ГЛАСНИК ШУМАРСКОГ ФАКУЛТЕТА, БЕОГРАД, 2013, бр. 108, стр. 67-80

BIBLID: 0353-4537, (2013), 108, p 67-80

Kovačević B., Miladinović D., Katanić M., Tomović Z., Pekeč S. 2013. *The effect of low initial medium pH on in vitro white poplar growth*. Bulletin of the Faculty of Forestry 108: 67-80.

Bravislav Kovačević
Dragana Miladinović
Marina Katanić
Zoran Tomović
Saša Pekeč

UDK: 58.085.2:581.165.7:582.681.81=111
UDK: 630*232.328:630*176.1 Populus alba L.=111
Оригинални научни рад
DOI: 10.2298/GSF1308067K

THE EFFECT OF LOW INITIAL MEDIUM pH ON *IN VITRO* WHITE POPLAR GROWTH

Abstract: The effect of low initial medium pH on shoot and root development of five white poplar (*Populus alba* L.) genotypes was tested. The shoot height, fresh mass of shoots per jar, dry mass of shoots per jar, number of roots, as well as the length of the longest root were measured and final pH of the media determined, after 35 days of culture *in vitro*. Three initial pH values of the medium were tested: 3.0, 4.0 and 5.5 as control. Agar solidification at pH 3.0 was not achieved after sterilization in autoclave, but it was successful after sterilizing in a microwave oven. The obtained results indicate that the tested genotypes are able to significantly influence the changes of media pH during culture. The effect of differences among the examined media was significant for biomass accumulation and final media pH. Generally, significantly higher values of fresh and dry shoot mass, shoot height and the longest root length were recorded on a medium with initial pH 3.0 then on a standard medium with pH 5.5.The implications of the obtained results for the improvement of *in vitro* propagation of white poplars are discussed.

Keywords: *Populus alba*, micropropagation, microwave sterilization

dr Branislav Kovačević, University of Novi Sad, Institute of Lowland Forestry and Environment, Novi Sad (branek@uns.ac.rs)
dr Dragana Miladinović, research fellow, Institute of Field and Vegetable Crops, Novi Sad
dr Marina Katanić, - research associate, University of Novi Sad, Institute of Lowland Forestry and Environment, Novi Sad
dr Zoran Tomović, Public Enterprise "Vojvodinašume", Petrovaradin
dr Saša Pekeč, University of Novi Sad, Institute of Lowland Forestry and Environment, Novi Sad

ЕФЕКАТ НИСКЕ ПОЧЕТНЕ pH ВРЕДНОСТИ ХРАНЉИВЕ ПОДЛОГЕ НА РАСТ БЕЛЕ ТОПОЛЕ *IN VITRO*

Извод: У истраживању је тестиран ефекат ниске почетне pH вредности хранљиве подлоге на развој избојака и корена пет генотипова беле домаће тополе (*Populus alba* L.). Након 35 дана култивације у култури *in vitro* мерени су висина избојака, свежа маса избојака по теглици, сува маса избојака по теглици, број коренова, дужина најдужег корена и одређена је коначна pH вредност хранљиве подлоге. Три почетне вредности pH медијума - 3,0, 4,0 и 5,5- испитане су као стандардна pH вредност медијума (контрола). Проблеми са очвршћавањем подлоге код подлога са ниском почетном pH након стерилизације, превазиђене су стерилизацијом подлоге у микроталасној пећници. Добијени резултати указују на то да испитивани генотипови имају могућност да утичу на промену pH подлоге током узгоја у култури *in vitro*. Већина испитиваних генотипова беле тополе остварила је значајно бољи раст и развој избојка и кореновог система, као и акумулацију биомасе на подлози са почетном вредношћу pH. У раду се дискутује о импликацијама добијених резултата за побољшање *in vitro* размножавања беле тополе.

