

THE BEHAVIOR OF THREE CABERNET SAUVIGNON CLONES IN VALEA CĂLUGĂREASCĂ AREA

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Abstract

Cabernet Sauvignon variety is one of the most widespread grapevine for wine grown in Romania. By application of clonal selection, the only method to avoid the degeneration phenomenon of varieties to biotypes, five clones of Cabernet Sauvignon have been obtained and homologated in Romania. The present study aimed to analyse the behaviour of three Cabernet Sauvignon clones, namely Cabernet Sauvignon 30 Vl., 131 St. and 54 Mn. in the Valea Calugareasca viticultural area, considering the extension of their culture. The experiments were carried out during 2016-2017 harvest seasons, and were referred to the phenotypic spectrum, the fertility and productivity characteristics, the biological potential, the maturation dynamics of the grapes, the qualitative and quantitative evaluation of the grapes at harvest. These three Cabernet Sauvignon clones showed a similar phenological cycle and the behaviour to biotic factors, resistance to the pathogen attack being good and very good. During ripening, the sugar accumulation was similar at all clones (between 2.38 and 2.63 g/day) the amount of sugar accumulated on maturation period being between 49.9g and 55.2g. The glucoacidimetric ratio recorded very high values, characteristic for exceptional years (over 60%), mainly due to the very low value of total acidity. Acids metabolism has been achieved on average with 1.59-1.73 g/day. In terms of grape weight, the clone Cabernet Sauvignon 30 Vl. was highlighted with a greater weight of bunches with 11.5% compared with the Cabernet Sauvignon 54 Mn. and with 12.41% compared with the Cabernet Sauvignon 131 St. From the point of view of the polyphenolic composition, Cabernet Sauvignon 54 Mn. was noted by the value of total polyphenol index and the anthocyanic potential.

Key words: biotypes, clonal selection, Cabernet Sauvignon, glucoacidimetric ratio, polyphenol index.

INTRODUCTION

Cabernet Sauvignon is one of the most widespread vinegrapes varieties for wine grown in Romania (Șerdinescu et al., 2006; Uruçu, 2014). By application of clonal selection, the only method to avoid the degeneration phenomenon of varieties to biotypes, five clones of Cabernet Sauvignon have been obtained and homologated in Romania. Many authors have reported the distinction of some Cabernet Sauvignon clones from the point of view of wines quality, distinctive flavour of fruit aroma, higher content of tannins, anthocyanins (Jones and Davis, 2000; Fidelibus et al., 2006; Dejeu, 1986).

Clones of one variety differ from the population in better features of the grape and

better quality of wines obtained (Stefanini et al., 2000; Tebeica et al., 2005). From a great number of Cabernet Sauvignon clones, wines with distinctive flavour of fruit aroma, higher content of tannins, anthocyanins etc. are produced in France (Catalogue, 2009), Italy (Fidelibus et al., 2006), Australia and other countries.

The present study aimed to analyse the behaviour of three Cabernet Sauvignon clones, in the Valea Calugareasca viticultural area, namely Cabernet Sauvignon 30 Vl. obtained by ICDVV (Research-Development Institute for Viticulture and Oenology, Valea Calugareasca) and approved in 2010, Cabernet Sauvignon 131 St., obtained by INCDBH (National Research and Development Institute for Biotechnology in Horticulture, Stefanesti Arges) and approved in

2000 and Cabernet Sauvignon 54 Mn. obtained by SCDVV (Research and Development Station for Viticulture and Oenology, Minis Arad) and approved in 2006, considering the extension of their culture.

MATERIALS AND METHODS

Three Cabernet Sauvignon clones, namely Cabernet Sauvignon 30 Vl., Cabernet Sauvignon 131 St., and Cabernet Sauvignon 54 Mn., were taken into study.

The vines, were grafted on the SO4 (Oppenheim Selection 4) rootstock, were planted in 2014 in the germplasm collection belonging to the Research and Development Institute for Viticulture and Oenology, Valea Calugareasca. 24 vines per genotype were taken into study. The evaluation of clonal accessions focused on the duration of their phenological cycles, grape fertility and productivity, resistance to diseases, quantity and quality of the grapes production.

The recording of the vegetation phenophases was carried out weekly, following the methodology defined by the OIV (International Organization of Vine and Wine) descriptor list for grape varieties and *Vitis* species (2009).

