PROPAGATION OF BLUE HONEYSUCKLE (*Lonicera caerulea* var. *kamtschatica* Pojark.) IN *IN VITRO* CULTURE

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A B S T R A C T

A method was developed for micropropagation of two blue honeysuckle cultivars. The sterilization procedure based on application of 70% ethanol followed by 10% calcium hypochlorite resulted in satisfactory percentage of uninfected shoots obtained (65.9% for 'Czelabinka' and 64.9% for 'Duet'). Proliferating medium according to Murashige and Skoog with salt reduced by ¼ gave the best multiplication rate while the best rooting of both blue honeysuckle cultivars shoots was achieved on WPM medium supplemented with 2.0 mg l^{-1} IBA and 5.0 mg l^{-1} IAA. Applying AgroAquaGel® during acclimatization of plantlets increased their quality by improving the roots system.

Key words: blue honeysuckle, media, propagation, rooting, hydrogel

INTRODUCTION

Propagation of honeysuckle species is done traditionally by dividing mother plants and by the semi-hardwood and hardwood cuttings. However, the hardwood cuttings sometimes root at low percentage – 40%. Satisfactory results may be obtained by using softwood cuttings (Kolasiński, 2007). The sexual method of propagation does not guarantee receiving uniform, true-to-type plants. Tissue culture offers alternative method of plant propagation which is independent of the vegetative season. High multiplication rate and good health status of micropropagated plants are the additional features of that method.

In the work presented *in vitro* propagation and acclimatization *ex vitro* were studied to provide an efficient plant production system for edible honeysuckle.

MATERIAL AND METHODS

The initial plant material (vegetative axillary buds) of honeysuckle cultivars 'Czelabinka' and 'Duet' was surface disinfected with 70% ethanol for 1 min followed by 10% solution of calcium hypochlorite for 10 min. After rinsing with sterile distilled water the buds were cultured in Erlenmayer flasks, each with 25 ml of MS medium (Murashige and Skoog, 1962) solidified with 0.7% (w/v) agar. Initial medium contained BA (6-benzylaminopurine) at concentration of 1.0 mg l^{-1}. The experiments on micropropagation were done on stabilized shoot culture (after six months culturing in *in vitro* conditions).

To determine favourable conditions for shoot multiplication IBA (indole-3-butyric acid) at concentration of 0.1 mg l^{-1} and BA at concentrations of 1.0 mg l^{-1} and 2.0 mg l^{-1} were applied. pH of medium was adjusted to 5.7 before autoclaving. Media MS of full strength of mineral salts, with salts reduced by 50% and by 25% were used at proliferation stage. Both proliferating and rooting media were supplemented with 73.4 mg l^{-1} FeNaEDTA.

Microcuttings (15-20 mm long) were rooted in MS medium of full and reduced to 50% strength salts and in WPM medium (Lloyd and McCown, 1980). Media for rooting were enriched with 2.0 mg l^{-1} IBA and 5.0 mg l^{-1} IAA (indole-3-acetic acid).

Culture conditions was a 16 h photoperiod provided by cool-white fluorescent lamps at 92.8 µmol m^{-2} s^{-1} and 23±1°C. The observations and measurement were recorded after four weeks of culturing. The experiment was conducted in five replications, each consisted of 5 flasks with 5 shoots. The experiment was repeated twice.

The rooted microcuttings were acclimated in peat substrate (control) and in peat substrate supplemented with AgroAquaGel® (ARTAGO) at a dose of 4 g dm^{-3}. The observations and measurements were recorded after four weeks of acclimatization.

The results obtained were analyzed by analysis of variance, followed by means separation using Duncan's multiply-range t-test at $p = 0.05$.

RESULTS

The results of sterilization and establishing initial culture are recorded in Table 1. The successful sterilization procedures enabled obtaining similar percentage of disinfected explants of both cultivars.

The results concerning proliferating stage are presented in Table 2. The increasing of fresh biomass and newly-formed shoot's number varied with the cultivar, media and concentration of BA.

