AGROBIOLOGICAL AND WINE QUALITY TRAITS OF *VITIS VINIFERA* cv. MERLOT CLONES SELECTED IN SERBIA

Dragan S. VUJOVIĆ[1], Dragoljub M. ŽUNIĆ[1], Blaženka S. POPOVIĆ[1], Milica M. PANTELIĆ[2] and Jelena B. POPOVIĆ-DJORDJEVIĆ[1*]

1 : Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia
2 : Innovation Center, Faculty of Chemistry Ltd, University of Belgrade, 11158 Belgrade, Serbia

Abstract

Aims: The selection of cv. Merlot clones performed in the Grocka winegrowing region, Republic of Serbia, and the study of agrobiological and technological traits of three selected clones and Merlot standard.

Methods and results: The selection of clones was done using the method of individual clone selection in four phases over 15-20 years (Cindrić, 2003). O.I.V. methods were used in determining the most important agrobiological and economic-technological traits of grapes. The results of studied traits of grape and wine suggest that the quality of the clones surpassed the quality of the standard. Although the highest values for most of the studied traits were obtained for clone 025, there was no statistically significant difference between the clones. Indeed, the significant distinction between the clones was obtained for total polyphenol content (TPC) and total anthocyanin content (TAC) in wines: the wine of clone 025 had the highest TPC and TAC values. According to the tasting scores, wines of the clones may be classified as « quality wines with geographical indications ».

Conclusion: The selected clones were characterised by good quality grapes and wines. Improved viticultural parameters, versus the mother vine, indicate the need for further research work on the chemistry of both grapes and wines, aiming to better understand the characteristics of the selected clones and their market potential.

Significance and impact of the study: The development of new clones (namely 022, 025 and 029) of better quality than the mother vine will, in the long term, lead to the introduction of the best clone(s) in viticultural practices and production. Until today, the clonal selection of Merlot has not been done in the Republic of Serbia and there are no recognised clones.

Key words: Merlot, clones, grape composition, phenols, anthocyanins

Résumé

Objectifs: La sélection de clones de la variété Merlot réalisée dans un vignoble de Grocka, République de Serbie, et l'étude des caractéristiques agrobiologiques et technologiques de trois clones ainsi qu'un standard Merlot.

Méthodes et résultats: Les clones ont été sélectionnés par une méthode de la sélection clonique en quatre phases pendant une période de 15-20 ans (Cindrić, 2003). Pour déterminer les caractéristiques agrobiologiques et économiques-technologiques du raisin les plus importantes, les méthodes O.I.V. ont été appliquées. Les résultats des caractéristiques étudiées du raisin et du vin ont montré que la qualité des clones a surpassé la qualité du standard. Bien que les valeurs les plus élevées pour la plupart des caractéristiques étudiées ont été obtenues pour le clone 025, il n'y avait pas de différence statistiquement significative entre les clones. En effet, la distinction significative entre les clones a été obtenue pour la teneur totale en polyphénols (TPC) et la teneur en anthocyanes totale (TAC) dans les vins: le vin du clone 025 avait la plus forte TPC et TAC. D'après les notes de dégustation, les vins de clones ont été caractérisés comme des « vins de qualité à indication géographique ».

Conclusion: Les clones sélectionnés montrent une bonne qualité du raisin et du vin. L'amélioration des paramètres viticoles, par rapport à la vigne mère, montre la réelle nécessité de poursuivre les travaux de recherche sur la chimie des raisins et des vins afin de mieux comprendre les caractéristiques des clones sélectionnés et leur potentiel de marché.

Signification et impact de l'étude: Le développement de nouveaux clones (soit 022, 025 et 029) de meilleure qualité que la vigne mère va mener, à plus long terme, à l'introduction d(u)es meilleur(s) clone(s) dans les pratiques viticoles et la production. Jusqu'à aujourd'hui, la sélection clonale de la variété Merlot n'a pas été faite en République de Serbie et il n'y a pas de clones reconnus.

Mots clés: Merlot, clones, composition du raisin, phénols, anthocyanes

manuscript received 2nd March 2015 - revised manuscript received 23rd October 2015

*Corresponding author: jelenadj@agrif.bg.ac.rs

INTRODUCTION

Vitis vinifera is one of the oldest agricultural crops. It includes a large number of varieties of different use values (Vivier and Pretorius, 2002) and only a few hundred varieties are used for the commercial production of wine (Pelsy *et al.*, 2010). Full reproduction, vegetative reproduction and somatic mutations are the most important processes for the development of grape varieties (This *et al.*, 2006). The best results have been achieved in individual clonal selections from single, best vines (Ivanišević *et al.*, 2012). This method eliminates the negative influence of mutations and prevents the reproduction of virus-infected vines (Ruhl *et al.*, 2004). Old vine varieties are not genetically homogeneous. All plants originated from the same mother plant represent clones (Keller, 2010). Clonal selection in viticulture began in the late 1950s (van Leeuwen *et al.*, 2013). The initial aim of clonal selection was the creation of a virus-free population from healthy mother plants (Lacombe *et al.*, 2004). Later on, other criteria for the selection of clones were introduced, such as yield, sugar content in grapes, and the content of polyphenolic compounds and sensory characteristics of wine (van Leeuwen *et al.*, 2013).

