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AMPELOGRAPHIC DESCRIPTION OF CLUSTER, BERRY AND SEED OF MERLOT CULTIVAR (*VITIS VINIFERA* L.) AND ITS SELECTED CLONES

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Abstract: During a four-year period, ampelographic experiments focusing on the berry cluster (average length of grape cluster, number of grape clusters per shoot, number of berries per grape cluster and length of peduncle), berry (length of berry and berry juice yield), length of pedicel and seed (length of berry seed) of Merlot cultivar (used as a relevant standard) and 11 clones (Nos. 022, 023, 025, 026, 027, 028, 029, 030, 031, 033 and 034) were performed in order to establish the differences among them. These experiments were actually conducted in the third phase of individual clonal selection of Merlot cultivar carried out in Serbia. The lengths of grape cluster and pedicel as well as berry must yields differed significantly among the examined clones. The cluster and principal component analyses classified 12 samples into three divergent clusters/groups, respectively. The clones belonging to the cluster II /the second group/ had significantly higher values of numbers of grape clusters per shoot and berries per grape cluster; lengths of peduncle and berry; berry must yield and length of pedicel, compared both to standard Merlot /the cluster I, the first group/ and the clones of the cluster III /the third group/. The phenological observations showed no significant differences in the beginnings and durations of phenological stages and vegetation period of the examined clones. The obtained results indicate the real need for further research work focused both on the agrobiological and technological properties of the grapes and wines aiming to better describe the selected clones.

Key words: Merlot, clone, length of grape cluster, length of berry, berry must yield.

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Introduction

Grapevine (*Vitis vinifera* L.) is one of the oldest agricultural crops (Vivier and Pretorius, 2002), native to Southern Europe and Western Asia. Today, it is cultivated in all temperate regions of the world. Considered as the most important fruit crop in the world, it plays an important role in the economy of many developed and emerging countries (Martínez-Esteso et al., 2013). Merlot is one of the most important grape cultivars in the wine industry. It is a French cultivar for the production of top quality red wines. It has been grown for ages in France (in the vicinity of Bordaux), Italy and other countries. It is believed that the quality of phenolic compounds (inherited from Cabernet Franc, its father) and early maturation coupled with fertility (inherited from Magdeleine Noire des Charentes, its mother) are the reasons for the rise and spread of this cultivar (Boursiquot et al., 2009). The multi-annual work on clonal selection in France has led to creating clones that are characterised by a series of positive characteristics important for the production of quality grapes and wines (Audeguin et al., 2000).

Merlot cultivar is very common in Serbia. Since it is adaptable to climatic conditions in the country, it is grown on more than 23% of vineyard areas (Statistical Yearbook of the Republic of Serbia, 2014). Merlot is a heterogeneous cultivar with several varieties that have not been studied sufficiently in Serbia. The vine of this cultivar has medium vigor of shoot growth. In terms of morphology and functionality, the flower is hermaphrodite. The berry is medium-sized, dark blue with a prominent stalk. The cluster of grapes is pyramidal or pyramidally cylindrical with one or two wings, medium compact, with an average mass of 100–130 g. It is a cultivar with high yields (5,000–10,000 kg ha^{-1}). Grapes mature after the 20^{th} of September (III epoch). Berry juice yield is around 60%, while must contains 20–24% of sugar and 7–9% of acids. Wine obtained from this cultivar is potable and harmonic containing 12–14% of alcohol. This wine is easily blended with other red wines. It is highly valued in the international market (Žunić and Garić, 2010).

The aim of this research was to examine some of the most important ampelographic characteristics of berry cluster, berry and seed of 11 clones (Nos. 022, 023, 025, 026, 027, 028, 029, 030, 031, 033 and 034) of Merlot cultivar (using International Organisation of Vine and Wine /O.I.V./ list for a description of cultivars) in order to establish the differences among them (Benz et al., 2006; 2007). In addition, the health condition of examined varieties versus the most important pathogens (*Botrytis cinerea*, *Plasmopara viticola* and *Uncinula necator*) and chlorosis as well as the phenological phases in the annual cycle of the vine development were evaluated. This study may facilitate the selection of the best clones to be entered into the last phase of multi-annual clonal selection.