For both the cultivars tested the highest increase of fresh mass was recorded on MS media with mineral salts reduced to ¾, though 'Duet' produced more fresh mass comparing to 'Czelabinka' (Tab. 2). The high multiplication rate of shoots was noted on the same media. 'Czelabinka' produced great number of new shoots

Table 1. Surface sterilization with 70% alcohol followed by 10% calcium hypochlorite

Cultivar	Total number of explants	% of contaminated explants	% of not contaminated explants that developed shoots
Czelabinka	44	34.1	65.9
Duet	37	35.1	64.9

Table 2. Fresh mass increase and number of newly-formed shoots of two blue honeysuckle cultivars (calculation for one flask)

Medium	Czelabinka		Duet	
	average fresh mass increase [g]	mean shoot number	average mass increase [g]	mean shoot number
50% MS 1.0 mg l^{-1} BA	2.25 ab*	42.9 a	2.22 abc	51.9 abc
50% MS 2.0 mg l^{-1} BA	2.09 ab	50.4 ab	2.89 abcd	63.5 cde
75% MS 1.0 mg l^{-1} BA	3.36 bcd	85.8f	5.93 e	69.6 e
75% MS 2.0 mg l^{-1} BA	3.91 cd	62.5 cde	4.39 d	97.2 g
100% MS 1.0 mg l^{-1} BA	2.79 abcd	66.0 de	2.85 abcd	55.7 bcd
100% MS 2.0 mg l^{-1} BA	2.46 abc	64.4 de	1.47 a	58.3 bcde

*means followed by the same letters do not differ significantly at $p = 0.05$

at concentration of BA 1.0 mg l^{-1}, while 'Duet' needed higher concentration of this hormone. Increasing mineral salts content to 100% or decreasing it to 50% resulted in a weaker multiplication rate.

Honeysuckle cultivars responded differently to media composition regarding the length of newly-formed shoots. 'Czelabinka' formed more shortest shoots on the media containing 2.0 mg l^{-1} BA comparing to 'Duet' (Tab. 3).

The high number of new shoots formed on media with BA at concentration 2.0 mg l^{-1} was associated with high percent of vitrified shoots (36.0%, in 'Duet' and 21.5% in 'Czelabinka') Especially 'Duet' cultivar showed high susceptibility to enhanced level of BA in media tested (Tab. 4).

Exposure to high concentration of IAA and IBA was effective for root induction (Tab. 5). The first roots were observed on 12th day after exposing shoots to high concentration of auxins and the highest percentage of rooted shoots for both 'Czelabinka' and 'Duet' was noted on WPM medium. The rate of root induction within following days depended on the type of media and on a cultivar.

WPM media was the most effective for inducing the roots of 'Czelabinka' (96% rooted shoots) and 'Duet' (92% rooted shoots).

Table 3. Length distribution of newly-formed shoots of two blue honeysuckle cultivars

Medium	Czelabinka			Duet		
	root length [mm]			root length [mm]		
	< 5 mm	5.1-15.0	> 15.0	< 5 mm	5.1-15.0	> 15.0
50% MS 1.0 mg l^{-1} BA	43.0%	38.0%	19.0%	40.5%	40.5%	19.0%
50% MS 2.0 mg l^{-1} BA	56.5%	32.5%	11.0%	18.5%	41.0%	15.5%
75% MS 1.0 mg l^{-1} BA	25.5%	56.0%	18.5%	23.0%	40.0%	37.0%
75% MS 2.0 mg l^{-1} BA	45.5%	28.0%	26.5%	42.0%	39.5%	18.5%
100% MS 1.0 mg l^{-1} BA	22.5%	39.5%	38.0%	7.5%	48.0%	44.5%
100% MS 2.0 mg l^{-1} BA	34.5%	48.0%	17.5%	0.5%	71.0%	28.5%

Table 4. Shoot vitrification of two blue honeysuckle cultivars cultured on various media

Medium	Vitrified shoots [%]	
	Czelabinka	Duet
50% MS 1.0 mg l^{-1} BA	12.5	21.5
50% MS 2.0 mg l^{-1} BA	21.5	21.5
75% MS 1.0 mg l^{-1} BA	13.5	2.0
75% MS 2.0 mg l^{-1} BA	20.0	36.0
100% MS 1.0 mg l^{-1} BA	4.0	6.0
100% MS 2.0 mg l^{-1} BA	14.0	26.0

