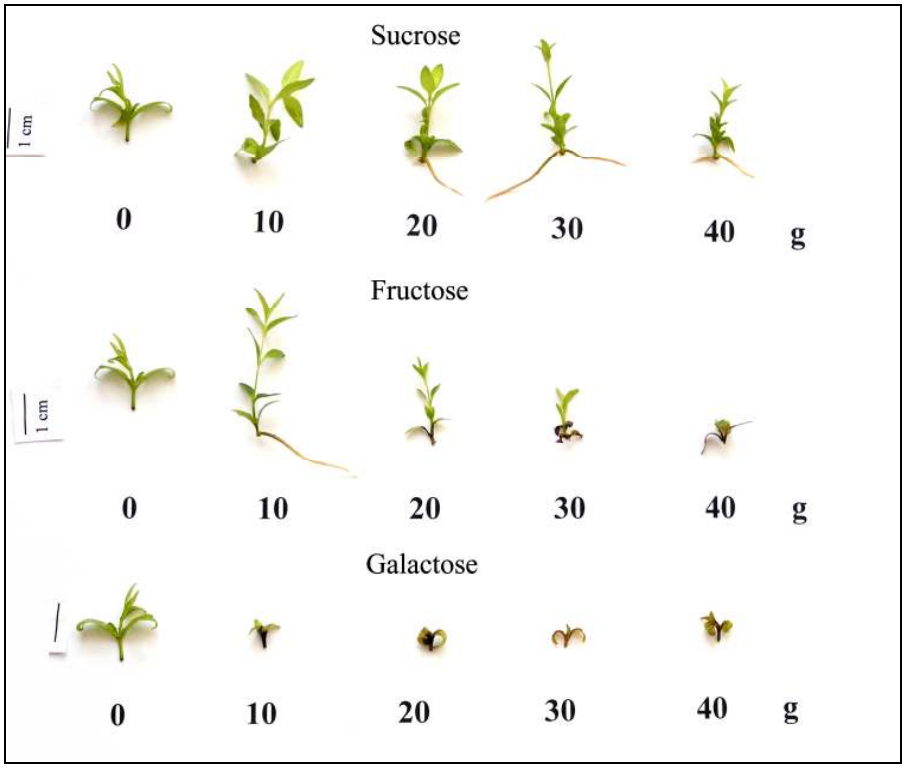
Table 2. The effect of carbohydrates and their concentration on *Clematis integrifolia* rooting *in vitro*

Type of carbohydrates	K	Sucrose				Fructose				Galactose			
Sugar concentration (g·dm^{-3})	0	10	20	30	40	10	20	30	40	10	20	30	40
Percentage of rooted shoots	3_a*	26_a	31_a	40_a	46_a	34_a	3_a	3_a	3_a	0_a	0_a	0_a	0_a
Mean	3_b	36_a				11_b				0_b			
No. of roots	0.2_a	0.7_a	0.9_a	0.9_a	1.1_a	0.8_a	0.2_a	0.2_a	0.2_a	0_a	0_a	0_a	0_a
Mean	0.2_b	0.9_a				0.5_{ab}				0_b			
Root length (mm)	2.4_a	5.9_a	6.2_a	6.8_a	5.4_a	10.5_a	1.8_a	2.2_a	0.9_a	0_a	0_a	0_a	0_a
Mean	2.4_{ab}	6.1_a				3.8_{ab}				0_b			
Root weight (mg)	0.7_a	2_a	1.7_a	2.3_a	1.9_a	3.2_a	0.4_a	0.6_a	0.4_a	0_a	0_a	0_a	0_a
Mean	0.7_{ab}	2.0_a				1.1_{ab}				0_b			

K – Control

* Means followed by the same letter do not differ significantly

Callusing of shoots was observed on all media supplemented with fructose and sucrose. There were no statistical differences; however, the highest percentage of shoots that formed callus occurred on the media supplemented with sucrose at the concentration of 30 mg·dm^{-3} (43%) and it was similar to the result obtained with fructose at the concentration of 30 mg·dm^{-3} (40%). On the media not supplemented with sugar only 3% of shoots formed callus and in the presence of galactose there was no callusing at all. Callus of the highest weight was obtained while fructose at the concentrations of 20 and 30 mg·dm^{-3} was added to the media (3.1 mg each). Callus of the smallest weight formed when sugar was not added to the medium (0.2 mg) – Tab. 2, Phot. 1.



