Electronic Journal of Polish Agricultural Universities is the very first Polish scientific journal published exclusively on the Internet, founded on January 1, 1998 by the following agricultural universities and higher schools of agriculture: University of Technology and Agriculture of Bydgoszcz, Agricultural University of Cracow, Agricultural University of Lublin, Agricultural University of Poznan, Higher School of Agriculture and Teacher Training Siedlce, Agricultural University of Szczecin, and Agricultural University of Wroclaw.

ELECTRONIC JOURNAL OF POLISH AGRICULTURAL UNIVERSITIES

2002
Volume 5
Issue 2
Series HORTICULTURE

LITWIŃCZUK W. 2002. PROPAGATION OF BLACK CHOKEBERRY (*ARONIA MELANOCARPA* ELLIOT) THROUGH *IN VITRO* CULTURE **Electronic Journal of Polish Agricultural Universities**, Horticulture, Volume 5, Issue 2.
Available Online http://www.ejpau.media.pl

PROPAGATION OF BLACK CHOKEBERRY (*ARONIA MELANOCARPA* ELLIOT) THROUGH *IN VITRO* CULTURE

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ABSTRACT

The aim of the present researchers was to find out solutions which can improve efficiency or/and save costs of chokeberry micropropagation.

The double-phase medium strongly stimulated shoot elongation in comparison to solid medium whereas did not significantly influence shoot proliferation. The reaction of both *Aronia* clones: 'Galicjanka' and 'Nero' on tested media as well as the coefficient of multiplication were generally the same. The best result for shoot rooting *in vitro* was achieved on medium with arginine (200 mg dm^{-3}). Shoots of 'Galicjanka' rooted significantly better than 'Nero' ones both *in vitro* and *in vivo*. The IBA treatment of shoots did not improve rooting efficiency *in vivo* but promoted the growth of obtained plants. Rooting shoots *ex vitro* should be recommended in chokeberry micropropagation.

Key words: chokeberry, *Aronia*, micropropagation, *in vitro*, double-phase medium, arginine, IBA

INTRODUCTION

The black chokeberry is a shrub native to North America although nowadays well-known in Poland. It bears fruits which are rich in macro- and micronutrients (Ca, Fe, Mo, Mn, Cu, B, J, Co), vitamins (P, C, B_2, B_6, PP, E, proA), saccharides, cellulose, pectins and anthocyanins. Therefore fruits of black chokeberry are used in food industry and pharmacy [6]. Chokeberries can be easily propagated by seeds but this method is not recommended, because obtained plants come late into bearing. They also grow too vigorously, are not uniform and then are not suitable for mechanical harvest [2]. Black chokeberry is comparatively young crop and only few cultivars or

breeding strains are known and grown. As micropropagation is far more efficient than other conventional cloning methods, it should improve breeding and rapid propagation of new, valuable strains of *Aronia*. The studies on black chokeberry multiplication *in vitro* were so far carried out by several authors [1, 7, 8, 9, 10, 11]. Because of that the aim of the present researchers was to find out solutions which can improve efficiency or/and save costs of micropropagation. The genera *Aronia* and *Malus* belong to the subfamily *Pomoideae*. Thus, some solutions which gave beneficial results in micropropagation of apple rootstocks, among others: application of double-phase medium to stimulate shoot elongation and/or proliferation [3] as well as addition of arginine to enhance shoot rooting *in vitro* [5] were tested in *Aronia* culture.

MATERIALS AND METHODS

Experiments were carried out on *in vitro* cultures of black chokeberry (*Aronia melanocarpa* Elliot) *cv* 'Nero' and breeding clone 'Galicjanka' ('Albigowianka').

Etiolated shoots of mother plants were surface sterilized and node explants were placed on double diluted MS medium [4] supplemented with MS vitamins, sucrose (20 g dm^{-3}), glucose (5 g dm^{-3}), fructose (5 g dm^{-3}), polyvinylpyrrolidone (PVP 360, 100 mg dm^{-3}), PPM^{TM} (0.5 ml dm^{-3}), 6-benzylaminopurine (BA, 0.5 mg dm^{-3}), indole-3-butyric acid (IBA, 0.05 mg dm^{-3}) and solidified with Kobe I^{TM} agar (5 g dm^{-3}). Cultures were grown in 16 h/8 h day/night photoperiod under cool-white light (OSRAM L36W/20) at 22.8 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD and 26±1°C temperature at each micropropagation phase.

