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RELATIONSHIP AMONG EASTERN COTTONWOOD GENOTYPES ACCORDING TO EARLY ROOTING TRAITS

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The relationship between twelve genotypes of eastern cottonwood (*Populus deltoides* Bartr.) was analyzed according to sixteen early rooting traits and cutting survival. Principal component analysis (PCA) and cluster analysis were used on data that were standardized by common and by one alternative way of standardization. Alternative way of standardization (standardization with within-genotype standard deviation instead of standard deviation of genotypes' means) was used in order to emphasize the contribution of genotype to the effect of differences among genotypes on

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total variation. After bought ways the first principal component had high correlation with the most of rooting traits and cutting survival, while the second was mainly related to the traits of root formation on the basal cut of cutting (wound roots). Three difficult-to-root genotypes (S6-7, S1-3, 129/81) were distinctly grouped against other examined genotypes, by bought principal component and cluster analysis. There was a slight difference in grouping of easy-to-root genotypes (B-229 and PE19/66) among examined ways of standardization.

Key words: multivariate analysis, *Populus deltoides,* root and shoot development

INTRODUCTION

Eastern cottonwood (*Populus deltoids* Bartr.) is a black poplar (section *Aigeiros* Duby) that is widely utilized in the intensive timber and biomass production, as well as in breeding programs through intra- and interspecies hybridization. Beside wood production, it is also used in the environmental protection and improvement projects (ZALESNY & ZALESNY, 2009).

The hardwood cuttings of black poplars are mostly characterized by good rooting. However, the problems in cuttings' rooting in eastern cottonwood could still compromise nursery production and the establishment of short rotation stands for the production of biomass (KOVACEVIC *et al*., 2009; ZALESNY & ZALESNY, 2009), That is why evaluation of cuttings' rooting ability is an important part of poplar breeding programs (ZALESNY *et al.*, 2005b; ZALESNY *et al.*, 2007; KOVACEVIC *et al.*, 2008; VANCE *et al.*, 2010).

Black poplars own the good cutting rooting to pre-formed primordia, discovered in poplars by Van der Lek in 1924. Primordia can be also initiated *de novo* and activated on cuttings' cut: on basal cut they form wound roots, and on upper cut adventitious shoots. Sufficient number and development of primordial and their timely activation improve chances for cutting's survival. The first three months seems to be critical for the success of cutting survival. During that time the cutting suffers the stress related to imbalanced shoot and root system growth and development (KOVACEVIC *et al.*, 2009; ZALESNY & ZALESNY, 2009). According to DESROCHERS & TREMBLAY (2009), the problem is more serious in planting stock types with shoot unpruned.

The activation of primordia and cutting rooting are influenced by many factors. On one side there are genetic sources of variation: differences among genotypes, among and within populations and families (ZALESNY *et al.*, 2005b, KOVACEVIC *et al.*, 2008). Then, differences among cuttings within genotype (C – effect): differences among ramets, cuttings from different positions on sprout, sprout age and cutting made in different time of year (ZALESNY *et al.*, 2003; KOVACEVIC *et al*, 2006).

There are also factors of the environment that acts directly or through interaction with genotype such as: soil characteristics, temperature, precipitation, store conditions, year conditions. They are thoroughly examined in order to optimize nursery and plantation establishment technology to the particular cultivar (cultivar

technology) as well as to adjust the breeding process (ZALESNY *et al.*, 2005a; ZALESNY *et al.*, 2005b; KOVACEVIC *et al.*, 2009). Also, different pre-treatments are used (DESROCHERS & THOMAS, 2003) and even transformation (DAI, *et al*, 2004) in order to improve cutting rooting of particular poplar genotype.

In classical poplar breeding it is necessary to reveal the significance of influence of genetic source of variance on cutting rooting traits, as well as to accurately and efficiently evaluate examined genotypes. In that sense, along the others, multivariate statistical methods are introduced recently in describing the relationship among rooting traits in poplars (KOVACEVIC *et al*., 2007; KOVACEVIC *et al*., 2008; KOVACEVIC *et al*., 2010).

The aim of this work was to analyze relationship among examined eastern cottonwood genotypes based on rooting traits in the early phases of cutting's rooting. Also, the effect of introduction of the information of the contribution of genotype variation to the total variation of particular traits in principal component analysis and cluster analysis and its relationship to cutting survival were analyzed.

