## EVALUATION OF OENOLOGICAL POTENTIAL OF CLONAL SELECTIONS OF OBTAINED AT THE STEFANESTI WINE CENTER

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#### Abstract

The aim of this study is to characterize and differentiate the main physico-chemical parameters, the evolution of the anthocyanin profile and the total polyphenol content of white and red wines obtained from clones of the varieties 'Feteasca Regala', 'Muscat Ottonel', 'Sauvignon Blanc', 'Burgund', 'Cabernet Sauvignon' and 'Merlot'. For the production of white wines, the clones 'Feteasca Regala' 72 St', 'Muscat Ottonel 16 St', 'Sauvignon Blanc111 St' and 3 other clones for the production of red wines, 'Burgund 86 St', 'Merlot 202 St' and 'Cabernet Sauvignon 131 St' were studied. The results showed that the red wines produced from the 'Burgund 86 St', Merlot 202 St' and 'Cabernet Sauvignon 131 St' clones outperformed the standard wines in terms of total anthocyanin and polyphenol content. In all the studied clones, the physico-chemical indicators, the alcohol concentration and the tannins in the wines, recorded higher values than the control varieties.

Key words: Vitis vinifera L., climate variability, clone, physico-chemical parameters.

### INTRODUCTION

Globally, the trends in viticulture are towards new vine varieties with genetic resistance and clonal selections of widespread varieties that are better adapted to climate change and that give quality grapes and better yields. The genetic diversity of the Vitis genus is a valuable resource for adapting to future climate change, but for the production of high-quality wines, both complex selection and aging programs are needed, as well as winemaking experiments with the resulting biological material (Faria, 2020). Conditions of increased water stress decrease grape production and winemaking yields, aspects that are further reflected in the economic part of the wine sector. At the same time, global warming increases the probability of extreme, unwanted climatic events (hail, floods, low temperatures during the flowering period, late spring frost, etc.). Under these conditions. appropriate vine adaptation strategies are needed to be able to continue producing high quality wines and to preserve varietal and terroir typicity in a changing climate. The quality of biological material

valuable remains а resource for the implementation of these strategies (Cornelis van Leeuwen and Darriet, 2016). For a centralized record of grapevine varieties in Europe, in 1984, it was proposed to establish the International Catalog of Vitis Varieties (VIVC), with the Institute for Grapevine Breeding Geilweilerhof, from Germany, as coordinator. The grapevine genetic resources database concept was supported by the International Board for Plant Genetic Resources (IBPGR) and the OIV. Later, other databases for this genre were created in Bulgaria, Czech Republic, France, Italy, Slovenia and Spain. Today, at the European level, the database presents information about approximately 23,000 varieties, hybrids and species of the genus Vitis vinifera existing in grapevine collections and/or described in specialized works. It is a useful source of information for the management of germplasm collections, as well as for vine growers and producers of wine and wine-derived products (Popescu et al., 2018). Through the clonal selection operations carried out over time, in Romania 54 clonal selections were obtained and promoted in practice, 9 from table varieties and 45 clonal selections from wine varieties. Among the clonal selections of winemaking varieties, about 35 clonal selections are from white wine varieties and 10 clonal selections are from red wine varieties (Marin et al., 2018). At NRDIBH Ștefanești, in the last decades, an intense activity of improving the vine was carried out, which led to the achievement of notable results. Following the long-term activity, at NRDIBH Ștefanești, new clones were obtained of most of the old varieties from the basic assortment, as well as new varieties in different production directions.

The present study focused on the analysis of three clones for white wines ('Muscat Ottonel 16 St', 'Fetească Regală 72 St', 'Sauvignon Blanc 111 St'), and 3 other clones intended for obtaining red wines ('Burgund 86 St', 'Merlot 202 St' and 'Cabernet Sauvignon 131 St') which are more spreaded in the Ștefănesti-Argeş wine-growing area. This study revealed information about the quality of white and red wines obtained from clones created at NRDIBH Stefanești, Romania, useful for their further promotion.

