Arch Biol Sci. 2020;72(2):153-163 https://doi.org/10.2298/ABS200114008I

Variability and relationships among rooting characteristics for white poplar hardwood cuttings

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Received: January 14, 2020; **Revised**: January 28, 2020; **Accepted**: February 20, 2020; **Published online**: February 24, 2020

Abstract: White poplar is a key species in wood production and afforestation, but also very important for biodiversity preservation and habitat improvement. Vegetative propagation from hardwood cuttings is the most desirable method of white poplar breeding. The present research analyzed the effect of various technological treatments on four white poplar clones. Selected treatments were powder formulations of indole butyric acid (IBA) and foliar treatment with urea. The variability of 14 morphometric characteristics on the rooting of cuttings was analyzed, as well as relationships between them. Differences in analyzed morphometric characteristics depended on genotype, while the effect of the examined technological treatments was detected only by Fisher's least significant difference (LSD) test. Significant variability between genotypes was found for traits describing rooting at the basal cut (R0 and R0p), the number of leaves (LN), the total number of roots (TRN), and cutting survival in the first part of the growing season (SURV07). Application of IBA had significant stimulating effects on rooting at the lower part of cuttings (R05) compared to controls. Variability between genotypes after foliar treatment with urea was not detected. The examined parameters were grouped into 5 groups by principal component analysis, where shoot traits and traits that describe rooting at the basal cut were in the same group with total number of roots, and cutting survival (SURV07) was in a separate group. These results suggest a need for further testing of the characteristics of hardwood cuttings in white poplars. More efficient clone technology is needed, using auxins and/or nitrogen fertilization designed to suit the specific requirements of particular clones.

Keywords: vegetative propagation; hardwood cutting; plant nursery; *Populus alba*

INTRODUCTION

White poplar is a vigorous tree species used in wood production and afforestation of marginal soils with high salinity, acidity, or low moisture content [1,2]. In Serbia, it is a dominant tree species in riparian zone forests, and a key component of biodiversity preservation and improvement strategies for these areas [3,4]. A principal advantage of most poplar species is that their hardwood cuttings can be successfully rooted. However, species from the section *Populus* (*Leuce* Duby), including white poplar, are difficult to root from hardwood cuttings, presenting problems in vegetative propagation, planting and production of target genotypes [5,6]. These problems may be avoided by selecting "easy-to-root" genotypes, coupled with optimization of nursery technology [7,8]. Unlike other poplars, activation of preformed primordia in *Leuce* species is difficult. Primordia are anatomical formations in primary bark formed during the previous growing season. In spring, roots from preformed primordia are initiated before wound roots, and are formed by primordia on the basal side of the cutting, providing a considerable advantage to the genotype with respect to production of reproductive and planting material [9,10]. Thus, activation of preformed primordia is important for white poplar breeding [11-13]. Root

How to cite this article: Igić D, Borišev M, Vilotić D, Šijačić-Nikolić M, Ćuk M, Ilić M, Kovačević B. Variability and relationships among rooting characteristics for white poplar hardwood cuttings . Arch Biol Sci. 2020;72(2):153-63.

formation of preformed primordia is influenced by many factors, including genotypic differences, environmental conditions and technological procedures [8,14-18]. Therefore, white poplar nursery technology must be optimized for each particular clone [4,10,19]. In addition to genotypic traits, hormonal regulation networks also influence poplar rooting during vegetative propagation [20]. For example, Zhao et al. [21] suggest that genetic and environmental factors, including exogenous interventions and treatments, as well as physiological properties, are all essential for development of successful rooting strategies. In fact, their findings concerning hormonal regulation of root formation in poplars could be developed for practical applications. For example, auxins are known to play a central role in adventitious root formation. A particular auxin, indole-3-butyric acid (IBA), has higher root-induction capacity and better light stability than the more common indole 3-acetic acid (IAA), and undergoes conversion to IAA in the plant [22]. Likewise, nitrogen supply is critical for adventitious root formation; and nitrogen can be stimulatory or inhibitory, depending on the plant species, environmental conditions and concentration of applied nitrogen [20].

The aim of the present study was to compare the rooting capacity of four white poplar clones, and to evaluate the effects of IBA and urea on adventitious root formation. Our results could provide a basis for improvement of nursery technologies.

MATERIALS AND METHODS

Experiments were conducted at “Ratno ostrvo” nursery, PC “Vojvodinašume”, in the vicinity of Novi Sad (45°17’05”N 19°54’38”E), on soil with a loamy texture surface layer (depth 0-20 cm) dominated by fine sand and silt (ca. 40%) [19]. Soil used for experiments is classified as poor due to high $CaCO_3$ content (10.62-11.03%), pH of 7.72-8.04, and low humus content (1.12-1.78%). Total nitrogen was 0.09%-0.05%; average available phosphorus was 3.44-10.79 mg per 100 g soil, and potassium was 2.30-8.41 mg per 100 g soil.