Time of bud burst was noticed when 50% of the buds are in green shoot tip stage C of Baggioolini, stage 7 to 9 of BBCH (Biologische, Bundesanstalt, Bundessortenamt und Chemische Industrie scale). Time of full bloom was noticed when 50% of flowers are open. When 50% of the red grape clusters show changes in colour, or when about 50% of the berries of the white cluster start softening was noticed the time of beginning of berry ripening (veraison). Time of physiological stage of full maturity of the berry is related to the maximum sugar content of the berry due to photosynthesis. Mean value of all bunches of 10 shoots was taken into account.

The behaviour to biotic factors was assessed by using OIV ampelographic descriptor method, the notation being done through attribution of figures depending on the level of expression.

Production and the quality of grape harvest was calculated based on fertility coefficient and productivity index, the average length and average weight of bunch grapes and berries, sugar content and the total acidity of must.

Absolute and relative fertility coefficients were calculated using computation formulas based on the number of inflorescences, total shoots and fertile shoots. The productivity of varieties was determined at full grape maturation, using absolute and relative productivity indices and the weight of the grapes (Pop, 2003). The average weight of grape at full maturity was calculated by weighting 50 grapes per clones.

The sugar concentration was determined by a hand held digital refractometer and the results were expressed as an absolute value and as a percentage by mass of sucrose (OIV-MA-AS2-02 method). Titratable acidity was determined by titration with 0.1M NaOH, with 1% phenolphthalein and the results were expressed in gL^{-1} H_2SO_4 (OIV-MA-AS313-01 method).

The behaviour to biotic factors was assessed by using OIV ampelographic descriptor method, the notation being done through attribution of figures depending on the level of expression.

The phenolic potential was assessed by the standard ITV (Institute Technique de la Vigne-France) method (www.vignevin-sudouest.com) based on the following analytical parameters: anthocyanins, total anthocyanin potential and total polyphenol index.

Extraction of phenolic compounds from the grapes was done with an aqueous acid solution. Maceration of the samples was done for one hour at room temperature. Fifteen milliliters of ethanol (95%) and 85 mL of 0.1% HCl were added to the 50 g of grape juice. The samples were shaken for one minute from quarter to quarter. Coloured extracts are filtered through glasswool (or quantitative filter paper) in order to obtain clear solutions.

The dilution of the samples to 1/100 was done in double-distilled water followed by the measurement of the absorbance at 280 nm in a 1 cm quartz vat, nm against a blank of distilled water and the total polyphenolic index (IPT) was determined as follow:

Total polyphenolic index (IPT) = $\text{DO}_{280} \times 100 \times [(\text{weight of marc} + 100) / \text{weight of marc}]$.

The samples were diluted 1/20 in 1% hydrochloric acid solution and absorbance was measured at DO_{520} nm against a blank of distilled water. The concentration of anthocyanin and the total anthocyanic potential were determined following formulas:

Anthocyanins (mg/l) = DO 520 x 22.75 x 20
Total anthocyanic potential (mg/kg) =
anthocyanins (mg/l) x [(weight of marc + 100)/
weight of marc].

RESULTS AND DISCUSSIONS

Compared to multiannual averages, the wine year 2016-2017 is characterized by a higher thermal regime, due to a relatively normal rainfall regime. During the winter, there were no minimum temperatures to affect the viability of the fruit buds, with an absolute minimum of -12.8°C, recorded in January, compared to -13.6°C multiannual average.

Both medium and absolute high temperatures were (with a few exceptions) much higher compared to multi-year averages. It should be noted the high temperature regime in the winter months, when active temperature values of 11.6°C were recorded in December (compared to 5.7°C multiannual average) and 15.3°C in February 2016 (versus 14.7°C multiannual average).

The precipitation rate was very low in the winter months, when it was 0.2 mm in December compared to 44.5 mm in the multiannual average, but it became surplus in the spring months (87.6 mm versus 35.3 mm multiannual average recorded in March) and in July, favouring the development of cryptogamic diseases. In the warmest months (June, July and August) there were 81.8 mm, 70.2 mm and 78.2 mm.

The sum of average temperature °C for 2010-2016 ranged from -4.3°C in January to 134.7°C in September.

