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Effect of IBA and TIBA on rhizogenesis of Wild cherry *in vitro*

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Abstract: Nowadays, considerable attention is paid to wild cherry (*Prunus avium* L.) due to its economic and ecological importance. This fast-growing noble tree species is highly valued in furniture industry, while preservation and restoration of its genetic variability is closely related to preservation of biodiversity in general. Along with its importance there is high interest in vegetative propagation of interesting wild cherry genotypes, especially by means of tissue culture. This technique provides relatively fast propagation and production of healthy planting material. Obtained plants are used for the establishment and improvement of seed orchards or the establishment of clonal plantations dedicated for wood production. Important phase in micropropagation of wild cherry is rooting.
In this work, results of application of indole-3-butyric acid (IBA) and 2,3,5-triiodobenzoic acid (TIBA) in rooting medium are presented, as well as the effect of medium pH. After thirty-five days of in vitro cultivation, results of analysis of variance suggested significant effect of examined treatments on variation of parameters of rooting and survival of wild cherry explants. The best rooting and shoot growth were obtained on treatments based on modified of MS medium with 20 μIBA and pH 5.8.

Keywords: *Prunus avium*, root, growth and development.

1. Introduction

Wild cherry (*Prunus avium* L.) is autochthonous, fast-growing noble tree species with broad areal that spans from Europe to northern Africa and western Asia. Its trees reach height of 15 to 30 meters. Beside its importance in fruit production, there is considerable interest in furniture industry in its wood, that is highly valued for its color and texture that resembles mahogany (Katičić-Bogdan et al. 2015). Beside economic this species has ecological significance because the preservation of genetic variability of this species is considered to be important part of preservation of biodiversity in general. To improve growth and the quality of timber of wild cherry, as well as tolerance to prevalent biotic and abiotic agents, long-term breeding projects have been established in a number of countries (Kobliha, 2002; Diaz and Merlo, 2008; Stanković-Nedić et al. 2018; Poljaković-Pajnik et al. 2019). Important step in wild cherry breeding is vegetative propagation of interesting genotypes (Tančeva-Crmarić and Kajba, 2016).

Conventional methods of vegetative propagation are usually time consuming and influenced by numerous hardly controllable agents. That is why micropropagation has broad application in forestry

and horticulture, providing means for relatively low-cost and efficient mass production of healthy planting material with clonal purity, in relatively short time (Vuksanović et al. 2017; Vuksanović et al. 2019a). Numerous studies were conducted on improvement of vegetative propagation of wild cherry *in vitro* (Tančeva-Crmarić and Kajba, 2016).

Auxins are well known plant growth regulators related to the formation of root system. The most used auxins for rooting in vitro as well as *in vivo* conditions are IAA and IBA (Mansseri-Lamrioui et al. 2011). Positive effect of IBA on rooting *in vitro* was found in numerous studies in different species of *Prunus* sp. (Buyukdemirci, 2008; Šiško, 2011; Canli and Demir, 2014; Sarropoulou et al. 2014; Xu et al. 2015; Kumar et al. 2020; Zamanipour et al. 2015). While 2,3,5-triiodobenzoic acid (TIBA) is well known as the inhibitor of polar transport of indol-acetic acid, it could, however, provoke rooting regarding site of application and concentration (McNamara and Mitchell, 1991; Kovačević et al. 2013a).

The aim of this work was to optimize procedure for rooting *in vitro* of autochtonous wild cherry 8A genotype interesting for preservation and improvement of biodiversity.

2. Material and methods

The wild cherry (*Prunus avium* L.) genotype 8A is used in this study. This genotype was chosen by positive selection from the natural stand in vicinity of village Molovin (45°10' N; 19°18' E) in Northwestern part of Serbia and transferred to the gene pool of the Institute of lowland forestry and environment, University of Novi Sad, Serbia. It is characterized by good growth in tissue culture and relatively good rooting potential. Micropropagation was based on axillary buds to avoid somaclonal variability and preserve clonal uniformity. Shoot tips, 10 mm high, where used for the establishment of the experiment. Concentrations of IBA and TIBA, as well as pH of media of examined treatments are presented in table 1. The pH on media with pH 4.0 was regulated with citric acid and sterilized in microwave oven (Kovačević et al. 2013b; Vuksanović et al. 2019b).

