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*Original scientific paper*

# THE CLUSTER ANALYSIS OF CLONES OBTAINED FROM AUTHOCHTHONOUS CULTIVAR KREACA (*Vitis vinifera L*)

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The purpose of this paper was to characterize the clones obtained from Kreaca, autochthonous grapevine cultivar of Banat. Based on examination of 6 important biological and technological properties, phenotypic and genetic divergence of 28 selected clones was established. The divergence was determined using ANOVA and hierarchical cluster analysis. Using variance analysis, for grape weight, yield, total acid content, sugar content and sugar/acid ratio very significant or significant differences were obtained between clones. The UPGA method was used and the Euclidean

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distance in order to determine the difference between the groups. Two clone groups were obtained on the dendrogram. The objective of clone differentiation was primarily cluster weight, although other properties were taken into account as well. As the most perspective clones for further investigation and production, that can be recommended, were clones 12/5/5, 56/11/7 and 69/11/7.

*Key words:* clone, cluster analysis, Kreaca, virus status

## INTRODUCTION

There are a large number of grapevine cultivars grown in Serbia, some of them are considered to be autochthonous. It is believed that Kraca is autochthonous cultivar of Banat region. In this area the most common synonyms of this cultivar are Kreazer and Banatski rizling. It has a moderate vigour, regular and high yield. The berry is egg-shaped, moderate size, with thin skin. The mesocarp is soft, juicy and colorless. Taste is neutral. Blending with cultivars such as Smederevka and Riesling Italico it gives a more complete wine.

Serbia is faced with rapid erosion of the autochthonous grapevine germplasm due to the introduction of the famous European cultivars. Unfortunately, clonal selection of these cultivars has never been done and there is no planting material of adequate quality standards.

As a first step in clonal selection, according to KONTIĆ *et al.* (2009) is mass positive selection. Clonal selection is considered to be a very important tool for grapevine genetic improvement. The best results are obtained when genetic and sanitary selection are carried out at the same time in order to propagate only clones naturally free from harmful viruses or made virus-free by artificial techniques. Clonal selection is based on genetic variability within cultivars. Possible explanations of this variability may be the polyclonal origin of cultivars and the accumulation of genetic mutations in their genotypes. On the other hand, virus diseases contribute to increase the phonotypical variability within grapevine populations (MANNINI, 2000).

The general purpose was to examine the main biological and technological properties of 28 clones *cv*. Kreaca and then by application of the ANOVA and cluster analysis to determine the level of genetic and phenotypic divergence. Besides establishing variability, the most promising clones are recommended for further investigation and spreading in production.

## MATERIALS AND METHODS

Mass positive selection as well sanitary selection of cv. Kreaca were carried out in grapevine plantations «Vrsac vineyards» in Gudurica. The 28 clones with a total area of 12 ha have been selected. The selection of clones was performed on the basis health and vitality of the vines, yield and quality of grapes. During the period 2006 – 2008 the next properties were examined: grape weight, yield per vine, *Botrytis* resistant, sugar and total acid content in must and sugar/total acid ratio. Average grape weight was calculated from the yield and total number of grape per

vine. The O. I. V. Code N°. 459 was used for assessment of the *Botrytis cinerea* resistance (O.I.V. 1983). On the bases of the appearance of symptoms on the grapes a resistance degree was determined. Evaluation was performed according to the following scale: 1 – 3 very small resistance; 5 medium resistance; 7 – 9 high and very high resistance

Sugar content was determined using refractometer (Atago Digital Brix Refractometer, PAL-1, 0-53%). Total acid content was determined by neutralisation with 0.1N NaOH. In order to detect the vines affected by severe virus diseases we provided ELISA (Enzyme Linked Immunosorbent Assay) screening. The presence of four economically important viruses: nepovirus grapevine fanleaf virus (GFLV), and tree clostero viruses: grapevine leafroll–associated virus 1 (GLRaV-1), grapevine leafroll–associated virus 2 (GLRaV-2) and grapevine leafroll–associated virus 3 (GLRaV-3) was tested.

