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Original scientific paper

The Influence of Drought on Growth and Development of White Poplar Shoots *In Vitro*

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Abstract: The study tested the effect of drought on growth and development of the shoots of five white poplar (*Populus alba* L.) genotypes (L-12, L-80, LBM, LCM and *Villafranca*) cultured *in vitro*. The effect of four different concentrations of polyethylene glycol (PEG 6000) (1 g/l, 10 g/l, 20 g/l and 50 g/l) in the rooting medium and the rooting medium without PEG (used as Control) were studied. After 35 days of cultivation, following characters were measured: height of shoot, number of roots, length of the longest root, the percentage of survival and rooting. According to the analysis of variance, effects of the media, genotypes and interaction genotype × medium were statistically significant for the most of examined characters. The best differentiation of genotypes was achieved on medium in which the PEG concentration was 50 g/l, so this medium was then used in drought tolerance evaluation. The best drought tolerance was achieved by the *Villafranca* genotype, which had the highest values of the height of shoot, the number of roots and percentage of rooting. Presented results suggest that testing of drought tolerance *in vitro* is a useful method for evaluation of white poplar genotypes. However, the research should be widening on the other parameters and set comparative experiments related to the tests in the field conditions.

Keywords: *Populus alba*, *in vitro* culture, drought tolerance.

1. Introduction

Climate changes, characterized by a trend of an increase in warm temperature extremes, are followed by a frequent alternation in precipitation patterns that can cause severe drought with drastic economic and environmental consequences (IPCC, 2007). Due to the climate change, plant species face new environmental conditions for which they are not adapted. Drought is one of the complex environmental factors affecting growth and yield of crops in arid and semi-arid areas of the world (Mengesha et al. 2016). It is an important abiotic stress, and losses in the production of woody species due to drought are increasing. In determining the optimal ecological conditions (Herpka, 1979) for the cultivation of selected poplar genotypes, in addition to the general potential of the habitat, it is necessary to determine the influence of the

humidification regime and the physiologically active soil profile, whose influence is dominant in some periods of plant's growth and development. Drought severely disturbs water balance of a plant and causes alterations in water uptake patterns (Mahmood et al. 2012). Considering advancing climate changes, the selection of genotypes tolerant to drought is increasingly gaining in importance.

Polyethylene glycol PEG 6000 is a nonpenetrating and nontoxic osmotic substance, which is used to lower the water potential of the culture medium. It is usually used to simulate drought stress in cultured plant tissues (Muhammad et al. 2010). White poplar presents tree species widely occurring throughout Europe, eastern Asia and northern Africa. However, in spite of its high adaptability, it is considered as a threatened species and indicator of biodiversity (Kovacević et al. 2010). There is also general interest in the utilization of white poplar in horticulture and landscape architecture. Besides "*pyramidal*" or "*fastigiate*" tree shape and male sex, the important feature is also tolerance to the environmental stresses. In addition, white poplar is one of the most interesting model tree species in biotechnology, where the tissue culture is an important propagation technique (Confalonieri et al. 2000). *In vitro* evaluation of abiotic stresses tolerance of interesting genotypes could be a useful method due to problems regarding the establishment of testing field trials with long-lived tree species (Vuksanović et al. 2017a; Khattab and El-Garhy, 2016; Vuksanović et al. 2017b). The aim of our research was to *in vitro* evaluate drought tolerance of five white poplar genotypes according to their reaction by morphometric characters on the presence of PEG in different concentrations in rooting medium.

2. Materials and Methods

2.1. Plant material

Five white poplar (*Populus alba* L.) genotypes were tested: widespread Italian selection *Villafranca*, and Serbian selections in experimental phase: LBM, LCM, L-12 and L-80. Five shoot tips were cultured per 190 ml jar with 25 ml of rooting medium. Mineral growing medium ACM (Aspen Culture Medium), described by Ahuja (1984), supplemented with $9gL^{-1}$ agar, $20gL^{-1}$ sucrose and with no growth hormones, was used in the experiment as rooting medium. The experimental design included four treatments/concentrations of PEG 6000: 1 g/l, 10 g/l, 20 g/l, 50 g/l and control with four repetitions/jars per treatment and genotype where we studied effect of PEG on five morphometric characteristics. Media were autoclaved at 120°C for 25 min. The explants were cultured at a temperature of 26±2°C, under a 16 h photoperiod (cool white fluorescent lamps, 3500 lx). After 35 days of cultivation the following morphometric characters were measured for every plant such as shoot height, number of roots and length of the longest root and for every jar: the percentage of survival and the percentage of rooting of survived explants as well.