## MATERIALS AND METHODS

The experiment was located at NRDIBH Stefanesti. During the research, 3 clones from white wine cultivars ('Fetească Regală 72 St', 'Muscat Ottonel 16 St' and 'Sauvignon Blanc 111 St') and 3 clones of three red wine cultivars ('Burgund 86 St', 'Merlot 202 St' and 'Cabernet Sauvignon 131 St') were studied. The varieties were grafted on the rootstock Oppenheim 4 (SO-4), the leading form being Guyot on the stem. Twelve plants from each clone were randomly marked in four rows. The analyzed white and red wines were produced under the same microvinification conditions at INCDBH Stefanesti, Arges County. After the second infusion, the wines obtained from the studied clones, the 2020 and 2021 production respectively, were filtered and bottled. The physico-chemical determination of the parameters of the wines established from the studied clones was carried out in the Oenology laboratory with the help of Automatic oenological analyzer MINDRAY BS-200 connected to the computer, hydrostatic balance

GIBERTINI, spectrophotometer UV/Visible SPECORD 205, electronic pH-meter, GIBERTINI digital still, Salleron ebulometer. The spectrophotometric analyses of the red and

white wines focused on the content of total anthocyanins and total polyphenols. The content in total polyphenols was determined by the Folin-Ciocâlteu method, and the expression was done in relation to a calibration curve with gallic acid, the results being expressed in mg (GAE) total polyphenols/l sample. The determination of the total anthocyanin content, expressed in mg/l, was carried out by the method of Ribereau Gavon-Stonestreet, 1976). The region is characterized by a humid temperate-continental climate, with a mean annual temperature (Tmean) of 9.6°C and an amount of precipitation of 671.8 mm for the period 1979-2019 not evenly distributed throughout the year. The weather data were collected from the meteorological platform NRDBH Stefănești, geographical coordinates 44°51'N and 24°57'E. Reference varieties were compared with their clonal selections using the individual t-test, for a statistical significance level of 5%. All data were entered into an excel spreadsheet and then imported, processed and interpreted using the statistical program SPSS.14.0.

## **RESULTS AND DISCUSSIONS**

Air temperature (lower thresholds, maximum thresholds, optimum level, critical moments) has a dominant influence on the vine according to the vine phenology dynamic. We had available for this analysis daily climatological databases for the experimental period (2020-2021), compared to the multi-year values (1979-2019). Average monthly temperatures were used to calculate a set of bioclimatic indices commonly used in viticulture. Table 1 describes the evolution of climate indicators from the experimentation period (2020-2021) compared to the multiannual average (1979-2019), at NRDIBH Stefanesti. In the study period (2020-2021) higher temperatures were recorded compared to the multi-year average, especially in terms of the maximum and minimum annual temperatures (Table 1), and during the winter period much higher temperatures from one year to another (Table 1). Compared to the long-term averages (1979-2019), the average annual air temperature was higher by 1.25°C in 2020, respectively by 0.82°C in 2022. From table 1, it can be seen that 2020 was an extremely dry year, the precipitation during the vegetation period being far below the multi-annual average. It has been noted that in temperate regions that generally do not suffer from drought, a certain controlled

deficit of water, during the ripening period, favors the organoleptic qualities of wine (Riou, 1994). However, in the study area, in the two analyzed years, the rainfall deficit in the vegetation period was 194.26 mm in 2020 and, respectively, 90.66 mm in 2022, compared to the multi-year average of 530.26 mm (2070-2019), influencing the main indicators of wine quality.

Table 1. Climatic indicators of the experimentation period (2020-2021) compared to the multiannual average (1979-2019), INCDBH Stefanesti - Arges

Climatic indicator	Multianual average (1979-2019)	2020	2021	
Average annual temperature, °C	10.98	12.23	11.8	
Average temperature in the growing season, °C (IV-X)	16.66	19.93	17.41	
Average temperature in summer (°C) (VI-VIII)	21.45	22.35	23.08	
Average annual minimum temperature (°C)	5.95	7.8	9.86	
Absolute minimum temperature (°C)	-18.72	-10.9	-13.9	
Average January minimum temperature (°C)	-7.58	-10.1	-10.9	
Average annual maximum temperature (°C)	21.29	23.72	23.84	
Average July maximum temperature (°C)	32.18	37.7	37.9	
Annual total precipitation, mm	783.1	380	770.4	
Total precipitation in the growing season, mm (IV-X), respectively for the years 2020-2021	531.26	337	440.6	
The total precipitation in summer (VI-IX)	307.74	231.2	213.2	

# Evaluation of the physico-chemical indicators of the white wines

In both years of the study, the wines obtained from the 'Fetească Regală 72 St' clonal selection presented a higher alcohol content of 13.73 % vol. (2020), respectively 12.83% vol. (2021), compared to the reference cultivar which registered an alcoholic content of 12.20 % vol. (Table 2).

