

Behaviour of several chokeberry cultivars (*Aronia melanocarpa*) at the *in vitro* micropropagation

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ABSTRACT

Successful micropropagation of black chokeberry (*Aronia melanocarpa*) cultivars 'Nero', 'Melrom' and 'Galicjanka' was realized using young shoots with the aim of an optimized hormones concentration for initiation, multiplication and rooting phases. Good potential of initiation was observed at 'Nero' and 'Galicjanka' cultivars, respectively of shoots regeneration at 'Galicjanka' and 'Melrom' cultivars. Results showed that the use of antioxidant solutions is crucially in the initiation phase in order to prevent explants oxidation. Good initiation rates were obtained at 'Nero' (57 %) and 'Galicjanka' (57%) cultivars, while for 'Melrom' the rate was only 33% by using a hormonal balance of 0.7 mg/L BAP and a reduced level of 0.3 mg/L GA₃. The *in vitro* shoots multiplication rate was relatively stable during the first subcultures. Best micropropagation rates were obtained at 'Galicjanka' and 'Melrom' cultivars followed by 'Nero' cultivar, using a 0.7 mg/L BAP and 0.5 mg/L GA₃. The study showed also that, rooting process is enhanced when the culture medium contains activated charcoal (1.2 g/L) and a lower IBA concentration (0.5 mg/L) compared with the application of higher auxin concentration of the same hormone (1.0 mg/L). Activated charcoal seems to promote a good rooting process, thus being very important in the rooting phase. Researches revealed that the genotype influences the capacity of inoculums regeneration, the multiplication capacity and the rooting ability.

Keywords: propagation, rooting, woody plant medium, 'Nero', 'Melrom', 'Galicjanka'

INTRODUCTION

Aronia spp. are an important group of plants because they have great potential as ornamental plants for landscape projects and as a novel fruit plant with nutraceutical properties (Brand, 2017). Black chokeberry (*Aronia melanocarpa*) is a shrub highly regarded for its rich fruits in anthocyanins, polyphenols and vitamins (Kulling and Rawel, 2008; Brand *et al.*, 2017). All cultivars of black chokeberry are really appreciated for their fruits, 'Viking' has the biggest fruit weight, 'Nero' is preferred for his quality and stability regarding nutrients, while 'Galicjanka' has lower polyphenols and anthocyanins in fruits according recent researches (Arus and Rätsep, 2022). Successful *in vitro* plant micropropagation from excised meristems have been reported for *A. melanocarpa* by several researches (Brand and Cullina, 1990; Kwak *et al.*, 2015; Şuţan *et al.* 2017). Somatic tissues can be a reliable source of explants (Borsai *et al.*, 2017; Rusea *et al.*, 2018). Cultivars 'Nero' and 'Viking' are the most *in vitro* micropropagated (Kwak *et al.*, 2015; Şuţan *et al.*, 2017) using different culture media as WPM and MS (Murashige and Skoog, 1962). The aim of the present research was to study the behaviour of several chokeberry *Aronia melanocarpa* cultivars regarding the initiation, multiplication, rooting and acclimatization by

optimizing all the micropropagation stages, in order to provide a modern improved technology.

MATERIALS AND METHODS

Young shoots of black chokeberry cultivars 'Nero', 'Melrom' and 'Galicjanka' were harvested in February from the field at Bistrița Fruit Research and Development Station. Uninodal shoots were washed for 10 minutes under running tap water, then disinfected by immersing in boscalid and pyraclostrobin (0,5%) fungal solution. Explants were washed three times in sterile de-ionized water on the plate with magnetic stirrer for 15 minutes. The following operations were carried out in the horizontal laminar air flow hood: firstly, shoots were disinfected for 1 minute in 70% ethanol and then for 20 min in 80% ACE bleach. 2-3 drops of Tween 20 were added and then were subsequently rinsed four times with sterile de-ionized water. In order to avoid oxidation in the first inoculation procedures, the uninodal shoots were kept in an antioxidant solution: citric acid 0,15 g/L and ascorbic acid 0,1 g/L.

All cultivars were inoculated on Woody Plant Medium (WPM) (Lloyd and McCown, 1980) supplemented with 0.7 mg/L BAP (6-benzylaminopurine), 0.3 mg/L GA3 (gibberellic acid), 36.7 mg/L FeNaEDTA, 0.5 mg/L, 0.5 mg/L PVP (polyvinylpyrrolidone) to prevent meristem oxidation, 30 g/L sugar as carbon source and 6 g/L Phytoagar for solidification. Shoots were forced in warmed spaced at 25°C for 4-5 days. Caulinar and apical apexes were used for culture initiation and after four weeks the initiation rate was determined. Multiplication medium was WPM supplemented with 0.7 mg/L BAP, 0.5 mg/L GA3, 0.5 mg/L PVP, 50 mg/L ascorbic acid, 30 g/L sugar and 6 g/L Phytoagar. After subcultivation at three-four weeks in three cycles, multiplication rate and shoots length were determined. The same WPM culture medium was used for rooting stage, supplemented with 40 mg/L phloroglucinol, 30 g/L sugar and 5.8 g/L Phytoagar. Regarding hormonal balance, two variants were tested: V1: 0,5 mg/L IBA (indole 3-butyric acid) with 1,2 g/L activated charcoal and V2: 1 mg/L IBA. Rooting rate and roots' length were determined. The *Aronia* cultures were incubated in the vegetative growth room at 25°C in artificial light conditions providing 4000 lux and 16/8-hour photoperiod. Acclimatization stage will be done first in perlite with water and after in mixture of soil:perlite:sand 1:1:1. Research data was analysed with the aid of XLSTAT statistical software (Addinsoft, France) ver. 2019 1.1, embedded in MS Excel, using Duncan's multiple range test.