Four white poplar clones were examined: Italian clone *Populus alba* cl. Villafranca and three experimental clones: *P. alba* cl. L-12, *P. alba* cl. L-80 and *P. alba* cl. L-100 of the Institute of Lowland Forestry and Environment, Novi Sad, Serbia.

Cuttings were prepared and planted in the second half of March, 2017. Nursery trials were randomized, and conducted in three repetitions, with 50 hardwood cuttings per plot and 20x155-cm spacing. Cuttings were 18-20 cm long with a diameter of 8-15 mm. The effects of combined application of IBA powder on the basal cut site before planting and foliar application of 2% urea solution were examined. Foliar treatment with urea solution was performed in mid 11.05.2017.

Technological treatments

The following treatments were applied: 06i – 0.6% IBA powder; 06iu – 0.6% IBA powder and foliar treatment with 2% urea; 2i – 2% IBA powder; 2iu – 2% IBA powder and foliar treatment with 2% urea; c – control; cu – foliar treatment with 2% urea. Plants were mechanically weeded and irrigated as needed. On 9 June 2017, the first ten cuttings were removed from the soil, washed and prepared for measurements. The remainder of the cuttings were left in the soil to record their survival.

Parameters measured for root cuttings

SH – shoot height; LN – number of leaves; RN0 – number of roots at the basal cut site; RN05 – number of roots 0-5 cm from the basal cut; RN510 – number of roots 5-10 cm from the basal cut; RN1020 – number of roots between 10 cm from the basal cut and the top cut; SURV07 – percentage of survived cuttings at the time of measurement. The following parameters were derived: RN5 – number of roots 5 cm from the basal cut (RN5=RN0+RN05); TRN – total number of roots. Parameters describing partitions of the number of roots at defined distances from the basal cut vs. the total number of roots were as follows: RN0p – (RN0/TRN)*100%; RN05p – (RN05/TRN)*100%; RN5p – (RN5/TRN)*100%; RN510p – (RN510/TRN)*100%; RN1020p – (RN1020/TRN)*100%.

Statistical analysis

The trial was completely randomized and consisted of six technological treatments on four genotypes of white poplar and their interactions for three repetitions. For further statistical analysis, average values calculated at the level of plot were considered to be a repetition. Data describing the number of leaves and roots were transformed by square transformation (($\sqrt{X+1}$)), and data for the partition over the total number of roots were transformed by arcsine transformation (arcsin $\sqrt{X}$). These transformations were performed in order to meet the normal distribution of frequencies that is a condition for the applied parametric tests. Data were analyzed by two-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) test to evaluate the significance of the effect of the examined controlled sources of variance and differences among treatments.

The relationship between the examined parameters was described by Pearson's correlation coefficient individually for each pair of parameters, while grouping of parameters was analyzed by principal component analysis (PCA). Grouping was done according to parameter loadings with principal components (PCs) selected by Kaiser's role (λ>1, where λ stands for the eigenvalue of a particular PC), and rotated by the Varimax method to maximize the variance of loadings between measured parameters and PCs within the PC. Analyses concerning the relationship between parameters were performed in the R programming language using a "psych" module.

RESULTS

A principal problem in white poplar nursery production is the difficulty of rooting white poplar hardwood cuttings. Based on two-way factorial ANOVA (Table 1), no statistically significant effect was found for technological treatments on any of the examined traits. However, the factor genotype had a statistically significant effect on the number of leaves (LN), the number of roots on the basal cut (R0) and its partition in the total number of roots (R0p), total number of roots (TRN), and the percentage of survived rooted cuttings at the time of measurement (SURV07). The examined genotypes showed relatively good survival at the time of measurement (SURV07), ranging from 40 to 70% (Table 2).

LSD test results show that some traits differed between interaction treatments. For traits RN5, TRN, R0p, R510p and SURV07, five or more homogenous interaction treatment groups were formed (Table 3).