The significance of differences was determined by the variance analysis. The monofactorial analysis of variance was used. A year is taken as a replication. According to these values components of variability were determined: variance of clone, variance of year and variance of error.

Taking into account all characteristics that had been analized using hierarchical cluster analyses phenotipical divergence between clones was determined. UPGA method was used, where there is a difference between the clones expressed through Eucladian distance.

## RESULTS AND DISCUSSION

The obtained results from ELISA test showed that all clones are virus free. As the presence Nepovirus Grapevine fanleaf virus (GFLV), and clostero viruses: Grapevine leafroll–associated virus 1 (GLRaV-1), Grapevine leafroll–associated virus 2 (GLRaV-2) and Grapevine leafroll–associated virus 3 (GLRaV-3) had not been detected, the manifested variability between clones was not under virus influence.

The evaluation of the Kreca germplasm showed a large variation in the all properties between the clones and between years (Table 1). The clone 46/10/7 had the smallest grape weight (93.2g) and sugar content (17.6%). The clone 9/2/8 had the smallest yield (1.9 kg/vine) while the clone 53/8/7 had the smallest total acid content (4.0g/l). The clone 12/5/5 had the largest grape weight (197.7 g), while the clone 29/10/7 had the largest yield (6.2 kg/vine). The clone 41/13/1 had the largest sugar content (22.6%) while the clone 69/11/7 had the largest acid content (6.1 g/l). The sugar/acid ratio was relatively high and varied from 3.6 to 5.6. Most of the clones had a moderate to a very high level of resistance to *Botrytis cinerea*. Only two clones had very small *Botrytis* resistance. These results were expected because the *Botrytis* resistance was one of the major criteria with the clonal selection. The largest grape weight, yield and total acid content and the smallest sugar content of the clones were in 2006. The smallest grape weight, yield and total acid content and the largest sugar content were obtained in 2008. The variability of important technological

characteristics of grapes were found by MARKOVIĆ *et al*. (2008) in the examined clones of autochthonous variety Prokupac and in Riesling Italico clones by NAKALAMIĆ *et al.* (1998).

*Table 1.- The average values of biological and techological properties of cultivar Kreaca clones*

