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Ampelographic characterization of *Vitis* cv "Prokupac" clones by multivariate analysis

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Abstract

Twenty-five Prokupac clones were included in the research. The aim of this study was to: examine the variability of morphological traits within the clone collection, calculate the correlation among characteristics, detect associations among clones, identify the most useful variables for discrimination among clones and recommend clones suitable for further exploitation.

Statistical analysis was performed based on the average values obtained from a three year long period. Relationship among the traits was presented with a correlation analysis which was done by using Pearson correlation coefficients. Cluster analysis was performed by applying Ward's method and Euclidean distances. The overall mean values showed high level of morphological variability. The highest level of variation was found in the number of berry seeds. Based on cluster analysis clones divided into three clusters I, II and III which include 7, 12 and 6 clones, respectively. Maximum Euclidean distance was observed between the clones 42/2 and 43/8, and minimum distance was observed between the clones 40/8 and 43/7. Parameters with high discriminating values were those related to bunch and berry size and structure. Most clones are perspective for further investigation and vine production, clones 40/4, 41/1 and 43/8 have large bunches and can be recommended as consummation grape.

Keywords: *Prokupac, clone, morphological variation, bunch weight, cluster analysis*

1. Introduction

Morphological properties of a grape cultivar are the most important factors based on which successful viticulture can be achieved. Therefore, the adequate choice of a clone is extremely important in the process of vineyard establishment.

Prokupac is Serbian autochthonous red wine variety whose precise origin has not been determined. It is often found under the synonyms: "Kameničarka", "Rekovačka crnka", "Nikodimka", "Rskavac", "Prokupka" and "Niševka". It is characterized with strong vigor and yielding capacity. Prokupac bunch is medium large, with cylindrical or conical form, medium compact. Berries are medium large, round or slightly snippy with thick and dark blue epidermis. Some Prokupac clones are characterized as highly resistant to *Botrytis cinerea* (ZIROJEVIĆ [1]; AVRAMOV & ŽUNIĆ [2]; ŽUNIĆ & GARIĆ [3]). Long-term cultivation in diverse agro ecological conditions has caused the Prokupac to become a mixture of clones (genotypes). Polyclonal origin and the accumulation of genetic mutations caused high variability within this cultivar. The heterogeneity of the cultivated population can create problems in production, so efforts have been made to identify clones with desirable properties. Also, autochthonous cultivars are in some cases at a high risk of extinction due to the introduction of famous European grapevine cultivars. Because of those reasons special attention should be pay to the clonal selection. In clonal selection the most important

parameters are: vigor, shape and bunch size, bunch and ampelographic composition of berry, grape and wine quality as well as disease resistance (KONRAD & al. [4]; RÜHL & al. [5]; WALKER & al. [6]; JACKSON [7]; CREASY & CREASY [8]; KELLER [9]).

MARKOVIĆ & al. [10] has allocated 25 Prokupac clones which were tested for this study. These clones were subjected to laboratory testing for presence of damaging viruses, thus confirming the "natural virus free" status. Natural virus free clones can be used for further multiplication and production of certified planting material. During the period of investigation of morphological characteristics of 25 clones, 13 clones were recognized by the Ministry of Agriculture as technologically better clones compared to standard variety: 40/5, 40/8, 41/1, 41/3, 41/4, 41/6, 42/1, 42/2, 43/2, 43/4, 43/5, 43/6, 43/7.

The objective of this study was (1) to examine the variability of morphological traits within the clone collection, (2) to calculate the correlation among characteristics, (3) to detect associations among clones, (4) to identify the most useful variables for discrimination among clones and (5) to recommend the clones suitable for further exploitation based on numerous desirable characteristics.

2. Material and Methods

Twenty-five Prokupac clones were included in the research which was carried out in the vineyard of experimental field "Radmilovac", University of Belgrade, Faculty of Agriculture (GPS coordinate E 44° 45' 15", N 20° 34' 56", altitude 126 m). Vineyard is planted in 2005. Vineyard is located on the southern exposure, lines extend in the direction east-southeast. The line spacing is 3 m and distance between vines in the row is 1m. Training system is double asymmetric cordon. In vineyard were applied standard agricultural and viticultural measures. For research of bunch and berries ampelographic composition, ten vines of each clone were selected and ten representative bunches from each clone were picked.