Compared to multiannual averages, the 2016-2017 wine years can be characterized as a year with reduced heliothermal resources on the background of rich water resources. The low heat regime recorded in April ($\Sigma^{\circ}t_{\text{actives}} = 226.7^{\circ}\text{C}$) caused a late start (about 10 days) and uneven vegetation in vineyard, a delay that was not recovered in May and June.

The average annual air temperature is 14.5°C and the monthly average is negative only in January (-3.3°C). The 18.4°C blooming temperature, the average July temperature of 21.2°C, the average August maximum temperature of 31.6°C and the average air humidity in August at 1:00 p.m. of 46-48%

indicates the presence of a relatively warm and drier climate. The active heat balance of the vegetation period records an average value of 3379°C and the thermal balance is 1779°C.

The precipitation from the vegetation period amounted to an average of 411.3 mm.

The high heliothermic regime in July, August and September, on a normal rainfall regime, even low in August and September, caused a good accumulation of sugars in grapes (Matei et al., 2009).

The development of the vegetation phenophases

Related to the climatic conditions of 2016 year, in Valea Calugareasca viticultural centre, it has been noticed that all the clones entered in vegetation at the end of March.

The beginning of budburst occurred on 30th March at Cabernet Sauvignon 30 Vl. and two days later in case of the other two clones. This phenophase ended in the second decade of the month of April, after sixteen days at Cabernet Sauvignon 30 Vl. and after fourteen days at the other two.

The two days difference was also observed in the case of blossoming, 50% of the inflorescences at Cabernet Sauvignon 30 Vl. being blossomed on 7th June, respectively on 9th June in case of the other two clones.

The ripening of the grapes started from 15th August. 50% of the grape clusters showed changes in colour on 21st August, date when was noticed the time of beginning of berry ripening (veraison), the same for the all clones. In comparison with Cabernet Sauvignon 30 Vl. which reached the maturation of the grapes at the end of September, the other two ended the maturation 6 - 7 days early, in the first decade of October.

Related to the climatic conditions of 2017 year, the budding occurred between 07 April and 27 April, 50% of the buds being in green shoot tip stage (stage C of Baggiolini, stage 7 to 9 of BBCH scale) (Baggiolini, 1952) were registered ten days later in comparison with 2016 year. Little differences were observed in the duration of budding and blossoming phases for the three evaluated clones. Difference was registered from ripening of grapes and harvest, full maturity of the berry being reached at the end of September for Cabernet Sauvignon 30

VI. and seven days later in case of the other two clones.

clones are characterized by medium fertility and productivity (Table 1).

Fertility and productivity

The high percentage of fertile branches in case of Cabernet Sauvignon 54 Mn. and Cabernet Sauvignon 131 St. ranged values between 70.90% and 77.08%, and Cabernet Sauvignon 30 VI., with values higher than 82%. All three

Diseases tolerance

According to the OIV norms, the Cabernet Sauvignon clones presented a good level of resistance to Mildew and a very good level to the attack of *Oidium* and *Botrytis cinerea* (Table 2).

Table 1. The fertility and productivity of the Cabernet Sauvignon clones in the Valea Calugareasca vineyard

Clones	Fertile branches (%)		Relative fertility coefficient		Absolute fertility coefficient		Relative productivity coefficient		Absolute productivity coefficient	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Cabernet Sauvignon 30 VI.	82.40	82.12	1.05	1.03	1.50	1.26	111.49	153.88	159.57	188.09
Cabernet Sauvignon 54 Mn.	70.90	71.80	1.07	0.98	1.50	1.37	140.14	131.52	196.85	188.69
Cabernet Sauvignon 131 St.	75.50	77.08	1.20	1.04	1.59	1.36	108.69	138.49	143.71	180.36

Table 2. Resistance to diseases at the Cabernet Sauvignon clones in Valea Calugareasca vineyard (years 2016 - 2017)

Clones	Diseases	Attack level (%)			
		Leaves		Grapes	
		2016	2017	2016	2017
Cabernet Sauvignon 30 VI.	Mildew	1,05±0.1	1.8±0.1	0,94±0.1	1.4±0.1
	<i>Oidium</i>	0,88±0.1	0.8±0.1	0,72±0.1	0.9±0.1
	<i>Botrytis</i>	-	-	1,00±0.1	0,6±0.1
Cabernet Sauvignon 54 Mn.	Mildew	0,77±0.1	1.9±0.1	1,15±0.1	1.3±0.1
	<i>Oidium</i>	0,95±0.1	0.8±0.1	0,99±0.1	0.8±0.1
	<i>Botrytis</i>	-	-	0,98±0.1	0,5±0.1
Cabernet Sauvignon 131 St.	Mildew	0,92±0.1	1.8±0.1	1,30±0.1	1.5±0.2
	<i>Oidium</i>	0,9±0.16	0.9±0.1	0,76±0.1	0.7±0.1
	<i>Botrytis</i>	-	-	0,95±0.1	0,5±0.1