Merlot is one of the most widespread grape varieties in red wine production. It is believed that the quality of the phenolic compounds, inherited from Cabernet Franc (father), and the early maturation and fertility, inherited from Magdeleine Noire des Charentes (mother), are the reasons for the rise and spread of this variety (Boursiquot *et al.*, 2009). The multi-annual work on clonal selection in France led to the creation of clones characterised by a series of positive characteristics important for the production of quality grapes and wines (Audeguin *et al.*, 2000).

The Merlot variety is very common in Serbia. However, the Merlot plantations have a heterogeneous population (Avramov *et al.*, 1996a). Therefore, the clonal selection of this variety is very important for obtaining clones with better characteristics than the variety population. It is also necessary to create a basic planting material from parent vines of selected clones. To date, there are no recognised Merlot clones in the Republic of Serbia.

The aim of the study was to examine and compare, over a five-year period, the agrobiological traits of grapes and the quality traits of wines obtained from new Merlot clones.

MATERIALS AND METHODS

1. Selection of the clones

The selection of clones was done using the method of individual clonal selection in four phases (lasting 15-20 years) (Cindrić, 2003). The first phase of the selection process started with thirty marked stem vines and included the study of agrobiological characteristics of grapes (yield, cluster weight, sugar content and total acids). On the basis of the obtained results, fourteen vines (potential clones) were selected. The next phase of the selection process was carried out in the « Radmilovac » experimental field of the Faculty of Agriculture in Belgrade. The site is located at 44° 45’ north latitude and 20° 35’ east longitude, at an altitude of 153 m above sea level. The study of the agrobiological properties of the fourteen vines (selected in the first phase) pointed out eleven « best » vines (Avramov *et al.*, 1996a). In the third phase of the selection process, these vines were planted on the above mentioned site. The clones were visually inspected for common symptoms of the most important pathogens (*Plasmopara viticola*, *Uncinula necator* and *Botrytis Cinerea*) and chlorosis. Merlot clones were very resistant to chlorosis (Code 401 - grade 9). In terms of disease resistance, they were **a)** very sensitive to *P. viticola* (Code 451 - grade 1), with leaves medium/highly resistant to *P. viticola* (Code 452 - grade 5/7) and clusters resistant to *P. viticola* (Code 453 - grade 7) ; **b)** very sensitive to *U. necator* (Code 454 - grade 1), with leaves resistant to *U. necator* (Code 455 - grade 7) and clusters resistant to *U. necator* (Code 456 - grade 7) ; and **c)** very sensitive to *B. cinerea* (Code 457 - grade 1), with leaves medium resistant to *B. cinerea* (Code 458 - grade 5) and clusters medium resistant to *B. cinerea* (Code 459 - grade 5). On the basis of the results gathered during the multi-annual period of studying agrobiological and technological characteristics (Avramov *et al.*, 1996b ; Vujović, 2003), three « best » clones stood out : 022, 025 and 029.

Before the beginning of the fourth phase of clonal selection, Merlot standard and selected clones were tested (in Bari, Italy) for Arabis mosaic virus (ArMV), grapevine fanleaf virus (GFLV), grapevine leafroll-associated virus 1 (GLRaV-1), grapevine flavescence dorée phytoplasma and grapevine bois noir. ArMV, GFLV and GLRaV-1 were tested using serological ELISA test and grapevine flavescence dorée phytoplasma and grapevine bois noir using molecular PCR test (OEPP/EPPO, 2008). Only virus-free vines were selected for further examination.

2. Experimental plot

In the fourth phase of the selection process, the experimental plot was planted with Merlot variety (standard) and three clones (022, 025 and 029). Both standard and clones were grafted using « tongue grafting » on the Kober 5BB rootstock. Each clone and standard were represented with 50 vines. The field experiment was set up according to the experimental scheme « random block system » in five replicates on the brown forest soil type (« Radmilovac » locality - Grocka winegrowing region). Planting distance was 3.0 m (between the rows) x 1.0 m (between the vines in a row). The training system was « high double asymmetric cordon ». Pruning was done uniformly leaving 20 buds per vine for all tested clones and standard.

3. Climatic conditions

Air temperature (at a height of 1.5 m above the ground) and rainfall were monitored at the automatic weather station Radmilovac-Vinča (Belgrade, Serbia) located less than 1.0 km from the experimental site.