Phot. 1. The effect of carbohydrates and their concentration on *Clematis integrifolia* growth and development *in vitro*

Carbohydrates used in the experiment influenced the rooting of *Clematis integrifolia* as well. The highest percentage of rooted explants was obtained in the media supplemented with sucrose at the concentration of 40 mg·dm^{-3} (46%). The was no rooting observed on the media supplemented with galactose. Sucrose at the concentration of 40 mg·dm^{-3} promoted the number of roots formed (1.1 per shoot). Roots were the longest and of the biggest weight were observed in presence of fructose at the concentration of 10 mg·dm^{-3} (10.5 mm and 3.2 mg respectively).

DISCUSSION

Several reports have demonstrated that the carbon source influences *in vitro* morphogenesis of different plant species [Taber *et al.* 1998, Biahoua and Bonneau 1999, Petersen *et al.* 1999, Fuentes *et al.* 2000].

In this study organogenesis was affected by the carbon source. The best results were obtained while sucrose was added to the media. Sucrose has been commonly used as a carbon source in tissue culture media [Petersen *et al.* 1999, Fuentes *et al.* 2000]. This

sugar is efficiently uptaken across the plasma membrane [Borkowska and Szczerba 1991]. Sucrose sugar occurred to be a suitable carbohydrate for many species, like *Vigna radiata* 'Wilczek' [Amutha *et al*. 2003], *Alnus acuminata* [Enrico *et al.* 2005] and *Prunus domestica* 'Węgierka Zwykła' [Nowak *et al.* 2004]. Sucrose is commonly used at the concentration of 20 and 30 $g \cdot dm^{-3}$ [Amutha *et al.* 2003, Nowak *et al.* 2004, Debnath 2005]. Fructose occurred to be a more suitable sugar for *Tibouchina* [Wnuk 2006] and *Morus latifolia* [Lu 2002].

CONCLUSIONS

1. Carbohydrates have an influence on the growth and development of explants in tissue cultures.
2. Sucrose is the most suitable sugar for micropropagation of *Clematis integrifolia.*
3. Sucrose at the concentration of 30 $gm \cdot dm^{-3}$ has the most positive effect on the formation of a good quality *Clematis integrifolia* axillary shoots.
4. The presence of sucrose in the media promotes formation of the roots.

REFERENCES

Amutha S., Ganapathi A., Muruganantham M. 2003. *In vitro* organogenesis and plant formation in *Vigna radiata* (L.) Wilczek. Plant Cell Tiss. Org. Cult. 72, 203–207.

Biahoua A., Bonneau L., 1999. Control of *in vitro* somatic embryogenesis of the spindle tree (*Euonymus europaeus* L.) by the sugar type and the osmotic potential of the culture medium. Plant Cell Rep. 19, 185–190.

Borkowska B., Szczerba J. 1991. Influence of different carbon sources on invertase-activity and growth of sour cherry (*Prunus cerasus* L.) shoot cultures. J. Exp. Bot. 42, 911–915.

Debnath S.C. 2005. Effects of carbon source and concentration on development of Lingoberry (*Vaccinium vitis-idaea* L.) shoots cultivated *in vitro* from nodal explants. In Vitro Cell. Dev. Biol.– Plant 41, 145–150.

Enrico R.J., Ramirez S.S., Mroginski L.A., Wall L.G. 2005. *In vitro* plant regeneration of *Alnus acuminata* H.B.K. ssp. acuminata and its root nodulation by Frankia. Plant Cell Tiss. Org. Cult. 80, 343–346.

Erwin J.E., Schwarze D., Donahue R. 1997. Factors affecting propagation of *Clematis* by stem cuttings. HortTechnology 7, 4, 408–410.

Fuentes S.R.L., Calheiros M.B.P., Manetti-Filho J., Vieira L.G.E. 2000. The effects of silver nitrate and different carbohydrate sources on somatic embryogenesis in *Coffea canephora*. Plant Cell Tiss. Org. Cult. 60, 5–13.