MATERIALS AND METHODS

Nursery experiments were established on the Experimental Estate of the Institute of Lowland Forestry and Environment near Novi Sad, Serbia, with 20 cm long hardwood cuttings of twelve genotypes Eastern cottonwood (*Populus deltoides* Bartr.): PE19/66, PE4/68, B-229, B-352, B-81, B-17, 124/81, 129/81, 182/81, 54/76-28, S1-3 and S6-7. Genotypes S1-3, B-229 and 182/81 are registered in Serbia. The others are currently in experimental phase. The precipitation for the period April-June (found to be critical for cutting survival) in 1996 and 1998 was near average for the region (193 and 183 mm, respectively) and in 1999 it was 235 mm (30% higher then an average).

The nursery experiments for examination of morphological characteristics were established in: 1996, 1998 and 1999, on April 15th on humofluvisol soil type (40% silt+clay content in surface horizon) at a spacing of 1.50 x 0.10 m between cuttings. There was no additional soil moisturizing in the period from April to June in order to determine more precisely the effect of the differences among years. Weeds were regularly treated mechanically.

The stem cuttings, 18-22 cm long and more than 8mm wide, were prepared by scissors, at the beginning of April, from the stems of one-year old rooted cuttings, 1.5 – 2.2 m high. The most of stem was used for the cutting preparation except its brittle top (too thin cuttings) and basal part (too small buds). The cuttings were not soaked before planting, in order to determine more precisely the differences among genotypes, which is especially important for genotypes of eastern cottonwood. Before the planting the cuttings were stored in trenches for not more then two weeks. The cuttings were planted in the soil manually in order to position the top of the cutting 0-5 cm beneath the soil surface. Twenty cuttings were planted per plot. Experiment was designed as completely randomized in four repetitions.

Five cuttings per plot were carefully dug out manually, cleaned and analyzed 40 days since planting (second half of May). According to KOVACEVIC *et*

al. (2009) this is the time when a significant decrement in the formation of new roots occurs, causing the imbalance in shoot and root system development. On each cutting with vital shoot, length of every first-order root and its distance from basal cut of cutting were measured, as well as height of dominant shoot (SH) and number of leaves on it (LN). The length of roots was measured only in the first three terms, because later we couldn't manage to dig up whole root system efficiently. At the base of measurements of root system total number of roots (TRN) and total length of roots (TRL) were derived. Also, five following parts of the cutting were taken in consideration: basal cut (wound roots), basal part (basal 5 cm of cutting without the roots of basal cut), middle part (5 th to 10 th cm from basal cut), upper part (over the 10th cm), basal cut with basal 5 cm and basal cut with basal 10 cm. For specified part, following traits were derived: number of roots (RN0, RN05, RN510, RN1020, RN5, RN10) and their ratio to TRN (RN0P, RN05P, RN510P, RN1020P, RN5P and RN10P). Average plot values were used in further statistical analysis. Average plot values were used for the statistical analysis.

The nursery experiments for examination of cutting survival were established on sandy and loamy fluvisol (30% and 62% silt+clay content in surface horizon, respectively), on April 15th in 1998 and 1999, at a spacing of 1.50 x 0.15 m between the cuttings. The cuttings were prepared and planted in the same way as in the experiments for morphological traits. Thirty cuttings were planted per plot in three randomized repetitions per clone. Experiment was designed as completely randomized. Cutting survival was determined at the end of growing period as the percent of cuttings with a viable shoot.

Data analysis

The variability of rooting traits was examined by two-way ANOVA, nested design:

$$X_{ijm} = \mu + g_i + y_{j(i)} + \varepsilon_{m(ij)} ,$$

where X_{ijm} stands for measured value, μ - average value, g_i - effect of genotype (G), $y_{j(i)}$ - effect of year within i^{th} genotype (Y), and $\varepsilon_{m(ij)}$ - effect of uncontrolled variation. Samples (number of repetitions) appeared to be unequal because, in some plots, no cutting had a vital shoot. The results of ANOVA were used in calculation of expected variances for examined sources of variation. Negative expected variances were considered to be zero (ALLARD, 1960).

Traits describing ratio of number of roots from cutting's portions to TRN were transformed by arcsine – transformation ($arcsin\sqrt{X}$, where X is a proportion, while all traits describing number of roots were transformed by square transformation ($\sqrt{X+1}$) to meet the normal distribution that was required by parametric statistical analysis. The effect of examined sources of variation were described by coefficients of variation:

$Cv = \frac{\sigma_A}{\overline{X}} * 100\%$, where σ_A - stands for expected standard deviation of A source of variation.