4. Agrobiological traits of grapes

International Organisation of Vine and Wine (O.I.V.) methods were used in determining the most important agrobiological and economic-technological traits of grapes. The analysed wines were produced using microvinification techniques. Grape yield, berry juice yield, berry weight, cluster weight and cluster structural indicator of Merlot standard and clones 022, 025 and 029 were examined in the period 2009-2013. The chemical composition of must (sugar content and total acids) and the quality of the wine through chemical composition (content of ethyl alcohol, total extract), color and wine tasting were studied as well. Each measurement was done in five replicates. In the last year of study, the content of phenols and anthocyanins was determined. Each measurement was done in three replicates. The harvest of both standard and clones was performed on the same day at the stage of full maturity. The sample for the determination of cluster weight and cluster structural indicator consisted of 10 clusters; the sample for the determination of berry weight consisted of 100 berries. Berry juice yield was measured on a 100-g berry sample without petioles. Berries were crushed in a blender (3000 rpm) and then the quantity of must was measured. Must sugar content (%) was measured using a digital refractometer (Pocket PAL-1, Atago, Japan). Total acid content (g/L) was determined by the neutralization method using n/10 NaOH solution.

5. Wine quality traits

Immediately after harvest, the grapes were processed in laboratory conditions, using microvinification technique. For the purpose of microvinification, 20 kg of grapes, both standard and clones, were used. Crushing was done manually using a grape crusher with rollers and supplement for stem removal (for separating the grapes from the stems). The must was supplemented with 100 mg/L potassium metabisulfite. The spontaneous fermentation took place in 10-L glass bottles at 20-25 °C. Upon completion of the fermentation, the wine was decanted from the lees, bottled and stored at 5-6 °C until chemical analysis and tasting. After two months of bottle aging, classic parameters of wines of standard and clones were analysed following O.I.V. standards (O.I.V., 2006). Ethyl alcohol was determined with a pycnometer in a distillate obtained by the distillation of a certain volume of wine. Wine total extract was determined by a specific densiometric method using a 50-mL pycnometer. Wine color intensity was determined by measuring the absorbance at 420 nm, 520 nm and 620 nm (Glories, 1984).

Wine tasting evaluation was done at the Faculty of Agriculture, University of Belgrade, by a five-member committee. The committee worked in an isolated, silent room (temperature between 18 and 22 °C) where the light could not affect the color of the wine. Each member evaluated the wine samples according to a pre-established order. Then, the average mark was calculated for all wines. The organoleptic properties of wines were evaluated by sensory analysis using a 20-point system, as follows: color - from 0 to 2 points; clarity - from 0 to 2 points; odor - from 0 to 4 points; and taste - from 0 to 12 points. Considering the overall score, wines were classified as table wines with geographical indications (≥15.00 points), quality wines with geographical indications (≥16.50 points), and high quality wines with geographical indications (≥18.50 points) (Official Gazette, 2003).

TPC was determined spectrophotometrically on a UV-Vis spectrophotometer (GBC UV-Visible Cintra 6), using the Folin-Ciocalteu method, with some modifications (Pavlović *et al.*, 2013). The absorbance was measured at 765 nm and was proportional to the total quantity of phenolic compounds. Gallic acid was used as a standard, and TPC was expressed as g gallic acid equivalent (GAE) per L of wine.

TAC was determined using the pH-differential method by measuring the absorbance of the sample at pH = 1 (KCl, 0.025 mol/L) and pH = 4.5 (NaOAc/HOAc, 0.4 mol/L) (Pavlović *et al.*, 2013). Measurements were performed at two wavelengths, 520 nm and 700 nm. TAC was calculated and expressed as mg malvidin-3-glucoside (mal-3-glu) equivalent per L of wine.

6. Statistical analysis

Data analysis was performed using the statistical package IBM SPSS Statistics 20 (Faculty of Agriculture, Belgrade, Serbia). Indicators of descriptive statistics (mean and standard error of the mean ($S\,x$)) were calculated for all the observed properties. In order to reach objective conclusions about the impact of the two factors - variety (standard versus clones) and year - on the observed changes, ANOVA and LSD test ($p<0.05$) were applied. The contribution of each factor was determined by partial eta squared coefficient, which was then classified according to Cohen's graduation (Cohen, 1988).

RESULTS AND DISCUSSION

1. Climatic conditions

The temperatures recorded at the experimental field of « Radmilovac » during the period of study (2009-2013) showed the rising trend in relation to the average annual temperature (10.9 °C) and average temperature during the growing season (16.6 °C) measured in the period 1951-2008. The highest average temperature during the growing season was recorded in 2012, the highest average annual precipitation in 2009, and the highest precipitation in the growing season in 2010. In the period 2011-2013, the total precipitation in the growing season was below 400 mm, which is less than the total precipitation in the growing season (410.5 mm) measured in the period 1963-2008. Data on temperature and precipitation during the period of study are shown in Figure 1.