During proliferation stage MS medium with 50% addition of Ca^{2+}, Mg^{2+} and Fe^{2+} salts as well as sucrose (30 g dm^{-3}), BA (1.0 mg dm^{-3}), IBA (0.05 mg dm^{-3}) and Kobe I^{TM} agar (6 g dm^{-3}) was used. The double-phase ('2F') medium was obtained by pouring the liquid MS solution (10 ml/jar) on the medium solidified with agar (50 ml/jar) at the beginning of subculture. Composition of both solid ('1F') and liquid medium was the same. At the proliferation stage the 300 ml capacity glass jars (5 per treatment) closed with transparent plastic 'Twist-off" caps and filled with solid (50 ml) or double-phase medium (50 ml + 10 ml) were used. Twelve nodal explants 1 cm long were planted in each jar. The subculture lasted 5 weeks. Thereafter the fresh weight of culture was determined and the numbers of short and long shoots (length about 5-15 mm and over 15 mm, respectively) were counted and the electric conductance (EC) and the acidity (pH) of medium were measured for each jar separately.

The double diluted MS medium with addition of sucrose (20 g dm^{-3}), arginine, proline or casein hydrolysate (100 and 200 mg dm^{-3}), IBA (0.05 mg dm^{-3}) and solidified with Kobe I agar (6 g dm^{-3}) was used for rooting shoots *in vitro*. The 100 ml capacity Erlenmayer flasks (5 per treatment) covered with aluminium foil and containing 20 ml of medium were used. Twenty healthy shoots 1.5 cm long were planted in each flask. The rooting passage lasted 3 weeks. Thereafter the number of rooted shoots was counted and magnitude of root system was evaluated.

At least 54 of healthy shoots about 2 cm long were dipped in water-ethanol (1:1, v/v) rooting solution of IBA (0, 1.5 and 3.0 mg dm^{-3}) and put down to peat and perlite (1:1, pH = 6.0) mixture watered with fertilizer 'Peters Starter' solution (0.8 g dm^{-3}) and sprayed with solution of 'Florovit' (1%), 'Previcur' (0.15%) and 'Rowral' (0.15%). They were grown at high air humidity in 16h/8h day/night photoperiod under sodium light at 64.4 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PPFD and 21±3°C temperature. The same conditions were maintained for adaptation of shoots rooted previously *in vitro*.

The data were subjected to ANOVA. The means were compared by LSD multiple test at the $P < 0.05$ significance level. The samples consisted of 60 cultures in proliferation stage, 100 and 54 shoots in rooting *in vitro* and *in vivo* stages, respectively and at least 64 plants during adaptation *ex vitro*.

RESULTS AND DISCUSSION

I. Initiation stage. Both *Aronia* clones readily adjusted to *in vitro* conditions and changed into rapidly growing cultures in second – third initation passage. From many other fruit species studied by the author, among others: sour and sweet cherries, apples, mulberries, strawberries, blueberries, actinidias, only blackberries had similar high adaptation potential (data not presented).

II. Proliferation stage. The distinct relationship between growth of cultures and kind of medium was found (fig. 1). The fresh shoot weight of both *Aronia* cultures grown on '2F' medium was significantly higher than '1F' control (tab. 1). The double-phase medium strongly stimulated shoot elongation whereas did not significantly

influence shoot proliferation. The total number of shoots of single culture remained similar both on '2F' and '1F' medium (tab. 1). The differences between two chokeberry clones in the growth intensity was not proved. Contrary to observation made by Litwińczuk [3] on 'M 26', 'MM 106' and 'P 14' apple rootstocks which behaved variously while were grown on '2F' medium the reaction of both *Aronia* clones on tested media was generally the same. The only exception was the elongation of shoots which was relatively more intense on double-phase medium in case of 'Nero' cultures compared to 'Galicjanka' ones (tab. 1). Differences in the acidity of the medium were not found (tab. 2) which may indicate that nutrient preferences of both clones were similar on solid and double-phase media. However, the conductance of double-phase media at the end of passage was lower than in the control (tab. 2). It suggests that a liquid layer of double-phase medium, through diffusion improvement, favours the uptake of medium components by growing cultures. Similar observations were made by Litwińczuk [3] on cultures of apple rootstocks. However, it should be mentioned that double-phase medium was 20% richer in nutrients than solid one. Thus the better growth of cultures on double-phase medium might be also a result of better culture nourishment.

