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Relationship among black poplar genotypes based on cutting rooting and survival – a multivariate approach

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SUMMARY

The relationship between 14 genotypes of black poplars (Sect. *Aigeiros* Duby) was analyzed with regard to the variability of sixteen early rooting characteristics and cutting survival. Principal component analysis (PCA) and cluster analysis were used in order to reduce the amount of data and to enable the presentation of relationships among the genotypes. Besides normal standardization, two alternative methods of standardization were used: standardization by standard deviation within genotype, and by standard deviation within genotype followed by indexing with the correlation between characteristics and cutting survival. Alternative standardization methods were used in order to emphasize the contribution of genotype to the effect of differences among genotypes on total variation and relationship of rooting characteristic with cutting survival. To preserve the effects of standardization, the principal component analysis was based on a matrix of covariances. Contribution to total variation rose from the first to the third method of standardization for the first principal component (PC), while the trend was the opposite for the second one. The distances between genotypes were more related to the survival of their cuttings than after normal standardization. There were no significant differences in loadings with the first two PCs among the considered types of standardization. The characteristics of the shoot and those describing rooting in the upper half of the cutting displayed the highest correlation with the first PC, while the characteristics with high loadings with the second PC mainly describe rooting on the basal part of the cutting.

Keywords: principal component analysis, data standardization, nursery and plantation establishment

1. Introduction

The hardwood cuttings of black poplars (section *Aigeiros* Duby) are characterized by good rooting due to pre-formed primordia, discovered in

poplars by Van der Lek in 1924. Primordia can be also initiated *de novo* and activated on the cuts of cuttings: they form wound roots on a basal cut, and adventitious shoots on an upper cut (Smith, Wareing, 1972). Sufficient number and development of primordia (Smith, Wareing, 1972, Jestaedt, 1977) and their timely activation (Okoro, Grace, 1976, Pallardy, Kozlowski, 1979) improve the chances of a cutting's survival.

The problems of rooting of cuttings, especially in eastern cottonwood (Sekavin, 1969), could still compromise nursery production and the establishment of short rotation stands for the production of biomass. For this reason, evaluation of cuttings' rooting ability has always been an important element of poplar breeding programs (Teisser du Cross, 1984, Zalesny et al., 2005b, Kovacevic et al., 2008).

The activation of primordia and cutting rooting are influenced by many factors. On one hand there are genetic sources of variation: differences among genotypes (Wilcox, Farmer, 1968, Guzina, 1987, Kovačević et al., 2005), among and within populations (Ying, Bagley, 1979). Then there are differences among cuttings within genotype (C-effect), such as differences among ramets (Stohlinger, Toliver, 1985, Herpka, Marković, 1969, according to Marković, Rončević, 1986, Li et al., 1994), differences in the position of the cutting on the sprout (Ying, Bagley, 1977, Martinez et al., 1994, Zalesny et al., 2003), the age of sprout (Smith, Wareing, 1972) and the season in which the cutting is made (Nanda, Anand, 1970, Fege, 1984).

There are also environmental factors, such as soil texture (Wilcox, Farmer, 1968, Kovačević et al., 2005), temperature and precipitation (Zalesny et al., 2005a), microrelief (Alkinani, 1972), storage conditions (Fege, 1984, Nanda, Anand, 1970) and nursery technology (Fege, 1983). Cutting rooting variability trials were usually carried out in one season, in field conditions (Ying, Bagley, 1977, Kovačević et al., 2001) or controlled conditions in soil substrate (Wilcox, Farmer, 1968, Ying, Bagley, 1977, Guzina, 1987), mostly concerning only the total number and length of first-order roots.

The aim of the present work was to analyze relationships among black poplar genotypes based on rooting characteristics in the first half of the vegetation period. Analysis was also made of the possibility of introduction of information on the contribution of genotype variation to the total variation of particular characteristics and its relationship to cutting survival in principal component and cluster analysis.

2. Material 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 two genotypes of Euramerican poplar (*Populus x euramericana* Duby): cl. Pannonia and cl. I-214 and 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 Pannonia, I-214 and S1-3 are registered in Serbia. The others are currently in an experimental phase of development.