J. Int. Sci. Vigne Vin, 2015, **49**, 4, 267-274

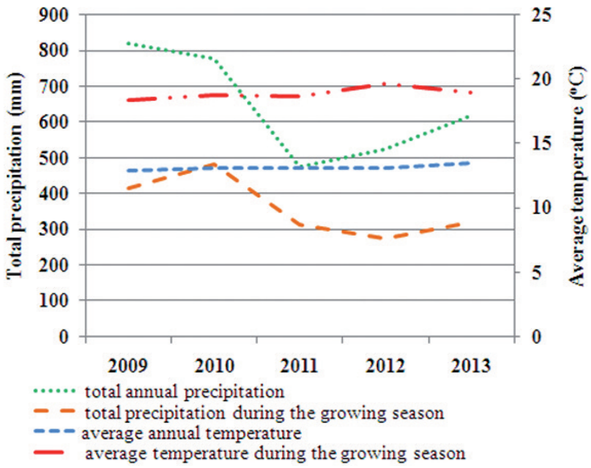

Figure 1. Temperature and precipitation at the experimental field of « Radmilovac » (Grocka winegrowing region) in the period 2009-2013.

2. Agrobiological traits

The effects of « year » and « clone » were significant for both agrobiological traits and enological parameters, while the clone x year interaction did not have any statistical significance (p>0.05). The contribution of each factor indicated that the climatic conditions (over the period of study) had a stronger influence on all studied agrobiological parameters (except sugar content in must) compared to the clones. The agrobiological characteristics of Merlot standard and clones 022, 025 and 029 are shown in Table 1. During the five-year period, the clones showed significantly higher values than the standard for all parameters, except yield. Clone 025 had the highest average grape yield, cluster content indicator, berry juice yield, berry weight and sugar content in must. Cluster weight was highest in clone 029, but there was no significant difference between clones for any of the studied agrobiological parameters. All yield components, except sugar content in must, varied significantly from year to year.

According to the study of Jones and Davis (2000), relief, temperature, sunlight and physico-chemical characteristics of the soil significantly affect the yield and quality of grapes, while the amount and distribution of rainfall affect the quality of grapes and wines (van Leeuwen and Seguin, 1994).

The average grape yield varied from 2.70 to 2.88 kg/vine, which is within the limits of the varietal characteristics (Žunić and Garić, 2010). There was no statistically significant difference between clones and the standard. However, the yield was significantly different between years; the highest/lowest average yield was recorded in 2012 and 2013, respectively. The climatic conditions over the years of study had ten times more influence on yield than the studied varieties (clones).

The highest average berry juice yield was recorded in clone 025 (56.41 mL) and the lowest in the standard (54.97 mL). The lowest berry juice yield was recorded in 2011 (not significantly different compared to 2009), while the differences were not significant in the other years.

The average cluster structural indicator was found to be greater in clones than in the standard. Clone 025 had the highest cluster structural indicator (31.61), which is coherent with the highest average berry weight found in clone 025. Both the standard and clones had the highest and lowest values of this parameter in 2011 and 2009, respectively. Regarding average annual precipitation, both the lowest and highest amounts were recorded in the aforementioned years.

The highest average cluster weight was recorded in clone 029 (112.08 g) and the lowest in the standard (101.96 g). Clone 025 had lower cluster weight compared to clone 029, but this was not related to lower grape yield. On the contrary, a higher average yield was recorded for clone 025, which was probably compensated by a greater number of clusters. With respect to year, the highest average cluster weight was recorded in 2011; significant difference was recorded only between 2009 and the other four years. The average berry weight varied from 1.18 g (standard) to 1.26 g (clone 025). This commercially important trait of grapes varies depending on environmental factors (Shiraishi *et al.*, 2010). The highest average berry weight was measured in 2012 (1.32 g) and was significantly different from all other years except 2010. Our study indicates that weather conditions significantly affected the berry weight. The highest grape yield and berry weight were recorded in years 2010 and 2012, while the lowest in 2011 and 2013, respectively. Cluster weight, cluster structural indicator and total acid content in must had the lowest values in 2009.

The most important elements of grape quality are sugar content and total acids, because the quality of wine depends on their content (Liu *et al.*, 2007). According to some authors, sugar content and berry weight are positively correlated and dependent on the variety (Ferrer *et al.*, 2014). The highest/lowest average sugar content in must was found in clone 025 (21.50 %)/standard (20.98 %). There was a significant difference between the standard and the clones, with no significant interannual differences. For this respective indicator, variety had a greater influence than year. Our results were in accordance with the findings of Ferrer *et al.* (2014). The temperatures in the period May-September (20.7-22.2 °C) in all years of study had a positive effect on sugar accumulation in grapes.