Figure 1. Growth of two chokeberry clones: 'Galicjanka' (V) and 'Nero' (Y) on solid (1) and double-phase (2) medium

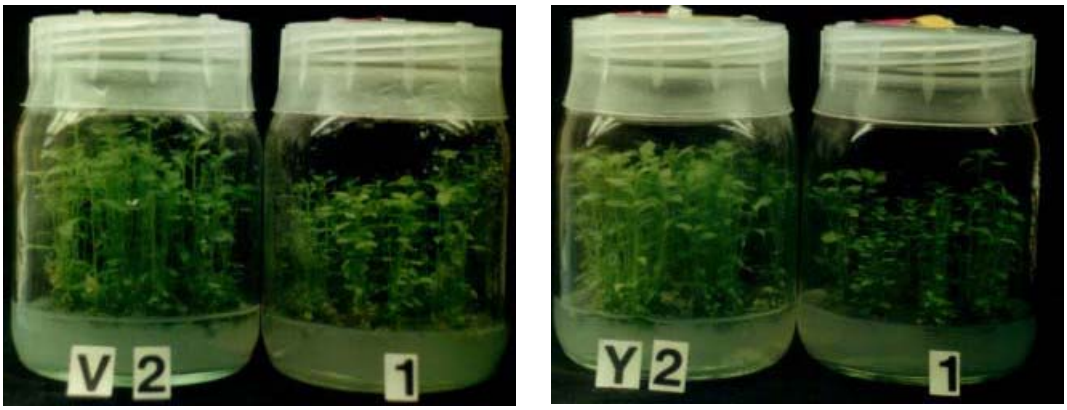

Table 1. Chokeberry culture growth depending on clone and type of medium

Object	Culture fresh weight [mg]	Number of short shoots [5-15 mm]	Number of long shoots [> 15 mm]	Total number of shoots [>5 mm]	Mean length of long shoot [mm]	Total length of long shoots [mm]
A. Clone						
'Nero' 'Galicjanka'	355.9 a[1)] 405.0 a	3.1 b 2.2 a	6.5 a 6.5 a	9.6 a 8.8 a	33.3 a 35.2 a	231.7 a 240.1 a
B. Medium						
1F 2F	330.0 a 429.2 b	3.0 b 2.3 a	5.8 a 7.2 b	8.8 a 9.5 a	30.7 a 37.5 b	183.9 a 285.0 b
A x B. Combination: Clone x Medium						
Nero 1F Nero 2F Galicj. 1F Galicj. 2F	291.0 a 420.7 b 369.0 ab 436.7 b	3.5 b 2.7 ab 2.5 a 2.0 a	5.3 a 7.7 b 6.3 ab 6.7 ab	8.8 a 10.4 a 8.8 a 8.8 a	28.5 a 38.0 c 32.9 b 37.1 c	159.8 a 303.6 c 208.0 ab 268.5 bc
A x B interaction SL[2)]	0.3687[-]	0.7628[-]	0.0733[-]	0.2033[-]	0.0380[*]	0.1052[-]

[1)] means in columns marked with diverse letter are significantly different at α = 0.05
[2)] SL – level of significance

Table 2. Acidity and electric conductance of media at the end of subculture depending on clone and type of medium

Object	pH	EC [mS]
A. Clone		
'Nero' 'Galicjanka'	5.2 a 5.0 a	4.0 a 3.6 a
B. Medium		
1F 2F	5.0 a 5.1 a	4.0 b 3.6 a
A x B. Combination: Clone x Medium		
Nero 1F Nero 2F Galicj. 1F Galicj. 2F	5.2 a 5.1 a 4.9 a 5.1 a	4.4 b 3.6 a 3.7 a 3.5 a
A x B interaction SL	0.6693^{-}	0.0325*