Table 1. F-test from two-way factorial ANOVA for traits of rooted cuttings in white poplar genotypes

Trait [a]	**F-test for genotypes (A)**		**F-test for technological treatment (B)**		**F-test for interaction A × B**	
SH	2.287		0.391		0.751	
LN	2.987	* [b]	0.571		0.697	
R0	3.806	*	1.083		1.507	
R05	2.312		1.577		1.342	
RN5	2.728		1.276		1.595	
R510	0.894		0.396		1.038	
R1020	0.847		1.515		0.447	
TRN	3.038	*	1.543		1.679	
R0p	9.539	**	0.936		0.904	
R05p	2.414		1.922		0.526	
RN5p	2.218		1.171		0.909	
R510p	2.035		1.888		1.265	
R1020p	0.769		1.738		0.565	
SURV07	16.924	**	1.111		0.540	

[a] Traits: SH – shoot height, LN - number of leaves, RN0 – number of roots on basal cut, RN05 – number of roots from the basal cut to the 5th cm from the basal cut, RN510 – number of roots from the 5th to the 10th cm from the basal cut, RN1020 – number of roots from the 10th cm from the basal cut to the top cut, RN5 – number of roots below the 5th cm from the basal cut (RN5=RN0+RN05), TRN – total number of roots, RN0P – (RN0/TRN)*100%, RN05P – (RN05P/TRN)*100%, RN5P – (RN5/TRN)*100%, RN510P – (RN510P/TRN)*100%, RN1020P – (RN1020P/TRN)*100%, SURV07 – percentage of surviving rooted cuttings at the time of measurement.
[b] Significance of F-test: * – $p<0.05$; ** – $p<0.01$

According to Pearson's correlation coefficients (Table 4), shoot height and the number of leaves are highly positively correlated: generally in moderate correlation with other examined characters. However, with the exception of R0p, weak correlation was found with most traits describing partitioning of the number of roots over different parts of the cutting with respect to the total number of roots. A positive but weak correlation was found between the number of roots at the middle (R510) and upper (R1020) parts of cuttings; while weak or no significant correlation was found between them and traits that describe the number of roots on the lower parts of the cuttings. Analogous traits that describe the partition in the total number of roots had a high negative correlation with R0p and RN5, and a low negative correlation with R05p. The correlation of RN5 with R0 was high and positive, but moderate with R05, suggesting a much stronger

Table 2. Results of the LSD test at the level of factor operation and factor genotype for traits of rooted cuttings in white poplar genotypes.

Trait [b]	**Technological treatment**						**Genotype**			
	06i [a]	06iu	2i	2iu	c	cu	L-100	L-12	Villafranca	L-80
SH	32.74 a	35.56 a	34.85 a	34.14 a	32.72 a	31.88 a	29.95 b	33.60 ab	36.74 a	34.28 ab
LN	20.05 a	21.37 a	20.16 a	20.76 a	21.27 a	19.19 a	18.56 b	21.19 a	22.19 a	20.02 ab
R0	5.42 a	5.65 a	7.00 a	6.97 a	5.04 a	5.15 a	4.29 b	5.52 ab	6.31 a	7.38 a
R05	2.89 a	2.40 ab	2.73 a	2.78 a	1.72 b	2.33 ab	2.09 b	3.13 a	2.47 ab	2.22 b
RN5	8.36 a	8.13 a	9.78 a	9.81 a	6.79 a	7.53 a	6.41 b	8.63 ab	8.87 a	9.65 a
R510	1.46 a	1.33 a	1.26 a	1.62 a	1.41 a	1.40 a	1.24 a	1.35 a	1.55 a	1.51 a
R1020	0.87 ab	0.64 b	1.34 a	1.30 a	0.94 ab	1.02 ab	0.97 a	0.81 a	1.05 a	1.20 a
TRN	10.73 ab	10.15 ab	12.54 ab	12.89 a	9.39 b	10.12 ab	8.79 b	11.01 ab	11.57 a	12.43 a
R0p	42.11 b	53.15 a	49.86 ab	47.29 ab	48.13 ab	46.36 ab	38.31 c	42.14 bc	50.62 b	59.96 a
R05p	31.93 a	26.30 ab	27.15 ab	22.00 ab	17.42 b	22.24 ab	27.60 ab	29.31 a	21.78 ab	19.60 b
RN5p	75.81 a	80.81 a	78.39 a	71.41 a	70.49 a	70.75 a	68.39 b	74.57 ab	75.07 ab	80.53 a
R510p	16.50 a	12.97 ab	10.22 b	16.80 a	18.93 a	14.51 ab	18.11 a	14.93 ab	15.04 ab	11.60 b
R1020p	6.36 ab	4.81 b	10.41 ab	10.87 ab	8.51 ab	13.60 a	11.36 a	8.54 a	8.61 a	7.07 a
SURV07	53.92 a	59.71 a	57.11 a	58.71 a	51.89 a	49.82 a	55.14 b	54.98 b	39.94 c	70.06 a

[a] Technological treatments: 06i – cutting treatment with 0.6% indole butyric acid powder, 06iu – cutting treatment with 0.6% indole butyric acid powder and foliar treatment with 2% urea solution, 2i – cutting treatment with 2% indole butyric acid powder, 2iu – cutting treatment with 2% indole butyric acid powder and foliar treatment with 2% urea solution, c – control, cu – foliar treatment with 2% urea solution.