Clone 022 had the highest (7.14 g/L) and the standard the lowest (7.02 g/L) average content of acids. The differences in total acids between the individual clones were not found to be statistically significant. The obtained values of sugar and acid content were within the limits of the studied variety (Žunić and Garić, 2010), indicating that the

Table 1 - Agrobiological traits of grapes of Merlot standard and clones

	Year	Standard	Clone 022	Clone 025	Clone 029	$\bar{X} \pm S_{\bar{X}}$
Yield (kg/vine)	2009	2.72	2.82	2.91	2.91	$2.83^{b}\pm0.06^{1}$
	2010	2.99	3.07	3.18	3.18	$3.08^{a}\pm0.06$
	2011	2.35	2.43	2.51	2.51	$2.43^{c}\pm0.05$
	2012	3.12	3.18	3.34	3.34	$3.22^{a}\pm0.08$
	2013	2.31	2.39	2.47	2.47	$2.39^{c}\pm0.05$
	$\bar{X} \pm S_{\bar{X}}$	2.70±0.07	2.78±0.09	2.88±0.08	2.80±0.09	2.79±0.04
Berry juice yield (mL)	2009	54.82	55.75	56.26	55.62	$55.61^{ab}\pm0.37$
	2010	55.91	56.49	56.96	56.29	$56.41^{a}\pm0.23$
	2011	52.82	55.68	55.36	55.02	$54.72^{b}\pm0.52$
	2012	55.85	56.26	56.94	56.75	$56.45^{a}\pm0.20$
	2013	55.44	55.36	56.51	56.19	$55.87^{a}\pm0.28$
	$\bar{X} \pm S_{\bar{X}}$	$54.97^{b}\pm0.40$	$55.91^{a}\pm0.27$	$56.41^{a}\pm0.24$	$55.97^{a}\pm0.31$	55.81±0.16
Cluster structural indicator	2009	29.13	30.61	30.67	30.61	$30.26^{c}\pm0.42$
	2010	30.60	31.83	31.88	31.95	$31.56^{ab}\pm0.38$
	2011	31.94	32.21	32.52	32.16	$32.21^{a}\pm0.23$
	2012	29.80	31.24	31.37	31.19	$30.90^{bc}\pm0.42$
	2013	30.44	31.59	31.59	31.82	$31.36^{ab}\pm0.36$
	$\bar{X} \pm S_{\bar{X}}$	$30.38^{b}\pm0.32$	$31.50^{a}\pm0.34$	$31.61^{a}\pm0.35$	$31.55^{a}\pm0.35$	31.26±0.17
Cluster weight (g)	2009	93.37	98.86	101.24	103.34	$99.20^{b}\pm1.88$
	2010	103.97	110.09	112.72	113.44	$110.05^{a}\pm2.19$
	2011	106.04	112.25	114.80	117.00	$112.53^{a}\pm1.68$
	2012	100.52	106.43	110.95	111.04	$107.24^{a}\pm2.58$
	2013	105.94	112.16	114.88	115.56	$112.13^{a}\pm1.86$
	$\bar{X} \pm S_{\bar{X}}$	$101.96^{b}\pm1.65$	$107.96^{a}\pm1.92$	$110.92^{a}\pm1.94$	$112.08^{a}\pm2.00$	108.23±1.03
Berry weight (g)	2009	1.18	1.25	1.27	1.24	$1.23^{b}\pm0.02$
	2010	1.23	1.30	1.31	1.28	$1.28^{a}\pm0.02$
	2011	1.11	1.18	1.20	1.18	$1.17^{c}\pm0.02$
	2012	1.29	1.32	1.34	1.32	$1.32^{a}\pm0.01$
	2013	1.07	1.14	1.16	1.13	$1.12^{c}\pm0.02$
	$\bar{X} \pm S_{\bar{X}}$	$1.18^{b}\pm0.02$	$1.24^{a}\pm0.02$	$1.26^{a}\pm0.02$	$1.23^{a}\pm0.02$	1.22±0.02
Sugar content in must (%)	2009	21.09	21.40	21.62	21.33	21.36±0.12
	2010	21.21	21.30	21.64	21.45	21.41±0.10
	2011	20.97	21.21	21.63	21.31	21.28±0.10
	2012	20.85	21.22	21.33	21.19	21.15±0.13
	2013	20.76	21.28	21.26	21.12	21.11±0.10
	$\bar{X} \pm S_{\bar{X}}$	$20.98^{b}\pm0.11$	$21.28^{a}\pm0.10$	$21.50^{a}\pm0.07$	$21.28^{a}\pm0.09$	21.26±0.05
Total acids in must (g/L)	2009	6.72	6.98	6.98	6.94	$6.91^{c}\pm0.04$
	2010	7.01	7.23	7.17	7.18	$7.15^{a}\pm0.03$
	2011	7.23	7.22	7.19	7.16	$7.20^{a}\pm0.02$
	2012	6.83	7.08	7.09	7.01	$7.00^{b}\pm0.04$
	2013	7.29	7.19	7.19	7.21	$7.22^{a}\pm0.02$
	$\bar{X} \pm S_{\bar{X}}$	$7.02^{b}\pm0.05$	$7.14^{a}\pm0.03$	$7.12^{a}\pm0.03$	$7.10^{a}\pm0.04$	7.10±0.02

1Data are expressed as mean ± standard error of the mean (n=5)

Values marked with different letters are statistically significantly different $p<0.05$ (ANOVA, LSD test)

environmental conditions of Grocka are suitable for Merlot.