Кључне речи: *Populus alba*, микропропагација, стерилизација микроталасима

1. INTRODUCTION

White poplar (*Populus alba* L.) is a tree species that is widely spread throughout Europe, eastern Asia and northern Africa. However, in spite of its high adaptability, it is considered to be a threatened species and an indicator of biodiversity (Kovacevic *et al.*, 2010a). Beside its use for wood and biomass production, this species has a wide implementation in horticulture and landscaping, especially the genotypes with a pyramidal tree shape (Eggens *et al.*, 1972; Kovacevic *et al.*, 2010b). Since the propagation of this species by stem cuttings is rather difficult and is one of the main obstacles for wider growing of this species, there is a considerable interest in its propagation by tissue culture (Ahuja, 1984; Guzina *et al.*, 1986). In addition, white poplar (*Populus alba* L.) is one of the most interesting model tree species in biotechnology, where the tissue culture is an important propagation technique (Confalonieri *et al.*, 2000). It is known that different genotypes of the same species differ in their requirements for growth in *in vitro* conditions. Thus, it is necessary to search for optimal culture conditions, in order to achieve satisfactory micropropagation rates and improve and optimize white poplar tissue culture procedures.

Medium pH is one of the important factors of physico-chemical environment during the development of plant tissues *in vitro.* It influences the utilization of medium components such as macro- and microelements and growth regulators (De Klerk *et al.*, 2008). The optimum pH of *in vitro* medium for different phases of the morphogenesis of woody species varies (Saborio *et al.*, 1997). However, the most effective pH value is specific to the individual plant species and even cultivars, and has to be determined experimentally (Ostrolucká *et al.*, 2010; Ruzic, Cerovic, 2001).

Tissue culture has also been extensively used to evaluate the abiotic stress tolerance of many species, since responses are relatively fast, the generation times are short, and the environment is controlled (Cui *et al.*, 2010, Lokhande *et al.*, 2010). It is a good tool to test the *in vitro* ability of the species to form roots and growth in the low pH conditions as most of the factors affecting *in vitro* growth are similar to those limiting the growth *in vivo* (Kovacevic *et al.*, 2010a). Thus, the effect of low medium pH on *in vitro* growth will be useful to understand their tolerance to low pH, without the interference of indirect factors.

In this research, the growth and development of shoots and roots of five different white poplar genotypes were observed with the aim to optimize the culture conditions for plant propagation. In addition, the ability of the tested genotypes to grow *in vitro* on media with low initial pH was investigated.

2. MATERIALS AND METHODS

2.1. Plant material

Five white poplar genotypes, considered interesting for wood production, horticulture and landscaping, were used in the experiment (Tab. 1).

Table 1. Examined white poplar genotypes
Табела 1. Испитивани генотипови беле тополе

Name **Име**	**Origin**[a)] **Порекло**	**Description** **Опис**
″Villafranca″	Italy Италија	Model genotype, straight, narrow tree shape Модел генотип, право стабло узане крошње
L-12	Serbia Србија	Experimental clone, vigorous straight tree shape Експериментални клон, вигорозано право стабло
L-80	Serbia Србија	Experimental clone, vigorous straight tree shape Експериментални клон, вигорозано право стабло
LBM	Serbia Србија	Horticultural genotype, straight pyramidal tree shape Генотип хортикултурног значаја, право ирамидално стабло
KA1	Serbia Србија	From natural stand, vigorous, slightly curved tree shape Из природне састојине, вигорозно слабо закривљено стабло

Legend/Легенда: a) All examined genotypes were selected in Institute of Lowland Forestry and Environment, Novi Sad, Serbia, except the clone "Villafranca", that was selected in Poplar Research Institute in Casale Monferrato, Italy./ a) Сви генотипови су селектовани у Институту за низијско шумарство и животну средину, Нови Сад, Србија, изузев клона "Villafranca", који је селекован у Istituto di Sperimentazione per la pioppicoltura, Casale Monferrato, Италија

Shoots of all five tested genotypes were multiplied by culture of axillary buds, as described by Ahuja (1984). The cultures were sub-cultured at 4-week intervals and kept at 26±2 ºC, under a 16 h photoperiod (cool white fluorescent lamps, 3500 lx), till their use in the experiment.