[b] Traits: as per Table 1.

[c] Values of a parameter with the same letter belong to the same homologous group according to the LSD test at the level of α = 0.05

Table 3. Results of the LSD test at the level interaction of technological treatment × genotype for traits of rooted cuttings in white poplar genotypes.

Trait [b]	**Technological treatment**											
	06i [a]				06iu				2i			
	Genotype											
	L-100	L-12	Villafranca	L-80	L-100	L-12	Villafranca	L-80	L-100	L-12	Villafranca	L-80
SH	21.17 b [c]	33.89 ab	35.69 a	40.21 a	37.14 a	35.86 a	35.72 a	33.50 ab	32.52 ab	34.74 a	36.72 a	35.44 a
LN	14.64 d	21.93 abc	22.71 abc	21.48 abc	21.98 abc	21.56 abc	22.18 abc	19.82 abcd	19.42 abcd	20.77 abc	20.46 abcd	20.01 abcd
R0	1.65 d	6.00 bc	6.84 abc	8.36 ab	6.73 abc	4.39 bcd	4.79 bcd	6.88 abc	5.73 bc	4.10 bcd	12.12 a	7.08 ab
R05	1.66 bcd	3.44 ab	3.24 bc	3.36 bc	2.90 bcd	1.84 bcd	2.88 bcd	2.04 bcd	2.41 bcd	2.62 bcd	3.71 ab	2.26 bcd
RN5	3.31 e	9.47 abcd	10.11 abcd	11.90 abc	9.64 abcd	6.28 cde	7.81 bcde	8.97 abcd	8.13 bcde	6.65 cde	16.01 a	9.37 abcd
R510	1.00 c	0.98 c	2.12 ab	1.83 abc	1.46 abc	1.52 abc	1.26 abc	1.10 abc	1.06 bc	1.03 c	1.26 abc	1.73 abc