Bunches were measured for their weight, length and width, and rachis (pedicel) from each berry was carefully cut off with scissors so that as little mesocarp as possible was left on stem. Number of berries per bunch was also determined and berry mass per bunch and mass of stems were measured on analytical balance. From each clone 100 berries were selected for the purpose of analysis and after measuring mass of berries, berry skin and seeds were separated. Mass of seeds and skin of 100 berries was measured on analytical balance, and number of seeds in 100 berries was determined by counting. Other parameters (table 1) were obtained by computation. Grape quality expressed in sugar content was determined with Oechsle mostwaage and values were determined using Dujardin-Salleron table, and total acid content was determined by titration method with n/4 NaOH. Glicoacidometric index was determined as ratio of sugar content and total acid content.

Statistical analysis was performed using the average values obtained from a three year long period. In order to show the relationship among the traits, a correlation analysis was performed using Pearson correlation coefficients. Cluster analysis was done in order to evaluate relationships among clones and it was performed by applying the Ward's method and Euclidean distances. Principal component analysis (PCA) was performed with the aim of summarizing numerous data into first principal component containing the highest possible variability of the data. To determine which of the PCs accounted for the greatest amount of variation, the Eigenvalues of 5 PCs were compared for each trait. Data analysis was done by using 'Statistica' (StatSoft, Inc., Tulsa, Oklahoma, USA).

3. Results and discussion

Results of ampelographic composition of bunch and berry are shown in table 1. The longest bunch recorded for clone 41/1 (15.54 cm) and the smallest for clone 41/2 (12.10 cm). Clone 43/4 was characterized by the largest bunch width (10.24 cm), and clone 43/6 had the smallest bunch width (6.4 cm). According to number of berries per bunch and bunch weight given in table 1, can be concluded that clone 43/8 has the highest value of these morphological characteristics (131.04 and 284.46 g, respectively). Bunch structure is expressed in percentages of bunch stem (rachis) and berries in a bunch. Clone 41/3 had the highest % of bunch stem (5.3%) but the lowest % of berries in a bunch (94.7%) compared to clone 43/3 which had the lowest % of bunch stem (2.73%) but the highest % of berries in a bunch (97.27%). The biggest number of seeds was recorded for clone 43/2 (3.28) which, together with clones 43/4 and 43/8, had the smallest seed mass (0.035 g). During a three year long testing period variations in berry weight were insignificant except that clone 40/8 had maximum berry mass (2.78 g). Based on berry structural composition, expressed in percentages of berry skin, berry meat and seeds, clones 40/6 (6.5% berry skin and 3.15% seed) and 43/8 (94.34% berry meat) singled out. Clone 43/2 was characterized with the highest level of accumulated sugar (22.05%), and the highest total acid content was noted for clone 40/5 (8.53 g/l). Glicoacidometric index ranged from 1.95 (40/4) to 3.95 (42/1).

Structure of bunch and berry is very important characteristic of wine cultivars. Berry skin is considered to be an important element of the structure because it contains phenolic compounds which are extracted into wine, thus giving it color and odor. Good knowledge of structural indices, thickness and percentage of berry skin, provides crucial information to a technologist in the process of maceration when making wine or other products such as grappa (LATAIEF & al. [11]; MATIJASEVIC & al. [12]; STOICA & al. [13]).

According to DOWNEY & al. [14] berry skin and mesocarp ratio in small sized berries is better in case of varieties with medium large and large berries. Increased ratio between pulp/berry skin causes “dillution“ of tanine and anthocyanin in juice. Other authors claim that berry composition is mostly affected by amount of water available to grapevine root system from soil (DE LA HERA-ORTIS & al. [15]).

The overall mean values of all traits showed high differences between the clones indicating high level of morphological variation. This was also confirmed by relatively high coefficients of variation established for the majority of examined traits (table 1). In general, the most significant variations were found in the number of seeds in a berry (CV= 21.30%), whereas insignificant differences between the clones were noted for percentage of berries in a bunch (CV= 0.62%) and berry meat (CV= 1.04%).

Based on mean values of all examined characteristics and by using hierarchical cluster analysis, a dendogram of phenotypic differences of examined Prokupac clones was constructed. Prokupac clones are related in many different ways which explains the existence of numerous hierarchical levels.

In this paper three distant clusters of Prokupac clones are presented (figure 1). Maximum Eucladean distance (d=118.4) was observed between the clones 42/2 and 43/8, and minimum distance (d=3.8) was observed between the clones 40/8 and 43/7. The lowest variability was observed within cluster I (d=33.6), cluster II had somewhat higher variability (d=49.4) and the highest variability was observed in cluster III (d=62.0).

The cluster I, which included 7 clones, can be divided into one subgroup (A) with two independent clones (41/2 and 42/2). Twelve clones were in cluster II. This cluster could be additionally divided into three subgroups (A, B and C). First and second sub-group includes

three clones and third subgroup includes 5 clones. Clone 41/6 was outgrouped and separated initially from all clones in cluster II. The remaining 6 clones were classified into cluster III.