3. Wine quality traits

Ideally, the quality of wine is based on visual and organoleptic characteristics (taste and aroma), which are above the average for certain types of wine. The organoleptic characteristics of the wine are affected by a broad spectrum of factors including grape variety, soil, cultural and enological conditions (production area factor) as well as climatic conditions (vintage factor) (Almela *et al.*, 1996; Jackson and Lombard, 1993). The results of selected quality traits of wines for the period 2009-2013 are presented in Table 2. Merlot standard was significantly different from all three clones, while the clones did not differ among themselves. The average alcohol content in wine samples varied from 12.42 % (standard) to 12.60 % (clone 029). The highest/lowest average alcohol content was measured in 2011 (not significantly different compared to 2009 and 2010) and 2012 (not significantly different compared to 2013), respectively. Wine of clone 025 had the highest average content of total extract(27.13 g/L), whereas the standard had the lowest (26.56 g/L). Interannual differences were not significant, which indicated that the variety factor had a greater influence on the differences than the year factor. The color of a red wine is closely related to the grape variety, degree of ripeness, and time and temperature of the maceration process (Briz-Cid *et al.*, 2014). The color intensity in wines of the clones and standard differed significantly.

Climatic conditions in 2012 (the highest average temperature and lowest precipitation in the growth period, compared to the other years) had a positive impact on the color intensity of the wines.

Different clones of a single grape variety may differ in yield and ability to give wines with different organoleptic characteristics (Zamuz *et al.*, 2007). The quality of red wine is also influenced by color intensity and anthocyanin content (Downey *et al.*, 2006). To obtain a more comprehensive picture of the quality of the wines, total phenolics and anthocyanins in wines of standard and clones were determined in the final year of study. The highest TPC and TAC was measured in the wine sample of clone 025 and significantly differed from wines of the other two clones and standard. The lowest content of these compounds was found in wine of clone 022 (Table 3).

Organoleptic evaluation completes the picture of wine quality, which is mainly defined by the chemical and physical properties of the wine. In the preliminary wine tasting evaluation, the average tasting scores ranged from 18.00 to 18.06 for clone 022 and 029, respectively (Figure 2). Interestingly, all three wines obtained from the new Merlot clones exhibited better tasting scores compared with the mother wine, but with no significant difference. The highest/lowest tasting scores were obtained in 2012/2009, presumably due to the year factor (the highest/lowest average temperature in the growing season was recorded in 2012/2009, while the highest average annual precipitation was observed in 2009). According to the tasting scores, wines of the clones were classified as « quality wines with geographical indications » (Official Gazette, 2003).

CONCLUSION

Vine yield components of Merlot standard and clones significantly differed from a technological point of view in favor of the clones. Clone 025 stood out regarding the higher TPC and TAC in wine, being statistically significantly different compared to the wines of the other two clones and standard. On the other hand, the wine of clone 022 had the lowest content of these compounds. The results obtained for most of the studied traits indicated no significant differences between the clones. Interannual differences were greater than the differences among the clones. Climatic conditions had a stronger influence on most studied agrobiological and wine quality traits, compared to the tested varieties. Variety had a greater influence on sugar content in must and total extracts in wine. Despite the fact that the wine of clone 029 had the

Table 2 - Selected quality traits of Merlot wines (standard and clones)

	Year	Standard	Clone 022	Clone 025	Clone 029	$\bar{X} \pm S_{\bar{X}}$
Ethyl alcohol (vol %)	2009	12.44	12.75	12.64	12.55	12.60[ab]±0.06[2]
	2010	12.57	12.50	12.70	12.68	12.61[ab]±0.04
	2011	12.66	12.62	12.75	12.72	12.69[a]±0.03
	2012	12.07	12.51	12.34	12.52	12.36[c]±0.08
	2013	12.33	12.46	12.52	12.55	12.47[bc]±0.06
	$\bar{X} \pm S_{\bar{X}}$	12.42[b]±0.07	12.57[a]±0.04	12.59[a]±0.06	12.60[a]±0.05	12.54±0.03
Total extract (g/L)	2009	26.04	26.70	26.81	26.57	26.53±0.18
	2010	26.46	27.13	27.13	26.94	26.91±0.19
	2011	26.76	26.90	27.18	27.30	27.04±0.16
	2012	26.69	27.09	27.23	27.30	27.08±0.14
	2013	26.82	27.28	27.29	26.93	27.08±0.11
	$\bar{X} \pm S_{\bar{X}}$	26.56[b]±0.04	27.02[a]±0.14	27.13[a]±0.14	27.01[a]±0.14	26.93±0.07
Color intensity	2009	1.59	1.66	1.66	1.64	1.64[e]±0.01
	2010	1.65	1.72	1.72	1.71	1.70[d]±0.01
	2011	1.77	1.80	1.80	1.83	1.80[b]±0.01
	2012	1.85	1.88	1.82	1.86	1.85[a]±0.01
	2013	1.71	1.78	1.78	1.75	1.76[c]±0.02
	$\bar{X} \pm S_{\bar{X}}$	1.72[b]±0.02	1.76[a]±0.02	1.76[a]±0.02	1.76[a]±0.02	1.75±0.01