2.2. Media preparation

At the initial stages of the experiment, the media were sterilized by autoclaving at 120^{o}C and 1.1 bar for 25 min. As there were problems with the solidification of the media with low initial pH (pH 3.0 and 4.0), the sterilization of growing media in a microwave oven was tested. 250 ml of the growing medium was poured into 400 ml Erlenmeyer flasks, sterilized at 800 W for 5 min, and then poured into autoclaved empty 190 ml jars. Agar and Gelrite in different concentrations were used as gelling agents (Tab. 2). As there were no problems with the solidification of microwaved low pH media, all media used in the experiment were sterilized in a microwave oven. Mineral growing medium ACM (Aspen Culture Medium) described by Ahuja (1984), supplemented with 9gL^{-1} agar, 20gL^{-1} sucrose and with no growing hormones in it, was used in the experiment. The pH of the medium was adjusted to 3.0, 4.0 or 5.5 before sterilization.

2.3. Influence of medium pH on shoot and root growth

In order to investigate the effect of initial medium pH on the shoot and root growth of the tested genotypes, 1.0-1.5 cm long shoot tips of the previously multiplied shoots were placed on the media with different initial pH values: pH 3.0, pH 4.0 and pH 5.5 (control). Five shoot tips were cultured per 190 ml jar with 25 mL of culture medium. There were three jars per examined medium within a genotype. The cultures were kept at 26±2 ºC in the white fluorescent light (3500 lx) with a 16-hour photoperiod.

After 35 days of culture, the shoot and root growth was assessed for shoot height (SH), the number of roots per rooted shoot (RN), the length of the longest root (LLR), the fresh shoot mass per jar (FM) and dry shoot mass per jar (DM).

2.4. Changes in media pH

For the purpose of determining the changes in media pH, the pH of the media was measured for each jar separately after 35 days of culture (final pH). The medium in the jars was squashed manually with a plastic fork and then mixed with a similar quantity of deionised water on a magnetic stirrer for 3 min. After that, the pH was measured by inserting a pH electrode in the obtained emulsion.

2.5. Statistical analysis

The examined characters were measured either per jar (FM, DM and final pH) or the average value per jar was calculated after the measurement of individual plants (SH,

RN and LLR). There were three repetitions within each genotype for each tested initial pH. The design of the experiment was totally randomized. The number of roots was transformed by square transformation ($\sqrt{x+1}$) in order to meet the normal distribution of frequencies. The obtained data were analysed by the two-way ANOVA and an LSD test with the STATISTICA 10 (StatSoft Inc., 2011) statistical program.

3. RESULTS

3.1. Media preparation

There were problems with the solidification of the autoclaved low pH media, especially when agar was used as a gelling agent. The autoclaved pH 3.0 medium with agar could not solidify, and the pH 4.0 medium was not sufficiently solid (Tab. 2). Better results were obtained with the Gelrite, but with the concentrations that were several times higher than the recommended one. On the other hand, microwaved media solidified well, regardless of the pH or the gelling agent (Tab. 2). That is why autoclaving was completely replaced by microwave oven sterilization, while agar was used for the solidification of media for shoot and root growth assessment.

Table 2. Solidification effect of gelatinous substance and means of sterilisation in low pH media
Табела 2. Ефекат средства гелификације и начина стерилизације на чврстоћу подлоге при ниском pH

Agar Агар (*g/L*)	**Gelrite Гелрит (*g/L*)**	**pH 3.0**		**pH 4.0**	
		Microwave oven Микроталасна пећница	**Autoclave Аутоклав**	**Microwave oven Микроталасна пећница**	**Autoclave Аутоклав**
9		+	- a)	+	-
10		+	-	+	+/-
12		+	-	+	+
14		+	-	+	+
	2	+	-	+	+
	4	+	+/-	+	+
	6	+	+	+	+
	8	+	+	+	+

Legend/Легенда: a) Medium solidification labels: ″-″ - not solidified; ″+/-″ - partially solidified; ″+″ - solidified / a) Ознак чврстоће подлоге: ″-″ – није чврста; ″+/-″ – делимично очврсла; ″+″ – чврста