Material and Methods

Experimental site

This research was carried out in the experimental field 'Radmilovac' that belongs to the Faculty of Agriculture of the University of Belgrade. The site is located at 44º 45' north latitude and 20° 34' east longitude, and at an altitude of 153 m. The locality is situated in the area of Šumadija and Velika Morava, in a subregion of Belgrade. The terrain exposition is southwest, while the rows are directed toward southeast-southwest. The training system was high double asymmetric cordon, with the tree height of 80 cm. Both the standard and clones were grafted using 'tongue grafting' on the rootstock Kober 5 BB. Planting distance was 3.0 m (between the lines) × 1.0 m (between the vines in a row). Winter cutting was the same for all clones: it consisted of leaving two canes with 10 buds each and two offshoots with two buds per vine. The total loading was 24 buds per vine. For the needs of the ampelographic description, each clone was represented by 10 vines of equal fertility and exuberance.

Plant material

Merlot cultivar (standard) and its clones (Nos. 022, 023, 025, 026, 027, 028, 029, 030, 031, 033 and 034) were examined. The ampelographic description of clusters, berries and seeds of standard Merlot and aforementioned clones was carried out according to the methodology of the 'Codes des Caractères Descriptifs des Variétés et Espèces de Vitis' (1983). The grape clusters, berries and seeds were described during picking. At least 10 clusters of grapes with 10 different vines were used for the description during full maturity; at least 100 berries were taken from 10 grape clusters and 10 different vines were used for the description at the same time; at least 100 seeds from 10 clusters and 10 different vines were used for the description at the aforementioned time for evaluation of mechanical contents of the grape cluster and berry. Phenological observations were carried out during a four-year research period using the descriptors of the O.I.V. (1983). All measurements were done in five replicates. The following indicators of descriptive statistics were calculated for all analysed characteristics of the tested varieties: mean, standard error of arithmetic mean and coefficient of variation. The analyses of the experimental data including the evaluation of their significance were accomplished by ANOVA and LSD test with a significance threshold of 5 and 1% (Hadživuković, 1977).

The dates of the following phenological phases in the annual cycle of development of the vine were observed and recorded: activation of buds beginning with the first single opening buds, over 50% of open mesh by vegetation; the beginning of flowering is when about 3–5% of ousted flowers caps, while the end

of flowering is when over 80–90% of flowers in inflorescences are opened; the veraison begins when the first berries soften getting a varietal characteristic colour; the full maturity of grapes was determined visually, based on their organoleptic and technological properties. The obtained data are used for the calculation of the average date of occurrence and duration of particular phenophases of standard Merlot and its varieties.

Soil characteristics

The type of soil of the experimental plot was a brown forest soil, with a good air capacity. Physical and chemical analyses of soil were conducted in the laboratory of the Faculty of Agriculture (University of Belgrade). The soil samples were taken at four depth levels: 30, 60, 90 and 120 cm. Chemical characteristics of soil pointed out a relatively well secured soil, provided with all elements necessary for the successful growth of the vine, neutral reaction of soil, whereas its pH slightly decreased with depth.

Results and Discussion

The clonal selection includes the examination of clones of a cultivar regarding agrobiological and enological characteristics, health status and varietal identity. The clones with best characteristics are then selected, aiming to maintain variation within the cultivar (Atak et al., 2014). Three international organizations, namely *Herbal and Genetic Resources Council* (IBPGR), *New Genetic Resources Protection Association* (UPOV) and *Vine and Wine International Office* (O.I.V.), have adopted a common set of methods for the description and differentiation of vine cultivars consisting of 128 characteristics/features.

The grape clusters were described according to the number of grape clusters per shoot (Code 201), length of grape cluster (Code 203), number of berries per grape cluster (Code 205) and length of peduncle (Code 206) (O.I.V., 1983). The indicators of descriptive statistics (average values) of the examined ampelographic characteristics of the grape cluster are presented in Table 1.