Most of the clones are characterized by moderate bunch size, good bunch and berry structure and satisfactory sugar and acid content, except the clones 40/4, 41/1 and 43/8 from third cluster, which have large bunches. Cluster analysis was used to evaluate the divergence of clones (FORVEILLE & al. [16]; SCALABRELLI & al. [17]) and grapevine cultivars (ASENSIO & al. [18]; MARTINES & al. [19]; ARADHYA & al. [20]; ORTIZ & al. [21]; FRANCO-MORA & al. [22]; VARGA & al. [23]; SABIR & al. [24]). By analyzing the properties based on which the clones were grouped it was concluded that grape weight was most influential; however, other characteristics should not be underrated. Similar results were obtained by RAKONJAC & al. [25] who also found that the grape weight had major impact on the grouping of Kreaca clones in clusters.

Table 1. Ampelographic composition of Prokupac clones bunch and berry

Clone	Bunch length	Bunch width	Number of berries per bunch	Bunch weight	Bunch structure		Number of seeds per berry	Seed weight	Berry weight	Berry structure			Sugar content	Total acid content	Glicoacid ometric index
					% of bunch stem	% of berry				% of berry skin	% of seed	% of berry meet			
40/1	13.83	7.87	93.60	190.36	4.04	95.96	1.46	0.042	2.4	5.13	2.50	92.38	18.75	6.91	2.76
40/2	14.66	9.99	111.78	244.77	3.67	96.33	1.27	0.040	2.18	4.59	2.11	93.30	18.58	7.57	2.40
40/3	14.53	8.12	94.51	217.92	3.89	96.11	1.75	0.039	2.47	4.86	2.55	92.59	19.03	6.80	2.84
40/4	13.45	9.20	115.60	261.93	4.36	95.64	1.36	0.035	2.42	5.08	1.90	93.02	16.80	8.38	1.95
40/5	14.50	9.13	110.40	239.90	3.92	96.08	1.51	0.038	2.4	5.54	2.50	91.96	17.82	8.53	2.37
40/6	14.85	8.05	86.70	188.17	4.29	95.71	1.58	0.038	2.00	6.50	3.15	90.35	17.90	6.00	2.99
40/7	14.09	7.83	91.84	222.28	3.23	96.77	1.75	0.039	2.62	5.57	2.52	91.91	19.67	6.68	2.97
40/8	13.50	7.60	93.47	203.75	3.37	96.63	1.76	0.042	2.78	4.89	2.52	92.59	18.55	6.15	2.98
41/1	15.54	8.24	96.93	263.34	3.17	96.83	1.84	0.040	2.63	4.41	2.66	92.93	19.09	6.87	2.75
41/2	12.10	6.64	77.13	180.52	3.49	96.51	1.73	0.044	2.52	5.79	2.62	91.59	20.03	6.75	3.44
41/3	13.38	7.69	92.80	211.19	5.30	94.70	1.98	0.040	2.77	5.42	2.64	91.95	19.32	8.02	2.50
41/4	12.70	7.40	87.90	194.73	4.23	95.77	1.79	0.036	2.35	5.53	2.98	91.49	17.03	8.12	2.20
41/6	13.90	8.14	73.22	210.09	3.90	96.10	1.73	0.039	2.28	4.82	2.46	92.72	18.57	6.47	2.97
42/1	12.56	7.54	99.09	232.93	3.36	96.64	1.70	0.047	2.60	5.77	2.92	91.31	20.10	5.34	3.95
42/2	12.94	7.81	99.69	170.37	4.72	95.28	1.52	0.038	2.17	5.39	2.30	92.30	19.10	6.43	3.82
42/3	12.87	8.19	99.89	220.92	3.84	96.16	1.54	0.037	2.33	5.15	1.55	93.30	18.93	5.67	3.79
42/4	12.24	6.93	94.11	199.66	3.78	96.22	1.62	0.040	2.22	5.41	2.70	91.89	18.38	6.92	3.04
43/1	13.09	7.37	89.29	217.13	3.33	96.67	1.45	0.037	2.53	4.86	1.34	93.79	18.57	6.95	3.11
43/2	14.36	6.84	88.76	188.50	3.05	96.95	3.28	0.035	2.17	3.50	2.23	94.27	22.05	6.22	3.81
43/3	14.80	8.20	103.30	220.52	2.73	97.27	1.68	0.042	2.73	4.76	2.56	92.67	19.00	6.42	3.02
43/4	12.67	10.24	98.76	207.59	3.60	96.40	1.70	0.035	2.37	4.35	2.36	93.29	18.05	5.37	3.47
43/5	12.90	6.80	87.84	194.02	3.27	96.73	1.98	0.037	2.65	5.40	2.75	91.85	21.77	6.56	3.48
43/6	13.24	6.40	98.44	217.70	3.24	96.76	1.60	0.036	2.5	3.84	2.40	93.76	21.38	6.79	3.38
43/7	14.02	6.62	93.11	207.12	3.11	96.89	1.89	0.039	2.47	4.98	2.96	92.06	18.22	7.25	2.61
43/8	13.00	8.08	131.04	284.46	2.97	97.03	1.75	0.035	2.51	3.43	2.23	94.34	16.48	6.58	2.61
CV	6.69	12.44	12.38	12.67	16.16	0.62	21.30	7.83	8.28	14.27	16.91	1.04	7.29	12.37	17.77