Two separate studies were conducted:

- a study of morphological characteristics of cutting rooting, and
- a study of cutting survival.

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 than average).

Study of morphological characteristics

The nursery experiments for examination of morphological characteristics were established in 1996, 1998 and 1999, on April 15, on humofluvisol soil type (40% silt+clay content in the 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 between years. Weeds were regularly treated mechanically.

The stem cuttings, 18–22 cm long and more than 8 mm wide, were prepared using scissors, at the beginning of April, from the stems of one-year-old rooted cuttings, 1.5–2.2 m high. In that time the plants are dormant, but the buds are close to flush. Most of the stem was used for cutting preparation except its brittle top (too thin cuttings) and basal part (too small buds). These parts of the stem are usually avoided in practical cutting preparation. Also in this way the influence of topophisis – differences among cuttings within the same stem, related to the differences in their position on the stem (Ying, Bagley, 1977, Martinez et al., 1994, Zalesny et al., 2003) – was weakened, thus increasing the precision of the experiment. 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 planting the cuttings were stored in trenches for not more than two weeks. The cuttings were planted in the soil manually, with the top of the cutting 0–5 cm beneath the soil surface. Twenty cuttings were planted per plot. The experiment was designed as completely randomized in four repetitions.

Five cuttings per plot were carefully dug out manually, cleaned and analyzed 60 days after planting (the beginning of June). The following morphological characteristics were analyzed: On each cutting with vital shoot, the length of every first-order root and its distance from the basal cut of the cutting were measured, as well as the height of the dominant shoot (SH) and its number of leaves (LN). The length of roots was measured only in the first three terms, because later it was not possible to dig up the whole root system effectively. Based on measurements of the root system the total number of roots (TRN) and total root length (TRL) were derived. Also the following five parts of the cutting were considered: basal cut (wound roots), basal part (the basal 5 cm of cutting without roots of the basal cut), middle part (between 5 and 10 cm from the basal cut), upper part (above 10 cm), basal cut with basal 5 cm and basal cut with basal 10 cm. For each part the following characteristics were derived: number of roots (RN0, RN05, RN510, RN1020, RN5, RN10), and its

ratio to TRN (RN0P, RN05P, RN510P, RN1020P, RN5P and RN10P). Average plot values were used in further statistical analysis.

Study of cutting survival

The nursery experiments for examination of cutting survival were established on sandy and loamy fluvisol (with respectively 30% and 62% silt+clay content in the surface horizon), on April 15 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 characteristics. Thirty cuttings were planted per plot in three randomized repetitions per clone. The experiment was designed as completely randomized. Cutting survival was determined at the end of growing period as the percentage of cuttings with a viable shoot.

Data analysis

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

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

where X_{ijm} is the measured value, μ the average value, g_i the effect of genotype (*G*), $y_{j(i)}$ the effect of year within the i*th* genotype (*Y*), and $\varepsilon_{m(ij)}$ the 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 to calculate expected variances for the examined sources of variation (KIRK, 1968). Negative expected variances were considered to be zero (ALLARD, 1960). Characteristics describing ratios of number of roots in portions of a cutting to TRN were transformed by an arcsine transformation ($arcsin\sqrt{X}$, where *X* stands for the value in %), while all characteristics describing number of roots were transformed by a square transformation ($\sqrt{X+1}$) to provide the normal distribution of frequencies required by the

statistical methods. The effects of the examined sources of variation were described by coefficients of variation:

$$Cv = \frac{\sigma_A}{\overline{X}} 100\% ,$$

where σ_A denotes the expected standard deviation of source of variation A.

Three methods of standardization were examined:

- normalization i.e. standardization of clone means with their standard variation
  $(X_j - \overline{X})/\sigma_G$, where X_j is the mean of the j*th* genotype, and σ_G is the standard deviation of genotype means;
- standardization with residual standard deviation $(X_j - \overline{X})/\sqrt{\sigma_g^2 + \sigma_{y(g)}^2 + \sigma_{err}^2}$, where σ_g^2 is the expected variance of genotype, $\sigma_{y(g)}^2$ the expected variance of year within genotype and σ_{err}^2 the expected variance of error;
- standardization with residual standard deviation and indexing with analog correlation coefficient with cutting survival $r \cdot (X_j - \overline{X})/\sqrt{\sigma_g^2 + \sigma_{y(g)}^2 + \sigma_{err}^2}$, where r denotes the correlation coefficient with cutting survival.