In terms of the number of clusters per shoot (Code 201), the examined clones were graded 2. The lowest and highest values were recorded for the standard Merlot (1.25) and the clone No. 022 (1.37), respectively. The examined sample material showed homogeneity and low variability (Cv = 3.08%). The outlined differences between the clones and standard were statistically significant ($p<0.01$).

The clones had short grape clusters (Code 203, grade 3). The lowest average lengths of grape clusters were recorded for the clones Nos. 026 and 027 (13.45 and 13.46 cm, respectively), while the highest one was found for the clone No. 025 (14.92 cm). However, there is no statistical significance among a large number of

the examined clones. Significant differences were defined between the clone No. 025 and all other clones (p<0.01), excluding the clones Nos. 029, 031 and 033.

Table 1. The ampelographic characteristics of grape cluster.

Clone No.	No. of grape clusters per shoot 201*	Length of grape cluster (cm) 203	No. of berries per grape cluster 205	Length of peduncle (cm) 206
	$\bar{x} \pm S_{\bar{x}}$			
Standard	1.25^{c}±0.024	13.47^{b}±0.347	74.81^{b}±7.137	3.88^{i}±0.021
022	1.37^{a}±0.020	13.90^{b}±0.305	88.40^{ab}±9.010	3.93^{i}±0.009
023	1.34^{ab}±0.009	13.88^{b}±0.606	74.66^{b}±9.169	4.10^{g}±0.011
025	1.34^{ab}±0.014	14.92^{a}±0.251	89.65^{a}±5.764	4.23^{f}±0.015
026	1.32^{ab}±0.011	13.45^{b}±0.206	83.10^{ab}±3.952	4.34^{e}±0.015
027	1.32^{ab}±0.005	13.46^{b}±0.325	80.00^{ab}±3.310	4.48^{d}±0.013
028	1.31^{ab}±0.007	13.90^{b}±0.191	93.54^{a}±3.285	4.61^{b}±0.015
029	1.34^{ab}±0.010	14.43^{a}±0.195	88.60^{ab}±3.118	4.75^{a}±0.012
030	1.34^{ab}±0.005	13.74^{b}±0.310	84.19^{ab}±3.318	4.55^{c}±0.008
031	1.29^{bc}±0.007	14.71^{a}±0.277	81.49^{ab}±2.434	4.01^{h}±0.015
033	1.32^{ab}±0.007	14.54^{a}±0.326	86.51^{ab}±2.445	3.81^{j}±0.013
034	1.28^{bc}±0.010	13.87^{b}±0.239	83.39^{ab}±2.639	3.74^{k}±0.020
Cv (%)	3.08	5.73	14.16	7.89
LSD p=0.05	0.034	0.877	14.456	0.011
p=0.01	0.047	1.154	19.029	0.054

Cv = Coefficient of variation; Different letters indicate a statistical difference. *Code No. (O. I. V.).

The differences in values for the number of berries per grape cluster (Codes 205) among a large number of analysed clones were not significantly different (p>0.05). The significant difference was found between clones No. 028 and No. 023 (93.54 and 74.66 berries per cluster, respectively), which is in line with the findings of Benz et al. (2006). All the clones had a small number of berries per cluster of grapes (Code 205, grade 3). Furthermore, the values for the length of peduncle indicated statistically significant differences between the examined clones (p<0.01), excluding standard Merlot and the clone No. 022 (no significant difference, p>0.05). The highest/lowest values were recorded for the clones Nos. 029 (4.75 cm) and 034 (3.74 cm), respectively. In addition, the examined clones had short-length peduncles (Code 206, grade 3).

The berry characteristics may influence its chemical composition (Roby et al., 2004). Grape seed contains high amounts of polyphenols, especially flavan-3-ol monomers and proanthocyanidins, which in great part contribute to bitterness and astringency of red wines (Kennedy et al., 2000). The mean values of the examined ampelographic characteristics of berries: length of berry, berry must yield, length of pedicel and length of berry seed (Codes 221, 233, 238 and 242, respectively) (O.I.V., 1983) are shown in Table 2.

Table 2. The ampelographic characteristics of berry and seed.