Table 3. Continued

R1020	0.53 b	0.57 ab	0.93 ab	1.56 ab	0.54 ab	0.53 b	0.66 Ab	0.84 ab	1.39 ab	1.27 ab	1.06 ab	1.66 ab
TRN	4.86 f	10.99 bcde	13.25 abcd	15.32 abc	11.71 abcde	8.36 def	9.74 cdef	10.92 bcde	10.72 bcde	8.97 cdef	18.33 a	13.01 abcd
R0p	25.83 f	46.57 abcdef	42.52 abcdef	54.49 abc	54.71 abc	46.67 abcde	48.52 abcde	62.57 a	39.49 bcdef	39.99 bcdef	62.79 a	57.15 abc
R05p	40.96 a	37.42 ab	24.86 abc	25.33 abc	27.95 abc	23.07 abc	34.12 ab	20.68 abc	30.96 abc	33.85 ab	24.84 abc	19.67 abc
RN5p	68.05 abcd	84.51 ab	67.92 abcd	81.12 abcd	83.35 abcd	70.91 abcd	84.10 abc	83.86 abcd	72.03 abcd	74.77 abcd	88.19 a	77.04 abcd
R510p	24.68 ab	10.41 cdef	20.92 abcde	11.75 bcdef	10.65 cdef	21.52 abcde	10.79 cdef	10.13 def	11.87 bcdef	8.92 ef	6.49 f	14.40 abcdef
R1020p	5.13 b	4.88 b	9.08 ab	6.73 ab	5.16 b	5.29 b	3.12 b	5.93 b	14.37 ab	15.71 ab	5.28 b	7.96 ab
SURV07	47.50 efghi	50.84 cdefghi	47.46 efghi	69.40 abc	56.77 bcdefgh	67.41 abcde	45.72 fghi	68.36 abcd	59.35 abcdefg	59.65 abcdefg	33.99 i	74.62 ab
Trait [b]	**Technological treatment**											
	2iu [a]				c				cu			
	Genotype											
	L-100	L-12	Villafranca	L-80	L-100	L-12	Villafranca	L-80	L-100	L-12	Villafranca	L-80
SH	30.53 ab [c]	39.13 a	36.44 a	32.11 ab	30.31 ab	29.12 ab	40.89 a	30.56 ab	28.01 ab	30.69 ab	34.98 a	33.85 ab
LN	19.21 abcd	24.06 ab	21.34 abc	19.65 abcd	19.84 abcd	19.50 abcd	25.46 a	20.53 abc	16.71 cd	20.37 abcd	21.15 abc	18.67 bcd
R0	4.63 bcd	8.87 ab	6.90 abc	8.43 ab	5.22 bcd	5.01 bcd	3.77 bcd	6.31 bc	2.72 cd	6.30 bc	4.77 bcd	7.32 ab
R05	2.01 bcd	6.35 a	2.33 bcd	2.11 bcd	1.42 cd	2.84 bcd	1.07 d	1.70 bcd	2.26 bcd	3.24 bc	1.92 bcd	1.99 bcd
RN5	6.64 cde	15.22 ab	9.25 abcd	10.57 abcd	6.62 cde	7.70 bcde	5.02 de	8.02 bcde	5.00 de	9.57 abcd	6.69 cde	9.30 abcd
R510	1.23 abc	1.62 abc	2.17 a	1.51 abc	1.79 abc	1.61 abc	0.98 c	1.31 abc	0.95 c	1.48 abc	1.60 abc	1.59 abc
R1020	1.33 ab	0.79 ab	1.77 a	1.20 ab	0.78 ab	1.16 ab	0.87 ab	0.97 ab	1.36 ab	0.63 ab	1.09 ab	1.04 ab
TRN	9.28 cdef	17.72 ab	13.28 abcd	13.40 abcd	9.37 cdef	11.20 abcde	6.92 ef	10.33 bcde	7.63 def	11.82 abcde	9.41 cdef	11.93 abcde
R0p	37.95 cdef	42.25 abcdef	45.01 abcdef	62.27 a	43.80 abcdef	32.01 def	53.98 abc	63.00 a	29.33 ef	45.72 abcdef	50.77 abcd	60.17 ab
R05p	21.35 abc	41.39 a	16.67 bc	16.87 bc	18.51 abc	19.64 abc	13.65 c	18.14 abc	27.48 abc	26.14 abc	18.65 abc	17.33 bc
RN5p	60.20 d	83.94 abcd	62.48 bcd	80.94 abcd	63.66 bcd	60.76 cd	74.17 abcd	81.85 abcd	61.03 cd	73.31 abcd	69.83 abcd	78.08 abcd
R510p	23.58 abcd	10.90 bcdef	21.21 abcde	11.04 bcdef	26.22 a	23.67 abc	15.41 abcdef	11.87 bcdef	14.36 abcdef	15.30 abcdef	18.17 abcdef	10.64 cdef
R1020p	15.42 ab	4.64 b	15.48 ab	7.61 ab	9.39 ab	11.10 ab	9.98 Ab	4.40 b	23.11 a	10.74 ab	11.50 ab	10.45 ab
SURV07	60.07 abcdef	57.66 abcdefgh	37.47 hi	77.81 a	53.65 cdefghi	48.31 defghi	38.87 ghi	66.50 abcdef	53.41 cdefghi	46.50 fghi	36.43 hi	62.96 abcdef

[a] Technological treatments: as per Table 2.

[b] Traits: as per Table 1.

[c] Values of a parameter with the same letter belong to the same homologous group according to the LSD test at the level of $\alpha=0.05$.

Table 4. Correlations among examined traits of white poplar rooted cuttings.

Trait [a]	**SH**	**LN**	**R0**	**R05**	**RN5**	**R510**	**R1020**	**TRN**	**R0p**	**R05p**	**RN5p**	**R510p**	**R1020p**	**SURV07**
SH	-													
LN	0.779 ** [b]	-												
R0	0.517 **	0.368 **	-											
R05	0.351 **	0.424 **	0.461 **	-										
RN5	0.532 **	0.435 **	0.954 **	0.704 **	-									
R510	0.404 **	0.250 *	0.349 **	0.115	0.320 **	-								
R1020	0.276 *	0.038	0.156	-0.052	0.106	0.245 *	-							
TRN	0.577 **	0.426 **	0.938 **	0.652 **	0.970 **	0.477 **	0.301 *	-						
R0p	0.362 **	0.345 **	0.749 **	0.063	0.620 **	0.062	-0.076	0.546 **	-					
R05p	-0.236 *	-0.062	-0.253 *	0.573 **	-0.009	-0.325 **	-0.311 **	-0.105	-0.445	-				
RN5p	0.171	0.324 **	0.537 **	0.515 **	0.605 **	-0.264 *	-0.378 **	0.438 **	0.680 **	0.329 **	-			
R510p	-0.163	-0.217	-0.473 **	-0.408 **	-0.517 **	0.418 **	0.047	-0.394 **	-0.585 **	-0.218	-0.817 **	-		
R1020p	-0.011	-0.241 *	-0.293 *	-0.379 **	-0.364 **	0.044	0.641 **	-0.209	-0.451 **	-0.348 **	-0.764 **	0.278 *	-	
SURV07	0.073	-0.013	0.124	0.103	0.136	0.053	0.011	0.127	0.166	0.045	0.169	-0.135	-0.084	-