Table 5. Rooting rate (%) of blue honeysuckle shoots on three rooting media

Media	Days after setting the experiment					
	12	13	14	15	16	17
	Czelabinka					
MS	44	48	56	68	68	68
50% MS	32	48	66	68	84	84
WPM	72	84	92	96	96	96
	Duet					
MS	44	64	76	76	80	80
50% MS	32	44	60	68	68	72
WPM	60	84	92	92	92	92

Within next two weeks the root number increased but the efficiency of rooting process did not change. The shoots on WPM medium formed the highest number of roots: on average 26.6 roots per a shoot in 'Czelabinka' and 24.2 roots per a shoot in 'Duet'. The secondary roots were formed well on primary roots. The height of rooted plants was 4.0 cm for 'Czelabinka' and 3.32 cm for 'Duet' (Tab. 6)

The media used for rooting affected the length of obtained roots. The greatest percentage of the longest roots were formed by 'Czelabinka' on MS medium and by 'Duet' on half strength MS medium (Tab. 7).

The plants acclimatized in peat substrate supplemented with Agro-AquaGel® exhibited higher mass of roots and shoots in comparison to the plants acclimatized in the peat substrate only (Tab. 8).

'Duet' exhibited stronger response to applied hydrogel. The higher relative root length index was obtained for the plants acclimatized in substrate supplemented with hydrogel (1.14) in comparison to the control (0.53).

DISCUSSION

The sterilization procedure was satisfactory, resulted in 64.9-65.9% of disinfected explants, depending on a honeysuckle cultivar. Sometimes sodium hypochlorite solution (Karhu, 1997a) or mercuric chloride (Sedlák and Paprštein, 2007) are used for sterilization procedures. In the study presented shoot proliferation was performed on MS medium only. The satisfactory growth of biomass and high number of new shoots was observed on medium containing 75% MS salts with BA at concentrations 1.0 mg l^{-1} and 2.0 mg l^{-1}. Boonnour et al. (1988) achieved superior growth of *Lonicera periclymenum* L 'Serotina' on WPM medium rather than on MS medium with low concentration of BA (0.1 mg l^{-1}). Karhu (1997a) achieved efficient production of high-quality microshoots of *L. caerulea* f. *caerulea* by supplementing nutrient media with 2.0 mg l^{-1} BA.

In the study presented high concentration of BA resulted in increased production of callus at the base of the shoots. Also, Karhu (1997a) reported elevated callus production at a higher concentration of BA in the media. High level of BA can lead to an increased risk of production of adventitiously regenerated shoots. Especially 'Duet' showed higher production of adventitious shoots, which were discarded during subculture. Lately Litwińczuk (2007) reported that propagation method has significant effect on nursery and field performance of blueberries and emphasises that adventitious shoots should be eliminated from micropropagation process. High level of BA caused vitrification of honeysuckle shoots, even up to 36.0% in 'Duet'. However, Sedlák and Paprštein (2007) reported the positive effect of high BA concentration on shoot proliferation of two genotype *Lonicera kamtschatica* (Sevast).

In the study discussed the rooting rate of microcuttings was satisfactory –

Table 6. Mean number of roots per a shoot and mean height of rooted shoots of two blue honeysuckle cultivars

Media	Mean number of roots for one shoot on the 17th day after setting experiment	Mean number of roots for one shoot after two next weeks	Mean height of rooted shoots [mm]
Czelabinka			
MS	7.8 a*	18.0 a	4.18 a
50% MS	9.5 a	23.8 ab	3.94 a
WPM	19.4 b	26.6 b	4.00 a
Duet			
MS	11.5 b	15.8 a	3.66 a
50% MS	7.8 a	17.6 a	3.68 a
WPM	16.2 c	24.2 b	3.32 a

*Explanation, see table 2

Table 7. Length distribution of roots of two blue honeysuckle cultivars (calculation for one flask)