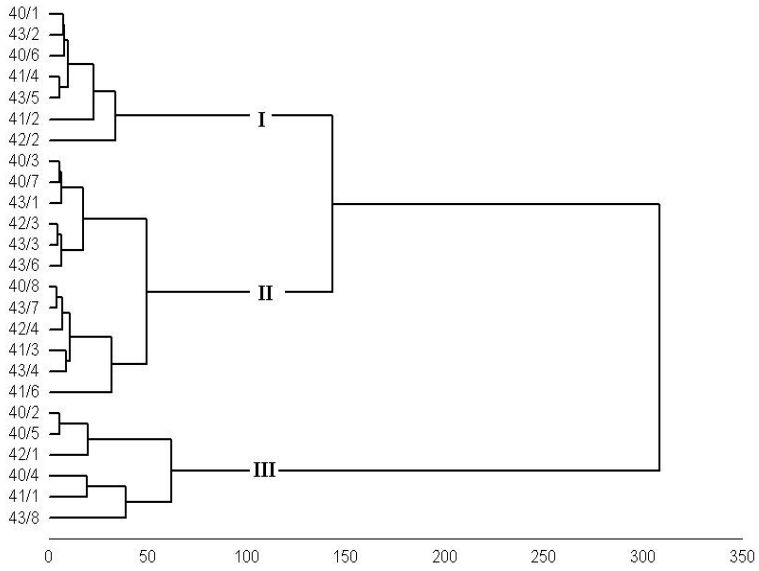

Figure. 1 Cluster dendrogram for 25 Prokupac clones

Principal component analysis (PCA) was used to identify the most significant variables and five principal components with Eigenvalues greater than 1 were isolated (table 2).

Table 2. Eigenvalues-proportion of total variability and correlation between the original variables and the first five principal components for Prokupac clones

Variable	PC1	PC2	PC3	PC4	PC5
Bunch length	0.277	-0.048	-0.344	0.489	-0.588
Bunch width	0.663	0.235	0.067	-0.256	-0.459
Number of berries per bunch	0.796	-0.116	-0.093	-0.202	0.015
Bunch weight	0.804	-0.195	-0.370	-0.097	0.030
% of stem	0.010	0.825	0.424	0.058	0.120
% of berry	-0.010	-0.825	-0.424	-0.058	-0.120
Number of seeds per berry	-0.363	-0.504	0.166	0.606	-0.035
Seed weight	-0.420	0.131	-0.617	-0.351	-0.022
Berry weight	-0.014	-0.227	-0.718	-0.074	0.569
% of skin	-0.449	0.768	-0.179	-0.213	-0.053
% of seed	-0.486	0.325	-0.491	0.305	-0.225
% of meet	0.544	-0.711	0.345	0.027	0.137
Sugar content	-0.635	-0.500	0.067	0.170	0.086
Total acid content	0.448	0.491	-0.121	0.512	0.397
Glicoacidometric index	-0.640	-0.487	0.318	-0.405	-0.131
Eigenvalue	3.852	3.720	1.931	1.452	1.166
% Variance	25.68	24.80	12.88	9.68	7.78
% Cumulative	25.68	50.48	63.36	73.04	80.82

The isolated components explained 80.82% of total number of variables examined. The obtained information is absolutely adequate considering the number of involved variables and purpose of the study. According to REIM & al. [26] this result indicates moderate genetic diversity between the clones and suggests evaluation of different morphological characteristics which is necessary for a meaningful characterization.