III-IV. Shoot rooting and adaptation stages. For rooting shoots *in vitro* six kinds of media supplemented with different doses of two aminoacids and casein hydrolysate were tested. In most cases the rooting efficiency was very high (tab. 3, fig. 2). The worst result was obtained while casein hydrolysate at 200 mg dm^{-3} was used (tab. 3). Rooting percentages of other media tested were similar. However, the best elongation of roots was observed on medium with arginine 200 mg dm^{-3}. Similar result was achieved by Orlikowska [5] for 'P 60' and 'P 2' apple rootstocks. The clone-specific reaction on tested media was not proved. In general shoots of 'Galicjanka' rooted significantly better than 'Nero' ones (tab. 3). The higher regeneration potential of 'Galicjanka' clone was also confirmed while shoots of both clones were rooted in non-sterile conditions (tab. 4). The IBA auxine treatment of shoots did not improve rooting efficiency *in vivo*. However, auxine promoted the growth of obtained plants (tab. 4).

Figure 2. Rooted *in vitro* shoots of 'Galicjanka' clone

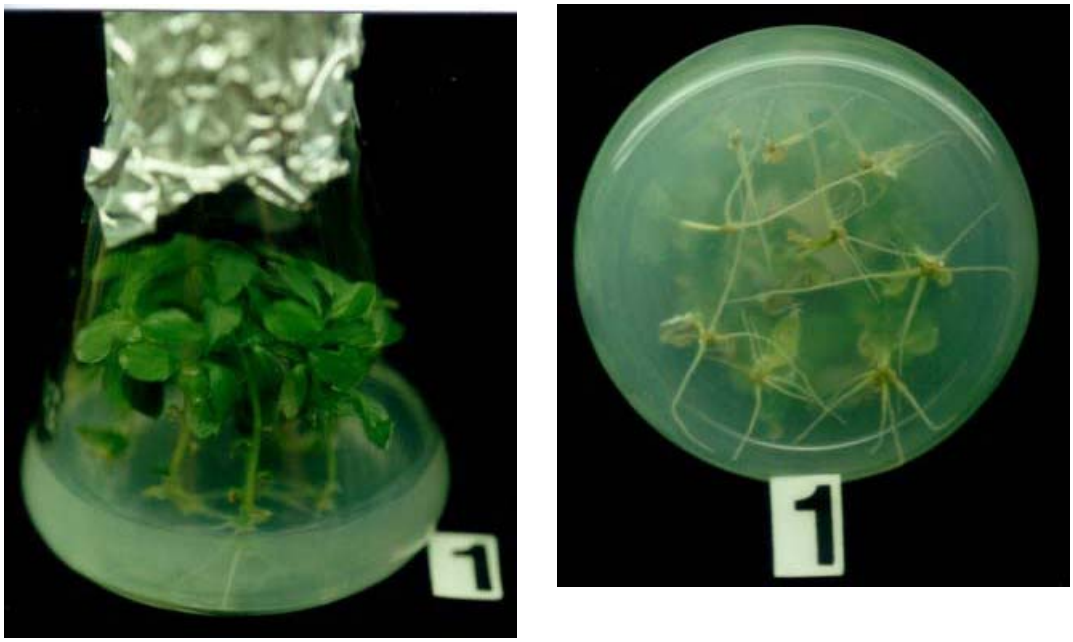

Table 3. Chokeberry shoot rooting *in vitro* depending on clone and kind of applied medium