The alternative methods of standardization were applied in order to introduce information on the significance of influence of genotype on total variation of the characteristics used, as well as information on their relationship with cutting survival.

Principal component analysis and cluster analysis were used in order to reduce the amount of data and to enable the presentation of relationships among genotypes. Principal component analysis was also used for grouping of examined characteristics according to their loadings with principal components, selected to satisfy the criterion $\lambda > 1$ (Kaiser 1958). Principal component analysis was based on a covariance matrix, and the obtained principal components were not rotated, in order to preserve the effect of standardization and indexing. Also the examined genotypes are grouped by cluster analysis based on standardized genotype means, using unweighted pair-group average

linkage (UPGMA). The software package STATISTICA 7.1 (StatSoft Inc., 2006) was used for the statistical analysis.

3. Results

According to the results of analysis of variance, the influence of the examined sources of variance on rooting characteristics varied, especially the effect of differences among genotypes. The contribution of genotype to the total variation was considerable for number of leaves (LN), number of roots on middle and upper part of cutting (RN510 and RN1020), total number of roots (TRN) and contribution of some parts of cutting to the total number of roots (RN510P, RN1020P, RN5P and RN10P). Also, coefficients of variation of the same characteristics were higher than coefficients of variation of other examined characteristics (Table 1).

The listed rooting characteristics and shoot height had high correlation with cutting survival, usually more than 0.66 (Table 2).

According to the results of the principal component analysis, the examined characteristics could be divided in two groups. The first has high loadings with the first principal component, where RN1020 and its contribution to the total number of roots (RN1020P) have the highest loadings. The other group has high loadings with the second principal component, where component number of roots at the basal part of cutting (RN5) has the highest loading. The grouping of characteristics was almost the same following all the methods of standardization, except that RN05 and RN10 moved to the second group following alternative standardization (Table 2).

The contribution of the first principal component to the total variation increased in the case of alternative standardization and indexing of data by correlation coefficient with cutting survival. Also the eigenvalues of both selected principal components decreased, as well as the eigenvalue of the second vs. first principal component ratio. The correlations of cutting survival

with analog principal components following standardization by the considered methods were similar (Table 2).

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

	Mean square			F-test		Coefficient of variation		
Characteristics[1)]	MS_G[2)]	$MS_{Y(G)}$	MS_{ERR}	F_G[3)]	$F_{Y(G)}$	Cv_G	$Cv_{Y(G)}$	Cv_{ERR}
LN	94.24	40.82	8.16	2.31*	5.00**	13.19	17.90	17.77
SH	550.76	537.40	63.00	1.02	8.53**	3.05	33.46	24.23
TRL	5171.75	4894.54	906.58	1.06	5.40**	5.66	38.09	36.09
RN0	0.63	2.38	0.11	0.26	20.80**	0.00	42.36	18.92
RN05	0.52	1.96	0.14	0.26	13.79**	0.00	27.11	15.06
RN510	0.67	0.15	0.08	4.54**	1.95**	11.50	7.41	15.07
RN1020	1.49	0.33	0.11	4.51**	3.13**	17.20	13.13	17.88
RN5	1.08	0.73	0.16	1.49	4.48**	5.73	12.48	13.30
RN10	1.47	0.84	0.17	1.75	5.03**	6.77	12.13	12.01
TRN	2.41	1.10	0.18	2.20*	5.97**	8.89	12.85	11.45
RN0P	259.21	1544.65	51.15	0.17	30.20**	0.00	85.76	31.54
RN05P	236.87	669.60	55.80	0.35	12.00**	0.00	30.68	18.38
RN510P	79.10	34.13	33.22	2.32*	1.03	7.92	1.96	23.45
RN1020P	436.74	81.52	53.17	5.36**	1.53	23.44	11.48	31.24
RN5P	469.47	92.26	52.29	5.09**	1.76*	10.47	5.91	13.43
RN10P	436.74	81.52	53.17	5.36**	1.53	8.21	4.02	10.94