3.2. The influence of medium pH on shoot and root growth

Table 3. The results of two-way analysis of variance for examined white poplar genotypes and initial pH a)

Табела 3. Резултати двофакторијалне анализе варијансе за испитиване генотипове беле тополе и почетна pH a)

Character Својство	Mean square Средина квадрата				F-test F-тест		
	Genotype Генотип (A)	Initial pH Почетна pH (B)	Interaction Интеракција A × B	Error	Genotype Генотип (A)	Initial pH Почетна pH (B)	Interaction Интеракција A × B
Root number Број коренова	9.634	2.259	2.259	1.402	6.873**	1.611	1.202
Shoot height Висина избојка	1474.802	328.414	328.414	101.332	14.554**	3.241	0.578
Length of the longest root Дужина најдужег корена	280.197	149.372	149.372	71.104	3.941*	2.101	2.590*
Fresh shoot mass Свежа маса избојка	0.160	0.678	0.678	0.061	2.602	11.058**	0.728
Dry shoot mass Сува маса избојка	0.001	0.005	0.005	0.001	1.519	5.962**	0.404
Final pH Крајњи pH	0.664	0.027	0.027	0.008	82.790**	3.380*	7.973*

Legend/Легенда: a) Degrees of freedom for genotype was DFA = 4, degrees of freedom for initial pH DFB = 2, degrees of freedom for interaction genotype × pH DFA×B = 8, degrees of freedom for error DFERR = 28 and degrees of freedom for total DFT = 42./ a) Степени слободе за генотип: DFA = 4, степени слободе за почетни pH DFB = 2, степени слободе за интеракцију A×B: DFA×B = 8, степени слободе за погрешку DFERR = 28 и степени слободе тотала DFT = 42.

The number of roots varied among genotypes from 3.31 (KA1) to 5.59 (L-12) roots per shoot, the length of the longest root from 26.64 mm (KA1) to 41.72 mm (L-80) and shoot height from 36.21 mm (L-80) to 71.47 mm (KA1) (Tab. 3). The LSD-test showed that there were no significant differences between the pH 4.0 medium and the control for all examined morphological traits in all genotypes (Tab. 4). The medium with the initial

pH 3.0 did not have significant effect on the number of roots and shoot height in all tested genotypes, while the positive effect of the same medium was observed for length of the longest root in Villafranca and L80, fresh mass in LBM, Villafranca and KA1, as well as for dry mass in KA1. The only negative effect of the initial pH 3.0 was recorded on the length of the longest root in KA1. Overall, the initial pH 3.0 medium had a mostly significant positive effect on all the examined traits except for the number of roots.

Table 4. LSD-test for measured morphological characters of examined white poplar genotypes a)
Табела 4. НЗР-тест за мерена морфолошка својства испитиваних генотипова беле тополе а)

