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VARIABILITY AND RELATIONSHIP AMONG CUTTINGS' ROOTING CHARACTERS FOR EASTERN COTTONWOOD

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The influence of genotype, year and genotype x year interaction on variation of 14 characters of hardwood cutting rooting ability in field conditions, as well as relationship among them was examined in four dates of observation during the first half of vegetation period for fifteen genotypes of *Populus deltoides* Bartr. are presented. The influence of year rose throughout the examined period, while influence of genotype x year interaction was usually weak. Most of characters had high heritability in second date of observation (second half of May). Beside total number of first-order roots, results signify number of roots on middle and basal part of cutting, as well as shoot characters (number of leaves and shoot height), while characters describing wound roots (roots on basal cut) appeared insignificant. Examined characters were grouped according to PCA

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(Principal Component Analysis) in two groups, defined by number of roots in the middle part of cutting and number of roots on basal part of cutting.

Key words: *Populus deltoides*, cutting's rooting, variability, PCA

INTRODUCTION

The hardwood cuttings of black poplars (section *Aigeiros* Duby) are characterized by good rooting (SEKAWIN, 1969) due to pre-formed primordia, discovered in poplars by Van der Lek in 1924 (according to SMITH and WAREING, 1972). Primordia can be also initiated and activated on cuttings' cut: on basal cut they form wound roots, and on upper cut adventitious shoots (SMITH and WAREING, 1972). Sufficient number and development of primordia (SMITH and WAREING, 1972; JESTAEDT, 1977) and their timely activation (OKORO and GRACE, 1976; PALLARDY and KOZLOWSKI, 1979) improve chances for cutting's survival.

The problems in cuttings' rooting, especially in eastern cottonwood (SEKAWIN, 1969), could still compromise nursery production and the establishment of short rotation stands for the production of biomass. That is why evaluation of cuttings' rooting ability was always an important part of poplar breeding program (TEISSER du CROSS, 1984).

The activation of primordia and cutting rooting are under the influence of many factors. On one side there are genetic sources of variation: differences among genotypes (WILCOX and FARMER, 1968; GUZINA, 1987, KOVAČEVIĆ *et al.*, 2005), among and within populations (YING and BAGLEY, 1979). Then, differences among cuttings within genotype (C – effect) as: differences among the ramets (STUHLINGER and TOLIVER, 1985; HERPKA and MARKOVIĆ in 1969, according to MARKOVIĆ and RONČEVIĆ, 1986; LI *et al.*, 1994), differences in the position of the cutting on the sprout (YING and BAGLEY, 1977; MARTINEZ *et al.*, 1994), the age of sprout (SMITH and WAREING, 1972) and the season when the cutting is made (NANDA and ANAND, 1970; FEGE, 1984).

There are also factors of the environment such as: soil texture (WILCOX and FARMER, 1968; KOVAČEVIĆ *et al.*, 2005), temperature and precipitation (ZALESNY *et al.*, 2005), microrelief (ALKINANI, 1972), store conditions (FEGE, 1984; NANDA and ANAND, 1970), nursery technology (FEGE, 1983). Cutting's rooting variability trials established in field conditions (YING and BAGLEY, 1977; KOVAČEVIĆ *et al.*, 2001) or in controlled conditions in soil substrate (WILCOX and FARMER, 1968; YING and BAGLEY, 1977, GUZINA, 1987) usually lasted one season, mostly concerning total number and length of first-order roots.

The aim of this work was to analyze variability and relationship of a group of regular and some alternative rooting characters of eastern cottonwood cuttings in the first half of vegetation period. These results were gained in a course of improvement of selection tests and cultivar-adjusted nursery technology.

MATERIAL AND METHODS

Fifteen genotypes of eastern cottonwood (*Populus deltoides* Bartr.) were examined in field conditions on humofluvisol (GUZINA *et al.*, 1997), in three years (1996, 1998 and 1999) in four replications, in completely randomized design: 665, PE19/66 PE4/68, B-229, B-352, B-81, B-17, B-447, 124/81, 129/81, 54/76-28, S1-3, S6-7, 181/81 and 182/81. After the nursery experiment establishment in the mid April, five cuttings per plot were dug up and carefully cleaned in four dates of observation during the first half of vegetation period: the beginning of May (the first date of observation), second half of May (second), first decade of June (third) and the middle of July (fourth). The experiment was designed in course of moderation of C-effect. 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 10^{th} cm) and basal part with basal cut. For specified part, following characters were derived: number of roots (RN0, RN5, RN510, RN1020 and RNB5) and their ratio to TRN (RN0P, RN5P, RN510P, RN1020P and RNB5P). Average plot values were used in further statistical analysis.