Alcohol content of the wines analyzed are much higher in 2020, compared to those obtained in 2021. This can be explained by the high temperatures that occurred in the climatic year 2020, and low precipitation until the end of autumn in the reference area with a deficit of 194.26 mm recorded (Table 1), allowing the accumulation of greater amounts of sugars in the grapes. Also, the clonal selection "Fetească Regală 72 St' recorded higher values of the non-reducing extract (18.42 g/l and 19.8 g/l respectively), compared to the control that reached a level of the non-reducing dry extract of 18.12 g/l (2020), respectively 18.32 (2021), the differences between them being statistically significant, in both years of study (Table 2). It is known that high values of non-reducing extract provide wines with consistency and personality (Hodor, 2011).

In the two analyzed years, the total acidity of the analyzed wine samples (Table 2) recorded values between 5.34 and 5.43 g/l tartaric acid in the clonal selection 'Fetească Regală 72 St' and 5.45-5.87 g/l tartaric acid in the reference cultivar the differences between them being significant. The clonal selection 'Muscat Ottonel 16 St' recorded a higher alcohol content and non-reducing dry extract than the analyzed control, the differences between them being significant (Table 3). The results obtained in 2020 regarding the acidity, respectively values of 4.85 highlighted in clone 'Muscat Ottonel 16 St' and over 4.59 g/l tartaric acid in the reference cultivar were similar to those presented by Chircu, 2014. Also, the results can be compared to those reported by de Bora et al. (2016) who reported a higher alcohol content, 'Fetească Regală' (13.80% vol.) and lower for 'Muscat Ottonel' (11.00% vol.).-The consistency of the wines given by the nonreducing dry extract and the concentration of the alcoholic (% vol) showed superior values for the clonal selection 'Sauvignon Blanc 111

St', compared to the reference cultivar, in both evaluation years (Table 4).

The volatile acidity of the wines obtained from the 'Fetească Regală', 'Muscat Ottonel' and 'Sauvignon Blanc' cvs. had values located within the accepted limits, but with higher values for their clones.

Cultivars/ clonal selection	Alcohol (% vol.)	Non- reducing dry extract, g/l	Zah. Nereduc., g/l	Total acidity, (g/l) tartric acid	VA, g/l acid acetic	рН		
			2020					
'Fetească Regală' control reference	12.20±0.20 <sup>b*</sup>	18.12±0.08 <sup>b</sup>	1.8±0.20 <sup>b*</sup>	5.87±0.03 <sup>b</sup>	$0.27 \pm 0.02^{b}$	3.28±0.02 <sup>b</sup>		
'Fetească Regală 72 St'	13.73±0.33ª	$18.42{\pm}0.08^{a}$	2.9±0.33ª	5.34±0.08 <sup>a</sup>	$0.23{\pm}0.02^{a}$	3.25±0.01 <sup>a</sup>		
	2021							
'Fetească Regală' reference	12,20±0.08ª	$18.32{\pm}0.8^{\rm b}$	1.3±0.22 <sup>b*</sup>	$5.45{\pm}0.08^{b^*}$	$0.42 \pm 0.42^{b^*}$	3.28±0.02 <sup>b</sup>		
'Fetească Regală 72 St'	12,83±0.08ª	19.8±0.12ª	2.7±0.28ª	5.43±0.38ª	0.21±0.033 a	3.25±0.01ª		

Table 2. Physico-chemical characteristics of the wines obtained from 'Fetească Regală 72St' clonal selection compared to the reference cultivar (Stefanesti vineyard, Arges, 2020-2021)

\*T-test for independent samples (p<0.05)

Table 3. Physico-chemical characteristics of the wines obtained from 'Muscat Ottonel 16 St' clonal selection compared to the reference cultivar (Stefanesti vineyard, Arges, 2020-2021)