Genotype Генотип	**Initial pH Почетна pH**	**Root number Број коренова**	**Shoot height Висина избојка (*mm*)**	**Length of the longest root Дужина најдужег корена (*mm*)**	**Fresh shoot mass Свежа маса избојка (*g*)**	**Dry shoot mass Сува маса избојка (*g*)**
L12	3.0	4.90^{abc}	51.00^{bc}	33.80^{abcde}	0.57^{bcd}	0.082^{bc}
L12	4.0	6.18^{ab}	54.50^{bcd}	25.48^{de}	0.49^{bcd}	0.077^{bc}
L12	5.5	5.67^{ab}	42.80^{abc}	30.53^{cde}	0.48^{bcd}	0.076^{bc}
LBM	3.0	5.13^{abc}	50.80^{bc}	36.20^{abcd}	0.74^{bc}	0.091^{abc}
LBM	4.0	6.53^{a}	50.53^{bc}	33.67^{bcd}	0.45^{bcd}	0.064^{bc}
LBM	5.5	5.00^{abc}	43.47^{bcd}	28.47^{de}	0.33^{d}	0.049^{c}
L111/81	3.0	4.60^{abc}	58.13^{ab}	46.27^{ab}	0.81^{b}	0.102^{ab}
L111/81	4.0	3.40^{cd}	44.15^{bc}	32.15^{cd}	0.40^{bcd}	0.064^{bc}
L111/81	5.5	4.83^{abc}	41.78^{bcd}	23.80^{de}	0.37^{cd}	0.058^{bc}
L80	3.0	3.20^{cd}	40.60^{cd}	49.13^{a}	0.71^{bcd}	0.086^{abc}
L80	4.0	4.27^{bc}	40.83^{cd}	42.70^{abc}	0.39^{cd}	0.060^{bc}
L80	5.5	3.22^{cd}	27.20^{d}	33.33^{bcd}	0.37^{cd}	0.059^{bc}
KA1	3.0	2.20^{d}	70.47^{a}	17.13^{e}	1.28^{a}	0.130^{a}
KA1	4.0	3.60^{cd}	72.87^{a}	28.53^{de}	0.58^{bcd}	0.085^{abc}
KA1	5.5	4.13^{bcd}	71.07^{a}	34.27^{bcd}	0.57^{bcd}	0.077^{bc}
Genotype Генотип	L12	5.59^{a}	48.49^{b}	30.02^{b}	0.51^{b}	0.083^{ab}
	LBM	5.56^{a}	48.27^{b}	32.78^{b}	0.51^{b}	0.068^{ab}
	L111/81	4.28^{b}	48.02^{b}	34.07^{ab}	0.53^{b}	0.076^{ab}
	L80	3.56^{b}	36.21^{c}	41.72^{a}	0.49^{b}	0.066^{b}
	KA1	3.31^{b}	71.47^{a}	26.64^{b}	0.81^{a}	0.094^{a}
Initial pH Почетна pH	3.0	3.94^{a}	54.43^{a}	36.70^{a}	0.84^{a}	0.099^{a}
	4.0	4.70^{a}	52.44^{ab}	33.01^{ab}	0.46^{b}	0.069^{b}
	5.5	4.57^{a}	45.26^{b}	30.08^{b}	0.42^{b}	0.069^{b}

Legend/Легенда: a) The differences among values of particular characteristic marked with the same letter are not significant at the level α0.05./a) Разлике међу вредностима посматраног својства обележених истим словом нису значајне на нивоу α0.05.

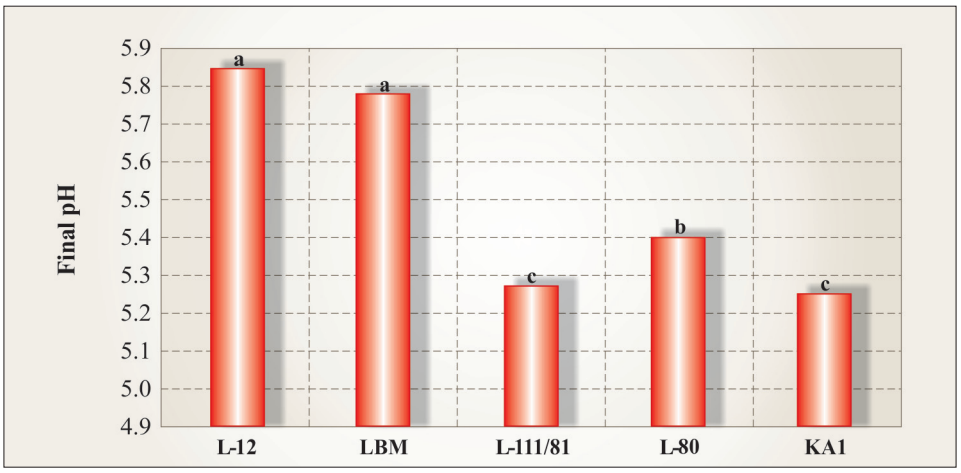

Diagram 1. Average final pH of the media after 35 days of cultivation of examined White poplar genotypes. The letters at the top of the bars correspond to the LSD-test, where the same letter represents the same homogenous group at the level α 0.05