First principal component showed variation of 25.68% of variables which had high absolute values (over 0.70), especially for the traits such as number of berries per bunch and

bunch weight. The second principal component explained 24.80% of total number of variables and featured bunch structure (% of bunch stem and % of berry), % of berry skin and % of berry meet. Finally, principal component PC3 explained 12.88% of variables with berry weight being the most dominating trait (table 2). Principal components in this paper could reduce the number of properties to be studied in grapevine clone selection. Those properties are related to bunch and berry size and structure.

The obtained results are in accordance with those of LEÃO & al. [27], [28] who analyzed wine and table grapes by applying PCA method. He discovered that most variations were found in the first four components where the principal features were berry weight and bunch weight. High correlation between the size of berry and bunch (PC1 and PC2) in different grape germplasm collection was also established by FATAHI & al. [29] and MATTHEOU & al. [30] [31].

Table 3 shows the results of correlation between ampelographic traits of bunch and berry in tested clones. Positive correlation ($P<0.05$) was determined for the following parameters: bunch width and number of berries, bunch width and mass, percentage of bunch stem berry skin, seed mass and percentage of berry skin, percentage of berry skin and seeds in a berry, bunch length and number of berries per bunch with % of mesocarp in a berry. Negative correlation ($P<0.01$ and $P<0.05$) was determined between the bunch width and number of seeds, bunch weight and number of berries per bunch with sugar content, bunch weight and percentage of berry skin, percentage of berries in a bunch and percentage of berry skin, seed mass and percentage of berry mesocarp, bunch weight and glycoacidometric index. High positive correlation ($r=0.66$; $P<0.01$) was determined for sugar content and glycoacidometric index, but acid content and glycoacidometric index showed high negative correlation ($r=-0.081$; $P<0.01$). High negative correlation was also determined between percentage of berry skin and seeds in a berry ($r=-0.92$; $P<0.01$), and percentage of berry seeds and berry mesocarp ($r=-0.73$; $P<0.01$).

Table 3. Pearson correlation coefficients for 16 morphological traits evaluated in 25 Prokupac clones

	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	0.30	0.09	0.28	-0.15	0.15	0.11	0.01	-0.05	-0.14	0.12	0.05	-0.02	0.17	-0.34
2		0.48*	0.45*	0.21	-0.21	-0.41*	-0.16	-0.19	-0.06	-0.27	0.16	-0.52**	0.09	-0.34
3			0.74**	-0.10	0.10	-0.29	-0.23	0.05	-0.39	-0.30	0.42*	-0.43*	0.21	-0.32
4				-0.26	0.26	-0.25	-0.11	0.27	-0.40*	-0.28	0.42*	-0.40*	0.24	-0.45*
5					-1.00**	-0.26	-0.09	-0.29	0.48*	0.05	-0.38	-0.28	0.38	-0.26
6						0.26	0.09	0.29	-0.48*	-0.05	0.38	0.28	-0.38	0.26
7							-0.18	0.01	-0.37	0.17	0.21	0.54**	-0.19	0.32
8								0.34	0.40*	0.35	-0.44*	0.17	-0.18	0.16
9									-0.08	0.06	0.03	0.17	0.05	-0.08
10										0.40*	-0.92**	-0.12	0.10	-0.04
11											-0.73**	0.07	-0.01	-0.09
12												0.06	-0.07	0.07
13													-0.34	0.66**
14														-0.81**

1-bunch length; 2-bunch width; 3-number of berries per bunch; 4-bunch weight; Bunch structure: 5-% of bunch stem and 6-% of berry; 7-number of seeds per berry; 8-seed weight; 9-berry weight; Berry structure: 10-% of berry skin, 11- % of seed and 12-% of berry meet; 13-sugar content; 14-total acid content; 15-glicoacidometric index

4. Conclusion

The results of this study show considerable morphological and biological diversity among 25 selected Prokupac clones. Morphological characteristics of berries and bunches showed great variations in CV which values ranged from 0.62-21.30%. The highest variation was observed in the number of seeds per berry, % of berry in a bunch and berry meat. After cluster analysis, three clusters were determined: clusters I, II and III which included 7, 12 and 6 clones, respectively. This classification was mostly influenced by the bunch weight. Results of PCA suggested that 25 morphological and biological characteristics that were studied could be reduced to three main characteristics: bunch size, bunch and berry structure and berry size. Correlation analysis showed high positive correlation between sugar content and glycoacidometric index, but on the other hand, high negative correlation was determined between acid content and glycoacidometric index, percentage of berry skin and seeds in a berry, percentage of seeds in a berry and berry mesocarp. All clones can be recommended for production, whereby clones 40/4, 41/1 and 43/8 from third cluster which have large bunches.

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