Two ways of standardization were examined:

- common standardization i.e. standardization of genotype means with its standard variation ($\frac{\overline{X}_j - \overline{X}}{\sigma_G}$), where X_j stands for mean of j^{th} genotype, and σ_G stands for standard deviation of genotype means;
- standardization with residual standard deviation ($\frac{\overline{X}_j - \overline{X}}{\sqrt{\sigma_g^2 + \sigma_{y(g)}^2 + \sigma_{err}^2}}$), where σ_g^2 stand for expected variance of genotype, $\sigma_{y(g)}^2$ stands for expected variance of year within genotype and σ_{err}^2 stands for expected variance of error.

The alternative way of standardization was performed in order to introduce the information of the significance of influence of genotype on total variation of used traits.

Principal component analysis and cluster analysis were used in order to reduce the data amount and to enable presentation of relationship among examined genotypes. The first two principal components were used for presentation of relationships. In order to preserve the effect of standardization principal component analysis was based on covariance matrix and gained principal components were not rotated. The examined genotypes are grouped by cluster analysis based on standardized genotype means, using unweighted pair group method with arithmetic mean (UPGMA).

The program package STATISTICA 10 (STATSOFT INC. 2011) was used for the statistical analysis.

RESULTS

The results of two-way ANOVA suggest that most of examined traits were significantly influenced by differences among genotypes. High contribution of genotype variance to the total variance was especially traits of: number of roots at the middle part of cutting (RN510), at the upper part of the cutting (RN1020), total number of roots (TRN), ratio of number of roots at the middle part of the cutting and total number of roots (RN510P) and ratio of number of roots at the lower part of cutting together with wound roots and total number of roots (RN5P). However, the differences among genotypes in traits of wound roots and shoot traits were weak. The influence of year within genotype was mostly considerable, except in traits of contribution of examined parts of cutting to the total number of roots (Tab. 1).

Most of traits of root system, as well as number of leaves (LN) and shoot height (SH) had considerable correlation with cutting survival (SURV). The opposite was for number of wound roots (RN0) and their ratio with total number of roots (RN0P).

The first two principal components were for the presentation of the relationship among examined genotypes. Their contribution to the total variance was more than 85% after every examined way of standardization. The ratio between eigenvalues for

the second and the first principal component were similar after alternative ways of standardization and smaller then after common standardization.

Also, eigenvalues were considerably smaller after alternative way of standardization, especially after the third one. Most of the traits had the highest loadings with the first principal component, except for traits of wound roots (Tab. 2).

Table 1. Results of two-way ANOVA, nested design, for examined morphological traits[*)]

	Contribution to the total expected variance (%)			F-test[2)]		Coefficient of variation (%)		
Traits[1)]	Genotype	Year (genotype)	Error	Genotype	Year (genotype)	Genotype	Year (genotype)	Error
LN	2,355	43,994	53,651	1,126	4,230**	5,243	22,659	25,022
SH	4,913	52,253	42,834	1,237	5,806**	8,978	29,277	26,508
TRL	20,938	21,987	57,075	2,726*	2,518**	28,926	29,641	47,757
RN0	0,000	45,980	54,020	0,330	4,353**	0,000	18,670	20,237
RN05	0,000	28,428	71,572	0,826	2,565**	0,000	11,525	18,287
RN510	29,672	13,765	56,563	4,171**	1,959*	13,798	9,398	19,050
RN1020	30,646	13,176	56,178	4,357**	1,924*	15,286	10,023	20,696
RN5	4,090	15,720	80,190	1,342	1,772*	4,221	8,275	18,691
RN10	16,409	24,363	59,228	2,253*	2,620**	9,170	11,174	17,422
TRN	28,076	22,872	49,052	3,390**	2,837**	12,859	11,607	16,997
RN0P	0,000	43,767	56,233	0,165	4,066**	0,000	108,354	122,819
RN05P	19,590	0,000	80,410	4,159**	0,934	16,203	0,000	32,828
RN510P	28,850	1,778	69,372	5,470**	1,101	29,240	7,260	45,342
RN1020P	9,090	0,000	90,910	4,001**	0,546	17,164	0,000	54,280
RN5P	23,440	0,000	76,560	8,553**	0,540	16,846	0,000	30,446
RN10P	9,090	0,000	90,910	4,001**	0,546	7,281	0,000	23,026