Графикон 1. Просечна коначна pH вредност хранљиве подлоге после 35 дана култивације код испитиваних генотипова беле тополе. Слова на врху бара одговарају LSD тесту, док исто слово представља исту хомогену групу на нивоу α 0.05

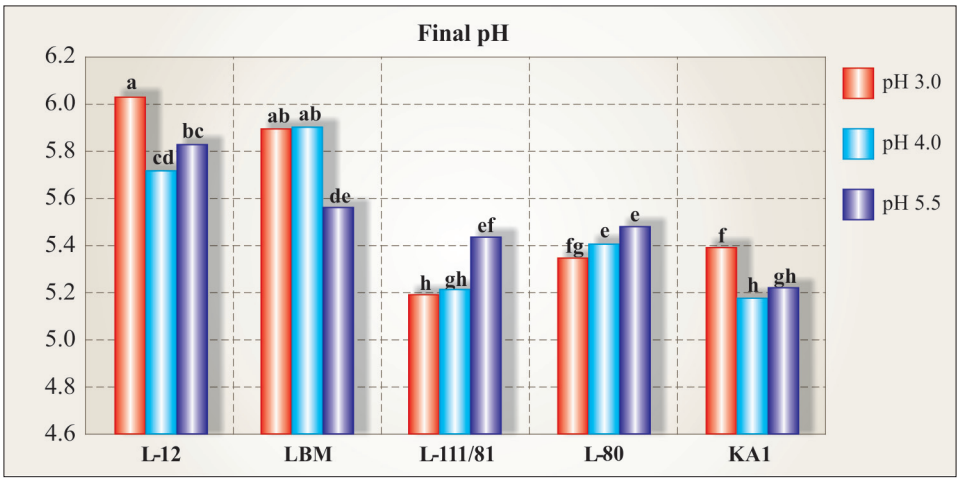

Diagram 2. Final pH of media after 35 days of cultivation for different initial medium pH and examined White poplar genotypes. The letters at the top of the bars correspond to the LSD-test, where same letter represent the same homogenous group at the level α0.05

Графикон 2. Коначна pH вредност хранљиве подлоге после 35 дана култивације за различите почетне pH вредности хранљиве подлоге код генотипова беле тополе. Слова на врху бара одговарају LSD тесту, док исто слово представља исту хомогену групу на нивоу α 0.05.

3.3. Changes in media pH

The LSD-test showed that there were significant differences, regarding the average final pH, among the tested genotypes. Generally, the average final pH varied among genotypes with the pH value ranging from 5.2 (KA1) to 5.9 (L-12) (Fig. 1). Considering initial and final pH, significant changes in media pH of the media with initial pH 5.5 (control) were observed in two out of five tested genotypes (KA1 - significant decrease, L-12 - significant increase) (Fig. 2). In the media with lower initial pH (3.0 and 4.0), there was a significant increase in pH in all tested genotypes, with the final pH values near the standard pH value (5.5).

4. DISCUSSION

One of the main obstacles in the research of the effect of low pH media on *in vitro* development of plant shoots and roots is the loss of the solidifying ability of agar at pH values lower than 4.5 (De Klerk *et al*., 2008). In our work, we have faced the same problem when autoclaving was used as the sterilization method. De Klerk *et al.* (2008) and Woodward *et al.* (2006) managed to solidify the autoclaved media with low pH with Gelrite, but the initial pH was not lower than 4.5. In our experiment neither 0.2% nor 0.4% Gelrite was enough to solidify the growing media with pH 3.0 after autoclaving. The optimal media consistency was obtained only with 0.6% and 0.8% Gelrite, which is several times higher than the recommended concentration (0.2%). However, according to Van Winkle *et al.* (2003), higher Gelrite concentrations could affect the *in vitro* growth, as the doubling of Gelrite concentration in the medium reduces the availability of nutrients by up to 20%. We have managed to overcome the problems with low initial medium pH solidification by sterilizing the media in the microwave oven. Thus, this way of sterilization could be recommended for the experiments on low initial pH solid media. Youssef, Amin (2001) used microwave sterilization for the sterilization of media for the culture of white poplar shoots, but with regular initial medium pH.