| Clone | Grape weight (g) | Yield (kg/vine) | Sugar content (%) | Total acid content (g/l) | sugar/acid ratio | Level of *Botrytis* resistance * |
|---|---|---|---|---|---|---|
| 6/2/8 | 149.7 | 3.4 | 19.5 | 4.8 | 4.2 | 7 |
| 9/1/8 | 119.3 | 2.1 | 19.6 | 4.2 | 5.0 | 7 |
| 9/2/8 | 110.3 | 1.9 | 21.0 | 4.1 | 5.4 | 7 |
| 10/-10/3 | 127.3 | 5.4 | 19.6 | 4.9 | 4.2 | 3 |
| 12/5/5 | 197.7 | 3.9 | 19.7 | 4.6 | 4.3 | 7 |
| 18/5/2 | 124.0 | 5.8 | 19.1 | 5.3 | 3.8 | 5 |
| 21/8/8 | 176.7 | 3.6 | 19.8 | 4.9 | 4.4 | 9 |
| 23/-5/8 | 115.3 | 2.1 | 19.4 | 5.2 | 4.0 | 9 |
| 29/5/5 | 141.3 | 4.3 | 20.2 | 5.1 | 4.2 | 7 |
| 38/3/7 | 121.3 | 2.3 | 20.6 | 4.3 | 5.0 | 5 |
| 41/13/1 | 164.0 | 3.4 | 22.6 | 4.5 | 5.3 | 5 |
| 49/10/6 | 116.7 | 3.4 | 18.9 | 4.4 | 4.4 | 5 |
| 49/17/8 | 124.3 | 1.9 | 19.7 | 4.2 | 5.1 | 7 |
| 54/16/1 | 184.3 | 4.6 | 18.9 | 4.5 | 4.4 | 7 |
| 56/11/7 | 168.3 | 4.5 | 18.9 | 4.8 | 4.1 | 5 |
| 69/11/7 | 115.0 | 4.2 | 21.9 | 6.1 | 3.7 | 9 |
| 70/2/8 | 114.0 | 2.4 | 22.0 | 4.2 | 5.4 | 7 |
| 76/17/7 | 132.3 | 3.0 | 20.3 | 4.4 | 4.9 | 5 |
| 92/6/3 | 129.0 | 2.9 | 22.3 | 5.5 | 4.2 | 5 |
| 99/1/1 | 125.0 | 2.5 | 19.6 | 4.7 | 4.5 | 5 |
| 1/7/5 | 163.3 | 4.1 | 20.3 | 4.8 | 4.5 | 5 |
| 10/10/2 | 119.7 | 3.2 | 17.8 | 5.1 | 3.6 | 5 |
| 12/11/8 | 147.3 | 3.9 | 18.4 | 4.6 | 4.1 | 5 |
| 29/10/7 | 189.0 | 6.2 | 21.1 | 5.1 | 4.5 | 7 |
| 46/10/7 | 92.3 | 2.2 | 17.6 | 5.0 | 3.7 | 7 |
| 46/3/1 | 108.0 | 2.8 | 19.8 | 4.7 | 4.4 | 5 |
| 48/3/8 | 163.3 | 4.8 | 20.0 | 4.2 | 5.1 | 3 |
| 53/8/7 | 112.0 | 3.1 | 20.5 | 4.0 | 5.6 | 7 |
| 2006 | 120.0 | 3.7 | 18.7 | 6.1 | 3.1 | - |
| 2007 | 157.1 | 3.6 | 20.4 | 4.1 | 5.2 | - |
| 2008 | 135.5 | 3.1 | 20.8 | 4.0 | 5.2 | - |

*1 – 3 very small; 5 medium; 7 – 9 high and very high

Analysis of variance (Table 2) showed that the differences among clones for yield and total acid content were very significant ($P < 0.01$) and significant ($P < 0.05$) for grape weight and sugar/acid ratio. Also, for most properties, except yield, a very significant difference ($P < 0.01$) between years of study were established. These results are in agreement with the results STEFANNINI *et al.* (2000). They found significant differences between clones and between years for yield and total acid content as well as sugar content and grape weight only between years in Cabernet Sauvignon clones.

*Table 2. - F-values obtained in the ANOVA for the biological and techological properties of Kreaca clones*

| Variable | DF | MS | F-value | p-value |
|---|---|---|---|---|
| | | Grape weight | | |
| Clone | 27 | 2350.3 | 2.09 | 0.010 |
| Year | 2 | 9704.0 | 8.66 | 0.000 |
| Error | 54 | 1120.8 | - | - |
| | | Yield | | |
| Clone | 27 | 4.268 | 2.94 | 0.000 |
| Year | 2 | 3.038 | 2.09 | 0.133 |
| Error | 54 | 1.452 | - | - |
| | | Sugars content | | |
| Clone | 27 | 4.616 | 1.01 | 0.498 |
| Year | 2 | 32.872 | 7.20 | 0.002 |
| Error | 54 | 4.565 | - | - |
| | | Total acid content | | |
| Clone | 27 | 0.663 | 3.83 | 0.000 |
| Year | 2 | 39.73 | 229.4 | 0.000 |
| Error | 54 | 0.173 | - | - |
| | | Sugar/acid ratio | | |
| Clone | 27 | 0.955 | 1.95 | 0.018 |
| Year | 2 | 41.276 | 84.18 | 0.000 |
| Error | 54 | 0.490 | - | - |

Therefore, the manifested differences between clones are the result of differences in hereditary basis (genotype) on one side, and environmental factors on the other hand that are characteristic of quantitative properties. For more precise analysis the components of phenotypic variability was calculated and the results are shown in Figure 1.