Grapes quality

As a result of the evolution of climatic factors in the maturation period, weight grains were 11.17% for the Cabernet Sauvignon 30 VI. clone and a maximum of 45.95% at the clone Cabernet Sauvignon 131 St.

The degree of sugar accumulation was close to Cabernet Sauvignon clones (between 2.38 and 2.63 g L⁻¹/day). Acid metabolism was averaged 1.59-1.73 g L⁻¹/day.

Compared to the other two clones, the average weight of a cluster at Cabernet Sauvignon 30 VI. was higher with 18.92% - 31.06% in the climatic conditions of 2016 year and with 11.04% - 13.11% in 2017. Cabernet Sauvignon 30 VI. was distinguished by a greater average weight of the berry (126.92 g - 145.44 g) compared to Cabernet Sauvignon 54 Mn. and Cabernet Sauvignon 131 St. (88.11 g - 128.68 g) (Table 3).

The differences in berry weight had an effect on their structure (Table 4). At Cabernet Sauvignon 30 VI. the skin ranged between, 16.4 - 18.6% and seeds between 4.2 - 4.7%, correlated with a higher content in pulp (77.2-78.9%).

Table 3. Mechanical analysis of grapes at harvest

Clone	Grape				Cluster		Berry	
	Weight (g)		Volume (ml)		Weight (g)		Weight (g)	
	2016	2017	2016	2017	2016	2017	2016	2017
Cabernet Sauvignon 30 VI.	130.97	149.40	120	136.67	4.05	3.97	126.92	145.44
Cabernet Sauvignon 54 Mn.	106.18	133.79	98.33	118.33	3.84	4.27	102.35	129.52
Cabernet Sauvignon 131 St.	90.29	132.91	78.33	118.33	2.18	4.23	88.11	128.68

Table 4. Berry structure

Clone	Skin (%)		Seeds (%)		Pulp (%)	
	2016	2017	2016	2017	2016	2017
Cabernet Sauvignon 30 VI.	16.4	18.6	4.7	4.2	78.9	77.2
Cabernet Sauvignon 54 Mn.	18.4	22.2	5.1	4.2	76.4	73.5
Cabernet Sauvignon 131 St.	25.8	20.0	5.8	4.8	68.5	75.2

Chemical composition of the stum

The potential for the accumulation of sugars in the stum, a characteristic of the variety, influenced by the climatic factors of the grape maturation period, was very high at all genotypes, in terms of acidity and low pH (Table 5). Grapes from the vintage 2017 had better sugar accumulation potential due to favourable weather conditions during the ripening stage.

Analysis of the polyphenolic potential of grapes

From the point of view of the polyphenolic composition, the Cabernet Sauvignon 54 Mn. clone is evidenced by the total polyphenol index and the anthocyanin potential.

The lowest values were obtained for clone 131 St. (average data over the two years of study) (Figure 1).

Table 5 Chemical composition of the stum

Clones	Weight of 100 berries (g)		Volume 100 berries		Sugar (g/l)		Acidity (g/l H ₂ SO ₄)		pH	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Cabernet Sauvignon 30 Vl.	110.86	120.84	100	105	245.9	223.6	3.28	3.61	3.22	2.17
Cabernet Sauvignon 54 Mn.	106.97	108.58	95	95	224.9	219.4	2.95	3.21	2.64	2.29
Cabernet Sauvignon 131 St.	106.68	129.81	95	115	227.9	227.9	2.99	3.36	2.62	2.20