[*)] Degrees of freedom: for genotypes = 11, for year within genotype = 24, for error = 106, for total = 141

[1)] Abbreviations of rooting traits: LN – number of leaves; SH – shoot height (cm) TRL – total root length (cm); RN0 – number of roots on the basal cut; RN05 – number of roots on basal portion of cutting (0. – 5. cm form basal cut); RN510 – number of roots on middle portion of cutting (5. - 10. cm); RN1020 – number of roots on upper portion of cutting (above 10. cm); RN5 = RN0 + RN05; RN10 = RN0 + RN05 + RN510; TRN – total number of roots; RN0P = RN0/TRN*100%; RN05P = RN05/TRN*100%; RN510P = RN510/TRN*100%; RN1020P = RN1020/TRN*100%; RN5P = RN5/TRN*100%; RN10P = RN10/TRN*100%

[2)] Significance of F-test: * - significant for α=0.05, ** - significant for α=0.01

The contribution of the first principal component to the total variation was higher after alternative way of standardization. Also, eigenvalues of bought selected principal components decreased, as well as the ratio between eigenvalues of the second and the first principal component. The Spearman's rank correlation coefficients between cutting survival and principal components were similar. The

eigenvalue of the second principle component did not meet the Keiser's criterion ($\lambda > 1$), but we included the second component in graphs in order to make comparisons with common standardization. The correlation of cutting survival with the second principal component was higher for alternative way of standardization, but still not statistically significant (Tab. 2).

Table 2. Correlations of examined rooting traits with cutting survival and results of their grouping according to principal components formed after examined ways of standardization

Traits[1)]	r_S[2)]	**After common standardization**		**After standardization with within-genotype standard deviation**	
		PC1	**PC2**	**PC1**	**PC2**
LN	0,78**[3)]	0,803[4)]	0,153	0,788	0,020
SH	0,92**	0,783	0,231	0,754	0,209
TRL	0,64*	0,959	0,098	0,957	0,205
RN0	0,48*	0,460	0,818	0,443	0,543
RN05	0,71**	0,799	0,152	0,781	0,506
RN510	0,54	0,976	0,001	0,984	-0,001
RN1020	0,60*	0,947	-0,208	0,954	-0,153
RN5	0,73**	0,817	0,374	0,797	0,587
RN10	0,69*	0,951	0,187	0,946	0,303
TRN	0,68*	0,987	0,026	0,987	0,118
RN0P	0,33	-0,141	0,849	-0,158	0,328
RN05P	-0,50	-0,904	0,107	-0,917	0,299
RN510P	0,39	0,940	-0,049	0,945	-0,036
RN1020P	0,59*	0,874	-0,402	0,885	-0,415
RN5P	-0,42	-0,934	0,261	-0,947	0,306
RN10P	-0,59	-0,874	0,402	-0,885	0,415
Eigenvalue (λ)		11,54	2,12	4,24	0,33
$\lambda / \Sigma \lambda$ (%)		72,15	13,27	84,37	6,55
$\lambda_{PC2} / \lambda_{PC1}$		0,184		0,078	
r_S with SURV		0,587*	0,294	0,552	0,406

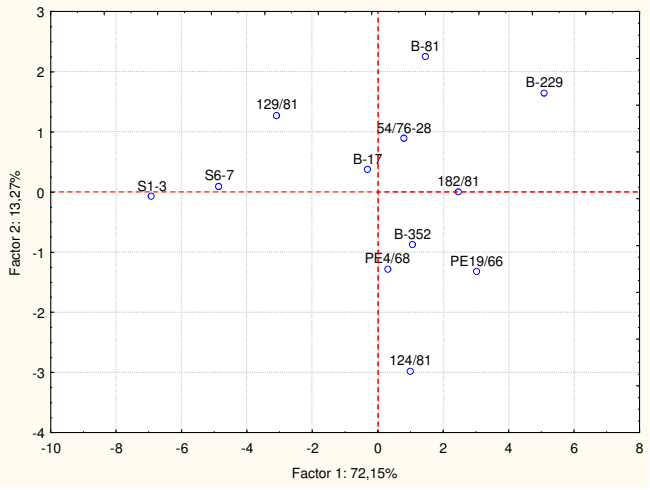
[1)] Abbreviations of rooting traits: LN – number of leaves; SH – shoot height (cm) TRL – total root length (cm); RN0 – number of roots on the basal cut; RN05 – number of roots on basal portion of cutting (0. – 5. cm form basal cut); RN510 – number of roots on middle portion of cutting (5. - 10. cm); RN1020 – number of roots on upper portion of cutting (above 10. cm); RN5 = RN0 + RN05; RN10 = RN0 + RN05 + RN510; TRN – total number of roots; RN0P = RN0/TRN*100%; RN05P = RN05/TRN*100%; RN510P = RN510/TRN*100%; RN1020P = RN1020/TRN*100%; RN5P = RN5/TRN*100%; RN10P = RN10/TRN*100%