[*)] Degrees of freedom for: genotypes df_G=13, year within genotype $df_{Y(G)}$=28, error df_{ERR}=124

[1)] Abbreviations of rooting characteristics: LN = number of leaves; SH=shoot height (cm); TRL = total root length (cm); RN0 = number of roots on basal cut; RN05 = number of roots on basal portion of cutting (0–5 cm from 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 of sources of variation: G=Genotype; Y(G)=Year within Genotype; ERR=error

[3)] Significance of F-test: * statistically significant for $\alpha_{0.05}$, ** statistically significant for $\alpha_{0.01}$

According to the factor scores the relationships among the examined genotypes remain similar, except that the distribution of genotypes was more narrow by the second principal component, and wider by the first. The grouping of genotypes was also similar, but distances are more influenced by the first

principal component and characteristics with high correlation with cutting survival (Figures 1–3).

Table 2. Correlations of examined rooting characteristics with cutting survival and results of their grouping according to principal components following standardization by the examined methods

Characteristics[1)]	r[2)]	N		S		SP	
		PC1	PC2	PC1	PC2	PC1	PC2
LN	0.89**[3)]	0.843	-0.064	-0.863	0.117	0.834	0.045
SH	0.82**	0.809	-0.115	-0.833	0.065	0.801	-0.007
TRL	0.74**	0.882	0.264	-0.845	0.321	0.850	0.318
RN0	0.17	0.195	0.931	-0.085	0.866	0.104	0.898
RN05	0.52	0.638	0.489	-0.571	0.614	0.581	0.610
RN510	0.63*	0.947	0.032	-0.920	0.167	0.937	0.153
RN1020	0.70**	0.951	-0.215	-0.972	-0.130	0.976	-0.127
RN5	0.42	0.502	0.843	-0.398	0.878	0.415	0.896
RN10	0.57*	0.753	0.637	-0.666	0.717	0.684	0.724
TRN	0.70**	0.930	0.344	-0.879	0.439	0.892	0.442
RN0P	-0.32	-0.449	0.790	-0.534	-0.633	-0.523	0.670
RN05P	-0.57*	-0.800	-0.012	-0.793	-0.006	-0.801	-0.013
RN510P	0.47	0.803	-0.283	-0.801	-0.145	0.817	-0.170
RN1020P	0.68*	0.901	-0.366	-0.942	-0.301	0.940	-0.296
RN5P	-0.64*	-0.885	0.447	-0.927	-0.349	-0.928	0.360
RN10P	-0.68*	-0.901	0.366	-0.942	-0.301	-0.940	0.296
Eigenvalue (λ)		9.97	3.64	4.36	0.88	2.06	0.27
$\lambda / \Sigma \lambda$ (%)		62.28	22.80	75.12	15.21	78.91	10.40
$\lambda_{PC2} / \lambda_{PC1}$		0.365		0.202		0.131	
Pearson sa surv		0.778**	0.031	-0.790**	0.163	0.765**	0.112
Spearman sa surv		-0.719**	0.020	-0.754**	0.143	0.705**	0.143

[1)] Abbreviations of rooting characteristics: LN = number of leaves; SH = shoot height (cm) TRL = total root length (cm); RN0 = number of roots on basal cut; RN05 = number of roots on basal portion of cutting (0–5 cm from 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 = Pearson's coefficient of correlation, N = data standardized by normalization, S = data standardized by within-genotype standard deviation, SP = data standardized by within-genotype standard deviation and indexed by correlation with cutting survival, PC = principal component

[3)] * and ** - statistically significant difference from 0 for $\alpha_{0.05}$ and for $\alpha_{0.01}$, respectively

There were four genotypes that were distinctly outside the main group of genotypes: S6-7, S1-3, 124/81 and Pannonia. Genotypes S6-7 and S1-3 had the poorest results for cutting survival, while Pannonia had the best cutting survival. As the first principal component had high correlation with cutting survival, the distribution of examined genotypes by the first principal component reveals their relationship according to cutting survival.