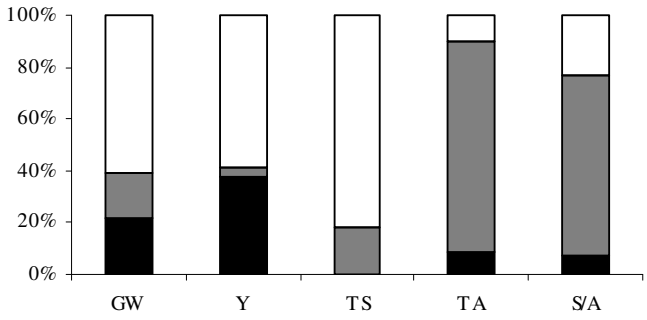


*Fig. 1. - Components of variability ($S^2_c$ – genetic variance, $S^2_y$ – variance of year; $S^2_e$ - variance of error) for grape weight (GW), yield (Y), total sugar content (TS), total acid content (TA) and sugar/acid ratio (S/A)*

In total phenotypic variance the moderate value of genetic variance had been established for grape weight (22%) and yield (38%), while for the remaining properties it was small (0-9%). Differences in years had produced the greatest impact on the total acid content (81%) and sugar/acid ratio (69%) variability. Variance of error was present the most in phenotypic variability for grape weight (61%), yield (59%) and total sugar content (82%).

Obtained results are partly in agreement with LEFORT and BRONNER (1981) results. They obtained that genetic variance participated with the greatest percentage in total variance for yield, while for sugar and acid content variance of year had the largest participation in phenotypic variance. High values of error variance in our work for grape weight, yield and total sugar content point significant clone x year interaction that it contained.

Hierarchical cluster analysis allowed the assessment of similarity or dissimilarity and clarified some of the relationships among Kreaca clones. Previously, cluster analysis had been used to evaluate divergence of clones (FORVEILLE *et al*. 1996; ROTARU and PETREA, 2006; SCALABRELLI *et al.* 2007) and grapevine cultivars (YUNCONG *et al*. 1995; ASENSIO *et al*. 2002; MARTINEZ *et al*. 2003; FRANCO-MORA *et al.* 2008; Also, multivariate analysis was applied to the diversity analysis of indiogenus genotypes from Serbia for Oblačinska sour cherry clones (NIKOLIĆ *et al.* 2005; RAKONJAC *et al.* 2010), vineyard peach genotypes (NIKOLIĆ *et al.* 2010) and cherry plum genotypes (NIKOLIĆ and RAKONJAC 2007). In our paper, based on mean value of all examined properties, by using UPGA procedure, dendrogram of the phenotypic differences of the examined clones was constructed (Figure2).

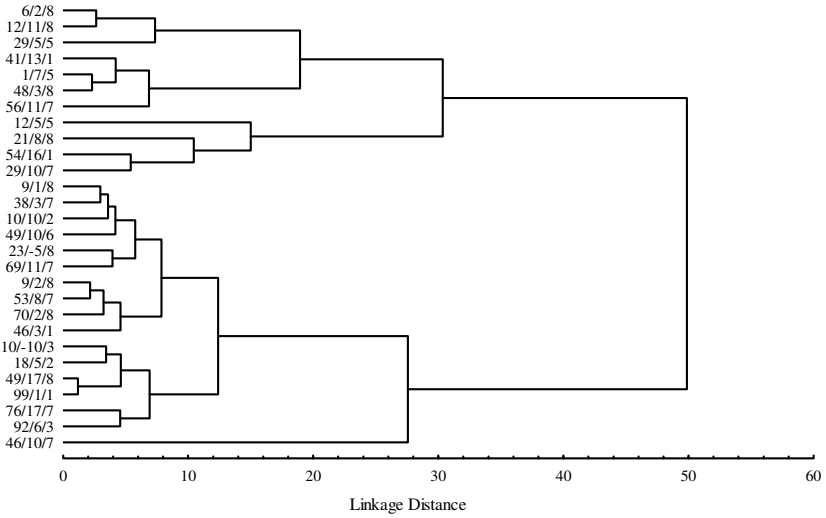


*Fig. 2. - Dendrogram of phenotypic differences of studied Kreaca clones by UPGA.*