The variability of rooting characters was examined by two-way ANOVA, random model: $X_{ijm} = \mu + g_i + y_j + gy_{ij} + \varepsilon_{mij}$, where X_{ijm} stands for measured value, μ - average value, g_i - effect of genotype (G), y_j - effect of year (Y), gy_{ij} - effect of $G \times Y$ interaction, and ε_{mij} - effect of uncontrolled variation. Samples (repetitions within GxY interaction) appeared to be unequal because, in some plots, no cutting had a vital shoot. Because of that, mean number of repetitions was calculated as harmonic mean by formula:

$$\tilde{n} = \frac{p * q}{\sum_{j=1}^{q}\sum_{i=1}^{p} 1/n_{ij}},$$

where p stands for number of treatments of the first factor, q for number of treatments of second factor, while n_{ij} stands for sample size (number of plots without ones with no cutting with vital shoot) for i^{th} treatment of first factor and j^{th} treatment of second factor (KIRK, 1968). This harmonic mean was used for calculation of expected variances for examined sources of variation. Negative expected variances were considered to be zero (ALLARD, 1960). Characters describing ratio of number of roots from cutting's portions to TRN were transformed by arcsine – transformation ($\arcsin\sqrt{X}$, where X stands for the value in %), while all characters describing number of roots were transformed by square transformation ($\sqrt{X+1}$) to meet normal distribution of frequencies that was required by statistical methods.

At the base of expected variances the coefficients of heritability in broad sense were calculated by the formula:

$$H = \frac{\sigma_G^2}{\sigma_F^2},$$

where σ_G^2 stands for expected genotype variance and σ_F^2 for phenotypic variance ($\sigma_F^2 = \sigma_G^2 + \sigma_{GY}^2 / q + \sigma_{ERR}^2 / \tilde{n} q$). The label *G* stands for the first examined factor of variation, the genotype, *GY* for genotype x year interaction, and *ERR* for error, while *q* stands for number of treatments of second factor (year), and $\tilde{n}$ for harmonic mean of number of repetitions. The Principal Component Analysis was used for grouping of examined characters according to their loadings with principal components, that were selected to meet criterion: λ > 1 (THURSTONE, 1969), and rotated by Varimax method (KAISER, 1958). Program package STATISTICA 6.0 (STATSOFT INC., 2001) was used for statistics.

RESULTS

Table 1. ANOVA for rooting characters of eastern cottonwood cuttings in field conditions – ratio between expected variances examined sources of variation and the expected total variance (%)

Rooting	1st date of observation				2nd date of observation				3rd date of observation				4th date of observation			
characters	G [2)]	Y	G x Y	Err	G	Y	G x Y	Err	G	Y	G x Y	Err	G	Y	G x Y	Err
LN	3,01	15,22	21,32	60,45	16,12	28,61	5,97	49,31	27,05	25,09	13,77	34,08	1,69	33,93	1,77	62,61
SH	26,09	6,42	19,56	47,92	17,83	37,39	4,54	40,24	9,01	43,31	12,00	35,68	2,89	61,21	0,09	35,80
TRL	21,25	0,00	21,50	57,26	19,21	0,00	23,52	57,27	9,17	27,84	21,74	41,25	-	-	-	-
RN0	0,00	0,00	0,00	100,00	0,00	28,12	14,68	57,20	0,00	67,64	14,74	17,62	0,72	82,78	2,37	14,13
RN5	23,35	0,00	10,58	66,07	11,15	6,59	12,75	69,51	6,57	68,32	0,51	24,60	2,84	81,43	0,00	15,73
RN510	49,68	4,20	4,36	41,76	30,86	4,84	10,24	54,06	20,41	10,56	4,79	64,24	21,49	4,78	2,81	70,93
RN1020	30,41	7,92	25,63	36,04	21,60	2,30	12,95	63,15	22,76	7,25	19,14	50,86	13,72	12,12	6,37	67,79
RB5	23,42	0,00	10,85	65,73	11,91	0,00	14,49	73,59	12,10	23,10	16,24	48,57	14,61	25,49	0,00	59,90
TRN	41,89	3,55	16,00	38,57	29,03	0,79	16,24	53,93	21,45	22,92	20,15	35,49	22,20	14,00	3,61	60,18
RN0P	0,00	0,00	0,00	100,00	0,00	35,59	4,51	59,90	0,00	78,86	3,24	17,91	0,00	86,06	0,73	13,21
RN5P	47,24	0,00	0,00	52,76	12,91	1,55	0,00	85,54	3,49	55,71	4,62	36,18	0,00	84,65	1,54	13,81
RN510P	22,85	0,00	12,42	64,73	28,15	1,58	0,00	70,27	4,00	0,00	4,69	91,30	8,18	10,17	10,50	71,14
RN1020P	22,51	1,92	0,00	75,57	7,44	1,17	0,00	91,39	14,42	0,00	5,53	80,05	3,22	18,08	2,01	76,69
RB5P	46,91	0,00	0,00	53,09	22,67	2,75	0,00	74,59	6,95	0,00	14,60	78,45	4,56	23,98	1,10	70,36