[2)] Abbreviations in table heading: r_S – Spearman's rank correlation coefficient, PC – principal component

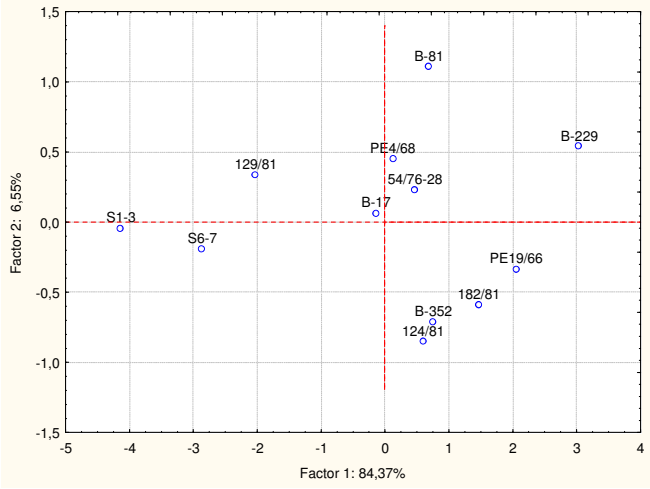
[3)] * - statistically significant difference from 0 for α=0.05, ** - statistically significant difference from 0 for α=0.01

[4)] underlined are the highest loadings of examined traits with the first two principal components

According to factor scores the relationship among examined genotypes remain similar, but the distribution of genotypes was more narrow by the second principal component, and broader by the first. Relations among genotypes were also similar, but distances were more influenced by the first principal component (Graph 1-2).



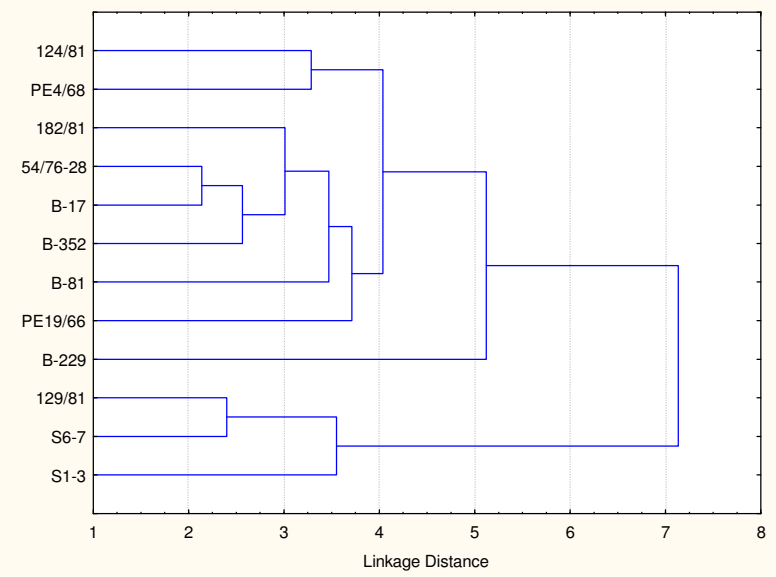
Graph 1. Relation among examined genotypes based on the first two principal components formed on data standardized by standard deviation of genotype means