Cultivars/ clonal selection	Alcohol (% vol.)	Non-reducing dry extract, g/l	Non-reducing sugar g/l	Total acidity, (g/l) tartric acid	VA, g/l acid acetic	рН	
			2020				
'Muscat Ottonel' reference	12.07±0.40 <sup>b</sup>	17.77±0.25 <sup>b</sup>	$1.47{\pm}0.40^{b}$	4.59±0.17 <sup>b</sup>	$0.50{\pm}0.08^{b}$	3.51±0.01ª	
' Muscat Ottonel 16 St'	12.87±0.12ª	18.27±0.15ª	1.17±0.12ª	4.85±0.36ª	0.46±0.01ª	3.52±0.02ª	
	2021						
'Muscat Ottonel' standard	$11.92{\pm}0.40^{b}$	18.07±0.25 <sup>b</sup>	1.9±0.40 <sup>b</sup>	4.39±0.12 <sup>b</sup>	$0.34{\pm}0.03^{b}$	3.57±0.04ª	
'Muscat Ottonel 16 St'	12.7±0.12ª	18.27±0.15ª	1.3±0.12ª	4.85±0.06ª	0.27±0.01ª	3.65±0.02ª	

\*T-test for independent samples (p<0.05)

Table 4. Physico-chemical characteristics of the wines obtained from 'Sauvignon Blanc 111 St' clonal selection compared to the reference cultivar (Stefanesti vineyard, Arges, 2020-2021)

Cultivar / clonal selection	Alcohol (% vol.)	Non- reducing dry extract, g/l	Non- reducing sugar g/l	Total acidity, (g/l) tartric acid	VA, g/l acid acetic	рН		
2020								
'Sauvignon Blanc' reference	13.97±0.55 <sup>b*</sup>	19.43±0.26 <sup>b</sup>	$4.20{\pm}0.55^{b^*}$	4.90±0.07 <sup>b</sup>	$0.47{\pm}0.02^{b}$	3.46±0.01ª		
'Sauvignon Blanc 111 St'	13.23±0.15 <sup>a</sup>	19.54±0.15 <sup>a</sup>	1.23±0.15 <sup>a</sup>	5.08±0.04 <sup>a</sup>	0.36±0.04ª	3.47±0.01ª		
	2021							
'Sauvignon Blanc' reference	13.8±0.55 <sup>b*</sup>	19.0±0.16 <sup>b*</sup>	$5.3{\pm}0.55^{b*}$	$6.08{\pm}0.07^{a}$	$0.37{\pm}0.08^{b}$	3.46±0.12 <sup>b</sup>		
'Sauvignon Blanc 111 St'	13.0±0.15 <sup>a</sup>	$19.8{\pm}0.08^{a}$	$1.5 \pm 0.15^{a}$	6.31±0.04 <sup>a</sup>	$0.23{\pm}0.02^{a}$	3.65±0.04ª		

\*T-test for independent samples (p<0.05

# Evaluation of physical-chemical indicators in red wines

All the clones for red wines exceeded the control variety in terms of the alcohol concentration in the wines, the non-reducing dry extract, as well as the total acidity in the wines.

From Table 5 it can be seen that the year of the study influenced the alcoholic concentration of the wines, total sugar and acidity.

The total acidity proved to be higher in the clonal selection 'Merlot 202 St' (5.13 AT g/l in 2020, respectively 6.0 AT g/l in 2021), than in the case of the standard cultivar (5.07 TA g/l, respectively 4.04 in 2021) (Table 6).

High temperatures accelerate decrease of grape acidity, mainly due to a faster degradation of malic acid (Buttrose et al., 1971).

The increase in grape sugar concentration and alcohol concentration is frequently reported in the literature (Bucur et al., 2019; Aloston et al., 2011; Duchêne and Schneider, 2016; Neethling et al., 2012).

The results obtained at the clonal section 'Cabernet Sauvignon 131 St' in 2020, of the total acidity in the wines (3.73) were also reported by Filip (2018) in a study on the chemical composition of three 'Cabernet Sauvignon' clones studied in the area of the Valea Calugareasca culture (Filip, 2018). These values obtained at NRDIBH Stefanesti for the 3 clones of red wines studied, compared with those obtained in other cultivation areas in the country, fall within the normal limits.