[1)] Abbreviations of rooting characters: LN – number of leaves; SH – shoot height (cm) TRL – total root length (cm); RN0 – number of roots on the basal cut; RN5 – 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); RNB5 = RN0 + RN5; TRN – total number of roots; RN0P = RN0/TRN*100%; RN5P = RN5/TRN*100%; RN510P = RN510/TRN*100%; RN1020P = RN1020/TRN*100%; RNB5P = RNB5/TRN*100%

[2)] Abbreviations of sources of variation: G - Genotype; Y - year; G x Y - interaction genotype x year; Err - error

The influence of year rose throughout the examined part of vegetation period, while the influence of genotype x year interaction was usually weak. The strongest influence of interaction (around 20%) had total number (TRN) and length of roots (TRL), especially in the first three dates of observation (Tab. 1. and Tab. 2).

Table 2. Heritability in broad sense (H) and significance of F – test for the influence of examined sources of variation 4)

Rooting 1)	*1st date of observation*				*2nd date of observation*				*3rd date of observation*				*4th date of observation*			
characters	*H 3)*	*G 2)*	*Y*	*G x Y*	*H*	*G)*	*Y*	*G x Y*	*H*	*G)*	*Y*	*G x Y*	*H*	*G)*	*Y*	*G x Y*
LN	14,06		**	**	71,57	**	**		78,26	**	**	**	22,45		**	
SH	62,13	*	*	**	77,72	**	**		56,05	*	**	**	48,80		**	
TRL	54,05	*		**	59,70	*		**	45,95		**	**	-	-	-	-
RN0	0,00				0,00		**	**	0,00		**	**	26,83		**	*
RN5	62,97	*		*	51,56	*	*	*	74,23	**	**		68,27	**	**	
RN510	86,86	**	*		78,91	**	*	*	74,17	**	**		75,72	**	*	
RN1020	63,57	*	*	**	68,42	**		*	67,93	**	*	**	63,72	*	**	
RB5	62,89	*		*	51,06			*	55,80	*	**	**	74,39	**	**	
TRN	76,42	**		**	73,92	**		**	68,73	**	**	**	78,02	**	**	
RN0P	0,00				0,00		**		0,00		**	*	0,00		**	
RN5P	87,15	**			62,61	*			42,91		**		0,00		**	
RN510P	60,78	*		*	81,64	**			29,88				46,35		**	*
RN1020P	69,31	**			47,46	*			62,34	*			31,18		**	
RB5P	87,01	**			77,13	**	*		37,46			*	42,07		**	

) and 2) Abbreviations of rooting characters and sources of variation as it is for table 1; 3) H - Heritability in broad sense (%); 4) Significance of F-test: * - $p < 0,05$, ** - $p < 0,01$

The influence of genotype on examined characters deferred among examined dates of observation. Only TRN and RN510 had high coefficients of heritability in all examined dates of observation, while the lowest were coefficients of heritability for the characters that describe wound roots (RN0 and RN0P). The characters describing the participation of number of roots on different parts of cutting in total number of roots had high coefficients of heritability in the first two dates of observation, and low in the last two. The characters of shoot had high heritabilities in the first three dates, except for number of leaves (LN) in the first date (Tab. 2).