2.2. Statistical analysis

The data for the number of roots were transformed by square transformation ($\sqrt{x+1}$), and the data for the percentage of survival and percentage of rooting by arcsine transformation ($\arcsin\sqrt{x}$) in order to meet normal distribution of frequencies. The significance of the influence of examined sources of variation and differences between treatments were tested by two-way ANOVA and Fisher's LSD test at the level of α=0.05. All statistical procedures were performed by STATISTICA for Windows version 13 (TIBCO Software Inc. 2017).

3. Results and Discussion

The results of variance analysis after 35 days of *in vitro* cultivation showed that in the most of the morphometric parameters tested, except for the length of the longest root, the influence of the tested medium treatments were statistically significant. The examined genotypes also showed a statistically significant influence on variation of shoot height, number of roots and length of the longest root. The interaction of the genotype × medium that describes the differences in genotypes' reaction on examined medium treatments demonstrated the statistically significant influence on the variation of shoot height, length of the longest root and especially of the percentage of rooting (Table 1).

Results of the LSD test show that there is a statistically significant difference between the examined treatments of drought (treatment with 50 g/l PEG in particular) and Control (without PEG), in total (Figure 1).

Drought achieved by the addition of polyethylene glycol at a concentration of 50 g/L caused 51% decrease in height of the shoot, and 31% decrease of a number of roots compared to the control treatment. In general, an increase of PEG concentration in rooting medium leads to decrease in values in all of the examined parameters (Figure 1A, 1B and 1C). By researching the influence of the drought on the tolerance of the cactus genotypes *in vitro* conditions, adding 0, 10, 20 and 40 g/L of PEG, Mengesha *et. al.,* (2016) of cactus yields the similar results that show that the increase in the PEG concentration reduces number of roots and shoot height. Also, Kulkarni (2007) in his research concludes that increasing the PEG concentration results in decrease in shoot height in tomatoes, as well as Hussein et al. (2017) who added polyethylene glycol (0.5, 1, 1.5 and 2%) to examine the effects of drought *in vitro* on the *Fragaria ananassa*. Earlier studies indicate that the root is first affected by the drought stress compared to other parts of a plant. For example, Mengesha et al. (2016) conclude that number of the roots on the cactus genotypes per plantlet gradually decreased with an increased level of PEG.

Table 1. The results of two-way analysis of variance for morphometric properties [a].

Character	Mean square			F-test		
	Genotype (A)	Medium (B)	Interaction A × B	Genotype (A)	Medium (B)	Interaction A × B
Shoot height (mm)	1069.9	1209.9	52.7	36.76**[b]	41.57**	1.81*
Number of roots	0.7	2.9	0.1	9.26**	37.49**	1.71
Length of the longest root (mm)	796.6	10.9	150.0	9.52**	0.13	1.79*
Percentage of survival	273.4	1428.0	159.6	1.77	9.25*	1.03
Percentage of rooting	127.6	650.0	176.5	2.26	11.50**	3.12**

[a] Degrees of freedom: for genotype was $DF_A = 4$, for medium $DF_B = 4$, for interaction genotype × medium $DF_{A\times B} = 16$, for error $DF_{ERR} = 75$ and for total $DF_T = 99$.

[b] Labels for F-test: * - significant at the level $\alpha_{0,05}$ ** - significant at the level $\alpha_{0,01}$

The significant difference between the control and medium with the highest polyethylene glycol concentration was observed for all examined characters except for the length of the longest root in most of the genotypes. The best differentiation of the genotypes was achieved on nutrient medium with 50 g/l PEG, so this concentration of the stress agent was used in further genotypes' drought tolerance evaluation.

There were differences between examined genotypes in their reaction to the applied drought treatments (Table 1). The weakest inhibitory effect to drought on the tested morphometric parameters was observed in *Villafranca*, L-12 and L-80, while LCM and LBM genotypes achieved significantly poorer results. According to the results of this treatment, the widespread Italian clone *Villafranca* achieved the greatest percentage of rooting and shoot height,

as well as number of roots, while it had the second greatest length of the longest root after L-12. The differences in percentage of survival were not statistically significant (Table 2).