[a] Traits: as per Table 1

[b] Significance of t-test: * – $p<0.05$, **– $p<0.01$

contribution from R0 to the variation of RN5. These two traits are also in strong positive correlation with the total number of roots (TRN), while the correlation of TRN with R05p and R1020p was weak and not statistically significant.

The examined traits were grouped into five groups according to their loadings with rotated first five principal components (Table 5). The traits that had their highest loadings, i.e. correlation coefficients, with the same PC were agglomerated in the same group.

DISCUSSION

Our results confirm the importance of genotype as a source of variability, in agreement with generally accepted theories describing the rooting process. In fact, various authors have proposed that species specificity, as well as genotype specificity, plays a major role in the propensity of poplars for adventitious root formation during vegetative propagation [21,23]. This process appears to be under strong genetic control, and clone (genotype) selection is an important factor in successful poplar vegetative propagation [24,25]. Interaction effects between technological treatment × genotype were not found to be statistically significant for any of the examined parameters. However, there were significant differences in the reaction of some genotypes according to the LSD test, suggesting that genotype × environment interactions should be further considered with respect to rooting poplar cuttings [21]. Kovačević et al. [4], after analyzing the effects of four white poplar genotypes and two cutting preparation terms (in mid-February and mid-March, planted in late April), found a significant genotype effect in RN1020 and TRN, a significant effect of the timing of the preparation of cuttings and planting in R0, R510p and R1020p, and a significant effect between the interaction of genotype × term of cutting preparations in R1020 and R1020p. They performed measurements in the beginning of June.

Table 5. Loadings of rotated principal components.

Trait [a)]	Rotated principal components				
	RC1	RC4	RC2	RC3	RC5
SH	0.772	0.062	0.118	0.053	-0.384
LN	0.652	-0.166	0.064	0.129	-0.556
R0	0.924	-0.078	-0.123	-0.123	0.185
R05	0.599	-0.136	-0.081	0.741	0.034
RN5	0.931	-0.112	-0.126	0.155	0.159
R510	0.433	0.087	0.822	-0.127	0.075
R1020	0.226	0.898	0.060	-0.099	0.017
TRN	0.951	0.075	0.023	0.106	0.168
R0p	0.684	-0.354	-0.376	-0.489	0.092
R05p	-0.088	-0.281	-0.237	0.895	0.054
RN5p	0.526	-0.566	-0.577	0.196	0.086
R510p	-0.467	0.121	0.820	-0.131	-0.073
R1020p	-0.296	0.877	0.132	-0.182	-0.061
SURV	0.174	-0.068	0.016	0.073	0.711
Eigenvalues	5.329	2.200	1.957	1.778	1.084
Proportion of total variance	0.381	0.157	0.140	0.127	0.077
Cumulative proportion of total variance	0.381	0.538	0.678	0.805	0.882

[a)] Traits: as per Table 1.

According to LSD test results, significant differences were observed between some technological treatments in R05, R1020, TRN, R0p, R05p, R510p and R1020p. The highest R05 and R05p was found in treatment 06i, while controls had the lowest. The highest R510p was observed for the control, while the lowest was in 2i. The highest R1020p was in cu and the lowest in 06iu. These results suggest that IBA application stimulates rooting in the basal part of the cutting, with no significant effects in the middle and upper parts of the cuttings. Eggens et al. [26] discussed changes in the physiological status of *Populus alba* var. *bolleana* cuttings throughout dormancy. In contrast to the stimulatory effects of IBA in early spring, they described necrosis and decay in cuttings treated with IBA in the late-term (April). Also, the importance of the effect of IBA concentration was not precisely determined in our research. Some authors [10] found a positive effect on rooting cuttings of eastern cottonwood after foliar treatment with $CoCl_2$, which inhibits ethylene accumulation induced by excess IAA. The same authors also found a positive effect on the rooting of cuttings after $CoCl_2$ addition to an IBA powder formulation for the white poplar clone Villafranca. Root stimulation by IBA in the basal part of the cuttings was also found in the present study. IBA is the most commonly used hormone for stimulating root formation in hardwood tree species, even more than IAA, and it is a reliable tool for this process [21]. Auxin is a major coordinator of root morphogenesis [28]. However, the efficacy of auxin, IAA or IBA treatment depends on the concentration applied [21], and it is possible that high concentrations of exogenous auxin, along with prolonged exposure, could have an inhibitory effect on adventitious root growth. Although IBA is more widely used for adventitious root development than IAA, IBA can also significantly stimulate formation and activation of root primordia in poplar cuttings, but its later influence on root development is not clear [29]. The effect of IBA also depends on its conversion to IAA in plant cells after IBA uptake [30]. The rate and scale of IBA conversion to IAA has not been fully defined for poplar hardwood cuttings, and it is likely that IBA exerts its effects after conversion to IAA [22].