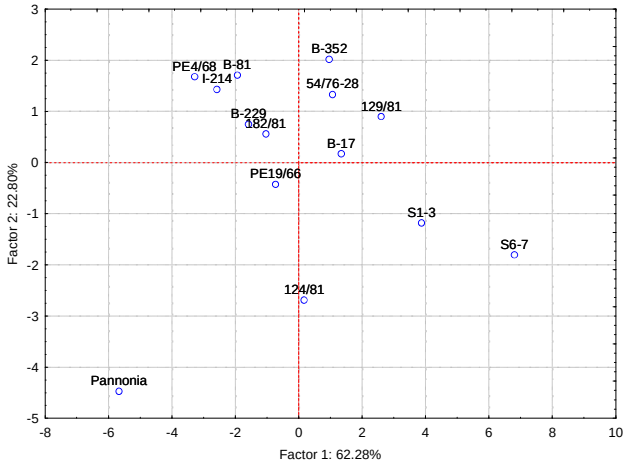

Figure 1. Relations of the examined genotypes based on the first two principal components formed on data standardized by standard deviation of genotype means

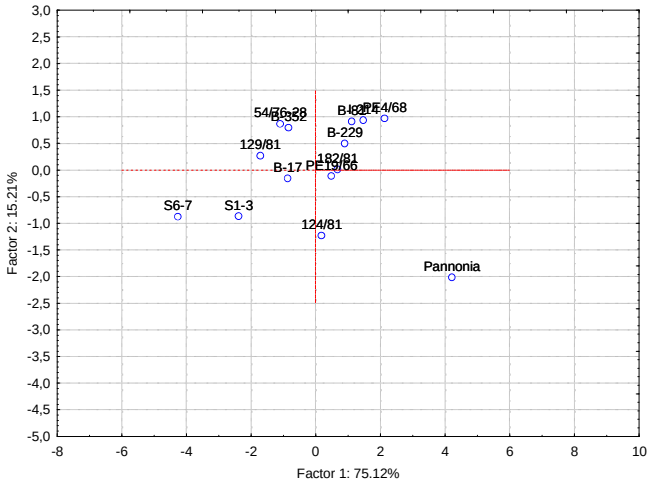

Figure 2. Relations of the examined genotypes based on the first two principal components formed on data standardized by standard deviation within genotype

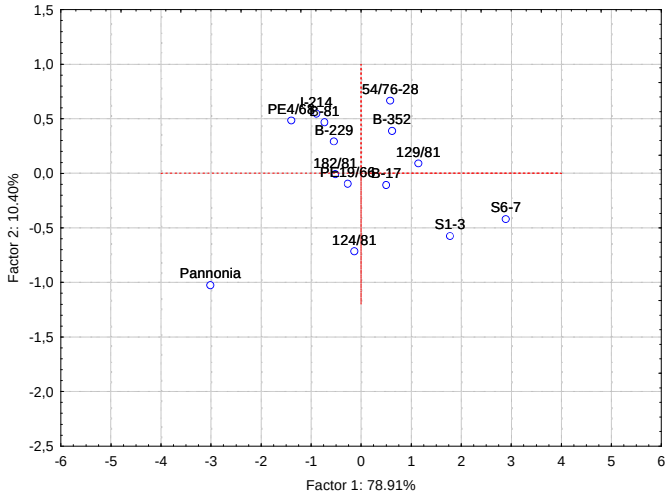

Figure 3. Relations of the examined genotypes based on the first two principal components formed on data standardized by standard deviation within genotype and indexed by coefficient of correlation of rooting characteristics with cutting survival