Chosen principal components described 80-90% of the total variation in all four dates of observation. According to loadings usually two groups of characters were defined: one with number of roots on basal part of cutting, and the other with RN510. The total number of roots and shoot characters were mostly in the group with characters that describe the roots on basal part of cutting (Tab. 3).

Table 3. Broad-sense heritability for examined rooting characters for eastern cottonwood cuttings in field conditions

Rooting [1) characters	***1^{st} date of observation***		***2^{nd} date of observation***		***3^{rd} date of observation***		***4^{th} date of observation***		
	PC 1[4)	***PC 2***	***PC 1***	***PC 2***	***PC 1***	***PC 2***	***PC 1***	***PC 2***	***PC 3***
LN	0,479	0,637	0,607	0,511	0,183	0,854	0.122	0.045	0.867
SH	0,434	0,713	0,727	0,412	0,120	0,911	0.291	-0.121	0.794
TRL	0,654	0,690	0,829	0,480	0,473	0,736	-	-	-
RN5	0,246	0,942	0,950	0,204	0,250	0,906	0.952	0.043	0.253
RN510	0,688	0,621	0,662	0,665	0,789	0,529	0.659	0.675	0.229
RN1020	0,796	0,519	0,633	0,695	0,842	0,435	0.535	0.292	0.684
RB5	0,241	0,943	0,966	0,199	0,356	0,880	0.933	-0.219	0.066
TRN	0,675	0,720	0,829	0,541	0,642	0,736	0.940	0.124	0.297
RN5P	-0,938	-0,296	-0,291	-0,936	-0,897	-0,127	0.420	-0.638	0.094
RN510P	0,825	0,422	0,522	0,729	0,875	0,184	0.142	0.961	-0.042
RN1020P	0,848	0,277	0,185	0,842	0,852	0,326	0.091	0.177	0.863
RB5P	-0,939	-0,294	-0,361	-0,922	-0,945	-0,228	0.013	-0.840	-0.490
λ [3)	5,689	4,767	5,466	4,926	5,395	4,915	3.684	2.692	3.055
$\lambda/\Sigma\lambda_i$	0,474	0,397	0,455	0,410	0,450	0,410	0.335	0.245	0.278

[1) – Abbreviations for rooting characters are explained in tab. 1,[2) – the highest loading of character is underlined,[3) - λ - the eigenvalue of principal component; $\lambda/\Sigma\lambda_i$ - ratio of variance of principal component and sum of variances of all principal components,[4) - PC X - X^{th} principal component

DISCUSSION

Influence of sources of the variation of cuttings' rooting characters is valuable in selection tests (TEISSER du CROSS, 1984) and in the design of the nursery technology adjusted to certain genotype (HEILMAN *et al.*, 1994). High dependence of variation of examined characters on differences among genotypes and low similarities to other characters were the criteria utilization in the evaluation of cuttings' rooting potential of interesting poplar genotypes.

Many of examined characters were under strong influence of error, suggesting that for those characters size or number of plots should be increased. Considerable influence of year was also considerable, which is in concordinance with the results of KOVAČEVIĆ *et al.* (2005). It became stronger during the first half of vegetation period, and gives the advantage to the earlier observation terms. The influence of genotype x year interaction was the strongest in the first three dates of observation. These results signify multiannual character of such trials, while interactions genotype x year could be the base for design of cultivar-adjusted technology.

Considerable genotype x year interaction in the first two dates of observation for shoot characters, TRN, TRL, RN1020 and RN510P, together with the influence of year suggest that significance of conditions that occurred during growth, setting of dormancy, dormant period, and the period of activation from dormancy should be more thoroughly examined.

Total number of roots (TRN) had high broad-sense heritability in all four dates of observation (mostly over 0,70). They were lower for TRL (over 0,45 - 0,60). These results are in concordinance with surveys of many authors (SIWECKI and GIERTYCH in 1965, according to TEISSER du CROSS, 1984; WILCOX and FARMER, 1968; YING and BAGLEY, 1979; GUZINA, 1987; HAN *et al.*, 1994; KOVAČEVIĆ *et al.*, 2001; KOVAČEVIĆ *et al.*, 2005).