Rooting media	Total number of roots for media	Length of roots [cm]		
		< 1.5	1.6-2.5	> 2.5
Czelabinka				
MS	90	8.9%	26.7%	64.4%
50% MS	119	15.1%	39.5%	45.4%
WPM	133	29.3%	34.6%	36.1%
Duet				
MS	79	12.7%	32.9%	54.4%
50% MS	88	9.0%	33.0%	58.0%
WPM	121	25.6%	46.3%	28.1%

Table 8. Acclimatization of blue honeysuckle shoots in two types of substrate

Measurements	Czelabinka		Duet	
	peat (control)	peat + AgroAquaGel®	peat (control)	peat + AgroAquaGel®
Relative root length index*	1.21	1.01	0.53	1.14
Total root mass after acclimatization [g]	1.05 g	1.07 g	0.98 g	1.13 g
Total shoot mass after acclimatization [g]	4.07 g	4.68 g	3.81 g	5,36 g

*relative root length index = (total root length before acclimatization – total root length after acclimatization)/total root length before acclimatization

92% for 'Duet' and 96% for 'Czelabinka'. The media with low nutrient concentration (WPM media) was found to be more effective in root induction than media rich in mineral salts. Karhu (1997b) suggests short auxin pulses for root induction rather than continuous treatment with auxin. Sedlák and Paprštein (2007) rooted honeysuckle shoots on MS medium supplemented with 2.5 mg l^{-1} IBA achieving 100% rooting and roots of good quality. Lately Karhu (2003) focused on quality of rooted plants than on the root number for the establishing the plants ex vitro. Some experiment proved the possibility for rooting of the two blue honeysuckle genotypes on media without auxins.

Acclimatizing plants originated from *in vitro* conditions is particularly difficult since their specific character. Using substrate supplemented with superabsorbent improved both plant growth and percentage of established plants (Pogroszewska, 1998; Szot, 1998). In current experiment positive effect of using superabsorbent during the acclimatization process was achieved. Especially 'Duet' plants reacted positive to peat substrate supplemented with AgroAquaGel®. However, the results obtained indicate that the method of hydrogel application for each plant species or even cultivar should be investigated separately in specified conditions.

In conclusion, MS medium with salts reduced by ¼ gave the best shoot multiplication rate of the two blue honeysuckle cultivars. The best rooting of both cultivars was achieved on WPM media sup-plemented with 2.0 mg l^{-1} IBA and 5.0 mg l^{-1} IAA. Applying Agro-AquaGel® at acclimatization of plantlets increased their quality by improving the root system.

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ROZMNAŻANIE SUCHODRZEWU SINEGO (*Lonicera caerulea* var. *kamtschatica* Pojark.) Z WYKORZYSTANIEM KULTUR *IN VITRO*

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STRESZCZENIE

Zastosowana procedura odkażania pąków wegetatywnych suchodrzewu jadalnego, polegająca na traktowaniu pąków 70% alkoholem, a następnie roztworem podchlorynu wapnia dała korzystny wynik w postaci zadowalającego procentu niezainfekowanych pąków (65,9% pąków odmiany ‘Czelabinka‘ oraz 64,9% pąków odmiany ‘Duet’). Najwyższy stopień namnożenia pędów dwóch odmian suchodrzewu jadalnego uzyskano po zastosowaniu pożywki według Murashige i Skoog (1962) z zawartością składników mineralnych zredukowanych do ¼. Ukorzenianie pędów na pożywce według Lloyd i McCown (1980) uzupełnionej auksynami IBA oraz IAA (odpowiednio w dawkach 2,0 mg l^{-1} i 5,0 mg l^{-1}) dało korzystny wynik w postaci 96% ukorzenionych pędów odmiany ‘Czelabinka’ oraz 92% pędów odmiany ‘Duet’. Wprowadzenie hydrożelu AgroAquaGel® do podłoża na etapie aklimatyzacji roślin poprawiło efektywność procesu dzięki poprawie jakości systemu korzeniowego.

Słowa kluczowe: suchodrzew jadalny, pożywki, namnażanie, ukorzenianie, hydrożel