[1]Data are expressed as mean ± standard error of the mean (n=5)
Values marked with different letters are statistically significantly different p<0.05 (ANOVA, LSD test)

Table 3 - Total polyphenol content (TPC) and total anthocyanin content (TAC) in Merlot wines (2013)

	Standard	Clone 022	Clone 025	Clone 029
	$\bar{X} \pm S_{\bar{X}}$			
TPC (g GAE/L)	1.27[b]±0.00	1.09[d]±0.00	1.46[a]±0.00	1.16[c]±0.00
TAC (mg mal-3-glu/L)	28.54[c]±0.07	24.31[d]±0.50	40.51[a]±0.12	35.58[b]±0.00

[1]Data are expressed as mean ± standard error of the mean (n=3)
Values marked with different letters are statistically significantly different p<0.05 (ANOVA, LSD test)

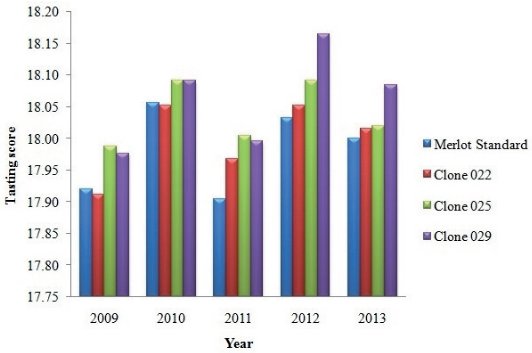

Figure 2. Average tasting scores of Merlot wines (standard and clones).

highest tasting score in the preliminary wine evaluation, it did not significantly differ compared to the wines of the other two clones and standard. Based on the wine tasting scores, the wines of clones may be classified as « quality wines with geographical indications ». A more comprehensive sensory evaluation (including more wine tasters and parameters) is planned.

The results of the studied traits of grape and wine suggest that the quality of the clones surpassed the quality of the standard. The selected clones were characterised by good quality grapes and wines and can be recommended for introduction in viticultural practices and production in similar agro-ecological conditions.

The improved viticultural parameters (versus the mother vine) indicate the need for further research work, which will be focused on the chemistry of both the grapes and wines in order to better understand the characteristics of the selected clones and their market potential.

Conflict of interest: The authors declare no conflict of interest.

Acknowledgments: This work was performed within the framework of the research projects Nos. 46001 and 46009 172017, supported by the Ministry of Education, Science and Technological Development, Republic of Serbia.

REFERENCES

Almela L., Javaloy S., Fernandez-Lopez J.A. and Lopez-Roca J.M., 1996. Varietal classification of young red wines in terms of chemical and colour parameters. *J. Sci. Food Agric.*, **70**, 173-180.

Audeguin L., Boidron R., Bloy P., Grenan S., Leclair P. and Boursiquot J.-M., 2000. L'expérimentation des clones de vigne en France. État des lieux, méthodologie et perspectives. *Rev. Fr. Œnol.*, **184**, 8-11.

Avramov L., Žunić D., Jovanović M., Radulović D. and Milanović D., 1996a. Production and technological characteristics of the F-1 clone generation of the variety Merlot in the "Vinaračko" and "Župa" vine district. *In: XI Savetovanje Vinogradara i Vinara Srbije sa Međunarodnim Učešćem*, Priština (Savezna Republika Jugoslavija), pp. 117-122.

Avramov L., Žunić D., Jović S., Gašić N., Vujović D. and Maletić R., 1996b. Production and technological characteristics of the F-2 clone generation of the variety Merlot in the "Grocka" vine district. *In: XI Savetovanje Vinogradara i Vinara Srbije sa Međunarodnim Učešćem*, Priština (Savezna Republika Jugoslavija), pp. 123-128.

Boursiquot J.-M., Lacombe T., Laucou V., Julliard S., Perrin F.-X., Lanier N., Legrand D., Meredith C. and This P., 2009. Parentage of Merlot and related winegrape cultivars of southwestern France: discovery of the missing link. *Aust. J. Grape Wine Res.*, **15**, 144-155.

Briz-Cid N., Figueiredo-González M., Rial-Otero R., Cancho-Grande B. and Simal-Gándara J., 2014. Effect of two anti-fungal treatments (metrafenone and boscalid plus kresoxim-methyl) applied to vines on the color and phenol profile of different red wines. *Molecules*, **19**, 8093-8111.

Cindrić P., 2003. Grapevine clone selection. *Contemp. Agric.*, **52**, 53-66.