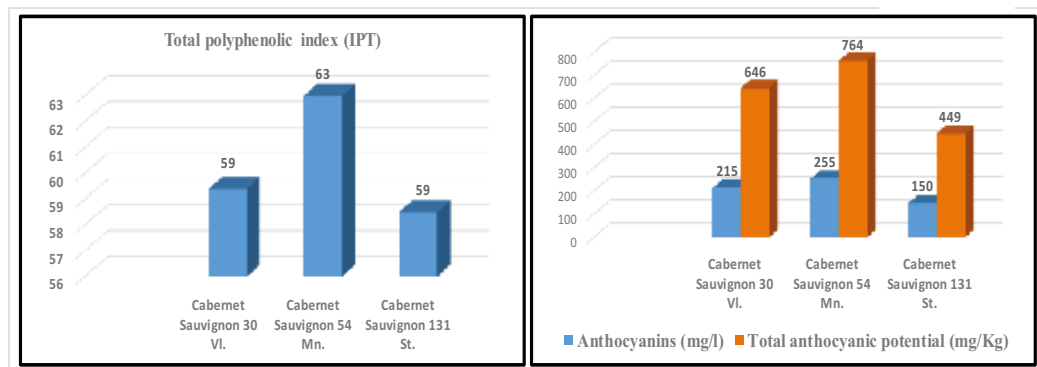


Figure 1. The polyphenolic potential of grapes (Average values 2016-2017)

The glucoacidimetric ratio records very high values of exceptional years (over 60%). The increase in this ratio is mainly due to the very low value of total acidity.

Between the degree of tolerance to cryptogamic diseases and the values of total polyphenol index (IPT) was recorded a very tight correlation.

The degree of mildew attack on grapes (Figure 2), with values ranging between 1.3% and 1.5%, influenced the value of the total polyphenolic index (IPT) of the grape must at harvesting by almost 80%, the value of the coefficient of determination R squared being 0.7961.

The intensity of the *Oidium* attack influenced the value of the total polyphenolic index to almost 90% ($R^2 = 0.898$) (Figure 3).

The degree of correlation shows a very high value in the case of the *Botrytis* attack (Figure 4), the value of the total polyphenolic index being determined almost entirely (99 %) by the frequency of the attack (Urucu, 2014).

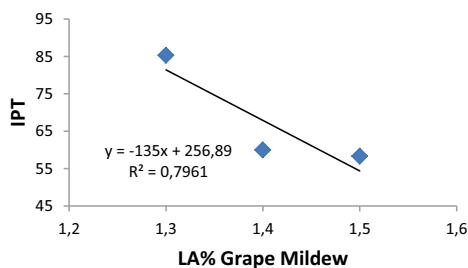


Figure 2. The Polyphenol Index (IPT) in Grape Mildew

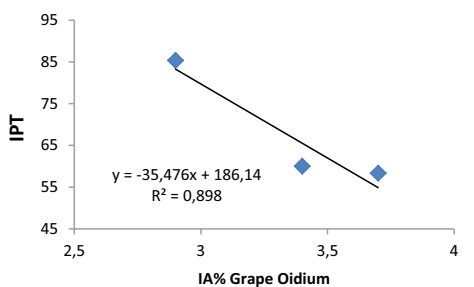


Figure 3. The Polyphenol Index (IPT) in *Oidium* attack

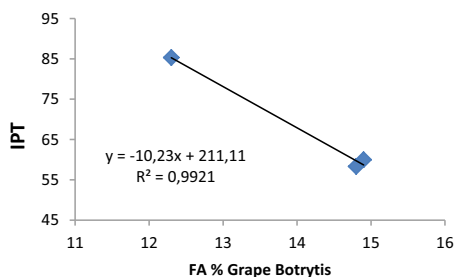


Figure 4. The Polyphenol Index (IPT) in *Botrytis* attack

CONCLUSIONS

The development of the vegetative phenophases depends on the climatic conditions during 2016 and 2017 harvest. Differences were registered concerning the maturation of the grapes which occurred 6-7 days later in case of Cabernet Sauvignon 54 Mn. and Cabernet Sauvignon 131 St.

Cabernet Sauvignon 30 Vl. highlighted by a greater weight of the cluster and berries.

The potential of sugar accumulation in the must was high in case of all clones, while the acidity and pH values were low.

Concerning the polyphenolic composition, Cabernet Sauvignon 54 Mn. was noted by total polyphenolic index (IPT) and Anthocyanin potential. The degree of correlation between polyphenolic index and frequency of the main diseases attack shows a very high value in the case of the *Botrytis* attack (99%).

Further studies will be done concerning the quality of wines in order to establish the degree of maintenance of wines tipicity.

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