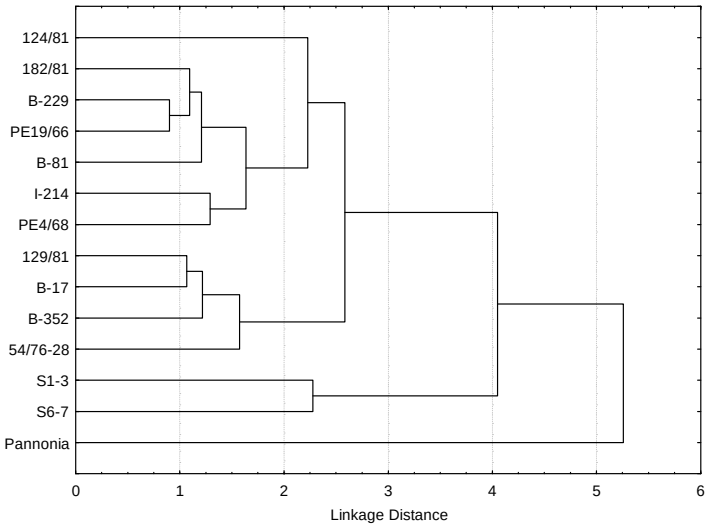

Figure 4. Relations of the examined genotypes based on cluster analysis – UPGMA linkage method on data standardized by standard deviation of genotype means

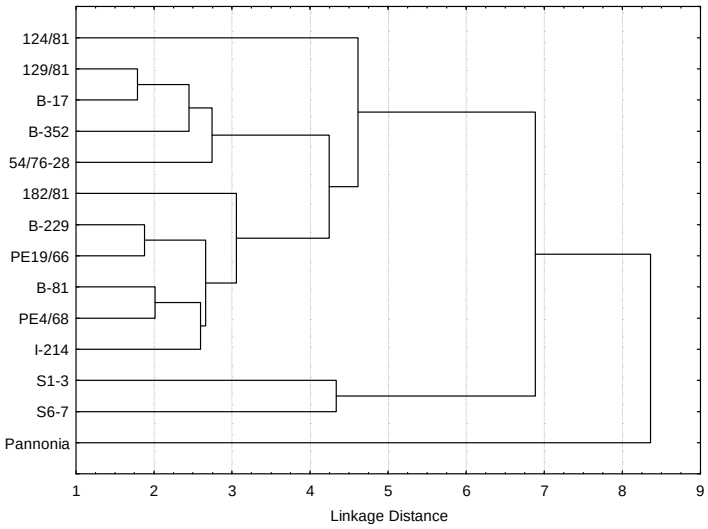

Figure 5. Relations of the examined genotypes based on cluster analysis – UPGMA linkage method on data standardized by standard deviation within genotype

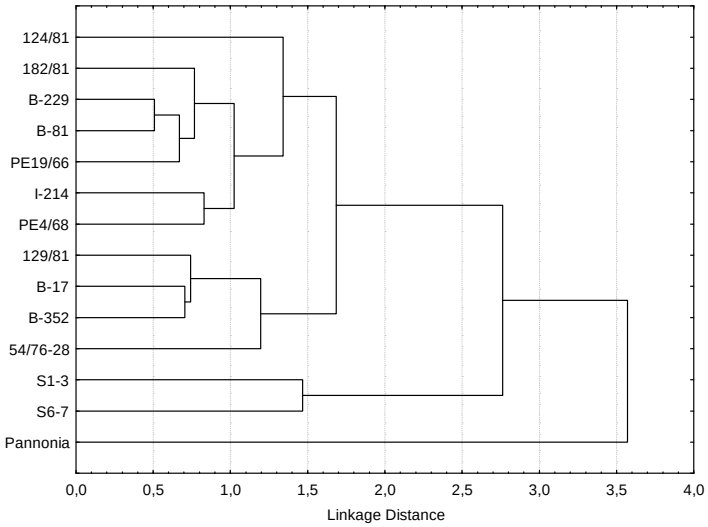

Figure 6. Relations of the examined genotypes based on cluster analysis – UPGMA linkage method on data standardized by standard deviation within genotype and indexed by coefficient of correlation of rooting characteristics with cutting survival

The cluster analysis showed a decrease in distances from data that is normalized to data standardized by within-genotype standard deviation and indexed by correlation coefficient with cutting survival. The grouping of genotypes remained similar (Figures 4–6).

4. Discussion

The relationship of genotypes is usually presented by multivariate methods such as cluster analysis and principal component analysis. However, rooting characteristics are rarely used (Khasa et al., 1995). The common basis for such an approach is standardized genotype means. Standardization of data is an important part of the procedure in methods of multivariate analysis, serving to overcome differences among characteristics in scale or measure. The usual method of standardization is normalization where all characteristics are transformed to have arithmetic mean $\overline{X} = 0$ and standard deviation $\sigma = 1$ (Sokal, Sneath, 1963).