Table 1. Concentrations of TIBA and IBA, with used mineral media and medium pH.

Medium	ACM1*)	ACM2	ACM3	ACM4	ACM5	ACM6	ACM7	K1	K2	K3
c(TIBA) (μM/l)	-	0.1	0.5	-	0.1	0.5	0.1	-	-	-
c(IBA) (μM/l)	-	-	-	-	-	-	5	10	20	25
pH	4.0	4.0	4.0	5.5	5.5	5.5	5.5	5.8	5.8	5.8

**) Media with ACM in their label are based on ACM mineral medium (Ahuja, 1984) and those with K in their label are based on modified MS mineral medium*

The treatments with ACM labels are based on ACM medium (Aspen culture medium, according to Ahuja, 1984). Treatments with K labels are based on K mineral medium obtained by modification of MS (Murashige and Skoog, 1962) with pH 5.8.

Table 2. Composition of mineral ACM (Aspen Culture Medium) (Ahuja, 1984).

Macroelements	mg/L	Microelements	mg/L	Vitamins and other ingredients	mg/L
NH_4NO_3	400	NaFe · EDTA	40	Thiamin	0.1
$Ca(NO_3)_2 \cdot 4H_2O$	556	$MnSO_4 \cdot H_2O$	22.3	Nicotine acid	0.5
K_2SO_4	990	$ZnSO_4 \cdot 7H_2O$	8.6	Pyridoxin	0.5
$CaCl_2 \cdot 2H_2O$	96	H_3BO_3	6.2	Sucrose	20000
$MgSO_4 \cdot 7H_2O$	360	KI	0.83	Agar	9000
KH_2PO_4	170	$Na_2MoO_4 \cdot 2H_2O$	0.25		
		$CuSO_4 \cdot 5H_2O$	0.025		
		$CoCl_2 \cdot 6H_2O$	0.025		

Table 3. Composition of modified MS medium.

Macroelements	mg/L	Microelements	mg/L	Vitamins and other ingredients	mg/L
NH_4NO_3	825*	NaFe · EDTA	37.3	Thiamin	0.1
$Ca(NO_3)_2 \cdot 4H_2O$	-	$MnSO_4 \cdot H_2O$	22.3	Nicotine acid	0.5
K_2SO_4	-	$ZnSO_4 \cdot 7H_2O$	8.6	Pyridoxin	0.5
$CaCl_2 \cdot 2H_2O$	220*	H_3BO_3	6.2	Glycine	2
$MgSO_4 \cdot 7H_2O$	370	KI	0.83	Myo-inositol	100
KH_2PO_4	85*	$FeSO_4 \cdot 7H_2O$	-*	Sucrose	20000
KNO_3	-*	$Na_2MoO_4 \cdot 2H_2O$	0.25	Agar	6000
		$CuSO_4 \cdot 5H_2O$	0.025		
		$CoCl_2 \cdot 6H_2O$	0.025		

** Modification of original MS medium (Murashige and Skoog, 1962)*

Cultures were cultivated at t = 26 ± 2°C under white light of LED lamps that provided 3500 lx/m^2 under the 16h/8h day/night photoperiod for 35 days.