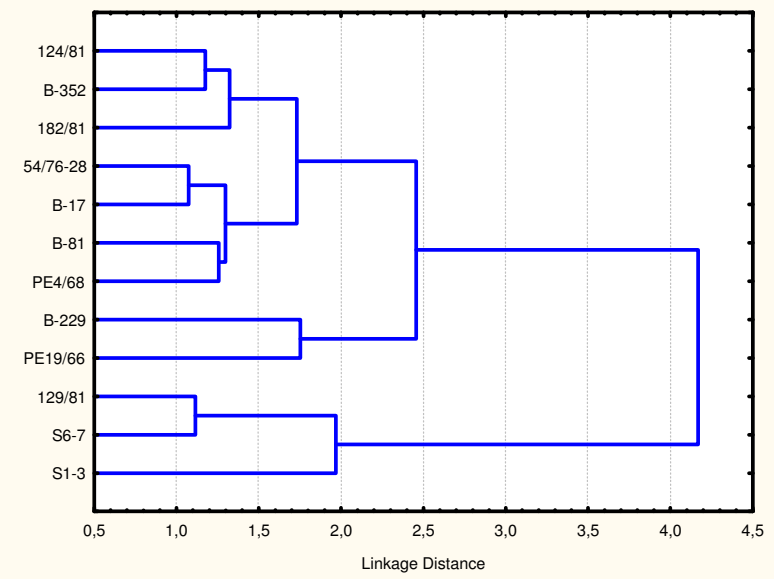
Graph 2. Relation among examined genotypes based on the first two principal components formed on data standardized by standard deviation within genotype

There were three genotypes that were distinctly off the main group of genotypes: S6-7, S1-3, 129/81. Also, the genotype B-229 was in all cases on the opposite side of graph. As the first principal component had high correlation with cutting survival after every examined way of standardization, the distribution of examined genotypes by the first principal component reveals their relationship according to cutting survival. The highest values for the second principal component had the genotype B-81, while the usual lowest score for the second principal component had 124/81.

The results of cluster analysis confirmed grouping of S6-7, S1-3 and 129/81 in a separate group. The genotype B-229 was distant from the main group: alone after common standardization and grouped with PE19/66 after the standardization with within-genotype standard deviation. The distances after alternative way of standardization were smaller then after common standardization. The grouping of genotypes remained similar after every examined way of standardization (Graph 3-4).



Graph 3. Relation among examined genotypes based on Cluster analysis – UPGMA linkage method on data standardized by standard deviation of genotype means



Graph 4. Relation among examined genotypes based on Cluster analysis – UPGMA linkage method on data standardized by standard deviation within genotype

DISCUSSION

Traits of cutting rooting and survival are rarely used in the multivariate description of relationship among genotype in poplars (KOVACEVIC *et al.*, 2007; KOVACEVIC *et al.*, 2010; ÖZEL *et al.*, 2010). However, cutting rooting is one of the most important properties of poplar clones and one of the reasons of fast improvement in poplar production and their worldwide cultivation. The information of rooting ability is significant in poplar breeding process in order to relate genotypes in concern (ZALESNY *et al.*, 2005b; KOVACEVIC *et al.*, 2008).

On the other side it is well known, and our results confirm it, that rooting traits are weakly inherited, under strong influence of environment. Considerable influence of year emphasizes the importance of multiannual character of research in cutting rooting. Coefficients of variation, as well as contribution to the total expected variance, show that rooting traits differed in influence of differences among genotypes on their variation. Some traits, like TRN, RN510, RN510P were under considerable influence of differences among genotypes and in high correlation with cutting survival. On the other side, shoot traits: shoot height (SH) and number of leaves (LN) were poorly influenced by genotype, but still in high correlation with cutting survival, as it was emphasized by KOVACEVIC *et al.* (2010). These facts make the research based on rooting traits to have a specific approach.

In this work we used rooting traits measured in the second half of May, when a significant decrement in the formation of new roots occurs and shoot and root system growth and development is imbalanced. This phase seems to be important for cutting survival since difficult-to-root genotypes suffer considerable cutting mortality in that time (KOVACEVIC *et al.*, 2009).

The relationship among genotypes is usually presented by multivariate methods such as principal component analysis and cluster analysis. The common basis for these methods is a matrix of standardized genotype means. Standardization of data is important procedure in methods of multivariate analysis in order to surpass differences among traits in scale or measure. Usual method of standardization is the transformation that converts all traits in order to have arithmetic mean $\overline{X} = 0$ and standard deviation $\sigma = 1$.

Gained results of principal component analysis and cluster analysis suggest one group of genotypes (S6-7, S1-3 and 129/81) that is distinct from others. Genotypes of this group are known to have problems in cutting survival (KOVACEVIC *et al.*, 2009).

However, by common standardization, the influence of variability within genotypes is not taken in consideration. It could be sufficient in taxonomic studies where every difference among taxa is equally important as far as it brings new discriminative information. In breeding, the traits that are highly inherited and under strong influence of differences among genotypes are much preferable, for the selection by these traits is more precise and effective. Thus, it could be interesting to introduce information about variability within genotype. By that the influence of traits that varies weakly within genotype on final results of PCA and Cluster analysis is enforced, while the relationship among traits is well preserved.