Object	Number of rooted shoots [%]	Number of roots [pcs]	Length of the longest root [cm]
A. Clone			
'Nero'	80.0 a	3.9 a	0.9 a
'Galicjanka'	89.0 b	4.3 a	1.0 a
B. Substance			
arginine 100 mg dm^{-3}	86.8 b	4.0 a	1.2 cd
arginine 200 mg dm^{-3}	93.8 b	4.1 a	1.5 d
proline 100 mg dm^{-3}	91.3 b	3.8 a	0.8 ab
proline 200 mg dm^{-3}	91.3 b	4.8 a	0.9 ab
casein hydrolysate 100 mg dm^{-3}	83.8 b	4.4 a	1.1 bc
casein hydrolysate 200 mg dm^{-3}	67.5 a	3.7 a	0.6 a
A x B. Combination: Clone x Substance			
N arginine 100 mg dm^{-3}	90.0 bcd	3.7 ab	1.0 bcde
N arginine 200 mg dm^{-3}	90.0 bcd	4.2 ab	1.6 f
N proline 100 mg dm^{-3}	86.7 bcd	3.3 a	0.8 abc
N proline 200 mg dm^{-3}	80.0 bcd	4.5 ab	0.6 ab
N casein hydrolysate 100 mg dm^{-3}	76.7 abc	4.1 ab	1.1 cde
N casein hydrolysate 200 mg dm^{-3}	56.7 a	3.7 ab	0.5 a
G arginine 100 mg dm^{-3}	84.0 bcd	4.3 ab	1.3 ef
G arginine 200 mg dm^{-3}	93.3 cd	3.8 ab	1.2 def
G proline 100 mg dm^{-3}	94.0 cd	4.1 ab	0.8 abc
G proline 200 mg dm^{-3}	98.0 d	4.9 b	1.0 bcde
G casein hydrolysate 100 mg dm^{-3}	88.0 bcd	4.6 ab	1.0 bcde
G casein hydrolysate 200 mg dm^{-3}	72.5 ab	3.9 ab	0.7 abc
A x B interaction SL	0.4671^{-}	0.9104^{-}	0.1556^{-}

Table 4. Chokeberry shoot rooting *in vitro* depending on clone and IBA concentration

Object	Number of rooted shoots [%]	Height of plant after 7 weeks [mm]	Height of plant after 18 weeks [mm]
A. Clone			
'Nero'	68.5 a	24.9 a	59.2 a
'Galicjanka'	77.0 b	25.2 a	59.3 a
B. IBA concentration			
0.0 g dm^{-3}	70.4 a	22.0 a	55.3a
1.5 g dm^{-3}	76.9 a	27.0 b	61.8 ab
3.0 g dm^{-3}	68.5 a	26.1 b	60.5 b
A x B. Combination of Clone and IBA conc.			
N 0.0 g/dm^{-3}	67.6 ab	22.1 a	57.9 ab
N 1.5 g/dm^{-3}	78.7 b	26.8 b	59.7 ab
N 3.0 g/dm^{-3}	59.3 a	25.9 b	60.0 ab
G 0.0 g/dm^{-3}	73.1 ab	21.7 a	52.8 a
G 1.5 g/dm^{-3}	75.0 b	27.3 b	64.1 b
G 3.0 g/dm^{-3}	77.8 b	26.3 b	60.8 b
A x B interaction SL	0.0911^{-}	0.9209^{-}	0.1867^{-}

Table 5. Comparison of the efficiency two schemes of *Aronia* shoot rooting and adaptation

Object	Stage III Number of shoots rooted *in vitro* [%]	Stage IV Number of plants adapted *in vivo* [%]	Stages III × IV (together) Number of obtained plants [%]	Stage III + IV Number of shoots rooted *in vivo* [%]
'Nero'	80.0 a	50.7 a	40.6 a	68.5 a
'Galicjanka'	89.0 b	57.5 a	51.2 b	77.0 b

Adaptation of *ex vitro* shoots rooted previously *in vivo* was unsatisfactory since their percentage did not exceed 60 (tab. 5). Probably the unknown mistake was commited because Staniene *et al.* [9] reached near 100% plant survival rate. Thus the efficiency of two separate stages of micropropagation (III. Rooting shoots *in vitro* and IV. Plant adaptation *in vivo*) was at about 20% lower than for the single combined stage (III+IV. Rooting shoots *in vivo*) (tab. 5). As the last technique is easier and cheaper it should be recommended in chokeberry micropropagation.

CONCLUSIONS

1. Both *Aronia* clones readily adjusted to *in vitro* conditions.
2. The double-phase medium strongly stimulated shoot elongation whereas did not significantly influence shoot proliferation. The reaction of both *Aronia* cultures on tested media was generally the same.
3. The best result for shoot rooting *in vitro* was achieved on medium supplemented with arginine (200 mg dm^{-3}).
4. Shoots of 'Galicjanka' rooted significantly better than 'Nero' ones both *in vitro* and *in vivo*.
5. The IBA treatment of shoots did not improve rooting efficiency *in vivo* but promoted the growth of obtained plants.
6. Rooting shoots *ex vitro* should be recommended in chokeberry micropropagation.

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