No significant effect was found for urea treatments added to any of the examined IBA treatments. However, treatment with urea alone resulted in the highest R1020p of all examined treatments, suggesting that urea has positive effects that should be further studied. The bioavailability of mineral nutrients is of vital importance in the rooting process. Although N and P clearly impact root formation [20], the effect of nitrogen on adventitious root formation remains unclear. For example, in *Pelargonium* spp. and *Euphorbia pulcherrima* Wild. this effect depends on the concentration of applied N, light intensity, and the ratio of N supply to carbon, or sugar availability. Depending on these other factors, N supply might have a stimulatory, neutral, or even inhibitory effect [31,32]. In the present study, LSD tests suggest no significant differences between technological treatments on the percentage of surviving rooted cuttings (SURV07). Thus, in these cases urea application as a N source likely did not affect the rooting process.

Significant differences between genotypes were found for most of the examined traits. For the majority of traits describing the number of roots, genotype

L-100 achieved the lowest values, while L-12 and L-80 were at the level of Villafranca or better. Considering the number of roots on the examined portions of the total number of cuttings, genotype L-80 achieved higher values of R0p and RN5p than L-100. Clone L-100 achieved higher values of R05p and R510p then L-80 with the small number of roots in this cutting and the small number of roots in total. Low values of R0 and RN5, as well as of TRN, probably contributed to the low values of shoot parameters in this clone, as has been reported [8,17] in black poplar genotypes, but this clone exhibited better survival than Villafranca.

The clone Villafranca showed a poor survival of cuttings (ca. 40% in total), although it had a higher total number of roots compared to L-100, which had the lowest TRN compared to all other clones. Villafranca also had higher values of shoot height (SH) and number of leaves (LN), as well as the number of roots on the basal cut (R0), and their partition in the total number of roots (R0p), suggesting that other traits that were not included in our study had a considerable effect. The poor survival performance of Villafranca compared to L-12 and L-80 was reported by Kovačević and Igić [19] after cutting preparation and planting in early April on an east-northeast-exposed plot: however, for other treatments it was at the level of L-12 and L-80. Some authors [10] reported a trend toward higher, albeit not statistically significant, survival of Villafranca cuttings over L-12 in nursery trials established in April. Clone L-12, which achieved the highest cutting survival (SURV07), was also dominant in R05 and R05p – traits that define the formation of roots from preformed primordia in primary bark on the lower portions of a cutting.

According to Kovačević et al. [17], rooting in the middle and upper portions of black poplar hardwood cuttings, measured in May and June, positively correlated to cutting survival, while this effect was weaker for root parameters measured in July. The authors proposed that root formation at the basal cut ("wound roots") could be a sign of insufficient root formation. Kovačević [16] recorded earlier root appearance at the middle part of the cutting than in other examined black poplar genotypes, suggesting that the presence of roots in the middle part of the cutting provides a survival benefit due to earlier root formation. According to some authors [4], a high total number of roots is associated with better rooting in the upper part of the cuttings in four examined white poplar genotypes. All of these mentioned studies focused on differences among genotypes. In the present study, significant differences among clones were found in traits describing the number of roots in the lower part of the cutting (R0, R05, RN5), but not in R510 and R1020, so that the effect of rooting in the middle and upper parts of the cutting could not be discussed.