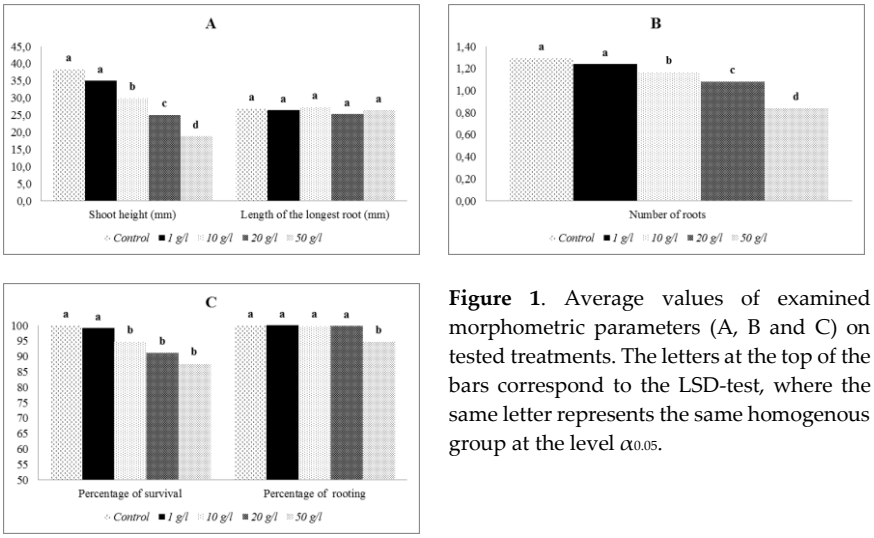

Figure 1. Average values of examined morphometric parameters (A, B and C) on tested treatments. The letters at the top of the bars correspond to the LSD-test, where the same letter represents the same homogenous group at the level $\alpha_{0.05}$.

The *Villafranca* genotype suffered the weakest impact of the drought on the medium with a 50 g/l PEG on the percentage of rooting, while the genotype L-12 achieved the longest root on the same medium. Fraser et al. (1990) reported that the reduction in the root length under drought stress may be due to an impediment of cell division and elongation, leading to tuberization. This improvement of root system through tuberization and lignification allow better exploitation of soil moisture and nutrients by plant. The length of the longest root is widely used in the tolerance studies (Turner and Marshal, 1972).

Table 2. LSD-test for measured morphometric characters of examined white poplar genotypes (only results for Control and medium with 50 g/l PEG are presented) [a)]

Genotype	PEG (g/l)	Shoot height (mm)		Number of roots		Length of the longest root (mm)		Percentage of survival		Percentage of rooting	
L-12	Control	42.6	bc	1.30	abcd	34.8	abc	100	a	100	a
L-80	Control	28.5	defghi	1.18	cdefgh	29.1	bcdef	100	a	100	a
LBM	Control	33.7	de	1.41	a	21.8	defg	100	a	100	a
LCM	Control	31.1	defg	1.29	abcd	24.3	cdefg	100	a	100	a
Villafranca	Control	56.0	a	1.29	abcd	24.7	cdefg	100	a	100	a
L-12	50	12.6	l	0.66	l	47.5	a	76	b	96	bc
L-80	50	18.9	jkl	0.96	jk	25.6	bcdefg	95	ab	100	a
LBM	50	16.4	kl	0.84	kl	13.2	g	95	ab	86	c
LCM	50	16.4	kl	0.69	l	13.1	g	88	b	71	d
Villafranca	50	30.0	defgh	1.00	ijk	32.8	bcde	80	b	100	a

[a)] The differences among values of particular characteristic marked with the same letter are not significant at the level of $\alpha_{0.05}$.

There were found significant differences between white poplar genotypes in this trait in vitro regarding medium pH (Vuksanović et al. 2016) and salt concentration (Vuksanović et al. 2017a). In this study, there were no significant differences between the length of the longest root on 50 g/l PEG medium and control in total or within examined genotypes. However, there were found significant differences in length of the longest root between genotypes on 50 g/l PEG medium, where L-12 and *Villafranca* had significantly longer the longest root than LBM and

LCM. This is important since root system with longer root length is useful in uptaking water, so early and rapid elongation of the root could be an important indication of drought tolerance (El Siddig et al. 2013).

The obtained results indicate that *in vitro* culture tests could help to narrow the selection of white poplar genotypes that are tolerant to water stress caused by PEG 6000 that could be used in dry habitats.

4. Conclusions

The study tested the effect of drought on the nutrient medium for rooting with four concentrations of PEG (polyethylene glycol) (1 g/l, 10 g/l, 20 g/l and 50 g/l) and Control without PEG on the growth and development of the rooted shoots of five white poplar genotypes on five morphometric characters (height of shoot, number of roots, length of the longest root, the percentage of survival and rooting), after 35 days of *in vitro* cultivation. Analysis of variance showed that there was the statistically significant effect of media, genotypes and interaction genotype × medium on the variation of the most of examined characters. Generally, statistically significant lower values in all studied morphological characters except length of the longest root have been recorded on the medium with 50 g/l PEG compared to the control, as well as in relation to the other tested media. The weakest inhibitory effect of drought on the tested morphometric parameters was observed in the *Villafranca*, L-12 and L-80, while the LCM and LBM genotypes achieved significantly poorer results.

The obtained results indicate that *in vitro* culture tests could help to narrow the selection of white poplar genotypes that are tolerant to water stress that could be used in the areas of drought.

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