Many authors have examined the effect of culture medium pH on the *in vitro* growth of different plant species (De Klerk *et al*., 2008; Ostrolucká *et al*., 2010; Anderson, Ievinsh, 2008; Bhatia, Ashwath, 2005; Liefert *et al*., 1995; Martins *et al*., 2011; Ruzic, 2004). However, as far as we know, no such research has been performed on white poplars. In our experiment, we have tested the effect of medium pH on shoot and root growth of five different white poplar genotypes in order to determine the optimum culture conditions for plant propagation. According to the obtained results, root number in all tested genotypes was not affected by medium pH, which is in agreement with the results of Martins *et al.* (2011) who found that medium pH had no effect on the number of roots of two *Plantago* species. Bennett *et al.* (2003) also observed that the pH of the medium did not affect the number of roots produced by *Eucalyptus globules* shoots. The length of the longest root was not affected by 4.0 pH, while 3.0 pH had the effect of a stimulant on this parameter. Consistently, Martins et al. (2011) found that in Plantago algarbiensis the roots obtained at 4.50 pH were longer than those obtained at 5.75

pH. However, in our work, the genotype KA1 showed the opposite reaction, suggesting significant variability within the species.

Generally, shoot height was significantly higher on the 3.0 pH medium, while there were no significant differences between the 4.0 pH and the control (pH 5.5). Martins *et al.* (2011) did not observe any significant differences in the shoot length of *P. algarbiensis* and *P. almogravensis* grown on low and normal pH media. Naik *et al.* (2010) observed that shoot regeneration of *Bacopa monnieri* was significantly affected by medium pH, obtaining the best results with pH 4.5. Culture on the pH 3.0 medium also had a stimulating effect on the fresh and dry mass produced per jar, as both fresh and dry biomass accumulation was by almost 60% higher on the medium with initial pH 3.0 than on the other two examined media. Several reasons for the stimulating effect of the low pH 3.0 on shoot and root development were suggested:

- the increased initial uptake of microelements at low pH (Van Winkle, Pullman (2003);
- the increased initial uptake of auxins at low pH, as suggested by De Klerk *et al.* (2008);
- the buffering capacity of plants *in vitro*, as suggested by Shang *et al.* (1991), as the higher pH is restored for further optimal uptake of macronutrients.

The final pH in all tested media was higher than the initial one, varying from pH 5.2 to nearly pH 6.0. The only exception was observed in the genotype KA1 on the medium with the initial pH 5.5, where pH decreased to final 5.2. This is not in agreement with the results of Woodward *et al.* (2006), who observed a considerable decrement in rooting media pH in *Eucalyptus marginata in vitro* culture - from the initial pH 5.5 to the final pH 3.0, after 28 days of culture. Martins *et al.* (2011) have also found that the pH significantly decreased on the media inoculated with *Plantago algarbiensis* and *Plantago almogravensis* cultures, after 6 weeks of culture, with the exception of *P.almogravensis* during proliferation.

There were significant differences among the tested genotypes regarding the final pH value, and all genotypes could be divided into three groups according to the final pH. The first group comprises L-12 and LBM with final pH higher than pH 5.5, the second L-80 with final pH near 5.5, and the third Villafranca and KA1 with the final pH lower than 5.5. A shift of a a wide range of initial pH to the same final pH value was observed in *Cucumis melo* callus liquid culture by Skrivin *et al.* (1986). This was also observed in the species *Plantago* on a solid medium by Martins *et al.* (2011), who also recorded differences among the examined *Plantago* species in the final pH on both rooting and proliferation media.