Kreaca clones are connected in different ways, which show the existence of numerous hierarchical levels. Considering values of the Euclidean distance and grouping way, two groups of similar clones are separated. This low level of phenotypic divergence is likely to result as the clonal selection was performed only in one locality. Further work should be spread on the wider area of growing cultivar Kreaca.

Analysing examined properties on grouping clones, it can be concluded that the grape weight had the biggest influence. The first cluster includes 11 clones, which had medium to large grape weight, from 141.3g to 197.7g. The second cluster included 17 clones with smaller grape weight, ranging from 92.3g to 132.3 g.

The degree of genetic variability of the Kreaca clones obtained by ANOVA was high, contrary to the aggregation within dendrogram done at a low degree of differences.

Of all examined clones for further collecting and spreading in production clones 12/5/5, 56/11/7 and 69/11/7 can be recommended. Clones 12/5/5 and 56/11/7 are characterized by very high *Botrytis* resistance, relatively large grapes, high yield and slightly lower sugar and acid content. The clone 69/11/7 has a small grape weight, moderate yield, high sugar and total acid content and high *Botrytis* resistance.

## CONCLUSION

The study showed considerable genetic and phenotypical diversity among selected Kreaca clones. All clones were virus free on four tested viruses: Nepovirus Grapevine fanleaf virus (GFLV), and clostero viruses: Grapevine leafroll–associated virus 1(GLRaV-1), Grapevine leafroll–associated virus 2(GLRaV-2) and Grapevine leafroll–associated virus 3(GLRaV-3). Using hierarchical cluster analysis, two groups of similar clones are separated on the obtained dendrogram. The first group included 11 clones, and second 17 clones. The separation of the clones in groups was primary because of the grape weight, but other properties influenced the separation as well.Clones 12/5/5, 56/11/7 and 69/11/7 can be recommended for the collecting and spreading in production.

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# KLASTER ANALIZA KLONOVA AUTOHTONE SORTE KREACA (*Vitis vinifera L*)

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I z v o d

U periodu od 2006 do 2008. godine, u zasadima vinove loze »Vršački vinogradi« u Gudurici, izvršene su sanitarna i masovna pozitivna selekcija sorte vinove loze Kreaca. Sa ukupne površine od 12 ha, na osnovu šest značajnih bioloških i tehnoloških karakteristika izdvojeno je 28 klonova. Primenom ELISA testa kod svih ispitivanih klonova nije utvrđeno prisustvo sledećih ekonomski značajnih virusa: Nepovirus Grapevine fanleaf virus (GFLV), clostero virusa, Grapevine leafroll–associated virus 1(GLRaV-1), Grapevine leafroll–associated virus 2(GLRaV-2) i Grapevine leafroll–associated virus 3(GLRaV-3). Primenom ANOVA i hijerarhijske klaster analize određena je divergentnost klonova. Analizom varijanse ustanovljene su veoma značajne ili značajne razlike između klonova za masu grozda, prinos, sadržaj ukupnih kiselina, sadržaj šećera i odnos šećera i kiselina. Fenotipska divergentnost između klonova ustanovljena je primenom hijerarhijske klaster analize. Korišćen je UPGA metod, pri čemu je razlika između grupa izražena preko Euklideanovog rastojanja. Na dobijenom dendrogramu izdvojene su dve grupe srodnih klonova. Prvu grupu čine 11 a drugu 17 klonova. Kao najperspektivniji, za kolekcionisanje i dalje širenje u proizvodnji, mogu se preporučiti klonovi 12/5/5, 56/11/7 i 69/11/7.

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