On the other hand it is well known that rooting characteristics are weakly inherited, under strong influence of the environment. The considerable influence of year confirms the importance of the multi-annual nature of research into cutting rooting (Kovacevic et al., 2005). Coefficients of variation show that rooting characteristics differed in terms of the influence of differences among genotypes on their variation. However in the usual method of standardization – normalization – the influence of variability within genotypes is not introduced.

This 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 characteristics that are highly inherited and under the strong influence of differences among genotypes are greatly preferable, since selection by these characteristics is more precise and effective. In that sense we introduced information about variation within genotype by standardization with standard deviation within genotype, as was suggested by Kovacevic et al. (1999). The covariance matrix was used in principal component analysis in

order to preserve this information. Borgognone et al. (2001) even propose a covariate matrix instead of the correlation matrix in all cases where the scales are the same for all attributes.

Compared with normal standardization, this alternative method decreased the eigenvalues of the principal components and total variation. The ratio between the eigenvalues of the second and first principal component was almost 3 times lower than with normalization. This could be expected, as the characteristics with the highest loadings with the first principal component were characterized by high contribution of genotype to the total variation. The variability within genotype in characteristics of the second group was relatively high compared with the variance of genotype means. Thus the variability of those characteristics decreased more, compared with the variability of characteristics in the first group. According to dendrograms the relationship among the examined genotypes was not very much disturbed, but it could be said that distances between them were more based on characteristics of the first than of the second group, relative to the results with normalization. For example, genotype 124/81, a genotype with moderate RN1020 but low RN5, was grouped within the main cluster, not outside it as was the case with normalization.

Indexing with cutting survival of data standardized by within-genotype standard deviation should introduce information about cutting survival in the standardized data, emphasizing the characteristics that are highly correlated with cutting survival. The introduction of survival information in data is important from the point of view of breeding. A good example is shoot height (SH). Unlike most of the examined characteristics, whose variation was poorly influenced by genotype, shoot height was significantly correlated to cutting survival. By indexing with correlation with cutting survival the influence of shoot height on the results of multivariate analysis was reinforced.

The eigenvalues of the first two principal components were the lowest out of all the examined methods of standardization, suggesting further decrease in total variance. The ratio between eigenvalues of the second and the first

principal component was higher than with the other alternative standardization method, but lower than with normalization. This is probably related to the fact that most of the characteristics are strongly correlated to the first principal component, which is significantly correlated to cutting survival. Thus indexing by correlation with cutting survival weakened the effect of characteristics of the second group (strongly correlated to the second principal component, but weakly to the first).

The presentation of factor scores of the first two principal components based on the examined methods of standardization showed similar relations among genotypes. The contribution of the first principal component eigenvalue to the total variation was high with each standardization method (more than 70%). Thus the distances among genotypes were less influenced by the second principal component. In that sense the genotype 124/81 appeared relatively closer to the main group following alternative methods of standardization.

Similarities were also observed in grouping by cluster analysis. This could be explained by the fact that most of characteristics that had high coefficients of variation among genotypes and contribution of genotype to the total variation also had high correlation to cutting survival. Also, according to the results of grouping based on principal component analysis, these characteristics are highly correlated among themselves. A more significant effect of the standardization with within-genotype standard deviation could be achieved if the characteristics were less correlated and more different in terms of contribution of genotype and within-genotype variation to the total variation.

The standard deviation within genotype or within population from other research could be used in alternative methods of standardization, with certain conventions. This could be also the case with indexing parameters in the second method of standardization. The results of our work could be interesting in cutting rooting studies and breeding. The relations among genotypes could be evaluated based on morphological rooting characteristics, avoiding the need for resource-intensive cutting survival experiments. For example, the rank correlation between distances formed by all characteristics related to those

formed only by standardized RN1020 (first group) and RN5 (second group) was over 0.90 for all methods of standardization. However, their correlations with distances formed on cutting survival were moderate (data not shown). Better results could be expected if only RN1020 or the first principal component based distances were compared to distances based on cutting survival.

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