However, heritability in our trial, highlighted some other rooting characters as well. The number of roots on the basal (RN5 and RB5), middle (RN510) and upper (RN1020) part of cutting had considerable high heritably in all four dates of observation. The group of characters describing participation of number of roots on different parts of cutting, except RN0P, had considerable heritability only in the first and second date of observation. On the other side, our results strongly indicate that influence of differences among genotypes on variation of characters describing wound roots (RN0 and RN0P) has no significance. These results are in concordinance with KOVAČEVIĆ *et al.* (2005).

Relatively high heritability in broad sense of shoot height (H = 0,50-0,80) are in concordinance with results of COPPER and RENDAL (1973), SIEWECKI and GIERTYCH in 1965 (according to TEISSER du CROSS, 1984) and WU *et al.* (1998). Results of KOVAČEVIĆ *et al.* (2001) for bought shoot characters were similar to ours, while the results of KOVAČEVIĆ *et al.* (2005) suggest stronger influence of year and interaction genotype x year, and weaker influence of genotype. High heritability of shoot characters and their strong relationship with TRN and TRL, suggest the significance of shoot characters in the evaluation of rooting potential of examined genotypes.

Results of PCA allowed us to conclude similarities between shoot characters and total number and length of roots, as well as the fact that number of roots on the middle part of the cutting and characters in its group gives us additional information about rooting of cuttings beside TRN and TRL. In spite of possibilities, PCA is rarely used in breeding of forest trees (KHASA *et al.*, 1995, SING *et al.* 2004).

According to coefficients of heritability in broad sense all of the first three dates of observation could be used for evaluation of cutting rooting, but the largest numbers of characters have respectable heritability at the second half of May.

The examination of root system formation is still important task in course of selection and utilization of clones of eastern cottonwood. Results of our work suggests that, beside total number and length of roots, that are regularly used in selection tests, shoot characters and characters that describe roots at the middle portion of cutting could be of high significance. Shoot characters could be particularly interesting for they could be measured without destroying the plant.

Utilization of these characters at the second half of May could be useful in breeding process of black poplars.

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VARIJABILNOST I MEĐUZAVISNOST SVOJSTAVA OŽILJAVANJA AMERIČKE CRNE TOPOLE

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Izvod

Ispitan je uticaj genotipa, godine i interakcije genotip x godina na variranje 14 svojstava ožiljavanja odrvenjenih reznica u poljskim uslovima 15 genotipova američke crne topole (*Populus deltoides* Bartr.), kao i međuzavisnost svojstava ožiljavanja. Ispitivanje je izvršeno u četiri roka posmatranja tokom prve polovine vegetacionog perioda. Uticaj ispitivanih izvora variranja je procenjivana na osnovu doprinosa očekivanih varijansi ukupnom variranju, dok je uticaj genotipa procenjivan i na osnovu koeficijenta heritabilnosti u širem smislu. Međuzavisnost ispitivanih svojstava je sagledana na osnovu njihovog grupisanja u analizi glavnih komponenata (PCA). Uticaj godine je jačao tokom vegetacionog perioda, dok je uticaj interakcije genotip x godina uglavnom bio statistički signifikantan, ali slab. Većina svojstava je imala značajan uticaj genotipa i visoke koeficijente korelacije u drugom (druga polovina maja) i trećem roku merenja (početak juna). Pored ukupnog broja korenčića prvog reda (TRN) rezultati ukazuju i na značaj broja korenova na srednjem (od 5.-10. cm reznica od donjeg reza - RN510) i donjem delu reznice (od 0. - 5. cm - RN5 i RB5), kao i svojstava izbojka (broj listova (LN) i visine dominantnog izbojka (SH)). Razlike među genotipovima u svojstvima korenova rane (korenovi na donjem rezu) nisu bile značajne. Ispitivana svojstva su se na osnovu analize glavnih komponenata grupisala u dve grupe: nosilac prve grupe je bio broj korenčića na srednjem delu reznice (RN510), a druge broj korenova u donjem delu reznice. Rezultati ovi istraživanja ukazuju da bi selekcioni testovi ožiljavanja u poljskim uslovima mogli da budu unapređeni uključivanjem broja korenova prvog reda na srednjem i donjem delu reznice, a pogotovo praćenjem svojstava izbojka, koji mogu da se mere i bez uništavanja ožiljene reznice. Optimalni rokovi merenja bi bili druga polovina maja i početak juna.

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