In our work all tested white poplar genotypes were able to achieve and maintain a particular pH (from pH 5.2 to 6.0), regardless of the initial pH (from pH 3.0 to 5.5). It is well known that standard tissue culture media are poorly buffered (De Klerk *et al.*, 2008; Woodward *et al.*, 2006; Owen *et al.*, 1991). Thus, it was found that medium pH could be influenced by media composition, storage conditions and autoclaving (Anderson,

Ievinsh 2008; Owen *et al.*, 1991; Sarma *et al.*, 1990). In addition, it was found that it could also be influenced by the selective uptake of anions and cations by explants (especially in the case of NH_4^+ and NO_3^-) from media (Woodward *et al.*, 2006; Bennett *et al.*, 2003; Schmitz, Lorz, 1990). Furthermore, the buffering of media by plant material was well described by Shang *et al.* (1991) in cotton cell suspension culture. Our results suggest the buffering capacity of examined genotypes, but further research should be performed in order to differentiate this effect on medium pH from others.

5. CONCLUSIONS

According to the data presented, it could be concluded that *in vitro* culture on a medium with initial pH 3.0 had a stimulating effect on the growth and development of white poplar shoots. From these results, it seems evident that all tested white poplar genotypes are able to tolerate an initial pH that is much lower than the commonly used one (pH 5.2-5.8). We have also managed to overcome problems with low pH media solidification by sterilizing the media in a microwave oven. Therefore, the obtained results could contribute to the improvement of *in vitro* propagation of white poplars, and in this way to a wider growing of this species, having in mind the difficulties in its propagation by stem cuttings. Furthermore, in our experiment the tested initial pH values (3.0 and 4.0) did not inhibit shoot and root growth of the tested genotypes, indicating that these genotypes have a certain degree of tolerance to low pH.

Acknowledgements: This paper was realized as part of the project "Studying climate change and its influence on the environment: impacts, adaptation and mitigation" (43007) financed by the Ministry of Education and Science of the Republic of Serbia within the framework of integrated and interdisciplinary research for the 2011-2014 period.

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Бранислав Ковачевић
Драгана Миладиновић
Марина Катанић
Зоран Томовић
Саша Пекеч

ЕФЕКАТ НИСКЕ ПОЧЕТНЕ pH ВРЕДНОСТИ ХРАНЉИВЕ ПОДЛОГЕ НА РАСТ БЕЛЕ ТОПОЛЕ *IN VITRO*

Резиме

Утицај pH подлоге на раст и развој биљака у култури *in vitro* је од значаја како у смислу оптимизације протокола микропропагације, тако и у домену испитивања реакције биљака на дате услове. У нашем раду је испитан је ефекат ниске почетне pH вредности хранљиве подлоге (pH 3.0, 4.0 и 5.5) на раст и развој избојка и корена код пет генотипова домаће беле тополе (*Populus alba* L.). Разлике у расту и развоју биљка међу третманима су праћене на основу висине избојка, свеже масе избојка по теглици, суве масе избојака по теглици, броја коренова, дужина најдужег корена након 35 дана култивације у култури *in vitro*. Такође је на крају овог периода испитана коначне вредност pH медијума. Три почетне вредности pH медијума су испитане: 3.0, 4.0 и 5.5, као стандардна pH вредност медијума (контрола). Проблеми са очвршћавањем подлоге код подлога са ниском почетном pH након стерилизације су превазиђене стерилизацијом подлоге у микроталасној пећници. Задовољавајући резултати су добијени како са агаром тако и са гелритом. Добијени резултати указују на испитивани генотипови имају могућност да утичу на промену pH подлоге током узгоја у култури *in vitro*. Иако је познато да минерлне подлоге које се користе у култури ткива имају слабу пуферну способност, добијени резултати указују да у будућим истраживањима, као што би то било тестирање толерантности према закишељеним срединама

овај проблем морао да се узме у обзир и реши. Већина испитиваних генотипова беле тополе је остварила значајно бољи раст и развој избојка и кореновог система, као и акумулацију биомасе на подлози са почетном вредношћу pH 3.0. Генерално, и сува и свежа маса избојка коју су испитивани генотипови остварили на подлози са pH 3.0 су били преко 60% већи него на подлози са стандардним pH 5.5. Добијени резултати указују на значај даљег рада на испитивању утицаја и практичне примене чврстих подлога са ниским pH у култури ткива беле тополе.