Table 5. Physico-chemical characteristics of the wines obtained from 'Burgund 86 St' clonal selection compared to the reference cultivar (Stefanesti vineyard, Arges, 2020-2021)

Cultivars/ clonal selection	Alcohol (% vol.)	Non- reducing dry extract, g/l	Non- reducing sugar g/l	Total acidity, (g/l) tartric acid	VA, g/l acid acetic	рН	
			2020				
Burgund reference	12.83±0.25 <sup>b*</sup>	28.93±0.06 <sup>b</sup>	1.93±0.25 <sup>b*</sup>	5.89±0.035ª	$0.27{\pm}0.006^{b}$	3.29±0.01 <sup>b</sup>	
Burgund 86 St	13.08±0.08ª	29.20±0.10 <sup>a</sup>	3.98±0.08ª	6.44±0.07 <sup>a</sup>	$0.25{\pm}0.007^{a}$	3.26±0.006ª	
2021							
Burgund reference	12.67±0.25 <sup>b*</sup>	22.9±0.06 <sup>b</sup>	1. 3±0.25 <sup>b*</sup>	5.93±0.035ª	$0.48{\pm}0.006^{b}$	3.42±0.01 <sup>b</sup>	
Burgund 86 St	12.8±0.08ª	26.85±0.10 <sup>a</sup>	3,85±0.08ª	5.86±0.07 <sup>a</sup>	$0.24{\pm}0.007^{a}$	3.3±0.006ª	

\*T-test for independent samples (p<0.05).

Tabelul 6. Physico-chemical characteristics of the wines obtained from 'Merlot 2020 St' clonal selection compared to
the reference cultivar (Stefanesti vineyard, Arges, 2020-2021)

Cultivars/ clonal selection	Alcohol (% vol.)	Non- reducing dry extract, g/l	Non- reducing sugar g/l.	Total acidity, (g/l) tartric acid	VA, g/l acid acetic	рН
2020						
Merlot reference	$10.80{\pm}0.28^{b^*}$	18.36±0.15ª	$3.50{\pm}0.10^{b^*}$	$5.07{\pm}0.038^{a}$	$0.71{\pm}0.04^{b}$	3.81±0.015ª
Merlot 202 St	11.80±0.11 <sup>a</sup>	18.30±0.17 <sup>a</sup>	2.80±0.02ª	5.13±0.07 <sup>a</sup>	0.48±0.03ª	3.82±0.012 <sup>a</sup>
2021						
Merlot	$10.80{\pm}0.10^{b^*}$	18.36±0.11ª	$3.35{\pm}0.18^{b*}$	$4.07{\pm}0.038^{a}$	0.71±0.09 <sup>a</sup>	3.59±0.015ª
Merlot 202 St	$11.80{\pm}0.08^{a}$	18.30±0.17 <sup>a</sup>	2.55±0.11 <sup>a</sup>	$6.00{\pm}0.066^{a}$	$0.48 \pm 0.01^{b}$	3.5±0.012 <sup>a</sup>

\*T-test for independent samples (p<0.05).

Table 7. Physico-chemical characteristics of the wines obtained from 'Cabernet Sauvignon 131 St' clonal selection compared to the reference cultivar (Stefanesti vineyard, Arges, 2020-2021)

Cultivars/ clonal selection	Alcohol (% vol.)	Non- reducing dry extract, g/l	Non- reducing sugar, g/l	Total acidity, (g/l) tartric acid	VA, g/l acid acetic	рН	
2020							
C. Sauvignon reference	12.77±0.26ª	18.47±0.21 <sup>b</sup>	$2.2{\pm}0.04^{b*}$	3.69±0.02ª	0.82±0.033 <sup>b</sup>	3.25±0.017 <sup>b</sup>	
C. Sauvignon 131 St	12.80±0.1ª	21.82±0.11ª	2.7±0.1ª	3.73±0.025 <sup>a</sup>	0.69±0.021ª	3.43±0.08ª	
2021							
C. Sauvignon reference	12.27±0.15 <sup>b*</sup>	22.6±0.30 <sup>b*</sup>	3.0±0.2ª	6.71±0.02 <sup>a</sup>	0.82±0.021ª	3.26±0.021 <sup>b</sup>	
C. Sauvignon 131 St	12.50±0.08ª	26.02±0.11ª	3.2±0.1ª	7.19±0.025 <sup>b</sup>	0.71±0.021 <sup>b</sup>	3.44±0.01ª	

\*T-test for independent samples (p<0.05).