As it was suggested by KOVACEVIC *et al.* (2010) we introduced that information by standardization of examined traits with their standard deviation within genotype. Also, in order to preserve this information in principal component analysis, the matrix of covariances was used as the entering data. BORGOGNONE *et al.* (2001) even proposed covariate matrix instead of correlation matrix in all the cases when the scales are same for all attributes.

Comparing to common standardization, this alternative way decreased eigenvalues of principal components and total variation. The ratio between eigenvalues of second and the first principal component was for more than two times lower then after common standardization. It seems that the distances among genotypes were influenced by second principal component less then after common standardization. As a result, the genotypes 124/81 and B-81, that were the most distant by the second principal component, appeared relatively closer to the main group after alternative ways of standardization. The first principal component remained closely related to cutting survival. This could be expected as the traits with the highest loadings with the first principal component were characterized by high contribution of genotype to the total variation and were closely related to cutting survival. Even shoot traits, whose variation was weakly influenced by differences among genotypes, remained highly correlated to the first principal component. Also,

that suggests that influence of shoot traits in principal component analysis was not much altered by alternative way of standardization. We assume that stronger effect of the standardization with within-genotype standard deviation would be achieved if traits were less correlated among themselves. According to KOVACEVIC *et al.* (2007, 2010), for rooting traits measured at the beginning of June, i.e. nearly after the end of stress caused by imbalanced growth of shoot and root system (KOVACEVIC *et al.*, 2009), traits of rooting on the lower part of cutting appeared to be more correlated to the second principal component and in different group than traits of rooting on the upper part of cutting. That suggests differences in reaction of examined genotypes on that stress.

According to gained dendrograms the relationship among examined genotypes was weakly changed by standardization with standard deviation within genotype. The difficult-to-root group of genotypes (129/81, S6-7 and S1-3) was relatively more distinct from the main cluster, within which was more similarity among genotypes. However, according to KOVACEVIC *et al.* (2010), genotype 129/81 was not grouped with S6-7 and S1-3 if rooting traits had been measured at the beginning of June, suggesting good reaction of this genotype on imbalanced growth stress. Genotypes PE19/66 and B-229 that showed the best rooting performance were also relatively further from the main group after alternative way of standardization. Thus, it seems that the result of implementation of this way of standardization was: relatively more similarity within clusters and less among them.

Results of our work, as well as the idea of implementation of multivariate methods in the description of relations among genotypes, could be interesting in cutting rooting studies and poplar breeding praxis in the future. It could be still the meter of discussion if this time of measurement should be used only, especially in the case of shoot traits. The relations among genotypes could be evaluated based on cuttings' rooting traits, skipping the establishment of resource consuming cutting survival experiments. In this sense, the special attention deserves shoot traits that could be measured quickly, by non-destructive means.

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ODNOS MEĐU GENOTIPOVIMA AMERIČKE CRNE TOPOLE PREMA OSOBINAMA RANOG OŽILJAVANJA

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I z v o d

Odnos između dvanaest genotipova američke crne topole (*Populus deltoides* Bartr.) su analizirane na osnovu šesnaest svojstava ranog ožiljavanja i preživljavanja reznicnica. Analiza glavnih komponenata (PCA) i analiza grupisanja su korišćene na podacima koji su bili standardizovani uobičajenom standardizacijom jednim alternativnim načinom standardizacije. Alternativni način standardizacije (standardizacija standardnom devijacijom unutar genotipa umesto standardnom devijacinom sredina genotipova) je korišćen kako bi se naglasio doprinos genotipa efektu razlika među genotipovima na ukupno variranje. Nakon oba načina standardizacije prva glavna komponenta je imala visoku korelaciju sa većinom svojstava ožiljavanja i preživljavanjem reznica, dok je druga bila uglavnom u vezi sa osobinama formiranja korena na donjem rezu reznice (korenovi rane). Tri genotipoa koji imaju problema sa ožiljavanjem (S6-7, S1-3, 129/81) su se jasno odvojila od stalih genotipova, i po analizi glavnih komponenata i po analizi grupisanja. Postojala je izvesna razlika u grupisanju genotipova koji se dobro ožiljavaju (B-229 and PE19/66) između ispitivanih načina standardizacije.

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