2.1. Statistical analysis

The experiment is designed as completely randomized in three repetitions. Five shoot tips were set per jar, with three jars per medium treatment. Following traits were examined: number of first order roots, shoot height (mm), length of the longest root (mm), survival rate (%), and rooting percentage (%), which describes the percentage of rooting of survived explants. In order to meet normal distribution of frequencies, which is required for used tests of parametric statistics, number of roots was transformed by square root transformation ($\sqrt{X+1}$) and survival rate and rooting percentage by arcsine transformation ($\arcsin\sqrt{X}$). Results were analyzed by one-way analysis of variance and difference between treatment means tested by Fisher's Least significant difference test (LSD) at the level of α=0.05. Relations between examined traits are presented by Pearson's correlation coefficient. Statistical analysis was performed by program package STATISTICA 13 was used (TIBCO, 2017).

3. Results and discussion

According to F-test, factor Medium achieved statistically significant effect on variation of all examined traits measured after 35 days of cultivation *in vitro*, except for length of the longest root (Table 4).

Table 4. Results of F-test of one-way factorial analysis of variance for measured traits after 35 days of *in vitro* culture in *P. avium cl. 8A*.

Measured traits	F-test [a)]
Number of first-order roots	30.277 **
Shoot height	5.307 **
Length of the longest root	1.5725
Survival rate	4.000 **
Rooting percentage	14.067 **

*[a)] * - Labels for the significance of F-test: significant at the level of α=0.05; ** - significant at the level of α=0.01*

According to the Fisher's Least significant difference test (LSD-test), the highest number of first-order roots was achieved on media K2 and K3, both based on modified MS medium with pH 5.8, where IBA was added in concentrations 20 μM (≈ 4 mg/l) and 25 μM (≈ 5 mg/l), respectively (Table 5). Except

for survival rate, values for all other traits were significantly lower on medium with 10 μM IBA (≈ 2 mg/l) (K1) than on K2 and K3. The best results on treatments based on ACM mineral medium was obtained on ACM4, the medium without plant growth regulators and with medium pH 5.5. Survival and rooting percentage as well as length of the longest root on this medium were similar to those of K2, but number of roots and shoot height were with significantly lower on ACM4. The smallest number of roots (0.37) was recorded on ACM7 medium, with 5 μM IBA, 0.1 μM TIBA in ACM medium with pH 5.5. The highest shoot height (28.93mm) was obtained on K2 medium (modified MS with 20 μM IBA), while the smallest shoot height value (17.0 mm) was recorded for K3 medium (modified MS with 25 μM IBA).

Table 5. Results of Fisher's least significant difference test for measured traits in *P. avium cl. 8A*.

Treat-ment	Number of first-order roots	Shoot height (mm)	Length of the longest root (mm)	Survival rate (%)	Rooting percentage
ACM1	0.56 d *)	18.16 bc	41.00 ab	100.0 a	44.8 bc
ACM2	0.44 d	18.38 bc	40.17 abc	100.0 a	29.2 cd
ACM3	0.59 d	20.40 bc	49.11 a	100.0 a	52.0 bc
ACM4	1.03 c	19.30 bc	36.13 abc	100.0 a	100.0 a
ACM5	0.65 d	21.93 b	44.19 a	100.0 a	73.8 b
ACM6	0.65 d	19.38 bc	41.44 ab	100.0 a	48.0 bc
ACM7	0.37 d	20.17 bc	37.50 abc	100.0 a	6.7 d
K1	1.27 b	20.33 bc	14.04 bc	100.0 a	60.6 bc
K2	1.67 a	28.93 a	33.93 abc	100.0 a	100.0 a
K3	1.58 a	17.00 c	11.73 c	90.7 b	100.0 a

**) Difference between values of the same traits that are labeled with the same letter are not statistically significant at the level α=0.05*

The smallest length of the longest root (11.73 mm) was achieved on K3 medium (modified MS with 25 μM IBA), and the longest (49.11 mm) on ACM3 medium (0.5 μM TIBA, ACM medium with pH4.0). Generally, treatments with ACM mineral medium, without plant growth regulators or with TIBA tend to have longer roots then treatments with IBA. On all examined media the 100% survival rate was recorded except on K3 where survival rate was 90.7%. The highest rooting percentage was achieved on K2 and K3, as well as on ACM4. Generally, media with TIBA or with pH4.0 achieved moderate or low rooting percentages. The lowest rooting percentage was recorded on ACM7 (6.7%).