# The content in total polyphenols in the analyzed white wines

Increasing temperatures negatively influence the accumulation of anthocyanins in grains (Montealegre et al., 2006), while strong solar radiation can have opposite effects. Also, higher concentrations of substances characteristic of muscat-type varieties (from the terpene family) were highlighted in naturally shaded grapes compared to grapes exposed to light radiation (Bertelli et al., 2002; Mattivi et al., 2006).



Figure 1. Content of white wines in total polyphenols from 'Fetească Regală', 'Mulscat Ottonel' and 'Sauvignon Blanc' clone selections compared to standard cultivars

Both the amounts of total polyphenols and the antioxidant activity recorded higher values in the wines obtained from the clonal selections studied, compared to the wines obtained from the control varieties, being higher in the 2020 crop year (Figures 1 and 3). The highest concentrations of total polyphenols were evident in the clone 'Fetească Regală 72 St' (470.91), in 2020, and the lowest were

evidently in the 'Sauvignon Blanc' cultivar (390.0 GAE/l) in 2021 (Figure 1).



Figure 2. Content of experimental red wines in total polyphenols from 'Burgun', 'Cabernet Sauvignon' and 'Merlot' control cvs. compared to clonal selections





The highest values regarding the concentration of wines in anthocyanins and polyphenols were evident in the clonal selection 'Merlot 202 St' followed by the reference cultivar. Even though the concentrations of these components in grapes can be affected by environmental conditions and the culture technology applied (Jackson and Lombard, 1993), the ratios between anthocyanin components and aromas are controlled by genotype.

'Cabernet Sauvignon' is one of the most widespread wine cultivars grown in Romania. In both experimental years the wines obtained from 'Cabernet Sauvignon 131 St' clone were highlighted by a higher content in total anthocyanins (they varied between 278.32 g/l in 2020 and 298.8 g/l in 2021) (Figure 4) and total polyphenols (2986.0 and 3352.77 mg GAE/I) (Figure 2) compared to the cultivar control. Similar results regarding the amounts of total anthocyanins in wine samples obtained from clone 54 of the 'Cabernet Sauvignon' cv. (255 g/l total anthocyanins) were also reported by Filip, 2018, in a study evaluating 3 'Cabernet Sauvignon' clones from the Valea Calugareasca area.



Figure 4. Content of experimental red wines in total anthocyanins from 'Burgun', 'Cabernet Sauvignon' and 'Merlot' control cvs. compared to clonal selections

## CONCLUSIONS

The suitable wines from the control varieties 'Fetească Regală', 'Muscat Ottonel' and 'Sauvignon Blanc' but also their clonal selection have acquired an alcoholic strength of over 12.0% vol. reaching up to 13.73% ('Fetească Regală 72 St'), in the year 2020 being able to recommend to be marketed as superior quality wines, according to the legislation in force.

The amounts of total polyphenols recorded higher values in the wines obtained from the clonal selections 'Fetească Regală 72 St', 'Muscat Ottonel 16 St', and 'Sauvignon Blanc 111 St' (470.90 mg GAE/l polyphenols, 449.3 mg GAE/l, respectively 418.32 mg GAE/l), compared to those obtained from the reference cultivars 'Feteasca Regală', 'Muscat Ottonel' and 'Sauvignon Blanc', in 2020.

The red wines obtained in the Stefanesti culture area are appreciated as highly extractive wines. This fact is confirmed by the results recorded at NRDIBH Stefanesti, where non-reducing extract values of over 19 g/l, were obtained in the 'Burgund 86 St' clonal selection (between 21.82 g/l and 26.02 g/l in the two years study), and 'Cabernet Sauvignon 131 St' (between 29.2 g/l in 2020 and, respectively, 26.85 g/l in 2021). These values ensure the wine's consistency and personality.

Among the clones for red wine, we emphasize the clone 'Merlot 202 St' and the reference variety from which it comes, which recorded the highest values of total anthocyanins and polyphenols.

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