Guan-Kai Y., Li-Zhi J., Li-Jing X., Kuan-Jian, Guan-K.Y., Li Z.J., Kuang J. 2002. A preliminary study on the introduction and cultivation of *Clematis.* Acta Botanica Yunnanica, 24, 3, 392–396.

Hlebionek G. 1994. Rozmnażanie powojnika górskiego (*Clematis montanta* 'Rubens') za pomocą sadzonek pędowych. Materiały z ogólnopolskiej konferencji „Postęp w rozmnażaniu roślin ozdobnych". Kraków, 16–17 września 1994, AR im. Hugona Kołłątaja, ss. 77–81.

Kreen S., Svensson M., Rumpunen K. 2002. Rooting of *Clematis* microshoots and stem cuttings in different substrates. Sci. Hort. 96, 131–357.

Luttman R., Florek F., Preil W. 1994. Silicone-tubing aerated bioreactors for somatic embryo production. Plant Cell Tiss. Org. Cult. 39, 157–170.

Lu M-C. 2002. Micropropagation of *Morus latifolia* Poilet using axillary buds from mature trees. Sci. Hort. 96, 329–341.

Mandegaran Z., Sieber V. K. 2000. Somatic embryogenesis in *Clematis integrifolia* × *C. viticella*. Plant Cell Tiss. Org. Cult. 62, 163–165.

Matthews V. 2002. The International Clematis Register and Checklist. The R.H.S. London, 129.

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

Nowak B., Miczyński K., Hudy L. 2004. Sugar uptake and utilisation during adventitious bud differentiation on *in vitro* leaf explants of 'Węgierka Zwykła' plum (*Prunus domestica*). Plant Cell Tiss. Org. Cult. 76, 255–260.

Petersen K.K., Hansen J., Krogstrup P. 1999. Significance of different carbon sources and sterilization methods on callus induction and plant regeneration of *Miscanthus* × *ogiformis* Honda 'Giganteus'. Plant Cell Tiss. Org. Cult. 58,189–197.

Pritchard J., Wyn-Jones R.G., Tomos A.D. 1991. Turgor, growth and rheological gradients in wheat roots following osmotic stress. J. Exp. Bot. 42, 1043–1049.

Taber R.P., Zhang C., Hu W.S. 1998. Kinetics of Douglas-fir (*Pseudotsuga menziesii*) somatic embryo development. Can. J. Bot. 76, 863–871.

Wnuk K. 2006. Rozmnażanie tibuchiny wykwintnej (*Tibouchina urvilleana*/DC./Cogn) *in vitro.* Praca doktorska, Akademia Rolnicza w Lublinie.

Streszczenie. Badano wpływ rodzaju i stężenia węglowodanów na wzrost i rozkrzewianie pędów powojnika całolistnego (*Clematis integrifolia* L.). Pędy wykorzystane w doświadczeniu pozyskiwano z ustabilizowanych kultur *in vitro* prowadzonych na zestalonej pożywce MS. Eksplantaty wykładano na pożywkę podstawową MS uzupełnioną 2 mg 2iP·dm^{-3} oraz 0,5 mg IAA·dm^{-3}. Celem doświadczenia było zbadanie wpływu węglowodanów: sacharozy, fruktozy i galaktozy w stężeniach: 0, 10, 20, 30 i 40 g·dm^{-3} na wzrost i rozkrzewianie eksplantatów powojnika całolistnego.

Na podstawie otrzymanych wyników stwierdzono, że węglowodany zastosowane w doświadczeniu wpłynęły na wzrost pędu głównego oraz rozkrzewianie powojnika całolistnego.

Na wzrost pędu głównego najbardziej korzystny wpływ miała fruktoza w stężeniu 10 mg·dm^{-3}. Rozkrzewianie pędu głównego w największym stopniu stymulowała sacharoza w stężeniu 30 mg·dm^{-3}, w której obecności najwięcej pędów tworzyło pędy kątowe. W obecności tego węglowodanu powstawało najwięcej pędów kątowych, o największej długości i masie.

Galaktoza we wszystkich użytych w doświadczeniu stężeniach wyraźnie hamowała wzrost i rozkrzewianie pędów powojnika całolistnego w kulturze *in vitro*.

Słowa kluczowe: węglowodany, powojnik całolistny, *in vitro*