The simulative effect of indol 3 butiric acid in modified MS medium on rooting of wild cherry shoot tips *in vitro* was also reported by Ďurkovič (2006) as well as by Tančeva-Crmarić and Kajba, (2016). According to Ďurkovič (2006), 73% of rooting was achieved on medium with 0.3 mg/l IBA. Much higher percentage of rooting achieved Fotopoulos and Sotiropoulos (2004) in *P. persica* × *P. amygdalus*. Our study is also in concordance with results of Ružić and Vujović (2008), who achieved 72.22% of rooting percentage on medium with 5 μM IBA.

Low pH media (pH 4.0) generally achieved negative effect on rooting percentage except for higher concentration of TIBA (ACM3) where it was at the level of rooting percentage at analog medium with pH 5.5 (ACM6). Lower rooting percentage on low pH is in accordance with results of Vuksanović et al. (2017) that they obtained also on media without growth regulators and the same pH in white poplar clones *in vitro*. Inhibitory effect of low pH was also reported by Long et al. (2017) in hydroponics. They found that high concentrations of H^+ ions can produce toxicity symptoms and direct damage of roots of citrus by influencing import of essential minerals and water. However, according to Kovačević et al. (2013b), in medium that was not buffered with citric acid but initially adjusted to pH3.0 with 1M HCl, the stimulative effect on rooting of white poplar microshoots could be obtained.

Pruski (2007) reports that enhanced root induction after treatment with 2 mg/l IBA *in vivo* was found in *Prunus tomentosa, Prunus fructicosa, Prunus verginiaca* and *Prunus pensylvanica*. According to Zhou et al. (2010) use of IBA in concentrations of 4.92 and 7.38 μM resulted in 100% rooting in *Prunus persica* × *P. davidiana in vitro*.

Inhibitory effect of TIBA, as an inhibitor of polar transport of auxins, is in concordance with general opinion (McNamara and Mitchell, 1991). Kovačević et al. (2013a) recorded inhibitory effect of low concentrations of TIBA (0.1μM) on rooting in two black locust genotypes *in vitro*. However, values of length of the longest roots were the highest on two media with TIBA: ACM3 and ACM6. Also, Kovačević et al. (2013a) did found positive effect of 1μM TIBA on number of roots in hard-to-root genotype Rózsaszín AC. They suggested that manipulations with auxins' accumulation site and sensitivity to auxins could increase intensity of the formation of root system, and proposed use of TIBA for improvement of microshoot rooting in some specific cases.

Table 6. Coefficients of correlation and significance of z-test for examined traits in *P. avium cl. 8A.* [a)]

	Number of first-order roots	Shoot height (mm)	Length of the longest root (mm)	Survival rate (%)
Shoot height	0.408			
Length of the longest root	**-0.724**	0.145		
Survival rate	-0.517	0.362	**0.659**	
Rooting percentage	**0.828**	0.319	-0.390	-0.423

[a)] *Bolded coefficients of correlation are significantly different from 0 at the level of α=0.05*

According to coefficients of correlation between measured traits, there is high negative correlation between number of roots and length of the longest root, and high positive correlation between length of the longest root and survival rate, as well as between number of roots and rooting percentage which is in accordance with the results of Vuksanović (2019c), obtained in rooting of five white poplar genotypes.

4. Conclusion

Results of the study suggest that both concentration and type of auxin influenced rooting percentage of wild cherry shoots *in vitro*. The best rooting and shoot growth were obtained on medium based on modified MS with pH 5.8 and 20 μM IBA (K2). Only favorable result obtained in media with TIBA was better root growth. Further research should be directed to a larger number of genotypes by implementation of same or similar methodology.

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