Clone No.	Length of berry (mm) 221*	Berry must yield (mL) 233	Length of pedicel (mm) 238	Length of berry seed (mm) 242
	$\bar{x} \pm S_{\bar{x}}$			
Standard	10.42^{b}±2.211	53.29^{k}±0.081	6.21^{h}±0.016	6.29^{bc}±0.010
022	12.84^{a}±0.026	56.49^{h}±0.062	6.76^{g}±0.023	6.15^{cd}±0.012
023	12.96^{a}±0.026	58.74^{e}±0.045	6.94^{f}±0.029	5.77^{ef}±0.116
025	12.95^{a}±0.078	60.19^{c}±0.062	7.12^{e}±0.025	5.64^{f}±0.032
026	12.91^{a}±0.052	62.48^{a}±0.057	7.42^{d}±0.026	6.34^{bc}±0.031
027	13.00^{a}±0.053	61.33^{b}±0.044	7.74^{bc}±0.032	6.26^{bc}±0.034
028	12.93^{a}±0.071	59.22^{d}±0.042	7.91^{b}±0.024	5.87^{def}±0.030
029	12.88^{a}±0.076	57.65^{g}±0.054	8.15^{a}±0.097	6.03^{cde}±0.034
030	12.90^{a}±0.043	55.19^{i}±0.048	7.61^{c}±0.039	6.33^{bc}±0.030
031	12.92^{a}±0.096	53.77^{j}±0.055	7.77^{bc}±0.023	6.57^{ab}±0.030
033	12.90^{a}±0.059	58.35^{f}±0.049	7.89^{b}±0.035	6.72^{a}±0.034
034	12.90^{a}±0.062	59.17^{d}±0.046	7.66^{c}±0.037	6.63^{ab}±0.221
Cv (%)	11.55	4.76	7.45	5.86
LSD p=0.05	1.170	0.156	0.112	0.218
p=0.01	2.339	0.208	0.149	0.291

Cv = Coefficient of variation; Different letters indicate a statistical difference. *Code No. (O. I. V.).

The average length of the berry (Code 221) of the examined varieties ranged from 10.42 mm (standard Merlot) to 13.00 mm (No. 027). In terms of the examined property, differences between the varieties were quite small (p>0.05), except for standard Merlot. All the clones had small berries (Code 221, grade 3).

The average values of berry must yield (Code 233) ranged from 53.29 (standard Merlot) to 62.48 mL (No. 026). The variability of the samples was small (Cv = 4.76%) confirming the homogeneity of the experimental material and allowing the use of parametric tests. On the other hand, the differences of berry must yields were highly significant between all the clones (p<0.01). Some clones (Nos. 025, 026 and 027) had a high berry must yield (Code 233, grade 7), while the others had a medium berry must yield (Code 233, grade 5).

In terms of the length of pedicel (Code 238), standard Merlot and the clone No. 029 recorded the lowest (6.21 mm) and highest (8.15 mm) values, respectively. Such results actually indicated the existence of a larger amplitude variation of this ampelographic characteristic between the clones (with a significant difference, p<0.01). The lack of a significant difference for this parameter was noticed between the varieties Nos. 027, 028, 031 and 033 (p>0.05), while the difference among the other examined clones was highly significant (Table 2). The examined clones had short pedicels (Code 238, grade 3).

All the clones had medium long berry seeds (Code 242, grade 5). The maximum and minimum values were observed for the clones Nos. 033 (6.72 mm)

and 025 (5.64 mm), respectively. The statistical difference was found (p<0.01) among some of the examined clones. However, no significant difference was found between the clones Nos. 031, 033 and 034; the clones Nos. 026, 027, 030, 031 and 032 in comparison with standard Merlot; the clones Nos. 022, 026, 027, 028, 029 and 030 in comparison with standard Merlot. The description of seeds herein was in good agreement with the literature data (Cindrić et al., 1994; Vujović, 1997).