RESULTS AND DISCUSSIONS

In vitro culture initiation

Initiation stage showed different success rates for all cultivars. Best initiation rates were obtained with cultivars 'Nero' (57%) and 'Galicjanka' (57%), while 'Melrom' rate was only 33% (Table 1), considered that genotype influences the regeneration capacity of inoculums. These results are in agreement with the results obtained by Sivanesan *et al.* (2016) and Clapa *et al.* (2021).

Table 1. The influence of cultivars on the initiation rate of several chokeberry cultivars (*Aronia melanocarpa*)

Cultivars	Initial number of explants	Number of explants after 30 days	Initiation rate (%)
'Nero'	7	4	57
'Melrom'	15	5	33
'Galicjanka'	7	4	57

***In vitro* multiplication**

Multiplication rate (Figure 1) was adequate for all cultivars, that indicate the culture medium and hormones used are suitable for multiplication protocols. The performed statistical analysis showed that 'Galicjanka' and 'Melrom' chokeberry cultivars behaved better than 'Nero' in the multiplication phase with 0.7 mg/L BAP and 0.5 mg/L GA₃.

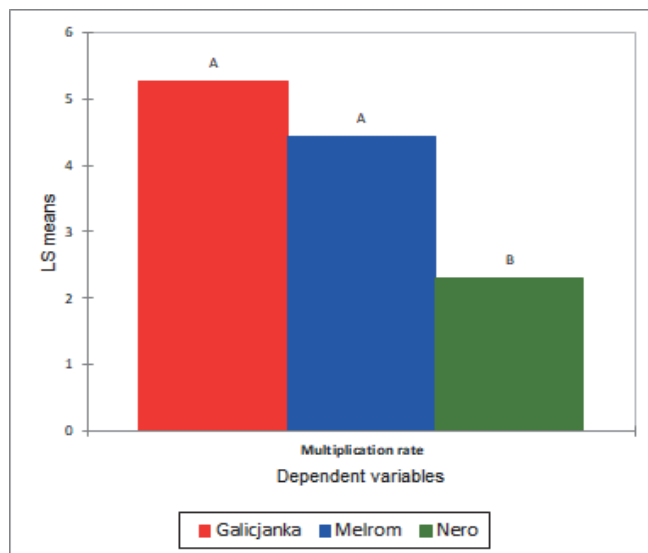


Figure 1. The influence of genotype and culture medium composition on the multiplication rate of 'Nero', 'Melrom', 'Galicjanka' chokeberry cultivars

All cultivars developed well on WPM culture medium: cultivar 'Nero' (Fig 2), 'Melrom' (Figure 3) and 'Galicjanka' (Figure 4). WPM medium supplemented with 0.7 mg/L BAP and 0.5 mg/L GA₃ is suitable for a proper developed of all cultivars, these results are in concordance with Pirlak and Almokar (2018).

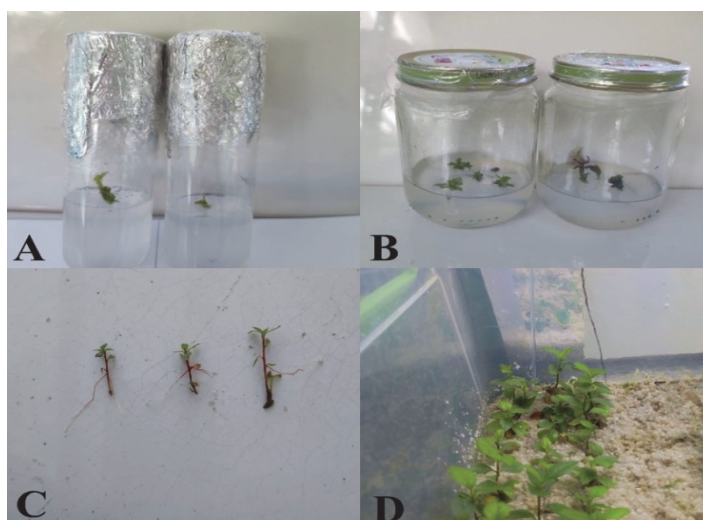


Figure 2. *In vitro* propagation of *Aronia melanocarpa* cultivar 'Nero': initiation stage (A), multiplication stage (B), rooting stage (C), acclimatization stage (D)