The LSD test at the level of interaction treatment suggests that the difference between them was rarely significant. Most of the homogenous groups (5 and more) were found in RN5, TRN, R0p, R510p and SURV07. The highest values of RN5, TRN and R0p, and the lowest values of R510p and SURV07 were recorded for Villafranca in the 2i treatment. The best survival was found in clone L-80 in the 2iu treatment (77.91%). There was no significant difference among treatments for any of the examined clones with respect to cutting survival (SURV07) and rarely for the other examined parameters. However, clone L-100 had higher SH values, more leaves, and higher R0 and RN5 values after 06iu treatment vs. 06i treatment, suggesting a positive reaction of this clone to urea treatment. The Villafranca clone had higher R510 and TRN values for 06i and 2iu treatments vs. control. In clone L-12, higher R05 values were found for 2iu and higher RN5p values after 06i treatment vs. control, while lower R510p values were obtained after 2i treatment vs. control. Together these results suggest that L-12 reacted to IBA treatment, as demonstrated by intense root formation at the lower part of the cutting. Some authors [10] reported differences in the reaction of L-12 and Villafranca to IBA treatment, as Villafranca achieved significantly better results after treatment with 0.6% IBA in combination with 100 mM $CoCl_2$, while L-12 showed no significant differences in cutting survival between these two treatments. These examples suggest a need for further research to design technology suited to the specific requirements of particular clones (clonal technology). It has already been shown that IBA concentrations should be optimized for successful root growth stimulation. Otherwise, IBA or other exogenous auxin applications might have an inhibitory effect on root formation [22,33]. Thus, optimization of the entire process, including the concentration of active substances, application techniques and exposure time, represents a practical challenge that depends on further elucidation of the functional properties and signaling pathways of hormonal action. Importantly, environmental factors, such as dark-light conditions, temperature, soil humidity, and water-table depth [34], etc. are highly correlated with the success of root formation in white poplar, and from cuttings of tree species in general [21,34].

The examined traits were grouped into five groups according to their loadings with the first five PCs rotated. The traits that had their highest loadings, i.e. correlation coefficient, with the same PC were agglomerated in the same group. Kovačević et al. [35] also achieved grouping of early rooting traits in eastern cottonwood in a study designed to assess relationships between examined eastern cottonwood genotypes, as well as between examined cuttings' rooting parameters.

The first group (RC1) consisted of SH, LN, R0, R05, TRN and R0p, suggesting a close relationship between shoot parameters, the total number of roots and rooting at the basal part of the cutting. Kovačević et al. [35] found that, based on loadings with the first two selected and rotated PCs, all examined parameters were grouped in the first group, except for R0 and R0p, which were grouped apart from others in the second group. The second group in the present study (RC4) included R1020 and R1020p, while the third group (RC2) consisted of R510, R510p and RN5p. Trait RN5p had similar loadings with the first and second PCs, suggesting moderate but not particularly close relationships with these two groups of parameters. The fourth group (RC3) included R05 and R05p, and the fifth group (RC5) consisted only of SURV07. Loadings of the other traits with the fifth PC were weak, except for weak and moderate loadings of SH and the number of leaves, respectively. Because these loadings were negative, we assumed that white poplar root cuttings with a high shoots and a large number of leaves could face survival difficulties during early phases of growth and development. This effect was not detected by Pearson's correlation coefficients, probably due to multicollinearity. However, according to some authors [8,17,36], in black poplars and other *Populus* clones high shoots were positively related to cutting survival. We assume that the reason for these differences between white and black poplar clones may be due to difficulties in rooting cuttings of white poplar species.

CONCLUSIONS

Significant differences in rooting and survival were found among cuttings from different white poplar genotypes. Fewer differences were found for examined technological treatments and between genotype × technological treatments. IBA treatments appear to have a positive effect on rooting in the lower parts of a cutting, while foliar applications of urea did not have significant positive effects on the examined traits. Shoot traits and traits that describe rooting on the basal cut were grouped with the total number of roots, while cutting survival (SURV07) was placed in a separate group from other traits. Thus, in white poplar genotypes, a strong relationship may exist between rooting at lower parts of a cutting in the first half of the growing season and the formation of a root system and shoot growth, but not with cutting survival. Therefore, further research is necessary to improve and optimize rooting of white poplar cuttings, a hard-rooting plant in vegetative propagation. New technology should be designed to suit the needs of particular clones (clonal technology).

Funding: This work is part of the projects "Studying climate change and its influence on the environment: impacts, adaptation and mitigation" (43007), a project of Biosensing Technology and Global Systems for Continuous Research and Integrated Management of Ecosystems (43002), financed by the Ministry of Education and Science of the Republic of Serbia and the project "Improvement of lowland forest management", financed by the PC Vojvodinašume.

Author contributions: Dušan Igić conducted field research, data organization and manuscript writing. Milan Borišev participated in the discussion of results. Dragica Vilotić and Mirjana Šijačić-Nikolić supervised activities and reviewed the manuscript. Miloš Ilić and Mirjana Ćuk participated in discussion of the results. Branislav Kovačević participated in the design of the study, data organization, analyses and writing of the manuscript.

Conflict of interest disclosure: This manuscript has not been published before and is not under consideration for publication anywhere else. The publication of this article was approved by all authors.

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