In terms of the examined ampelographic characteristics, the clones differed both from standard Merlot and among themselves. This was observed by Hierarchical Clustering Analysis (HCA) and multivariate data analysis using the method Principal Component Analysis (PCA) utilising all the relevant data. In the dendrogram of dissimilarity (1.25), three clusters of the samples were clearly distinguished: the cluster I (including standard Merlot only), different from all the examined varieties; the cluster II (including the varieties Nos. 22, 23, 25, 26, 27, 28, 29 and 30); the cluster III (including the varieties Nos. 31, 32 and 33) (Figure 1).

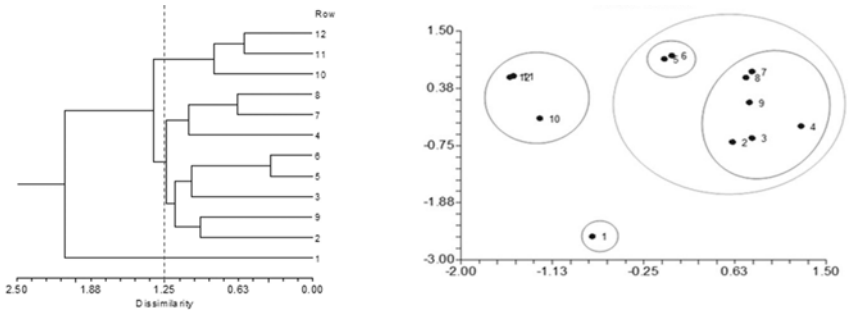

Figure 1. HCA and PCA diagrams of ampelographic characteristics of standard Merlot and the examined clones.

PCA analysis gave the same distribution of the clones within three groups: the first group (standard Merlot); the second group (the varieties Nos. 22, 23, 25, 26, 27, 28, 29 and 30); the third group (the varieties Nos. 31, 32 and 33). The greatest influences on the disjunction of the sample groups were as follows: the first group – length of grape cluster; the second group – numbers of grape clusters and berries per shoot and grape cluster, respectively, lengths of peduncle and berry, berry juice yield and length of pedicel; the third group – lengths of pedicel and berry seed, respectively (Figure 1). The clones belonging to the cluster II or the second group respectively had significantly higher values of the aforementioned parameters compared to both standard Merlot (the cluster I, the first group) and the clones belonging to the cluster III (the third group).

The health of the examined clones was visually estimated in terms of the most important pathogens (*Plasmopara viticola, Uncinula necator* and *Botrytis cinerea*) (Bovey et al., 1980) and chlorosis. Merlot clones were very highly resistant to chlorosis (Code 401, grade 9). In terms of disease resistance, the grape clusters were: highly resistant to *P. viticola* (Code 453, grade 7) and *U. necator* (Code 456, grade 7); medium resistant to *B. cinerea* (Code 459, grade 5) (Table 3). These results were in accordance with the previous evaluation data described in the literature (Blaich, 1990; Cindrić et al., 1994).

Table 3. Resistance of clones to chlorosis and the most common diseases.

Clone No.	Chlorosis 401*	*Plasmopara viticola* 453	*Uncinula necator* 456	*Botrytis cinerea* 459
Standard	9	7	5	7
022	9	7	5	7
023	9	7	5	7
025	9	7	5	7
026	9	7	5	7
027	9	7	5	7
028	9	7	5	7
029	9	7	5	7
030	9	7	5	7
031	9	7	5	7
033	9	7	5	7
034	9	7	5	7

*Code No. (O. I. V.).

The phenophases (bud opening, flowering, growth of berries, grape maturation, full maturity and falling of leaves) as well as the starting and ending dates of each phenophase were clearly observed. The following results on phenological characteristics of standard Merlot and clones were obtained: average duration of the development of buds in the examined clones ranged from the 24th (No. 022) to the 29th of April (No. 031); of full flowering, from the 28th (No. 022) to the 30th of May (No. 026); of the beginning of ripening of berry (ripening phenophase), from the 20th (No. 023) to the 23th of July (No. 028); of full maturity of berry, from the 18th (Merlot standard) to the 21st of September (No. 026). Before the falling of leaves, shoots matured and converted in brown-yellowish color. This phase takes, on the average, 60–70 days. The beginning of shoot maturation for the studied clones was observed in the mid of July and lasted until the end of September. The average duration of phenophases was almost identical in all the examined Merlot clones. From the beginning of bud activation to the beginning of flowering 32–36 days passed on an average; from the beginning to the end of flowering, 14–18 days; from the ending of flowering phase to ripening phase, 52–56 days; from the ripening phase to the grape picking phase, 59–60 days passed.