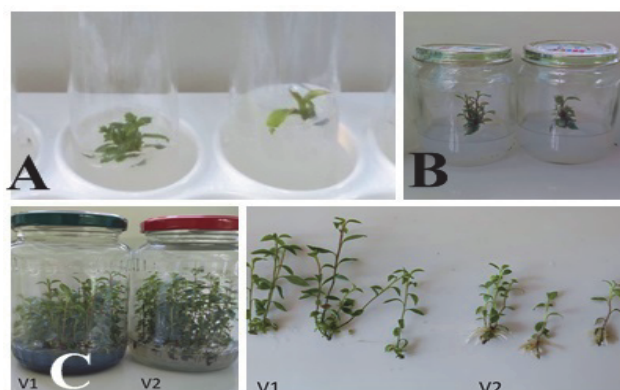


Figure 3. *In vitro* propagation of *Aronia melanocarpa* cultivar 'Melrom': initiation stage (A), multiplication stage (B), rooting stage (C)

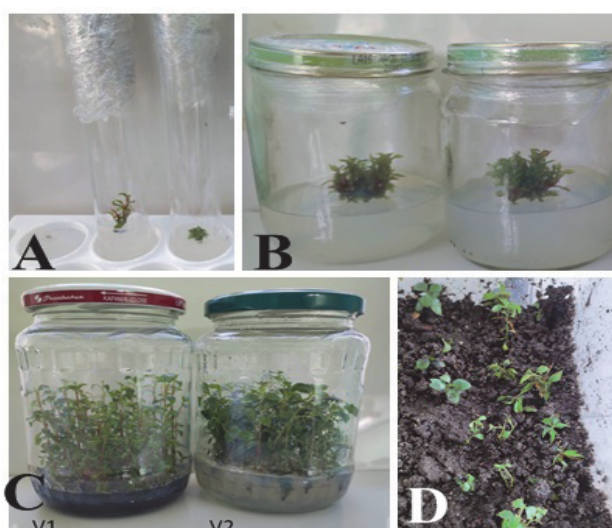


Figure 4. *In vitro* propagation of *Aronia melanocarpa* cultivar Galicjanka: initiation stage (A), multiplication stage (B), rooting stage (C), acclimatization stage (D)

Results regarding the shoots' length (Table 2) showed that 'Galicjanka' and 'Melrom' achieved a shoot length ranged between 2.290-6.510 cm while 'Nero' cultivar achieved 3.933 cm in the multiplication process. The two cultivars 'Galicjanka' and 'Melrom' shoot length were statistically higher that of the cultivar 'Nero'.

Table 2. Shoots length of 'Nero', 'Melrom', 'Galicjanka' during multiplication stage

Variant/Cultivar	Shoots length (cm)
'Galicjanka'	6.510 ^a
'Melrom'	6.290 ^a
'Nero'	3.933 ^b
<i>Pr>F</i>	<0.0001
<i>Significant</i>	Yes

*Different lowercase letters indicate significant differences between the multiplication rates obtained on different medium according to Duncan's test ($p \leq 0.05$)

***In vitro* rooting**

Researches regarding the roots' length (Table 3), showed that 'Nero' had the longest roots ranged between 2.65-2.84 cm in the beginning of rooting process followed by 'Galicjanka' cultivar (2.020 cm) in variant V1. The rooting media composition which contained activated charcoal, showed a good effect in the rooting process. Results showed that smaller roots length was registered in higher IBA hormone concentration application in the other cultivars 'Galicjanka' and 'Melrom'.

Table 3. Roots length of 'Nero', 'Melrom', 'Galicjanka' cultivars

Variant / Cultivar	Roots length (cm)
V1-'Nero'	2.844 a
V2-'Nero'	2.656 a
V1-'Galicjanka'	2.020 b
V1-'Melrom'	1.570 c
V2-'Galicjanka'	1.540 c
V2-Melrom'	1.270 c
<i>Pr > F(Model)</i>	<i>< 0.0001</i>
<i>Significant</i>	<i>Yes</i>

*Different lowercase letters indicate significant differences between the multiplication rates obtained on different medium according to Duncan's test ($p \leq 0.05$).

CONCLUSIONS

The influence of genotype proved to be very important in the *in vitro* micropropagation of *Aronia melanocarpa* cultivars 'Nero', 'Galicjanka' and 'Melrom', revealing different behaviour in the whole multiplication process. Good potential of cell initiation was observed at 'Nero' and 'Galicjanka' and shoot regeneration at 'Galicjanka' and 'Melrom' cultivars respectively. The *in vitro* shoot multiplication rate was relatively stable during the first subcultures. Results showed that it is crucially to use antioxidant solutions in the initiation phase in order to prevent oxidation of explants and to use optimized hormone concentrations. Best micropropagation rates were obtained at 'Galicjanka' and 'Melrom' cultivars followed by 'Nero' cultivar using the optimized hormonal balance of medium concentration of BAP in order to prevent vitrification and a proper GA₃ dosage to promote elongation of shoots. The study showed also that the rooting process is enhanced when the culture medium contains activated charcoal and a lower auxin (IBA) concentration when compared with higher concentration of IBA. Activated charcoal seems to promote a good rooting process in combination with an optimized auxin concentration.

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