Cohen J., 1988. *Statistical Power Analysis for the Behavioral Sciences, second ed.* Lawrence Erlbaum Associates, Hillsdale, NJ.

Downey M.O., Dokoozlian N.K. and Krstic M.P., 2006. Cultural practice and environmental impacts on the flavonoid composition of grapes and wine: a review of recent research. *Am. J. Enol. Vitic.*, **57**, 257-268.

Ferrer M., Echeverría G. and Carbonneau A., 2014. Effect of berry weight and its components on the contents of sugars and anthocyanins of three varieties of *Vitis vinifera* L. under different water supply conditions. *S. Afr. J. Enol. Vitic.*, **35**, 103-113.

Glories Y., 1984. La couleur des vins rouges - 1re partie. Les équilibres des anthocyanes et des tannins. *Conn. Vigne Vin*, **18**, 195-217.

Ivanišević D., Korać N., Cindrić P., Paprić Dj., Kuljančić I. and Medić M., 2012. Riesling Italico subclones. *Genetika-Belgrade*, **44**, 299-306.

Jackson D.I. and Lombard P.B., 1993. Environmental and management practices affecting grape composition and wine quality - a review. *Am. J. Enol. Vitic.*, **44**, 409-430.

Jones G.V. and Davis R.E., 2000. Climate influences on grapevine phenology, grape composition, and wine production and quality for Bordeaux, France. *Am. J. Enol. Vitic.*, **51**, 249-261.

Keller M., 2010. *The Science of Grapevines. Anatomy and Physiology*. Academic Press: New York.

Lacombe T., Boursiquot J.-M. and Audeguin L., 2004. Prospection, conservation et évaluation des clones de vigne en France. *Bull. OIV*, **77**, 799-809.

Liu H.-F., Wu B.-H., Fan P.-G., Xu H.-Y. and Li S.-H., 2007. Inheritance of sugars and acids in berries of grape (*Vitis vinifera* L.). *Euphytica*, **153**, 99-107.

OEPP/EPPO, 2008. Schemes for the production of healthy plants for planting : pathogen-tested material of grapevine varieties and rootstocks. *Bull. OEPP/EPPO Bull.*, **38**, 422-429.

Official Gazette of the Republic of Serbia and Montenegro, 2003. Regulations on quality and other requirements for wine. N° 56.

O.I.V., 2006. *Compendium of International Methods of Wine and Must Analysis*. Vol. 1, International Organisation of Vine and Wine, Paris.

Pavlović A.V., Dabić D.Č., Momirović N.M., Dojčinović B.P., Milojković-Opsenica D.M., Tepić Ž.Lj. and Natić M.M., 2013. Chemical composition of two different extracts of berries harvested in Serbia. *J. Agric. Food Chem.*, **61**, 4188-4194.

Pelsy F., Hocquigny S., Moncada X., Barbeau G., Forget D., Hinrichsen P. and Merdinoglu D., 2010. An extensive study of the genetic diversity within seven French wine grape variety collections. *Theor. Appl. Genet.*, **120**, 1219-1231.

Ruhl E., Konrad B., Lindner B. and Bleser E., 2004. Quality criteria and targets for clonal selection in grapevine. *Acta Hortic.*, **652**, 29-33.

Shiraishi M., Fujishima H. and Chijiwa H., 2010. Evaluation of table grape genetic resources for sugar, organic acid, and amino acid composition of berries. *Euphytica*, **174**, 1-13.

This P., Lacombe T. and Thomas M.R., 2006. Historical origins and genetic diversity of wine grapes. *Trends Genet.*, **22**, 511-519.

van Leeuwen C. and Seguin G., 1994. Incidences de l'alimentation en eau de la vigne, appréciée par l'état hydrique du feuillage, sur le développement de l'appareil végétatif et la maturation du raisin (*Vitis vinifera* variété Cabernet Franc, Saint-Émilion, 1990). *J. Int. Sci. Vigne Vin*, **28**, 81-110.

van Leeuwen C., Roby J.-P., Alonso-Villaverde V. and Gindro K., 2013. Impact of clonal variability in *Vitis vinifera* Cabernet franc on grape composition, wine quality, leaf blade stilbene content, and downy mildew resistance. *J. Agric. Food Chem.*, **61**, 19-24.

Vivier M.A. and Pretorius I.S., 2002. Genetically tailored grapevines for the wine industry. *Trends Biotechnol.*, **20**, 472-478.

Vujović D., 2003. Ampelographic and technological characteristics of Merlot noir clones. *Ph.D. Thesis*, University of Belgrade.

Zamuz S., Martinez M.-C. and Vilanova M., 2007. Primary study of enological variability of wines from different clones of *Vitis vinifera* L. cv. Albariño grown in Misión Biológica de Galicia (CSIC). *J. Food Compos. Anal.*, **20**, 591-595.

Žunić D. and Garić M., 2010. *Ampelografija*. Poljoprivredni fakultet Univerziteta u Prištini-Kosovska Mitrovica.