Based on phenological observations during a four-year study, no significant differences were recorded in the beginning and duration of individual phenophases. Phenological characteristics of Merlot clones were in accordance with the descriptions of phenological characteristics of the cultivar population (Avramov, 1995; Cindrić et al., 1994; Vujović, 1997).

Conclusion

Clonal selection is one of the tools used for grapevine improvement. It is important to characterise the variation and identify superior clones for future propagation. Once such clones have been characterised, they should be registered and propagated for commercial production. In terms of the screened ampelographic characteristics, the examined clones differed both from standard Merlot and among themselves. This was especially true for the lengths of grape cluster stalk and pedicel as well as for berry must yield. HCA and PCA analyses classified 12 samples into three divergent clusters/groups, respectively. The clones belonging to the cluster II /the second group/ had significantly higher values of numbers of grape clusters per shoot and berries per grape cluster; lengths of grape cluster stalk and berry; berry must yield and length of pedicel, compared to standard Merlot cluster I /the first group/ and the clones of the cluster III /the third group/. Resistance to *Plasmopara viticola* and *Uncinula necator* of the examined clones was high, while resistance to *Botrytis cinerea* was medium. Finally, no significant differences in the duration of individual phenophases and vegetation period of the examined clones of Merlot cultivar were observed. The elapsed time from the moment when winter buds were activated to the moment of grape harvest was 142–148 days in total.

The obtained experimental data indicate that further research work needs to be focused both on the agrobiological and technological properties of the grapes and wines aiming to better understand characteristics of the selected clones (among the best ones) to be entered into the last phase of clonal selection.

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AMPELOGRAFSKI OPIS GROZDA, BOBICE I SEMENKE SORTE MERLO (*VITIS VINIFERA* L.) I ODABRANIH KLONOVA

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R e z i m e

Tokom četvorogodišnjeg perioda urađen je ampelografski opis grozda, bobice i semenke jedanaest klonova (022, 023, 025, 026, 027, 028, 029, 030, 031, 033 i 034) i sorte Merlo (kao standarda) u cilju utvrđivanja razlike među njima. Datim ispitivanjem (sprovedenim u Srbiji) obuvaćene su sledeće karakteristike: broj grozdova po izdanku, dužina grozda, broj bobica po grozdu, dužina stabljike grozda, dužina bobice, randman soka bobice, dužina peteljčice bobica i dužina semenke. Eksperimenti su urađeni u toku treće faze individualne klonske selekcije sorte Merlo. Proučavani klonovi značajno su se razlikovali među sobom, a posebno prema dužini stabljike grozda, randmanu soka bobice i dužini peteljčice bobice. Dobijeni rezultati obrađeni su hijerarhijskom klasterskom analizom (HCA) i multivarijantnom analizom podataka (pomoću analize glavnih komponenata – PCA). Shodno tome, dati uzorci (standard i varijeteti) svrstani su u tri klastera (HCA), odnosno grupe (PCA). Klonovi obuhvaćeni klasterom II /druga grupa/ odlikovali su se značajno većim vrednostima broja grozdova po izdanku, broja bobica po grozdu, dužine stabljike grozda, dužine bobice, randmana soka bobice i dužine peteljčice bobice kako u odnosu na Merlo standard, tako i u odnosu na klonove klastera III /treća grupa/. Kao takvi, mogu biti obuhvaćeni daljim proučavanjima u okviru klonske selekcije. Sa fenološkog aspekta, pak, među datim klonovima nisu uočene značajnije razlike u početku i trajanju pojedinačnih razvojnih fenofaza, kao i u dužini vegetacionog perioda.

Ključne reči: Merlo, klon, dužina grozda, dužina bobice, randman soka bobice.

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